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IFPA Meeting 2010 Workshops Report II: Placental pathology; Trophoblast invasion; Fetal sex; Parasites and the placenta; Decidua and embryonic or fetal loss; Trophoblast differentiation and syncytialisation

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ABSTRACT

Workshops are an important part of the IFPA annual meeting. At IFPA Meeting 2010 diverse topics were discussed in twelve themed workshops, six of which are summarized in this report. 1. The placental pathology workshop focused on clinical correlates of placenta accreta/percreta. 2. Mechanisms of regulation of trophoblast invasion and spiral artery remodeling were discussed in the trophoblast invasion workshop. 3. The fetal sex and intrauterine stress workshop explored recent work on placental sex differences and discussed them in the context of whether boys live dangerously in the womb.4. The workshop on parasites addressed inflammatory responses as a sign of interaction between placental tissue and parasites. 5. The decidua and embryonic/fetal loss workshop focused on key regulatory mediators in the decidua, embryo and fetus and how alterations in expression may contribute to different diseases and adverse conditions of pregnancy. 6. The trophoblast differentiation and syncytialisation workshop addressed the regulation of villous cytotrophoblast differentiation and how variations may lead to placental dysfunction and pregnancy complications.

1. Placental pathology and clinical correlates (accrete/percreta)

Chairs: Abdulluh Al-Khan, Carolyn Salafia
Speakers: Alexandre Borbely, Sally Collins, Gladys Ramos

1.1. Outline

This workshop focused on new clinical and basic science insights into the mechanisms underlying abnormal trophoblast invasion and/or uterine pathology that result in placenta accreta/percreta. Discussion centered on the epidemiology, clinical presentation, and complications of these disorders, as well as medical costs. One of the goals was to identify areas of research in need of more intensive investigation.

1.2. Summary

Gladys Ramos discussed sonographic findings associated with the diagnosis of placenta accreta. A number of sonographic signs have been described as indicative of placenta accreta although the accuracy of these signs in predicting invasive placentation is not known. A diagnostic accuracy study in which cases of pathology-proven placenta accreta were compared to cases of placenta previa matched by gestational age was performed whereby sonographic images were reviewed and ultrasound signs of placenta accreta (previa, loss of myometrial interface, chaotic intraplacental blood flow, 'swiss cheese appearance', placenta bulging into bladder, distance of bulge into the bladder, invasion into the bladder, and color Doppler crossing vessels) were coded as present, absent or indeterminate. The most common and sensitive sonographic finding associated with placenta accreta was loss of myometrial interface. If present all signs were highly specific (90–100%) and had a high positive predictive value (90–100%) for the diagnosis of placenta accreta. No combination of sonographic signs could accurately predict all placenta percretas. However, placenta percretas often had more than one sonographic sign. It was concluded that the presence of a previa with loss of myometrial interface is the most common finding in patients with placenta accreta.

Alexandre Borbely discussed expression of biglycan in normal human term placenta and in highly invasive pathologies. Decorin and biglycan are members of the small leucine-rich proteoglycan family and are important factors in the control of cellular proliferation, migration and invasion. Several placental pathologies include increased invasive activity of the trophoblast. These pathologies can be lethal for both mother and fetus and they can develop during gestation as placenta accreta and invasive mole or after gestation, like classic choriocarcinoma. The expression and localization patterns of decorin and biglycan in normal term placenta, placenta accreta, invasive mole and choriocarcinoma were determined. In normal term placenta, decidual cells were immunopositive for decorin whereas the extravillous trophoblast cells (EVT) were negative. In placenta accreta and invasive mole, EVT were positive for decorin whereas the surrounding matrix was negative. In choriocarcinoma, only cytotrophoblast and metastatic cells were immunoreactive for decorin. Biglycan displayed similar results to decorin, with exception of the matrix-type fibrinoid in normal term placenta and the EVT surrounding matrix in placenta accreta, which presented strong staining for biglycan. These results suggest that the expression patterns of biglycan in highly invasive cells from placenta accreta, invasive mole and choriocarcinoma might play a role in modulating trophoblast migration and invasion. The expression pattern presented by decorin suggests that this proteoglycan might exert another role than migration/invasion modulation.

In a comment from the floor, Peeyush K. Lala mentioned how previous studies have shown that decidua-derived decorin is a paracrine regulator of extravillous trophoblast (EVT) invasiveness during normal pregnancy. It serves as a storage device for inactive TGF-β in the decidual extracellular matrix, until the decorin-TGF-β complex is cleaved by an EVT-derived proteinase cascade that also activates TGF-β to exert its anti-invasive action. In addition, decorin exerts anti-proliferative, anti-migratory and anti-invasive actions on EVT independent of TGF-β, because of its binding as an antagonistic ligand to several tyrosine kinase receptors such as EGFR, IGF-1R, MET and VEGF-R2 on the EVT. Since normal EVT never shows immuno-detectable decorin, whereas EVT in placenta accreta does, it was suggested that careful ultrastructural localization and *in situ* hybridization are needed to ascertain the source and location of decorin in placenta accreta.

Sally Collins discussed "spirals" and "spurts" and small for gestational age (SGA) babies.Inadequate transformation of the spiral arteries has long been implicated in adverse pregnancy outcomes.A novel technique for measuring the spiral artery waveform has recently been developed and it appears to be altered in SGA babies.Women were recruited prospectively to have seven extra ultrasound scans throughout pregnancy. Customized birth weight centiles were calculated with SGA defined as $<$10th centile. A mixed model statistical analysis was used which takes into account the longitudinal nature of the data. In the study 66 women were recruited, 9 of which had SGA babies. The pulsatility index of the spiral artery 'jets' was increased in SGA pregnancies compared to those with normal birth weights. A similar trend was

seen in uterine arteries. Increased pulsatility in blood flowing forward into the intervillous space probably reflects the persistence of muscularity in the spiral artery wall. Despite this, no significant difference was seen in the uterine artery, adding weight to the theory that intra-myometrial shunts play an important part in the placental circulation.

1.3. Conclusions

Placenta accreta and percreta are increasingly important and common obstetric catastrophes about which relatively little is understood. While the rise in cesarean sections has been blamed for the increased incidence, the exact mechanism (basalis defect resulting from surgery or an abnormal vascularization resulting from repair of surgery) remains elusive. Multidisciplinary approaches to this life-threatening disorder are clearly needed.

2. Trophoblast invasion

Chairs: Charles Graham, Peeyush Lala

Speakers: Ivraym Barsoum, Georgina Cerchi, Tiziana Cotechini, Thierry Fournier, Paula Headley, Charles Graham, Geeta Godbole, Peeyush Lala, Ellen Menkhorst

2.1. Outline

This workshop aimed to discuss (a) mechanisms involved in trophoblast invasion, and (b) mechanisms involved in uterine vascular remodeling and the association between the two events.

2.2. Summary

Peeyush Lala discussed whether mechanistic studies of trophoblast invasion identify early markers for pre-eclampsia. Highly regulated trophoblast invasion of the decidua and the uteroplacental arterioles are key events in the development of the fetoplacental unit during early pregnancy. Compromised placental perfusion resulting from inadequate trophoblast invasiveness during early gestation has been linked with the origin of fetal growth restriction and pre-eclampsia. There are many factors involved in regulating trophoblast invasion to maintain uteroplacental homeostasis, including growth factors, growth factor binding proteins, proteoglycans, enzymes, lipids and extracellular matrix (ECM) components. Trophoblast invasiveness can be promoted by autocrine or paracrine mediators: *e.g.* EGF and VEGF family (paracrine), IGF2 (autocrine), HGF (paracrine), uPA (autocrine), IGF-BP-1 (paracrine), IET-1 (autocrine/paracrine), IL-8, IP-10, and PGE2 (paracrine). On the other hand, trophoblast invasiveness is controlled primarily by paracrine (decidua-derived) mediators, including TGF-β, TNFα, and decorin. Decorin can be an antagonistic ligand for multiple tyrosine kinase receptors EGFR, IGFR-1, MET, and VEGF-R2 expressed by EVT cells. A derangement in any or a combination of these regulators may underlie pre-eclampsia, which is a multifactorial disease. Some of them may result in maternal serum markers in early-mid pregnancy, which may be of predictive value in longitudinal studies; whereas other markers reported during clinically identified disease may represent effects of the disease. Identification of early markers that can be harnessed for the prevention or management of early-onset pre-eclampsia is urgently needed.

Ellen Menkhorst discussed the relative roles of decidualized and non-decidualized stromal products in regulating trophoblast adhesion. EVT must adhere, migrate and invade through the decidua during placental formation. Abnormal decidualization of endometrial stromal cells leads to unregulated trophoblast invasion and pregnancy failure in mice. Recent evidence in humans indicates pre-eclampsia may be associated with impaired decidualization. The mechanisms by which decidual cells interact with EVT and the importance of adequate decidualization in this interaction remain largely unknown.The regulation of EVT protein expression and adhesion by factors secreted by non-decidualized and decidualized endometrial stromal cells was assessed. EVT showed increased adhesion to decidualized compared to non-decidualized endometrial stromal cells. Non-decidualized and decidualized endometrial stromal cell conditioned media stimulated the expression of distinct proteins in isolated EVT, some of which are associated with placental pathologies including pre-eclampsia. These data suggest that cell surface and secreted decidual cell factors regulate EVT adhesion and proteins. Adequate decidualization may be critical for appropriate EVT function and therefore placental development in humans.

Georgina Cerchi discussed the activity and expression of matrix metalloproteinases in a human uterine fibroblast-trophoblast co-culture system. Successful implantation is the result of complex molecular interactions between the hormonally primed uterus and mature blastocyst. Ethical restrictions and limited availability of human placental tissue limits human implantation studies. An *in vitro* co-culture model using human uterine fibroblasts and trophoblast explants, both from term placenta, was established to study maternal–fetal interaction. Matrix metalloproteinase (MMP) protein expression and activity was assessed by Western blot and zymography respectively. Pro-MMP-2 expression was up-regulated in uterine fibroblast cells after co-culture with trophoblast compared with controls; in contrast, MMP-2 expression was down-regulated. In trophoblast explants from co-culture, MMP-2 expression was up-regulated. Pro-MMP-2 and MMP-2 activity were up-regulated in co-culture conditions while MMP-2 activity was down-regulated. The model established may prove a good tool for further study of the role of MMPs during implantation.

Geeta Godbole discussed the role of decidual homeobox (HOX) A10 in controlling trophoblast invasion by regulating paracrine factors. The decidua is a major regulator of trophoblast invasion, which dictates the degree of placentation. However, the genetic/molecular players within the decidua regulating this invasion have not been identified. HOXA10 is a major regulator of decidualization and was hypothesized to be involved in regulation of trophoblast invasion. To investigate the role of decidual factors and decidual HOXA10 on trophoblast invasion, supernatants from decidualized and HOXA10 silenced decidual-endometrial stromal cells were used to investigate their effects on invasion and expression of invasion-related markers on trophoblast cell lines JEG-3 and ACH-3P.Decidual supernatants activated invasion of trophoblast cell lines, and elevated expression of MMPs; the loss of HOXA10 in the decidual cells further augmented this response. Supernatants derived from HOXA10 knockdown cells increased the invasive ability of the trophoblast cells and activated expression of MMPs and integrins. These observations indicate that HOXA10 in decidual cells may balance the expression of pro- and anti-invasive factors secreted by the decidua for controlled invasion and that loss of this factor in the decidual cells disrupts this balance leading to over invasion. Additional data suggests that HOXA10 might be critical for transformation of endometrial stromal cells into functional decidual cells.

Thierry Fournier discussed how invasion of CMV-infected human cytotrophoblasts is decreased in a PPARγ-dependent manner. Human implantation involves a major invasion of the uterine wall and complete remodeling of uterine arteries by EVT. Abnormality in these early steps of placental development leads to poor placentation and fetal growth defects and is often associated with pre-eclampsia. It has previously been shown that activation of the

ligand-activated nuclear receptor PPARγ with synthetic (rosiglitazone) or natural (15deoxyPGJ2) agonists inhibits trophoblast invasion. Interestingly, it was demonstrated that for its own replication human cytomegalovirus (HCMV) activates PPARγ transcriptional activity in infected cells. It has also been shown that PPARγ antagonists dramatically impair virus production and that the major immediate-early promoter contains PPRE able to bind PPARγ as assessed by EMSA and ChIP. By activating PPARγ, HCMV dramatically impaired early human trophoblast migration and invasiveness, as assessed by using EVT primary cultures and the EVT-derived cell line HIPEC. These data provide new clues to explain how early infection during pregnancy could impair implantation and placentation and therefore embryonic development.

Paula Hedley discussed leptin and soluble leptin receptors in first trimester pregnancy sera as markers for pre-eclampsia. The maternal serum concentration of leptin is increased in the second and third trimesters of pregnancies afflicted by pre-eclampsia. Leptin, its soluble receptor, and the free leptin index in first trimester sera from 123 women who developed pre-eclampsia and 285 controls were assessed. Maternal serum leptin levels increased in pre-eclampsia compared to controls, while soluble receptor concentrations were reduced. There was no correlation between leptin or soluble receptor levels and the severity of pre-eclampsia. In severe pre-eclampsia cases there was a negative correlation between TNFα and free leptin index, whereas in mild pre-eclampsia cases, this correlation was positive. There was no correlation between TNFα and free leptin index in control pregnancies. These data indicate a role for leptin and free leptin index as early screening markers for pre-eclampsia and a prognostic role of TNFα and free leptin index in differentiating severe from mild pre-eclampsia

Charles Graham discussed the link between aberrant maternal inflammation and deficient utero-placental perfusion leading to pregnancy complications. Trophoblast invasion and remodeling of the uterine spiral arteries is required for the establishment of adequate utero-placental perfusion. Trophoblast invasion is finely regulated and requires the participation of locally produced molecules, some of which stimulate invasion, whereas others, such as TGF-β, inhibit invasion. Various complications of pregnancy (*e.g.* pre-eclampsia and fetal growth restriction) are characterized by deficient remodeling of the spiral arteries. These complications are also often associated with aberrant maternal inflammation linked to macrophage infiltration around the spiral arteries. Recent research has revealed that activated macrophages inhibit trophoblast invasiveness *in vitro* via a mechanism dependent on TNFα. Studies with rats indicate that aberrant maternal inflammation leads to utero-placental vascular abnormalities resulting in impaired utero-placental blood flow and fetal morbidities. Deficient utero-placental perfusion resulting from maternal inflammation may be linked to poor remodeling of the spiral arteries and/or maternal coagulopathies. These findings provide insight on the role of inflammation in pregnancy complications.

Tiziana Cotechini discussed how inflammation-mediated fetal growth restriction in a rat model is associated with shallow trophoblast invasion. There is evidence that fetal growth restriction is causally linked to deficient remodeling of the uterine spiral arteries leading to inadequate placental perfusion. This pregnancy complication is also characterized by aberrant maternal inflammation. Using a model in which pregnant rats are injected with repeated low doses of lipopolysaccharide (LPS), it was shown that abnormal maternal inflammation disrupts trophoblast invasion and placental hemodynamics leading to fetal growth restriction. Pups from rats receiving daily LPS injections beginning at 10 μg/kg on gestational day (GD) 13.5 followed by 40 μg/kg on GD 14.5, 15.5 and 16.5 were growth-restricted on GD 17.5. Compared to control rats, immunohistochemistry revealed fewer interstitial trophoblast cells invading the mesometrial triangle in LPS-treated animals. Ultrasound analysis revealed a decrease in spiral artery flow velocity following LPS exposure. These data indicate that inflammation-associated fetal growth restriction may be a consequence of deficient trophoblast invasion and remodeling of the spiral arteries.

Ivraym Barsoum discussed whether nitric oxide donors can prevent pre-eclampsia. Pre-eclampsia is associated with increased circulating levels of pro-inflammatory molecules such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng). In hypoxic placentas sFlt-1/sEng are released into the circulation and are associated with the hypertension and proteinuria that characterize pre-eclampsia. Low concentrations of the nitric oxide (NO) mimetic nitroglycerin inhibited the accumulation of hypoxia-inducible factor 1α (HIF-1α), an important mediator of adaptations to hypoxia, and thereby interfered with hypoxic effects. Therefore, it was hypothesized that NO mimetics can inhibit the hypoxia-induced release of sFlt-1/sEng from hypoxic placentas.Data indicated that nitroglycerin inhibits the expression and release of sFlt-1/sEng from placental explants exposed to hypoxia. The nitroglycerin-mediated inhibition of expression and release of sFlt-1 and sEng was due to diminished HIF-1α accumulation. This study provides evidence that hypoxia, through HIF-1α, induces placental release of both sFlt-1 and sEng, and this effect is inhibited by nitroglycerin. These findings indicate that NO mimetics may have potential applications in the treatment and/or prevention of pre-eclampsia.

2.3. Conclusions

Studies of cellular and molecular mechanisms regulating trophoblast invasion continue to provide new insights into the physiology of utero-placental homeostasis and its breakdown under a wide range of pathological conditions that compromise fetal-maternal health. While diseases associated with poor trophoblast invasion (pre-eclampsia and fetal growth restriction) were highlighted in this workshop, trophoblast hyper-invasive disorders such as placenta accreta, gestational trophoblastic neoplasias, and choriocarcinomas deserve equal attention. For example, choriocarcinomas exhibit refractoriness to the anti-invasive action of decidua-derived TGF-β as well as decorin. While their TGF-β resistance was shown to be, at least in part, due to loss of smad-3 expression, the reasons for the resistance to decorin remain to be explored. Further studies into why trophoblast hypo-invasiveness is shared by many, but not all, cases of fetal growth restriction and pre-eclampsia should be valuable. Consensus is needed regarding the choice of early serum markers for longitudinal screening of pregnant mothers that can predict disease and also help in devising strategies for prevention and intervention. In this regard, therapeutic use of certain animal models of pre-eclampsia may be helpful for possible application in the human.

It is also important to emphasize that the fine balance between pro-invasive and anti-invasive factors at the fetal-maternal interface can be disrupted by alterations in the local microenvironment. Among such alterations is scarring of the uterine wall (*e.g.* due to repeated caesarean deliveries) leading to a hyper-invasive trophoblast and placenta accreta/percreta, as discussed in the pathology workshop. Furthermore, the presence of high levels of pro-inflammatory molecules resulting from aberrant maternal immune activation may result in shallow invasion and the impaired utero-placental perfusion associated with various complications of pregnancy.

3. Fetal sex and intrauterine stress: boys live dangerously in the womb

Chairs: Vicki Clifton, Rohan Lewis

Speakers: Luciana Lassance, Rohan Lewis, Richard Saffery, Colin Sibley, Owen Vaughan

3.1. Outline

Sex differences in the placenta have important implications for the developmental origins of adult disease (DOHaD). Determining the relationship between sex and placental function is crucial for understanding how poor intrauterine environment contributes to the risk of chronic disease in later life. This workshop explored recent work on placental sex differences and discussed them in the context of the boys living dangerously in the womb hypothesis. The aim was to better our understanding of how males and females respond to suboptimal intrauterine conditions and how this may affect their health throughout life.

3.2. Summary

Luciana Lassance discussed how fetal sex determines insulin and IGF2 effects on the feto-placental endothelium. Insulin and insulin-like growth factor (IGF) 2 are central growth factors for the development of the placenta and the fetus. Their receptors are expressed on the placental endothelium but their cellular effects remained elusive. They can activate different signaling pathways and regulate different cellular effects in arterial and venous placental endothelial cells. These effects may also depend on fetal sex and on oxygen levels. Therefore, insulin and IGF2 effects on arterial and venous endothelial cells isolated from placentas derived from male and female fetuses were investigated by microarrays. The role of insulin and IGF2 in cell signaling was analyzed in arterial endothelial cells from both sexes under 12% and 21% oxygen. As 21% oxygen represents a hyperoxic situation, these experiments were performed in the presence or absence of the anti-oxidant NAC. Microarray analyses of the effects of insulin and IGF2 revealed differential gene expression in a sex dependent way even in the absence of the growth factors. Insulin and IGF2-stimulated signaling showed more Akt and GSK3α/β phosphorylation in arterial endothelial cells isolated from male placentas than in those isolated from female placentas. This activation was oxygen dependent occurring only at 21% oxygen and in the presence of NAC but not at 12% oxygen. These data suggest that fetal sex can modulate insulin and IGF2 effects on feto-placental endothelial cells, with the male endothelium being more susceptible to these effects.

Rohan Lewis discussed sex differences in placental gene expression. Sex differences in placental gene expression may underlie differences in the responses of male and female placentas to environmental stimuli. Analysis of genes involved in amino acid transport and metabolism identified sex differences both in average placental gene expression between male and female and in the relationships between placental gene expression and maternal factors. Sex differences were observed in the average level of expression of 8 genes including those commonly used as housekeeping genes. Sex differences were primarily observed in the most stably expressed genes, suggesting that this may be a more general phenomenon but that in many genes it is obscured by other sources of variation. Analysis of target genes suggests that there are sex dependent relationships between maternal factors, including maternal smoking, diet and parity, and mRNA levels in the placenta. These genes are therefore good candidates for further investigation of sex differences in the placenta.

Richard Saffery discussed sex-specific differences in the placental epigenome of newborn twins. Epigenetic modifications, such as DNA methylation, play a major role in controlling gene expression. Mounting evidence suggests a unique epigenetic profile in human placenta and purified cell subtypes, with evidence for considerable inter-individual variation. However, mechanisms underlying this variation remain unclear. It has been speculated that this results from a combination of genetic and environmental factors, which combine during pregnancy to modulate the level of methylation present at specific gene promoters. As part of the Peri/Postnatal Epigenetics Twins (PETS) study, placental tissue from over 150 twin pregnancies has been collected. In the current study, DNA methylation profiling of placental tissue from sixteen twin pairs was analyzed at over 27,000 genomic CpG sites, associated with 14,500 genes. Cluster analysis revealed that fetal sex was the biggest determinant of overall placental methylation profile. The majority of this effect was due to X-chromosome-associated changes resulting from X-inactivation in females. However, significant differences were also found on autosomal chromosomes (~900 probes), highlighting the role of sex-specific epigenetic change in placental genome regulation.

Owen Vaughan discussed sex differences in efficiency and amino acid clearance in the late gestation mouse placenta. It is well documented that the extent to which the intrauterine environment programs adult phenotype is dependent upon fetal sex. For instance, male offspring show increased incidence and severity of hypertension in human and animal models of fetal growth restriction. The supply of nutrients across the placenta is a major fetal growth signal and programming stimulus but differences in placental transport characteristics between the sexes have not been investigated. Conceptus growth and materno-fetal amino acid clearance in the mouse in late gestation was assessed. In normal pregnancies, the female placenta is more efficient, as determined by the number of grams of fetus produced per gram of placenta, and exhibits higher amino acid clearance per gram than that of the male; these functional differences may be related to differences in placental architecture and expression of nutrient transporters. Sex specificities in total placental nutrient supply and the combination of substrates provided to the fetus may render the male placenta less able to adapt to altered maternal environment and thus the fetus is more susceptible to adverse outcomes.

Colin Sibley discussed how corticosteroids stimulate the activity of the Na^+/H^+ exchanger (NHE) in the syncytiotrophoblast in placentas from female babies. NHE regulates intracellular pH (pH_i) in the syncytiotrophoblast; its activity in this epithelium is reduced in fetal growth restriction. During a study testing the hypothesis that corticosteroids acutely stimulate NHE in the syncytiotrophoblast, it was shown that the observed effects were dependent on the sex of the fetus. Villous fragments from term placentas were loaded with a pH sensitive fluorescent dye and the Na^+-dependent recovery of pH_i from an acid load was taken as a measure of NHE activity. In placental villi from female babies aldosterone significantly increased NHE activity but there was no effect in villi from placentas of male babies. Simultaneous application of cortisol with carbenoxolone (11-β-HSD-2 blocker) onto the villi again significantly increased NHE activity only in placentas from female babies. It is speculated that these sex dependant effects of corticosteroids on NHE activity may be related to differences in placental 11-β-HSD-2 activity.

3.3. Conclusions

This workshop was an interesting exploration of a new body of evidence identifying that the placenta does function in a sex-specific manner in both animal models and humans. Data

presented at this workshop demonstrated that sex differences are observed in the placenta at multiple levels, from epigenetic modification of DNA, through gene expression to functional changes in the way in which the placenta responds to hormones and transports nutrients. It is clear that these changes become established in the placenta via mechanisms such as DNA methylation, and are not simply due to direct effects of fetal sex hormones. Future work is required to establish the basis of sex differences and where there are common underlying mechanisms. The discussion raised a number of interesting issues including the basis of sex differences from an evolutionary perspective and how different evolutionary pressures may shape the sex differences in specific species.

4. Parasites, infections and the placenta

Chairs: Henning Schneider, Ulrike Kemmerling

Speakers: Ashley Davey, Ricardo Fretes, Thierry Fournier, Stefan R. Hansson, Ulrike Kemmerling, Cindy Morris, Demba Sarr, Henning Schneider

4.1. Outline

This workshop addressed various inflammatory responses as a sign of interaction between placental tissue and parasites like *Plasmodium falciparum*, *Trypanosoma cruzi* as well as cytomegalovirus. Changes in morphology and function of the placenta as a result of infection were discussed. The significance of different tissue reactions were also discussed in the context of defensive or toxic effects. Congenital transmission of infectious agents can lead to miscarriages and low birth weight newborns. Data on molecular mechanisms involved in the transmission of the organisms from the maternal into the fetal compartment were presented.

4.2. Summary

Ulrike Kemmerling discussed the possible mechanisms of *T. cruzi* infection in congenital Chagas' disease. Chagas' disease, one of the major public health concerns in Latin America, is caused by the hemoflagellate protozoan *T. cruzi*. In vector-related diseases, it is second to malaria in prevalence and mortality. In the past few years congenital transmission of *T. cruzi* has become more important, and partly responsible for the "globalization of Chagas' disease", constituting a public health problem of increasing relevance. Diverse pathogens, including *T. cruzi*, are able to cross the placental barrier and infect both the placenta and fetus. However, knowledge about the mechanism of tissue invasion and infection of human placenta by this parasite is scarce. *T. cruzi* induces syncytiotrophoblast destruction and detachment, selective disorganization of the basal lamina and of collagen I in the connective tissue of villous stroma and apoptosis in the chorionic villi. These results suggest that the penetration of this parasite in the placenta is a consequence of its proteolytic activity on the basal lamina and on the connective tissue. Together with the induction of apoptosis these may be part of the mechanisms of infection and tissue invasion by this parasite.

Ricardo Fretes discussed analysis of the human placental chorionic villi and trophoblast infection by *T. cruzi in vitro*. Chorionic villi of human placenta may actively participate in the control of congenital Chagas transmission. In accordance with the low transmission rate of *T. cruzi* to the fetus, there is a low productive infection of *T. cruzi* in placental tissue with no viable parasites in culture media. It was questioned whether syncytiotrophoblast and cytotrophoblast have the same susceptibility to infection by *T. cruzi*. Isolated cytotrophoblast and trypsin-denuded chorionic villi were very susceptible to infection, contrary to isolated syncytiotrophoblast and the complete chorionic villus barrier.In addition, *T. cruzi* induced detachment of the syncytiotrophoblast associated with nitrative-oxidative stress and increased expression of eNOS mRNA and protein. These data support a resistance of the complete placental barrier to infection by *T. cruzi* and a possible mechanism that this parasite might employ to infect the chorionic villi.

Ashley Davey discussed how differences in binding of human cytomegalovirus to cultured syncytiotrophoblast and Caco2 epithelial cells are due to differential expression of maternal human cytomegalovirus (HCMV) receptors. Active HCMV infections increase the risk of fetal transmission; however, this is not correlated with viral load in maternal blood or urine.It has been proposed that infectious HCMV persists and is protected in placental syncytiotrophoblast through reversible binding. This will maintain the maternal infection leading to negative vascular effects as has previously been shown and increase the risk of fetal transmission. Inoculum and not progeny HCMV reversibly binds to heparan sulfate proteoglycans on cultured syncytiotrophoblast and is protected from degradation for $>$48 h. Interestingly, Caco2, microvilliated epithelial cells, show equivalent reversible binding but a threefold higher infection efficiency compared to syncytiotrophoblast. These differences could be attributed to differential expression of HCMV receptors. Thus, even though the placenta acts like a viral reservoir, with infectious HCMV persisting in syncytiotrophoblast, a strong protective barrier to syncytial infection is maintained.

Thierry Fournier discussed how activation of PPARγ by human cytomegalovirus for *de novo* replication impairs trophoblast invasion. The HCMV, a virus of the beta herpes family, which generally has no effect on an individual's health, may cause serious disorders in immunodeficient individuals and lead to miscarriage and fetal injury in pregnant women. Indeed, an initial infection, or reinfection of a mother during pregnancy may be responsible for miscarriage, low birth weight, mental retardation or serious sensory problems in newborn babies. It is known that infection of the fetus is always preceded by infection of the placenta. It has been previously shown that activation of the ligand-activated nuclear receptor PPARγ inhibits trophoblast invasion. Interestingly, when infecting trophoblast cells, HCMV activates and uses PPARγ for its own replication, thus upsetting some of the cellular mechanisms such as invasion and migration involved in the physiological process of anchoring the placenta to the uterine wall. This can lead to abnormal development of the placenta resulting, in turn, in insufficient nutrition of the fetus and, as a consequence, the appearance of growth and neurological developmental disorders, independent of infection of the fetus itself by HCMV.

Cindy Morris discussed differential permissiveness of extravillous trophoblast-like cell lines to human cytomegalovirus infection. HCMV infection at the fetal-maternal interface may result in poor pregnancy outcomes for both mother and child. The exact mechanism(s) required for HCMV binding and entry into EVT is unclear. To determine how HCMV binds and enters EVT, studies were performed to compare infection kinetics between 2 EVT-like cell lines, SGHPL-4 and SGHPL-5. Unlike SGHPL-4 cells that are highly permissive to HCMV infection, the SGHPL-5 cells had minimal viral protein expression at high multiplicity of infection. Flow cytometric and Western blot analyses demonstrated similar surface receptor expression patterns on both cell lines at different levels; therefore the published fibroblast receptors for HCMV are likely not the reason for the lack of permissiveness of the SGHPL-5 cells. Experiments are ongoing to determine whether the HCMV gH/gL/UL128-131 pentameric complex plays a role in HCMV binding and internalization in cytotrophoblasts, as seen in endothelial and epithelial cells.

Demba Sarr discussed how the malaria parasite toxin, hemozoin, activates MAP kinases and promotes a chemotactic and immunostimulatory secretory response in primary human syncytiotrophoblast. Malaria during pregnancy is characterized by sequestration of parasitized erythrocytes and accumulation of the parasite hemoglobin metabolite, hemozoin, in the intervillous space. Primary syncytiotrophoblast cells respond immunologically to cytoadherent *P. falciparum* but their responsiveness to hemozoin has not been reported. *In vitro* exposure of syncytiotrophoblast to hemozoin induced ERK1/2 and JNK mitogen-activated protein kinase phosphorylation, secretion of chemokines, and release of cell surface receptors. The stimulated cells also elicited the specific migration of peripheral blood mononuclear cells. Finally, conditioned medium from hemozoin-stimulated syncytiotrophoblast induced upregulation of intercellular adhesion molecule-1 on primary monocytes. The dependence of the hemozoin responses on mitogen-activated protein kinase stimulation was confirmed by inhibition of chemokine release in syncytiotrophoblast pre-treated with MEK1/2 inhibitor. These findings expand the cell types known to be responsive to native hemozoin to include fetal syncytiotrophoblast and provide further evidence that syncytiotrophoblast cells can influence the local maternal immune response to placental malaria.

Henning Schneider discussed adherence of *P. falciparum* infected red cells to the trophoblast and placental inflammatory response studied by dual *ex vivo* perfusion of an isolated cotyledon. The pathogenesis of placental malaria is associated with accumulation of malaria-infected erythrocytes in the intervillous space. The *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) mediates the adherence of red cells infected with the Var2CSA strain of the parasite via binding to chondroitin A sulfate in the microvillous membrane of the syncytiotrophoblast. After closed loop dual *ex vivo* perfusion of an isolated cotyledon with malaria-infected erythrocytes in the maternal circuit, only the schizont stage of the Var2CSA showed a marked decline indicating sequestration in the placenta. The ring and early trophozoite stages, which do not express PfEMP-1, and all stages of the non-adherent 3D7 did not drop. As an inflammatory response, the trophoblast in perfusions with malaria-infected erythrocytes compared to perfusions with plain medium or with uninfected erythrocytes releases the macrophage chemokines MIF and MIP-1α into the maternal perfusate at a significantly increased rate. Microarray-based gene expression shows substantial upregulation of several pro-inflammatory cytokines (IL-1β, TNFα, IL-6) and the chemokines IL-8, MIP-1α, CCL3. Only the release of MIF and MIP-1a and the upregulation of the transcription factor c-fos seem to be malaria induced, whereas the changes in expression of other chemokines and pro-inflammatory cytokines is an unspecific response to stress related to *ex vivo* perfusion.

Stefan Hansson discussed links between pregnancy-associated malaria and pre-eclampsia. Pregnancy-associated malaria results in sequestration of parasites in the placenta, causing placental inflammation and impaired placental function. Placental malaria has been associated with an increased risk of pre-eclampsia, but the mechanisms linking these conditions are not known. Pre-eclampsia is associated with placental over-expression of hemoglobin and increased plasma concentrations of free fetal hemoglobin and the heme scavenger, alpha-1-microglobulin (A1M). In a longitudinal prospective study in Korogwe, northeast Tanzania, 1000 pregnant women were followed throughout their pregnancy with clinical and parasitological examination and collection of blood samples at 4 antenatal visits, emergency visits, and at delivery. Plasma from women with positive rapid diagnostic test and women developing pre-eclampsia, or eclampsia were analyzed for free HbF/A1M.

4.3. Conclusions

The present understanding of infection of the placenta by *T. cruzi* (Chagas' disease), human cytomegalovirus (HMCV) and *P. falciparum* (Malaria) was reviewed. The impressive diversity of mechanisms of infection and penetration of the placental barrier on the part of the infectious organism and of the defensive responses on the part of the placenta became readily apparent.

5. Decidua, embryonic and fetal development and loss

Chairs:Elisa Cebral, Alicia Jawerbaum

Speakers: Elisa Cebral, Vicki Clifton, Ana Franchi, Cristina Ibarra, Michael Soares, Stacy Zamudio

5.1. Outline

Embryos and fetuses are highly susceptible to adverse changes in the maternal environment, and the differences between their capacity to adapt to those changes and their damage and loss may be related to the adverse effects exerted in the decidua and the placenta. Throughout intrauterine development both the cells and the extracellular matrix components interact with complex mechanisms to determine the proper development that will lead to fetal survival at term. Underlying the pathologies of pregnancy, dysregulation of these mechanisms may lead to embryo or fetal loss. In this workshop, the aim was to discuss the latest information on key regulatory mediators in the decidua, the embryo and the fetuses and to explore their alterations in different diseases and adverse conditions. A main focus was to distinguish between those adverse situations that lead to embryonic/fetal survival and those that threaten embryonic/fetal life. An important question addressed was if there is a limit in the quantity or quality of damage that induces adaptive responses compatible with embryonic/fetal development when compared to those that cause severe developmental damage or lead to embryonic/fetal loss.

5.2. Summary

Michael Soares discussed decidual development in the subfertile brown Norway rat. The brown Norway inbred rat strain exhibits a subfertility phenotype and possesses a growth-restricted placentation site. Subfertility in the brown Norway rat is associated with ovarian and uterine dysfunction. Brown Norway rats ovulate fewer eggs and their ovaries show disruptions in organization and in ability to produce steroid hormones. These ovarian anomalies contribute to uterine dysfunction. The brown Norway rat uterus is resistant to progesterone, which leads to an attenuated decidual reaction and a less than optimal maternal milieu for placental/fetal development. Collectively, these maternal factors and potential intrinsic differences in brown Norway rat trophoblast development contribute to growth-restricted placentas. There are a wealth of tools developed for the brown Norway rat that can facilitate genetic dissection of pathophysiology, which makes the brown Norway rat an ideal model organism for investigating the genetics of pregnancy failure.

Elisa Cebral discussed early embryo loss, growth restriction and abnormal regulatory mechanisms after periconception alcohol ingestion. It was addressed whether moderate alcohol consumption prior to gestation and up to mid-gestation in mice is able to cause early retarded growth-differentiation, alter morphogenesis and cause embryo loss. Trophoblast cells, inner cell mass in blastocysts and cylinder eggs, and the mesenchymal and neuroepithelial cells in organogenic embryos are potential targets for alcohol-induced early embryo loss.It was discussed

how the embryonic cells, molecules and developmental processes such as proliferation and growth, migration, invasion, timing of differentiation and morphogenesis can be altered by direct alcohol action and indirectly through defective placentation. Deregulated differentiation, defective trophectoderm expansion/migration, delayed trophoblast growth, implanting embryo loss, embryo nuclear alterations, defective neural tube, disorganized neurepithelium, microcephaly, disruption of metabolic, cellular and molecular pathways in organogenic embryos, were some of the main effects found after alcohol exposure. Periconception ethanol intake exerts a strongly negative influence on the early embryo leading to fetal growth restriction and skeletal malformation in mouse fetuses at term.

Stacy Zamudio discussed links between hypoxia and fetal loss. Among humans 78/100 of all conceptions fail to result in a live birth.The rates at which embryonic/fetal loss occurs at various time points in pregnancy and their known causes were examined.In addition, the time course for oxygen changes in placenta and fetus and whether abnormalities of oxygen tension or transport are or are not likely to be causally involved in miscarriage/fetal loss/stillbirth was also assessed. This analysis allows identification of key time points in pregnancy when oxygen-related issues should be discounted or considered as a consequence of other pathological processes rather than a proximate cause of pregnancy failure. These key time points include the first trimester of pregnancy when profound fluctuations in placental oxygen tension occur, the late second trimester in which other pathological processes may impair placental oxygen diffusion, and late in gestation when the fetus, for a variety of reasons, can outgrow its supply line.

Ana Franchi discussed the role of the endocannabinoid system and early pregnancy loss. Endocannabinoids are unsaturated fatty acid derivatives which act as endogenous ligands for cannabinoid receptors and mimic the effects of natural existing cannabinoids. It has been clearly demonstrated that endocannabinoid signaling influences female reproduction during early pregnancy. Anandamide, the major endocannabinoid studied so far, has a role in implantation and embryo development. However, high levels of this molecule correlate with fetal weight loss and abortion.The participation of anandamide in a murine model of early pregnancy loss in order to develop useful tools to prevent miscarriage was assessed.It was observed that anandamide is involved in the synthesis of the pro-inflammatory molecules such as nitric oxide and prostaglandins that contribute to embryonic resorption. It was also observed that the metabolism of anandamide and nitric oxide synthesis are regulated by progesterone.

Vicki Clifton discussed maternal asthma and fetal development. It has been questioned how a fetus can respond to an adverse maternal environment in order to survive and the impact of maternal asthma during pregnancy has been investigated to address this question. Asthma is a highly common complication of human pregnancy and exerts significant effects on fetal development. Uncontrolled asthma, regardless of its severity, exerts sex-specific effects on fetal growth. The female fetus reduces growth at the end of gestation to cope with the presence of the asthma while the male fetus continues to grow normally. If asthmatic mothers' had an acute, asthma exacerbation during pregnancy, the male fetus was at risk of a low birth weight outcome, preterm delivery or stillbirth. The female fetus was unaffected by an asthma exacerbation and more likely to deliver at term. These findings raise the question of whether fetal sex should be considered when assessing fetal growth and survival in an adverse environment.

Cristina Ibarra discussed the role of nitric oxide in shiga toxin 2-induced premature delivery of dead fetuses in rats. Shiga toxin-producing *Escherichia coli* infections are a possible cause of fetal morbidity and mortality. It has been previously reported that intraperitoneal injection of rats in the late stage of pregnancy with a combination of shiga toxin 2 and LPS induces premature delivery of dead fetuses. Now it has been demonstrated that there is an increase in nitric oxide production and high nitric oxide synthase (iNOS) expression in placental tissues from rats injected with shiga toxin 2/LPS and killed 12 h after treatment. Aminoguanidine, an inhibitor of iNOS, caused a significant reduction of shiga toxin 2/LPS effects on the feto-maternal unit but did not prevent the premature delivery. Placental tissues from rats treated with aminoguanidine/shiga toxin 2/LPS presented normal histology that was indistinguishable from controls. These results reveal that placental damage and fetus mortality is mediated, at least in part, by an increase in nitric oxide production by iNOS.

5.3. Conclusions

A complex interaction of molecular signals guides embryonic/fetal development. In an adverse environment, embryonic/fetal susceptibility is highly dependent on the developmental stage as well as on other factors such as fetal sex. Furthermore, it is clearly relevant that embryonic/fetal developmental damage is in part induced by the adverse effects exerted by different pathologies on the decidua and the placenta. Damaging mechanisms have common features in different pathologies and can result in embryonic and fetal adaptations or loss. The similar insults that can lead to different effects on embryonic/fetal survival or loss illustrate the narrow gap delimiting intrauterine life and death, a key point that deserves future research.

6. Trophoblast differentiation and syncytialization

Chairs: Yoshiki Kudo, Neal Rote

Speakers: Irving Aye, Jesica Flores-Martin, Takahiro Nobuzane, Neal Rote, Ambika Singh, Tharini Sivasubramaniyam

6.1. Outline

Successful human placentation is dependent upon the transition from mononuclear villous cytotrophoblast into multinuclear syncytiotrophoblast. Major differentiation events include, but are not limited to, cellular fusion, production and secretion of hCG, development of relative resistance to apoptosis, and loss of proliferative capacity. This workshop addressed advances in the study of all components of the villous cytotrophoblast differentiation process, their interrelationships, and how variations may lead to placental dysfunction and pregnancy complications.

6.2. Summary

Nobuzane Takahiro discussed whether syncytin and its receptor ASCT2 have an influence on the fusion activity in human placental BeWo cells. Syncytin, the human endogenous retrovirus envelope protein, is highly expressed in the placental syncytiotrophoblast layer and contributes to placental trophoblast fusion. ASCT2 is a syncytin receptor and known to be identified as the amino acid transporter B0. To investigate the role of syncytin and ASCT2 on fusion activity RNA interference techniques were used in human placental BeWo cells. BeWo cells were treated with siRNA for syncytin-1 and ASCT2, and then incubated with forskolin to induce fusion. The effect of those specific siRNA treatments on the expression of mRNA and protein was evaluated by means of real-time RT-PCR and Western blotting, respectively. Cell fusion activity was evaluated by flow-cytometry and hCG secretion. The expression of both mRNA and protein of syncytin-1 and ASCT2 was reduced after incubation of specific siRNA treatment. The secretion

of hCG was also reduced after treatment with siRNA for syncytin and ASCT2, but fusion activity was only reduced after treatment with siRNA for ASCT2. This result suggests that syncytin has a different effect on fusion activity and hCG secretion compared with ASCT2 in BeWo cells.

Tharini Sivasubramaniyam discussed a novel role for Partitioning defective protein 6 (Par6) in trophoblast cell fusion. Human placental development is dependent upon the establishment of proper trophoblast cell differentiation events including fusion and invasion, shaping proper organogenesis. This study examined the contribution of polarity to trophoblast cell fusion by examining Par6, a key regulator of cell polarity. Human placental tissues across gestation, pre-eclamptic placentae and age-matched controls were collected. To establish a role for Par6 in trophoblast fusion, primary isolated trophoblast cells were cultured at 3% and 20% oxygen and Par6 expression examined spatially and temporally in conjunction with a polarity marker, Zona Occludin-1 (ZO-1). Par6 expression was assessed following forskolin treatment in BeWo cells. Par6 siRNA strategy was employed in BeWo cells and ZO-1 expression examined. Additionally, the role of Par6 on trophoblast fusion was assessed by examining the fusogenic marker GCM-1 following Par6 silencing. Early in gestation, Par6 localized mainly to the nuclei of cytotrophoblast cells while, with advancing gestation, Par6 expression increased and its localization shifted to the cytoplasm where it was found at the interface between cytotrophoblast cells and the syncytium. In primary trophoblast cells, Par6 levels were increased after 48 h of exposure to 3% oxygen and localized to tight junctions while at 20% oxygen, Par6 expression remained cytoplasmic. Silencing Par6 in BeWo cells resulted in a disruption of ZO-1 localization and this was associated with an increase in GCM-1 expression. Following forskolin treatment, Par6 expression decreased in association with an increase in syncytin expression. Interestingly, Par6 expression was decreased in pre-eclampsia relative to age-matched controls. These findings provide novel insights into a role for Par6 in regulating trophoblast cell fusion via its effect on polarity during human placental development, which may be disrupted and contribute to the pathogenesis of pre-eclampsia.

Ambika Singh discussed how ceramide, acid sphingomyelinase and ceramidase exert differential effects on trophoblast differentiation and fusion. Although trophoblast syncytialisation has been actively investigated for decades, various contradicting theories describing the fundamental nature of trophoblast differentiation and fusion exist.A novel role of ceramide, and sphingolipid synthesis/metabolic enzymes in trophoblast differentiation has recently been described. The distinct roles of ceramide in differentiation and fusion, and the signaling pathways involved in this process were investigated. Cytotrophoblast cells, isolated from term human placentas were allowed to differentiate over 7 days in culture, as assessed by measuring hCG secretion, placental alkaline phosphatase (PLAP) activity and E-cadherin expression and immunostaining. Exposure of trophoblast cells for 72 h to short chain ceramide, acid sphingomyelinase and a ceramidase inhibitor (B13) enhanced hCG secretion, indicating a role for ceramide in differentiation but not fusion. Syncytialised trophoblast expressed high levels of ceramidase, suggesting that this enzyme may be involved in maintaining the syncytial phenotype. Consistent with this view, B13 treatment reduced fusion as shown by enhanced E-cadherin expression. Inhibition of the ceramide-responsive pathways JNK-II and PP2A did not abolish the effects of ceramide, and in fact increased hCG production while having divergent effects on fusion (PLAP and E-cadherin). This study highlights the complexity of the pathways that regulate trophoblast differentiation and fusion (syncytialisation). Different agents (e.g. ceramide) can regulate differentiation independently of effects on fusion, while regulators of fusion can have no or even opposite effects on differentiation. Pregnancy complications such as pre-eclampsia, where a moderate increase in syncytialisation is observed with no effect on differentiation, may be the end result of perturbations in one or more of these pathways.

Irving Aye discussed how oxysterols inhibit trophoblast syncytialisation by activating the liver X receptor. Oxygenated cholesterol metabolites known as oxysterols display potent biological activities ranging from regulation of lipid homeostasis to cytotoxic and pro-apoptotic effects, and have been linked to lipid disorders such as atherosclerosis. Oxysterols have previously been shown to inhibit invasion of first trimester trophoblast cells, an effect which involves activation of the nuclear liver X receptor, suggesting a link between oxysterols and pre-eclampsia. In this study, the effect of several oxysterols on trophoblast syncytialisation (differentiation and fusion) in term placental trophoblast cells was investigated. Primary trophoblast cells were isolated from term placentas and allowed to syncytialise *in vitro*. Trophoblast cells were treated with various oxysterols [25-hydroxycholesterol (25-OHC), 7-ketocholesterol (7-ketoC), 22(R)-hydroxycholesterol (22R-OHC)] and the synthetic liver X receptor agonist (T0901317) at non-toxic doses, with or without pre-treatment with liver X receptor antagonist geranylgeranyl diphosphate (GGPP). Trophoblast differentiation was monitored by measuring GCM-1 mRNA expression, hCG secretion and placental alkaline phosphatase activity, while cell fusion was determined by E-cadherin immunostaining and quantification of syncytialised nuclei. Trophoblast differentiation and fusion were both significantly inhibited by all oxysterols used. GCM-1 expression was reduced between 30 and 50%, hCG secretion between 20 and 35%, alkaline phosphatase activity between 15 and 25% and cell fusion between 30 and 50%. The synthetic liver X receptor agonist T0901317 also potently inhibited trophoblast differentiation and fusion. Moreover, treatment with the liver X receptor antagonist GGPP abrogated the inhibitory effects of oxysterols and T0901317 on trophoblast syncytialisation. These findings suggest that oxysterols impair differentiation and fusion of term trophoblast cells via a liver X receptor dependent mechanism. Excessive oxysterol exposure may impair placental formation and regeneration via inhibition of syncytialisation, thereby contributing to placental pathologies.

Jésica Flores-Martin discussed how down-regulation of StarD7 by RNA interference increases β-hCG production and secretion in JEG-3 cells. StarD7 is a member of the START-domain protein family whose function remains poorly defined. It has recently been reported that StarD7 is partially relocated from the cytoplasm to the plasma membrane during *in vitro* cytotrophoblast differentiation into syncytiotrophoblast. Furthermore, it has been shown that β-catenin activates human StarD7 expression through Wnt/β-catenin signaling.To explore its function in JEG-3 cells StarD7 expression was down-regulated by siRNA. Silencing of StarD7 expression led to a marked decrease of β-catenin, Cnx43, iNOS, MBD2, ABCG2 and TGFbRII mRNA levels, all of them associated with Wnt signaling. In contrast, expression of syncytial formation markers, such as β-hCG protein production and secretion, as well as β-hCG mRNA levels, was increased by StarD7 siRNA. In addition, immunostaining for desmoplakin suggested that there was a reduction of intercellular desmosomes between adjacent JEG-3 cells after knockdown of StarD7 expression. These findings indicate that the inhibition of StarD7 transcript level alters the expression of several critical genes suggesting that it may play a role in placental development.

Neal Rote discussed how silencing of caspase 8 in BeWo suppresses β-hCG induction. Development of the human syncytiotrophoblast from mononuclear villous cytotrophoblast results in increased resistance to apoptosis. It has previously been reported

that forskolin-induced differentiation and syncytialization of the BeWo cell line resulted in increased expression of both protein and mRNA for the anti-apoptotic protein Bcl-2 and increased resistance to apoptosis. However, in cells treated with solvent alone approximately 10% underwent spontaneous intercellular fusion, and in forskolin-treated cells approximately 20% did not fuse. It was investigated whether increased expression of Bcl-2 was a result of forskolin-induced differentiation or the result of syncytialization. BeWo treated with DMSO or with forskolin for 72 h were evaluated by immunofluorescence for E-cadherin (to identify fused cells) and Bcl-2.The immunostaining intensity for Bcl-2 was similar in mononuclear cells from cultures treated with DMSO or forskolin. Compared with mononuclear cells, staining for Bcl-2 was increased by 17% in spontaneously fused cells in DMSO-treated cultures and by 30% in fused cells in forskolin-treated cultures. Forskolin treatment enhanced the production of Bcl-2 by 8% in fused cells. Thus, increased production of Bcl-2 in BeWo appears to be related more closely to syncytialization, whether spontaneous or forskolin-induced, than to differentiation.

6.3. Conclusions

Trophoblast cell differentiation and syncytial formation are two key processes in the development of the placenta. Several key regulatory molecules of these processes were discussed in this workshop. What became clear is that these two processes are differentially regulated and this perhaps highlights a need to consider these processes as being linked but quite distinct.

Conflict of interest

The authors have no conflict of interest.