HHS Public Access

Author manuscript

Placenta. Author manuscript; available in PMC 2015 December 17.

Published in final edited form as:

Placenta. 2013 March ; 34(0): S6–10. doi:10.1016/j.placenta.2012.11.020.

IFPA Meeting 2012 Workshop Report II: Epigenetics and imprinting in the placenta, growth factors and villous trophoblast differentiation, role of the placenta in regulating fetal exposure to xenobiotics during pregnancy, infection and the placenta

M.S. Ahmed[a], **L.M. Aleksunes**[b], **P. Boeuf**[c,d], **M.K. Chung**[e], **G. Daoud**[f], **G. Desoye**[g], **P. Díaz**[h], **T.G. Golos**[i], **N.P. Illsley**[j], **K. Kikuchi**[k], **R. Komatsu**[l], **T. Lao**[e], **D.M. Morales-Prieto**[m], **T. Nanovskaya**[a], **T. Nobuzane**[l], **C.T. Roberts**[n], **R. Saffery**[o], **I. Tamura**[p], **K. Tamura**[q], **N.G. Than**[r], **M. Tomi**[s], **A. Umbers**[c,t], **B. Wang**[u], **M.S. Weedon-Fekjaer**[v], **S. Yamada**[w], **K. Yamazaki**[x], **M. Yoshie**[y], and **G.E. Lash**[z,*,1]

[a]Department of Obstetrics and Gynaecology, University of Texas Medical Branch, Galveston, TX, USA

[b]Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA

[c]Department of Medicine (RMH), The University of Melbourne, Parkville, Victoria, Australia

[d]Victorian Infections Disease Service, Melbourne Health, Melbourne, Victoria, Australia

[e]Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong

[f]Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon

[g]Department of Obstetrics and Gynaecology, Medical University of Graz, Graz, Austria

[h]Maternal and Fetal Health Research Centre, University of Manchester, Manchester, UK

[i]Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI, USA

[j]Division of Maternal-Fetal Medicine and Surgery, Department of Obstetrics and Gynaecology, Hackensack University Medical Center, Hackensack, NJ, USA

[k]Department of Molecular Medicine and Anatomy, Nippon Medical School, Tokyo, Japan

[l]Department of Obstetrics and Gynaecology, Hiroshima City Hospital, Hiroshima, Japan

[m]Placenta-Labor, Department of Obstetrics, University Hospital Jena, Jena, Germany

[*]Corresponding author. Tel.: +44 191 222 8578; fax: +44 191 222 5066. gendie.lash@ncl.ac.uk, g.e.lash@ncl.ac.uk. .

[1]GEL edited this manuscript based on contributions from the other authors.

Conflict of interest statement

None of the authors have any conflict of interest to declare.

[n]Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, South Australia, Australia

[o]Cancer, Disease and Developmental Epigenetics Group, Murdoch Childrens Research Institute, Royal Children's Hospital Melbourne, Parkville, Victoria, Australia

[p]Department of Obstetrics and Gynaecology, Yamaguchi University Graduate School of Medicine, Ube, Japan

[q]Department of Endocrine Pharmacology, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan

[r]Department of Obstetrics and Gynaecology, Wayne State University, School of Medicine, Detroit, MI, USA

[s]Faculty of Pharmacy, Keio University, Tokyo, Japan

[t]Papua New Guinea Institute for Medical Research, Vector Borne Disease Unit, Madang, Papua New Guinea

[u]Department of Obstetrics and Gynaecology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

[v]The Biotechnology Centre of Oslo, Blindern, Oslo, Norway

[w]Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan

[x]Department of Obstetrics and Gynaecology, Hamamatsu University School of Medicine, Hamamatsu, Japan

[y]Department of Endocrine Pharmacology, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan

[z]Institute of Cellular Medicine, 3rd Floor, William Leech Building, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

Abstract

Workshops are an important part of the IFPA annual meeting as they allow for discussion of specialized topics. At IFPA meeting 2012 there were twelve themed workshops, four of which are summarized in this report. These workshops related to various aspects of placental biology: 1) epigenetics and imprinting in the placenta; 2) growth factors and villous trophoblast differentiation; 3) role of the placenta in regulating fetal exposure to xenobiotics during pregnancy; 4) infection and the placenta.

Keywords

Epigenetics; Trophoblast differentiation; Placenta; Xenobiotics; Infection

1. Epigenetics and imprinting in the placenta

Chairs: Richard Saffery and Claire Roberts.

Speakers: Kunio Kikuchi, Diana Morales-Prieto, Richard Saffery, Isao Tamura, Susanne Weedon-Fekjaer.

1.1. Outline

Epigenetic regulation underpins all gene expression and therefore plays a critical role in cellular differentiation and development during pregnancy and beyond. Epigenetic mechanisms include DNA methylation, histone modification, non-coding RNAs and chromatin modifiers. The placenta displays many unique epigenetic features thought to underpin its equally unique function and physiology, and this workshop aimed to explore the interplay between several such mechanisms. Initial discussion focused on microRNAs (miRNAs) in the placenta and their role in placenta-associated disease. Further discussion explored the regulation of miRNAs by DNA methylation and some of the unique features of human placenta methylation more generally. Finally, the interplay between DNA methylation and histone modification in the regulation of specific genes involved in placental function was explored.

1.2. Summary

Diana Morales-Prieto discussed the miRNA signature of trophoblast cells. MiRNAs are small regulatory RNA molecules and their expression is regulated in a tissue-specific manner. The expression of 754 miRNAs in isolated trophoblast cells from first and third trimester placentas was analyzed and compared with the expression in four trophoblast-like cell lines. Three miRNA clusters dominated the miRNA signature: C19MC, C14MC and the miR-371-3 cluster. Expression of C19MC and C14MC members changes throughout pregnancy and can differentiate between primary cells and trophoblast-like cell lines. The reported similarities and differences in the miRNA fingerprints of primary trophoblast cells and trophoblast-like cell lines may be involved in the regulation of their different behavior.

Susanne Weedon-Fekjaer discussed the differential placental expression of miRNAs in pre-eclampsia. Pre-eclampsia is a heterogeneous disease which can be subdivided into early-onset (delivery < 34 weeks) and late-onset disease (delivery ≥ 34 weeks). Differential expression of miRNAs was identified by high throughput sequencing in early-onset pre-eclampsia and late-onset pre-eclampsia compared to control placentas. After correction for multiple testing (EdgeR) it was found that 51 genes were differentially expressed between early-onset pre-eclampsia and controls while only 4 miRNAs were differentially expressed between late-onset pre-eclampsia and controls. This suggests that miRNAs could potentially be useful for molecular sub-classification of pre-eclampsia phenotypes.

Kunio Kikuchi discussed methylation of the C19MC CpG-island in trophoblast cell lines. MiRNAs derived from the chromosome 19 miRNA cluster (C19MC-miRNAs) are exclusively expressed in the human placenta. The expression of C19MC-miRNAs correlated with methylation status about 18 kb upstream of these genes in trophoblast cell lines. These *in vitro* findings suggest that the methylation status of the CpG-island regulates C19MC-miRNA expression levels, and is altered with trophoblast differentiation along the invasive pathway.

Richard Saffery discussed regulation of the placental epigenome, by sex specific, genetic and environmental factors subject to temporal change during pregnancy. The placenta encounters a myriad of environmental exposures with the capacity to alter placental function and fetal development. Many of these effects are likely to be mediated by epigenetic change, which is itself potentially regulated by maternal and fetal genetic factors. Using DNA methylation profiling of placental biopsies obtained from (i) full term monozygotic and dizygotic twin pregnancies and (ii) first and second trimester elective terminations, it has been confirmed that the unique placental epigenome in humans is subject to a variety of different influences throughout pregnancy. The resulting functional relevance to the developing pregnancy will require case-by-case examination with appropriate functional and other studies.

Isao Tamura discussed the idea that induction of insulin-like growth factor binding protein-1 (IGFBP-1) by cAMP is associated with histone acetylation in the absence of DNA methylation changes. Many genes are up- or down-regulated in endometrial stromal cells undergoing decidualization. IGFBP-1 and prolactin are preferentially expressed during decidualization and are recognized as specific markers of decidualization. The involvement of epigenetic mechanisms in the regulation of IGFBP-1 and prolactin in endometrial stromal cells was investigated by comparing them with non-endometrial control cells. High histone acetylation status of the promoter regions of IGFBP-1 and prolactin was associated with the induction of these genes by making the promoter regions accessible to transcriptional factors. DNA methylation may not play a role in the cell specific expression of these genes.

1.3. Conclusions

The investigation of epigenetic modifications in the placenta remains in its infancy. Studies to date have revealed many unique epigenetic features, including distinct miRNA and DNA methylation profiles, but much remains to be learned about how different epigenetic processes interact to control placental function and pregnancy outcome. Only when a concerted effort to profile additional epigenetic regulators is undertaken in a range of purified placenta-derived cell types, in association with specific exposures and phenotypes, will we begin to fully understand the complexities and role of epigenetics in placental function.

2. Growth factors and villous trophoblast differentiation/signalling for regulation of placental formation

Chairs: Kazuhiro Tamura and Nandor Gabor Than.

Speakers: Georges Daoud, Paula Díaz, Takahiro Nobuzane, Nandor Gabor Than, Mikihiro Yoshie.

2.1. Outline

The multinucleated syncytiotrophoblast plays essential roles throughout pregnancy, such as feto–maternal gas, nutrient and waste exchange, synthesis of steroid and peptide hormones required for fetal development, and generation of an immunological barrier between the

mother and her semi-allogeneic fetus. The syncytiotrophoblast layer is maintained by a continuous turnover process including the proliferation and functional differentiation of mononuclear cytotrophoblasts, and their fusion into the syncytiotrophoblast in the villous differentiation pathway. Inadequate formation, injury, or dysregulation of this unique tissue layer may result in several disorders of pregnancy such as fetal growth restriction or pre-eclampsia. This workshop aimed to highlight the recent advances in our understanding of biochemical aspects of villous trophoblast differentiation with an emphasis on signaling pathways and secretory regulators including growth factors.

2.2. Summary

Mikihiro Yoshie discussed the potential role of Epac on syncytialization and functional differentiation of trophoblast cells. The cyclic AMP-mediated intracellular signaling pathway is indispensable for syncytialization and endocrine differentiation of trophoblast cells. The role of exchange protein directly activated by cAMP (Epac), a cAMP signaling mediator, in the differentiation of BeWo cells was examined. Epac-selective cAMP analog (CPT) stimulated the production of hCG and progesterone, markers of trophoblast differentiation. CPT increased the number of syncytialized cells even in the presence of a protein kinase A inhibitor. Furthermore, knockdown of Epac (subtype 1) or Rap1, a downstream mediator of the Epac pathway, repressed the syncytialization. These data suggest that cAMP-mediated Epac signaling may regulate syncytialization via Rap1 and may be partially involved in functional differentiation in human trophoblasts.

Paula Díaz discussed the proposal that activation of intermediate conductance calcium activated potassium channels (IK_{Ca}) impairs cytotrophoblast syncytialization *in vitro*. Cytotrophoblasts isolated from term placentas were used to assess trophoblast differentiation. The role of IK_{Ca} in cytotrophoblast differentiation was explored, as IK_{Ca} regulates cellular turnover and differentiation in several tissues. IK_{Ca} function was demonstrated, since channel modulators activate/inhibit ^{86}Rb efflux from cytotrophoblast cells, and IK_{Ca} protein expression was confirmed. IK_{Ca} openers inhibit syncytialization and hCG secretion, thereby disrupting morphological and biochemical differentiation. The lack of understanding of factors that activate IK_{Ca} *in situ*, such as intracellular calcium, were discussed. The suggestion that inappropriate activation of IK_{Ca} underlies altered cytotrophoblast turnover in complications of pregnancy is currently being explored.

Georges Daoud discussed the role of MAPK and SFK pathways in trophoblast differentiation. Trophoblast differentiation plays multiple roles in pregnancy including implantation and nutrient exchange between the mother and the fetus. The role of MAPK and SFK in trophoblast differentiation was evaluated using human term placenta. These studies showed that ERK1/2 and p38 control the onset of cytotrophoblast differentiation into syncytiotrophoblast. Moreover, the role of SFK in trophoblast differentiation was discussed and showed that, depending on the isoform, SFKs can either promote or inhibit trophoblast differentiation. Finally, the crosstalk between MAPK and SFK pathways was discussed.

Takahiro Nobuzane discussed the role of syncytins and other fusogenic proteins in syncytialization. Two human endogenous retroviral proteins, HERV-W (syncytin-1) and HERV-FRD (syncytin-2) have been found to be relevant to human trophoblast

syncytialization. Each has a different localization and receptor in the placenta. To investigate the role of syncytin-1, syncytin-2, and the syncytin-1 receptor (ASCT2), specific siRNAs were used in BeWo cells. The fused cell-count was decreased in syncytin-1 siRNA treated cells, overlapping with syncytin-2 treated cells. In addition, β-hCG mRNA expression was suppressed in syncytin-1 siRNA treated cells. Silencing of ASCT2 had no effect on cell fusion. These data suggest that syncytin-1 has a more important role in cell fusion, compared with syncytin-2, and ASCT2 may only have a regulatory role on cell fusion as a receptor.

Nandor Gabor Than discussed villous trophoblast differentiation, placenta-specific gene expression and pre-eclampsia. Human placenta-specific galectins have emerged during primate evolution and may confer maternal–fetal immune tolerance. These galectins are highly expressed in the syncytiotrophoblast and their expression is induced during villous trophoblast differentiation. Computational and experimental data suggest that the co-option of transposable repetitive elements, which harbor binding sites for key transcription factors of villous placental development, into the 5′ UTR of these galectin genes led to their lineage-specific placental expression. The expression of placenta-specific galectins is down-regulated in preterm pre-eclampsia, in parallel with the down-regulation of some of these key transcription factors, suggesting disturbed villous trophoblast differentiation and/or syncytialization.

2.3. Conclusions

During the morphological and biochemical differentiation of the villous trophoblast, categorical reprogramming of the transcriptome occurs, leading to remarkable structural, functional, and metabolic changes in the trophoblast. These processes are tightly regulated at various levels, including growth factors and hormones, secondary messenger molecules, kinase signaling pathways, epigenetic modifications, key transcription factors binding to intergenic regulatory DNA elements, fusogenic proteins and their receptors, and placenta-specific proteins that may have autocrine functions. Dysregulation in the fine balance of these molecules and pathways has been observed in complications of pregnancy such as pre-eclampsia.

3. The role of human placenta in regulating fetal exposure to medications during pregnancy

Chairs: Mahmoud S Ahmed and Lauren M Aleksunes.

Speakers: Gernot Desoye, Nicholas Illsley, Tatiana Nanovskaya, Masatoshi Tomi.

3.1. Outline

The goal of the workshop was to discuss and come to a consensus on the role of human placenta in regulating fetal exposure to xenobiotics, including medications, environmental toxins and intermediary metabolites.

3.2. Summary

Mahmoud Ahmed presented a case study using glyburide (a drug prescribed for patients with gestational diabetes) as an example of a medication that is biotransformed by the human placenta to metabolites that are structurally identical to those formed by hepatic enzymes but in different ratios. These data suggest that the major placental metabolite, which is different from that formed by the liver, would be more accessible to the fetal circulation. It was pointed out that the placental enzyme responsible for metabolizing glyburide was cytochrome (CYP) 19, also known as aromatase, as opposed to the hepatic CYP3A4, CYP2C8 and 9 isoforms.

Nick Illsley discussed placental metabolism. Metabolism is essential for the placenta, supplying energy and the building blocks for growth and development. Metabolism is also integral to the tasks performed by the placenta, including the barrier function and materno–fetal transport. As a result, the molecular profile to which the fetus is exposed is acutely dependent on placental metabolism. The ways in which this profile can be modified and the potential for modulation of fetal growth and development by placental metabolism were discussed.

Gernot Desoye discussed gestational diabetes and the placenta. Obesity and/or diabetes (‘diabesity’) in pregnancy increases maternal and offspring risk for developing clinical features of the metabolic syndrome. This condition is recognized as one contributor to the global diabesity epidemic. The various changes (metabolic, endocrine, inflammatory, stress) in the maternal and fetal circulations affect the placenta in many ways and depend on gestational age of the diabetic insult, severity of the disease, quality and modality of treatment, maternal body mass index as well as other factors. Pharmacotherapy of the patient for treatment or prevention of gestational diabetes must be carefully considered for several reasons namely: maternal to fetal transfer of the pharmacon with as yet unknown consequences on the fetus (especially the pancreas) and/or placenta (e.g. such as those documented for treatment with glipizide). Whether these changes could modify placental functions and indirectly fetal growth is unknown but must be considered.

Tatiana Nanovskaya discussed *in vitro* techniques used in studying the role of trophoblast efflux transporters and metabolizing enzymes in placental disposition of medications during pregnancy. The discussion was focused on the advantages and limitations associated with the use of brush border membrane vesicles, subcellular fractions, and the dually perfused placental lobule in evaluating the disposition of pravastatin, 17-α-hydroxyprogesterone caproate, and bupropion (as examples). Applicability of data obtained from *in vitro* experiments to the *in vivo* condition was also considered.

Masatoshi Tomi discussed the role of anion transporters on trans-placental drug transport and its physiological significance. Organic anion transporter (OAT) 4 located at the basolateral membrane of syncytiotrophoblasts are thought to be involved in placental estrogen synthesis by facilitating the uptake of steroid sulfates as precursors from the fetal blood. Some drugs such as bumetanide and methotrexate have been shown to be transported by OAT4, implying a modulating role of OAT4 on fetal exposure to anionic drugs. These

data also support the hypothesis that OAT4 substrate drugs influence placental estrogen synthesis by modulating the uptake of estrogen precursors.

3.3. Conclusions

This workshop highlighted novel research in the field of placental transport and identified knowledge gaps for future investigations. One theme of the final panel discussion was the use of complementary techniques to evaluate maternal–fetal drug disposition. Another theme was to provide a hierarchical approach to investigating placental biotransformation/metabolism of medications and their bidirectional transfer (uptake and efflux by the tissue). Going forward, it is crucial to integrate biochemical, physiological, pharmacological, pathological, and clinical endpoints in evaluating the role of human placenta in limiting fetal exposure to drugs and environmental chemicals.

4. Infection and placenta/placental barrier and intrauterine infection (HIV, HBV, CMV, Listeria, Malaria)

Chairs: Terence Lao and Philippe Boeuf.

Speakers: Man-Kin Chung, Thaddeus Golos, Reina Komatsu, Terence Lao, Alexandra Umbers, Bo Wang, Souichi Yamada, Kaori Yamazaki.

4.1. Outline

Fetal infection can occur through the placenta, giving rise to adverse outcomes from congenital malformation such as congenital rubella syndrome, to pregnancy loss and preterm birth. Little is known about placental defense against infections, despite their long-term health implications. It is not known why maternal infection would result in fetal infection in some but not every pregnancy, and why asymptomatic chronic infection such as that of hepatitis B virus (HBV) could contribute to the high prevalence of HBV infection in endemic areas. This workshop aimed to examine mechanisms of placental defense and directions of future research.

4.2. Summary

Man-Kin Chung discussed expression of placental toll-like-receptors (TLR)-3, -7 and -8 upon placental hepatitis B virus infection. Hepatitis B is endemic in Hong Kong affecting 10.1% of pregnancies. Vertical HBV transmission is thought to be responsible for this high prevalence, yet little is known about fetal HBV infection and the associated adverse pregnancy outcomes. The effects of HBV infection on placental TLR-3, -7 and -8 were investigated. HBsAg/HBV DNA positive placentas showed increased expression of TLR-7 and TLR-8 (but not TLR-3), which positively correlated with HBV DNA load, suggesting that this increased TLR expression is a consequence of HBV infection that warrants further investigation.

Bo Wang discussed the role of viperin, a highly conserved antiviral protein constitutively expressed by human first trimester trophoblast cells, in cytomegalovirus (CMV) infections. TLR-3 is involved in host defense against CMV and its activation in trophoblast cells

induces viperin via an IFN-β-dependent pathway. Induction of viperin is one of the immune responses provoked by trophoblast cells upon CMV infections, and Swan cells infected with CMV significantly increase their viperin expression. However, pre-treatment of trophoblast cells by the TLR-3 ligand poly (I:C) cannot inhibit CMV replication in primary trophoblast cells. The actual role of viperin in CMV infection needs further investigation.

Souichi Yamada discussed congenital CMV infection (cCMV) that occurs in ~0.3% of all births in Japan, and causes birth defects and developmental abnormalities. To determine the effects of cCMV at different stages of pregnancy, a guinea pig model was used, and pathological and virological analyses of infected placentas were performed, and placental gene expression characterized. It was found that animals in early and mid-term pregnancies were more susceptible to infection and that, despite normal placental histology, the expression of several genes (especially those relating to cell differentiation and metabolism) was affected by infection.

Reina Komatsu reported on rare cases of intrauterine herpes simplex virus (HSV) infection. The prevalence of intrauterine neonatal HSV infection is low but its prognosis is poor. It is estimated that 2% of pregnant women acquire HSV during pregnancy, leading to subclinical viremia. Most of these infections are prevented by syncytiotrophoblast villous barriers and maternal immune mechanisms. However, trans-placental HSV infection through extravillous trophoblast leads to placental dysfunction, and then to miscarriage, fetal growth restriction and/or pregnancy induced hypertension. Though intrauterine HSV infection is rare, its symptoms and mechanisms should be better studied. In particular when a pregnancy is complicated with severe fetal growth restriction, clinicians should be alert to the fact that severe invasive HSV disease can occur without genital HSV infection.

Alexandra Umbers discussed malaria infection. Infections in pregnancy, such as malaria, are important contributors to maternal mortality, particularly in developing countries. There is an incomplete understanding of the dynamics of malaria over pregnancy, especially at the placental interface, termed placental malaria, where malaria-infected erythrocytes accumulate in the intervillous spaces. Therefore, the placental binding characteristics of malaria-infected erythrocytes collected from various gestational ages, using a flow cytometry-based adhesion assay were investigated. Pilot data demonstrate this assay can be applied to study placental adhesion characteristics of malaria-infected erthrocytes from the second trimester onwards. Further studies are required to determine whether a malaria vaccine aimed at blocking placental adhesion of malaria-infected erythrocytes would protect women from malaria over the duration of pregnancy.

Kaori Yamazaki discussed the association between funisitis stage III and fetal inflammatory response syndrome (FIRS). The aim of this study was to investigate the association between funisitis III, including subacute necrotizing funisitis (SNF), and onset of FIRS. Singleton deliveries were retrospectively examined from medical records and all placentas and umbilical cords histologicaly examined. Among 551 deliveries, funisitis III was observed in 22 cases. Among these 22 cases, there were two cases of SNF. One case of SNF was intact, and only one case of FIRS showed SNF. It was suggested that SNF or funisitis III may be necessary, but not sufficient, in the onset of FIRS.

Thaddeus Golos presented data on a nonhuman primate model of *Listeria monocytogenes* infection. Maternal infection by Listeria can be catastrophic for the fetus and in order to better understand the precise sequence of events leading to fetal demise and pregnancy loss, *in vivo* infection of a cynomolgus monkey at d40 of pregnancy (d165 = term) was performed. There was rapid (<8 days) fetal infection and demise with maternal intragastric infection in early pregnancy, and this outcome was associated with rapid placental as well as decidual infection and histopathology. It is proposed that this model will allow a precise understanding of the sequence of events that take place at the maternal–fetal interface to result in adverse outcomes in human pregnancy.

4.3. Conclusions

In this “Infection and placenta” workshop, all presentations highlighted our limited understanding of the pathogenesis of viral, bacterial and parasitic infections in pregnancy. A recurrent issue was the inherent associative nature of human studies conducted at delivery. Better models are needed to identify causative links between pathogen, infection and disease. Animal models would only be relevant if both the pathogen and the host recapitulate the characteristics of the infection in humans. Relevant models should advance our knowledge of the pathogenesis of these diseases and allow the design and validation of intervention strategies aimed at controlling infections in pregnancy.