Introduction

Development of ultrasonography

Recent achievements in high-resolution ultrasound technology over the past two decades enabled the study of the sono-anatomy and function of the embryo and fetus, greatly improved our knowledge of normal embryonic and fetal development, thus refined the prenatal diagnosis of complex congenital malformations. Ultrasonography has become an integrated part of current obstetric care.

The guidelines of the Hungarian Society of Ultrasound in Obstetrics and Gynecology (MSZNUT) clearly define the requirements for obstetric screening examinations. The prime objective of ultrasound screening during pregnancy is the detection of abnormal embryonic/fetal morphology and function. In Hungary pregnant patients are scheduled for screening at the 12th, between the 18 to 20th, 30 to 32nd, and at the 36th weeks of gestation.

Two-dimensional (2D) ultrasound is visualizing tomographic images, the number of planes is restricted, photo-realistic surface or transparent delineation of structures is not feasible, volume measurements are inaccurate, reproduction of images is difficult, dysmorphologic evaluation is limited.

Three-dimensional (3D) ultrasound has revolutionized imaging, because the visualizing capacity of this technique is practically unlimited. The most spectacular result of 3D fetal imaging is the „sculpture-like" picture. Surface rendering is helpful in outlining fetal surface structures. Fetal skeleton can be studied with the application of transparent maximum mode. The introduction of 3D ultrasound technique resulted in significant improvement of diagnostic accuracy with shorter exam period. With spatial presentation the anatomy of the embryo and fetus can be studied in detail, thus several congenital malformations may be diagnosed earlier. Fetal volume measurements in combination with 3D perfusion studies allow us to examine physiologic organ-function and pathologic conditions of the developing fetus. With the application of power Doppler-based color histogram analysis quantitative organ and tissue perfusion studies can be done. We can update our knowledge in fetal physiology. In the second and third trimesters we can better predict high-risk pregnancies. Digital 3D imaging and data storage (archivation) enables further „off-line" assessment without the presence of the patient: re-evaluation of already diagnosed malformations, detection of new subtle defects. We can take advantages of interdisciplinary domestic or international consiliary consultation via telemedicine.

At the beginning of the XXIst century „real-time 3D", also known as „live-3D" or 4D ultrasound technique has been introduced into clinical practice. With the advent of continuous „real-time" spatial presentation we can study the intrauterine development, behaviour, personality, and individualism of the embryo and fetus.

With the implementation of endovaginal 3D sonography in gynecologic diagnosis volumetry of the ovaries and uterus under normal or pathologic conditions may be completed with individual 3D spatial flow-studies, color histogram analysis and with quantitative tissue-/organ-perfusion measurements.

At the Department of Obstetrics and Gynecology of University of Pécs we perform 3D/4D ultrasound examinations as of the year 2000.

New approaches for the prevention of respiratory distress syndrome

Respiratory distress syndrome (RDS) is one of the most common causes of neonatal morbidity and mortality in premature infants. Antenatal maternal corticosteroid (ANCS) treatment is indicated for the prevention of RDS in expectation of preterm birth. ANCS therapy is only effective if delivery occurs at least 24-48 hours after the first dose was given. In human preterm infants, repeated ANCS therapy resulted in intrauterine growth restriction (IUGR). Recent data suggest that in pregnancies complicated with preeclampsia (PE) and/or IUGR the incidence of RDS and the effectiveness of ANCS is a controversial issue. Potential maternal and fetal side effects of glucocorticoids have also been reported.

A single direct fetal injection of betamethasone has been shown to improve ventilatory and cardiovascular functions and augment postnatal metabolic adaptive responses in prematurely delivered animals. Animal studies demonstrated, that direct fetal intramuscular corticosteroid (DFCS) administration induced lung maturation without apparent effect on fetal growth. Ultrasonography-guided DFCS injection for the prophylaxis of RDS was first applied by our perinatal group in high-risk human pregnancies, when preterm delivery was expected to occur within 24 to 48 hours.

The introduction of colored power angiography (CPA) has opened new perspectives for the analysis of tissue blood perfusion in the human fetus. By means of 2D-CPA it was shown that there is an increase in fetal lung perfusion after ANCS administration. The impact of DFCS therapy for the prophylaxis of RDS on feto-placental circulation in high-risk human pregnancies has not yet been investigated.

New observations in polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrinopathy affecting women of reproductive age. The syndrome is characterized by polycystic ovarian morphology, clinical and biochemical features of hyperandrogenism and chronic anovulation. Key features include menstrual cycle disturbance, hirsutism, obesity, infertility and biochemical evidence of hyperandrogenemic anovulation. At a recent ASRM/ESHRE consensus meeting, refined ultrasonographic definition of PCOS was agreed.

For the treatment of infertility associated with PCOS ovulation induction with clomiphene citrate and/or insulin sensitizers seems to be the simpliest method. In the event of „clomiphene failures", the next step may be stimulation of the ovaries by exogenous gonadotropins or surgical manipulation of the ovaries. Laparoscopic unipolar electrocautery was found to be effective in restoring ovulation for at least 6 months. As a consequence of profound changes in gonadotropin secretion in response to ovarian surgery, resumption of normal menstrual cyclicity with subsequent ovulation has been observed.

Vascular and flow-patterns of angiogenesis in reproductive tissues clearly represent morphology and function. Thus alterations in ovarian vascularity and perfusion may correlate with changes of consequtive cyclic events involving follicular growth and hormone production.
The availability of endovaginal CPA has made the study of pelvic organ perfusion possible. Three-dimensional sonographic characterization of polycystic ovaries have been performed recently. The impact of laparoscopic treatment on both ovarian volume and vascular flow-patterns assessed by 3D-CPA, however, have not yet been elucidated.

Objectives

There were three main objectives of this thesis:

1. Complex 3D/4D ultrasound examination of normal and abnormal embryonic and fetal development:

- investigation of surface defects,
- volume measurement of internal organs,
- transparent delineation of the fetal skeleton,
- spatial presentation of blood flow.

A detailed analysis of testing:

- the capability of 3D/4D ultrasound examination to confirm or rule out suspected clinical diagnosis achieved by the conventional 2D ultrasound scan,

- the sensitivity and specificity of 3D/4D ultrasound in detection of congenital malformations in the light of embryo/feto-pathologic or postnatal clinical examinations as compared with 2D ultrasound.

2. Two-D/3D ultrasound examination of the hemodynamic changes of feto-placental circulation after DFCS treatment:

Investigation of the impact of ultrasonography-guided DFCS therapy for the improvement of postnatal cardio-pulmonary adaptation of extremely-low birth weight premature infants:

- on umbilical and middle-cerebral artery circulation by 2D Doppler-flow-velocimetry,
- on fetal lung perfusion by quantitative 3D Doppler-flow studies and with the application of color histogram analysis.

3. Transvaginal 3D ultrasound examination of the ovaries of infertile patients with polycystic ovary syndrome:

Three-dimensional ultrasound assessment of ovarian volume using „VOCAL" (Virtual Organ Computer-aided AnaLysis) software before and after:

- laparoscopic electrocautery,
- gonadotropin-releasing hormone (GnRH) agonist analogue treatment,
- metformin therapy.

Evaluation of the ovarian vascular flow-patterns using 3D-CPA and histogram analysis before and after:

- laparoscopic electrocautery,
- GnRH agonist analogue treatment,
- metformin therapy.

Determination of serum gonadotropin and androgen levels as well as urinary steroids before and after surgery or medical (GnRH analogue, metformin) treatment.

Comparison of the hormonal (serum and urinary) and clinical effects of laparoscopic surgical or medical (GnRH analogue, metformin) treatment with 3D sonographic findings.

Patients and Methods

Complex 3D/4D ultrasound examination of normal and abnormal embryonic/fetal development

At the Department of Obstetrics and Gynecology of University of Pécs between October, 12, 2000 and December 31, 2003 we performed 36841 transabdominal and 9253 transvaginal 2D

ultrasound examinations in pregnant women. These included routine screenings, diagnostic and follow-up scans, as well as ultrasonography-guided invasive prenatal diagnostic procedures. Two-D scans were done using *Hitachi EUB 555 (Hitachi Med. Co., Tokyo, Japan)* and *Sonoace 6000 C (Medison Co., Seoul, South Korea)* „real-time scanners" equipped with 3.5 MHz (Hitachi) and 3-7 MHz (Sonoace) convex abdominal and with 5-6.5 MHz (Hitachi) and 4-9 MHz (Sonoace) endovaginal transducers, respectively.

During the investigation period 6080 complex 3D/4D ultrasound examinations were performed using *Kretz Voluson 730D (Kretztechnik, Zipf, Austria)* and *Sonoace 8000 Live (Medison Co., Seoul, South Korea)* „real-time 3D scanners" equipped with compatible 4-8 MHz abdominal volume-, and 3-7 MHz (Kretz Voluson) and 5-8 MHz (Sonoace) vaginal volume-transducers, respectively. Three-D/4D scans were done at the 12th, 20th, and 28-32nd weeks of singleton pregnancies and between the 22nd and 24th weeks of multiple pregnancies.

The 3D/4D scan at the 12th week of gestation included surface reconstruction, volumetric, transparent, and 3D-CPA-based flow-examinations of the embryonic face, profile, orbits, nose, maxilla, mandibula, and of brain, followed by detailed analysis of the embryonic thorax, abdomen, spine, and extremities. We drew special attention to follow the development of central nervous system, neural tube, and uropoetic system. For the measurement of embryonic nuchal fold thickness the median sagittal plane of the multiplanar 3D image was selected.

The 3D/4D scan at the 20th week of gestation integrated assessment of the fetal face, profile, orbits and eyes, the nose, nostrils, phyltrum, upper and lower lips, the chin, cheeks, and ears. We analysed specific movement and mimic of the fetal face and evaluated intracranial anatomy in detail (multiplanar imaging, volumetry). Fetal thorax was studied with surface and transparent maximum modes, fetal heart was observed with „live-3D". Three-D sonography of the fetal lungs was done with volumetry and transparent minimum mode. With the application of 3D color histogram analysis quantitative measurements of the pulmonary blood perfusion were accomplished. Examination of the fetal abdomen included assessment of the abdominal wall, esophagus, stomach, duodenum, liver, kidneys, ureters, and urinary bladder using volumetry and transparent minimum mode, occasionally multiplanar imaging and perfusion studies. For visualization of the fetal extremities, hands and feet, fingers and skeleton (babygram) 3D transparent maximum and X-ray modes were applied. External genitals were depicted using 3D surface reconstruction. The 3D/4D scan between the 28th and 32nd weeks of gestation included the protocol of the previous screening and was supplemented with CPA-based organ-perfusion studies of the feto-placental circulation.

Ultrasound examinations were carried out by OB&GYN specialists licensed with level „B" and „C" sonography certificate issued by the MSZNUT.

Two/three-dimensional ultrasound examination of the hemodynamic changes of fetoplacental circulation after DFCS therapy

We analyzed the perinatal data of 60 neonates born to mothers with severe PE and/or IUGR between the 24th and 32nd weeks of gestation from May 1, 1996, to December 31, 2002, at our perinatal unit. All fetuses were treated with DFCS for the prophylaxis of RDS, because termination of pregnancy for combined maternal/fetal interest was inevitable and fetal lung maturity tests showed immature index values.

Fetal steroid injection and Doppler examinations

Written consent was obtained from every patient. After 2D transabdominal ultrasound examination Doppler-flow-velocimetry of the umbilical and middle-cerebral arteries (UA, MCA) and 3D-CPA of fetal lung perfusion was carried out. Patients underwent amniocentesis and amniotic fluid samples were assayed immediately for fetal lung maturity tests. The needle was then moved to the fetal thigh and a single shot of intramuscular betamethasone was given to the fetus under continuous ultrasound-guidance in a dose of 0.5 mg/kg estimated fetal weight. After maternal consent fifteen fetuses were given 1 ml of isotonic NaCl-solution prior to the fetal intramuscular betamethasone shot.

Doppler-flow examinations were performed using *Hitachi EUB 555 (Hitachi Med. Co., Tokyo, Japan)* „real-time" scanner equipped with convex transabdominal 3.5 MHz transducer and with pulsed and color Doppler-options. Measurements were made during fetal quiescence. UA waveform was measured at placental insertion site, MCA waveform was recorded at a point approximately 1 cm from its origin at the circle of Willis. In 25 pregnancies Doppler flow examinations of the UA and in 20 pregnancies Doppler measurements of the MCA were available one day before, immediately, and one day after DFCS administration.

For statistical analysis Student's t-test was applied. Data are represented as mean ± SD. P values of < 0.05 were considered to be statistically significant.

Spatial presentation of fetal lung perfusion

Examinations were performed using Kretz Voluson 730D (Kretztechnik, Zipf, Austria) „real-time" 3D-scanner, equipped with transabdominal 4-8 MHz volume transducer. In all patients identical preinstalled instrument settings were used. After visualizing the fetal heart and

lungs at cross section in 2D B-mode the mobile sector for angio mode was switched on and set up to cover an (arbitrarily) standardized region of interest (ROI) in the right upper lobe of the fetal lung (margins: wall of the right ventricle of the heart, clavicula, and the first rib), then the 3D volume mode was engaged. The volume sector angle was preset to 60° and the fast volume acquisition setting was selected to avoid motion artifacts. The acquired 3D lung volumes were stored immediately in the machine. All stored volumes were analysed later by myself using the VOCAL^{TM} imaging program (Virtual Organ Computer-aided AnaLysis), which is integrated into Voluson 730D ultrasound system. The contour mode in the VOCAL program was set to manual. The longitudinal view was used as a reference image and the rotation step was selected as 30°, resulting in the definition of six contours for each lung. Once a contour was defined in all image planes, the volume of the standardized fetal lung tissue portion was obtained. After definition of the contour the VOCAL program automatically calculates index values for gray-scale and color-scale voxels (smallest unit of volume). According to these values *vascularisation flow index (VFI)* was calculated, which measures the ratio of the mean value of color voxels and all the voxels in the defined lung-portion contour, and is a feature of both vascularization and flow. Three-dimensional-CPA of fetal lung perfusion was carried out in 15 pregnancies before NaCl and DFCS injection and in every two minutes for 10 minutes after procedure, then in every hour thereafter up to 26 hours.

Transvaginal 3D ultrasound examination of the ovaries of patients with PCOS

Subjects

Twenty four patients (age: 18-36 years) with PCOS were recruited from infertility clinic of University of Pécs. The diagnosis of PCOS was based on the "Rotterdam consensus statement". All patients had failed to ovulate in response to clomiphene citrate administered for 3 months. Ten infertile patients, who desired to conceive were counseled to proceed to laparoscopic ovarian unipolar electrocautery. In another group of eight patients with PCOS and hirsutism women were treated with monthly intramuscular injections of GnRH analogue (Decapeptyl Depot®, Ferring, Germany) for 3 months. In the third group of six patients with PCOS and associated hirsutism and oligo-amenorrhoea women received twelve-month metformin therapy in a dose of 2x850 mg/day (Merckformin 850mg-Merck, Germany), because the combined OAC therapy with ciproterone-acetate was contraindicated for thrombophylia (protein C and S defficiency, MTHFR-mutation).

Serum gonadotropin (FSH, LH), androgen (T), and estradiol (E2) levels were determined before and after surgery or medical (GnRH analogue, metformin) treatment. To evaluate 5α-

reductase enzyme activity in the skin and liver, specific ratios of steroids (An/Et, and aTHF/THF) were calculated from 24-hour urine samples by column gas chromatography before and after surgery or medical (GnRH analogue) treatment. Changes in the ratio of androgen metabolites to cortisol metabolites (AM/CM) before and after surgery or medical (GnRH analogue) treatment were also evaluated.

Three-dimensional-CPA and histogram analysis of intraovarian circulation

Examinations were performed using Kretz Voluson 730D ultrasound scanner equipped with transvaginal 3-7 MHz volume transducer (100° field of view). In all patients identical preinstalled instrument settings were used. After visualizing the ovary in 2D B-mode, the mobile sector for angio mode was switched on and set up to cover the ROI, then the 3D volume mode was engaged. The volume sector angle was preset to 90° and the fast volume acquisition setting was selected to avoid motion artifacts. The acquired 3D volumes were stored immediately in the ultrasound machine. All stored volumes were analysed later by myself using the VOCAL^{TM} imaging program which is integrated into Voluson 730D ultrasound system. The contour mode in the VOCAL program was set to manual. The longitudinal view was used as a reference image and the rotation step was selected as 30°, resulting in the definition of six contours for each ovary. Once a contour was defined in all image planes, the volume of the ovary was obtained. After definition of the contour the VOCAL program automatically calculates index values for gray-scale and color-scale voxels. According to these values four indices are calculated: *vascularisation index (VI), flow index (FI), VFI,* and *mean grayness (MG)*. VI is thought to represent the presence of blood vessels in the tissue-ROI (vascularization) and is expressed as a percentage value. FI (scale 0..100), the mean value of the color voxels, is thought to express the average intensity of flow in the vessels. VFI is a feature of both vascularisation and flow. MG (scale 0..100) expresses the gray-scale brightness or echogenicity of the tissue. Three-dimensional-CPA and histogram analysis of intraovarian circulation was carried out in all patients before laparoscopic electrocautery or medical (GnRH analogue, metformin) treatment, plus one week, and one and 4 months after laparoscopic electrocautery, as well as one, two, and three months after GnRH analogue treatment, and monthly after metformin therapy for one year (if menstrual cycle resumed, on cycle-day 3). All quantitative measurements were done by myself. Doppler settings were not changed during examination. The ROI covered the whole ovary. Ovaries were examined bilaterally, and the average value from both sides was used as a single parameter.

For statistical analysis of the ovarian volume and flow parameters Student's t-test was applied and data were expressed as mean ± SD. Serum and urinary hormone values are represented as mean ± SEM. Since normal distribution of the data could not be assumed, the non-parametric tests for paired (Wilcoxon test) and unpaired (Mann-Whitney U-test) samples were employed. P values of < 0.05 were considered to be statistically significant. Clinical outcome was also evaluated concerning resumption of menstrual cyclicity, ovulation and pregnancy rates during a one-year follow up.

Results

The efficacy of ultrasound screening for fetal malformations with the application of 2D and 3D sonography

At the Department of Obstetrics and Gynecology of University of Pécs between October, 12, 2000 and December 31, 2003 we detected 340 cases of fetal malformations with routine 2D ultrasound exam. Of them suspected diagnosis by 2D ultrasound was confirmed with 3D sonography in 242 cases. Feto-pathologic evaluation or postnatal clinical assessment proved congenital malformations in 246 cases. Two-D ultrasound examination was false positive in 94 cases (27.6%) compared with 98.3% sensitivity of 3D sonography.

Suspected anomalies by 2D sonography were confirmed in 100% of cases of anencephaly, gastroschisis, and omphalocele, in more than 90% of cases of cystic hygroma (94.5%), and of anomalies of the uropoetic system (93.5%). An acceptable detection rate of cases of hydrocephaly/ventriculomegaly (87.9%) and skeletal anomalies (83.4%) was achieved by 2D ultrasound screening. The efficacy of 2D screening was poor in the detection of fetuses with intestinal dilatative leasions and with neural tube defects (NTD).

Congenital anomalies diagnosed by 3D sonography were fully confirmed in cases of anencephaly, ventriculomegaly, holoprosencephaly, cleft lip, cystic hygroma, diaphragmatic hernia, gastroschisis, omphalocele, and of anomalies of the uropoetic system, of ovarian cysts, and skeletal malformations. We emphasize the excellent sensitivity of 3D sonography in the detection of fetuses with NTD (96%) and compare it with the poor pick-up rate of 2D screening (43.4%). This fact is of value and concern, because NTD-s together with other CNS malformations account for the most frequent congenital birth defects during the investigation period. Given the high expences of 3D ultrasound instrumentation and examination, and the lack of sufficient domestic experience with this technique routine ultrasound screenings during pregnancy were always done first with high-resolution 2D machines, then we proceeded to

3D/4D sonography in the event of a suspected anomaly. In another words 3D/4D sonography was already performed for diagnostic purposes, and this could considerably contribute to better efficacy of 3D technology.

Hemodynamic changes of feto-placental circulation after DFCS therapy

Mean gestational age at birth and birth weight were 29.7 weeks and 1125 g, respectively. The mean time interval between fetal intervention and delivery was 2.1 days. The incidences of RDS and intraventricular hemorrhage were 35.0% and 20.0%, respectively. Thirty newborn infants (50.0%) required ventilatory support for an average of 7.1 days. Bronchopulmonary dysplasia developed in 3 neonates (5.0%). Ophthalmologic examination revealed 6 cases of retinopathy of prematurity (10.0%). Eight extremely-low-birth weight infants were lost (mean gestational age 25.6 weeks, birth weight 755 g), giving a 28-day survival rate of 86.7%.

In 25 pregnancies pulsatility index (PI) significantly increased in the UA immediately after DFCS injection (1.00 ± 0.08 to 1.30 ± 0.12, $p<0.01$) and returned to the initial value one day after intervention.

In 20 pregnancies semiquantitative measurements of fetal cerebral blood flow in the MCA showed significant decrease in both peak systolic/end-diastolic velocity ratio (S/D), resistance index (RI), and PI values one day after treatment (from 3.90 ± 0.62 to 3.35 ± 0.70, $p<0.05$; from 0.71 ± 0.03 to 0.62 ± 0.05, $p<0.01$; from 1.49 ± 0.22 to 1.20 ± 0.20, $p<0.01$, respectively).

The mean ± SD volume of the standardized fetal lung tissue portion was 54.0 ± 3.1 cm^3. Consequtive volume measurements in the same fetus served as autocontrols to estimate accuracy and reproducibility of the 3D contour examination. With 3D-CPA within eight minutes after DFCS injection a 3.13-fold increase of the mean initial VFI value was observed, that sustained for 26 hours (mean ± SD initial, 8-minute-, and 26-hour-VFI values: 1.529 ± 0.142, 4.793 ± 0.783, 4.260 ± 0.820, respectively, $p < 0.05$).

Changes of hormone levels and intraovarian circulation in PCOS

<u>The effect of laparoscopic electrocautery</u>

Serum LH and T levels decreased significantly one week after laparoscopy (from 9.9 ± 2.1 to 7.9 ± 1.8 IU/L, and from 3.4 ± 0.7 to 2.8 ± 0.6 nmol/L, respectively, $p < 0.05$). Both serum LH and T concentrations remained low one and 4 months after ovarian surgery. Specific ratios of urinary steroids reflecting 5α-reductase enzyme activity significantly decreased one week after

ovarian drilling (An/Et: from 1.7 ± 0.7 to 1.4 ± 0.6; aTHF/THF: from 0.8 ± 0.2 to 0.6 ± 0.1; $p < 0.05$), as did the PCOS-characteristic elevated ratio of androgen metabolites to cortisol metabolites (AM/CM: from 1.0 ± 0.3 to 0.8 ± 0.2, $p < 0.05$). Serum FSH levels increased significantly 7 days after laparoscopic ovarian electrocautery (from 4.7 ± 1.5 to 12.9 ± 2.7 IU/L, $p < 0.05$ and remained high one and 4 months after surgical treatment.

Seven out of 10 (70%) women had documented biochemical evidence of ovulation with clinical resumption of menstruation within the next 4 months following laparoscopic surgery. Five pregnancies concieved within 1 year following ovarian diathermy.

Mean ± SD ovarian volume significantly decreased one week after surgery (from 13.436 ± 2.874 to 10.164 ± 2.013 cm^3, $p < 0.05$. We detected a mild, but continuous increase of VI throughout the 4-month follow-up period after laparoscopy, but it did not reach statistical significance. Both FI and VFI increased significantly 7 days after surgical intervention (mean ± SD of FI before and 1 week after electrocautery: 14.249 ± 2.357 and 18.248 ± 2.811, respectively, $p < 0.05$; mean ± SD of VFI before and 1 week after electrocautery: 0.114 ± 0.046 and 0.207 ± 0.048, respectively, $p < 0.05$). The difference between the mean initial and 4-month FI and VFI values was even more pronounced.

Serum LH, T concentrations and specific ratios of urinary steroids, reflecting 5α-reductase enzyme activity, have changed parallel with ovarian volume, and inversely with FI and VFI values following laparoscopic electrocautery. In contrast, serum FSH levels and the subsequent chance for resumption of menstrual cyclicity within 4 months have changed parallel with ovarian FI and VFI values after surgery. An inverse correlation was found between serum FSH levels and ovarian volume after laparoscopy.

<u>The effect of medical treatment</u>

GnRH analogue

Serum LH and T levels decreased significantly after 3-month GnRH analogue treatment (from 12.0 ± 2.5 to 0.5 ± 0.1 IU/L, $p < 0.01$; and from 3.7 ± 0.8 to 2.6 ± 0.5 nmol/L, $p < 0.05$, respectively). Specific ratios of urinary steroids reflecting 5α-reductase enzyme activity significantly decreased after 3-month GnRH analogue treatment (An/Et: from 2.10 ± 0.25 to 1.6 ± 0.2; aTHF/THF: from 1.1 ± 0.1 to 0.8 ± 0.1; $p < 0.05$), as did the PCOS-characteristic elevated ratio of androgen metabolites to cortisol metabolites (AM/CM: from , 0.8 ± 0.2 to 0.5 ± 0.1, $p < 0.05$). We detected a mild, but continuous increase of serum FSH levels throughout the 3-month GnRH analogue treatment, but it did not reach statistical significance. Serum E2 concentrations

decreased to postmenopausal values shortly after the first GnRH analogue injection was given, and remained at low levels throughout the 3-month therapy. PCOS-associated hirsutism has improved moderately in each patient in response to 3-month GnRH analogue treatment.

Mean ± SD ovarian volume significantly decreased after 3-month GnRH analogue treatment (from 9.771 ± 2.089 to 6.912 ± 1.368 cm^3, $p < 0.05$. After an initial „flare-up" effect both VI, FI, and VFI values, representing intraovarian vascularisation and circulation decreased gradually throughout the 3-month GnRH analogue treatment (mean ± SD of VFI before and 3 months after GnRH analogue treatment: 0.153 ± 0.061 and 0.015 ± 0.006, respectively, $p < 0.05$). In response to GnRH analogue treatment serum LH, T concentrations, and specific ratios of urinary steroids, reflecting 5α-reductase enzyme activity, as well as AM have decreased parallel with ovarian volume and VFI values. Inversely, serum FSH levels mildly increased.

Metformin

Serum LH and T initial levels remained high after 12-month metformin therapy (10.7 ± 2.2 vs 10.5 ± 2.2 IU/L, $p > 0.05$; and 3.6 ± 0.8 vs 3.4 ± 0.7 nmol/L, $p > 0.05$, respectively).

Serum FSH levels have not changed as a result of one-year metformin therapy (mean ± SEM FSH values before and after twelve-month metformin therapy: 4.1 ± 1.3 and 4.2 ± 1.4 IU/L, respectively; $p > 0.05$). We observed a mild, but continuous increase of E2 levels throughout the 12-month metformin therapy, but it did not reach statistical significance. (mean ± SEM E2 values before and after one-year metformin therapy: 174 ± 7.8 and 248 ± 10.4 pmol/L, respectively; $p > 0.05$).

PCOS-associated hirsutism has not improved within 12 months after continuous metformin treatment. Two out of six (33%) patients with secondary amenorrhoea developed oligomenorrhoea by the end of one-year treatment, and in one patient (17%) menstrual cycle resumed as of the fourth month of metformin therapy. All of these three women had documented biochemical evidence of ovulation.

Mean ± SD ovarian volume has not changed significantly during one-year metformin therapy, although its initial value slightly increased 12 months after continuous metformin treatment (from 12.205 ± 2.610 to 16.0 ± 3.422 cm^3; $p > 0.05$). VI values, representing intraovarian vascularisation significantly increased in response to 12-month metformin therapy (mean ± SD of VI values before, three, and 12 months after continuous metformin treatment: 0.178 ± 0.097%, 0.184 ± 0,100%, and 1.663 ± 0.912%, respectively; $p < 0.01$). FI and VFI values, representing intraovarian circulation significantly increased as a result of metformin

therapy (mean ± SD of FI values before, three, and 12 months after continuous metformin treatment: 21.171 ± 3.261, 34.595 ± 5.329, and 29.8 ± 4.590, respectively; $p < 0.05$); (mean ± SD of VFI values before, three, and 12 months after continuous metformin treatment: 0.047 ± 0.018, 0.069 ± 0.027, and 0.505 ± 0.203, respectively; $p < 0.01$).

Twelve-month metformin treatment has not influenced serum LH, T, and FSH concentrations. Serum E2 levels and ovarian volume slightly increased, subsequently, parameters reflecting intraovarian vascularisation (VI) and circulation (FI, VFI) significantly increased.

Conclusions

The significance of 3D/4D ultrasonography in prenatal diagnosis

Three-D ultrasound technology revolutionized sonographic imaging, because the visualizing capacity of this technique is practically unlimited. New 3D/4D ultrasound systems are capable to show multiplanar views or reconstruct surface and transparent volume-images.

The most significant advantages of 3D/4D ultrasound technology are diagnostic accuracy, fast scanning, coronal views, spatial presentation and photo-realistic „sculpture-like" imaging of small structures, excellent reproducibility. Fetal volume measurements in combination with power Doppler-based color histogram analysis enable the study and quantitative assessment of both morphology and fetal blood perfusion. Demonstration of fetal surface defects (surface mode), transparent delineation of the fetal skeleton (maximum mode), outlining of hypoechoic non-reflective structures (minimum mode), 3D-volumetry of internal organs, spatial presentation and quantitative evaluation of fetal blood flow result in complex analysis of the embryonic/fetal anatomy and physiology, thus several congenital malformations may be diagnosed earlier and with better accuracy. Isolated „minor anomalies" suspicious for chromosomal abnormality may be detected easier with rotation of 3D portrait-like volume-images or with „live-3D" in real-time motion. In the third trimester we can better predict high-risk pregnancies.

Digital 3D imaging and data storage enables further „off-line" assessment without the presence of the patient: re-evaluation of already diagnosed malformations and detection of new subtle defects is feasible. At the same time archivation decreases scanning time and unnecessary ultrasound exposure to the embryo/fetus. With „electric scalpel" artifacts or redundant structures may be removed from the rendered volume. We can take advantages of interdisciplinary domestic or international consiliary consultation via telemedicine.

Disadvantages and limits of 3D/4D ultrasound technology include oligohydramnios, rapid fetal movements, qualified personnel, high-capacity and expensive hardware. Detailed analysis of the volume is still time consuming.

Suspected prenatal diagnosis of all detected congenital malformations by the routine screening 2D-, versus targeted 3D-scan compared with the feto-pathologic evaluation or postnatal clinical assessment revealed 72.4% sensitivity of 2D, and 98.3% sensitivity of 3D ultrasound, respectively. Definite diagnosis of fetal anomalies by the 2D ultrasound may be further refined by the „second-opinion" 3D/4D ultrasound scan.

Three-D ultrasonography has great potential to become the first choice of diagnostic imaging in future obstetric care and prenatal diagnosis. It should be available at every perinatal center. For the time beeing, however, screening ultrasound examinations of pregnant women should be done at perinatal units with high-resolution 2D machines by obstetricians licensed with level „B" and „C" sonography certificate issued by the MSZNUT. Further capacity building of the accumulated knowledge in obstetric 3D/4D ultrasonography is warranted, that is available at domestic and international courses.

The significance of 2D/3D ultrasound examinations in the evaluation of hemodynamic changes caused by DFCS therapy

ANCS therapy has been proven to be the gold-standard for the prevention of RDS in preterm infants.

The data of our perinatal group suggest, that DFCS treatment between the 24th and 32nd weeks of gestation is a promising novel method for the prophylaxis of RDS in high-risk pregnancies, if:

1. preterm delivery is expected to occur within 24 to 48 hours,
2. ANCS therapy is contraindicated (diabetes mellitus),
3. termination of pregnancy for combined maternal/fetal interest (severe PE and/or IUGR) is inevitable, but fetal lung maturity tests show immature index values.

Since the introduction of DFCS treatment in 1996, follow-up examinations of preterm newborns failed to show any major adverse effect in the postnatal development. DFCS treatment represents an alternative to maternal administration of corticosteroids and theoretically should be the ideal mode of delivery of the drug since the fetus represents the main therapeutical target. Fetal route of drug administration may be the choice, if maternal treatment is contraindicated.

DFCS treatment caused immediate, but transient increase of placental vascular resistance, as measured by 2D Doppler-flow-velocimetry, that may be related to decreased fetal heart-rate observed immediately after the invasive procedure. One day after DFCS injection, however, we could not show any significant hemodynamic change in UA vascular resistance.

Fetal cerebral circulation was not influenced shortly after DFCS therapy, however, an increased blood flow was observed in the MCA one day after treatment. This possible reduction in the MCA 2D Doppler-PI value may be interpreted as a sign of ongoing centralization („brain-sparing") due to hypoxaemia during the process of deterioration, but a higher brain stem activity due to corticosteroid binding may also result in similar changes.

As a result of DFCS therapy blood flow in the fetal lung, as measured by 3D-CPA, is significantly increased. The observed pronounced increase in the fetal lung perfusion may partly be explained by the effect of DFCS on the vascular tone of pulmonary veins and arteries. In animal studies DFCS treatment potentiates isoproterenol and prostaglandin E_2, as well as nitric oxide-mediated relaxation of these vessels.

DFCS treatment may increase fetal cerebral and pulmonary perfusion but has no permanent influence on umbilical blood flow as measured by 2D Doppler and 3D-CPA.

This novel modality may be an alternative to ANCS administration for the prevention of RDS in high-risk pregnancies complicated with PE and/or IUGR.

New observations with the application of 3D ultrasound in PCOS after surgical or medical treatment

Polycystic ovarian volume decreased significantly and 3D-CPA showed increased intraovarian flow intensity following laparoscopic ovarian electrocautery.

Hormonal changes (serum and urinary) after laparoscopic surgery are in accordance with changes of 3D sonographic features, in another words they may correlate with alterations of measurable 3D volume and vascular flow data of the polycystic ovaries. Decreased ovarian volume is in accordance with the decreased androgen production, whereas increased ovarian blood flow (FI↑, VFI↑) and partly neovascularisation (VI↑) around the developing follicles make ovarian tissue accessable to higher circulating levels of FSH. Subsequently, follicular development is triggered and resumption of menstrual cyclicity and fertility may be achieved.

Changes in ovarian volume and blood flow parameters following laparoscopic ovarian electrocauterization contribute to and partly explain hormonal alterations and the consequent clinical results.

PCOS-associated hirsutism decreased in response to 3-month GnRH analogue treatment, however, for significant improvement long-term or combined treatment is required.

Ovarian volume, as measured by endovaginal 3D sonography decreased significantly after 3-month GnRH analogue treatment. After an initial „flare-up" effect both intraovarian vascularisation and circulation decreased gradually throughout the 3-month GnRH analogue treatment in patients with PCOS.

In response to GnRH analogue treatment serum LH, T concentrations, and specific ratios of urinary steroids, reflecting 5α-reductase enzyme activity, as well as AM have decreased parallel with ovarian volume and blood flow parameters, thus hormonal changes (serum and urinary) may be reflected in alterations of measurable 3D volume and vascular flow data of the polycystic ovaries. Decreased ovarian volume is in accordance with the decreased androgen production, whereas decreased ovarian vascularisation and blood flow (VI↓, FI↓, VFI↓) represents suppressed ovarian function and subsequent inhibition of follicular development (E_2↓, secondary amenorrhoea) in response to GnRH agonist therapy.

Ovarian blood flow parameters, quantified by the non-invasive 3D-CPA histogram analysis are probably one of the most accurate variables reflecting ovarian function or quiescence.

Continuous increase of E2 levels was observed throughout the 12-month metformin therapy in patients with PCOS, however initial T levels remained high. Parallel with changes of E2 concentrations as of the fourth month of the implementation of metformin therapy biochemical evidence of ovulation was documented. Theoretically, best ovulation results may be achieved with metformin therapy in severely insulin-resistant infertile patients with PCOS.

Twelve-month metformin treatment has not influenced serum LH, T, and FSH concentrations, neither has PCOS-associated hirsutism improved.

Mean ovarian volume has not changed significantly during one-year metformin therapy, although its initial value slightly increased 12 months after continuous metformin treatment.

VI values, representing intraovarian vascularisation significantly increased in response to 12-month metformin therapy as did FI and VFI values, reflecting intraovarian circulation. This may be explained by improved follicular development, that is in accordance with elevated E2 concentrations.

Transvaginal 3D ultrasonography, equipped with color Doppler angiography-based, computer aided histogram analysis option, may be a useful adjunct and non-invasive method for correlating clinical parameters with the blood flow alterations in PCOS patients.