

**Results:** Comparing our microarray data with previous analyses of the human transcriptome, we found that the gene expression patterns of KRT7-positive cells were similar to those identified in placental tissue. We identified 259 upregulated genes commonly expressed in all four KRT7-positive groups, including well-known TB markers. Among these, we identified seven genes whose placental expression and functions have not been previously characterized. To identify whether these genes could be candidate novel TB-related genes, we confirmed their expression in primary placental tissue by immunohistochemical staining. As a result, we found that only XAGE2 and KCNQ2 were expressed in TB layers.

**Conclusions:** BMP4-treated KRT7-positive cells have similar characteristics to those of human placental tissue. Our approach allowed for the identification of novel genes possibly involved in placentation.

#### 17. CHARACTERISTICS OF PHYSIOLOGICAL DEVELOPMENT OF PLACENTAL VILLOUS VASCULATURE USING SUPERB MICRO-VASCULAR IMAGING

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**Objective:** Superb Micro-vascular Imaging (SMI) is a new technology for observing low blood flow profiles and providing high-resolution Doppler images. We aimed to analyze the characteristics of the physiological development of placental chorionic villous vessels (VVs) using SMI.

**Material and Methods:** The subjects were 5 cases of normal singleton pregnancy. The observations were made longitudinally, at 19-21, 29-31 and 36-38 weeks. The VVs were defined as primary (P), secondary (S) and tertiary (T), according to branching structures. In each observation, branching characteristics of VVs were analyzed qualitatively. The distance between P-VVs on the chorionic plate were measured. Among the VVs, one branching vessel clearly delineated from P to T VVs was sampled, and the length of each VV was measured. The gestational age-related characteristics of VVs were analyzed qualitatively using the Kruskal-Wallis one-way ANOVA on ranks test and followed up with the Dunn's test. The significance was set at  $P < 0.05$ .

#### Results

- 1) The distances between the P-VVs at 19-21 weeks (median: 14.8mm) became elongated at 29-31 weeks (23.2mm), but no change thereafter at 36-38 weeks (23.1mm).
- 2) The lengths of the P/S-VVs at 19-21 weeks (8.15mm / 5.55mm) extended at 29-31 (12.6mm/ 9.6mm), but showed no change thereafter at 36-38 weeks (11.35mm/ 8.0mm).

**Conclusions:** The distance between the P-VVs and the length of the P/S-VVs extended to 29-31 weeks and no further changes thereafter. This suggested that the basic structure of villus vasculature was completed at around 30 weeks' gestation.

#### 18. UMBILICAL CORD EDEMA WITH UMBILICAL CORD HEMANGIOMA IN PREGNANCY, A CASE REPORT

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In addition to the circulating disorders and inflammations, edema of the umbilical cord has an association with congenital anomalies and perinatal death. Here we report a case having umbilical cord edema with umbilical cord hemangioma who gave birth to a healthy infant vaginally. A 26-year-old, 3 gravida 1 para female conceived naturally and received periodical check-up at a local obstetrical clinic. She was pointed out

umbilical cord cysts at 30 weeks of gestation and referred to our institution. Ultrasonography revealed edematous umbilical cord with cystic structures. The fetal growth was appropriate, no morphological abnormalities. Her pregnancy course was uneventful and she gave birth to a 2,965g of female infant vaginally at 39 weeks and 2 days of gestation. The fetal status was reassuring throughout the parturition. The total length of the umbilical cord was 70 cm and the cord was edematous over 53 cm from the umbilical ring. Histopathological examination revealed no urachal sinus in umbilical cord. Instead, there was a lobulated and circumferential hemangioma-like hyperplasia around the umbilical artery, leading to a diagnosis of umbilical cord hemangioma.

Hemangioma is benign endothelial cell neoplasms that affect the skin and the other organs. It may form part of a syndrome or occur in association with other malformations. Unlike the placental hemangioma, umbilical cord hemangioma is very rare and may not be detected prenatally. It is recommended to rule out associated malformations and hemangiomas in other locations for the fetus with umbilical cord edema.

#### 19. PROGESTERONE SUPPRESSES THE ENHANCEMENT OF INFLAMMATION ON THE FETAL MEMBRANE

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**Objective:** Progesterone (P4) has been known to be important in maintaining pregnancy by anti-inflammatory effect in the myometrium, however, this effect is less understood in the fetal membrane. We previously reported that mice with dental *Porphyromonas gingivalis* (*Pg*) infection could be useful as a model of preterm birth. In this model, inflammation in the fetal membrane via toll-like receptor 2 (TLR2) is thought to result in preterm birth. The aim of this study was to investigate the effect of P4 on the fetal membrane.

**Methods:** *Pg* mice were injected subcutaneously with (*Pg* + P4 mice) or without (*Pg* mice) 1mg P4 daily at day 15.5-17.5 of gestation. We performed Western blot analysis for detection of MAPK and NF- $\kappa$ B on the fetal membrane at day 18 of gestation. We also evaluated inflammatory cytokines (IL-1 $\beta$ , IL-8, TNF- $\alpha$ ) and TLR2 at the same tissues using RT-PCR.

**Results:** The enhancement of MAPK and NF- $\kappa$ B expression levels was decreased in *Pg* + P4 mice, compared with in *Pg* mice. The treatment with P4 significantly reduced the enhancement of the expression of IL-1 $\beta$ , IL-8, TNF- $\alpha$  and TLR2.

**Conclusion:** P4 prevented preterm birth by suppressing the activation of inflammatory signaling pathways via TLR2 in the fetal membrane of preterm birth mouse model.

#### 20. THE CONTRIBUTION OF PLACENTAL PATHOLOGY TO THE PREDICTION OF, AND PROTECTION AGAINST, CEREBRAL PALSY (CP)

Masayoshi Arizawa. *Tokyo Metropolitan Ohtsuka Hospital*

**Object:** As we know, cerebral palsy (CP) can be caused by intrauterine hypoxia, hypoxia in delivery, and premature birth. In this study I investigate how to predict CP through examination of the placenta for signs of hypoxia.

We looked at what kind of hypoxic states can be found through an examination of the placenta, looking at Villous edema, thrombosis of the villous vessels, decidual degeneration, Diffuse Chorioamnionic Hemiosiderosis (DCH), Chorioamnionitis (CAM) and abruptio placentae.

**Method:** I examined 37 placentas from CP cases by microscope.

The cases were chosen from clinical records and diagnosis by pediatricians.

**Results:** From 37 cases I found.

Edematous villi - 19 cases

Villous vessels occlusion - 15 cases

Dysmature villi - 5 cases

Decidual degeneration - 11 cases

DCH - 5 cases

Sever CAM - 4 cases

Abruptio placentae - 2 cases

Delivery of all 37 cases was 22 to 40 gestational weeks

**Conclusions:** There are many premature deliveries which present hypoxia leading to ventricular hemorrhage and PVL. These brain damages can lead to CP. Through placental pathology we found bad circulation between the placenta and the baby. We can definitely conclude that placental pathology can contribute to our understanding of CP prediction and protection.

## 21.

### FINDINGS REGARDING THE HIGH RATES OF CASES OF DECIDUAL PATHOLOGIC LESIONS AT 34 GESTATIONAL WEEKS OR EARLIER DEVELOPING INTO HYPERTENSION DISORDER OF PREGNANCY (HDP)

Masayoshi Arizawa. *Tokyo Metropolitan Ohtsuka Hospital*

**Object:** I examined the placentas in cases of Hypertension Disorder of Pregnancy (HDP) at 34 gestational weeks or earlier in order to help discover causes and treatment

**Method:** I examined the placentas of 118 cases of HDP at 34 gestational weeks or earlier - defined by standard diagnosis - by microscope.

**Results:** Out of 118 cases, atherosclerosis, thrombosis, and decidual degeneration was found in 98 cases –83.1 %

Ischemic villi was found in 90 cases –76.3%

Villous vessels abnormality was found in 40 cases - 33.9%

Severe Chorioamnionitis(CAM) could not be found

**Conclusions:** Problems with decidual vessels from the mother to the placenta at 34 gestational weeks and earlier leads to a high rate of HDP.

According to the Amsterdam Conference 2014, the definition of MVM is bad circulation leading to ischemic villi, syncytial knots increase, and the development of fibrin deposition. This definition of MVM applies to bad maternal circulation affecting the placenta.

We also found FVM which resulted from MVM, as the degeneration of the terminal villi led to occlusion in the central villous vessels. This developed into other parts of the terminal villi.

According to the Amsterdam conference, MVM and FVM are different phenomena. However this study shows that the two are connected.

Also in this study I found no sign of CAM. I suspect the reason is immunological relations.

## 22.

### EXTRAPLACENTAL INTER-TWIN ANASTOMOTIC VESSELS IN MONOCHORIONIC DIAMNIOTIC TWINS

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**Objective:** Inter-twin anastomotic vessels that were observed on the outside of placental parenchyma could contribute to residual anastomotic vessels during fetoscopic laser photocoagulation (FLP). The objective of this study was to elucidate the prevalence of extraplacental inter-twin anastomotic vessels and to examine the relative factors.

**Methods:** We conducted retrospective study of MD twin cases whose placentas were inspected postnatally. Cases that underwent FLP or with fetal demise were excluded. An anastomotic vessel which was located out of placental parenchyma by macroscopic inspection was defined as extraplacental inter-twin anastomotic vessels. The prevalence of extraplacental inter-twin anastomotic vessels was examined and association with factors including umbilical cord insertion was analyzed with Fisher's exact test.

**Results:** One-hundred and twenty eight cases were included. The prevalence of extraplacental inter-twin anastomotic vessels was 5.5% (seven cases). The relative factor of extraplacental inter-twin anastomotic vessels was velamentous cord insertion of both fetuses (P=0.035). Extraplacental

inter-twin anastomotic vessels were found in two of six cases with VCI of both fetuses.

**Conclusion:** Extraplacental inter-twin anastomotic vessels were found in 5% of MD twins, therefore it might be recommended to observe placenta during FLP in light of anastomotic vessels that were difficult to be found. It might be useful for the prediction of extraplacental inter-twin anastomotic vessels to understand umbilical cord insertion preoperatively.

## 23.

### ELEVATION OF ANGIOGENIC FACTORS IN PREGNANCY WITH MIRROR SYNDROME CAUSED BY FETAL CARDIAC FAILURE

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**Introduction:** Mirror syndrome (MS) is characterized by the combination of maternal generalized edema, fetal hydrops and placental hypertrophy. Elevation of soluble Flt-1 or soluble Endoglin in preeclampsia is also reported to be related to the onset of MS.

We experienced a MS case due to fetal cardiac failure with elevation of angiogenic factors.

**Case:** A 27-year-old woman, gravida1, para0, had prenatal care in a local clinic. At 26 weeks of gestation, small amount of fetal ascites was observed. At 28 weeks of gestation, she had dyspnea with weight gain and fetal ascites apparently increased. She was transferred and admitted in our hospital. Fetal ultrasound examination revealed ascites, pericardial fluid ascites, subcutaneous edema, and placental hypertrophy. Hypokinetic biventricular movement and the thinning of the myocardium were detected in the fetus, suggesting the cardiac dysfunction.

She had an emergency cesarean section on the same admission day. The placenta was edematous and weighed 460 g. In microscopic examination, stromal edema was observed in the majority of the villi.

Elevation of soluble Flt-1 (7580pg/ml) and soluble Endoglin (25.4 ng/ml) was detected in the maternal serum on the admission day.

**Discussion:** We experienced a MS case due to fetal cardiac failure. A rapid progress of fetal cardiac failure and hydrops makes placenta blood flow disrupted. Disruption of placental blood is related to elevation of angiogenic factors and clinical presentation such as maternal edema. The angiogenic factors may be a key of MS pathogenesis.

## 24.

### LACTOBACILLUS CRISPATUS PROMOTES TROPHOBLAST INVASION

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Human vaginal cavity has a unique microbiome dominated by Lactobacilli. Recent reports show non pregnant uterine cavities and placentae also possess unique microbiome dominated by Lactobacilli. Their reduction frequently observed in the patients with bacterial vaginosis (BV) as well as uterine infection such as endometritis and chorioamnionitis are great concern for reproductive health. In the presented study, we examined tissue-bacterial interactions of immortalized human trophoblasts and Lactobacilli in order to analyze their roles on trophoblast functions.

**Methods:** HTR-8/SVneo cells were plated on Matrigel chambers with or without Lactobacillus crispatus (LC), Lactobacillus acidophilus (LA), Escherichia.coli and Staphylococcus aureus. The invasive activity was directly evaluated using the microscopy and the time-lapse imaging using by the IncuCyte® system. Genes induced by Lactobacilli were screened by microarray and then verified with real time PCR and ELISA.

**Results:** Matrigel invasion of HTR-8/SVneo cells was up-regulated significantly by LC while others were not significantly promotive or cytotoxic.