GENERAL ABSTRACTS

G-1

Viral ssRNA induces a trophoblast pro-inflammatory and antiviral response in a TLR8-dependent and TLR8-independent manner

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Problem: There is growing interest in the role of viral infections and their association with adverse pregnancy outcomes. While the trophoblast is permissive to viruses, little is known about their impact on the placenta. We previously established that the Toll-like receptor 8 (TLR8) agonist, viral ssRNA, induces a pro-inflammatory cytokine response in first trimester trophoblast. Thus, we sought to determine the mechanisms involved, and whether viral ssRNA could induce an antiviral response.
Methods of study: The human first trimester trophoblast cell line, HTR8, was stably transfected to express either a TLR8 dominant negative (DN) or MyD88-DN. The wildtype, TLR8-DN or MyD88-DN cells were treated with or without viral ssRNA (5 μg/mL). After 12 hr, RNA was extracted and qRT-PCR performed for pro-inflammatory cytokines [IL-8, IL-6]; type I interferons [IFNα, IFNβ], antiviral factors [2′,5′-oligoadenylate synthetase (OAS), Myxovirus-resistance A (MxA) and apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G)]; and antimicrobial peptides [secretory leukocyte protease inhibitor (SLPI), human beta defensin-1 (HBD1) and HBD2].
Results: Treatment of wildtype trophoblast with ssRNA significantly increased mRNA for IL-6 by 8.3-fold; IL-8 by 1.3-fold; IFNα by 2.6-fold; IFNβ by 3.6-fold; OAS by 1.4-fold; MxA by 2.5-fold; APOBEC3G by 2.2-fold; and SLPI by 2.3-fold, compared to untreated controls ($P < 0.05$). ssRNA had no effect on HBD1/2 expression. The TLR8-DN significantly inhibited ssRNA-induced IL-6 by 70.1%; IL-8 by 61.7%; IFNα by 48.1%; and SLPI by 58.6%, compared to wildtype cells ($P < 0.05$). Similarly, the MyD88-DN significantly inhibited ssRNA-induced IL-6 by 69.4%; IL-8 by 70%; IFNα by 64.6%; and SLPI by 55.4%. Viral ssRNA-induced upregulation of IFNβ, OAS, MxA and APOBEC3G was unaffected by either the TLR8-DN or MyD88-DN.
Conclusions: These findings demonstrate that viral ssRNA induces a trophoblast inflammatory cytokine, IFNα, and SLPI response through TLR8 and MyD88. In contrast, the ssRNA-induced IFNβ and subsequent antiviral response occurs independently of the TLR8/MyD88 pathway.

G-2

Antiphospholipid antibodies alter trophoblast exosome release and syncytin-1 expression through a TLR4-independent mechanism

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Problem: Women with antiphospholipid antibodies (aPL) are at high risk for preeclampsia. aPL recognizing β_2GPI can directly alter placental trophoblast function, in part, through activation of TLR4. During normal pregnancy, placental exosomes are shed into the maternal circulation. The human endogenous retroviral protein, syncytin-1, is normally expressed in trophoblast exosomes and modulates immune cell activation. Since patients with preeclampsia exhibit increased placental shedding and altered immune activation, we sought to investigate whether aPL could alter trophoblast exosome release and exosome-associated syncytin-1 expression.
Methods of study: Human first trimester trophoblast cell lines (HTR8 & Sw.71) were treated with or without aPL [anti-human β_2GPI mAb, IIC5] (20 μg/mL) or an IgG control (20 μg/mL) for 72 hr, in the presence and absence of the TLR4 antagonist, LPS-RS (10 μg/mL). Exosomes were isolated from supernatants using Exoquick-TC. Exosome release was determined by visualizing pellet size and quantified by protein assay. Cellular and exosome expression of syncytin-1

protein (60 kDa) and its gylcosylated form (80 kDa) was determined by Western blot and densitometry.
Results: aPL significantly increased levels of exosomes released from trophoblast by 3.2 ± 1.5-fold, compared to untreated controls ($P < 0.05$). The IgG control had no effect on exosome release. aPL-upregulation of exosome release was unaffected by LPS-RS. aPL had no effect on the cellular levels of trophoblast syncytin-1 or its gylcosylated form. However, after aPL treatment, exosome expression of syncytin-1 (60 kDa) was significantly reduced, while expression of the glycosylated form (80 kDa) was unaffected. Both forms of exosome syncytin-1 were unaffected by the IgG control. LPS-RS had no effect on the ability of aPL to downregulate exosome syncytin-1 (60 kDa) levels.
Conclusions: These findings demonstrate that aPL alter trophoblast exosome release and syncytin-1 content in a TLR4-independent manner, without modulating cellular syncytin-1 expression. Through this mechanism, aPL may alter the crosstalk between the trophoblast and the maternal immune system.

G-3

Interleukin 17A expression in plasma and endometriotic lesions from women with endometriosis and post laparoscopic removal of endometriotic lesions

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Problem: Endometriosis is a chronic painful inflammatory condition characterized by growth of endometrial lining outside of the uterine cavity. Even though the pathogenesis of endometriosis remains elusive, the abnormal behavior of the immune system has been theorized to play a central role in the development of the disease. Indeed, numerous inflammatory cytokines are elevated in women with endometriosis. Interleukin 17A (IL-17A) produced by Th17 cells is implicated in the pathogenesis of rheumatoid arthritis and psoriasis. However, its role in the pathogenesis of endometriosis is unclear. We hypothesized that IL-17A may be involved in endometriosis development by induction of cellular proliferation and neovasculature growth.
Method of study: The concentration of IL-17A was measured from the plasma, endometrial and endometriosis tissue samples from women with endometriosis prior to and post-surgical removal of lesions. Plasma and eutopic endometrium from women without endometriosis were used as controls. The effect of IL-17A on endometrial epithelial carcinoma cell (EECC) survival was measured *in vitro* using a WST-1 cell proliferation assay and a Propidium Iodide cell cycle assay. The effects of IL-17A stimulation of EECCs were assessed by screening the supernatants for growth factors and angiogenic cytokines.
Results: Expression of IL-17A is detected in plasma and endometrial samples as well as in lesions from endometriosis patients. The concentration of IL-17A decreased post-surgical removal of lesions. Interleukin-17A did not have proliferative or apoptotic effect on EECCs *in vitro*. However, EECCs stimulated with IL-17A led to significant increase in G-CSF, VEGF, PDGF-AA and SDF-1 when compared with PBS/media controls.
Conclusions: These studies indicate the potential implication of IL-17A in the development of endometriosis. Not only is IL-17A expressed in patients with endometriosis, *in vitro* studies show that IL-17A induces expression of cytokines involved in angiogenesis and chemotaxis of leukocytes. *In vivo* as well as co culture experiment using endometrial stromal cells and epithelial cells are necessary steps to assess the effect of IL-17A in future studies.

G-4

Comparative analysis of Th1, Th2 and Th17 cytokine levels in postmenopausal women

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Problem: Little is known about the role some cytokines and cells (like IL-12, IL-17, Foxp3+ Treg, NK) in postmenopause. In the present study we investigated Th1 and Th2 cytokines with especially focusing on IL-17 producing and Foxp3+ regulatory cells (Treg) and to compare between cytokines levels changing in postmenopausal women.
Method of study: Blood samples were taken from fertile women (were used as controls, ages 29 ± 5)

and postmenopausal women (ages 62.1 ± 3; ≥10 years, since their last menstruation). Percentage of NK (CD56) and Foxp3+ Treg cell were measured by flow cytometry and cytokines IL-4, IL-6, IL-10, IL-12, IL-1β, TNF-α, IL-2, IFN-γ, IL-17 were measured by ELISA. Statistical analysis was performed by one way ANOVA followed by Tukey HSD. A value of $P < 0.05$ was considered to indicate statistical significance.

Results: Levels of IL-4, IL-6, IL-12, IL-1β, IL-17 and NK(CD56) in postmenopausal women were significantly increased than in fertile women. However IL-2 and IL-10 were significantly lower in the postmenopausal women than that of controls. TNF-α, IFN-γ and Foxp3+ Treg were not different between two groups.

Conclusion: According to our results, increased innate immunity and Th1 and Th17 mediated adaptive immunity in postmenopausal women may explain increasing prevalence of chronic inflammatory diseases in postmenopause.

G-5

Relationship of iron supplementation during pregnancy with the opsonization and complement components

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Problem: Iron and other micronutrients have immunomodulating functions that influence the susceptibility of a host to infectious diseases. The objective of the present study was to confirm whether iron supplementation during pregnancy can alter the immunologic aspects especially phagocytosis.

Method of study: Blood samples were obtained from healthy 85 women, aged 20–35, chosen from the intake to the Department of Gynecology and Obstetrics Center at Private Bogazici Hospital. They had applied for regular checkups. Randomized controlled study, pregnant women were randomly allocated to iron replacement and non-replacement group. (i) nonpregnant control group (late luteal phase, $n = 25$) (ii) iron supplemented pregnant group (IS) ($n = 35$) and (iii) non-iron supplemented pregnant group (NIS) ($n = 25$). Material and methods: Complete Blood Count (CBC), iron parameters, IL-1, IL-1B, CD19, IgM, IgG, C3a, C5a levels and opsonization capacity were assessed. CD19 levels were assessed by flow cytometry method (BD Biosciences USA). IL-1 and IL-1B levels were measured by ELISA method (Anogen, Canada). Nephelometric method and ELISA kits were used for the measurements of serum IgG and IgM levels (Beckman Coulter, Inc. and IMMAGE Immunochemistry Systems). C3a and C5a were from Calbiochem (Germany). Phagocytic activity was assessed by Nitroblue tetrazolium chloride (NBT). Opsonization was shown by the Iodination procedure.

Results: All of the serum iron and complement parameters were found to be better and statistically significant in replacement group ($P < 0.05$).

Conclusion: Our research results showed serum iron parameters and non-specific immune system parameters are found to be in favor of iron supplemented group compared to control group. Iron replacement therapy may improve the cellular immunology in pregnant women. From a biological point of view, we propose that various level iron replacement therapy may contribute to different aspects of the reproductive immunology. We thought that one of the best ways for pregnant women is to lead a healthy, balanced iron replacement.

G-6

Trophoblast promote LPS tolerance in macrophages by enhancing the expression and function of TAM receptors

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Problem: One of the most abundant immune cells present throughout pregnancy are decidual macrophages, which play an important role in contributing to the immunoregulatory environment at the maternal/fetal interface. In early pregnancy, macrophages migrate into the decidualized endometrium and are found in close proximity to trophoblast cells suggesting a cross-talk between these two cell types. We have previously shown that trophoblast induce a unique population of CD14+/CD16+ macrophages with an M2 phenotype and tolerant response to LPS. The objective of this study is to characterize the molecular pathway regulating the response to LPS in

trophoblast-derived macrophages (TDM). Here we demonstrate that trophoblast factors induce the expression of TAM signaling components, well-defined negative regulators of inflammation.
Method of study: Peripheral blood monocytes were treated with or without 50% conditioned media (CM) from the first trimester trophoblast cell line, Sw.71. Phenotypic changes were determined by flow cytometry and gene array and LPS cytokine response and signaling by multiplex analysis and Western Blot.
Results: TDM's express high levels of the Mer receptor and its ligand GAS6. Furthermore, these cells constitutively secrete high levels of IL-10 while constitutively expressing p-stat3, a downstream target of TAM signaling. In concurrence with this phenotype TDM's produce high amounts of RANTES and IP-10 and dampened levels of TNFα and IL-6 in response to LPS.
Conclusion: We demonstrate that trophoblast secreted factors can induce monocyte differentiation into a unique macrophage that express Mer, GAS6, and p-stat3, major regulators of inflammation. Alterations of this phenotype and therefore function of these cells may predispose to complications later in pregnancy. These findings support how the microenvironment of the placenta can modulate the phenotype of macrophages present at the decidua.

G-7

Estradiol primes vaginal dendritic cells to induce potent Th17 responses through an IL-1 dependent pathway

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Problem: Antigen presenting cells (APCs) such as dendritic cells (DCs) and macrophages play an important role in priming inflammatory and tolerant responses on mucosal surfaces. We examined the phenotype and function of APCs from the lung, vagina and small intestine to determine if there were unique functional adaptations depending on the microenvironment.
Methods of study: Tissue resident APCs from the vagina, lungs and intestine were isolated, phenotyped and sorted by flow cytometry. OVA-Tg CD4+ T cells were co-cultured with total or sorted APC populations pulsed with OVA peptide and examined functionally. Ovariectomized (OVX) estradiol (E2) - or control mice were immunized intranasally with HSV-2 TK- and challenged 3 weeks later intravaginally with WT HSV-2 and T cell responses examined.
Results: Vaginal mucosa has an enriched population of CD11c+ CD11b+ MHCII- DCs, unlike the lung or small intestine. Functionally, mucosal APCs from all tissues induced similar proliferative and Th1/Th2 cytokine responses in co-cultures with OVA Tg CD4+ T cells. However, vaginal CD11c+ DCs induced 10–20 fold higher Th17 responses compared to lung or intestinal CD11c+ DCs. The IL-17 responses were almost completely abrogated in APC-T cell co-cultures where APCs were from IL-1α KO and IL-1β KO, but not IL-6 KO animals, and the Th17 response was restored by addition of exogenous IL-1β. Vaginal DCs from OVX mice and estradiol-receptor KO mice were unable to prime the Th17 responses, showing that E2 plays a critical role in priming vaginal DCs. *In vivo* experiments showed that E2-treated, immunized mice had significantly higher frequency of Th17 cells in the vaginal tract on Days 1, 3 and 5 post-HSV-2 challenge, that were absent in OVX controls.
Conclusion: These results indicate that vaginal tract has a unique microenvironment regulated by E2, which primes CD11c+ DCs to induce significantly higher Th17 responses, through an IL-1-dependent, but an IL-6-independent mechanism.

G-8

Carbon monoxide inhibits bacterial-induced IL-1beta expression via inhibition of caspase-1 activity

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Problem: Inflammation is a common cause of preterm birth linked to bacterial infection that stimulates the production of pro-inflammatory cytokines,

such as IL-1β. IL-1β is reported to induce preterm labor in animal models. We have previously shown that low dose of carbon monoxide (CO) has potent anti-inflammatory properties and it can inhibit E.coli-induced secretion of IL-1β in placental explants. However it is unclear if this effect of CO is mediated at the level of transcriptional or post-translational level. It is also unclear if CO has direct effects on trophoblast cells or if the effects are mediated through other placental cell types. Therefore we evaluated the effects of CO on transcription and post-translational modifications of IL-1β in placental explants and isolated trophoblast cells.

Method of study: Cultures of placental explants and isolated trophoblasts were established from tissues harvested from second-trimester elective terminations of pregnancy. Cultures were stimulated with heat killed *E. coli* and incubated under either room air or 250 ppm CO with 5% CO2. Tissues and trophoblast cells were harvested, quantified for mRNA levels by real-time PCR and for protein levels by ELISA and Western blotting using anti-IL-1β and anti caspase-1 antibodies. Placenta tissues were fixed and immunostained for IL-1β.

Results: Elisa analysis showed that treatment of placental explants with E.coli increased total IL-1β expression by three fold ($P < 0.001$). However, CO did not inhibit total IL-1β Expression. Similarly, the stimulation of placenta explants with E.coli induced the expression of mRNA IL- 1β but CO did not. *E. coli* stimulated the expression of both precursor and active form of IL-1β, however exposure to CO inhibited the formation of active form by 30% ($P = 0.022$). Further, CO reduced the expression of the active form of caspase-1(interleukin-1 converting enzyme) by 35% ($P = 0.001$) which is an enzyme that proteolytically cleaves precursor forms of IL- 1β into active mature form of IL-1β. Immunohistochemistry showed that the active form of IL-1β was highly expressed in placental Hofbauer cells after E.coli stimulation.

Conclusion: The exposure to CO inhibits active IL1β - formation through caspase-1 cascade. Since blockade of IL-1β is an important step to limit bacterial induced inflammation, these results will contribute to our understanding of how CO interferes with the formation of active pro-inflammatory cytokine, IL1β-. This finding has an important implication in the development of therapeutic interventions using CO to prevent inflammation-induced preterm labor.

G-9

Effect of sulforaphane on placental HO-1 expression and cytokine production

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Problem: Sulforaphane (SFN) is a non-nutritive component of cruciferous vegetables that activates nrf-2, which in turn, increases the production of heme oxygenase-1 (HO-1), an enzyme with potent anti-oxidant and anti-inflammatory properties. However, the effects of SFN on HO-1 or cytokine production by placental tissues are unknown. Therefore, we evaluated the effects of SFN on basal and bacteria-stimulated HO-1 and cytokine production using a well-established placental explant culture system.

Method of study: Second trimester placental explant cultures were incubated for 18 hr in the presence of 0, 5, or 10 μM SFN and then stimulated with or without 107 CFU/mL heat-killed *E. coli* for an additional 18 hr. Conditioned medium was assayed for IL-1 beta, TNF-alpha, IFN-gamma, IL-10 and IL-1ra and HO-1 levels in the remaining tissues were quantified by western blotting. In additional experiments, placental cultures were cultured in 8, 16, 32, 64, or 128 μM SFN for 48 hr and relative viability of the tissues was ascertained with the MTT assay.

Results: 5 μM SFN increased basal expression of HO-1 placental explants ($P = 0.008$). HO-1 production by *E. coli*-stimulated cultures was also stimulated by 5 ($P = 0.058$) and 10 μM ($P = 0.012$) SFN. As expected, *E. coli* increased the expression of IL-1 beta, TNF-alpha, IFN-gamma, IL-10 ($P < 0.001$ ea.) and IL-1ra ($P = 0.046$). Treatment with 10 μM SFN reduced *E. coli*-stimulated production these cytokines ($P < 0.001$) except for TNF-alpha. 10 μM SFN also reduced ($P < 0.001$) the secretion of these cytokines by unstimulated cultures. Culture of tissues with up to 32 μM SFN had no detectible effect on their viability. MTT activity was slightly (<10%) reduced for cultures treated with 64 ($P = 0.004$) and 128 μM ($P < 0.001$) SFN, however.

Conclusion: High concentrations of SFN reduce the viability of placental explant cultures. However, at

subtoxic levels, SFN promotes HO-1 expression and reduces the production of cytokines associated with preterm birth.

G-10

Uterine radial arterial resistance index and auto-immune abnormalities in women with recurrent pregnancy losses

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Problem: Ultrasonographic evaluation of uterine blood flow using Doppler technique may reflect angiogenesis and vascular remodeling at fetal-maternal junction. However, underlying etiologies for increased uterine radial artery resistance index (U-RI) have not been thoroughly studied. In this study, we aim to investigate a possible relationship of U-RI and auto-antibodies in women with recurrent pregnancy losses (RPL).

Method of study: A retrospective medical record review was carried out in 189 women with RPL, who consecutively enrolled Reproductive Medicine program at Department of Obstetrics and Gynecology, Chicago Medical School at Rosalind Franklin University of Medicine and Science. Correlations between U-RIs and auto-antibodies were analyzed in women with RPL (≥2).

Results: U-RIs are higher in RPL women with anti-phospholipid antibodies (APA) than those without APA ($P = 0.018$). U-RIs are higher in RPL women with APA-IgG and APA-IgA ($P = 0.015$ and $P = 0.030$, respectively) as compared to those without APA.U-RIs of RPL women with APA-IgG and APA-IgA are higher than those of APA-IgG and APA-IgA negative respectively ($P = 0.025$ and $P = 0.049$, respectively). Significant differences in U-RIs were found in women with anti-phosphatidic acid antibody ($P = 0.045$) and anti-phosphatidylinositol antibody ($P = 0.048$) as compare to those of APA negative women. U-RIs of ANA positive women are higher, but not significantly different ($P = 0.235$) as compared to ANA negative women. U-RIs in women with other auto-antibodies, such as anti-thyroperoxidase antibody, anti-thyroglobulin antibody, auto-antibodies to dsDNA, ssDNA, histone, and Scl70 are higher than those of auto-antibody negative women, but these are not significantly different.

Conclusions: Higher U-RI and decreased uterine arterial blood flow are associated with APA in women with RPL. Further studies are needed to elucidate the underlying immune pathologies associated with impaired uterine perfusion in women with APA.

G-11

Maternal recognition of pregnancy: role of PreImplantation Factor (PIF*)

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Abstract: Maternal recognition followed by maternal adaption is essential for pregnancy success. While the knowledge on maternal adaptation is well established, the timing and elements involved in maternal recognition are limited. There is evidence that shortly post-fertilization, maternal recognition initiates, evidenced by observed changes in the maternal immune milieu i.e. platelet emargination, and an increase in circulating Tregs. This requires presence of a viable embryo and embryo-derived specific signaling. The secretion of PIF a 15 aa peptide by viable embryos (2-cells murine, 4-cells human, 6-cells bovine) might represent this essential embryo-specific, essential signal: Placenta derived PIF levels in maternal circulation correlate with good pregnancy outcome. Beyond PIF autotrophic effect on the embryo development as an immune signal regulates global maternal immunity; minimal effect on innate and maximal on activated immunity. Primary target are CD14+ cells- antigen presenting cells through increased B7H1 expression on T cells. PIF blocks activated PBMC MLR, proliferation, creating Th2/Th1 cytokine bias gene/proteins. In patients with history of recurrent miscarriage PIF blocks NK cells toxicity by reducing pro-inflammatory NK CD69 expression. *In vivo* PIF acts by blocking activated macrophages/neutrophils migration and extravasation. Priming of the endometrium is required prior to implantation; through adaptive mechanisms PIF promotes beta integrin, at implantation pro-inflammatory, pro-adhesive, apoptosis regulating genes/proteins, phosphorylated kinases post-implan-

tation protection against environmental toxins. In parallel, PIF enhances trophoblast invasion balancing TIMP/integrin ratio as well up-regulating trophoblastic pro-tolerance HLA-G expression. Finally, *in vivo* PIF administration maximizes the number of implantation sites that successfully reach the fetal stage (murine). Collective data corroborates that PIF-initiated maternal recognition orchestrates maternal adaptation that is required for successful pregnancy outcome.
*PIF Proprietary

G-12

IL-33-responsive group 2 innate lymphoid cells are present in mouse uterine tissue and may play roles in healthy pregnancy

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Problem: Group 2 innate lymphoid cells (ILC2s) that are responsive to IL-33 drive helminth immunity, type 2 immune responses, and tissue pathology and homeostasis in mucosal organs, such as lungs and skin. Considering their biological effects, ILC2s may also play a role in the placenta and be involved in fetus-protective type 2 immunity. Indeed, recent reports have implicated changes in levels of both IL-33 and soluble IL-33 receptor (i.e. sST2) in spontaneous abortion and pre-eclampsia. Here, we sought to examine the presence of ILC2s in uterine tissue and investigate the effects of ST2 deficiency on successful pregnancy outcomes in mice.
Methods of study: Single cell suspensions of murine uteri were examined by flow cytometry for the presence of ILC2s. Dynamic changes in ILC2s in uteri were examined in IL-5-reporter mice by administering IL-33 systemically. To examine the role of IL-33/ST2 signaling in healthy pregnancy, ST2−/− females (on a Balb/c background) were mated with ST2−/−, MHC-matched Balb/c and MHC-mismatched C57B6 males. Balb/c, C57B6 and Balb/c × C57B6 pairs were used as controls. Litter sizes and numbers of non-viable pups (survival less than 24 hr) were examined.
Results: Lineage-negative, CD25+ and CD44+ ILC2s were found in normal murine uteri. Systemic administration of IL-33 to naïve Balb/c mice increased the ILC2 numbers in uteri and induced IL-5 production by them *in vivo*, suggesting that IL-33 affects the number and activity of uterine ILC2s. Litter sizes were not significantly different among the pairings irrespectively of their genotypes. However, total numbers of non-viable pups, percent of litters with at least one non-viable pup and percent of non-viable pups per litter were significantly increased in ST2−/− × C57B6 pairs when compared to control pairs.
Conclusion: IL-33-responsive ILC2s are present in murine uterine tissue and may play pivotal roles in successful reproduction.

G-13

Interleukin-16 enhances ovarian tumor associated neoangiogenesis

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Problems: Ovarian cancer (OVCA), a lethal malignancy of women, disseminates locally in the peritoneal cavity. Tumor microenvironment plays important roles in OVCA metastasis. Tumor associated neo-angiogenesis (TAN) is a hall mark of OVCA progression and cytokines may stimulate the establishment of early TAN. Interleukin (IL)-16, a pro-inflammatory cytokine is associated with OVCA development. The goal of this study was to examine whether IL-16 stimulates ovarian TAN during OVCA development.
Method of study: Four years old White Leghorn laying hens with (n = 17) or without (n = 20) ovarian tumors were selected by ultrasound scanning and OVCA stages was determined following euthanasia. Ovarian tissues and serum samples from hens were processed for immunoassay, immunohistochemistry (IHC), proteomic and gene expression studies for IL-16 and SMA-expressing micro vessels. Normal ovarian epithelial cells were treated with IL-16 to determine expression of IL-8, an angiogenic factor. HUVEC cells were examined for CD9 (receptor for IL-16) expression. Differences in IL-16 expression and micro vessel frequencies among normal and OVCA hens were determined by one-way ANOVA and paired T-tests.
Results: OVCA were limited to the ovaries in eight hens (early stage) and metastasized in nine hens (late stage). IL-16 expression was significantly

($P < 0.01$) high in hens with early stage OVCA and increased further in late stage OVCA. Frequency of SMA-expressing micro vessels were significantly ($P < 0.001$) high in OVCA hens than normal hens. Increase in IL-16 expression in OVCA hens was positively correlated with the frequencies of SMA-expressing micro vessels. A strong band for IL-8 was detected in IL-16 treated cells and HUVAC cells expressed CD9 proteins.

Conclusion: The results suggest that changes in IL-16 expression were associated with increased frequency of SMA-expressing micro vessels. IL-16 enhanced expression of IL-8, possibly through its receptor CD9. These results suggest a novel role of IL-16 and may be useful in designing antitumor immune therapeutics targeting IL-16 for OVCA prevention.

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G-14

Chorioamnionitis induced by inactivated group B streptococcus: from placental lesions to autism spectrum disorders features

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Problem: A high incidence of neurobehavioral disorders, such as autism spectrum disorders, occurs in children born to mothers who experienced infections during pregnancy. According to our hypothesis group B streptococcus (GBS) induced maternal immune activation plays a role in placental lesions and offspring subsequent neurobehavioral disabilities.

Methods of study: We designed a new pre-clinical animal model in which dams were injected every 12 hr with inactivated GBS (109 CFU) from serotype 1a GBS, versus saline, from gestational day (G) 19 to G22. Some dams gave birth naturally at G23 (behavioral studies with pups) and C-sections were performed at G22 on other dams to remove placentas for immunohistochemical studies and proteins analysis.

Results: GBS-exposed placentas presented cystic lesions and polymorphonuclear infiltration located within the decidual/maternal side of the placenta. Interestingly, preliminary results showed higher level of PMN infiltration and expression of MMP8 in placentas associated with male than those associated with female fetuses. Surprisingly, cardinal features of human autism were found predominantly in males, characterized mainly by social interactions impairments, lack of exploratory behavior and singular sensory processing.

Conclusions: Our results show for the first time that materno-fetal inflammatory response to GBS plays a role in the induction of placental insults and neurobehavioral disabilities in offspring. Placental lesions and changes in placental proteins may spread through a fetal inflammatory reaction syndrome (FIRS) affecting developmental processes of the offspring's brain, thereby increasing its susceptibility to ASD, especially for males.

G-15

Distinct microRNA and their putative target mRNA expression in endometrial lymphocytes, endometrium and trophoblast during healthy and abortive porcine pregnancy

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Problem: Approximately 25–40% genetically normal conceptuses are spontaneously lost during peri-attachment and mid-pregnancy in pigs. A deficit in vasculature is one of the major factors associated with conceptus loss. During early pregnancy, endometrial lymphocytes are uniquely recruited to the maternal-fetal interface by conceptus derived signals. They adopt a specialized phenotype that regulates placental angiogenesis but precise mechanism is not known. microRNAs are emerging as bio-regulatory molecules of various processes including angiogenesis. We hypothesize that microRNAs are involved in development of endometrial lymphocytes and their angiogenic functions at the maternal-fetal interface.

Methods of study: Laser capture micro dissected endometrial lymphocytes, endometrium, and trophoblasts associated with healthy and spontaneously

arresting conceptus attachment sites (CAS), at two well defined periods of fetal loss (gestation days 20 and 50), were screened for miRNAs involved in immune cell development and pro- or anti-angiogenic functions using targeted miRNA-PCR arrays. In addition, the levels of putative mRNA targets using Q-PCR and subsequent functional clustering of genes were studied in order to decipher biological mechanisms affected during pregnancy loss.

Results: Compared to healthy CAS, miRNAs such as ssc-miR-150, ssc-miR-296-5P and ssc-mir-19a were significantly up-regulated in endometrial lymphocytes associated with arresting CAS at gd20 ($P < 0.05$). Significant differences also found in endometrium for microRNAs such as ssc-miR-20b, ssc-miR-17-5P, and ssc-miR-18a. In trophoblasts associated with arresting CAS, ssc-miR-15b-5P, ssc-miR-18a, and ssc-miR-122 were significantly down-regulated. Finally, selected mRNA targets showed differential expression in endometrial lymphocytes, endometrium, and trophoblasts from arresting CAS compared with healthy CAS. Functional clustering to predict the pattern of genes revealed altered biological processes potentially affecting the fetal viability.

Conclusions: Our data strongly suggest microRNAs are involved in endometrial lymphocyte development and their angiogenic functions at the maternal-fetal interface. Further, we provide evidence that microRNAs interact with potential target mRNAs to modulate important biological processes during successful or abortive porcine pregnancy.

G-16

In vitro differentiation of bovine macrophages

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Problem: Monocytes differentiate into macrophages and dendritic cells to elicit an immune response. Macrophages are broadly classified as either M1 or M2 based on expression of proteins. There is limited information on protein expression to classify bovine macrophages. The objectives of this project were to: determine an optimal serum free culture method for bovine monocytes, differentiate bovine monocytes into M1 or M2 macrophages *in vitro*, and determine protein 'markers' for M1 and M2 macrophages.

Method of study: In this study, media, cell number, and time in culture were tested for optimum conditions. Macrophages were then treated with GMCSF, GMCSF + IFNG + LPS, MCSF, MCSF + IL4 or MCSF + IL10. Expression of cell type-specific genes was determined using quantitative polymerase chain reaction (qPCR). The mRNAs quantified were Tumor Necrosis Factor (TNF), Interleukin 1-beta (IL1B), and inducible Nitric Oxide Synthase (NOS2) to identify M1 macrophages, and Mannose Receptor 1 (MRC1), Kruppel Like Growth Factor 4 (KLF4), and Cluster of Differentiation 163 (CD163) to identify M2 macrophages.

Results: The culture medium that best supported the attachment, growth and viability of monocytes was Xivo-10 media supplemented with insulin, transferrin, selenium and gentamycin. Macrophages treated with IL4 exhibited a giant cell morphology as described in other species. Treatment with MCSF + IL4 or MCSF + IL10 resulted in greater steady state concentration of CD163 mRNA, indicative of M2 macrophages, while treatment with GMCSF + IFNG + LPS resulted in greater concentration of TNF, IL1B, and NOS2 mRNAs, indicative of M1 macrophages.

Conclusion: Bovine monocytes can be successfully cultured in serum free medium, and exhibit morphological characteristics consistent with differentiation to macrophages. This culture system can be used to produce *in vitro* differentiated macrophages and provides the information needed to classify bovine macrophages as M1 or M2.

G-17

The role of fetomaternal MHC class II histoincompatibility in regulating tolerance of the semi-allogenic fetus

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Problem: Viviparous pregnancy represents a physiological state in which the maternal immune system must tolerate a semi-allogenic fetus expressing 'non-self' antigens derived from the paternal genome. While a critical role for paternal antigen-specific regulatory T (Treg) cells in regulating fetal tolerance is now well established, roles for specific classes of paternal antigens in regulating Treg cells are not defined.

Method of study: Literature review.
Results: Studies in animals and humans support a role for MHC class II allele disparity (histoincompatibility) between mother and fetus in preventing fetal rejection that can cause abortion, fetal growth restriction, and preeclampsia. Although MHC class II molecules are not present on the surface of trophoblasts and likely do not serve as targets for maternal anti-fetal rejection, they are present on sperm, in seminal fluid, and intracellularly in trophoblasts where they are likely released into maternal circulation within exosomes. Paternal class II antigens are therefore subject to indirect presentation by maternal tolerogenic antigen-presenting cells to generate paternal class II-specific Treg cells. MHC class II disparity promotes tolerance of allografts at other immune-privileged sites including liver and cornea, and expression of donor class II antigens through tolerogenic routes promotes acceptance of allografts at non-immune-privileged sites. MHC class II, but not MHC class I, alloantigens are capable of triggering linked suppression of the full complement of alloantigens present in an allograft, including minor H antigens (mHAgs) which are present on trophoblasts and represent likely targets of anti-fetal alloresponses. Evidence for MHC class II-induced linked suppression during human pregnancy comes from studies showing that fetomaternal MHC class II histocompatibility regulates amelioration of maternal autoimmunity.
Conclusions: We propose a model whereby allogenic paternal MHC class II antigens trigger linked suppression of fetal mHAgs and a lack of fetomaternal class II disparity facilitates effector immune responses to mHAgs mediating fetal rejection.

G-18

Impact of gestational nicotine exposure on intrauterine and fetal infection in a rodent model

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Problem: Based on recent epidemiological studies, women who smoke were at significantly higher risk for chorioamnionitis. With the increased use of electronic nicotine delivery systems among young reproductive age women, the role of nicotine exposure and its relative risks independent of smoking is of concern. However, few if any studies have examined the interaction between prenatal nicotine exposure and infection.
Method of study: We investigated the interaction between prenatal nicotine exposure and intrauterine infection using an established, well-defined rodent model. At gestation day (GD) 6, pregnant rats were implanted with an osmotic minipump delivering saline or 6 mg/kg/day nicotine (NIC). At GD 14, NIC-pump rats received 105 CFU *M. pulmonis* (Nic+MP) or sterile broth (Nic only); saline-pump rats received *M. pulmonis* (MP only) or sterile broth (controls). At GD 18, rats were necropsied, maternal and fetal tissues were cultured and processed for histological lesions.
Results: Nic+MP rats had extensive mucoid exudate present in the uterine lumen, which was not observed in MP only rats. Nicotine exposure did not impact colonization rates of maternal sites, but significantly increased ($P \leq 0.02$) the percentage of amniotic fluids and fetuses that were infected. Prenatal nicotine exposure reduced the threshold of placental microbial load that was required for infection of the amniotic fluid and fetus ($P < 0.004$).
Conclusions: Prenatal exposure to nicotine increased the risk for intrauterine infection, lowering the infectious dose required to establish placental loads sufficient to breach the placental barrier and infect the amniotic fluid and fetus, and also altered the pathology associated with maternal and fetal sites, increasing the severity of lesions associated with fetal inflammation.

G-19

Seasonal trends in early-onset severe preeclampsia

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Problem: It has been postulated that seasonal variation influences the manifestation of some immune disorders. Considering the immune etiology of preeclampsia, we investigated whether early-onset severe preeclampsia (sPE) varies by season of conception or season of delivery.

Methods of study: We queried an electronic database at Brigham & Women's Hospital for pregnant women who were delivered for early-onset sPE (20–32 weeks GA) from 2000 to 2010. Pregnancies with multiple gestation or chromosomal abnormality were excluded. Months of conception and delivery, and indication for delivery were abstracted. Conception and delivery dates were also grouped by season: Fall (9/22–12/20); Winter (12/21–3/19); Spring (3/20–6/20) and; Summer (6/21–9/21). Indications for delivery were categorized as fetal (non-reassuring fetal heart tracing, abnormal umbilical artery dopplers, etc.), maternal (severe hypertension, headache, pulmonary edema, HELLP, etc.), mixed (maternal and fetal) or unspecified. Seasonal sPE rates and delivery indications were compared to normal distribution by the McNemar test, with alpha=0.05.

Results: In total, 143 women with early-onset severe preeclampsia met inclusion criteria. Among sPE cases, the most common months for conception were March and November while January and September were the most common months for delivery. The largest month-to-month decrease in sPE conceptions occurred at the warm-weather transition between winter and spring (March to April); meanwhile, the greatest month-to-month increases in sPE deliveries occurred during cold-weather transitions (Summer to Fall & Fall to Winter). Overall, the distribution of sPE deliveries by season was: Fall (19.6%), Winter (23.1%), Spring (31.4%) & Summer (25.9%); the distribution of sPE by season of conception was: Fall (29.4%), Winter (25.2%), Spring (19.6%) & Summer (25.9%). The overall distribution of sPE cases by season of conception or season of delivery did not vary significantly from normal distribution (p=NS). Delivery indications for sPE were: fetal (27.3%), maternal (55.9%), mixed (12.6%), and unspecified (4.2%). The distribution of sPE subjects delivered for fetal indications was: Fall (21.6%), Winter (24.3%), Spring (32.4%) &, Summer (21.6%); the distribution of sPE deliveries for maternal indications was: Fall (17.1%), Winter (21.9%), Spring (30.5%) &, Summer (30.5%). Seasonal distribution of sPE cases by delivery indication was not significantly different from normal distribution (p=NS).

Conclusion: Despite a large cohort of early-onset sPE cases no clear seasonal trend was appreciated for delivery or conception, even when stratified by delivery indication. The higher rate of deliveries for sPE during cold-weather seasonal transitions is intriguing but the difference was not significant.

G-20

MiR-21 and miR-519d regulate proliferation, migration invasion and apoptosis in trophoblastic cells by targeting phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4)

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Problem: MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression by binding to the 3′-untranslated regions (3′UTRs) of mRNAs. MiRNAs are abundantly expressed in the placenta, many of which tissue-specifically, and contribute to the regulation of its development. However, the function of most placental specific miRNAs remains to be investigated. In previous studies, our group demonstrated that miR-21, mostly considered as being an oncomir, is the highest expressed miRNA out of 762 analyzed miRNAs in 1St trimester trophoblast, while miR-519d is one of the highest expressed miRNAs in 3rd trimester trophoblast. Therefore, the aim of this study is to investigate the functions of miR-21 and miR-519d and their targets by using trophoblastic cell lines as models.

Methods of study: The immortalized human trophoblast cell line HTR-8/SVneo and the choriocarcinoma cell line JEG3 were transfected with either a miR-21 or miR-519d inhibitors (for silencing) or mimics (for overexpression). Total RNA was extracted and RNA quality and quantity were determined at a spectrophotometer. MiRNA-21 and miR-519d expression levels were quantified by quantitative PCR. Cell growth and invasion were measured after treatment with miR-21 or miR-519d inhibitor or mimic for up to 72 hr by using a colorimetric cell proliferation and a Matrigel invasion assay. Migration was assessed by a transwell migration assay and apoptosis by flow cytometry. The expression of the potential targets phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4) of miR-21 and miR-519d was analyzed by RT-PCR and Western blotting.

Results: Constitutive expression of miR-519d was high in JEG3 cells, but absent in HTR-8/SVneo cells. MiR-21 expression was higher in HTR-8/svneo cells than in JEG-3 cells. MiR-519d and miR-21 inhibition in JEG3 or HTR-8/SVneo cells significantly decreased proliferation compared to control cells, while overexpression resulted in a significantly increased proliferation. Invasiveness of JEG-3 cells was significantly reduced after silencing of miR-519d and miR-21. Overexpression of miR-21 in HTR-8/SVneo cells significantly induced migration compared to controls, but no change was observed in JEG-3 cells. Inhibition of miR-21 or miR-519d expression increased apoptosis in JEG-3 cells, but not in HTR-8/Svneo cells. Silencing of miR-21 induced expression of PDCD4 in JEG-3 cells, while PTEN expression was increased in HTR-8/SVneo. Inhibition of miR-519d expression in JEG-3 cells increased the expression of PTEN.
Conclusion: Our results confirmed that both cell lines express miR-21 at different levels and miRNA-519d is expressed in JEG-3 choriocarcinoma, but not in HTR-8/SVneo cells. PTEN is a potential target of miR-21 that may regulate proliferation and migration in HTR-8/SVneo cells. PDCD4 is a possible target of miR-21 and miR-519d and associated with regulation of apoptosis in JEG-3 cells. MiR-21 and miR-519d seem to be involved in the regulation of trophoblast cell behavior and functions.

G-21

Antiphospholipid antibodies disrupt the mitochondria in the human syncytiotrophoblast

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Problem: Antiphospholipid antibodies are autoantibodies that are major risk factors for preeclampsia and recurrent miscarriage/still-birth. Exactly how these antibodies induce obstetric complications remains unanswered. We recently demonstrated that antiphospholipid antibodies are internalised into the syncytiotrophoblast in an antigen-dependent, receptor-mediated process. Once internalised the antibodies induce aberrant cell death in the syncytiotrophoblast leading to the extrusion of necrotic trophoblast debris but the mechanism by which they do this is not clear.
Methods of study: Normal first trimester placental explants were exposed in duplicate to the monoclonal antiphospholipid antibody ID2, or control antibody, for 2 min. The subcellular location of ID2 in the syncytiotrophoblast was determined and quantified by semi-correlative fluorescent-electron microscopy.
Results: ID2 was 14.6-fold more abundant than control antibody in the syncytiotrophoblast of placental explants after two or 30 min exposure. ID2 was associated with mitochondria and other structures. ID2 was present on the both outer mitochondrial membrane and the inner mitochondrial membrane of normal and swollen mitochondria in the syncytiotrophoblast. Of 102 mitochondria counted in ID2-treated explants, 46% had a normal morphology and 54% were swollen. In comparison, of the 104 mitochondria counted in the syncytiotrophoblast of placental explants treated with control antibody, 82% had a normal morphology and 18% were swollen. Chi-squared contingency table analysis demonstrated that the distribution of normal and swollen mitochondria in the syncytiotrophoblast of placental explants treated with ID2 and control mAb was significantly different ($P = 1 \times 10^{-7}$).
Conclusions: Antiphospholipid antibodies are rapidly internalised by the syncytiotrophoblast and once internalised the antibodies associate with the mitochondria where they alter mitochondrial morphology, correlating with reported functional consequences. Since the mitochondria are key regulators of cell death this may explain how antiphospholipid antibodies induce aberrant cell death in the syncytiotrophoblast and extrusion of necrotic trophoblast debris with consequent adverse effects on maternal physiology and/or placental function.

G-22

Protein misfolding and aggregation: a novel mechanism for preeclampsia

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Problem: The etiology of preeclampsia (PE) is unclear, and early detection and treatment of PE are lacking. We recently reported that the protein

transthyretin (TTR) - transporter of thyroxine and retinol- undergoes protein misfolding and aggregation, and plays a causative role in preeclampsia. This study aims to develop a sensitive and high-throughput assay to detect TTR aggregates in the sera of women with PE and test the hypothesis that the endoplasmic reticulum (ER) stressors such as hypoxia and inflammation may induce TTR aggregation at the maternal-fetal interface in preeclamptic women.

Method of study: Sera and placental tissue from preeclamptic women and normal pregnancy women (32–38 weeks) were evaluated for the presence of TTR aggregates using an ELISA-based assay and dual immunofluorescent staining with TTR antibody and ProteoStat dye, a rotor dye that specifically binds to aggregated proteins, respectively. TCL-1 cells were treated with normoxia/hypoxia (1%) or vehicle/inflammatory cytokines over a time-course. Dynamic alterations in various players in the unfolded protein response (UPR) and autophagy-lysosome degradation pathways (ALDP) were analyzed in normoxia- and hypoxia-treated cells using Western blotting and immunoflurescence.

Results: The level of TTR aggregates in sera from preeclamptic women was 3–4 fold higher than that in normal pregnancy sera. Robust deposition of TTR aggregates was observed in the placenta of preeclamptic women. Continued treatment with hypoxia and inflammatory cytokines induced the accumulation of TTR aggregates and overwhelmed the capacity of UPR and ALDP pathways.

Conclusions: Our results suggest that persistent ER stress leads to impairment of the UPR and cellular protein degradation machinery. This may result in accumulation of misfolded and aggregated proteins such as TTR in the placenta and circulation and may contribute to the onset of PE. Importantly, our findings provide evidence for an ELISA-based assay for early detection of TTR aggregates in sera and for a possible therapeutic option.

G-23

Expression of human endogenous retrovirus gene mRNA in local endometriosis lesions

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Problem: We reported the expressions of Toll-like receptor (TLR) 7 and TLR9 in local lesions of endometriosis, and those mRNA expression levels correlated with microsomal PGE2 synthase-1 (mPGES-1) mRNA. Recent studies have revealed that TLR7 and TLR9 are involved in the activation of dendritic cells and autoreactive B cells through the recognition of endogenous DNA- or RNA-containing antigens and subsequent development of autoimmune responses against nuclear autoantigens. Recently, other researchers reported the expression of human endogenous retrovirus gene (HERV) or the envelope gene belonging HERV in endometriosis tissues. HERVs form a part of the human genome. However, the role of HERVs in pathogenesis of endometriosis is not clarified. We investigate into the expression of HERVs mRNA in local lesions of endometriosis, and its relationships toward TLR7, 9 and PGE2 synthases.

Methods of study: Endometriosis samples were obtained from 35 patients of endometrial cyst. Endometrial tissues were obtained from patients undergoing operations for benign gynecological conditions. Informed consents were obtained from all the patients participating in this study, and Institutional Review Board (IRB) approval was obtained. Tissue samples were stored at −80°C until analysis. We determined the mRNA expression of HERV-w, HERV-k, TLR7, TLR9, mPGES-1, and Cyclooxygenase2 (COX2) by real-time reverse-transcription PCR.

Results: The expressions of HERV-w, HERV-k mRNA were observed in eutopic endometrium and endometriosis lesions. The expression levels of HERV-k mRNA in endometriosis samples were higher than eutopic endometrium of proliferative phase of endometriosis patients. In addition, the expression levels of HERV-k mRNA in peritoneal endometriosis were higher than eutopic endometrium of proliferative phase of endometriosis patients. There are relationships between expression levels of HERVs mRNA and COX2 mRNA in endometriosis lesion.

Conclusions: The expressions of HERV-w, HERV-k mRNA of endometriosis samples indicate that enhanced autoimmune reaction may have a role in part of the pathogenesis of endometriosis.

G-24

Intramuscular (IM) and oral micronized progesterone may have immunosuppressive advantages over vaginal progesterone or IM 17-hydroxyprogesterone in the prevention of pre-term labor

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Problem: To compare the effect of three different routes of administration of progesterone and 17-hydroxyprogesterone on the levels of serum progesterone induced blocking factor (PIBF). A low level of PIBF has been associated with pre-term labor and delivery.

Method of study: Now that the 34 kDa immunomodulatory serum protein PIBF has been synthesized by recombinant DNA technology monoclonal antibodies have been created leading to the development of a research ELISA assay for PIBF. In women whose ovulation was blocked by estrogen (and thus a corpus luteum was inhibited) the effect of IM progesterone 100 mg per day, oral progesterone (200 mg/day), vaginal progesterone (Crinone® vaginal gel 8% – 90 mg daily) and 17-hydroxyprogesterone (250 mg – one injection) on subsequent serum PIBF levels were determined after 3 days of progesterone or the third day from the 17-hydroxyprogesterone injection.

Results: The serum PIBF levels were as follows: intramuscular 720.5 ng/mL, oral – over 760, vaginal 41, and 17-hydroxyprogesterone – 11.7 ng/mL.

Conclusions: Intramuscular and oral progesterone seemed to be the best vehicle to increase PIBF which among other functions inhibits natural killer cell cytotoxicity. Pre-term labor has been found to be associated with low PIBF levels. Pre-term labor may in part be an immune rejection phenomenon. Previous studies have found that both IM progesterone and vaginal progesterone are equally efficacious in advancing the endometrium to a late secretory histologic pattern. However oral progesterone proved markedly inferior not reaching the endometrium because of being metabolized by first pass through the liver. Some studies suggest twice weekly injection of 17-hydroxyprogesterone can delay delivery in those with pre-term labor. Based on its effect on raising PIBF levels IM progesterone would seem to be the vehicle of choice. If not tolerated perhaps the combination of vaginal and oral would be the next most efficacious method.

G-25

A comparison of follicular versus luteal phase serum levels of the immunomodulatory protein the progesterone induced blocking factor (PIBF) as determined by ELISA assay

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Problem: Now that a sensitive ELISA assay has been developed it is important to determine how much influence will endogenous progesterone exposure have in causing a rise of the PIBF a unique 34 kDa protein that stabilizes perforin granules in natural killer cells and thus markedly inhibits their cytotoxicity and causes a shift of TH1 to TH2 cytokines.

Method of study: Using a research ELISA assay with a monoclonal antibody to PIBF, five women had serum PIBF measured 1–3 times during the follicular phase when the serum progesterone level was <2 ng/mL and then obtained again in mid-luteal phase. The cycles were completely natural without follicle maturing drugs or progesterone supplementation.

Results: The average follicular phase serum PIBF (ng/mL) levels versus single level in the luteal phase for the five women were as follows: patient 1 – 76.5 versus 323 (progesterone 9.6 ng/mL), 2 – 70.7 versus 370 (14.0), 3 – 122 versus 365 (17.1), 4 – 116 versus 754 (11.7), and 5 – 127 versus 403 (13.0 ng/mL).

Conclusions: Using a sensitive ELISA with a monoclonal antibody to PIBF compared to a relatively insensitive immunocytochemistry assay using a polyclonal antibody to PIBF which had been used many years ago, it is clear that this unique 34 kDa protein is present even in the follicular phase. The increase in endogenous progesterone secretion that occurs with ovulation is associated with a precipitous rise in serum PIBF. The next step is to study PIBF levels in mid-luteal phase in infertile women in natural cycles to determine if some discriminatory level can be determined below which pregnancies are less likely to occur or miscarriages are more likely to happen. Studies of endometrial histology and endometrial molecular markers and serum progesterone levels have been disappointing as to their ability to determine luteal phase deficiency.

G-26

The effect of lymphocyte immunotherapy (LIT) on mid-luteal phase serum levels of the immunomodulatory protein the progesterone induced blocking factor (PIBF) using a new research ELISA assay

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Problem: A previous study found that LIT increased the expression of the immunomodulatory protein, the PIBF, performed by a relatively insensitive immunocytochemistry technique with only a polyclonal antibody available. The purpose of the present study was to corroborate or refute this aforementioned study now using a much more sensitive ELISA assay with a monoclonal antibody to PIBF.
Method of study: Three women volunteered to participate. They agreed not to try to conceive on the cycle of LIT. PIBF was tested in the follicular phase and in mid-luteal phase in two cycles. In the second cycle the women were injected intradermally with her male partner's lymphocytes. Mid-luteal phase PIBF and progesterone levels were obtained in the cycle before and the cycle of receiving the lymphocyte immunotherapy.
Results: The average follicular phase serum PIBF (ng/mL) was 19. The mid-luteal serum PIBF level (progesterone ng/mL) for the pre-LIT cycle were 370 (19.0), 119 (20.0), and 63 (4.5) (average 186 ng/mL) versus 114 (14.0), 28 (18.0), and 6 (5.8) (average 47).
Conclusions: In contrast to the previous study measuring serum PIBF by an immunocytochemistry technique, the new ELISA test failed to show any beneficial effect of lymphocyte immunotherapy in raising mid-luteal phase serum PIBF in endogenous cycles without P supplementation. These data are consistent with a previous presentation at the 2013 ASRI meeting showing no requirement for an allogeneic stimulus to cause a rise in PIBF. At least in this small study lymphocyte immunotherapy does not seem to enhance endogenous PIBF secretion. These data do not necessarily refute the possibility that lymphocyte immunotherapy may aid successful conception through some other mechanism.

G-27

Nitric oxide signaling inhibits tumor necrosis factor (TNF) release by differentiated THP-1 macrophages

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Problem: Complications of pregnancy including fetal growth restriction (FGR) and pre-eclampsia (PE) are often linked to an aberrant maternal inflammatory environment and decreased nitric oxide (NO) signaling. Evidence links the presence of classically activated macrophages at the fetal-maternal interface with elevated levels of pro-inflammatory cytokines including tumour necrosis factor (TNF). Our previous work revealed a causal role of TNF in the pathogenesis of FGR and features of PE using a rat model. Here we investigate whether activation of NO signaling is able to inhibit the release of TNF from activated macrophages *in vitro*.
Method of study: Human monocytic leukemia THP-1 cells were differentiated into macrophages using 50 ηg/ml phorbol 12-myristate 13-acetate (PMA) over a 24-hr period. Following a 24-hr washout period, differentiated cells were exposed to two concentrations (10 ηm and 1 μM) of various NO-mimetics

(glyceryl trinitrate, GTN; 8-bromo-cGMP; isosorbide dinitrate, ISDN) for 24 hr. Cells were then treated with 100 ηg/mL lipopolysaccharide (LPS) ± fresh NO-mimetic for 4 hr. Media were collected and analyzed for TNF by ELISA.

Results: Treatment of PMA-differentiated THP-1 cells with LPS significantly increased TNF release. Pretreatment of differentiated THP-1 cells with GTN (10 ηm and 1 μM) prevented LPS-induced TNF release while 8-Br-cGMP was only effective at preventing TNF release at 10 ηM and not at 1 μM. Pretreatment with the NO donor ISDN did not inhibit TNF release. No difference in cell viability was observed between treatment conditions within experiments. To determine whether the effects of GTN are mediated by protein kinase G (PKG), cells were additionally treated with the PKG-inhibitor KT5823. The effect of LPS-induced TNF release from cells treated with GTN was not affected by PKG inhibition.

Conclusion: Our results indicate that activation of cGMP-dependent NO signaling may be a novel immunotherapeutic strategy for the management of inflammation associated with complications of pregnancy.

G-28

Is superfertility associated with recurrent pregnancy loss?

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Problem: A recent hypothesis has implicated superfertility as a cause of recurrent pregnancy loss. Clinical support for the concept comes from one report that 40% of women experiencing recurrent miscarriages had monthly fecundity rates of 60% or greater and thus were designated as superfertile.

Method of study: To confirm or refute this finding, clinical histories of 201 women with a history of recurrent pregnancy loss were reviewed and months to desired pregnancy, karyotypes of their products of conception as well as results of laboratory tests including antiphospholipid antibodies and circulating natural killer cells were recorded.

Results: The prevalence of superfertility was 32% (64/201) among recurrently aborting women compared with 3% of the general population according to the model of Tietze ($P < 0.0001$). Fifty-nine of the 201 (30%) study patients displayed presence of APA, LA, elevated CD56+ cells or elevated NK cytotoxicity and were designated as having an immunologic risk factor. Of the 192 karyotypes of products of conception from women with a history of recurrent miscarriage, 153 (80%) had a normal chromosome complement and 38 (20%) were abnormal. Among the normal karyotypes, 86 (56%) were 46XX and 67 (44%) were 46XY.

Conclusion: Recurrent pregnancy loss is associated with superfertility in 32%, immunologic risk factors in 30% and a 20% frequency of chromosomally normal pregnancy losses. Thus, implantation failure can result from too much or too little implantation.

G-29

Ghrelin and visfatin serum levels and genetic polymorphisms in patients with gestational diabetes mellitus

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Problem: The incidence of obesity is increasing worldwide and it is an important risk factor for gestational diabetes mellitus (GDM). It is still unclear how adipokines are involved in the development of GDM and what is the adipokine profile of obese women who develop GDM. Ghrelin and visfatin are adipokines involved in insulin resistance. This study aimed to assess adipokine serum levels and genetic polymorphisms in high-risk pre-obese and obese women and correlate findings with the diagnosis of GDM.

Method of study: This prospective cohort study recruited healthy overweight pregnant women (pre-pregnancy BMI > 25 kg/m^2) before 20 weeks′ gestation. Blood was collected to assess ghrelin and visfatin levels and evaluate Leu72Met (rs696217) ghrelin and -3186C/T (rs11977021) visfatin genes polymorphisms. All participants underwent a 75 g oral glucose tolerance test before 20 weeks′ gestation; those with one or more abnormal values were diagnosed with GDM according to IADPSG criteria. Serum concentrations were measured by ELISA and genetic polymorphisms by PCR-RFLP. We compared findings between women with and without GDM.

Results: A total of 61 overweight women were recruited: 21 developed GDM and 40 had a healthy

pregnancy. Ghrelin and visfatin levels were similar in women who developed GDM compared to those who had a healthy pregnancy (Ghrelin: 140.5 ± 61.88 pg/mL versus 156.0 ± 43.49 pg/mL respectively, $P = 0.93$; Visfatin: 61.09 ± 16.49 ng/mL versus 69.86 ± 12.13 ng/ml respectively, $P = 0.24$). There was no association between GDM and Leu72Met, and between GDM and -3186*C/T* genetic polymorphisms. No significant differences were found in genotypic (Ghrelin: $P = 0.60$, Visfatin: $P = 0.46$) and allele (Ghrelin: $P = 1.00$; Visfatin: $P = 0.71$) frequencies when comparing healthy women versus those who developed GDM.

Conclusions: Ghrelin and Visfatin levels and genetic polymorphisms are similar in overweight pregnant women who develop GDM compared to those who do not.

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G-30

Evaluation of Killer cell Immunoglobulin like Receptor (KIR) and HLA-C genotypes in recurrent pregnancy losses

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Problem: During human pregnancy semi-allogeneic extravillous trophoblast invades the endometrial mucosa that is highly populated with NK cells. The activity of NK cells is controlled by the balance between activating and inhibitory signals from cell surface receptors including KIR. Trophoblast expresses HLA-C molecules, which are main ligands for KIR on NK cells. NK cell recognition of trophoblast is important for placentation as it mediates trophoblast migration and spiral artery remodeling. Both KIR and HLA-C genes belong to families of genes with large population diversity. The goal of this study was to analyze the HLA-C, KIR genes and KIR haplotype frequencies in the North American cohort of women with recurrent pregnancy losses (RPL) to evaluate whether there is an immunogenetic susceptibility to RPL based on KIR repertoires along with cognate HLA-C ligand co-expression in women with RPL and the HLA-C status of their partners.

Method of study: In a pilot study comprised of sixty seven mainly Caucasian patients of the Reproductive Medicine Center, KIR genotyping was performed using Lifecodes KIR SSO Typing kit (Gen-Probe). KIR genotypes were classified into haplotypes based on the following scheme: expression of KIR2DL1, KIR2DL3, KIR3DL1 and KIR2DS4 comprise the AA KIR haplotype and all other combinations of KIR genes comprise the Bx KIR haplotype (non AA KIR haplotype). HLA-C genotyping was performed using LABType® SSO HLA C Locus kit and Micro SSP Allele Specific DNA Typing Kits for higher resolution. Data for KIR haplotype and HLA-C allele frequencies were compared with data for corresponding populations obtained from Allele Frequency Net, a database and online repository for immune gene frequencies in worldwide populations.

Results: RPL patients revealed higher frequency of the AA KIR haplotype (38.8%, 26 patients out of 67) when compared to frequencies from the database for North American populations (average - 29.6%, median – 30.1%) or US Caucasians (average – 30.5%, median – 30.5%), while no differences were revealed for individual KIR gene frequencies. HLA-C genotyping indicted that in RPL patients 51% of alleles belong to HLA-C C2 group. This is higher as compared to North American populations (average – 39%) or US Caucasian (average – 37%). Interestingly, in KIR2DS1 positive patients (44.8%, 30 patients out of 67), the frequency of HLA-C C2 alleles (C2 is cognate ligand for KIR2DS1) was higher (58%) than C1 group (42%).

Conclusions: The preliminary results suggest that KIR AA haplotype and HLA-C C2 frequencies are increased in RPL patients. If this relationship holds true in a larger cohort, KIR and HLA-C genotyping have a potential to be a useful test for women with immune related infertility or RPL. Further studies with increased cohort size are warranted.

G-31

Decidual CXCR4-expressing Natural Killer cells induce Immune tolerance by an interleukin-4-dependent Th2 differentiation and conversion of CD4+CD25+Foxp3+ T regulatory cells

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Problem: Natural killer (NK) cells accumulate at the maternal–fetal interface in large numbers, but their exact roles in successful pregnancy remain poorly defined.

Method of study: Decidual immune cells were obtained by magnetic activated cell sorting (MACS) from human early pregnancy. The phenotype and cytokine expression profile were determined by flow cytometry (FCM) assay. The source of IL-4 from different decidual immune cells was determined by FCM and ELISA. Cell co-culture system of CXCR4+/− dNK and CD4+ Naïve or conventional T cells was set up and CD4+T cell subtype differentiation was observed. The phenotype of NK cell and CD4+T subset from normal pregnancy and miscarriage were also compared. Finally, we observed the pregnancy outcome and CD4+T subset after deletion of NK cells.

Results: A subtype of CXCR4-expressing dNK cells are preferentially accumulated at the maternal-fetal interface in human early pregnancy. These CD56brightCXCR+NK cells show inhibitory phenotype and are the main source of IL-4 at the materno-fetal interface. Furthermore, we have demonstrated that CD56brightCXCR+NK cells induce interleukin-4-dependent Th2 bias and CD4+CD25+FoxP3+ T (Treg) conversion from CD4+CD25− T cells in the deciduas in human early pregnancy. In addition, IL-4-expressing CXCR4+NK cells are decreased in patients with recurrent spontaneous abortions, which are accompanied by CD4+ T subsets disorder. Moreover, deletion of NK cells in pregnant mice disturbs CD4+ T number and function, resulting in increased embryo resorption.

Conclusions: Our data identified a type of CXCR4+CD56brightNK cells as key regulatory cells at the maternal–fetal interface by inducing Th2 bias and Treg conversion, contributing to successful pregnancy.

G-32

Dendritic cell subsets in the endometrium of women with recurrent pregnancy loss

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Problem: Dendritic cells (DCs) are a heterogeneous population of antigen-presenting cells that are key regulators of immune response and immunologic tolerance in numerous organs. DC subsets have been poorly characterized in the endometrium of women with recurrent pregnancy loss (RPL).

Method of study: Endometrial specimens from patients with RPL in the mid-luteal phase were analyzed by immunohistochemistry, immunofluorescence and real-time PCR.

Results: A predominance of CD11c+ and CD209+ DCs and strong HLA-DR, IL-23p19, IL-17 infiltration was observed in the RPL endometrium, while the density of CD1a+ DCs significantly decreased in the RPL compared with the control. There were no significant differences in mature CD83+ DC density between RPL and control. Significant increased mRNA level of proinflammatory cytokines IL-23p19, IL-6, IL-17 and TNF-α could be demonstrated in the RPL endometrium but not in control. Furthermore, Th17-inducing cytokine IL-23p19 was produced by CD11c+ but not CD1a+ DCs.

Conclusions: The increased density of CD11c+IL-23+ DCs in RPL endometrium suggests that the inflammatory DCs induce the Th17/Treg imbalance and may lead to pregnancy loss.

G-33

Tenofovir-Diphosphate concentrations in human female reproductive tract cells in culture

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Problem: Little attention has been paid to the intracellular levels of tenofovir- diphosphate (TFV-DP) within female reproductive tract (FRT) tissues either as a function of location (ectocervix, ECX; endocervix, CX; endometrium, EM) or cell type (epithelial cells, fibroblasts and immune cells) that comprise these tissues. We hypothesized that there would be differences in intracellular TFV-DP depending on FRT location and cell type.

Method of study: FRT tissues were obtained following hysterectomy for benign reasons from HIV-negative women. Epithelial cells, fibroblasts and CD4+ T cells were isolated by enzymatic digestion and/or magnetic bead purification. Cells were treated with tenofovir for 24 hr and intracellular TFV-DP concentrations measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in FRT cells and blood CD4+ T cells.

Results: There was a significant variation in TFV-DP concentration in different tissues and cell types. EM-EC had 2-fold higher TFV-DP than EC from the CX and ECX. TFV-DP in fibroblasts from EM, CX and ECX were comparable. Concentrations of TFV-DP in EC from EM, CX and ECX were ~5-fold greater than that seen in fibroblasts at each site. Analysis of CD4+ T cells from the EM, CX and ECX indicated that TFV-DP concentrations were comparable to that measured in blood CD4+ T cells and ~125-fold less that that measured in EC. Overall, these findings indicate the potential of a gradient in which exposure of individual FRT cell types results in TFV-DP concentrations EC>fibroblasts>CD4+ T cells.

Conclusions: These results demonstrate that TFV-DP can be produced in multiple cell types throughout tissues of the FRT and that intracellular concentrations vary with the cell type analyzed. Our results suggest that TFV-DP concentrations in epithelial cells and fibroblasts represent a repository of TFV-DP which, when converted to TFV, is available for uptake and protection of CD4+ T cells and macrophages in the FRT.

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G-34

Transcriptional profiling of innate and adaptive immune response gene expression pathways in the rat testis following *P. aeruginosa* lipopolysaccharide-induced inflammation

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Problem: Inflammation of the male reproductive tract by microbes contributes to male infertility. Experimentally, bacterial pathogens are known to suppress androgen production. Research on immune responses and antimicrobial properties of the testis has advanced an understanding of specific genes and proteins involved in the detection and clearance of invading microbes. The goal of this work was to determine the effects of lipopolysaccharide (LPS)-induced inflammation on innate and adaptive immune response gene expression pathways of the rat testis and to determine possible roles of affected genes in inflammatory responses of the testis.

Method of study: Inflammation in Sprague-Dawley rats was accomplished via i.p. administration of LPS from *P. aeruginosa* (5 mg/kg body weight) for 3 or 6 hr ($n = 4$ animals/time point). RNA was isolated from testes and cDNA synthesized for analysis by qPCR. The RT2 Profiler PCR Array Rat Innate and Adaptive Immune Responses Signaling Pathway (Qiagen) was used to evaluate expression of 83 genes. Only genes showing a 3-fold (up-regulated or down-regulated) change in expression with a P-value of <0.05 were categorized as statistically significant.

Results: Array results demonstrated that 11 genes (Cc12, Cc13, Cd14, Cxcl10, Icam1, IL10, IL-1b, IL6, Nfkbia, Tlr2, and Tnf) were up-regulated after 3 hr of LPS-induced inflammation and expression of six genes (Ccl12, Cc13, Cd14, Nfkbia, Tlr2, Tnf) remained elevated after 6 hr. Five genes were up-regulated at 6 hr only (C3, Jak2, Nlrp3, Slc11a1, and

Tlr1). No genes were down-regulated after 3 or 6 hr following LPS administration.

Conclusions: Genes involved in cytokine-mediated signaling comprised a major functional category of genes up-regulated in the testis following LPS-induced inflammation. The specific functions of these genes will be investigated to elucidate the molecular mechanisms involved in the immune response to LPS in the testis.

G-35

Trichomonas vaginalis endosymbionts as immunity modifiers facilitating HIV infection

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Problem: *Trichomonas vaginalis* (TV) causes the most common nonviral sexually transmitted infection associated with increased risks of adverse pregnancy outcome and HIV-1 transmission. Although it is known that TV infection increases HIV-1 shedding in the female genital tract, which correlates with sexual and perinatal transmission, no clear molecular mechanism has been identified to explain the trichomoniasis-attributable HIV-1 infections. We hypothesize that TV and its endosymbionts, mycoplasma and dsRNA *Trichomonas vaginalis* viruses (TVVs), increase the vulnerability of the vaginal mucosa to HIV-1 by inducing inflammatory responses that assist in HIV-1 entry and replication.

Methods of study: Human vaginal epithelial cells and peripheral blood mononuclear cells (PBMCs) were stimulated with purified TVV virions or live TVV-and mycoplasma-infected TV, followed by supernatant collection at multiple time intervals. The PBMCs pre-stimulated for 3 days with TV, TVV or vaginal epithelial supernatants were exposed to primary isolates of CXCR4- and CCR5-tropic HIV-1 virus. Cytokines /chemokines were tested using Meso Scale Discovery multiplex assays, and HIV-1 detection was measured by a p24 ELISA. ANOVA was performed using GraphPad Prism and $P < 0.05$ was considered significant.

Results: Live TV, TV-stimulated supernatants and purified TVV virions induced immune stimulation of CD4-positive cells evident by up-regulation of Th1/Th2 and proinflammatory cytokines. TV and TVV induced IFNβ, IL6, IL-8, IL17, IL10, IL12p70 and TNFα. Moreover, HIV-1 p24 levels were also increased in the cells pre-exposed to either TVV-infected TV ($P < 0.001$), supernatants from TV exposed cells or purified TVV virions ($P < 0.05$). Over 20-fold increase in p24 levels was observed 5 days post-infection. TV isolates infected by both TVV and Mycoplasma hominis induced the strongest proinflammatory response.

Conclusions: Our data support the hypothesis that TV and its endosymbionts can lead to activation of HIV-1 host cells and inflammatory responses that can increase the risk of HIV-1 infection and propagation in the mucosal environment.

G-36

Systemic inflammation in the extremely low gestational age newborn following maternal genitourinary infections

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Problem: Gestational genitourinary infections are associated with preterm delivery and late adversities in the surviving offspring that might result from perinatal inflammation (*e.g.* cerebral palsy, autism, epilepsy, schizophrenia, asthma, and mental retardation). However, the association of these maternal infections with inflammation manifested in the newborn soon after birth is unknown.

Method of study: This study utilized specimens from the multi-center US cohort ELGAN (Extremely Low Gestation Age Newborns) study. As part of a structured interview administrated after delivery before the 28th week of gestation, mothers reported if they had a cervical/vaginal infection (CVI), and separately, a urine/bladder/kidney infection (UTI). Concentrations of 25 inflammation-associated proteins were quantified in blood spots collected from 914 newborns at three time points a week apart, starting within 3 days of birth. Logistic and multinomial regression models adjusted for gestational age compared children whose mother reported CVI and UTI,

CVI only, or UTI only, to those whose mother denied all of these infections.

Results: Infants born to mothers who reported UTI or CVI were more likely than others to have inflammation-associated protein elevations defined as concentrations in the top quartile for gestational age and the day the specimens were collected. For all whose mother reported a CVI, whether alone or in combination with UTI, the systemic inflammation was most evident within three days of birth and included elevated blood concentrations of CRP, SAA, MPO, IL-1 , IL-6, IL-6R, TNF-alpha, RANTES, ICAM-3, E-selectin and VEGF-R2. For all whose mother reported a UTI, inflammation was most evident between postnatal days 5 and 8, and included elevated concentrations of MPO, IL-6R, TNF-R1, TNF-R2, and RANTES. These associations were no longer evident at days 12–15 after birth.

Conclusions: A gestational genitourinary infection reported by the mother increases the very preterm newborns' risk of systemic inflammation detectable by elevated blood concentrations of inflammatory mediators shortly after birth.

G-37

The soluble placental fraction guides a trophoblast stem-cell-like population via chemokine receptor 1 and 3 (CCR1/3) to rapidly migrate and cover distressed villous areas

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Problem: Human trophoblast-stem-cell-like populations migrate toward placental explants, the mechanism of which has not been identified.

Method of study: We formed spheroids of HTR8/svneo (immortalized first trimester trophoblast) cells in hanging drops, which thus adopt trophoblast-stem-cell-like characteristics. Spheroids and explants were stained with fluorescent markers and brought into contact within hanging drops supplemented with/out a specific blocker of chemokine receptor 1 and 3 (CCR3), UCB35625. Confrontation products (n = 200) were analyzed after 10, 24 and 48 hr by laser scan microscopy. To test if observed phenomena are cell-contact independent, explants and spheroids were co-cultured without contact within culture wells. Spheroid swarming was assessed per conventional microscopy. Spheroid lysates were layered on phospho-kinase arrays to screen for the regulation of CCR1/3-mediated signaling molecules via soluble factors within explant supernatants.

Results: All HTR8/svneo cells leave their spheroids (10 hr), which completely disintegrate into the placental explants, and cover villi within 48 hr, but never when CCR1/3 is blocked. When spheroids and explants are cocultured without contact, cells slowly migrate and leave the spheroids (8 days), but not in the presence of CCR1/3 inhibitor. Stimulation of HTR8 cells with media conditioned by placenta explants induces phosphorylation of several signaling molecules, but in the presence of CCR1/3 inihibitor, only glycogensynthase-kinase 3 (GSK3) and signal transducers and activators of transcription 1 (STAT1) are inactivated.

Conclusion: At least one chemokine derived from placental villi binds to CCR1/3, which possibly signals via GSK3 and STAT1 to direct trophoblast-stem-cell-like populations to migrate and cover/replace distressed placental villi. Several possible soluble CCR1/3 candidate ligands are present in human placentae. STAT1 is a known mediator of trophoblast migration and differentiation. GSK3 has attracted recent attention since its inhibition is related to maintenance of pluripotency in human embryonic stem cells.

G-38

Does immunoglobulin or intralipid work as an immune-modulator for recurrent pregnancy loss women with abnormal NK cells?

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Problem: Recently, there are some reports about the effectiveness of intralipid for recurrent pregnancy loss (RPL) women with abnormal NK cell such as higher NK cell cytotoxicity. For RPL women with abnormal NK cells, intravenous immunoglobulin treatment (IVIG) is clinically available. However, IVIG treatment is expensive and the effect of IVIG

for RPL is controversial. The purpose of this study is to evaluate the effects of immunoglobulin and intralipid for NK cell cytotoxicity.

Method of study: Peripheral blood sample taken from women with RPL ($n = 4$) or controls ($n = 10$) were collected and analyzed for NK cell cytotoxicity using immunofluorescent labeled K562 cells as targets and flow cytometry. Suppression of NK cell cytotoxicity by immunoglobulin (2 and 4 mg/mL) or intralipid (10 and 20 mg/mL) was also analyzed using immunofluorescent labeled K562 cells as targets and flow cytometry.

Results: NK cell cytotoxicity (Target: Effector cell ratio 1:50) without any treatment in women with RPL was 12.2 ± 7.3% and that in controls was 12.0 ± 9.2%. By addition of immunoglobulin, NK cell cytotoxicity was suppressed in dose dependent manner both women with RPL and controls [in women with RPL: immunoglobulin 2 mg/mL; 12.1 ± 4.8%, 4 mg/mL; 5.3 ± 1.9%, in controls: immunoglobulin 2 mg/mL; 5.9 ± 3.4%, 4 mg/mL; 2.5 ± 0.6% ($P < 0.05$)]. However, by addition of intralipid, NK cell cytotoxicity was not suppressed but increased both women with RPL and controls [in women with RPL: intralipid 10 mg/mL; 28.6 ± 10.7%, 20 mg/mL; 26.2 ± 10.6%, in controls: immunoglobulin 10 mg/mL; 25.4 ± 17.3% ($P < 0.001$), 20 mg/mL; 20.7 ± 16.5% ($P < 0.001$)].

Conclusions: Elevated levels of circulating NK cells have been linked to reproductive failure such as RPL or implantation failure. Intravenous immunoglobulin worked to suppress the NK cell cytotoxicity using in-vitro assay. However, intralipid did not work for that kind of study. Therefore, intravenous immunoglobulin may be an effective treatment for RPL women with abnormal NK cells. Further evaluation may be needed to clarify the effect of intralipid.

G-39

The function of uterine and peripheral blood NK22 cells in women with reproductive failures

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Problem: A new type of NKp46+ NK cell, which produces IL-22, was reported. These cells are called NCR22 cells or NK22 cells. It is considered that IL22 production by NK22 cells might mediate mucosal immune defense such as skin, lung and gastrointestinal tract. NK22 cells may become a key factor for reproduction, but this has not been elucidated yet. On the other hand, NKp46 receptors on NK cells have a function not only in cytotoxic activity, but also cytokine production by NK cells. The purpose of this study is to evaluate the relationship between the expression of NKp46 and the production of IL-22 and other cytokines such as IFN-gamma, TNF-alpha, IL-4, and IL-10 in women with reproductive failure.

Method of study: The expression of NKp46 on endometrial (EM) and peripheral blood (PB) NK cells and the cytokines production (IFN-gamma, TNF-alpha, IL-4, IL-10, IL-22) by EM and PB NK cells were analyzed by multi-color flow cytometry in women with reproductive failure such as recurrent pregnancy loss or implantation failure.

Results: The percentages of IL-22 producing NK cell in women with reproductive failure in both EM ($P = 0.052$) and PB ($P < 0.05$) group were higher than controls. According to the production of IL-22, NK cells could be divided into two groups, IL-22 high group and IL-22 low group. In both EM and PB, the percentages of TNF-alpha and IFN-gamma producing CD56bright NK cell in the IL-22 high group were significantly lower than that in the IL-22 low group (all $P < 0.05$). There was significantly negative correlation between NKp46+ NK cells and IL-22 producing NK cells ($r = -0.33$, $P < 0.001$).

Conclusions: We previously reported lower production of NKp46 on NK cells in reproductive failure. From this study, lower expression of NKp46 may cause higher production of IL-22 by NK cells because of the negative correlation between NKp46+ NK cells and IL-22 producing NK cells. Actually, the percentage of NK22 cells in women with reproductive failure was higher than controls and higher production of IL-22 may cause lower production of IFN-gamma and TNF-alpha. Taken together, NK22 may work as a regulator of pregnancy for women with reproductive failure.

G-40

Natural cytotoxicity receptors expression and cytokines production of natural killer cells in peripheral blood and peritoneal fluid in patients with endometriosis

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Problem: The pathogenesis of endometriosis remains to be elucidated. However, it is reported that cytotoxic activity of natural killer (NK) cells decrease and various kinds of inflammatory cytokines increase in patients with endometriosis. However, the differences of natural cytotoxicity receptors (NCRs) expression on NK cells and cytokines production by NK cells have not been clarified. The purpose of this study is to investigate participation of the NK cells in women with endometriosis by analyzing NCRs expression and cytokines production of NK cells in the peripheral blood (PB) and peritoneal fluid (PF).
Methods of study: NK cells in the PB and PF were collected from patients with endometriosis ($n = 31$) and controls without endometriosis ($n = 48$). Endometriosis group was divided into two groups; hormonal treated (GnRH agonist or low dose estrogen progestin) endometriosis group ($n = 10$) and non-treated endometriosis group ($n = 21$). NCRs expression (NKp46, NKp44 and NKp30) and CD16 on NK cells (CD56dim and CD56bright) in the PB and PF were analyzed using multi-color flow cytometry. Cytokines (IFN-gamma, TNF-alpha, IL-4, IL-10, GM-CSF, and TGF-beta) producing NK cells in PB and PF was also analyzed.
Results: In PF, the percentage of CD56+/NKp46+ cell in endometriosis group was 22.84 (11.58 – 46.34) [median (interquartile range)] and that in controls was 30.20 (18.72 – 51.20). The percentage of CD56dim/NKp46+ cell in endometriosis group was 16.67 (8.95 – 33.09) and that and that in controls was 26.12 (14.60–41.14). CD56+/NKp46+ cells ($P < 0.05$) and CD56dim/NKp46+ cells ($P < 0.01$) in endometriosis group were significantly lower than those in controls. In addition, the percentage of CD56dim/NKp46+ cells in non-treated endometriosis group [9.1 (3.4–18.9)] was significantly lower than that in controls [24.3 (17.2–42.1), $P < 0.05$]. In PB, the percentage of CD56+/NKp30+ in non-treated group [6.00 (3.66–8.35)] was significantly lower than that in controls [9.97 (7.58–12.35), $P < 0.05$]. TNF-alpha producing NK cells in PF in endometriosis group [33.87 (25.21–60.73)] were significantly higher than those in controls [25.42 (17.56–30.43), $P < 0.05$]. IFN-gamma producing NK cells in PF in endometriosis group [50.04 (26.98–71.33)] were significantly higher than those in controls [19.41 (6.01–38.37), $P < 0.05$].
Conclusions: The differences of NCRs expression on NK cells, TNF-alpha, and IFN-gamma production by NK cells in patients with endometriosis may become one of the pathogenesis and development of endometriosis. In addition, NCRs expression might change by hormonal treatment.

G-41

Altered levels of endogenous proteases in genital tract of post-menopausal women: implications for immune defense against HIV

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Problem: The female reproductive tract (FRT) secretes immune mediators protective against sexually transmitted infections, including HIV. Many of these are hormone responsive hence genital tract immune responses in postmenopausal women might be affected as a result of sex hormone loss. Proteases are little-studied critical mediators in the FRT that can activate/deactivate antimicrobials and therefore modulate their biological activity. The Cathepsin family of proteases can directly regulate anti-HIV innate immune factors including human beta defensin-2 (HBD2), SLPI, and MIP3α. In this study we determined levels and activity of Cathepsins B, D, and G in cervical vaginal lavage (CVL) of premenopausal and postmenopausal women and correlated them to levels of HBD2, MIP3α, and SLPI.
Method of study: CVL samples were collected from 20 HIV-negative premenopausal and postmenopausal women. Whereas each postmenopausal woman provided only one sample, each premenopausal woman provided three samples, collected during proliferative, ovulatory, and secretory stages of the menstrual

cycle. Commercially available ELISA kits were used to assess levels of Cathepsin B, D, G, HBD-2, MIP3α and SLPI. Fluorescence-based enzymatic assay was used to determine Cathepsin B activity.
Results: We observed Cathepsin D to be significantly lower in postmenopausal CVL compared to those from premenopausal women (1.0 versus 15,936 pg/mL). Whereas Cathepsin B levels did not differ between pre and postmenopausal samples, decreasing trend from proliferative to ovulatory to secretory phase was observed (26,970, 20,946 and 14,878 pg/mL). Cathepsin B levels correlated significantly with Cathepsin B activity as well as with HBD2 levels. Cathepsin G was undetectable in all but one sample.
Conclusions: Our data indicates that protease levels and activity are important determinants of the immune condition of the FRT and its ability to fight against pathogens including HIV. We also find differences between pre and postmenopausal women thereby pointing out the importance of developing specific therapeutic interventions for older women.

G-42

Increased circulating levels of alarmins in human high-risk pregnancies

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Problem: Inflammation during pregnancy has a devastating impact on the developing fetus, mainly through its effect on the placenta, leading to stillbirth, preterm birth and fetal growth restriction (FGR). While infections are an important cause of inflammation, a significant proportion of pregnancies present signs of inflammation without infection and identification of non-pathogenic causes of inflammation in high-risk pregnancies is greatly needed. Our objective was to determine the circulating levels of endogenous inflammatory stimuli, known as alarmins, in human high-risk pregnancies as possible contributors/initiators of inflammation.
Methods of study: We determined the levels of uric acid, high mobility group box 1 (HMGB1) and cell-free fetal DNA (cffDNA) in maternal serum from high-risk pregnancies associated with reduced fetal movements (RFM) (n = 19–40). We also assessed, by immunohistochemistry, (IHC) the localization of HMGB1 within the placenta.
Results: When compared to normal pregnancies, circulating levels of uric acid were elevated in pregnancies presenting with RFM (288 versus 219 nmoL/mL, $P < 0.05$), and were further increased in those with preterm birth/FGR (341 nmoL/mL, $P < 0.001$). Elevated levels of cffDNA (by 4-fold) and HMGB1 (by 7-fold) were also detected in maternal serum from pregnancies associated with RFM when compared to controls. IHC revealed placental HMGB1 expression was mainly localized to the syncytiotrophoblast and was increased in RFM pregnancies. Moreover, the cellular distribution of HMGB1 in RFM pregnancies was cytoplasmic and nuclear, as compared to a predominantly nuclear localization in tissues from normal term pregnancies.
Conclusion: Globally, these results suggest that the placenta might be a source of alarmins in high-risk pregnancies and that alarmin detection in maternal serum could provide new diagnostic markers of compromised maternal-fetal environment.

G-43

Alarmins alter human third trimester trophoblast function and induce an inflammatory cytokine profile

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Problem: Intrauterine inflammation has deleterious effects on fetal development, and on pregnancy outcome; and inflammation of the placenta may be a critical factor. Although infection can be a major cause of inflammation at the maternal-fetal interface, there are a significant number of high-risk pregnancies where an infectious element is not confirmed. Alarmins or damage-associated molecular patterns (DAMPs) are endogenous inducers of inflammation and are elevated in pathological pregnancies. However, their effect on the placenta is poorly understood. Thus, in this study we investigated the capacity of DAMPs to alter trophoblast function.

Methods of study: Human third trimester trophoblast cells were isolated from normal term placenta delivered by caesarean section without labor (n = 3–5). Cells were treated for 48 hr with or without either monosodium urate (MSU; 100 μg/mL) or high mobility group box 1 (HMGB1; 1 μg/mL). Cytokine secretion was measured by ELISA and multiplex analysis. Syncytialization and apoptosis was determined by immunohistochemistry.
Results: Treatment of trophoblast with MSU significantly increased the secretion of IL-1 β, IL-6, IL-10, G-CSF, RANTES, TNF-α, GRO-α, MIP-1α, MIP-1β and MCP-1, compared to the untreated control ($P < 0.05$), while HMGB1 significantly increased the secretion of IL-1 β, IP-10, MIP-1α, MIP-1β and MCP-1 ($P < 0.05$). Treatment with MSU or HMGB1 increased the number of apoptotic M30+ cells (by 2.8 and 3.7 fold, respectively), and abrogated syncytialization, reflected by a significant decrease in the number of multinucleated cells (20.9% and 20.0% respectively versus 41.4% in control, $P < 0.05$).
Conclusion: Our findings demonstrate that the DAMPs, MSU and HMGB1, are potent initiators of placental inflammation by triggering distinct trophoblast cytokine/chemokine profiles. Furthermore, these DAMPs modulate trophoblast differentiation and promote cell death. Since these alarmins are known to be elevated in high-risk pregnancies, a better understanding of their role in the pathogenesis of adverse pregnancies, and the mechanisms by which they impact placental function is warranted.

G-44

Human endometrial cell infection *in vitro* with *Listeria monocytogenes*: strain- and cell line-dependent differential infection of epithelial and endothelial cells

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Problem: Infection during pregnancy with the bacterium *Listeria monocytogenes* (LM) can be catastrophic for the fetus, causing miscarriage, fetal demise, preterm birth and neonatal infection. Although placental infection is well-recognized, the contribution of the decidua to fetal infection is poorly understood.
Methods of study: To determine whether the endometrium might be a target for *L. monocytogenes* infection, we infected Ishikawa and ECC1 endometrial epithelial cells, or HUVEC or HEEC-1 endothelial cells with LM strains WS1 (human spontaneous abortion isolate) or 10403s (standard laboratory strain). Intracellular bacterial replication was monitored in cell lysates, and lactate dehydrogenase in the culture medium was used to estimate cell death.
Results: There was differential infection among the cell lines tested. Ishikawa and ECC1 epithelial cells were both readily infected, with peak bacterial recovery at 6–12 hr, and enhanced replication with the WS1 strain. However viable bacteria remaining after 18–24 hr was dramatically reduced. Infection with internalin mutant strains demonstrated that Internalin A binding to E-cadherin was the primary mechanism of epithelial cell infection. There was greater sensitivity of cell death with ECC1 cells compared to Ishikawa cells at 18–24 hr post infection, and cell death also was significantly greater with the WS1 strain. With HEEC1 endometrial endothelial cells, there was rapid cell death with infection with either strain, and this rapid death appeared to preclude bacterial replication since little if any viable bacteria were recovered from cell lysates following 1 hr of infection. Initial experiments with HUVEC cells suggest modest early invasion, but increasing bacterial replication 18–24 hr post infection.
Conclusion: While intracellular bacterial replication is limited to <24 hr in culture in epithelial cell lines, HUVEC (but not HEEC-1) appear to support more sustained replication. This may be related to more limited initial invasion of HUVEC cells by LM. Further work is needed to determine the pathway(s) of endothelial cell invasion, and the mechanisms by which epithelial and HEEC-1 cell death are effected by LM infection.

G-45

Reduction of folate receptor (FR)-β expression in hofbauer cells by herpes infection: a novel signature of viral action in placenta

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Problem: The role of placental fetal macrophages (Hofbauer cells, HBCs) in the regulation of innate immune response at the placental/fetal interface remains understudied. Mounting evidence implicates placental viral infection (e.g. by herpes virus) in preterm birth (PTB), yet biochemical markers of this process remain to be elucidated. In the current study, we examined the effect of herpes infection on HBC levels of folate receptor (FR)-β, and CD163, key HBC-specific cell-surface proteins involved in the transport of folate and scavenging of hemoglobin, respectively.

Method of study: HBCs were purified from human term placentas using Percoll gradients and negative immunoselection ($n = 5$). HBCs were infected for 1 hr with murine herpes virus (MHV)-68 and were then maintained for an additional 48 hr at which time levels of FR-β protein and mRNA were determined by Western blotting and qPCR, respectively.

Results: Western blotting and qPCR revealed that MHV-68 infection reduced FR-β protein and mRNA expression 5–10-fold. Levels of CD163 protein and mRNA were largely unaffected by MHV-68 infection. The presence of 1 μg/mL Poly (I:C), a viral mimetic and TLR3 agonist, for 48 hr promoted approximately a 7-fold reduction in FR-β mRNA and protein levels suggesting that MHV-68 effects on FR-β levels were mediated through TLR3.

Conclusions: We demonstrated that levels of FR-β are specifically down-regulated by infection of HBCs with herpes virus. This provides a novel signature of viral action in placenta, and suggests that viral-associated PTB may be characterized by suppression of folate-driven biosynthetic pathways in HBCs.

G-46

Cytokines with key roles in reproduction may be modulated by psychopathology adversely affecting IVF outcome

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Problem: Anxiety, depression and emotional stress have been associated with infertility and pregnancy loss. We hypothesized that levels of cytokines (circulating and/or local) with putative roles in reproduction may mediate the untoward effects of psychological stress and psychopathology.

Methods of study: We studied 45 couples undergoing IVF. Serum, cervico-vaginal lavage (CVL), follicular fluid (FF) and semen were collected at the time of egg retrieval and tested for cytokines. Standardized psychological questionnaires were administered (SA-45 self-report inventory, Visual Scale Analog for stress, depression, and anxiety, Daily Stress Inventory, Life Stressors and Social Resources Inventory). The outcome measures were % fertilized eggs, implantation, clinical pregnancy and live birth. We used data mining and linear and logistic regression models while adjusting for multiple covariates including age and embryo quality to calculate effect estimates, 95% confidence intervals, and two-sided Wald P-values. $P < 0.05$ was significant.

Results: Female partners had higher perception of stress, depression and anxiety. Female personal experience of stress was associated with lower live birth and clinical pregnancy rates. These findings correlated with partner's psychopathology and stress ratings. Male stress had negative association with clinical pregnancy rates. Female psychopathology was associated with lower levels of TGF in FF, CVL and serum, and higher levels of FF and serum IL-6, and CVL IL1, with elevations associated with impaired implantation and no live birth. High serum IL-6 levels coupled with self-reported daily stress were associated with no pregnancy. Lower TGF levels in male serum correlated positively with lower rates of implantation and clinical pregnancy.

Conclusions: Female stress/psychopathology had a negative impact on IVF and was associated with both systemic and local changes in immunoregulatory mediators TGF and IL-6. Male stress and systemic immunity also impacted IVF outcome underscoring the potential utility of designing psychological interventions for both partners.

G-47

Interleukin 2 (IL-2), Interleukin 12 (IL-12) concentration in seminal plasma and their association with other semen parameters of intracytoplasmic sperm injection (ICSI) patients

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Problem: The objectives of this study were to find out if IL-2, IL-12 were present in human seminal plasma of patient undergoing ICSI therapy and to determine the relationship of these interleukins to other semen and sperm parameters and fertility potential.
Methods of study: One hundred and sixty five patients were included in this study. Semen samples were evaluated according to the WHO guideline (1999). Sperm membrane integrity (HOS-Test), maturity (Chromomycin CMA3) and, nuclear DNA integrity (TUNEL) were investigated. IL-2, IL-12 in seminal plasma was evaluated by ELISA technique.
Results: IL-2 concentration in seminal plasma was (12.53 ± 2.7 pg/mL) and IL-12 level was (8.26 ± 1.83 pg/mL). The concentration of WBC (2.63 ± 0.55), the concentrations of other semen parameters were as follow. Sperm motility (27.9 ± 15.4%), vitality (36.0 ± 16.3%), chromatin integrity (32.9 ± 11.8%), DNA fragmentation (16.11 ± 4.22%), mean number of fertilized oocyte (4.7 ± 3.1%) and embryo transferred rate (1.9 ± 0.09%). In seminal plasma, IL-2 found to be correlated positively significant with white blood cells concentration ($r = 0.59$; $P = 0.001$) and negatively with sperm vitality ($r = -0.07$; $P = 0.55$), sperm membrane integrity ($r = -0.1$; $P = 0.22$), fertilization rate (-0.17; $P = 0.20$) and Embryo transfer ($r = -0.20$; $P = 0.26$). Whereas, a negative significant correlation was shown between IL-12 and the mean age of the patients ($r = -0.10$; $P = 0.36$). Embryo transfer rate demonstrated a negative significant correlation with IL-12 ($r = 0.0$; $P = 0.77$).
Conclusion: Interleukins (IL-2, IL-12) was found in seminal plasma and showed a positive correlation with white blood cells concentration which indicated that both IL-2 and IL-12 produced from leukocytes in seminal plasma and abnormal spermatozoa. Besides, IL-2, -IL-12 demonstrates a negative correlation with sperm motility, DNA, and membrane integrity as well as embryo quality. Therefore, the elimination of leukocytes and abnormal sperm of the ejaculate is recommended and could improve sperm quality and fertilization capability.

G-48

Polychlorinated biphenyls (PCBs) impair placental angiogenesis by targeting delta-like ligand 4-Notch signaling pathway

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Problem: We have previously demonstrated that Aroclor 1254, a mixture of >100PCB congeners, caused preterm delivery, affected spiral artery remodeling, and reduced litter size and fetal weight in IL-10 null (IL-10−/−) mice. Importantly, Aroclor 1254 inhibited placental content of the water channel protein, aquaporin 1 (AQP1), a protein associated with angiogenesis and regulation of amniotic fluid. However, the direct anti-angiogenic effects of PCB congeners and the underlying mechanisms at the maternal-fetal interface remain poorly described.
Method of study: C57BL/6 wild type or their IL-10−/− counterpart were exposed to PCBs from gd4 to 12. Uteroplacental units were collected on gd 13 and used for further analysis. To mimic endovascular interactions, a three-dimensional assay of vessel formation involving first trimester trophoblast cells and endothelial cells was used. Importantly, *in vivo* matrigel plug assay was used to test the trophoblast invasion capacity. The aortic ring assay was performed to assess the ability of PCBs to inhibit sprouting endothelial cells.
Results: We show that treatment with Aroclor 1254 blocks trophoblast invasion and angiogenesis as determined by *in vivo* matrigel plug assay in mice. A complementary rat aortic ring assay also confirmed Aroclor 1254-mediated inhibition of endothelial cell

sprouting. Furthermore, we demonstrated coplanar congener PCB126, not non-coplanar PCB153, disrupted three-dimensional tube formation between HUVEC cells and trophoblast cells. The axis of the delta-like ligand 4 (Dll4) and its cognate Notch receptor has been shown to modulate VEGFR2 expression. We found that treatment with Aroclor 1254 resulted in significant reduction of VEGFR2induction of transcription factor Hey-2, Dll4, and Notch 4 in the IL-10−/− placenta.
Conclusions: The Notch/Dll4 signaling pathway is a novel target of PCBs and its activation is linked to an anti-angiogenic activity at the maternal-fetal interface.

G-49

Effect of TLR ligand on the production of PlGF and sVGFR1 from primary trophoblast

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Problem: Tool like receptors (TLRs) may play an important role of innate immune response during pregnancy. Placenta growth factor (PlGF) is a vascular endothelial growth factor derived from placenta. Soluble vascular endothelial growth factor receptor 1 (sVEGFR1) is its soluble receptor and regulate the PlGF function. To elucidate the action of TLR ligand to angiogenic and antiangiogenic factor in trophoblast, we examined the effect of TLR ligand on the production of PlGF and sVGFR1 from primary trophoblast.
Method of study: Villous tissues were obtained from healthy pregnant women who asked artificial abortion at 7–11 weeks' gestation with the informed consent. The trophoblasts were isolated from early pregnant villous tissues and cultured with serum-free medium. Subsequently, trophoblasts were treated with TLR ligand (TLR 1-9) for 24 hr. The levels of PlGF and sVEGFR1 were measured by ELISA.
Result: The PlGF production increased by adding TLR2 and decreased by TLR4 ligand. Increased sVEGFR1 production was found by adding TLR1, 5, 8 and 9 ligand.
Conclusion: TLR ligand may relate the production of PlGF and sVEGFR1. TLR ligand may work for placentation and maintenance of pregnancy.

G-50

Interaction between peritoneal M2 macrophages and endometrial stromal cells in the development of endometriosis via Stat3 activation

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Problem: The importance of peritoneal macrophages in the development of endometriosis has been known during the past three decades. Endometrial glandular and stromal cells in the refluxed menstrual blood also have been suggested to be the crucial constituents in this disorder. We previously demonstrated that activation of signal transducer and activator of transcription-3 (Stat3) is involved in interactions between peritoneal macrophages and ovarian cancer cells. Stat3 is a key signal transducer and regulator of macrophage activation associated with several oncogenic signaling pathways, such as cell proliferation and survival, angiogenesis, and immunosuppression. On the basis of these data, we hypothesized that interactions between peritoneal macrophages and endometrial stromal cells (ESCs) may be important in the development of endometriosis and that Stat3 activation may be associated with interactions between peritoneal macrophages and ESCs.
Method of study: Macrophage phenotypes in peritoneal fluid from patients with endometriosis and those without endometriosis were determined via immunostaining. Proliferation and activation of Stat3 in ESCs cocultured with macrophages were evaluated by immunostaining and 5-Bromo-2-deoxyuridine labeling assay. Stat3 activation in human endometriotic lesions and normal endometrium was investigated by immunostaining. The soluble factors in ascites and conditioned medium from macrophages and ESCs were quantified by means of the Cytokine Array Kit. Effects of soluble factors and the Stat3 inhibitor Corosolic acid (CA) on proliferation and activation of Stat3 of ESCs were evaluated. All patients provided written informed consent for participation in the study.
Results: The endometriosis group had a significantly-higher total number of macrophages in ascites compared with the control group, but the ratios of

CD163+ alternatively activated macrophages (M2) in the two groups did not differ significantly. Coculture with M2 macrophages significantly up-regulated ESCs proliferation and Stat3 activation in ESCs *in vitro*. Proliferation of ESCs was suppressed after Stat3 was down-regulated by small interfering RNA. Stat3 was activated in epithelial cells and ESCs in human endometriotic lesions and normal endometrium. The results of the cytokine array significantly showed up-regulated GM-CSF, RANTES, IL-1RA, and MCP-1 production in conditioned medium from co-cultured cells (c-CM) compared with single cultured medium. c-CM induced ESC proliferation and Stat3 activation in ESCs. GM-CSF significantly and dose-dependently induced ESC proliferation. However, GM-CSF did not induce Stat3 activation in ESCs. CA inhibited both ESC proliferation and Stat3 activation induced by c-CM

Conclusions: Our findings from *in vitro* and *in vivo* studies indicated that the interactions between M2 macrophages and ESCs via Stat3 activation may play indispensable roles in the development of endometriosis. Targeting Stat3 signals or the regulation of macrophage function may aid the treatment of patients with endometriosis.

G-51

Altered levels of soluble immune mediators in HIV-negative postmenopausal women: implications for HIV acquisition in the elderly

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Method of study: CVL samples were collected from 20 HIV-negative premenopausal and postmenopausal women. Whereas each postmenopausal woman provided only one sample, each premenopausal woman provided three samples, collected during proliferative, ovulatory, and secretory stages of menstrual cycle. Commercially available ELISA kits were used to assess the levels of IL-6, IL-8, TNFα, Elafin, HBD-2, MIP3α/CCL20 and SLPI. Samples were analyzed for their anti-HIV activity against HIV-1 IIIB and BaL strains via the TZM-bl assay.

Results: We observed significantly lower levels of critical immune mediators in CVL from postmenopausal women compared to those from premenopausal women: TNFα (11.59 versus 51.46 pg/mL), MIP3α (1.00 versus 93.77 pg/mL), SLPI (39,598 versus 239,184 pg/mL) and HBD-2 (626 versus 6821 pg/mL). Levels of IL-6 and IL-8 displayed a trend toward lower levels in postmenopausal samples whereas Elafin levels remained unchanged. Inhibition of HIV-1 infection was observed for X4/IIIB and R5/BaL strains in both pre and postmenopausal samples with inhibition of BaL stronger in premenopausal samples (54.2 versus 37.6%).

Conclusions: Our findings indicate that levels of critical mucosal immune factors and anti-HIV-1 activity in CVLs are affected by the hormonal status of healthy HIV-negative women. This suggests the need for specific therapeutic interventions to boost genital tract immunity against HIV in older women.

G-52

A2V-ATPase regulates autophagy and linked with inflammation-induced preterm labor

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Problem: The a2 isoform of V-ATPase (a2V) is a crucial molecule required for normal implantation, placental development and spermatogenesis. Our previous studies showed that for the successful implantation and placentation, a definite innate immune response is necessary, which is regulated by the a2V with the concurrent infiltration of macrophages in uterus and placenta. V-ATPase is crucial candidate for maintenance of low acidic pH in intracellular compartments such as lysosome endosomes, Golgi complexes, etc. which is critical for cell survival via regulating autophagy. The objective of the present study was to identify the role of a2V and its relationship to the regulation of autophagy and inflammation during infection-induced preterm labor in mouse.

Method of study: A mouse model of inflammation-induced preterm labor was used to collect gestational

tissues 8 hr after intrauterine injection of the combination of peptidoglycan (PGN, a toll-like receptor (TLR) 2 agonist) and polyinosinic:cytidylic acid (poly (I:C), a TLR3 agonist) modeling Gram positive bacterial and viral infection, respectively) on day 14.5 of pregnancy. RT-PCR, western blot analysis, immunohistochemistry and immunofluorescence were performed for a2Vand molecules involved in autophagy.

Results and conclusion: The expression of a2V and lysosome activity was drastically reduced in the uterus and placenta of PGN+poly(I:C) treated animals. Microtubule-associated protein light chain 3 (LC3B) is a widely used marker to monitor autophagy and correlated with the number of autophagosomes. The level of LC3BII, phosphorylated-NF-κB p65 and various cytokines like TNF, IL1-β and iNos were significantly increased in PGN+poly (I:C) treated uteri and placenta compared to controls. Using RAW 264.7 mouse macrophages cell line it was shown that the level of LC3BII was increased due to reduced lysosomal activity by blocking of a2V. Moreover, this blocking of a2V enhanced the PGN+poly(I:C) induced effect on LC3BII, phosphorylated-NF-κB p65 and secretion of various cytokines. These results suggest that a2V might be a bridge between autophagy and inflammation, and may be a useful therapeutic target for the prevention of preterm labor.

G-53

Anti-Müllerian hormone concentration as a biomarker of pregnancy success or failure

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Problem: The aim of this retrospective study was to determine serum anti-Müllerian hormone (AMH) concentration influence on pregnancy outcome.

Method of study: In this study we investigated sixty one infertile women (aged 27–44 years). We determine ovarian reserve measured by AMH concentration. Patients were divided in three groups according to their serum AMH concentration (2.5 ng/mL respectively). Main outcomes measure(s): clinical pregnancy rate due to AMH concentration. In addition, anti– thyroid antibodies (anti-TG and/or anti-TPO) positivity and insulin concentration were correlated with AMH concentration and pregnancy outcome in the study groups.

Results: We found no statistical differences between AMH concentration regarding number of pregnancies (42.3%; 41.1 %; 38.9% respectively in study groups; $P = 0.7$) and miscarriage rate in all study groups ($P = 0.19$). We found that anti-thyroid positivity is more frequent in women with lower AMH concentration (23.1%; 11.7%; 3: 5.5% respectively). Patients with lower serum AMH had higher serum insulin concentration ($P = 0.03$).

Conclusions: It seems that AMH concentration might not reflect oocyte quality and the chance of pregnancy. Moreover, it cannot be excluded that presence of anti-thyroid antibodies and increased insulin serum concentration may be connected to diminished ovarian reserve measured by AMH concentration.

G-54

Bedside assessment of amniotic fluid interleukin-6 in preterm prelabor rupture of membranes

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Problem: To test the efficiency of bedside assessments of interleukin-6 with respect to the presence and absence of microbial invasion of the amniotic cavity and histological chorioamnionitis in pregnancies complicated by preterm prelabor rupture of membranes.

Method of study: One hundred twenty-four women with singleton pregnancies were included in this study. The amniotic fluid was sampled by transabdominal amniocentesis at the time of admission. Interleukin-6 levels were assessed with an immunoassay.

Results: The presence of microbial invasion of the amniotic cavity, histological chorioamnionitis, or both microbial invasion of the amniotic cavity and histological chorioamnionitis was associated with higher amniotic fluid interleukin-6 levels upon crude analyses and analyses adjusted for gestational age. The amniotic fluid interleukin-6 level of 1000 pg/mL was determined to be the best cutoff value for identifying pregnancies complicated by both microbial invasion of the amniotic cavity and histological chorioamnionitis (sensitivity of 60%, specificity of 94%, positive predictive value of 75%, negative predictive value of 88%, and likelihood ratio of 9.4).
Conclusions: The bedside assessment of amniotic fluid interleukin-6 seems to be an easy, rapid, and inexpensive method for identifying pregnancies complicated by both microbial invasion of the amniotic cavity and histological chorioamnionitis.

G-55

Changes in myeloid lineage cells in the endometrium of dairy heifers during the estrous cycle and early pregnancy

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Problem: Embryo loss during early pregnancy in dairy heifers contributes to lower fertility. However, little is known about the myeloid lineage cells within the endometrium during this critical period. This study compared gene and protein expression in endometrial macrophages and dendritic cells on Day 17 of the estrous cycle versus Days 17 and 20 of pregnancy in Holstein dairy heifers.
Method of study: Uterine tissue sections labeled with CD45, MHC II and CD172a antibodies were analyzed for immunostaining via ImageJ software (n = 5 heifers/treatment). Genes expressed by the myeloid lineage cells, including MHC II, CD80, CD86 and CD163 were analyzed by quantitative PCR (n = 7–9 Day 17 cyclic, n = 6 Day 17 pregnant and n = 5 Day 20 pregnant). Results were analyzed using GLM procedures of SAS and with preplanned orthogonal comparisons.
Results: Endometrial labeling for CD45 was greater (P = 0.04) in cyclic compared to pregnant animals, with a tendency (P = 0.06) toward greater staining in the deep glands. MHC II staining tended (P = 0.06) to be less in endometrium of cyclic heifers than pregnant heifers, with the greatest difference (P = 0.02) observed in shallow glands. Similarly, overall CD172a expression tended (P = 0.06) to be less in endometrium of cyclic heifers than pregnant heifers, but was greater (P = 0.04) in luminal epithelium and tended (P = 0.09) to be less in both shallow and deep glands in cyclic heifers. Results of qPCR indicated less (pripts in endometrium of Day17 cyclic compared to pregnant heifers. CD163, a marker for tolerogenic myeloid cells, was greater (P = 0.03) in pregnant than cyclic heifers.
Conclusion: Changes in protein and gene expression in myeloid lineage cells in pregnant compared to cyclic heifers supports the hypothesis that conceptus signals affect resident immune cells at the very early stages of pregnancy. CD80 and CD86 were previously shown to be elevated in presence of type I interferons and the bovine embryo expresses the type I IFN, interferon tau, during early pregnancy. Higher expression of CD163 is an indication of a tolerogenic environment in the pregnant endometrium. Thus, during early pregnancy in cattle, embryonic signals, including IFN tau, may promote development of tolerogenic macrophages and dendritic cells as well as changes in gene expression for successful establishment of pregnancy.

G-56

Medroxyprogesterone acetate enhances HIV-1 uptake and transcytosis, but not replication, in primary human genital epithelial cells

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Problem: Women constitute more than half of all people living with HIV/AIDS worldwide, yet the early events of HIV-1 infection in the female genital tract (FGT) are poorly understood. The FGT is lined by genital epithelial cells (GECs), the first cells to

encounter HIV-1 during sexual transmission. Whether HIV-1 can infect the GECs remains controversial. Furthermore, whether endogenous female sex hormones or hormonal contraceptives, particularly medoxyprogesterone acetate (MPA), which has been correlated with increased HIV susceptibility and transmission can regulate GEC susceptibility or permissiveness to HIV-1 is unknown. In this study we examined the effect of female sex hormones on HIV entry and replication in GECs.

Methods: Primary GEC cultures were prepared from human genital tract tissues and grown in the presence or absence of physiological concentrations of estrogen (E2), progesterone (P4) or medroxyprogesterone acetate (MPA) prior to HIV-1 exposure. GEC monolayers were acid-washed to remove excess virus and HIV uptake by GECs was measured by p24 assays on disrupted cells. Various inhibitors were used to determine the cellular pathways involved in viral uptake. HIV infection and replication was determined by RT-PCR to measure HIV transcription, pro-viral DNA and spliced RNA.

Results: HIV uptake, as measured by p24 levels, was significantly increased within endometrial and cervical epithelial cells grown in the presence of MPA, but not other hormones. HIV-1 uptake by GECs was partly mediated by heparin sulphate (HS) since treatment with heparinase III partially blocked HIV association with GECs. HIV uptake into GECs was predominantly via endocytosis, since blocking endocytic inhibitor DYNASORE, significantly decreased HIV association with GECs. Despite uptake into GECs, no early or late reverse transcription products, integrated HIV DNA or spliced HIV RNA transcripts were measured, regardless of hormone exposure. Some of the virus taken up via endocytosis was recycled apically or transcytosed to the basolateral compartment within hours. Interestingly, HIV-1 transcytosis was significantly increased among GECs grown in the presence of P4 and MPA.

Conclusions: These results suggest that HIV-1 is taken up by GECs via endocytosis but does not result in a productive infection. Female sex hormones, particularly MPA, regulate HIV transcytosis across the epithelium and HIV uptake into GECs, but not replication. Ongoing studies are investigating whether enhanced transcytosis in the absence of productive infection, under the influence of MPA, would result in increased HIV infection.

G-57

mRNA stability: TIS11 family at the porcine maternal-fetal interface

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Problem: The TIS11 gene family are mRNA destabilizing genes that bind to cytokines and mediate their degradation. Previous reports from our laboratory confirmed that elevated pro-inflammatory cytokines such as TNF-α and IFN-γ and lower levels of VEGF are associated with conceptus arrest during porcine pregnancy. TIS11 family is of primary interest with respect to porcine spontaneous fetal loss since they are associated with posttranscriptional regulation of several pro-inflammatory genes including TNF-α and IFN-γ. This study aims to investigate association of TIS11 with early and mid-gestational porcine fetal loss.

Methods of study: Endometrial and fetal trophoblast samples were collected from gestation day 20 (gd20) and gestation day 50 (gd50) sows. Transcript expression levels of TIS11 family members were quantified using plate-based real-time PCR. Western blotting and immunohistochemistry were employed for detection of protein levels and cellular localization, respectively. Additional mechanistic studies are currently in progress in JEG-3 and JAR cell lines.

Results and conclusions: With the exception of TTP in endometrium, all TIS11 transcripts were elevated in healthy tissues as compared to arresting tissues at gd20 (pripts were expressed in arresting gd50 trophoblast compared to healthy counterparts and in arresting endometrium (pript expression was observed for all family members between gd20 and gd50 on the fetal side. TIS11 protein is also expressed at both sides of maternal-fetal interface. This shift may be due to a variety of factors and is currently being investigated in our planned mechanistic studies in cell lines.

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G-58

Possible role of placental endotoxin tolerance in protecting the fetus from exaggerated inflammatory responses

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Problem: Placental infection induces increased levels of pro-inflammatory cytokines, which have been implicated in the pathogenesis of preterm labor. Endotoxin tolerance is a phenomenon in which exposure to a dose of endotoxin makes tissue less responsive to subsequent exposures, with decreased expression of pro-inflammatory mediators. Endotoxin tolerance has been shown to play a role in multiple disease settings, including sepsis and cancer. However, the possible role of endotoxin tolerance in maintaining a pro-pregnancy, anti-inflammatory environment at the maternal-fetal interface during pregnancy has not been studied. The objective of our study is to determine if repeated exposure to endotoxin will induce a tolerant phenotype in normal human second trimester placental tissue.

Method of study: Second trimester placental explants from elective termination of pregnancy were cultured and exposed to endotoxin (lipopolysaccharide, LPS). After 24 hr, the media was collected for analysis, and the explants were re-exposed to LPS after adding fresh media. This process was repeated for a total of 4 days. The media was collected from each day and analyzed for cytokine levels using Bio-Plex and Milliplex arrays.

Results: After 24 hr, LPS treatment stimulated the secretion of the pro-inflammatory cytokines IL-1β and TNF-α. However, their production was significantly diminished with repeated LPS doses, with the lowest levels achieved on day 4. In contrast, the secretion of the anti-inflammatory cytokine IL-10 was up-regulated after the first dose of LPS and remained high over 4 days. Production of IL-1ra, an anti-inflammatory cytokine that is a naturally occurring IL-1 receptor antagonist, was also stimulated by the first LPS treatment, but was diminished with repeated LPS doses. Although the absolute level of IL-1ra decreased, the IL-1ra/IL-1β ratio became progressively more anti-inflammatory with repeated LPS doses.

Conclusions: Repeated LPS exposure of second trimester placental tissues induced endotoxin tolerance, characterized by down-regulation of pro-inflammatory cytokines, increased IL-10 production, and a shift in the IL-1ra/IL-1β ratio toward an anti-inflammatory state. We speculate that endotoxin tolerance at the maternal-fetal interface will protect the fetus from exaggerated inflammatory responses after repeated infectious exposure, and may prevent the onset of preterm labor.

G-59

Carbon monoxide modulates *porphyromonas gingivalis*-induced proinflammatory cytokine milieu in human placenta

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Problem: Several reports have established a significant correlation between periodontal infections and preterm labor. Microbial invasion of the amniotic cavity by *Porphyromonas gingivalis* (*P. gingivalis*), a common oral pathogen, was detected in women presenting with preterm labor. However, the biological effects of *P. gingivalis* on placental tissue remain unclear. We have previously demonstrated that carbon monoxide (CO) in very low, non-toxic concentrations can confer potent anti-inflammatory effects and reverse infection induced preterm labor in an animal model. We hypothesize that *P. gingivalis* will induce pro-inflammatory responses consistent with that seen in preterm labor in human placenta that can be modulated by low dose CO.

Method of study: Normal second trimester human placental samples (14–20 weeks gestation) were obtained after elective termination. Placental explant culture model was utilized. Explants were treated with various doses of *P. gingivalis* with or without exposure to CO (250 ppm) for 18 hr. Conditioned media were collected and analyzed for an array of pro- and anti-inflammatory cytokines using ELISA.

Results: As expected, the basal levels of IL-1 beta were low in normal second trimester samples, how-

ever it was significantly up-regulated by *P. gingivalis* stimulation. Although CO treatment did not change basal IL-1 beta levels, it significantly decreased its production after *P. gingivalis* stimulation when compared to control ($P < 0.01$). Similarly, CO tended to reverse the induction of TNF alfa after *P. gingivalis* stimulation, but it didn't reach statistical significance ($P < 0.1$). Interestingly, CO exposure significantly increased the basal production of the pro-pregnancy, anti-inflammatory cytokine IL-10 but not after *P. gingivalis* stimulation.

Conclusions: We believe we are the first to report that *P. gingivalis* exposure induces a pro-inflammatory profile in second trimester placenta. Moreover, our data indicate that CO can effectively inhibit placental *P. gingivalis*-induced proinflammatory mediators pointing to a potential role of CO in treatment of infection-induced preterm labor.

G-60

The effect of IgG (AT1-AA) from preeclampsia sera on the production of sEng and TGF-β from primary trophoblast cells

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Problem: The soluble endoglin (sEng) is an antiangiogenic protein inhibiting TGF-β1 signaling and eNOS activation. The levels of sEng increase in sera from preeclampsia. The factors increasing the sEng in preeclampsia have not been known well. In preeclampsia, many autoantibodies such as anti phospholipid, angiotensin II type IA receptor autoantibody (AT1-AA) have been reported. As we know, Losartan is AT1-receptor antagonist. To investigate factors increasing sEng in preeclampsia, we examined the effect of IgG from preeclampsia sera on the production of sEng from primary trophoblast cells with or without losartan. We also analyzed the production of TGF-β with or without losartan since sEng attenuates TGF-β signaling.

Method of study: Serum samples were from women with normal pregnancies and those with preeclampsia. Villous tissues were obtained from healthy pregnant women having artificial abortion from 8 to 11 weeks gestation. To study the effects of IgG fraction from preeclampsia sera on the production of sEng and the expression of sEng mRNA, IgGs isolated from normal pregnancy sera and preeclampsia were eluted by Protein G affinity chromatography. The primary trophoblast were cultured with IgG fraction for 24 hr, and the sEng levels in supernatants and expression of sEng mRNA were measured. To investigate the possibility of AT1-AA, the production and expression of sEng mRNA were measured with losartan treatment. We also analyzed the production of TGF-β with or without losartan since sEng attenuates TGF-β.

Results/Conclusion: The addition of preeclampsia IgG into primary trophoblast led to increased release of sEng and increased expression of Eng mRNA. The increased production and expression of sEng and sEng mRNA were suppressed by the treatment of losartan.

G-61

Expression of leptin & its long form receptor at the porcine maternal-fetal interface*

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Problem: Spontaneous conceptus loss in swine has been associated with multiple factors including an imbalance of angiogenic and/or immunologic factors. Leptin (LEP), an endocrine hormone that regulates adipose tissue mass, has been also implicated to promote angiogenic processes via activation of leptin receptor (OB-Rb) in endothelial cells. In the present study, LEP and its long form receptor (OB-Rb) were evaluated at the porcine maternal-fetal interface to assess their level of expression and their potential role in successful conceptus development.

Method of study: Leptin and OB-Rb genes were quantified by Real Time PCR in the endometrium of non-pregnant Yorkshire sows and in the endometrium and trophoblast of pregnant sows at gestation days 20 and 50 (gd20 and gd50). Leptin and OB-R protein expression was quantified by Western Blotting and detected by immunofluorescence staining.

Data were analyzed by a 3-way ANOVA testing the effects of conceptus health, tissue type, gestation day and their interaction.

Results: Leptin and OB-Rb transcripts were significantly higher ($P < 0.05$) in pregnant than in non-pregnant sows. Greater LEP ($P < 0.001$) was detected in the endometrial tissue of conceptus attachment sites at gd20 compared with gd50. At gd50, OB-Rb was greater ($P < 0.001$) in the endometrium of arresting conceptus attachment sites, and in the trophoblast of healthy conceptus attachment sites as compared with the other tissues examined. The lowest LEP protein ($P < 0.01$) was detected in the trophoblast at gd50, while OB-R protein was lower ($P < 0.01$) at gd50 in the trophoblast than in the endometrium collected from gd20 and gd50 conceptus attachment sites, both irrespective of conceptus health. Immunofluorescence confirmed the expression of these proteins at both gestation days and in both tissue types.

Conclusions: Changes in the expression patterns of leptin and OB-R between gd20 and gd50 suggest a role for the LEP/OB-R complex at the early stages of porcine pregnancy, possibly affecting the attachment process.

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G-62

The central role of interleukin 10 (IL-10) in the induction of vascular endothelial growth factor (VEGF) in placental explants model

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Problem: VEGF plays a pivotal role in vascular growth of the placenta. There is convincing evidence for the role of VEGF in the pathogenesis of perinatal complications such as preeclampsia and preterm labor. Interestingly, these same conditions are linked to low levels of IL-10, an important anti-inflammatory molecule for sustaining pregnancy. However, factors regulating placental VEGF production are largely undefined. In addition, it is unclear whether placental inflammation associated with bacterial infection seen in several pathologic pregnancies will modulate VEGF production and possibly placental angiogenesis. The objective of this study is to determine the role of IL-10 and bacterial inflammation in placental VEGF production.

Method of study: Normal pregnancy samples from second trimester and term placentas were collected following elective pregnancy terminations or non laboring elective c-section, respectively. Placental tissues were processed and cultured for 18–24 hr in DMEM and exposed to various treatments such as IL -10 (100 ng/mL), LPS (100 ng/mL) and IL-10 antibody (10 μg/mL). HTR-8 cells (throphoblast cell line) were also cultured and exposed to similar treatments. VEGF production was quantified by ELISA. Immunohistochemistry was used to identify the specific cell type responsible for placental VEGF production.

Results: Placental VEGF production was significantly increased with IL-10 treatment. Interestingly, IL-10 neutralizing antibody was able to block placental VEGF. Placental explants exposed to LPS alone showed an increase in VEGF but with the addition of IL-10, VEGF expression was more pronounced. In HTR-8 cells (known to have no IL-10 production), VEGF levels were minimal detected but with the addition of IL-10 there was a significant increase in VEGF. LPS exposed HTR-8 cell had similar VEGF expression as the controls but with the addition of IL-10, VEGF expression was significantly higher. Immunohistochemistry suggest that cytotrophoblasts are the major site for placental VEGF production.

Conclusions: These findings demonstrate that IL-10 plays a central role in inducing placental VEGF production. We speculate that low IL-10 is etiologically linked to abnormal VEGF production observed in some pathologic pregnancy such as preeclampsia and preterm labor.

G-63

Vitamin D receptor is overexpressed in women with RPL and vitamin D deficiency

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Background: Vitamin D deficiency is prevalent among women with recurrent pregnancy losses (RPL). We observed that in women with RPL and low serum levels of 25-OH vitamin D3, the cytotoxic activity of peripheral blood NK cells (NK cell cytotoxicity) is significantly elevated as compared to those of RPL women with normal levels of 25-OH vitamin D3. Moreover, NK cell cytotoxicity in these women were significantly suppressed by 1,25-(OH) 2-vitamin D3 *in vitro*.

Purpose: The goal of these study was to evaluate the vitamin D receptor (VDR) gene expression in NK cells from women with RPL and to reveal if the effect of 1,25-(OH)2-vitamin D3 on NK cell cytotoxicity is associated with levels of VDR expression.

Methods: Lymphocyte populations were sorted from peripheral blood mononuclear cells (PBMC) obtained using Leucosep protocol. NK cells were purified with anti-CD56 magnetic beads and MS separation column (Miltenyi Biotec) according to manufacturer's instructions. NK cells mRNA was isolated using Qiagen RNeasy micro kit, and then converted to cDNA on Applied Biosystems StepOne for subsequent RT-PCR with primers for VDR. Data were analyzed using the StepOne software by examining expression of VDR and GAPDH (internal control).

Results: The comparative Ct method ($\Delta\Delta C_t$) was utilized to assay relative mRNA expression levels in NK cells. The ΔC_t value was calculated by comparing the mean Ct value of VDR and GAPDH for each sample in duplicate. The C_t values for VDR vary from 7.85 to 12.26 in NK cells from women with RPL and vitamin D deficiency, indicating presence of VDR in peripheral blood NK cells. The ΔC_t value for VDR mRNA expression in peripheral blood NK cells, commonly used for calculation of relative mRNA expression levels by the $\Delta\Delta C_t$ method, was 2.06–6.47 approximately 138% higher than that in normal controls (ΔC_t = 5.78).

Conclusion: Women with RPL and vitamin D deficiency have increased VDR expression in peripheral blood NK cells. Greater suppression of cytotoxicity in NK cells from women with RPL and vitamin D deficiency by 1,25-(OH)2-vitamin D3 may be associated with VDR expression.

G-64

Trophoblast immune response is modulated by TAM receptor

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Problem: Inflammation is an important part of human response to pathogen to recruit immune cells and remove infected or damaged cells. However, an uncontrolled proinflammatory response may lead to extensive tissue damage and organ failure. Therefore, regulatory mechanisms are necessary to modulate the proinflammatory responses. During pregnancy, inflammation is necessary for several events, including implantation and defense against microorganism. Conversely, excessive inflammation may lead to pathologic conditions such as preterm labor. TAM receptors kinase have been reported to exert negative regulation of innate immune response by modulating TLR mediated inflammatory responses. The objective of our study was to evaluate the role of TAM receptor in modulation of inflammatory response in trophoblasts.

Methods of study: Immortalized first trimester trophoblast cell line, Swan71 cells were treated with human recombinant Gas6 and interferon β (IFNβ). Gene expression of cytokine gene profile and TAM receptors and ligands were evaluated by qPCR. Cytokine profile from the supernatant was analyzed by multiplex (Luminex). To assess involvement of TAM receptor in the placental response to lipopolysaccharides (LPS), Axl−/−Mer−/− mice were treated with either PBS or low dose LPS (20 μg/kg) at day E15.5 and observed for preterm birth within 48 hr.

Results: First trimester Trophoblast Swan71 cells expressed TAM receptors (Axl and Mer) and the

ligand Gas6 . Interestingly, Axl, Mer and Gas6 were upregulated by IFNβ stimulation. Treatment of trophoblast cells with Gas6 enhanced the anti inflammatory cytokines secretion. Axl−/−Mer−/− pregnant mice treated with low doses of LPS, known to cause no effect on WT mice, underwent preterm labor (50% Axl−/−Mer−/− versus 0% WT).

Conclusion: In the present study, we demonstrate that TAM receptors may play an important role regulating the inflammatory responses during pregnancy. Alterations on the expression and function of TAM receptor pathways may be associated with an abnormal inflammatory response leading to preterm labor.

G-65

Significance of HLA-DR expression on natural killer cells in terms of natural killer cell cytotoxicity

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Problem: HLA-DR is known as an activation marker of lymphocytes and is expressed on the surface of natural killer (NK) cells. We investigated whether NK cell cytotoxicity is correlated with HLA-DR expression on NK cells.

Method of study: Study subjects consist of 39 women with recurrent pregnancy loss and 48 fertile control women. Peripheral blood was taken in the early or mid-follicular phase of menstrual cycle. Peripheral mononuclear cells (PBMCs) were isolated and stained with anti-CD3, anti-CD56, anti-CD16, and anti-HLA-DR monoclonal antibodies. NK cell cytotoxicity assay was also performed at three different effectors to target cell ratios of 50:1, 25:1, and 12.5:1. Flow cytometric analysis was done.

Results: The level of CD3-CD56+ HLA-DR+ NK cells showed strong correlation with NK cell cytotoxicity (r = 0.388, 0.355, and 0.378; P = 0.000, 0.001, and 0.000, at three different E:T ratios). However, the level of CD3-CD56+ HLA-DR- NK cells was not correlated with NK cell cytotoxicity. CD3-CD56-dimHLA-DR+ NK cells demonstrated stronger relationship with NK cell cytotoxicity as compared to CD3-CD56brightHLA-DR+ NK cells. (r = 0.373, 0.338, and 0.359 versus 0.293, 0.299, and 0.341 at each E:T ratio) HLA-DR expression had very strong correlation with CD16 and CD56 expression (r = 0.794 and 0.778; P = 0.000 and 0.000, respectively).

Conclusions: HLA-DR expression on NK cells had significant correlation with NK cell cytotoxicity. Further studies are warranted to elucidate whether HLA-DR can be a representing marker of NK cell cytotoxicity.

G-66

RANKL/RANK interaction induces decidual M2 macrophage differentiation and maternal-fetal tolerance through AKT/STAT6 signal pathways in early pregnancy

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Problem: Fetal-derived extravillous trophoblasts come in direct contact with maternal decidual leukocytes (DLCs). The interaction plays an important role in maintaining the Th2 type immune bias at the maternal-fetal interface. Decidual macrophages (dMϕs), comprise the second largest decidual leukocyte population in early pregnancy (10–20%) next to decidual NK cells (50–70%). However, their function and differentiation remain unclear. RANKL represents the essential molecule that controls osteoclast cells differentiation. It also plays an important role in the immune regulation. Therefore, this study was conductive to explore whether RANKL in maternal-fetal interface modulates phenotype and function of dMϕs and herein participates in the formation and maintenance of maternal-fetal immune tolerance.

Methods of study: We analyzed expression of RANKL/RANKL expression in trophoblasts and DSCs by immunhistochemistry, ELISA and flow cytometry, respectively. And then, co-culture model of primary trophoblasts, DSCs and dMϕs (Tros-DSCs-dMϕs) was performed to investigate the effects of embryonic trophoblasts and maternal DSCs-derived RANKL on dMϕs.

Results: Trophoblasts and DSCs co-expressed RANKL and its receptor RANK. The expression of RANK on dMϕs was higher than it on peripheral monocytes. Further phenotypic analysis showed that both RANK+ peripheral monocytes and dMϕs expressed significantly higher levels of IL-10, the scavenger receptor CD163, and the phagocytic receptors, CD206 and CD209, compared with RANK- cells. Adding recombinant human OPG (rhOPG) or anti-human RANKL neutralizing antibody (α-RANKL) to the co-culture unit of Tros-DSCs-dMϕs, the percentage of CD80+ and CD86+ dMϕs, IL-12 and IL-23 levels in the supernatant of co-culture system were obviously increased. Instead, IL-10 concentration in co-culture unit was declined. In addition, treatment co-culture unit with rhOPG or α-RANKL inhibited the phosphorylation of AKT(pS473) and STAT-6 signal pathways and the transcription of IRF4 and JMJD3 in dMϕs, but not influenced the activation level of AKT(pT308), ERK1/2, JNK, p38, STAT1, STAT3, STAT4, STAT5, and mRNA level of IRF5. Subsequently, we over-expressed RANKL expression of Human placental choriocarcinoma cell line, JEG-3 cells and DSCs by plasmid transfection, and found that co-cultured dMϕs with these RANKL-over-expressed JEG-3 cells and DSCs not only significantly stimulated IL-10 production and decrease IL-12 and IL-23 secretion levels in the supernatant of co-culture unit, but also increased the mRNA level of IRF4 and JMJD3 in dMϕs, in contrast to co-culture with control JEG cells and DSCs. But these effect induced by RANKL over-expression of JEG-3 cells and DSCs could be partly reversed by AKT signal inhibitor LY294002. Furthermore, co-cultured decidual naïve T cells with trophoblasts and DSCs-derived RANKL instructing dMϕs up-regulated the secretion of IL-4 and IL-10, and down-regulated the production of TNF-α and IFN-γ in supernatant of co-culture system.

Conclusion: Our results indicated that RANKL derived from DSCs and trophoblasts inhibits the expression of CD80 and CD86 on decidual macrophage, facilitates the secretion of IL-10, and drives dMϕs polarized to M2 phenotype through activating Akt and downstream STAT6 signal pathways and enhancing the transcription of IRF4 and Jmjd3 in dMϕs. These educated dMϕs can induce decidual naïve T cells to Th2 bias, which is beneficial to immune tolerance at maternal-fetal interface.

G-67

Maternal-fetal immuno-tolerance instructed by trophoblasts

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The maternal-fetal interface is composed of embryo-derived trophoblast and maternal decidua, and the decidua contains decidual stromal cells and lymphocytes in the early pregnancy. The trophoblasts express a lot of membrane surface molecules and secret a series of soluble cytokines that instruct the decidual lymphocytes, NK cells, macrophages, dendritic cells and T cells. The trophoblasts recruit NK cells expressing CXCR4 into the decidua from the peripheral by secreting CXCL12, and recruit monocytes and T cells expressing CXCR6 via secreting CXCL16, respectively. The trophoblasts down-regulate the expression of perforin, CD16 and NKp44, and up-regulate the expression of KIR2DL1 in peripheral NK cells, and thus down-regulate cytotoxicity of NK cells by way of interaction of CXCL12 and CXCR4. The trophoblasts inhibit Th1 cytokines production, and promote Th2 cytokines production in either decidual or peripheral NK cells, which favors Th2 bias at maternal-fetal interface. The trophoblasts can secret TSLP that instructs the decidual dendritic cells secret IL-10 and CCL17 and in turn recruit naïve T cells which are differentiated Th2 cells, and result in regulatory T cells expansion. The expanded Treg cells improve invasiveness of trophoblasts in humans, and the trophoblasts express higher HLA-G that suppresses the cytotoxicity of NK cells. Therefore, crosstalking at the maternal-fetal interface is contributed by the embryo-derived trophoblasts that play central roles in the formation of maternal-fetal immunotolerance, which guarantees gestation smoothly.

G-68

The adverse influence of the time between the last injection of paternal lymphocyte immunization and the subsequent pregnancy on the pregnancy outcome in couples with recurrent spontaneous abortion

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Problem: Recurrent spontaneous abortion (RSA) is partially caused by immunologic disturbance. Paternal lymphocyte immunization (PLI) has been proposed as an effective therapeutic option for these patients, for restoration of Th1/Th2 balance. Our aim was to determine whether the time between the last dose of PLI and the following pregnancy impacts on subsequent pregnancies outcome.
Method of study: A descriptive cross-sectional study was conducted involving women with a history of primary RSA, whose flow cytometry crossmatch (FCXM) test against their partners was negative and were submitted to 1–3 doses of PLI with fresh lymphocytes were given at 15-day intervals. The women who became pregnant after the treatment were divided into two outcome groups: successful pregnancy (SP: live birth $\geq$ 21 weeks of gestation) and spontaneous abortion (SA). The time interval (months) between the final PLI injection and the women's last menstrual date was compared into two groups.
Results: A total of 85 women were included: 68 women in the SP group and 17 in the SA group. The mean of women age was 33 years (25–42 years) in the SP group and 35.4 years (28–43 years) in the SA group ($P = 0.073$). The mean of number of previous miscarriages was 2.6 (range 2–4) for both groups. The mean gestational age at delivery into the SP group was 36.5 weeks (24–40 weeks), with 13% of prematurity (9/68), of which, 1 neonatal death. The interval in months between the final PLI injection and the woman's last menstrual period was 3.0 ± 1.9 in the SP group and 6.6 ± 3.2 in the SA group ($P = 0.037$).
Conclusions: Our results demonstrated that the success pregnancy rate increases with the decrease of the time interval between the last dose of PLI and the following pregnancy. It's important to be discussed with the RSA couples.

G-69

The role of endothelin-1 in endotoxin-triggered release of placental pro-inflammatory cytokines

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Problem: Premature delivery occurs in 12% of all births and accounts for nearly half of long-term neurological morbidity and 60% to 80% of perinatal mortality in non-anomalous infants. Despite advances in the field, the rate of premature delivery has increased approximately 12% since 1990. Our lab and others have shown that endothelin-1 (ET-1) plays a critical role in the pathogenesis of inflammation-associated preterm birth (PTB).
Methods: Chorionic villous explants were prepared from term placentae obtained from Albert Einstein Hospital, Bronx, NY under aseptic conditions. The explants were divided into four groups: sham; lipopolysaccharide (LPS); LPS plus low concentration BQ-123, an ET-1 receptor antagonist; and LPS plus high concentration BQ-123. Explant supernatants from all groups were collected 24 hr after the addition of LPS and evaluated for changes in levels of interleukin 1 beta (IL-1B) and tumor necrosis factor alpha (TNFa) by Western blotting analysis and ET-1 by enzyme linked immunosorbent assay (ELISA).
Results: Villous explants treated with LPS released significantly higher amounts of IL-1B and TNFa into culture media than sham explants. This pro-inflammatory response was reversed, in a concentration-dependent fashion, with BQ-123. LPS exposed explants also secreted higher amounts of ET-1 as compared to sham explants. BQ-123 treatment suppressed this LPS-induced rise in ET-1.
Conclusion: IL-1B and TNFa, pro-inflammatory cytokines closely linked to infection-associated PTB, are ET-1 dependent. The mechanism of action of ET-1 blocking agents in tocolysis involves decreased ET-1-dependent release of these mediators. ET-1 itself is regulated by a positive feedback loop. Further evaluation of inflammatory mediators affected

by ET-1 may lead to the identification of novel targets for preventive therapy for PTB.

G-70

Infection-induced preterm delivery does not involve a shift in macrophage polarization from the M2→M1 phenotype but implicates a maternal pro-inflammatory state

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Problem: Macrophages are prevalent in the gestational tissues during late pregnancy and may have regulatory roles in the events of term and preterm delivery. Our aims were to investigate whether there is a shift in macrophage polarization from the M2→M1 phenotype during infection-induced preterm delivery and to evaluate whether this polarization is associated with changes in T cell phenotypes.

Method of study: Pregnant (16.0 days post-coitum) FVB/NJ mice were injected intraperitoneally with either 30 μg/200 μL of LPS or 200 μL of PBS as a control (n = 10–11 each). Peripheral blood was collected and cell suspensions from the decidua, myometrium, fetal liver and placenta were prepared 24 h after injection. M1 (CD11b+ F4/80+ iNOS+) and M2 (CD11b+F4/80+ Arg1+) macrophages, CD4+ and CD8+ T-bet+ cells, Th17 cells, CD4+ regulatory T cells, and neutrophils were analyzed by flow cytometry. Additionally, the total number of T cells, macrophages, and neutrophils were determined in all tissues, except the fetal liver, from mice treated with LPS or PBS (n = 5 each).

Results: (i) There was no change in the relative proportion of M2 or M1 polarized macrophages in the maternal or fetal tissues after LPS injection; (ii) Uterine and decidual macrophages as a proportion of total leukocytes were fewer in LPS-treated than in control mice ($P < 0.0001$ each); (iii) Uterine and decidual neutrophils were greater in proportion and number in LPS-treated than in control mice ($P < 0.016$); (iv) Maternal circulating T cell numbers and proportions were fewer in LPS-treated than in control mice ($P = 0.05$ and 0.032); (v) Maternal circulating CD4+ Tbet+ Th1 cell proportions were greater in LPS-treated than in control mice ($P = 0.036$); (vi) No changes were observed in the fetal tissues upon endotoxin administration.

Conclusions: Endotoxin treatment does not cause a shift in macrophage polarization from the M2 to M1 phenotype at the maternal-fetal interface; however, it promotes a pro-inflammatory state reflected by altered T cell phenotypes in the mother.

G-71

Lactobacillus crispatus, but not *L. iners*, downregulates vaginal epithelial immune response to *Gardnerella vaginalis* and *Atopobium vaginae*

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Problem: Vaginal colonization with hydrogen-peroxide producing lactobacilli is associated with lower rates of preterm delivery. We hypothesize that this is due to an anti-inflammatory effect of these bacteria.

Method of study: Immortalized vaginal epithelial cells (VK2) were cultured in keratinocyte serum-free media with *Lactobacillus crispatus*, *L. iners*, *Gardnerella vaginalis* and/or *Atopobium vaginae*. Interleukin 6 (IL6) and IL8 were measured in supernatant by ELISA. Fold-change in IL6 and IL8 over untreated cells was compared between conditions using Mann-Whitney test.

Results: In comparison to *L. crispatus*, *L. iners* was associated with higher median expression of IL8 (3.4 versus 1 fold change; $P = 0.009$), as were *G. vaginalis* (34-fold; $P < 0.0007$), *G. vaginalis* (6 versus 1; $P < 0.0001$), and *A. vaginae* (4 versus 1; $P < 0.001$). IL8 was lower when *L. crispatus* was co-cultured with *G. vaginalis* (8 versus 34 fold change; $P < 0.0001$) or *A. vaginae* (3 versus 14 fold change; $P < 0.0001$). There was no decrease in IL8 after co-culture with *L. iners* for either *G. vaginalis* (16 versus 34 fold change; $P = 0.68$) or *A. vaginae* (8 versus 14 fold change; $P = 0.83$). After co-culture with *L. crispatus*, *G. vaginalis* induced slightly lower IL6 (6 versus 4 fold change; $P = 0.32$), as did *A. vaginae* (4 versus 3 fold change; $P = 0.29$). Co-culture with *L. iners* induced slightly higher IL6 expression with both *G. vaginalis* (26 versus 6 fold change; $P = 0.17$) and *A. vaginae* (10 versus 4 fold change; $P = 0.75$).

Conclusion: *L. crispatus* downregulates vaginal epithelial IL8 response to two bacterial vaginosis associated bacterial species, while *L. iners* does not

G-72

Evaluation of haemostatic parameters and circulating pro-coagulant factors in pre-eclamptic women at delivery and post-partum

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Problem: Women affected by pre-eclampsia (PE) are at an increased risk for developing chronic hypertension and cardiovascular disease later in life. While the etiology of PE remains unknown, there is evidence that defective placentation is important in the pathogenesis of the disease. Women with PE often exhibit elevated circulating placental microvesicles, increased maternal inflammation and a hypercoagulable state. We hypothesize that women with PE exhibit haemostatic parameters indicative of hypercoagulability and elevated circulating microvesicles and pro-inflammatory cytokines. Furthermore, we propose that these changes extend into the post-partum phase.

Method of study: Blood was collected from non-pregnant women, women with uncomplicated pregnancies and women with PE at delivery/term, 6 weeks and 6 months post-partum. Thromboelastography (TEG), a measurement of global haemostasis, was performed on whole-blood and plasma was frozen until further use. Plasma will be used to assess the number and origin of circulating microvesicles and to evaluate the systemic pro-inflammatory cytokine profiles of each participant.

Results: Coagulation index (CI), a TEG parameter that is indicative of cloth strength and time and rate of clot formation, revealed that at delivery/term, women with PE are more hypercoagulable compared to women with uncomplicated pregnancies ($P = 0.0534$) and non-pregnant control women ($P < 0.0001$). Evaluation of post-partum CI revealed that the hypercoagulable state observed in women with PE normalized to non-pregnant values.

Conclusions: Our findings suggest that at delivery/term, women with PE are more hypercoagulable than non-pregnant women and women with uncomplicated pregnancies. Further work will investigate the profile of systemic cytokines and circulating microvesicles in these women.

G-73

Human chorionic gonadotropin increases regulatory T cells at the maternal-fetal interface in late gestation

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Problem: Human chorionic gonadotropin (hCG) has been implicated in the maintenance of uterine quiescence by down-regulating myometrial gap junctions during pregnancy; and, hCG has been proposed as a strategy to prevent preterm birth after the occurrence of preterm labor. Spontaneous preterm labor has been attributed to a breakdown of maternal-fetal immune tolerance, which is maintained by a tight equilibrium between regulatory and effector immune cells. Our aim was to determine whether hCG has an effect on these immune cells in late gestation.

Methods of study: Pregnant B6 mice were injected with either hCG (10 IU/500 μL) or PBS as a control on the 13, 15, and 17 day- post-coitum ($n = 10$ per group). Prior to delivery, the decidual tissues, thymus, spleen, and lymph nodes were harvested, and cell suspensions were prepared. Proportions of regulatory T cells (CD4+ or CD8+/CD25+/FOXP3+), macrophages (F4/80+), dendritic cells (CD11c+), neutrophils (Ly6G+), and NK cells (CD49b+) were analyzed by flow cytometry. Plasma progesterone concentrations were quantified by ELISA. A P value of <0.05 was considered significant.

Results: (i) Pregnant mice injected with hCG had a higher proportion of decidual CD4+ regulatory T cells ($P = 0.029$) than the control mice; (ii) Pregnant mice injected with hCG had lower proportions of decidual macrophages ($P = 0.005$), dendritic cells ($P = 0.015$), and neutrophils ($P = 0.028$) than the control mice; (iii) No changes were observed in the lymphoid tissues; and (iv) Plasma progesterone con-

centrations did not differ between the mice injected with hCG and the controls.
Conclusions: hCG increases the proportion of CD4+ regulatory T cells and reduces the proportion of effector immune cells at the maternal-fetal interface. These events are independent of plasma progesterone concentrations. These findings provide insights into the immunological mechanisms whereby hCG can inhibit preterm labor.

G-74

Expression of angiotensin receptors type1(AT1), type2(AT2) mRNA in local endometriosis lesions

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Problem: The presence of angiotensin receptors has been demonstrated in the endometrial tissue. Angiotensin II receptors can be classified angiotensin (AT) 1, AT2 and non AT1/AT2 receptors. Angiotensin II in endometrial stromal cells was mediated via AT1 receptor. We investigated into the expression of AT1, AT2 receptors in local lesions of endometriosis.
Methods of study: Endometriosis samples were obtained from 35 patients of endometrial cyst. Endometrial tissues were obtained from patients undergoing operations for benign gynecological conditions. Institutional Review Board (IRB) approval was obtained, and informed consents were obtained from all the patients participating in this study, and Tissue samples were stored at −80°C until analysis. The expression of AT1, AT2 receptors and PGE2 synthases mRNA was examined by real-time reverse-transcription PCR. Additionally, immune-histochemial staining was performed for AT1, AT2 receptors protein in eutopic endometrium and endometriosis lesions. We investigate into the relationships between expressions of AT1, AT2 receptors and PGE2 synthases.
Results: the expression level of AT1 receptors mRNA of secretory phase was significantly higher than that of proliferative phase in non-endometriosis control. The expression level of AT1 receptors mRNA of endometriosis sample was significantly increased compared to eutopic proliferative endometrium of non-endometriosis control. Interestingly, there was a relationship between expression of AT1 receptor mRNA and COX-2 mRNA in endometriosis samples. Positive cells for AT-1 and AT-2 were detected in both eutopic endometrium and endometriosis lesions by Immunohistochemical analysis.
Conclusions: the expressions of AT1, AT2 receptors mRNA of endometriosis samples indicate that RAS may have important role in the pathogenesis of endometriosis.

G-75

Impaired autophagy and transthyretin protein aggregation in preeclampsia

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Problem: We reported that transthyretin (TTR), a transporter of thyroxine and retinol, undergoes protein aggregation in preeclampsia and the aggregated TTR cytotoxically deposits in the placenta. Aggregated TTR also causes preeclampsia-like features in a serum-based 'humanized' mouse model. It is, however, unknown what induces and maintains the TTR aggregate phenotype in preeclampsia. We hypothesize that TTR aggregation is caused by hypoxia-like triggers and is by maintained by impaired autophagy, a process which maintains protein homeostasis by destroying bulky targets including organelles.
Methods of study: We used the human hepatocyte cell line, HepG2, which expresses TTR. Additionally, to confirm the role of autophagy, extravillous trophoblast (EVT) cells defective in autophagy were also used in this study. The expression of TTR was detected by immunocytochemistry or western blotting. Aggregated proteins were verified by Proteostat dye staining. Chemical inhibitors used were as follows; chloroquine for inhibiting lysosome-autophagosome fusion, 3-methyladenine for autophagy inhibition, and MG132 for proteasome inhibition.
Results: 3-methyladenine or chloroquine enhanced TTR aggregation in HepG2 cells. Immunocytochemistry analysis also showed that TTR aggregates were partially co-localized with p62, a substrate of auto-

phagy, in HepG2 cells treated with chloroquine. Western blotting showed that the expression of cytoplasmic TTR in autophagy-defective cells was higher than that in autophagy-normal cells. These and other results suggest that degradation of dysregulated TTR is mediated mainly by autophagy.
Conclusions: Autophagy is essential for normal placentation and pregnancy. Its defective phenotype may lead to TTR aggregation and onset of pre-eclampsia-like symptoms.

G-76

Different roles of IL-13 for the fetal loss induced by IL-12 treatment or 33D1 + DC depletion in mice

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Problem: Dendritic cells (DCs) seem to provide an appropriate fetal/maternal balance during pregnancy. Such balance appears to be regulated mainly by two distinct DC subsets, DEC-205+ DCs and 33D1+ DCs. Indeed, we have demonstrated that depletion of 33D1+ DCs during the perinatal period caused substantial fetal loss via augmented IL-12 secretion during pregnancy. (Negishi, et al., Immunobiol. 217 (2012) 951–961). Actually, we have found that the secretion of IL-12 was continuously seen throughout the pregnant state. However, it should be noted that the level of IL-12 reached maximum in the early phase of pregnancy followed by temporal elevation of IL-13 in the normal pregnancy of syngeneic mating (BALB/c X BALB/c).
Method of study: On the basis of these findings, we examined the effect of IL-13 injection on the miscarriage of mice induced by IL-12 administration or 33D1 + DC depletion. Female BALB/c mice were allowed to mate with male BALB/c mice. 33D1 antibody (0.5 mg/mouse, on gestational day (Gd) 5.5, 6.5 and 7.5) or murine recombinant IL-12p70 (0.2 mg/mouse, on Gd 9.5 and 10.5) was injected into pregnant mice to induce the fetal loss. Murine recombinant IL-13 (0.05 mg/mouse, on Gd 9.5 and 10.5) was also injected into each pregnant mouse.
Results: As expected, percentage of miscarriage induced by IL-12 inoculation was decreased by simultaneous injection of IL-13. However, the miscarriages induced by 33D1 + DC depletion were increased by the IL-13 treatment.
Conclusions: These findings suggest that IL-13 seems to have regulatory roles for the pregnancy, and IL-13 might have a capacity to prevent fetal loss through the 33D1 + DCs. These results indicate that IL-13 might be one of the critical cytokines, which could control the pregnancy.

G-77

Increased susceptibility to primary HSV-2 infection during early gestation leads to implantation failure and adverse pregnancy outcome in a mouse model

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Problem: Primary HSV-2 infection during pregnancy is associated with adverse pregnancy outcomes. However the mechanisms underlying these outcomes remain largely unknown. In this study we examined the dose dependent effects of primary HSV-2 infection during early pregnancy and its effects on pregnancy and fetal outcomes in a mouse model.
Method of study: C57BL/6 female mice positive for vaginal plugs were infected intravaginally (IVAG) with 103/104/105 PFU/mouse of HSV-2 (333) or PBS (control) on gestational day (gd) 5. For comparison, female mice in diestrus stage were infected with HSV-2 at the same doses. Pathology scores and vaginal viral shedding were measured post-infection. Maternal serum was collected for multiplex cytokine analysis. Vaginal tissue, implantation site, placenta and fetus were examined by histology.
Results: Minimum viral inoculation dose for infection in pregnant mice was 103 PFU of HSV-2, compared to 100-fold higher dose required to infect diestrus mice (105 PFU). There was a dose-dependent increase in number of resorptions with increasing dose of viral inoculum and all of the mice succumbed to infection by gd 13–15 in 104 and 105 PFU group. There was evidence of abnormal placen-

tal morphology and necrotic fetal tissues in HSV-2 infected, pregnant mice compared to controls. In the 103 PFU group, all mice showed local viral shedding, but 75% survived the infection. However, there was a significant increase in implantation failure compared to mock-infected group, which correlated with significant increase in eotaxin, IL-6 and RANTES in serum.

Conclusions: These results indicate a 100-fold increase in susceptibility to HSV-2 infection during early pregnancy. At higher inoculation doses, IVAG HSV-2 infection spread systemically resulting in poor fetal development and maternal mortality. At lower inoculation dose, the infection was localized in the reproductive tract and resulted in increased inflammation and implantation failure. This model will help to understand pathological mechanisms underlying adverse outcomes following primary HSV-2 infection in pregnancy.

G-78

Low dose carbon monoxide attenuates bacteria-induced endothelin-1 expression in second trimester placental explants

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Problem: Preterm birth (PTB) is defined as any birth occurring prior to 37 weeks' gestation, and currently accounts for 11–12% of all births in the United States. Maternal infection attributes to 40% of PTBs, causing a cascade of pro-inflammatory events which ultimately lead to expulsion of the fetus. The pro-inflammatory mediator and potent vasoconstrictor Endothelin-1 (ET-1) is known to be expressed in the placenta. It has been shown that blockade of the ETA receptor prevents infection mediated PTB in mice, implicating its important role in pregnancy. Recently, we demonstrated that very low, non-toxic doses of carbon monoxide (CO) also prevented infection-induced preterm birth in mice. However the effect(s) of CO on human gestational tissues is yet to be fully explored. We hypothesize that CO will have a protective role against inflammation-induced *E. coli* by down-regulating the ET axis in placental explants.

Method of study: Twenty placentas from elective termination of pregnancy in the second trimester were analyzed with or without exposure to heat killed *E. coli* over the course of 30 hr. Placental ET-1, along with its biologically inactive precursor BIG ET-1, and Endothelin Converting Enzyme-1(ECE-1, responsible for the cleavage of Big ET-1 to ET-1), were analyzed by ELISA. Gene expression for ET-1 (EDN1), ECE-1 and the ETA receptor (EDNRA) were analyzed using qPCR, using Beta Actin as the house keeping gene. Localization of ET-1 expression was also demonstrated using immunohistochemistry.

Results: *E. coli* significantly increased ET-1 transcription and secretion of BIG ET-1 and ET-1 in a time dependent manner which was ameliorated when exposed to CO at later time points. Incubation of explants in the presence of *E. coli* showed increased localization of ET-1 to Hofbauer cells and trophoblast layer. In the presence of CO, mRNA levels of ECE-1 were significantly reduced at 3 and 24 hr, while EDNRA was significantly reduced at 6 and 18 hr.

Conclusions: Up-regulation of ET-1 production in human placenta in the setting of infection can be attenuated by low doses of CO. Our results further explore the anti-inflammatory and regulatory mechanism(s) of CO on the ET axis components at the maternal fetal interface.

G-79

Uterine MyD88-dependent and -independent TLR4 signaling shift during early mouse pregnancy

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Problem: The uterus creates an immune milieu advantageous to the success of pregnancy. It is currently unclear the mechanism by which the uterus allows the inflammatory process of embryo implantation to occur for the establishment of pregnancy, yet fight pathogen-induced infections. It is likely that the uterine immune environment undergoes substantial modifications during the transition of the uterus from pre- to post-implantation states.

Method of study: Analysis of TLR4-mediated MyD88-dependent and-independent (Trif-mediated) pathways by qPCR and/or in situ hybridization in

the preimplantation uteri and post-implantation uterine sites having embryos before and after treatment of endotoxin.

Results: Analysis of MyD88 and Trif gene expression in the pre-implantation uteri and post-implantation embryo implantation sites revealed that Trif mRNA expression was significantly greater in embryo-implantation sites while MyD88 mRNA expression was significantly greater in pre-implantation uteri. These data indicate that while MyD88-dependent pathway is supportive of the uterine receptive immune environment, MyD88-independent pathway is helpful for the uterine decidual microenvironment. Since LPS is a known TLR4 agonist, and an inducer of early pregnancy complications by immune disturbance, we investigated which TLR4 signaling pathway is induced at embryo implantation sites in response to LPS. To study this, we compared MyD88 and Trif gene expression at embryo implantation sites following LPS exposure. We found that LPS specifically induces mRNA of MyD88 and its downstream effectors such as TNFα, IL6 and IL1β at embryo implantation sites.

Conclusions: Our findings demonstrate that (i) the process of embryo implantation induces MyD88-independent immunological changes at implantation sites, and (ii) endotoxin-induced defects at embryo implantation sites are the result of a shift in TLR4 signaling pathways from independent of MyD88 to dependent on MyD88. Supported by NIH grant HD044741.

G-80

Spheroid formation patterns of different granulosa cell types

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Problem: 3-dimensional cell culture models improve the *in vivo* conditions and influence particularly morphology, growth, proliferation and differentiation, as well as the regulation of several cell functions and gene expression. Formation of spheroids by using the 'hanging drop' method is one possibility to study cells in 3D environment. Although a number of cell types and lines have been studied throughout the last decades, nothing is known yet about granulosa cell spheroids. Our aim was to form spheroids using the two well-studied immortalized human granulosa cell lines COV434 and KGN, as well as from primary human granulosa cells, which could be used for further analyses.

Methods of study: Spheroids from primary granulosa cells isolated from follicular fluid of patients undergoing IVF or ICSI, as well as from COV434 and KGN cell lines, were formed according to the 'hanging drop' method. Different media, including standard culture medium, oocyte conditioned medium and follicular fluid, as well as standard culture medium supplemented with methylcellulose were tested. Cell numbers ranged from 1 × 103 to 5 × 104. All 3D cultured cells were microscopically monitored for 3 days.

Results: Although COV434 cells formed 3D aggregates, stable spheroids could not be established under the different 'hanging drop' conditions. In contrast, KGN and primary granulosa cells formed stable spheroids. Alterations of the spheroid shape depending upon the influence of different media and their different size induced by variation of applied cell numbers have been observed.

Conclusions: Our preliminary data obtained using the 'hanging drop' method of 3D culturing demonstrate the formation of 3D aggregates by COV434 cells and stable spheroids by the KGN cell line and primary human granulosa cells, as previously indicated for several other cell lines. We have established a method for generation of granulosa cell spheroids which can be used for further investigations, such as confrontation cultures with immune cells or endometrium cells.

G-81

Differential cytokine expression before and after surgical removal of endometriosis lesions

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Problem: In women with endometriosis, the growth of ectopic endometrial tissue in the peritoneal cavity

has been associated with abnormal regulation of local and systemic immune modulators. Cytokines are signaling molecules secreted by immune cells to direct cell recruitment, angiogenesis, and cell proliferation. Aberrantly expressed cytokines could contribute to the implantation and growth of ectopic endometrial tissue in endometriosis. As cytokines can be stably detected in serum and peritoneal fluid samples, they could be used as non-surgical diagnostic markers of endometriosis. In this study, we investigated the cytokine profile of women with endometriosis before and after surgical removal of endometriotic lesions.

Methods: Plasma samples (pre and post-surgery), eutopic endometrium, ectopic endometrium and peritoneal fluid were obtained from (n = 14) patients who underwent laparoscopic surgery at Greenville Hospital (South Carolina, USA). Eutopic endometrium and peripheral blood plasma from normal control women were also collected (n = 8). Protein extracts from eutopic and ectopic tissues, plasma, and peritoneal fluid samples were screened for 27 metabolites (including major cytokines and growth factors) using Bio-Plex multiplex cytokine assay.

Results: Our results showed that cytokine levels in plasma samples from endometriosis patients decreased significantly after surgery and remained low three months after surgery. Compared to pre-operative samples, levels of PDGF-bb, TNF-alpha, IFN-gamma, IL-1b, IL-1ra, IL-5, and IL-6 were significantly lower in post-operative and three month post-operative plasma samples ($P < 0.05$). No significant differences were observed in levels of other metabolites. . VEGF, MCP-1, RANTES IP-10, (a chemokine that had been shown to inhibit angiogenesis), had decreased expression in ectopic endometrium compared to eutopic endometrium ($P < 0.05$).

Conclusions: Based on these preliminary results, we conclude that removal of ectopic lesions resulted in decreased inflammatory response as evident from the reduced levels of several important inflammatory cytokines. Further studies are in progress to delineate expression patterns of various cytokines in eutopic, ectopic, plasma and peritoneal samples obtained from same patients.

G-82

Placental viral infection dysregulates type I interferon and sensitizes the trophoblast to bacteria: A potential mechanism for preterm birth during polymicrobial infection

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Problem: The placenta supports pregnancy and avoids fetal rejection by promoting an anti-inflammatory environment through the majority of term. Conversely, it must be capable of inflammatory responses during labor or infection. Placental inflammation is tightly regulated to avoid responses to normal microflora found in the upper reproductive tract, but during infection-related preterm birth, this regulation is overcome. Previous findings demonstrated virus sensitized the placenta to bacteria; therefore we hypothesized viruses affect cellular mechanisms regulating inflammation within the trophoblast, meaning women harboring a placental viral infection have higher risk of preterm birth. To test this we (i) Defined key pathway(s) modulating trophoblast inflammation and (ii) Determined how virus dysregulates placental responses to bacteria.

Method of study: Human trophoblast SW.71 cells and E15.5 mouse placenta were analyzed with microarrays to identify pathways regulating responses to pathogens. Type I interferon (IFN) and STAT3 pathways were identified, and BX-795 and BP-1-102 inhibitors were used to characterize function. Interferon and targets were further evaluated using overexpression, luminex and mice lacking IFN receptor (IFNAR-/-).

Results: Viral infection downregulated IFN in mouse placenta and SW.71 while increasing the inflammatory response to LPS in these cells/tissues. To determine if reduced IFN caused the sensitized response, the IFN pathway was inhibited in SW.71, mimicking viral infection, which resulted in high IL-1B. Treating IFNAR-/- mice with low-dose LPS at E15.5 caused inflammation and preterm birth in 50% of animals while wild-type suffered no effect. Exogenous IFN-beta activated STAT3 and transcriptional repressors of NF-kB responses in SW.71; indeed the repressor Twist was positively correlated with IFN expression and STAT3 phosphorylation, while overexpressing Twist reduced IL-1B.

Conclusion: We demonstrate IFN regulates trophoblast inflammation by activating STAT3 and inducing transcriptional repressors of NF-kB. Furthermore, IFN is downregulated by viral infection and therefore may be part of the mechanism of preterm birth associated with polymicrobial infection.

G-83

Inhibition of prostaglandin D2 decreases susceptibility of mice to LPS-induced preterm birth

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Problem: Preterm birth (PTB) occurs in approximately 12% of all pregnancies with up to 70% of these births associated with an underlying infectious process. As an infection enters the gestational tissues, a complex set of immunological responses occurs, including the enhanced production of prostaglandins (PGs). Because labor is driven by these inflammatory mediators, perturbations of their delicate balance can result in PTB. Recently, lipocalin-type prostaglandin D2 synthase (L-PGDS), the enzyme responsible for PGD2 synthesis, has been found in human gestational membranes, villous placenta, and amniotic fluid. PGD2, the product of L-PGDS, is found in relative abundance in gestational tissues and fluids, the choriodecidua, and is preferentially up-regulated in response to lipopolysaccharide (LPS) when compared to PGE2 and PGF2α. In addition, PGD2 has been proposed as a mediator of a Th2 immunological state contributing to the maintenance of pregnancy. Although current data strongly supports a role for the synthesis of PGD2 and its derivatives, via L-PGDS, in labor and delivery, the precise regulatory role for this arm of the PG cascade is lacking.
Method of study: Using transgenic L-PGDS overexpressors, L-PGDS knockout mice, and C57BL/6 control colonies we examined how modulation of PGD2 affects gestational outcomes after LPS-induced infection. In addition, DP1 and DP2 agonists were delivered to control mice via Alzet® pump and gestational outcome determined in response to LPS exposure.
Results: L-PGD2 knockout mice ($n = 10$) had a 50% decrease in LPS-induced preterm birth when compared to L-PGDS transgenics ($n = 15$) and a 30% decrease compared to controls ($n = 17$). In addition, control mice exposed to DP1 (BW A868C) and DP2 (11-deoxy-11-methylene PGD2) antagonists, were 50% less likely to experience adverse outcomes when compared to vehicle alone.
Conclusions: We believe inhibition of DP1 and DP2, the physiological receptors of PGD2, may represent a novel therapeutic site for the treatment of infection-induced PTB.

G-84

Porphyromonas gingivalis strain specific effects on the placental bed

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Problem: *Porphyromonas gingivalis* (Pg) is a common periodontal pathogen of humans that is also implicated in intrauterine growth restriction, preeclampsia, spontaneous preterm birth, and low birth weight. The mechanism by which Pg promotes adverse pregnancy outcomes (APO) remains elusive. Our objective was to determine if the local presence of Pg within the placental bed/metrial triangle induced uterine stromal pathology attributed to APO.
Method of study: Using a rodent model of infection, we examined gestation day (GD) 18 utero-placental tissues from Sprague-Dawley dams that were intravenously inoculated with sterile vehicle, Pg strain W83, or Pg strain A7436 at GD14. The extent of metrial triangle pathology on H & E stained sections, macrophage (CD68+) density, and trophoblast (cytokeratin 7) invasion was evaluated by morphometry. The in situ location of Pg within utero-placental tissues was determined by immunofluorescent histology.
Results: Compared to control animals, the metrial triangle of Pg inoculated dams exhibited greater amounts of uterine spiral arteritis composed of mononuclear infiltrates with or without granulocytes ($P < 0.05$) and occasional uterine thrombosis. Both Pg W83 and Pg A7436 were observed within the extracellular matrix of the uterine stroma, within fibroblasts, and CD68+ cells. However, only Pg A7436 infected metrial triangles exhibited a significant

influx of CD68+ cells around uterine vessels ($P < 0.01$), and a significant decrease in interstitial trophoblast invasion ($P < 0.01$).

Conclusions: The presence of Pg within the metrial triangle induces uterine vascular pathology similar to disorders of deep placentation in women (i.e. arteritis and shallow trophoblast invasion resulting in inadequate spiral artery remodeling). Thus, these findings suggest that certain Pg strains may be contributing to APO by disrupting placentation.

G-85

15-epi-lipoxin A4 reduces the mortality of prematurely born pups in a mouse model of infection-induced preterm labour

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Problem: Preterm birth remains the leading cause of neonatal mortality and morbidity. Given that labour, both at term and preterm, is an inflammatory event, there is growing interest in examining the potential of anti-inflammatory agents to treat preterm labour related preterm birth, and to improve survival. We investigated the potential of the anti-inflammatory and pro-resolution mediator, 15-epi-lipoxin A4, as a novel therapeutic agent in a mouse model of infection-induced preterm labour (PTL).

Method of study: On D17 of gestation, CD1 mice were pre-treated with an intra-peritoneal injection of 15-epi-lipoxin A4 (12.5 ng or 125ng) or vehicle (PBS+1% ethanol) followed 1–2 hr later with intra-uterine administration of 20mcg of LPS (from *E. coli* 0111:B4) or PBS ($n = 9–12$). Time to delivery and the number of live/dead pups were recorded for each treatment group and mortality rate per dam was calculated. In a second cohort, utero-placental tissues were collected 6 h post-LPS administration for qRT-PCR analysis of inflammatory mediators ($n = 3–5$).

Results: Mice treated with LPS delivered significantly earlier than PBS control mice (27.5 h ± 6.3 versus 55.4 h ± 6.4; mean ± SEM; $P < 0.001$). 15-epi-lipoxin A4 pre-treatment did not delay LPS-induced PTL. The mortality rate of prematurely delivered pups (defined as delivery within 36 h of surgery) was significantly reduced in mice treated with 15-epi-lipoxin A4 prior to intrauterine LPS, compared to LPS alone (mortality 0.55 ± 0.12 versus 0.97 ± 0.02; $P < 0.05$, respectively). QRT-PCR analysis of tissues harvested 6 h post-surgery demonstrated that 15-epi-lipoxin A4 increased Cox-2 expression ($P < 0.01$) and decreased 15-Hpgd expression ($P < 0.05$) in the placenta and uterus.

Conclusions: 15-epi-lipoxin A4 pre-treatment reduced pup mortality in a mouse model of LPS-induced PTL, possibly via a mechanism involving altered utero-placental prostaglandin production. Hence, 15-epi-lipoxin A4 may be useful in protecting the fetus from the adverse effects of infection-induced preterm birth and warrants further investigation as a potential novel therapeutic option in the treatment of PTL.

G-86

Levels of cytokines and chemokines in different fractions of the ejaculate of fertile men

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Problem: Seminal plasma has been ascribed an immunomodulatory role for the female response already at intercourse, related to presence of specific proteins and peptides including cytokines and chemokines. For analyses, human semen is routinely collected in a single tube despite its fractionated character, and the contents of cytokines are hence presented as the total concentrations. This study aimed at determining levels of selected cytokines/chemokines in two fractions of the ejaculate; the one non-coagulating, holding most spermatozoa (I) and the other coagulating, holding most proteins and few spermatozoa (II).

Method of study: Two fractions of the ejaculate was provided via masturbation by ten healthy human sperm donors with documented fertility, following documented consent. Six of them delivered up to four ejaculates at 2 month intervals. Seminal plasma

was harvested after two consecutive centrifugations (10,000 g/10 min) and immediately frozen (−70°C) until analyses for the content of pro-inflammatory cytokines [IL-6, IL-8 and monocyte-recruiting protein 1 (MCP-1)], Th1-associated cytokines (inteferon (IFN)-γ, IP-10), Th2-associated cytokines (MDC), Th17-associated cytokines (IL-17, GRO), growth factor (GM-CSF), anti-inflammatory (IL-10), immune-deviating TGF-β1, TGF-β2, TGF-β3 and IL-15 (inducer of NK cell proliferation) using multiplex bead assay.

Results: All above listed cytokines/chemokines were detected in seminal plasma, at various concentrations. Those having the highest levels were TGF- β1, MCP-1, IP-10 and GRO (0.7–2900 ng/mL). Between fractions, Fraction I had higher levels of IL-8 ($P = 0.021$), IL-15 ($P = 0.0001$), TGF-β3 ($P = 0.049$) and MDC ($P = 0.048$), but lower levels of GM-CSF ($P = 0.031$) compared to fraction II. Concentrations did not significantly vary between ejaculates (1–4).

Conclusion: Human seminal plasma contents of cytokines differs between fractions, possibly owing to temporal differences during sperm transport and eventual signaling to the female immune system.

G-87

Advanced maternal age disrupts neonatal T cell phenotypes

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Problem: Aging disrupts the immune system by causing an imbalance in regulatory T cells (*J Immunol* 2008; 181: 1835–48), which play a central role in the success of pregnancy. The aims of this study were to evaluate the effects of maternal age on the duration of labor, perinatal outcomes, and neonatal T cell phenotypes.

Methods of study: Older (20–24 weeks old, $n = 23$) and younger (8 weeks old, $n = 14$) pregnant B6N-Foxp3-GFP mice were monitored using a camera, and the following parameters were evaluated: weight gain, gestational length, duration of labor, total litter size, pup viability, and survival at 1, 2, and 3 weeks postpartum. Additionally, splenocytes collected from neonates (8 days old) of aged and young dams ($n = 6$–10 per group) were isolated, and flow cytometric analysis was performed to determine the following T cell subsets: CD4+ Tregs (CD4+ CD25+ Foxp3+), Th1 (CD4+ IFN+), Th2 (CD4+ IL4+), Th17 (CD4+ IL17+), and Th9 (CD4+ IL9+).

Results: (i) Older mothers had a longer duration of labor than younger mothers (2.05 ± 0.77 h versus 1.4 ± 0.71 h; $P = 0.05$); (ii) Neonatal mortality was higher in offspring of older dams than those of younger dams (71.93% versus 11.2%; $P = 0.001$); (iii) Neonates of older dams had lower proportions of splenic CD4+ Tregs ($P = 0.02$) compared to those of younger dams; (iv) Neonates of older dams had increased proportions of splenic CD8+ IL17+ T cells ($P = 0.001$), CD8+ IL9+ T cells ($P = 0.007$), CD8+ IFN + T cells ($P = 0.005$), and CD4+ IFN + T cells ($P = 0.002$) as compared to those of younger dams.

Conclusions: Offspring of older mothers have reduced CD4+ Tregs and increased effector T cells. The disruption in T cell phenotypes may explain the high rate of mortality for the offspring of older mothers.

G-88

Galectin-3 blood levels in endometriosis patients are increased. Its direct correlation with the number of Treg cells infiltrating endometriotic lesions

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Problem: It has been reported that Galectin-3, a 31KD carbohydrate-binding protein, is expressed ectopic endometrium. We investigated whether this protein may be used as a biomarker for endometriosis diagnosis, and its potential impact on the immune cells, in particular Treg cells, infiltrating endometriosis lesions.

Methods of study: A total of 35 women with suspect of endometriosis were enrolled for the study, from January 2013 through December 2013. Blood samples were collected from each woman to assess serum levels of galectin-3 by ELISA test (VIDAS, BioMrieux), as controls sera of 20 women without endometriosis were used. All 35 women underwent operative laparoscopy in order to diagnose and eventually remove endometriosis lesions. Endometriosis lesions collected during laparoscopy were fixed in

formalin and paraffin embedded to perform immunohistochemistry for Galectin-3 and FoxP-3 using commercial available monoclonal antibodies.
Results: Galactin-3 serum levels were statistically significant higher in endometriotic women with respect to controls (18.2 ± 6.6 versus 6.7 ± 1.3 ng/mL; $P < 0.001$). When a cut off of 13.5 ng/mL was used (according to ROC analysis) we were able to diagnose with Galectin-3 test 91.3% (21/23) of women showing endometriosis at laparoscopy, whereas 91.7% (11/12) of women tested negative did not have endometriosis ($P < 0.001$).
Conclusions: Galectin-3 may be used as a marker for the diagnosis of endometriosis, as well as of its activity. Furthermore, this protein, secreted by ectopic epithelial cells, seems promote Treg cell infiltration of endometriotic lesions.

G-89

Pregnancy hormones contribute to fetal tolerance by modulating adaptive immune responses

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Problem: The survival of the semi-allogeneic fetus within the uterus is ensured by both intensive hormonal changes and mechanisms that regulate the maternal immune response towards the fetus. The interaction between the endocrine and immune system allows that the fetus is tolerated rather than rejected. Regulatory T cells (Treg) and tolerogenic Dendritic cells (DCs) have been reported to favor fetal acceptance. Here, we aimed to investigate to which extent different pregnancy hormones modulate Treg and DCs and thereby affect pregnancy outcome in a murine model of disturbed fetal tolerance.
Methods of study: DBA/2J-mated CBA/J females known to spontaneously present high abortion rates were treated either with human Chorionic Gonadotropin (hCG), Luteinizing Hormone (LH), Progesterone (P4) or PBS on different pregnancy days. Pregnancy outcome as well as the number and phenotype of Treg and DCs were evaluated in the periphery and locally.
Results: hCG or LH application totally prevented fetal rejection in CBA/J females while P4 did not. The pregnancy protective effect of hCG and LH was associated with a Treg augmentation in the periphery and directly at the fetal-maternal interface. In addition, both hormones sustained a tolerogenic phenotype of peripheral and local DCs. By contrast P4 neither affected Treg number nor DC phenotype.
Conclusion: Our results suggest a differential regulation of Treg and DCs by various pregnancy hormones resulting in a different pregnancy outcome.

G-90

Obstetric outcomes of immune cause recurrent spontaneous abortion patients who successfully treated with immune modulating agent during early pregnancy

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Problem: To investigate whether treatment with immune modulating agent in early pregnancy can brings poor obstetric outcomes in patients who diagnosed to immune cause RSA.
Material and methods: The obstetric outcomes such as the incidence of pregnancy induced hypertension (PIH), gestational diabetes (GDM), small or large for gestational age (SGA or LGA), preterm birth, preterm rupture of membrane (PROM), oligohydramnios, birth weight , APGAR score and rate of major or minor congenital anomaly of newborns delivered by women who diagnosed to immune cause RSA and have been successfully treated with immune modulating agent such as intravenous immunoglobulin (IVIG) and/ or prednisolone (PDS) and/ or low-molecular-weighted-heparin (LMWH) were analyzed. The outcomes were compared to those of delivered by health control who were inspected to without history of take any medication during pregnancy.
Results: Rate of preterm delivery, SGA, LGA and PROM was similar in both groups. The incidence of pregnancy induced hypertension (PIH) was 1.7% in control group and 2.5% in RSA group, but the difference was not statistically significant. The incidence of GDM and oligohydramnios were similar in two group (control group versus study group, 1.7% versus 2.5%, 7.5% versus 10.0). Gestational age at delivery (weeks), APGAR score (1 min, 5 min) and

baby weight (g) were similar in both groups (control group versus study group, 39.0 ± 1.9 versus 39.4 ± 1.0, 7.9 ± 1.1 versus 8.1 ± 0.9, 8.7 ± 0.9 versus 8.9 ± 0.5, 3245.9 ± 488.7 versus 3240.3 ± 637.9) too. Also, the rate of major or minor congenital anomaly of newborns was similar in both groups (control group versus study group, 2.5% versus 1.2%).

Conclusion: Immune modulating agents can be used safely during early pregnancy without afraid of inferior obstetric outcomes in patients who diagnosed to immune cause RSA.

G-91

Chorioamnionitis and the effect of maternal glucose supplementation on neurodevelopmental outcomes in offspring

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Objective: Chorioamnionitis or intrauterine infection at term, are implicated in the pathogenesis of cerebral palsy. Corollary to that, we observed in a mouse model that exposure to maternal intrauterine inflammation (associated with the maternal infection) leads to adverse neurobehavioral outcomes in offspring. Currently there is no therapy to prevent cerebral palsy associated with chorioamnionitis. The objective of this study was to determine whether maternal glucose supplementation ameliorates perinatal brain injury in exposed neonatal mice.

Method of study: We utilized a mouse model of intrauterine inflammation at term (n = 27 dams). At E18 gestation, mice were randomized to receive either normal saline (NS) or lipopolysaccharide (LPS) intrauterine. 12 LPS mice received 10% dextrose (D10) intraperitoneally. Animals received 0.2 mL of D10 at 2,3,4,5 and 6 hr after LPS infusion. A standardized behavioral scoring system was used to evaluate brain neurocognitive outcome at PND 5, 9 and 13. Pups were tested in the following groups: NS (n = 30), LPS (n = 15) and LPS + D10 (n = 45).

Results: There was significant difference in LPS, NS and LPS + D10 survival (100% in NS, 50% in LPS compared with 85% of LPS + D10; $P < 0.05$). Pups in the LPS + D10 demonstrated overall improvement from LPS group on cliff aversion, open field test, geotaxis and forelimb grasp ($P < 0.001$ for all) and were similar to NS group ($P < 0.05$).

Conclusion: Our data indicate that glucose administration after LPS enhanced birth survival and improved neurobehavioral development. Clinically, these data suggest that once chorioamnionitis is diagnosed, maternal glucose supplementation may be beneficial for decreasing adverse neurobehavioral outcomes in the offspring.

G-92

Paternal antigen specific-regulatory T cells are induced by tolerogeneic DCs after seminal plasma priming

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Problem: Fetus is a semi-allograft to maternal immune system. Recently data show that regulatory T cells (Tregs) play a central role for feto-maternal tolerance. We analyzed the paternal antigen-specific Tregs (PA-Treg) by T cell receptor (TCR) level , and examined the role of seminal fluid and sperms for induction of Treg cells and/or tolerogenic DC.

Method of study: (i)To detect the paternal antigen specific Tregs, female BALB/c mice were mated with male DBA/2 mice (allogenic mating), and analyzed the kinetics of Vβ6+ Ki67+ Tregs in spleens, systemic lymph nodes , draining lymph nodes and uterus using the flow cytometry. T cell receptor Vβ6 recognize Mls1a antigen that is expressed on the surface of DBA/2 mice derived cells. Therefore, Vβ6+ Ki67+ Foxp3+ Tregs can be identified as paternal antigen specific Treg cells (PA-Tregs). (ii) To analyze the role of seminal fluid and sperm in Treg expansion, we resected the seminal vesicle of male DBA/2 mice (SVX) and vasectomized male DBA/2 mice (VAS). Female BALB/c mice were mated with male DBA/2 mice (SVX, VAS), and we analyzed the kinetics of PA-Tregs by using the flow cytometry. (iii) Antigen specific Treg cells are increased after antigen presenting by Dendritic cell (DCs).The character of DCs in spleens, superficial lymph nodes, draining lymph nodes and uterus on day3.5 and day5.5 were analyzed by using the flow cytometry.

Results: (i)The frequency of PA-Tregs in Tregs increased in draining lymphnodes on day 3.5 pc (before-implantation) and day5.5 pc (after-implantation) in BALB/c×DBA/2 allogeneic pregnant mice. The PA-Tregs in uterus increased after day 5.5 pc in allogeneic pregnant mice, but did not changed in syngeneic pregnant BALB/c. (ii)The frequency of PA-Tregs did not increase in draining lymphnodes on day 3.5 pc (before-implantation) and day5.5 pc (after-implantation) in BALB/c×DBA/2 (SVX) allogeneic pregnant mice. On the other hand, PA-Tregs of BALB/c mated with DBA/2 (VAS) were increased equally with BALB/c mated with DBA/2 (Wild), suggesting that seminal plasma play an important role for the induction of PA-Treg cells. (iii) CD86 and MHC class II expressions on DCs were significantly decreased in uterus on day3.5 pc and day5.5 pc in allogeneic pregnant mice . These molecules were not decreased in SVX allogeneic pregnant mice and in syngeneic pregnant mice. On the other hand, B7-DC expression on DCs were significantly increased in uterus on day 3.5 pc and day5.5 pc in allogeneic pregnant mice. B7-DC expression did not increase in SVX allogeneic pregnant mice and in syngeneic pregnant mice. These DCs might be tolerogenic DCs.
Conclusions: In allogeneic mating, the PA-Tregs were increasing in uterine draining lymph nodes just before the implantation. Seminal fluid priming is important for the induction of tolerogenic DC resulting in inducing the PA-Tregs in the implantation and pregnancy maintenance.

G-93

Identification of a novel site of epigenetic regulation of IP-10 in the human decidua

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Problem: Preterm birth is a major public health issue resulting in serious neonatal morbidity and mortality. Infections complicate forty percent of preterm births and may be demonstrated by inflammatory lesions in the placenta. Chronic chorioamnionitis, a common precursor to preterm birth, is characterized by large infiltrates of CD8 T cells. IP-10 is a chemoattractant for T-cells, and increased levels of IP-10 are associated with pregnancy complications. The decidua may be a major source of IP-10 production during an infection in pregnancy. The objective of this study is to characterize the regulation of IP-10 in the human decidua. We hypothesize that IP-10 expression is inhibited by histone methylation to maintain a normal pregnancy. In this study, we demonstrate that the trimethylated histone, H3K27me3 binds to the IP-10 promoter in the decidua.
Method of study: Chromatin immunoprecipitation was performed on decidua tissue and a first trimester trophoblast cell line and the binding region of H3K27me3 was determined by PCR. In addition, IP-10 levels were determined by quantitative PCR and Multiplex assay in trophoblast cells and in human decidua stromal cells incubated with estradiol and medroxyprogesterone acetate.
Results: Decidualized human stromal cells secrete low levels of IP-10 under basal conditions, while trophoblast cells constitutively secrete IP-10. Tri-methylated H3K27 binds to the promoter region of IP-10 at a specific novel site only in decidua tissue but not in trophoblast cells.
Conclusions: We identified a novel site of epigenetic regulation for the human IP-10 gene in the decidua. Histone H3K27me3 binds to a unique site in the promoter region of IP-10, maintaining low levels of IP-10. Alterations in IP-10 expression may indicate an infectious inflammatory process, thereby being useful as a biomarker to triage pregnant patients at risk of preterm delivery. These findings further support an immune regulatory role for the decidua stroma. Additional studies are underway to determine the factors regulating IP-10 histone methylation.

G-94

Cervical mucus as a barrier against infection during pregnancy: the role of cervical mucins

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Problem: Intrauterine infection is a common cause of spontaneous preterm birth. At the heart of the problem lies the increased passage of microbes through the cervical mucus barrier, the primary physical barrier between the colonized vagina and the sterile uterus. However, the mechanisms that lead to increased microbial passage through the cervical barrier are unknown. This information could be valuable for the design of effective treatments. Our goal is to understand how the cervical mucus barrier prevents ascending infection in normal pregnancy but permits ascending infection in preterm birth.
Methods of study: Mucins, the gel-forming building blocks of mucus, show consistent differences in O-glycosylation at ovulation, which result in an increased permeability of the mucus barrier to permit the passage of sperm. With glycan arrays and selected molecular tools we have begun to characterize the glycosylation profile of cervical mucus, with the goal to identify changes that are relevant for high-risk pregnancy.
Results: We previously showed that cervical mucus from women at high risk for preterm birth is characterized by an increased permeability and altered mechanical properties, suggesting that the gel-forming mucin polymers have undergone changes in molecular properties. Here, we present a set of tools that enables us to assess changes in composition of cervical mucus glycosylation. Our early data show that we can measure signature changes in glycosylation that correlate with the physiological state of the cervical mucus.
Conclusions: Our results indicate that the profiling of glycosylation in cervical mucus may provide a valuable strategy to predict pathogenesis within the cervical mucus barrier. We discuss the implications of altered mucus glycosylation for microbial growth and virulence in cervical mucus during pregnancy.

G-95

Isolation and characterization of macrophages in the bovine corpus luteum

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Problem: Macrophages are the most abundant immune cell within the corpus luteum (CL). However, the specific phenotype of luteal macrophages has not been determined. The objective of this study was to develop a protocol for isolation of luteal macrophages and evaluate the gene expression of macrophages isolated from functional and regressing luteal tissue.
Method of study: Luteal macrophages were isolated using antibodies targeting CD115, CD14, or CD45. CD45+ cells isolated from functional (midcycle) and regressing (8 hr after i.m. injection of prostaglandin F2α) CL were frozen immediately or cultured for 48 hr ± lipopolysaccharide (LPS). Culturing CD45+ cells allowed for adherence of macrophages and removal of nonadherent cells, providing a macrophage-only population. Genes expressed by macrophages (CD163, MRC1, IDO, NOS2, IL10 and TNF) were analyzed by quantitative PCR followed by SAS PROC Mixed analysis.
Results: Luteal macrophages isolated by CD115, CD14, and CD45 antibodies resulted in 0.14, 0.48, and 4.0 million cells/g of luteal tissue, respectively. The CD45+ cells were allowed to adhere to effectively isolate macrophages. Quantitative PCR revealed lesser concentrations of TNF ($P = 0.048$) and CD163 ($P = 0.047$), but a greater concentration of NOS2 ($P = 0.020$) mRNAs in freshly isolated CD45+ cells from regressing compared to functional CL. Treatment with LPS resulted in lesser concentrations of CD163 ($P = 0.036$) and MRC1 ($P = 0.014$) mRNA in macrophages from functional CL but greater concentrations ($P = 0.004$ and $P = 0.008$, respectively) of these mRNAs in macrophages from regressing CL. Cultured, LPS-treated macrophages from regressing CL contained greater concentrations of IL10 ($P = 0.044$) and MRC1 ($P = 0.007$) mRNAs and tended ($P = 0.063$) to have greater concentrations of CD163 mRNA compared to LPS-treated macrophages isolated from functional CL.
Conclusion: Isolation of luteal CD45+ cells followed by adherence resulted in the greatest yield of macro-

phages. Freshly isolated CD45+ cells from regressing CL expressed genes characteristic of M1-like macrophages. LPS induced differential responses in macrophages from functional and regressing CL, with genes characteristic of M2 phenotype being downregulated in functional CL but upregulated in regressing CL.

G-96

Humanized mouse model: new approach to study immune responses in female reproductive tract

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Problem: The female reproductive tract (FRT) is one of the major locations for natural HIV infection and any efforts aimed at preventing HIV transmission to women must successfully establish protection at this specific portal of virus entry. Despite advances obtained through studies using ex vivo tissue explants of the human FRT and non-human primates, vaginal HIV transmission in humans is still not fully understood. Over the past two decades, the construction of humanized animal models through the transplantation and engraftment of human tissues or progenitor cells into immunocompromised mouse strains has allowed for the development of a reconstituted human tissue scaffold in a small animal system.
Method of study: We use newborn NOD-SCID-gamma c-/- (NSG) mice (gamma c - common gamma chain of IL-2 receptor family) that are transplanted with human fetal liver hematopoietic CD34+ stem cells to generate humanized mice. The stage of estrous cycle in humanized mice was assessed by vaginal cytology method (based on the type and proportion of cells in the smear). Human immune cell engraftment and phenotype was monitored by multi-color flow cytometry.
Results: Consistently with other reports, humanized mice generated in our hands demonstrated high levels of human immune cell reconstitution in different compartments (blood, spleen, thymus, bone marrow, lymph nodes, small and large intestine, vagina and uterus). Particularly, we found extensive reconstitution of lymphoid tissue within the female reproductive tract (FRT), including vagina, ectocervix, endocervix and uterus. The number and activity of immune cells in reproductive tract of animals and humans vary significantly throughout the phases of the reproductive cycle. By examining humanized mouse uterine and vaginal tissues we show that adaptive and innate immunity varies with the stage of the estrous cycle.
Conclusion: Our data validates NSG humanized mice as a functional model to study female reproductive immune responses.

G-97

N,N-dimethylacetamide controls infection-associated preterm birth in a murine model

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Problem: Premature birth (PTB) causes high rates of neonatal morbidity and mortality. Failure to identify pharmacologic agents to prevent PTB has led to an intensification of research efforts focused on novel putative tocolytics. In this study, we present *in vivo* and *in vitro* evidence for the function of N′N-dimethylacetamide (DMA) in the prevention of PTB.
Methods of study: C57BL/6 mice were administered lipopolysaccharide (LPS) (50 mg/kg) intraperitoneally (ip) at E15.5 to induce PTB. Increasing doses [0.2 (n = 7), 0.39 (n = 8), 0.78 (n = 10), 1.56 (n = 6), 3.2 g/kg (n = 8)] of DMA or phosphate buffered saline were administered ip 30 min prior to, and 10 hr after LPS injection. Histological sections of placenta from sham, LPS control and LPS plus DMA treated groups were analyzed for inflammatory infiltrates. Western blot analysis, ELISA and immunohistochemistry were performed to compare levels of placental pro-inflammatory cytokines among the three groups. The effect of DMA on nuclear factor kappa B (NF kB) translocation and chemotaxis was tested in RAW 264.7 and MDCK(NBL-2) cells, respectively.
Results: DMA significantly reduced the incidence of LPS-induced PTB in a dose dependent manner. DMA reduced expression of the pro-inflammatory cytokines interleukin-1B, tumor necrosis factor a, and interleukin-6, and increased expression of the

regulatory inflammatory cytokine interleukin-10 in the placenta. Histological analysis of placental sections revealed a decrease in circulating polymorphonuclear neutrophils. DMA suppressed macrophage function and prevented nuclear translocation of NF kB in RAW 264.7 cells. Finally, DMA did not affect migration of MDCK cells at 10 μM, a concentration at which cell viability is not affected.

Conclusions: DMA was effective in preventing inflammation-associated PTB and showed a dose response relationship. The data suggest that DMA mediates its effect by suppressing proinflammatory cytokines and inflammation. Further studies are required to determine DMA's exact mechanism of action and its efficacy to toxicity ratio.

G-98

Antimicrobial host defence peptide, LL-37, as a potential vaginal contraceptive

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Problem: LL-37, a cationic antimicrobial host defence peptide, exerts its microbicidal effects through the disruption of microbial cytoplasmic membranes following its interaction with microbial surface anionic phospholipids. ALL-38 (an LL-37 close analog: LL-37+ Ala at the N-terminus) is produced in the vagina 2–6 hr post-intercourse from its precursor hCAP-18, a seminal plasma component. At this time, motile sperm have already swum into the uterine cavity, thus unexposed to ALL-38. Since sperm contain a substantial amount of acidic sulfogalactosylglycerolipid (SGG) on their surface, treatment of sperm with LL-37 may cause their membrane disruption in an analogous manner to that occurring on microbial membranes. If positive results are obtained, our long-term objective is to develop LL-37 into a vaginal contraceptive.

Method of study: Mouse/human sperm treated (2–30 min) with LL-37 in a physiological concentration range (up to 10.8 μM) were assessed for SGG-dependent binding to biotinylated LL-37 using Aelxavidin as a probe, and anti-SGG antibody to block SGG on the sperm surface prior to LL-37 treatment. Parameters relevant to fertilizing ability were assessed in LL-37-treated sperm, i.e., motility by videomicroscopy, the acrosomal status by Coomassie blue staining of acrosome intact mouse sperm or the exposure of an inner acrosomal membrane protein, CD46, of acrosome reacted human sperm, and the intactness of the sperm plasma membrane by hypo-osmotic swelling response (curling of sperm tails), exclusion of Sytox Green (a membrane impermeable fluorescent DNA dye), and electron microscopy. The ability of LL-37 treated mouse sperm to fertilize eggs was also evaluated. *In vitro*, the presence of 2-pronuclei in mature eggs, 6 h following insemination, was used as evidence of fertilization. Finally, the efficacy of LL-37 to inhibit sperm fertilizing ability *in vivo* was determined by the pregnancy outcome of female mice transcervically injected with LL-37 treated sperm or untreated sperm ($n = 26$ each for LL-37 treatment and no treatment), using sperm retrieved from the epididymis of 26 males.

Results: Biotinylated LL-37 bound to both mouse and human sperm and the binding was partially dependent on sperm surface SGG. Mouse and human sperm became immotile and underwent a premature acrosome reaction upon treatment with LL-37 at 3.6 μM and 10.8 μM, respectively. The initial action of LL-37 on both mouse and human sperm was through permeabilization/disruption of sperm surface membranes evidenced by the loss of hypo-osmotic swelling response, Sytox Green staining and ultrastructural damages. Mouse sperm treated with 3.6 μM LL-37 lost the ability to fertilize eggs both *in vitro* and *in vivo*. All 26 female mice inseminated with sperm and LL-37 did not become

pregnant, while pregnancy occurred in 24 of 26 females injected with control sperm. No apparent damage to the reproductive tract of LL-37-inseminated mice was observed as revealed by histological characterization and these females resumed fecundity following mating with fertile males.

Conclusion: Our results reveal selective inhibitory effects of LL-37 on sperm fertilizing ability. In mice, LL-37 exerted contraceptive effects with no apparent impairment to the female reproductive tract. LL-37 is therefore a promising candidate to be developed into a vaginal contraceptive with microbicidal activity. Funded by CIHR, and Bill & Melinda Gates Foundation

G-99

Contribution of IL-35 to maintaining the testicular immune privilege

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Problem: Testis is known as one of the immunologically privileged organs. In particular, blood-testis barrier formed by Sertoli cells protects autoimmunogeneic spermatozoa and spermatid from attack by the self-immune system. Moreover, it was demonstrated that Sertoli cells, Leydig cells and a few population of testicular macrophages exhibit immunosuppressive activity. Recent studies also suggest a possibility that some cytokines in the testis contribute to maintaining the immune privilege. Interleukin (IL)-35 is a heterodimeric cytokine composed of Epstein-Barr virus-induced gene 3 (EBI3) and the p35 subunit of IL-12. Although it is IL-35 has an important role in immunosuppression, its role in the testis remains unknown.

Method of study: In the present study, we investigated the role of intra-testicular IL-35 by histochemistry, immunohistochemistry and real-time RT-PCR using wild-type C57BL/6 mice and EBI3- and p35-deficient mice.

Results: EBI3 expression was detected in a part of CD163-positive macrophages and acrosomal regions of spermatids in testis of wild-type mice. Intriguingly, p35 expression was coincidently detected in the EBI3- and F4/80-positive macrophages, and also in basal lamina of seminiferous tubules, endothelial cells and acrosomal region of spermatids. A significant increase in the number of seminiferous tubules with spermatogenic disturbance was observed in both EBI3- and p35-deficient mice, compared with that in wild-type mice. Especially, p35-deficient mice showed severe spermatogenic disturbance. Moreover, CD4-, CD8- and B220-positive infiltrating cells were detected in the testicular interstitium of EBI3- and p35-deficient mice, but not of wild-type mice. Intra-testicular mRNA expression of interferon-gamma was significantly increased in EBI3- and p35-deficient mice. A similar increase in the expression of IL-10 was observed only in p35-deficient mice. Finally, autoantibodies to spermatids were detected in sera obtained from EBI3- and p35-deficient mice, but not from wild-type mice.

Conclusions: In testis, there are EBI3- and p35-double positive macrophages, possibly producing immunosuppressive IL-35. And, lack of either EBI3 or p35 causes infiltration of lymphocytes into testis and spermtatogenic disturbance. These results indicate that IL-35 plays an important role in maintaining the testicular immune privilege.

G-100

Interaction of decidual NK cells with extravillous trophoblasts leads to trogocytosis of HLA-G

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Problem: Trogocytosis is the uptake of plasma membrane fragments and molecules by lymphocytes after immune synapse formation with either antigen presenting cells (APC) or target cells such as tumor cells. Activated peripheral NK cells (pNK) have been shown to acquire HLA-G from tumor cells, and upon this acquisition pNK are no longer cytotoxic, and behave as suppressor cells. Thus trogocytosis of HLA-G induced a pNK cell phenotype that shares many similarities with the phenotype of decidual NK cells (dNK) that are found at the fetal-maternal interface during human pregnancy.

Methods of study: In a novel *in vitro* co-culture system human first trimester dNK and pNK were co-cultured with freshly isolated human fetal HLA-G+

extravillous trophoblasts (EVT). Trogocytosis of HLA-G was analyzed by multicolor flowcytometry. Furthermore, phenotypic and functional changes in dNK and pNK were analyzed by confocal microscopy, western blot and a flowcytometry based degranulation assay to determine cytotoxicity.

Results: In this study HLA-G protein was detected on freshly isolated human dNK cells in the absence of mRNA transcript. Furthermore, contact-dependent acquisition of HLA-G by pNK and dNK cells from primary EVT was demonstrated. Confocal imaging of co-cultures reveals immune synapse-like contacts between the fetal and maternal cells, in which HLA-G can be enriched. However, acquisition of HLA-G could not be blocked by addition of either anti-KIR2DL4 or anti-ILT2 the two main HLA-G receptors expressed by dNK cells. Although IL-15 activated pNK or dNK were not able to directly kill EVT, acquisition of HLA-G did not change the cytolytic capacity of either NK cell types in a redirected killing assay.

Conclusions: Our data indicate that trogocytosis of HLA-G by dNK cells may prevent direct killing of EVT cells but does not directly contribute to a general suppression of dNK cells at the fetal-maternal interface.

G-101

KIR2DS1+ decidual natural killer cells show ex-vivo activation and cytotoxicity but not cytokine secretion

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Problem: Decidual natural killer cells (dNK) are the most abundant lymphocytes at the fetal-maternal interface. dNK have been shown to have limited cytotoxic capacity but to secrete high levels of cytokines to facilitate extravillous trophoblast (EVT) invasion. Previous studies have shown that mothers expressing the activating Killer Immunoglobulin-like Receptor-2DS1 (KIR2DS1) are protected from pregnancy complications when carrying a fetus expressing HLA-C2 (KIR2DS1 ligand) (Hiby *et al.,* 2010). This study aimed to dissect the mechanisms associated with this protective effect.

Method of study: dNK from first trimester pregnancy terminations and peripheral NK (pNK) from blood donors were characterized by flow cytometry for expression of HLA-C receptors and cytotoxic granules. Activation state of NK cells was assessed using BD Phosflow® to detect SYK/ZAP70 phosphorylation. A degranulation assay was used to measure cytotoxicity of NK cells. Cytokine secretion of NK cells after co-culture with EVT was detected by Bioplex® assay.

Results: A significantly higher percentage of dNK expressed KIR2DS1 and KIR2DL1 than pNK. dNK and pNK expressed similar levels of perforin and granzyme B, but dNK contained markedly increased granulysin levels. Although pNK were more cytotoxic than dNK against HLA- targets, this cytotoxicity was inhibited by HLA-C1+ and HLA-C2+ targets. In contrast, the cytotoxicity of dNK from KIR2DS1+ mothers was not efficiently inhibited by HLA-C2+ targets. Moreover, higher phosphorylation of ZAP70/SYK in dNK from KIR2DS1+ mothers than KIR2DS1- mothers was showed. Contrary to stimulation of NK cell receptors with antibodies or HLA-C+-cell lines, stimulation of dNK and pNK with EVT revealed no specific production of NK cytokines such as IFN-γ or IL-8.

Conclusions: The higher phosphorylation of SYK/ZAP70 in dNKs from KIR2DS1+ mothers show an activation state that may dominate over inhibition through KIR2DL1+ dNK. KIR2DS1+ dNK activity was associated with cytotoxicity but not cytokine secretion. The association between cytotoxicity and protection from pregnancy complications is currently being addressed.

G-102

Decidual CD8+ effector T cell responses in healthy and complicated pregnancy

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Problem: During pregnancy maternal and fetal mechanisms prevent a destructive immune response of the mother towards the allogeneic fetus. Despite these immune regulatory mechanisms, CD8+ effector-memory T cells are present at the fetal-maternal interface throughout gestation. Thus far, no data are available on factors that prevent detrimental CD8+ T cell responses at the fetal-maternal interface and the antigen-specificity of decidual CD8+ (dCD8+) T cells

is unknown. Therefore, we aim to investigate CD8+ T cell responses at the fetal-maternal interface.
Methods of study: First trimester decidual and peripheral CD8+ T cells were separated into subsets based on CD45RA, CCR7, CD27 and CD28 expression. Perforin and granzyme B expression was assessed for each subset. Co-culture experiments of dCD8+ T cells with fetal EVT, VT and decidual macrophages (dMϕ) were performed. Subsequently, dCD8+ T cells were stimulated with anti-CD3/28 and perforin and granzyme B expression and production of IFN-γ was determined.
Results: Similar to term pregnancy, the presence of highly differentiated, effector-memory CD8+ T cells in first trimester decidua was demonstrated. dCD8+ T cells have reduced expression of perforin, but equal levels of Granzyme B when compared to peripheral blood CD8+ T cells. Fetal EVT, VT and dMϕ showed no direct effect on CD8+ differentiation and production of cytotoxic molecules. However, upon stimulation with anti-CD3/28, dCD8+ T cells increased expression of perforin and granzyme B and secreted IFN-γ. In addition, preliminary data demonstrated that anti-CD3/CD28 activated dCD8+ T cells reveal cytotoxic capacity towards EVT.
Conclusions: dCD8+ T cells have limited cytotoxic potential, but do not seem be anergic and are able to elicit an immune response when stimulated. The limited cytotoxic potential is not directly attributable to suppression by EVT, VT or dMϕ. Further research is necessary to understand the mechanisms that prevent detrimental dCD8+ T cell responses as well as dCD8+ antigen-specificity.

G-103

Effects of early pregnancy on endometrial cytokines and other immune related genes in dairy heifers

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Problem: A substantial portion of embryo loss occurs during early pregnancy contributing to low fertility in dairy cattle. Conceptus signaling can alter immune function in the endometrium. However, the effects of conceptus signals on uterine immune related genes are poorly understood. The objective of this study was to determine if expression of uterine immune related genes and proteins are altered by the presence of a conceptus.
Method of study: Endometrial tissue RNA was isolated from Day 17 cyclic (n = 5–9), and Day 17 (n = 5–7) and 20 (n = 4–5) pregnant Holstein dairy heifers. Steady-state concentrations of mRNA for IL10, IFNG, IL15, IL6, TGFB1, Gal-1, GZMA, T-bet and GATA3 were estimated using quantitative PCR. Results were analyzed using GLM procedures of SAS with preplanned orthogonal contrasts. Immunofluorescence studies conducted on frozen uterine sections were used for studying IFNG protein expression and were analyzed using ImageJ.
Results: Expression of IL10 mRNA was greater (P = 0.03), and GATA3 (P = 0.06), GZMA (P = 0.08) and IL15 (P = 0.09) tended to be greater, in endometrium from pregnant compared to cyclic heifers. Gal-1 mRNA tended (P = 0.06) to be less in the pregnant endometrium compared to the cyclic endometrium. There was an increase in the endometrial gene expression of GZMA (P = 0.02) and a tendency (P = 0.06) for increase in GATA3 from Day 17 to Day 20 of pregnancy. There were no differences in the concentrations of IFNG, TGFB1, IL6, VEGF or T-bet mRNA. The percentage of area labeled for IFNG protein was less (P = 0.01) in the shallow glands of the pregnant compared to the cyclic endometrium.
Conclusion: Both tolerogenic (IL10) and cytotoxic (GZMA) genes are regulated by conceptus signals during early pregnancy. Increased expression of GZMA and IL15 in pregnant endometrium corresponds to higher percentages of CD8+ T cells and NK cells observed in prior studies. Increased IL10 and GATA3 gene expression may suggest a possible regulatory role for those factors in the endometrium. Thus, during early pregnancy in cattle, conceptus signaling alters cytokine secretion in the endometrium for successful establishment of pregnancy.

G-104

Gardnerella vaginalis: inflammatory impact and strain-dependent variability

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Problem: Inflammation within the uterus during pregnancy can cause preterm birth and can be attributed in part to the actions of decidual

macrophages. *Gardnerella vaginalis* (*G. vaginalis*) is a Gram- variable, rod-shaped bacterium associated with bacterial vaginosis, pelvic inflammatory disease, bacteremia, preterm birth, and is a risk factor for HIV acquisition. Clinical isolates in cases of bacterial vaginosis demonstrate up-regulation in Toll-like Receptor 2 and 4 transcript levels and increased release of IL-1β, which itself is correlated to preterm birth. Further, IL-1β is among a few cytokines that are regulated by the Nod-like receptor family leading to inflammasome formation, Caspase-1 activation, and pyroptosis. As inflammatory characteristics of *G. vaginalis* have not been characterized yet in this model, we studied three closely related ATCC strains of *G. vaginalis* looking for cytokine release, inflammasome recruitment, and cell death.

Method of study: THP-1 cells, THP-1 NLRP3 knockdown cells, THP-1 ASC-YFP expressing cells, and Human Peripheral Blood Monocytic Cells (PBMCs) were used to study the effects of a *G. vaginalis* co-culture. Cytokines were quantified by ELISA, and Nod-like receptors were quantified by FLICA assay and confocal microscopy. Cell death was characterized by Annexin-V assay and western blot.

Results: A significant increase in IL-1β, IL-18, and TNF-α activity was produced by only two *G. vaginalis* strains over untreated samples but was not significantly different compared to LPS/ATP. NLRP3-knockdown caused a decrease in IL-1β release, but did not prevent an increase in IL-1β over control. Ac-YVAD-cmk, a caspase-1 inhibitor, prevented elevation of IL-1β all together. Cell death consistent with pyroptosis was observed in ATCC 14018-treated samples. Finally, visualization of NLRP3 and NLRC4 by confocal microscopy showed NLRP3 and NLRC4 both co-localize with ASC after stimulation with *G. vaginalis* for 12 hr.

Conclusion: *G. vaginalis* exhibits strain-dependent variability in inflammation with ATCC 14018 producing the highest sustained inflammation and a requirement for NLRP3 and caspase-1 leading to IL-1β secretion. This suggests one reason why variability is observed between clinical cases, with some strains being highly problematic and other remaining benign.

G-105

High glucose exposure primes human placental explants to induce an exaggerated inflammatory response to infection

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Problem: Hyperglycemia during pregnancy alters embryonic and feto-placental development leading to increased neonatal morbidity and mortality. Although epidemiologic studies suggest a link between maternal hyperglycemia and preterm labor, the mechanism is unknown. In this study we investigate the effects of hyperglycemia on human placental production of pro- and anti-inflammatory cytokines with or without exposure to infection.

Methods of study: Second trimester and term human placental explant culture model was utilized and lipopolysaccharide (LPS) was used to mimic infection. Explants were pretreated with high glucose (25 mM) or normal glucose (5.5 mM) in DMEM for 24 hr. Explants were then treated with LPS for an additional 18 hr in the presence or absence of human recombinant IL-10. In addition, a subset of placental explants were treated with bacterial organisms (such as *E. coli* and *P. gingivalis*) related to preterm labor. The culture media was collected and analyzed for pro- and anti-inflammatory mediators and caspase-1 p20 subunit using ELISA.

Results: High glucose treatment alone for 24 hr did not change the secretion of inflammatory cytokines in both 2nd trimester and term placental explants. As reported previously, LPS induces IL-1β and IL-10 secretion in a dose-dependent manner. More interestingly, pretreatment with high glucose significantly increased IL-1β and decreased IL-10 secretion with LPS exposure. Similar changes in the cytokine secretion profile were also found in the placentas treated with other organisms. This suggests that high glucose treatment renders a pro-inflammatory milieu in 2nd trimester and term placental explants. Caspase-1 levels were higher in explants pretreated with high glucose, suggesting that increased IL-1β secretion in response to glucose is regulated through the caspase-1 pathway. Exogenous IL-10 reduced the effects of high glucose on the LPS-induced IL-1β secretion.

Conclusion: To our knowledge this is the first report demonstrating that high glucose exposure primes the human placenta to produce an exaggerated inflammatory response after infection. We speculate that longstanding hyperglycemia might contribute to preterm labor by up-regulating IL-1β and down-regulating IL-10, leading to an exaggerated inflammatory reaction which drives the local immune system at the maternal-fetal interface towards anti-fetal responses and labor.

G-106

Immune responses governed by the gas composition in the human vaginal environment

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Problem: Gas composition in the vaginal mucosal environment can affect bacterial colonization patterns and be conversely affected by the growth of vaginal bacteria. The healthy vaginal mucosal environment is low in oxygen but oxygenation that affects the host gene expression levels happens during menstruation. The impact of these changes on the vaginal immune responses is not well understood although intercourse during menses has been associated with higher risk of sexually transmitted infections.

Method of study: Human ectocervical and endocervical epithelial cells were exposed to facultative vaginal anaerobes, e.g. Lactobacillus spp and *Gardnerella vaginalis*, a synthetic TLR2/6 ligand (MALP-2) and tumor necrosis factor-alpha (TNF-α) under controlled aerobic and anaerobic conditions. Cell culture supernatants, were collected at multiple time points for measurement of innate immunity mediators e.g. the chemokine interleukin-8 (IL-8), which is a well-established indicator of vaginal inflammations, using the Meso Scale Discovery electrochemiluninescence multiplex platform. Cell viability was tested by MTT and total protein content was determined by a BCA assay. ANOVA was performed by Prism Graphpad. $P < 0.05$ was considered significant.

Results: Under non-stimulated aerobic and anaerobic conditions extracellular levels reached peaks between 6 and 24 h and were relatively lower under anaerobic conditions. The extracellular chemokine secretion in response to TNF alpha and MALP-2 was blunted under anaerobic conditions as compared to aerobic conditions in both endocervical and ectocervical epithelial cells in the absence of cell toxicity. Lactobacillus caused no significant increase in chemokine levels above the baseline at both aerobic and anaerobic conditions. Different isolates of *G. vaginalis* caused a massive proinflammatory response, which occurred faster under aerobic conditions with some isolates reaching a 100-fold increase within 3 h.

Conclusions: The differences in immune responses mounted under anaerobic versus aerobic conditions may be critical for the vaginal mucosal barrier and may contribute to an enhanced vulnerability to reproductive and sexually transmitted infections and exacerbate the inflammatory squeal to infection during menses.

G-107

Immunosuppression of myeloid derived suppressor cells during early development of endometriosis

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Problem: Suppressed immune surveillance in peritoneal cavity plays an important role in facilitating the immune escape of ectopic endometrial cells in endometriosis. However, the underlying mechanism of the suppressed immune system in endometriosis remains elusive.

Methods of study: The early change of peritoneal immune cells and cytokines/chemokines from experimental endometriosis mouse model was analyzed by flow cytometry and cytokines antibody array. The characteristics and function of the abnormal immune cells and cytokines/chemokines were investigated. Then, the cross talk between immune cells and cytokines was analyzed *in vitro* and *in vivo*.

Results: Most of the peritoneal immune cells decreased after the endometrium transplantation, except myeloid derived suppressor cells (MDSCs). MDSCs were significantly increased in the peritoneal cavity as early as 6 hr and maintained at high level when the ectopic endometrial fragments attached

and proliferated in the following days. Isolated peritoneal MDSCs significantly suppressed T cell proliferation, reactive oxygen species generation and activated arginase activity, suggesting the immunosuppression of MDSCs during early development of endometriosis. Depletion of MDSCs significantly reduced the growth and development of endometriosis. Concurrently, CXCL1, CXCL2 and CXCL5 were significantly increased as early as 6 hr after transplantation. Supplementation of CXCL1, CXCL2 and CXCL5 in control mice induced the accumulation of MDSCs in peripheral blood and peritoneal cavity. Furthermore, the common receptor of these three ligands CXCR2 was expressed by more than 95% MDSCs. Inhibition of CXCR2 significantly attenuated migration of MDSC *in vitro*. This suggested that the recruitment of MDSCs in endometriosis was mediated by CXCR2 and its ligands.

Conclusions: MDSCs promoted the development of endometriosis through similar immunosuppression mechanisms as in cancer. The disruption of MDSCs chemotaxis by targeting CXCR2 may potentially effective for treatment of endometriosis.