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Lectures

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10 P PEDIATRICS: NOTES FOR THE FUTURE

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The Workshop has been organized with the patronage of the Italian Society of Neonatology (SIN), the Italian Society of Pediatrics (SIP), the Italian Society of Perinatal Medicine (SIMP), the Italian DOHaD (Developmental Origins of Health and Disease) Society, the Union of European Neonatal and Perinatal Societies (UENPS), the Union of Mediterranean Neonatal Societies (UMENS), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the Union of Middle-Eastern and Mediterranean Pediatric Societies (UMEMPS), and lastly the European Association of Perinatal Medicine (EAPM).

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LECT 44

DOES THE RABBIT NEPHROGENIC ZONE IN CULTURE REFLECT CAUSES FOR IMPAIRED NEPHROGENESIS?

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Intrauterine as well as extrauterine influences are held responsible to cause prematurity of renal parenchyma and impaired nephrogenesis in preterm infants, leading to a high incidence of severe kidney diseases later in life. Although involved noxae and resulting molecular effects are quite different, all of them converge to the nephrogenic zone, that is restricted to the outer cortex of a developing kidney. Covered by the organ capsule, it consists of aligned ureteric bud-derived collecting duct (CD) ampullae, containing epithelial stem cells, nephrogenic mesenchymal stem cells, renal vesicles and S-shaped bodies. Due to the complex composition of the nephrogenic zone and the different noxae, it is appropriate to investigate impaired nephrogenesis by an adequate *in vitro* system. In this case, microsurgical isolation and culture of the nephrogenic zone from neonatal rabbit kidney is particularly well suited. In fact, it shows a microarchitecture which is largely comparable with the human specimen. Moreover, a decisive advantage is that it can be easily and quickly isolated in original composition by microsurgical techniques and pieces of the explant are consequently available for a variety of advanced culture experiments. Formation of renal spheroids can be used for drug toxicity testing. Mounting in a tissue carrier makes it possible to register functional differentiation of the CD epithelium. Perfusion culture within an artificial interstitium enables to investigate spatial development of parenchyma. On the one hand these recent findings define the route to solve future tasks, on the other hand actual pathologic data inform about intrinsic cell biological risks during generation of renal parenchyma.

LECT 45

IS THE PLACENTA AN INNOCENT BYSTANDER IN PERINATAL PROGRAMMING?

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During pregnancy, a well functioning placenta is needed to ensure appropriate growth and development of the fetus [1]. Indeed, a mal-functioning or "insufficient" placenta has been recognized as the "cause" of Intrauterine Growth Restriction (IUGR) [2], leading to decreased oxygen delivery as well as altered placental transport of nutrients, mainly amino acids and lipids, but also micronutrients such as iron and folate. A number of previous studies from our lab support this hypothesis, demonstrating a specific placental phenotype of IUGR [3], recently confirmed with decreased levels of placental Transferrin Receptor (TFRC – mediating cellular iron uptake) or of Sodium-coupled Neutral Amino acid Transporter 2 (SNAT2) in IUGR *versus* controls [4, 5] (summarized in **Tab. 1**). Maternal nutritional status, diet and exposure to environmental factors are increasingly acknowledged as potentially affecting placental gene expression, thus modifying placental function. These epigenetic associations link intrauterine environment to adverse perinatal outcomes reprogramming the fetal epigenome with several mechanisms, such as methylation or miRNA, thus affecting gene expression and activity in preeclamptic (PE) and IUGR tissues [6]. Changes in miRNA expression pattern have been observed in placental tissue and associated with several pregnancy pathologies as preeclampsia (↓ miR-21, ↑ miR-155, ↓ miR-223), GDM (↓ miR-132), IUGR (↓ miR-21, ↓ miR-210) and preterm birth (↑ miR-493, ↑ miR-338) [7]. In this context, an active placental metabolism is crucial to support both trophoblast invasion and placentation [8]. Alterations in early implantation may lead to mismatches in oxygen (O_2) delivery to different areas of the placenta, with less O_2 exchange between the uterine and the umbilical circulations [9]. Mitochondrial DNA (mtDNA) copy number is positively correlated with the number of mitochondria. We have previously demonstrated altered mitochondrial content in IUGR placentas [10], with higher mtDNA levels in IUGR maternal blood [11]. Moreover, we measured the functionality of the respiratory complexes (RCC) by high-resolution respirometry (HRR), in order to assess potential alterations in placental energy metabolism [12] (summarized

Table 1 (LECT 45). Research findings in normal and abnormal placental development.

References	Topic	Study Description	Results
Mandò et al., Pediatr Res, 2013 [4]	Sodium-Coupled Neutral Amino acid Transporter 2 (SNAT2)	SNAT2 gene expression and its intron-1 levels methylation	mRNA levels significantly decreased in intrauterine growth restricted (IUGR) placentas, with reduced umbilical blood flows. Methylation levels were steadily low in both IUGR and controls
Mandò et al., Placenta, 2011 [5]	Transferrin Receptor 1 (TFRC), cellular iron uptake on trophoblast membranes	TFRC mRNA and protein expression and localization in 50 IUGR and 56 control placentas	TFRC gene and protein expression significantly lower in IUGR placentas vs controls ($p < 0.05$), especially in the most severe IUGR group. TFRC protein was predominantly in syncytiotrophoblasts
Lattuada et al., Placenta, 2008 [10]	Mitochondrial DNA (mtDNA) content in control and IUGR placentas	mtDNA content (Real-Time PCR) in 26 control pregnancies (AGA) and 24 IUGR	Increased mtDNA content ($p = 0.004$), inversely related to oxygen (O_2) tension in the umbilical vein
Colleoni et al., Am J Obst Gynecol, 2010 [11]	mtDNA content in maternal circulation of pregnancies complicated by IUGR	MtDNA in 13 non-pregnant women; 45 control pregnancies, in 3 trimesters; and 12 complicated by IUGR	Highly significant progressive reduction in circulating mtDNA in pregnant women of I-II-III trimesters compared to non-pregnant women ($p < 0.001$). mtDNA content was significantly increased in women with IUGR fetuses compared control ($p < 0.001$)
Mandò et al., Am J Phys Endocr Metab, 2014 [12]	mtDNA and nuclear respiratory factor 1 (NRF1) levels in placental tissue and cytotrophoblast cells; gene and protein expression of respiratory chain complexes (RCC) and their O_2 consumption	mtDNA, NRF1, RCC expression (Real-Time, Western Blotting), O_2 consumption (HRR) in 8 IUGR, 6 PE, and 8 pregnancies	Lower mRNA levels of mt complexes II, III, and IV in IUGR cytotrophoblast cells; no differences at the protein level. mtDNA increased in IUGR placentas ($p < 0.017$). Both mtDNA and NRF1 expression lower in isolated cytotrophoblast cells ($p < 0.05$). RCC activity was increased in placentas of IUGR fetuses ($p < 0.017$)
Mandò et al., Stem Cells Transl Med, 2016 [17]	Characterization of cells isolated from placental membranes and basal disc (pMScs) of IUGR and physiological placentas	Viability and proliferation of cell culture. Hematopoietic, stem, endothelial, and mesenchymal markers (flow cytometry). Multipotency of pMSCs and expression of mt genes (Real-Time PCR)	Cell viability high in all samples, proliferation rate lower in IUGR compared to controls. Multipotency of IUGR pMSCs was restricted because their capacity for adipocyte differentiation was increased, whereas their ability to differentiate toward endothelial cell lineage was decreased. Mitochondrial content and function higher in IUGR pMSCs

in **Tab. 1**). Preliminary observations suggest similar changes in placental mitochondria, DNA content and function of obese pregnant women. These pregnancies are characterized by low-grade inflammation and oxidative stress [13]. Moreover, dysregulated mt genes methylation (D-loop and CO1 hypomethylation) might expand our findings of higher mtDNA content in fetal cord blood of IUGR and PE [14]. These preliminary data may indeed suggest a compensatory attempt of fetuses to increase energy production through higher mtDNA content and RCC (CO1) expression, representing a further link between epigenetic changes and perinatal programming of diseases. Another issue is related to the placental hormonal function. The placenta as a source of a wide array of hypothalamic or pituitary hormones was a hot topic in the 60-70s, then neglected because of the radioactive techniques needed at that time. Steroid hormones, and in particular estrogens, are important for uterine/placental vascular adaptations to pregnancy, but also essential for trophoblast cells syncytialization in placenta. During pregnancy, the feto-placental unit is a source of estrogens through its aromatase enzyme Cytochrome P450 (CYP19) involved in estradiol (E2) production [15]. Interestingly, CYP19 levels appeared significantly higher in IUGR placentas that we recently analyzed. We might speculate that the CYP19 alterations have an estrogen-related protective action in more severe IUGR placentas, which we showed to be characterized by increased mtDNA [16]. Ongoing analyses will evaluate if these placental molecular alterations result in E2 hormone altered production. Placental mesenchymal stromal cells (p-MSCs) may also represent an interesting point to evaluate in order to understand normal and abnormal placental development. In IUGR pregnancies, p-MSCs have lower proliferation rate with earlier shift towards homogeneity than in controls. *In vitro* findings also demonstrate that multipotency of IUGR-

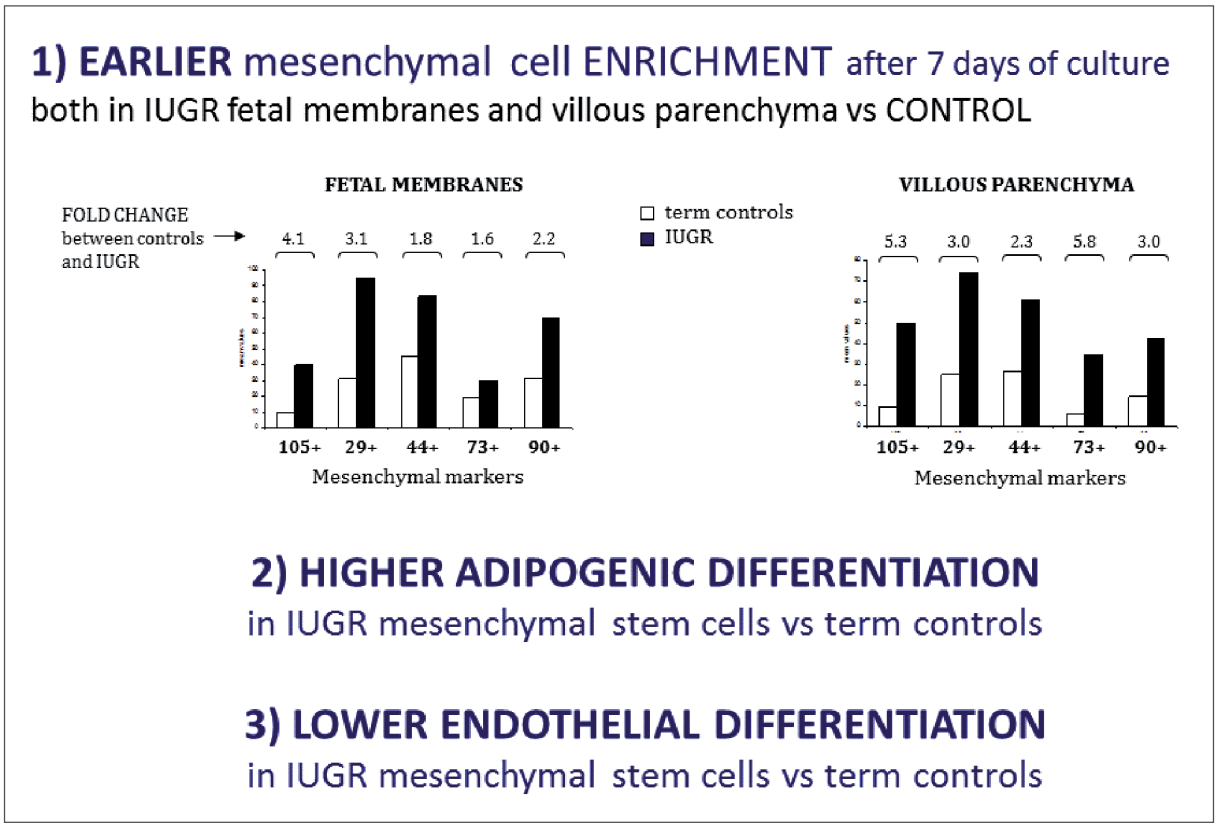

Figure 1 (LECT 45). Analysis of mesenchymal stem cells isolated form Intrauterine Growth Restriction (IUGR) and control human placentas.

derived p-MSCs is restricted, as their capacity for adipocyte differentiation is increased, whereas their differentiation ability towards endothelial cell lineage is decreased (**Fig. 1**) [17]. These findings are indicative of changes that may also be reflected in the developing fetus (summarized in **Tab. 1**). The potential role for p-MSCs in pregnancy pathologies, as well as the striking mitochondrial changes involved in energy production, open new perspectives for understanding the development of the diseases and potential routes of prevention and treatment.

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LECT 46

INSULIN SENSITIVITY IN IUGR: FROM PLACENTA TO ADOLESCENCE

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Subjects born with intrauterine growth restriction (IUGR) have an increased incidence of cardiovascular disease, hypertension and type 2 diabetes mellitus in adulthood compared with the general population. These features are shared with obese subjects, and derive primarily from increased insulin resistance. Fetal growth is driven mainly by the IGF system as experiments in knockout mice have shown, however, insulin is a well known determinant of fetal growth as well, and the IGF system is of importance in regulating glucose metabolism and insulin sensitivity. We reported that human IUGR subjects present increased insulin-like growth factor (IGF)-2, IGF binding protein (IGFBP-1) and IGFBP-2 content in placenta, if compared with appropriate for gestational age (AGA) newborns. The increase in IGF-2 could be a compensation for reduced insulin bioactivity in placenta. Moreover, we described increased IGFBP-2 cord serum concentrations in IUGR compared with AGA, and a positive relationship of serum IL-6 with IGFBP-2, although IL-6 concentration did not show any changes in the two situations. We described instead increased IL-6 mRNA and protein concentrations in placental lysates from IUGR. Finally, we reported a negative effect, although not a major effect, of IL-6 placental concentration on birth size. In detail, whereas the amount of total insulin receptor (IR) was similar in both AGA and IUGR, activated IR was significantly higher in IUGR. Total IR substrate-1 (IRS1) was increased in IUGR, whereas total IRS2 and activated IRS1 were similar. AKT content was reduced and activated AKT was undetectable in IUGR placentas. C-Jun N-terminal kinase content was reduced in IUGR. Total and activated ERK1/2 were similar in IUGR and AGA groups, and total SOCS2 was increased in IUGR. IL6 lysate concentrations correlated with AKT content and activated IR. SOCS2 correlated negatively with all growth parameters at birth. Close relationships of insulin action in placenta with fetal growth were shown. Insulin resistance and type 2 diabetes are related by association with high serum concentrations of proinflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, and low adiponectin concentrations, as studies in obese subjects have confirmed. As markers of insulin sensitivity in placenta we further studied adiponectin and resistin contents in placentas from IUGR and AGA newborns. Adiponectin was significantly lower in lysates from IUGR, insulin and resistin concentrations were positively correlated, and placental adiponectin concentration was positively correlated with the weight of the placenta, birth weight and head circumference. Fetal programming of the endocrine axis related to intra-uterine growth and events occurred during pregnancy contribute to the timing of puberty and to future reproductive capacity. Pubertal development disorders influence not only sexual maturity, but also adult height, bone mineral density and reproductive health. Adipokines play a significant role in the metabolic syndrome and in cardiovascular diseases, have implications in regulating insulin sensitivity and inflammation, and significant effects on growth and reproductive function. Precocious pubarche and precocious adrenarche have been shown to be more frequent in subjects with a low birth weight. Girls with previous prenatal growth restriction have been described to have more frequently than the general population idiopathic functional ovarian hyperandrogenism