ORAL PRESENTATIONS

1

INTERLEUKIN-10 IN PLACENTAL DEVELOPMENT AND LPS TOLERANCE IN PREGNANCY: LESSONS FROM NULL MUTANT MICE. Sarah A. Robertson, Christine A. White, Claire T. Roberts, Alistair J. Ramsay University of Adelaide Obstetrics and Gynaecology, S.A. Medical School, Adelaide, Australia.

Interleukin-10 (IL-10) is an anti-inflammatory and immune-deviating cytokine expressed in the endometrium and placenta. IL-10 is implicated in maternal immune tolerance as well as regulation of trophoblast invasion via inhibiting MMP-9 activity and NO production. We have employed mice with a null mutation in the IL-10 gene to analyse the physiological significance of IL-10 in pregnancy. Immune and inflammatory parameters, implantation success, placental development and reproductive outcome were examined in IL-10 null mutant C57B/66 (IL-10-/-) and control (IL-10+/+) mice. Endometrial inflammation was amplified early in pregnancy in IL-10-/- mice, with a 70% increase in leukocytes in the endometrial stroma at implantation. Despite this, no evidence of abnormal type 1 / type 2 skewing was seen in T-lymphocytes from draining lymph nodes. IL-10-/- females mated with IL-10-/- males had similar or slightly increased numbers of implantation sites compared with pregnant wild-type (IL-10+/+) mice. Similar data were obtained in allogeneic pregnancies. IL-10 deficiency altered placental structure, with a 28% increase in the cross sectional area of the placenta, principally due to an enlarged labyrinth. The proportion of maternal blood space in the labyrinth was increased by 26% and trophoblast surface area was 41% larger. Fetuses gestated in IL-10-/- mothers had an altered growth trajectory, being approximately 10% larger late in gestation and at birth, but with growth impairment evident from early postnatal life into adulthood. In addition, IL-10-/- mice were highly susceptible to bacterial LPS, with higher rates of pregnancy failure and fetal resorption induced after low dose LPS injection on day 10 of pregnancy, accompanied by dramatically increased levels of TNFalpha in serum, uterine and conceptus tissues. This study shows that IL-10 is not essential for maternal immune tolerance or successful pregnancy irrespective of fetal MHC disparity. However, maternal IL-10 deficiency is associated with abnormal uterine inflammatory parameters and decreased resistance to LPS challenge. In the absence of IL-10, structural correlates of placental function are enhanced leading to altered growth trajectory in progeny persisting into adult life. We conclude that IL-10 has a dual role in pregnancy, acting to regulate placental morphogenesis and to modulate placental resistance to inflammatory stimuli.

2

TOLL-LIKE RECEPTORS IN HUMAN ENDOMETRIUM. Steven L. Young, Rebecca L. Jorgensen, Terri D. Lyddon, Michael L. Misfeldt University of Missouri School of Medicine, Columbia, MO.

BACKGROUND: Cytokines are key regulators of endometrial function and dysfunction. Toll-like receptors (TLRs) regulate cytokine expression in immune cells, but endometrial expression and function of TLRs has not been explored.
AIMS: (1) Determine whether human endometrial cells express any of the ten known TLR species (TLR1 – TLR10) or the related molecule, CD14. (2) Determine function of endometrial TLR3. (3) Determine the effects of estradiol and progesterone on TLR3 function.
METHODS: The following human tissues and cells were evaluated for TLR expression by endpoint RT-PCR: endometrium, endometrial stromal and epithelial cells, the endometrial epithelial cell lines, Ishikawa and RL95-2. Immune cell lines, U937, THP-1, and SKW-6.4, were used as controls. Cytometric bead array (CBA) and ELISA were used to quantitate cytokine production.
RESULTS: Whole endometrial samples demonstrated expression of each TLR except TLR7, 8 and 10 which were at the limit of detection. Stromal cells each expressed some of the TLRs expressed in whole endometrium, but neither demonstrated definitive expression of TLR8 nor 10. Each epithelial cell line expressed a distinct subset of the TLRs expressed by endometrial epithelium. TLR3 and TLR9 were detected in RL95-2, but not Ishikawa cells. Functional relevance of RT-PCR findings was tested by measuring cytokine production in response to the TLR4 ligand, lipopolysaccharide (LPS), and the TLR3 ligand, poly(I:C). Poly(I:C) stimulated IL-6 and IL-8 production by TLR3 positive RL95-2 cells, but not by TLR3 negative Ishikawa or U937 cells. LPS stimulated IL-8 production by TLR4 positive U937 cells, but not by TLR4 negative Ishikawa and RL95-2 cells. Treatment of RL95-2 cells with estradiol and progesterone inhibited the poly(I:C) stimulation of IL-6.
CONCLUSIONS: We report the following novel findings: (1) Characterization of TLR expression in endometrium and endometrial cells; (2) Evidence of

TLR3 function in an endometrial cell line; (3) Evidence for reproductive steroid hormone regulation of TLR3 function. The demonstrated expression and function TLRs suggests that endometrial epithelium can detect and respond to a range of ligands associated with infection and cell damage.

3

UTERINE DEFENSE MECHANISMS IN THE MARE. Elaine Denise Watson University of Edinburgh, Roslin, Midlothian.

Endometritis is a major cause of subfertility in mares. Endometritis is a normal physiological event after mating, but if the inflammation persists, the resulting uterine environment is not compatible with establishment of pregnancy. The inflammation is often, but not always accompanied by accumulation of ultrasonically-visible intrauterine fluid. It is now thought that a defect in myometrial contractility may contribute to post-mating endometritis. This impaired myometrial activity may result from a primary muscular defect or from dysfunctional hormonal control of contractility. Not only has evacuation of the uterus shown to be defective in these mares, but sperm transport to the oviduct is also retarded. Persistence of inflammation can occur in these mares in the absence of infection, and the spermatozoa themselves are chemotactic for equine neutrophils, possibly via complement activation.

Post-mating endometritis can develop into chronic uterine infection, or mares can present with a primary complaint of chronic uterine infection. The major pathogens involved in equine uterine infection are b haemolytic streptococci, E coli, and yeasts. In the USA, Pseudomonas aeruginosa and Klebsiella pneumoniae are relatively common isolates, but in the UK they are relatively rare. Of these organisms, S zooepidemicus accounts for around 66% of infections. This organism is a normal commensal of horse skin and is a common contaminant of the uterus after mating. Whether infection is established or not depends on the efficacy of the mare's uterine defense system. Neutrophils are the first line of defense, and although they function suboptimally when placed in inflammatory uterine secretions, when the uterine neutrophils are washed, their function is normal. Although specific antibody titres in uterine secretions appear to be normal in mares infected with S zooepidemicus, the opsonic activity of these antibodies appears to be lower in mares prone to chronic uterine infection than in normal mares. No specific deficiencies have been identified in the uterine cellular immune system in mares with chronic uterine infection, although endometrial macrophage numbers do not increase as much as would be expected, perhaps leading to problems in antigen processing and handling at the uterine level.

4

GENDER AND SEX HORMONES IN THE RESPONSE TO IMMUNOSTIMULATORY CPG OLIGODEOXYNUCLEOTIDES. Daniela Verthely Food and Drug Administration Division of Therapeutic Proteins Bethesda, MD.

The innate immune response is triggered within minutes of infection and serves to limit pathogen spread. Innate immune recognition relies on a limited number of germ-line encoded receptors, such as Toll-like receptors (TLRs), that evolved to recognize conserved molecular patterns of microbial origin (such as lipopolysacharides, proteoglycan, CpG-DNA, dsRNA etc.) that are absent on the host cell. Unmethylated CpG motifs present at high frequency in bacterial but not vertebrate DNA are recognized by Toll-like receptor 9 (TLR 9) expressed by B cells and plasmacytoid dendritic cells (pDC). The interaction of TLR 9 with CpG motifs triggers an immune cascade resulting in improved antigen uptake/presentation by antigen presenting cells and the secretion of polyreactive Ig, chemokines and cytokines by B cells, NK cells, dendritic cells, and monocytes. Recent studies indicate that CpG DNA can act as a potent adjuvant in a vaccine combination or act alone as an anti-microbial agent.

We examine the effects of sex hormones on the in vitro response of mouse spleen cells and human PBMC to CpG ODN and other TLR targets and show that estrogen levels modulate the innate immune response. The impact of gender and sex hormones on the immunoprotective effects of CpG ODN in vivo confirm the role of sex hormones in the innate immune response. The impact of this interaction on infection and autoimmune disease will be discussed.

5

STEROID HORMONE REGULATION OF EMBRYO-UTERINE INTERACTIONS. Indrani C. Bagchi, Yong-Pil Cheon, Quanxi Li, Milan K. Bagchi University of Illinois at Urbana-Champaign Urbana, IL.

Implantation of the embryo to the uterine wall is regulated by a timely interplay of the steroid hormones, progesterone (P) and estrogen (E). The cellular actions of P and E are mediated via its cognate nuclear receptors, which are well-studied gene regulators. It is postulated that hormone-occupied steroid receptors trigger the expression of specific gene networks in the uterus and the products of these genes mediate the

hormonal effects during implantation. The identities of gene networks regulated by the P receptors (PRs) and E receptors (ERs) during implantation, however, remain largely unknown.

Aim of this study is to identify the P- and E-induced genes that are critical regulators of implantation.

MATERIALS AND METHODS: We have utilized high density oligonuleotide microarrays corresponding to 11,000 mouse genes to identify the P and E-regulated genes in the uterus during implantation. To identify the P/PR regulated genes, we employed RU486, a well-characterized PR antagonist which suppresses PR-regulated gene expression in the uterus. To identify the E/ER-regulated genes, we utilized a delayed implantation mouse model in which embryo attachment to uterus is dependent on E administration to the P-primed pregnant animals.

RESULTS: Using the microarray methodology we have isolated a number of genes that are potentially regulated by P and E in the uterus during implantation. The P-regulated genes include growth and transcription factors, enzymes, cell adhesion molecules, protease inhibitors, and molecules involved in immune response. The E-regulated genes can be broadly categorized into three main categories such as transcription factors, regulators of cell cycle and proteases. We have confirmed the steroid receptor regulation of several of these genes using PR and ER null mice and have examined the spatio-temporal expression of these genes in the uterus during early pregnancy. The functional analysis of a subset of P- and E- regulated genes is currently ongoing.

CONCLUSION: We have identified several novel PR- and ER-regulated gene pathways that are operative in the uterus during implantation. We believe that the identification of the steroid-regulated gene networks in the uterus is an important step towards understanding how P and E regulate the physiological events leading to implantation.

6

ROLE OF PROGESTERONE AND POTENTIAL ROLE OF PROSTAGLANDINS IN MODULATING THE UTERINE RESPONSE TO INFECTIOUS BACTERIA IN POSTPARTUM ANIMALS. Gregory S. Lewis USDA, Agricultural Research Service, Dubois, Idaho.

Postpartum uterine infections reduce reproductive efficiency and have significant animal welfare and economic consequences. In dairy animals, the incidence can range from 10 to 70% of the cows in a herd, and uterine infections are not uncommon in other livestock. Even though uterine infections are classified as non-specific, Arcanobacterium pyogenes and Escherichia coli are usually associated with them in cattle and sheep. Pyometra seems to be the most prevalent classification in dairy cattle. Pyometra is detected almost exclusively in cows with active corpora lutea, and induced or spontaneous luteolysis often resolves the infection. Defining the role of ovarian progesterone is, therefore, essential for formulating methods for reducing the incidence and severity of uterine infections. Indeed, ovarian progesterone seems to be the primary regulator of the uterine response to infectious bacteria in cattle, sheep, and pigs. Progesterone also regulates uterine prostanoid synthesis, and prostanoids can regulate immune cell functions in vitro. Thus, we have hypothesized that prostanoids mediate the effects of progesterone and that exogenous PGF2a can initiate events that up regulate immune functions and allow the uterus to resolve an infection, even when progesterone concentrations are increased. Our studies to test that hypothesis have indicated that postpartum cows and sheep, estrual sheep and pigs, ovariectomized sheep and pigs, and seasonally anestrous sheep were resistant to intrauterine infusions of A. pyogenes and E. coli, unless progesterone concentrations were increased. Animals receiving endogenous and(or) exogenous progesterone developed infections after intrauterine A. pyogenes and E. coli infusions. In sheep and pigs, exogenous PGF2a stimulated uterine production of PGF2a and allowed the uterus to resolve A. pyogenes-E. coli-induced infections, even when progesterone was maintained at luteal phase concentrations before and after treatment. Exogenous PGF2a enhanced unstimulated, lipopolysaccharide-stimulated, and concanavalin A-stimulated lymphocyte proliferation, regardless of progesterone concentrations. Using our induced infection models, we are now conducting studies to determine the effects of exogenous PGF2a on various cellular and molecular measures of uterine immunity, with the goal of developing methods for enhancing host immunity at strategic times postpartum to reduce the incidence and severity of uterine infections in livestock and reduce the unnecessary use of antibiotics.

7

NEUROHORMONAL REGULATION OF IMPLANTATION AND PLACENTATION. Errol R. Norwitz, Joong Shin Park, Ursula B. Kaiser Harvard Medical School, Boston, MA.

Successful implantation and placentation is the end result of complex molecular interactions between the hormonally primed uterus and a mature blastocyst. Multiple signals synchronize uterine receptivity. For example, implantation requires a preovulatory surge in

estradiol-17B, which stimulates proliferation and differentiation of uterine epithelial cells. Downstream effectors of steroid hormone actions include peptide hormones, growth factors, and cytokines. The blastocyst actively participates in implantation. Evidence of signaling between the blastocyst and uterus comes from mice in which implantation is delayed indefinitely by hormonal manipulation.

Adequate production of hCG by syncytiotrophoblast – and thereby maintenance of progesterone production by the corpus luteum – is critical to the survival of early pregnancy. Although the regulation of hCG is poorly understood, there is evidence that GnRH may be involved, acting via its heptahelical, G-protein coupled receptor, the GnRH receptor (GnRHR). Recent studies have identified a second receptor for GnRH (GnRHR-II), which binds and is activated by a structurally related ligand, GnRH-II. Recent data have shown that placental tissues bind GnRH-II with a greater affinity than GnRH-I. We have identified mRNA for GnRHR-II as well as GnRHR-I and their cognate ligands (GnRH-I and GnRH-II) in placental tissues throughout gestation. We are currently investigating the hypothesis that activation of GnRHR-II by its cognate ligand, GnRH-II, may maintain placental hCG production and thereby progesterone synthesis by the corpus luteum.

Progesterone receptor antagonists readily induce abortion before 7 weeks' gestation. Similarly, early surgical removal of the corpus luteum results in pregnancy loss. These data suggest that adequate progesterone production by the corpus luteum is critical to early pregnancy success. The mode of action of progesterone is not well understood, but appears to be partially independent of interaction with either progesterone or glucocorticoid receptors. We have shown that progesterone inhibits decidual prostaglandin production by upregulating secretory component of immunoglobulin A, an endogenous inhibitor of phospholipase A2 activity. In summary, normal implantation and placentation is critical for successful pregnancy. A better understanding of the molecular mechanisms responsible for these processes will improve clinicians' ability to treat disorders that occur along this continuum, including infertility, recurrent pregnancy loss, and preeclampsia.

8

TH1/TH2 BALANCE AT IMPLANTATION SITE IN HUMAN. Shigeru Saito Department of Obstetrics and Gynecology Toyama Medical and Pharmaceutical University, Toyama 930-0194, Japan.

Successful human pregnancy seems to be an immunological paradox. One of the important mechanism involves the down regulation of the cellular immune response, which has been shown to be dependent upon the suppression of Th1-type cells. However, localization of Th2 and Tc2 cells in decidua has not been reported, because of the difficulty in detecting intracellular cytokines in tissue. We have developed a novel method to detect Th2 and Tc2 cells by staining a chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). Interestingly, Th2 and Tc2 cells did not localize equally in decidua. Th2 and Tc2 cells are significantly increased at implantation site compared with those far from the implantation site. It is likely that Th2 and Tc2 cells migrate into the materno-fetal interface by attraction of chemotactic factors specific for Th2 and Tc2 cells such as TARC and prostaglandin (PG) D2. Indeed, trophoblast, endometrial gland cells and uterine epithelium all express TARC and hematopoietic PGD2 synthase (hPGDS) supporting this idea. If accumulation of Th2 and Tc2 cells at implantation site is one of the important mechanism for maintenance of pregnancy, Th1/Th2 balance might be disturbed in unexplained recurrent spontaneous abortion (URSA) of normal embryos. Our study showed that neither Th2 nor Tc2 cells accumulated in the decidua basalis in URSA with normal embryo. On the other hand, accumulation of Th2 cells was present in the decidua basalis in URSA with abnormal chromosomal content. The number and percentage of Th2 and Tc2 cells in the decidua parietalis were similar in normal pregnancy and URSA with normal embryo and with abnormal chromosal content. On the other hand, numbers of endometrial Th2 and Tc2 cells during peri-implantation period in URSA patients resembled those in control. Our data suggest accumulation of Th2 and Tc2 cells at implantation site occurs after pregnancy and this accumulation is important for successful pregnancy.

9

REPEATED PREGNANCY WASTAGE. Zeev Blumenfeld Technion-Faculty of Medicine, Haifa, Israel.

The enigma of repeated pregnancy wastage [RPW] has preoccupied obstetricians, immunologists, endocrinologists, hematologists, embryologists, geneticians,and many other disciplines due to the discrepance between a plethora of theoretic information, on one hand, and the remaining of more than half of the cases of an unknown etiology, on the other.The possible described autoimmune mechanisms for RPW include the APLA, ANA, ACL, LAC, A-DNA, and antibodies against thyroid, sperm, ovary, beta-GP-1, phosphatidyl-serine, -ethanolamine, -glycerol, and other APLA. An

attractive recent hypothesis suggest annexin-V as a possible target of the APLA.Thus the endogenous antithrombotic effect of annexin-V, at the endothelial-trophoblast interface is compromised by the APLA or other autoantibodies leading to placental thrombosis and coagulation. The thrombophilic factors associated with RPW are: FV-Leiden, AT-III, PT-G20210A, MTHFR-mutations and proteins C, S, & Z deficiencies. The genetic etiology of parental balanced translocations and fetal dyskaryosis have been also suggested.The in-vitro toxic effects of sera from patients experiencing RPW on placental explants and on mice embrya has added another tool for the study of habitual abortions. The suggested treatment of RPW also lacks a consensus. Since placental thrombosis may be the final common pathophysiologic pathway, in most women experiencing RPW, prophylactic antithrombotic therapy is indicated in patients with heritable thrombophilia and APLS and is probably more effective than the previously used modalities. Whereas previous treatments favourized glucocorticoids with or without low-dose aspirin, more recently, the pendulum has moved towards low-molecular weight heparin with or without aspirin. The immunization of patients with spouse leukocytes and iv-gamma globulin remain equivocal in respect of their scientific rationale and their clinical efficiency. Whereas many of the recognized thrombophilic factors and mutations were discovered in the last decade, it is probable that additional mutations or mechanisms associated with RPW/thrombosis may be diagnosed in the following years to come. Thus, an unanswerred question is whether patients with RPW may benefit empirical anticoagulant treatment, in their next gestation, or restrict treatment only to unequivocally diagnosed thrombophilic and proven autoimmune causes.Due to the complicated multifactorial, and emotional character of RPW, carefully designed prospective randomized studies are mandatory for providing answers to these practical questions.

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CONTROLLED DELIVERY SYSTEMS FOR MUCOSAL VACCINES IN THE REPRODUCTIVE SYSTEM. W. Mark Saltzman, Hong Shen, Michael Dutt Biomedical Engineering, Yale University.

The development and distribution of vaccine technology is perhaps the greatest advance in modern medicine. But we still suffer from high rates of occurrence of certain diseases, particularly sexually transmitted diseases (STD), which can be fatal. Better technology for disease prevention is urgently needed [1]. Immunization typically involves the injection of vaccine proteins, which stimulate an immune response through their action on the ensemble of cells and molecules that comprise the immune system. Most commonly, a local immunization produces a biological response throughout an organism; a successful immune response must be distributed throughout the organism and overcome the well-known barriers to transport of large molecules (such as antibodies) and cells through tissues. As an alternative, we developed methods for passive immunization by continuous delivery of pre-formed antibodies to spatially-defined regions of the body. We tested the local, passive immunization concept by inserting polymer rings containing antibodies into the lower reproductive tracts of female mice. Small polymer rings released active antibody for 30 days; antibodies released from the polymer were able to diffuse freely through unstirred layers of mucus and penetrated locally into epithelial tissue. Using antibodies directed against herpes virus, we have demonstrated that this approach can protect mice from genital herpes infections [2]. Extensions of this approach have been used to produce vaccination strategies that are orally active. Our work has identified two areas of opportunity for biomedical engineers: design of new materials for vaccine delivery and identification of the mechanisms of transport of immune molecules and cells. Our current work is exploring the influence of local vaccinogen release on mucosal immunity [3].

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CLINICAL PROTEOMICS: APPLICATIONS AT THE BEDSIDE. Emanuel Frank Petricoin FDA, Bethesda, MD

The field of molecular medicine is moving beyond genomics to proteomics. While DNA is the information archive, proteins do all the work of the cell. The challenge and opportunity within proteomics is much more than just developing a list of all the proteins. The true scientific goal of proteomics is to characterize the information flow within the cell and the organism. This information flow is mediated through and by protein

pathways and networks. The cause of most human disease lies in these functional derangement of protein-protein interactions. Understanding the role that protein networks play in disease will create enormous clinical opportunities, since these pathways represent the drug targets of the next decade. In the future, entire cellular networks, not just one disregulated protein, will be the targets of therapeutics. The next technologic leap will be the application of proteomic technologies to the bedside. It will soon be possible to analyze the state of protein signal pathways in the disease-altered cells, before, during, and after therapy. This can herald the advent of true patient-tailored therapy.

Our program is focused on the understanding of mechanisms of carcinogenesis, identification of new drug targets, and discovery of new biomarkers for early detection in actual human tissue tumor specimens. Tissue-based proteomics requires technology that can overcome the complex cellular heterogeneity one encounters when studying disease in tissue specimens. To that end, we employ the use of Laser Capture Micro- dissection (LCM) for the proteomic analysis of microdissected subpopulations of human solid tumors (prostate, breast, ovary, and esophageal) and human serum specimens for biomarker discovery, therapeutic monitoring and new drug target discovery. These studies encompass and employ:

a. High-throughput serum proteomic pattern diagnostics using artificial-intelligence based datamining tools coupled with high resolution mass spectrometry for ovarian, breast, prostate, colon, lung and pancreatic cancer early detection
b. Focused proteomic approaches through the use of a new type of protein microarray developed in our program. We are employing this array for multiplexed phospho-specific signal pathway profiling using biopsy of patients before, during and after molecular targeted therapy where dozens of signaling events can be analyzed at once.

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WHY DO TROPHOBLAST ANTIGENS MODULATE LYMPHOCYTE PROLIFERATION?. David Bainbridge, Jodi Taylor, Shirley Ellis, Ian Sargent University of Cambridge, Cambridge, Cambridgeshire.

For some years it has been suggested that patterns of mammalian trophoblast antigen expression may alter maternal immune responses to promote acceptance of the semi-allogeneic fetus. We hypothesise that HLA-G is a candidate for such an immunomodulatory antigen in humans – it is an intriguingly unusual MHC class I molecule, expressed primarily on human trophoblast cells, and which exhibits almost no genetic polymorphism. Indeed, it has previously been demonstrated that HLA-G may suppress natural killer and cytotoxic T-cell responses. The aim of these experiments was to investigate the effects of HLA-G on T-lymphocyte proliferative responses. The MHC class II-bearing human B-cell line C1R was stably transfected with full-length MHC class I cDNA, and irradiated for use as stimulator of a one-way mixed lymphocyte reaction (MLR). The presence of HLA-G, but not the classical class I molecule HLA-A2, on the surface of stimulator cells markedly suppressed thymidine incorporation by peripheral blood mononuclear responder cells from a class I-similar, class II-dissimilar male subject. HLA-G did not, however, alter the relative populations of CD4+ and CD8+ T-cells generated by MLR-stimulation. The suppressive effect of HLA-G on the MLR persisted after depletion of phagocytes and CD8+ T-cells from the responder population, but the MLR was entirely abolished by depletion of CD4+ T-cells, regardless of which stimulators were used. These results suggest that HLA-G exerts a novel direct suppressive effect on CD4+ T-lymphocytes, even in the absence of the CD8+ cells with which other MHC class I molecules interact. Thus, we propose that HLA-G may allow the fetus to escape maternal immune attack by modulating CD4+ T-cell activity. These results will be discussed in the context of other studies, including those into the effects of HLA-G on lymphocyte cytokine production. Also, we have undertaken investigations into the alterations in proliferative responses in pregnant sheep, a species with relatively non-invasive placentation, in an attempt to determine which features of human pregnancy immunobiology are simply epiphenomena of the invasive mode of implantation undertaken by the human conceptus.

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AROMATASE IN ENDOMETRIOSIS WHAT BASIC SCIENCE HAS BROUGHT TO CLINICAL MEDICINE FOR PATIENTS WITH ENDOMETRIOSIS. Serdar Bulun, MD Dept of Ob/Gyn, University of Illinois at Chicago, USA.

Estrogen favors the growth of endometriosis. Treatment of patients with gonadotropin releasing hormone (GnRH) agonists that block ovarian estrogen production provides pain relief only in half of the patients after drug withdrawal. Recent findings from several laboratories regarding local estrogen biosynthesis in endometriosis may be the basis for the lack of response to GnRH agonists in a large number of patients.

Estrogen biosynthesis takes place in three major sites in a woman's body with endometriosis: 1) in the ovaries in cyclic fashion; 2) in the peripheral tissues

such as fat and skin in a continuous fashion; and 3) in the endometriotic implants in a continuous fashion. The identical enzyme, named aromatase, catalyzes the formation of estrone from androstenedione in all three sites. We found that the absence of aromatase expression in the normally located eutopic endometrium and its presence in the diseased endometriotic tissue are mediated by inhibitory transcription factors (e.g., COUP-TF) in endometrium and aberrantly expressed stimulatory transcription factors (e.g., SF-1) in endometriosis. Interestingly, the inflammatory substance prostaglandin (PG) E2 is an extremely potent inducer of aromatase activity in endometriosis. To add a further twist, estradiol stimulates COX-2 and thus the formation of PGE2 in endometriosis. Therefore, a positive feedback cycle involving aromatase and COX-2 favors continuous production of estradiol and PGE2 in endometriosis. The clinical relevance of these laboratory findings was exemplified by the successful use of an aromatase inhibitor to treat postmenopausal woman with persistent endometriosis after hysterectomy and oophorectomy, loss of kidney function due to ureteral endometriosis and resistance to all other treatments.

In summary, others and we uncovered a number of aberrant pathways in endometriosis compared with its disease-free counterpart eutopic endometrium. Aberrant aromatase expression in endometriosis (but not in normal eutopic endometrium) gives rise to increased local estradiol concentrations in this tissue. High concentrations of estradiol in turn stimulate the growth of endometriotic tissue. Our current research focuses on various aspects of this hypothesis.

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CHLAMYDIAL INFECTIONS OF THE UPPER REPRODUCTIVE TRACT AND THE IMMUNE RESPONSES TO THEM. Morris D. Cooper, Melissa H. Roberts SIU School of Medicine, Springfield, IL.

Chlamydia trachomatis is the most common STD world wide and the annual incidence in the US is in excess of 4 million cases. Serovars D-K are the etiological agents of inclusion conjunctivitis, nongonococcal urethritis, cervicitis, salpingitis, pelvic inflammatory disease and endometritis and infertility. The precise events involved in the attachment of infection of chlamydia to host cells are unresolved. Current theories implicat that glycosaminoglycan (GAG) molecules are involved in attachment of chlamydia to the surface of susceptible host cells. These are negatively charged molecules due to their sulfate and carboxyl groups. The location and number of these groups are used to help classify the molecules. Several serovars of *C. trachomatis* are thought to use a GAG dependent mechanism for attachment to host cells.

MATERIAL AND METHODS: HeLa cell monolayers were used to grow chlamydia and assess the attachment induced by the various GAGs. Chinese Hamster Ovary (CHO) cells which are sufficient in their production of GAGs as well as the mutant CHO cell lines pgsD-677, psgA-745 which are deficient in GAGs were used in these studies. Hec-1B, vaginal, ectocervical and endocervical epithelial, as well as, Fallopian tube epithelial cells were also used. *C. trachomatis* serovars L-2 and E were used in these studies. Cell surface GAGs on the cell lines were quantitated by flow cytometry as were the infectivity studies.

RESULTS: Previously mentioned cells lines were examined for expression of GAGs on their cell surface. The GAG with the highest cell surface concentration was keratan sulfate. Heparan sulfate was found in relatively small concentrations on reproductive tract tissue with the fallopian tube epithelium having the lowest amount of heparan sulfate. Infectivity of cell lines which had been enzymatically treated to remove GAGs, as well as CHO mutant cells indicated that they were infected in a GAG independent manner by serovar E C. trachomatis.

CONCLUSION: *C. trachomatis* serovar E demonstrates multiple mechanisms for attachment and infection of reproductive tract tissues. We conclude that serovar E does not use a GAG dependent mechanism as its primary means of attachment.

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HAEMOSTASIS AND HUMAN REPRODUCTION: BALANCE BRINGS BABIES. Christian J. Thaler, Tina Buchholz University of Munich, Munich, Germany.

Successful pregnancies require an even balance of coagulation and fibrinolysis, in order to secure stabilization of the basal plate as well as adequate placental perfusion. Pregnancy results in profound changes of haemostatic and fibrinolytic systems with a procoagulatory net effect. Under a teleological perspective, this might be an effort of biology, to counteract the significant risk of maternal haemorrhage during pregnancy. On the other hand, the development of thrombotic disorders is an additional maternal threat, representing a major cause of pregnancy-related disorders and harm for the conceptus. A wide spectrum of acquired and inherited conditions affecting haemostasis have been described. Many of them remain asympotomatic until additional boosting factors arise.

Procoagulatory changes during pregnancy increase the thrombotic risk and, while often not noted during early gestation, may be preset as early as during implantation. The differentiation between several factors in the coagulation and the fibrinolytic pathway and their relation to recurrent miscarriages or other pregnancy-related disorders is important, because effective early treatment is available. Furthermore synergistic effects of two or more polymorphisms or mutations may multiply the risk for individuals and lead to an individual risk assessment. Intriguingly some genetic variations with moderate thrombophilic potential are rather common in certain populations and they might indeed provide particular advantages for specific aspects of reproduction.

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PLACENTAL DYSFUNCTION IN PREGNANCY COMPLICATIONS: ALTERATIONS IN REGULATION OF TROPHOBLAST APOPTOSIS?. D. Michael Nelson Washington University School of Medicine, St. Louis, MO.

OBJECTIVE: The trophoblast component of the human placenta mediates invasion into the basal plate in early pregnancy and maternal-fetal exchange of nutrients and wastes in the second and third trimesters. The proliferation, differentiation, and apoptosis of trophoblast are regulated throughout gestation by an orchestrated array of transcriptional mediators, and these endogenous regulators are modulated by numerous exogenous stimuli within the uterine environment. This session will overview the biology of human trophoblast and relate pregnancy complications with placental dysfunction as a result of abnormalities in trophoblast differentiation and apoptosis.
METHODS: Primary cultures were used to examine the differentiation and apoptotic pathways of human trophoblast in response to hypoxia, fibrin matrix and homocysteine. Differentiation was assessed by hormonal analysis of media HCG and confocal microscopic analysis of the formation of syncytiotrophoblast. Apoptosis was quantified using immunohistochemistry, western analysis, cellular ATP levels, and media LDH concentrations.
RESULTS: Work done by others has shown that cytomegalovirus alters apoptosis of trophoblast in the basal plate of early implantation sites. Work done in our lab shows that hypoxia hinders trophoblast differentiation, with an up regulation of cyclooxygenase-2, while enhancing trophoblast apoptosis, with an up regulation of p53. Hypoxia down regulates the system A amino acid transporters and reduces the uptake of amino acids transported by system A. In contrast, a fibrin matrix and ligands for PPARg enhance differentiation and limit apoptosis of trophoblast. Homocysteine-thiolactone, a cell permeable oxidation product of homocysteine, induces apoptosis and up regulates the pro-apoptotic protein Bak.
CONCLUSION: Viral infection, hypoxia, fibrin matrix, and homocysteine are a few of the agents that modify the biology of trophoblast. I speculate that the trophoblast response to these signals in vivo manifests as histopathological lesions indicative of villous injury and reflecting altered trophoblast differentiation and apoptosis. This injury yields placental dysfunction manifested as pregnancy complications such as spontaneous miscarriage, altered fetal growth, or preeclampsia. Understanding the signaling pathways involved with the trophoblast responses to exogenous stimuli will likely identify new therapeutic avenues for modulation of the differentiation and apoptotic pathway in trophoblast to facilitate placental function. NIH HD 29190

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ROLE OF GLUCOCORTICOIDS IN PLACENTAL DYSFUNCTION. Seth Guller NYU School of Medicine, New York, New York.

INTRODUCTION: Intrauterine growth restriction (IUGR) is a leading cause of perinatal mortality and morbidity and is often associated with maternal preeclampsia (PE). Enhanced production of the anti-fibrinolytic compound plasminogen activator inhibitor–1 (PAI-1) by syncytiotrophoblasts (SCTs) is implicated in aberrant periplacental fibrin deposition in these pregnancies. Fibrosis is attributed to over-expression of interstitial collagens (Col I and III) and other extracellular matrix (ECM) proteins by placental mesenchymal cells (PMCs). These changes are suggested to disrupt placental architecture and restrict the flow of nutrients between mother and fetus.
AIM: Since pregnancies associated with IUGR are also characterized by elevated levels of glucocorticoids (GCs) and transforming growth factor (TGF)-β in fetal and/or maternal sera, the aim of the current study was to test the hypothesis that GCs and TGF-β are key regulators of placental PAI-1 and ECM protein expression.
MATERIALS AND METHODS: Levels of PAI-1 were examined in HTR-8/SVneo cells (i.e. an immortalized trophoblast cell line) and primary cultures of cytotrophoblasts (CTs) and SCTs isolated from human term placentas. ECM protein expression was studied using PMCs isolated from human term placentas. Cells were maintained in serum-free medium for 1 to 4 days with and without 10-7 M dexamethasone (DEX) and

1-2 ng/ml TGF-β. PAI-1 and ECM protein gene expression were analyzed using ELISA, Northern blotting, promoter activity, mRNA stability, RNA UV-cross-linking and real-time PCR procedures.
RESULTS: We noted that GCs enhanced PAI-1 expression in TGF-β -treated CTs, SCTs and HTR-8/SVneo cells. The cellular mechanism involved (1) a 4 to 10-fold induction of PAI-1 mRNA (2) a protein intermediate, and (3) a non-transcriptional mechanism resulting in altered PAI-1 mRNA stability and expression of PAI-1 mRNA binding proteins. In addition, we observed that the combined action of GCs and TGF-β enhanced ECM protein and mRNA expression 4 to 20-fold in PMCs.
CONCLUSION: Our results indicated that GCs enhanced levels of PAI-1 in trophoblasts and ECM proteins in PMCs by augmenting the actions of TGF-β. These cell-type specific changes in gene expression may be responsible for pathological changes in placental structure noted in pregnancies with IUGR and PE that compromise fetal health.

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ANTI-HIV EFFECTS OF HCG. C.V. Rao University of Louisville Health Sciences Center, Louisville, KY

hCG is a multifunctional hormone that is capable of inhibiting HIV-1 replication. This property may at least partly explain the lower incidence of vertical HIV-1 transmission from mother to fetus. Consistent with this possibility, urinary purified as well as recombinant hCG, can inhibit the infection of human placental explants (Polliotti et al, 2002, Trophoblast Res. 16: S102-S106). De et al. (1997, J. Clin. Invest. 99: 1484–1491), have developed an HIV transgenic mouse model which mimics human disease to investigate the therapeutic potential of hCG. The homozygous pups appear normal at birth, but they begin to show symptoms of wasting syndrome, which rapidly progress resulting in death within 45 days. The progression of symptoms and death can be prevented by starting hCG α therapy soon after birth. While LH and hCG-β subunit can mimick dimer hCG, hCG- subunit, FSH, TSH, estradiol, progesterone and corticosteroids had no effect. The hCG treatment resulted in an up-regulation of its receptors, which coincided with an elimination of virus from skin keratinocytes which harbor high levels of levels which are α virus. Homogzymous animals have greatly elevated TNF- decreased by hCG treatment (De et al, 2002, J. Virol. 76: 11710–11714). Since the death of animals can also be prevented by immunoneutralization of TNF- TNF activates NF-kB and AP-1, which transactivate genes required for viral replication, hCG effect on TNF induced NF-kB and AP-1 activation was examined in MCF-7 cells used as a model. The results revealed that hCG can inhibit TNF induced NF-kB and AP-1 activation in a dose and time dependent member. The hCG physphorylation and degradation, which are α treatment also inhibited IkB prerequisite for NF-kB activation. These hCG actions can be blocked by dideoxyadenosine and H-89, suggesting that adenyl cyclase and protein kinase A activation are required. In summary, hCG has anti-HIV effects and perhaps they could be exploited to fight AIDS. The hCG is inexpensive to use, nontoxic, has few physiological side effects, if any, and do not harm if for some reason it does not help.

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HIV SHEDDING IN THE FEMALE GENITAL TRACT: BIOLOGIC AND IMMUNOLOGIC CORRELATES. Susan Cu-Uvin The Miriam Hospital/ Brown University, Providence, RI.

BACKGROUND: The initial barrier to female sexual transmission of HIV is the cervico-vaginal epithelium. Direct transmission may be mediated by phagocytic pathway, through Langerhan cells (LC) and dendritic cells (DC) that express CD4 receptors and CCR5 co-receptors, or through DC that express DC-SIGN which carries the virus to regional lymph nodes. There are numerous CD4 T cells, DC, macrophages in the lamina propia to infect. There is evidence that the genital tract is a separate compartment from the blood, that HIV dynamics in the genital tract may not always parallel that of the blood.
METHODS: This discussion will review the current data on the biologic and immunologic correlates of HIV in the genital tract.
RESULTS: Most studies have shown a positive correlation between HIV plasma viral load and genital viral load and a negative correlation with CD4 cell count. However, studies have also reported HIV shedding in the genital tract despite below detectable HIV levels in blood plasma. As in the blood, effective antiretroviral therapy decreases HIV in the genital tract. HIV detection is increased with cervical inflammation, vaginal discharge, presence of STDs, and genital ulcers. Treatment of STDs decreases HIV shedding. It is hypothesized that the release of proinflammatory cytokines (IL 1B, IL6, TNF) and immunoregulatory cytokines (IL10, IL12) modulate cervico-vaginal HIV replication. Hormonal changes during the menstrual cycle may also affect HIV shedding. Cytokines including IL8, RANTES, MIP 1B, TGF B, IL10, IL4, IL6 and IL1 B are elevated in the genital tract during menses in HIV-positive women and is correlated with increased levels of HIV detection

in the genital tract. Anti-HIV-gp120 IgA and IgG titers have been measured in cervicovaginal lavages of HIV-infected women. CD4 count correlated negatively with total IgA and positively with specific IgA activity. Women with BV had 5 fold lower anti-gp120 IgG titer. HIV specific antigen reactive T cells may be detected in cervicovaginal specimens and are less numerous in women on effective antiretroviral therapy.
CONCLUSIONS: Much work needs to be done to understand HIV infection of the genital tract and to further characterize the effects of biologic and immunologic correlates on HIV shedding.

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LISTERIA MONOCYTOGENES AS A PROBE FOR IMMUNE FUNCTION IN THE PLACENTA. Ellen M. Barber, Ari M. Mackler, Jeffrey W. Pollard Albert Einstein College of Medicine, Bronx, NY.

The interface between the mother and fetus poses an immunological conundrum: How does an infection get resolved without the loss of the allogenic fetus? This conundrum suggests that there must be strong local immunomodulation to activate the immune system to repel an invading organism while at the same time suppressing cells, particularly T-cells, that might reject the fetus. To explore this question we have infected mice with Listeria monocytogenes a Gram-positive intracellular facultative bacterium that has a predilection for replication at the maternal-fetal interface. In these studies, we have revealed a chronology of the immune response in the placenta. In the first phase the growth factor, Colony Stimulating Factor-1 (CSF-1), stimulates trophoblastic cells to synthesize mouse IL-8 homologues that in turn recruit neutrophils to the site of infection in the decidua basalis. These cells resolve the majority of the infection. In the second phase, the Th-1 cytokines TNFa and IL-12 are produced that in turn, appear to induce IFNg. TNFa and IFNg are both essential for the final resolution of the placental infection because in their absence the bacterium eventually gains hold and replicates profusely.

Surprisingly, the abundant population of uterine NK cells is not required for either the production or response to these Th-1 cytokines. Instead, IFNg induces the enzyme indoleamine 2, 3, dioxygenase (IDO) in decidual stromal and endothelial cells. We hypothesize that IDO acts to limit Listerial infection through its well-established role in inhibiting bacterial replication while at the same time suppressing the activation of T-cells in the placenta that might be cytotoxic to the fetus. These date help explain the paradox of pathogen rejection but fetal survival.

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SHARED EPITHELIAL TUMOR ANTIGENS FOR ACTIVE AND PASSIVE IMMUNOTHERAPY OF BEAST CANCER. Olivera J. Finn University of Pittsburgh, Pittsburgh, PA.

Molecular characterization and isolation of tumor antigens that are potentially immunogenic in humans is an important goal of tumor immunologists. The incentive is the eventual ability to use purified antigens, preferably those shared among many different tumors, to create effective cancer vaccines or as targets for immunotherapy. There are several well-studied epithelial tumor antigens that are being tested in animal models and in clinical trials. One such antigen is the MUC1 mucin. Expressed on normal ductal epithelial cells as a lumenal, highly O-glycosylated molecule, composed almost exclusively of 20aa tandem repeats, this molecule undergoes changes in glycosylation on tumor cells and assumes a new 3-D structure. Circulating, soluble MUC1 produced by tumor cells in vivo cannot be processed and presented by patient's APC, resulting in the lack of MUC1-specific T helper cells, low frequency of CTL and low titers of IgM. A synthetic form of MUC1 that can be introduced through vaccination can be processed by DC and elicits MUC1-specific helper T cells, higher frequency of CTL and multiple IgG antibody isotypes. Based on these properties, MUC1 has been tested as a vaccine in animal models and in cancer patients in phase I clinical trials. Furthermore, T cell receptor specific for an epitope on the native MUC1 molecule on the surface of tumor cells has been cloned and used for tumor therapy in a combination of gene therapy/immunotherapy protocol in the setting of bone marrow transplantation.

The second shared antigen, candidate for cancer vaccines and immunotherapy is Cyclin B1 that our group recently described as a target of tumor-specific T cell responses. Tumors that aberrantly express Cyclin B1 all have functionally inactive p53. Inactivation of p53 function and resulting overexpression of Cyclin B1 are very early changes in the transformation process in many breast cancers as well as premalignant lesions. This suggests the possibility of using a vaccine to eliminate these lesions and prevent their transformation to cancer.

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DEVELOPMENT OF A CONTRACEPTIVE VACCINE TARGETING SPERM. Rajesh K. Naz Medical College of Ohio, Toledo, Ohio.

Development of a vaccine(s) targeting sperm antigens represents a promising approach to contraception. The

utility of an antigen in immunocontraception is contingent upon its sperm/testis-specificity and involvement in the fertilization process. Using hybridoma technology, subtractive hybridization technique, and differential display technology, our laboratory has delineated several sperm antigens. These antigens have testis-specific expression and have a role in fertilization. Among these, the fertilization antigen (FA-1), contraceptive vaccinogen (CV) and testis-specific antigen (TSA) are particularly interesting. Recently, using the FliTrx phage peptide display library, a novel dodecamer sequence, designated as YLP12 peptide, that has testis-specific expression and is involved in human sperm-ZP recognition/binding, was identified. A synthetic 12-mer peptide was generated based on this sequence. In the hemizona assay, YLP12 peptide and its monovalent Fab antibodies specifically and significantly inhibited human sperm- human ZP binding. Furthermore, the presence of specific antibodies reactive with YLP12 peptide were identified in the serum and seminal plasma of immunoinfertile but not fertile men. A vaccine was prepared by conjugating the synthetic YLP12 peptide with the binding subunit of recombinant cholera toxin. Vaccination of female mice by i.m. or intranasal routes without any additional adjuvant induced high titer antibody response in serum and vaginal tract that caused a long-term contraceptive state. Antibodies raised after vaccination were testis/sperm- specific. Fertility was fully regained when antibody reactivity disappeared at 305–322 days. The contraceptive effect could also be reversed voluntarily by immunoneutralization of antibodies via intravaginal administration of the peptide. Antibodies affected fertility at the prefertilization stage by inhibiting sperm capacitation/acrosome reaction, and sperm-oocyte binding. The peptide sequence is an epitope of a 50 ± 5-kDa membrane protein localized on the acrosome and tail regions of spermatozoa. Thus, the sperm-specific YLP12 peptide, along with other sperm-specific antigens such as FA-1 antigen, is an attractive candidate for contraceptive vaccine development. (Supported by NIH grant HD 24425 to R.K.N.).

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TROPHOBLAST CELLS SECRETE AN ACTIVE FORM OF FASL: NEW INSIGHTS INTO IMPLANTATION. Vikki M. Abrahams, Shawn L. Straszewski, Gil Mor Yale University School of Medicine Obstetrics and Gynecology New Haven, CT.

BACKGROUND: Fas ligand (FasL) is expressed by trophoblast cells throughout pregnancy and is thought to protect the placenta from immune rejection by inducing Fas-mediated apoptosis in maternal immune cells. However, the role of FasL on immune privilege has been challenged by studies showing that surface FasL triggers rejection rather than protection. We have recently identified an active form of FasL, which is secreted via microvesicles from first trimester trophoblast cells. We propose that such release of FasL may represent the mechanism for the induction of immune privilege.
METHODS: First trimester trophoblast cells were isolated from normal placentas obtained from elective pregnancy terminations. Microvesicles were isolated from the supernatants of first trimester trophoblasts cultured in vitro. FasL expression was characterized by Immunocytochemistry (ICC) and Western blot. Bioactivity of the microvesicle-associated FasL was assessed by a cell viability assay, flow cytometry and Western blot using the Fas-expressing Jurkat T cell line as a target.
RESULTS: ICC of first trimester trophoblast primary cultures and cell lines revealed FasL to be localized to the cell cytoplasm. Confocal microscopy showed association of the intracellular FasL with the lysosomal marker, Lamp-1. Western blot analysis demonstrated a 37kDa protein, corresponding to whole FasL, in microvesicles isolated from trophoblast culture supernatants. Treatment of Jurkat cells with an agonistic anti-Fas mAb significantly reduced cell viability ($17.28 \pm 1.11\%$, $p < 0.0001$). FasL released from these microvesicles induced a significant reduction in Jurkat cell viability ($13.5 \pm 1.1\%$, $p < 0.0001$). Flow cytometry, using propridium iodide and Hoechst dye, confirmed that the reduction in Jurkat cell viability, following treatment with the secreted FasL was a result of the induction of apoptosis. Western blot analysis of caspase activation confirmed the apoptotic signal to be Fas-mediated.
CONCLUSION: We demonstrate, for the first time, that FasL expressed by first trimester trophoblast cells is localized to secretory lysosomes and is released via microvesicles. Furthermore, this secreted FasL is able to induce Fas-mediated apoptosis in T cells. We propose that secreted FasL provides a mechanism whereby trophoblast cells can establish immune protection through the elimination of trophoblast-specific Fas-expressing maternal immune cells.

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DECIDUAL NATURAL KILLER CELLS AND PREGNANCY LOSS IN IL-10 DEFICIENT MICE. Shaun Murphy*[1], Loren Fast[2], Nazeeh Hanna[3], and Surendra Sharma[1] [1]Departments of Pediatrics and Pathology, Women and Infants Hospital-Brown University, Providence, RI; [2]Department of Medicine, Rhode Island Hospital-Brown University, Providence,

RI; [3]Department of Pediatrics, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ

PROBLEM: IL-10 is thought to play a role in fetal immunoprotection and development. In order to directly investigate the role of IL-10 in pregnancy, matings between mice genetically deficient in IL-10 were performed. As the predominant immune cell type in the decidua are NK cells, their proportional presence and functional activity were evaluated in IL-10 deficient and congenic wild type mice.
METHODS: IL-10-deficient mice as well as congenic wild type controls were mated allogeneically or syngeneically and screened for copulation plug to determine day 0 or pregnancy. Implantation success was confirmed by i.v. injection of pontamine blue dye to visualize blastocyst implantation sites. Pregnancy success was assessed in terms of litter size. Additionally, decidual mononuclear cells were isolated from animals at gestational days 8-11, and NK cell proportions and cytotlytic activity were assessed.
RESULTS: Matings of virgin IL-10-deficient mice result in compromised pregnancy outcome as compared to wild type mice. This compromised pregnancy outcome does not appear to result from failed implantation as assessed by both dye injection and detection of decidual NK cells. Decidual, but not splenic, NK cell proportions from IL-10-deficient mice were approximately 50% that of wild type and displayed much greater cytotoxicity.
CONCLUSIONS: The increased cytotoxicity of decidual NK cells in IL-10-deficient animals may represent a mechanism for primigravid pregnancy compromise. To further elucidate this potential role, antibody depletion of NK cells prior to and during matings will be performed. It is speculated that subsequent matings in IL-10 deficient mice may result in successful pregnancy outcome due to acquired immune tolerance and/or normal recruitment of decidual NK cells.

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ACTIONS OF TUMOR NECROSIS FACTOR-α ON OOCYTE MATURATION, EMBRYONIC DEVELOPMENT AND APOPTOSIS IN BOVINE PREIMPLANTATION EMBRYOS. Paolete Soto, Roger P. Natzke, Peter J. Hansen University of Florida Department of Animal Sciences Gainesville, FL.

Infertility can accompany mastitis in cattle. Involvement of tumor necrosis factor-α (TNF-α) in this phenomenon is suggested by observations that circulating concentrations of TNF-α are elevated after intramammary infection or infusion of endotoxin. Here, it was hypothesized that 1) TNF-α acts on the oocyte during maturation to decrease the percent of oocytes that cleave and develop following fertilization; 2) exposure of embryos to TNF-α after fertilization reduces development to the blastocyst stage; and 3) TNF-α increases the proportion of blastomeres that undergoes apoptosis in a stage-of-development dependent manner. In experiment 1, oocytes were matured with various concentrations of TNF-α and then fertilized and cultured without TNF-α. Addition of TNF-α to maturation medium did not affect the proportion of oocytes that cleaved. However, the percent of oocytes that developed to the blastocyst stage at day 8 after insemination was reduced ($P = 0.05$) at all TNF-α concentrations (20, 13, 12, 15 and 13% for 0, 0.1, 1, 10, and 100 ng/ml TNF-α). In experiment 2, embryos were cultured with TNF-α for 8 days beginning after fertilization. There was no significant effect of TNF-α on the proportion of oocytes that became blastocysts. In experiment 3, embryos were collected at the 2 or 4-cell stage (at 28–30 hours after insemination) or when >8 cells (at day 4 after insemination) and cultured $+/-$ TNF-α for 24 h. For 2- and 4-cell embryos, there was no effect of TNF-α on the percentage of cells labeled with the TUNEL procedure. For embryos >8 cells, 10 and 100 ng/ml TNF-α increased ($P < 0.05$) the percent of blastomeres labeling as TUNEL-positive (12, 12, 11, 27, and 30% for 0, 0.1, 1, 10 and 100 ng/ml TNF-α). In conclusion, TNF-α can have deleterious actions on oocyte maturation that compromise development of the resultant embryo. While exposure of fertilized embryos to TNF α did not inhibit development to the blastocyst stage, TNF-α increased the percentage of blastomeres undergoing apoptosis when exposure occurred for embryos >8 cells. The implications of increased blastomere apoptosis for embryo survival in utero needs to be determined. (USDA 2002-35203-12664).

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DIRECT AND STROMAL MEDIATED EFFECTS OF ESTRADIOL ON TRANSEPITHELIAL RESISTANCE (TER) AND MOUSE UTERINE EPITHELIAL CELL CYTOKINE RELEASE IN CULTURE. Catherine Sarah Grant, Charles R. Wira Dartmouth Medical School Physiology Lebanon, NH.

PROBLEM: Estradiol-17b (E2), acting via the estrogen receptor (ER), stimulates uterine epithelial cell proliferation and is critical for normal uterine morphogenesis, differentiation, and secretory function. E2 can act directly on the epithelium via epithelial ER or indirectly via the ER-positive underlying stroma. A primary role for epithelial-stromal interactions has been established for mediating steroid hormone action

in the uterus. The current study was undertaken to determine the mode of E2 action in regulating epithelial cell monolayer integrity and cytokine release in the uterus.
METHOD OF STUDY: Mouse uterine epithelial and stromal cells were isolated and cultured separately. E2 and ICI 182,780, a pure estrogen antagonist, were dissolved in ethanol, evaporated to dryness, and re-suspended in the appropriate concentration in medium. After epithelial cells reached confluence on cell culture inserts, cells were fed with media containing $1 \times 10\text{-}8M$ E2 or $1 \times 10\text{-}8M$ E2 + ICI 182,780. In addition, epithelial cells were also co-cultured with stromal cells and treated with E2. Supernatants collected were assayed for TGFb and TNFa by bioassay and ELISA, respectively. TER was monitored with an EVOM voltohmmeter.
RESULTS: E2 treatment of epithelial cells led to a significant decrease in TER. This effect was reversed by the addition of ICI 182,780. In contrast, the amount of TNFa released when epithelial cells were treated with E2 was not affected. However, when epithelial cells were co-cultured with stromal cells and treated with E2, apical TNFa release decreased significantly, compared to control cells in the absence of hormone. TGFb release by epithelial cells was not affected by E2 when grown alone or in the presence of stromal cells.
CONCLUSIONS: These studies demonstrate that E2 has both direct and indirect effects on uterine epithelium. Whereas epithelial monolayer integrity is directly influenced by E2 and ER mediated, E2 effects on TNFa release requires the presence of stromal cells, indicating paracrine communication is necessary for TNFa hormone responsiveness. Greater knowledge of E2 action and epithelial-stromal interactions in the uterus is necessary to develop a better understanding of the mucosal immune system in the female reproductive tract. Supported by AI-13541 from NIH.

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ANTICOAGULANT HEPARAN SULFATE PROTEOGLYCANS IN MAMMALIAN REPRODUCTIVE FUNCTION. Ji-Cui Dong, Marc Princivalle, Nicholas Shworak, Didier Chardonnens, Ariane I. De Agostini Geneva University Hospital Geneva, Switzerland 1205.

Antithrombin III-binding heparan sulfate proteoglycans (aHSPG) possess the canonical 3-O-sulfated pentasaccharide sequence responsible of the anticoagulant activity of heparin. In addition to the vascular endothelium, aHSPGs are also present in discrete extravascular compartments, and we have shown that rat ovarian granulosa cells synthesize aHSPGs under hormonal control by gonadotrophins, with a maximal expression in the preovulatory stage. In the follicle, aHSPGs co-localize with serine protease inhibitors of the serpin family, antithrombin III (AT) and protease nexin-1, providing a potent thrombin inhibition potential. Injection of variant inactive 393Cys-AT in the rat ovarian bursa decreased ovulation efficiency and increased fibrin deposition in luteinizing follicles. These data suggest that aHSPGs play an important role in ovarian physiology.

Further studies have shown that aHSPGs are expressed in additional compartments of the murine genital tract, mainly associated with epithelial basement membranes. The function of aHSPGs in this locale has been examined in knockout mice deficient in 3-O-sulfotransferase-1 (3-OST-1), the key biosynthetic enzyme of aHSPGs. 3-OST-1 null mice have strongly decreased amounts of aHSPG in their genital tracts, and their fertility is severely impaired. Analysis of these mice has shown the appearance of multiple reproductive phenotypes both in males and females. The mechanism underlying these phenotypes is currently under scrutiny in our laboratory.

Taken together, the data cu.

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THYROID IMMUNITY, THYROID DYSFUNCTION AND RECURRENT ABORTION: DIAGNOSTIC AND THERAPEUTICAL APPROACH. Natalia Lazzarin, Elena Vaquero, Francesco Altomare, Herbert Valensise, Domenico Arduini "Tor Vergata" University Obstetrics and Gynecology Viale Vigna Pia Rome, Italy.

AIM OF THE STUDY: The aim of this study focuses on evaluating the role of mild thyroid abnormalities, such as the presence of thyroid antibodies and subclinical underlying hypothyroidism, on recurrent aborters and to assess the effect of two different therapeutical protocols on these patients.
METHOD: A prospective study in patients with recurrent spontaneous abortion (RSA) associated to mild thyroid abnormalities evaluating obstetric outcome in 53 patients. Twenty seven, thyroid antibodies positive, patients were treated with thyroid replacement therapy, while 11 patients received intravenous immunoglobulins (IVIG). Moreover 15 patients characterized by negative thyroid antibodies and having underlying thyroid pathology, were treated with thyroid replacement therapy.
RESULTS: Among patients with thyroid antibodies 6 out of the 11 pregnancies (54.5%) treated with IVIG ended in live births. This live birth rate was significantly lower with respect to that observed among

patients treated with thyroid supplementation. In this group 23 out of 27 (85%) gestations ended in live birth. Finally only 1 pregnancy loss occurred among patients with mild underlying thyroid pathology treated with thyroid replacement therapy.
CONCLUSIONS: Mild thyroid abnormalities are associated with an increased rate of miscarriage. Thyroid immunity seems to reveal an underlying, functional thyroid disorders rather than a more generalized abnormal stimulation of the immune system. The poor obstetrical prognosis seems to be related to an impaired thyroid adaptation to pregnancy. Thyroid replacement therapy result to be more effective than IVIG in preventing a new miscarriage.

rrently available suggest that aHSPGs could serve to modulate protease inhibition by heparin-binding serpin inhibitors in the reproductive tract, and thus be involved in the control of blood clotting, fibrin deposition, and tissue remodelling.

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EXPRESSION OF THE ANGIOTENSIN RECEPTORS AND NITRIC OXYDE SYNTHASE IN PREGNANT MICE WITH PE-LIKE SYMPTOMS. Ana Claudia Zenclussen, Ricarda Alcira Joachim, Stefan Fest, Mareike Tometten, Burghard Klapp, Petra Clara Arck Charité Biomedizinisches Forschungszentrum Berlin.

We recently reported a mouse model for pre-eclampsia (PE), in which increased blood pressure, proteinuria and abnormal pregnancies were observed after adoptive transfer of activated Th1-like cells into pregnant mice. The aim of the present study was to investigate if the PE symptoms were accompanied by abnormal expression of molecules closely related to the regulation of blood pressure, i.e. Nitric Oxide Synthase Enzymes (eNOS and iNOS) and angiotensin receptors (AT1 and AT2). We analyzed the expression of eNOS and iNOS in the kidneys of (1) PBS-treated non-pregnant mice, $n = 10$, (2) Th1-cell-treated non pregnant mice, $n = 10$, (3) PBS-treated pregnant mice, $n = 6$ and (4) pregnant mice which received Th1 cells and showed PE-like symptoms, $n = 8$ and at the feto-maternal interface of all pregnant animals using immunohistochemistry (IHC). We further used IHC to detect AT1 and AT2 at the kidneys of mice from all groups.

We observed increased expression of eNOS, iNOS and AT1 in the kidneys of PE mice (4) compared to control pregnant mice (3) or to non-pregnant mice (1 or 2). No differences in the expression of eNOS, iNOS or AT1 could be observed between PBS-treated and cell treated non-pregnant mice (1 vs. 2). No changes could be observed for the expression of AT2 in the kidneys of any group. The expression of eNOS was augmented at the placenta of PE mice compared to control pregnant mice (3), whereas no differences in the expression of iNOS could be observed between both groups. In decidua cells the expression of eNOS and iNOS was comparable in both groups (3 and 4).

NOS regulate the synthesis of NO, a mediator of blood pressure and parturition. NOS expression is increased in patients with PE. ATs mediate the effect of the vasoconstrictor angiotensin II, and particulary the AT1 receptor appears to be a candidate involved in the pathogenesis of PE. In the present work, we observed increased expression of eNOS and AT1 in the kidneys of pregnant mice with PE, compared to control pregnant mice and to non-pregnant mice. Thus, the augmentation of eNOS and AT1 may contribute to the pre-eclampsia symptoms in our model.

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NEURAL-IMMUNE INTERACTIONS IN PREGNANCY: EFFECTS OF PSYCHOSOCIAL STRESS AND MATERNAL ETHNICITY. Mary Coussons-Read, Mischel Schmitt, Scott Giese University of Colorado at Denver Denver, Colorado.

Poor pregnancy outcomes such as low birth weight (LBW) and prematurity are a significant problem in the United States, especially for women who are members of minority groups. Psychosocial stress contributes to poor pregnancy outcomes, and interactions between the endocrine and immune systems may be involved in these effects. The present study tested the hypothesis that high levels of psychosocial stress during pregnancy alter maternal endocrine and immune function in a manner which contributes to poor birth outcomes, and that these interactions contribute to the increased occurrence of LBW and other suboptimal pregnancy outcomes in minority women. Psychosocial stress and social support were measured in an ethnically-diverse group of women once during each trimester of pregnancy and 6 weeks postpartum. Levels of serum interleukin 10 (IL-10), IL-6, IL-8, tumor necrosis factor alpha (TNF-α), and estriol were assessed concurrently with stress and support measurements. Higher levels of proinflammatory cytokines were associated with shorter gestational age at birth and with increased occurrence of complications. High stress was predictive of high levels of IL-6 and TNF-α, and with low levels of IL-10. High social support was associated with low stress scores, high IL-10, and low TNF-α. Examination of the relationship between maternal ethnicity and the outcome measures revealed significantly higher rates of complications and shorter gestational lengths in

minority women. Minority women had significantly lower levels of circulating IL-10 than Caucasians, and Hispanic women tended to have higher levels of circulating TNF- α than Caucasian and African American women. Moreover, there were trend significant interactions between minority status and stress on levels of TNF-α and IL-10. These data suggest that maternal minority status may be related to circulating levels of cytokines that are involved in successful pregnancies and that that the effects of stress on maternal immunity in pregnancy may be more pronounced in minority women. These findings provide initial support for our hypothesis that stress-related neural immune interactions may contribute to pregnancy complications and poor outcome, and that these effects may contribute to the disproportionate occurrence of poor pregnancy outcomes in minority women.

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IN VIVO TRANSIENT GENE TRANSFER TO THE UTERINE ENDOMETRIUM IN MICE USING HEMAGGLUTINATING VIRUS OF JAPAN (HVJ) ENVELOPE VECTOR. Tadashi Kimura, Kazuhide Ogita, Shinsuke Koyama, Tateki Tsutsui, Qing Zhang, Masayasu Koyama, Fumitaka Saji, Yasufumi Kaneda, Yuji Murata Osaka University Graduated School of Medicine Osaka 565-0871, Japan.

The uterus plays crucial roles at implantation and in maintaining the fetus and expelling the fetus at the appropriate time for parturition. To elucidate molecular mechanism of implantation in the uterus, gene targeting strategy often has given unsatisfactory result because of redundancy or fetus lethality. In order to clarify the local mechanisms of reproductive physiology and to establish a localization therapeutic strategy for reproduction, we applied Hemaggulutinating Virus of Japan envelope (HVJ-E) vector system for in vivo gene transfer into the uterine cavity. The HVJ-E vector which contains the reporter gene was injected into mouse uterine cavity on day 1.5 post coitus (p.c.). The reporter gene was expressed in the efficiency of 120 times more than the procedure using cationic liposome (LipofectAMINE,). The expression of the introduced gene continued for more than 3 days. The introduced plasmid was localized in the endometrial epithelium layer whereas introduced oligodeoxynucleotides were distributed throughout the epithelium, stromal cells and myometrium. HVJ-E vector did not affect the pregnancy rate, course of pregnancy, litter size, fetal growth, or parturition, and did not transfer the DNA into the fetus. These results indicate that gene transfer into uterus using HVJ-E vector is highly efficient and safe during pregnancy.

Further, we focused the function of nuclear factor kB (NFkB) that is thought to be upregulated in the glandular epithelium during implantation period. We introduced IkBa expression plasmid, an inhibitor of NFkB into the uterine cavity on day 1.5 p.c. Overexpression of IkBa by the CMV promoter in the plasmid into the mouse uterine cavity successfully abolished the NFkB binding activity in the uterus on day 4.5 p.c. The effect of downregulation of the NFkB activity at the implantation window on the pregnant rate is now under investigation. We consider that this procedure is widely applicable for investigations of reproductive physiology as well as for methods of local gene therapy in the uterus.

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A CONTROLLED GENE DELIVERY MATRIX FOR TOPICAL IMMUNIZATION AT THE FEMALE GENITAL TRACT. Hong Shen, Alaina R. Steck, W. Mark Saltzman Yale University New Haven, CT.

The mucosa of female genital tract is unique in protecting the body from invasion of bacteria and viruses. Unlike skin, which is tough and offers mechanical protection from the external environment, the mucosal surface is moist and permeable, and therefore, a wide variety of bacterial and viral pathogens can override the protection mechanisms of the mucosal surface. One approach in preventing the spread of pathogens is the development of vaccines that induce local production of pathogen- neutralizing antibodies at the mucosal surface of the female genital tract. In this study, a biocompatible polymer matrix was designed to release active plasmid DNA in a controlled pattern to the mucosal surface at the female genital tract. The released DNA efficiently transfected the reproductive tissues and induced specific IgA and IgG immune responses in vaginal secretions to the encoded protein; we examined the duration of protection using a variety of antigens including Hepatitis B surface antigen. When an oligonucleotide containing CpG motif was co-encapsulated into the matrix with the plasmid DNA, higher levels of antibody response were observed both in the vaginal secretions and in serum.

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PROGESTERONE-MEDIATED GESTATIONAL DOWNREGULATION OF TOLL-LIKE RECEPTOR 4 IN PERIPHERAL BLOOD LEUKOCYTES. Edyta Pawelczyk, Stella Nowicki, Audrey Hart, Pawel Goluszko, Gary Hankins, Garland Anderson, Gregory

Locksmith, Gail Olson, Bogdan Nowicki University of Texas Medical Branch Departments of Obstetrics and Gynecology and Microbiology & Immunology Galveston, Texas.

OBJECTIVE: The excessive maternal immune responses to infections during pregnancy are known to have a deleterious effect on the semiallogeneic conceptus. Toll-like receptor 4 (TLR4), a component of innate immunity, regulates the immune responses to bacterial lipopolysaccharide (LPS). We hypothesize that pregnant women downregulate the expression of TLR4 to prevent aggressive inflammatory responses. To test the hypothesis we compared TLR4 mRNA levels in peripheral blood leukocytes (PBL) of age-matched pregnant and nonpregnant women. We also evaluated whether TLR4 expression is regulated by progesterone.
METHODS: Total RNA was purified from PBLs of 39 nonpregnant and 55 pregnant women at gestational ages between 7 to 40 weeks. The levels of TLR4 mRNA were evaluated by reverse transcriptase-polymerase chain reaction (RT-PCR) with ribosomal 18S RNA as control and quantitated by spot densitometry. To assess the effect of progesterone on TLR4 transcript and protein levels, THP-1 human monocyte cell line was treated with progesterone (10-5M and 10-6M) at different time period from 6 to 24 hours. After treatments, RNA was purified and RT-PCR done. TLR4 protein levels were evaluated by Western blot and immunofluorescence staining.
RESULTS: All 39 nonpregnant women expressed similar, relatively high levels of TLR4 ranging from 0.64 to 1.23 integrated density value (IDV) with a median of 0.88. In contrast, 52 of 55 pregnant women tested (94%) had significantly decreased TLR4 transcripts ($p < 0.0001$) ranging from 0.15 to 0.67 IDV with a median of 0.33. Significant decrease in TLR4 mRNA in progesterone treated THP-1 cells was observed as early as 6 hours. TLR4 transcripts correlated with the decreased levels of TLR4 protein in whole cell lysates detected by Western blot and decreased staining of surface TLR4 by immunofluorescence staining.
CONCLUSIONS: The expression of TLR4 mRNA in maternal blood was decreased in the majority of pregnant women when compared to nonpregnant controls. Progesterone was shown to have an inhibitory effect on TLR4 transcript and protein levels in THP-1 cells. These findings support our hypothesis that the ability to decrease TLR4 expression may be one of the mechanisms that regulate inflammatory responses during pregnancy.

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DISRUPTION OF THE MOUSE CD46 CAUSED AN ACCELERATED SPONTANEOUS ACROSOME REACTION IN SPERM. Naokazu Inoue, Masahito Ikawa, Tomoko Nakanishi, Misako Matsumoto, Midori Nomura, Tsukasa Seya, Masaru Okabe Osaka University Yamadaoka 3-1 Japan.

Human membrane cofactor protein (MCP, CD46) is a ubiquitously expressed protein known to protect cells from complement attack. Interestingly, when we examined the expression of mouse CD46, which we cloned recently, the message was found only in testis and the protein was found on sperm inner acrosomal membrane. In order to elucidate the function of CD46, we have produced mice carrying a null mutation in the CD46 gene using homologous recombination. Despite the absence of CD46, the mice were healthy and both sexes were fertile. However, to our surprise, the fertilizing ability of males appeared to be facilitated by disruption of the CD46 gene, as the average number of pups born from CD46 -/- males was significantly greater than that of wild-type males. It was also revealed that the incidence of spontaneous acrosome reaction doubled in CD46-/- sperm compared to wild-type sperm. It was assumed that this increase caused the heightened fertilizing ability found in CD46-/- sperm. These data suggest that CD46 may have some roles in regulating sperm acrosome reaction.

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LEUKEMIA INHIBITORY FACTOR INDUCES STAT3 DNA-BINDING ACTIVITY IN JEG-3 CHORIOCARCINOMA CELLS. Florian Corvinus, Luciana Berod, Tobias Pöhlmann, Karlheinz Friedrich, Udo R. Markert Friedrich-Schiller-Universität Dept. Obstetrics, Placenta-Lab Jena, Germany.

OBJECTIVES: Phosphorylation and dimerization of Stat3 (Signal Transducer and Activator of Transcription 3) is related to the malignancy of tumor cells and potentially to their invasive properties. Extravillous trophoblast cells resemble cancer cells in this aspect since they invade decidual tissue, although only up to a well-regulated limit. In an earlier investigation, we found that Stat3 DNA-binding activity was increased in invasive cells (choriocarcinoma, 1st trimester trophoblast) and absent in non-invasive (term trophoblast). Now, we aimed at identifying factors involved in triggering Stat3 activation.
METHODS: Jeg-3 choriocarcinoma cells were cultured under standard conditions and were supplemented with various factors known to induce trophoblast proliferation, migration or invasiveness: granulocyte-

macrophage colony stimulating factor (GM-CSF), insulin-like-growth-factor-2 (IGF-2), hepatocyte growth factor (HGF), interleukin-6 (IL-6) and leukemia inhibitory factor (LIF). Stat3 expression and tyrosine phosphorylation were studied by Western blot analysis, specific DNA-binding activity of Stat3 was tested by an electrophoretic mobility shift assay (EMSA).
RESULTS: Whereas all other factors tested had no detectable effect, LIF strongly induced DNA-binding activity of Stat3 in Jeg-3 cells.
CONCLUSION: LIF is involved in the regulation of Stat3 activity in chorioncarcinoma cells. It possibly plays a role in the malignancy of choriocarcinoma and in trophoblast invasiveness.

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A STUDY OF UTERINE NK CELLS IN THE PREGNANT UTERUS OF MICE WITH THE DELAYED IMPLANTATION OR DELAYED PATURITION . Keiji Kokubu, Noriko Sakaguchi, Eiichi Hondo, Yasuo Kiso Yamaguchi University The United Graduate School of Veterinary Science Yamaguchi Prefecture, Japan.

Murine uterine NK (uNK) cells, which emerge in the metrial gland and the decidua basalis, differentiate at each implantation site during early pregnancy. The number of uNK cells drastically increases from implantation to placentation period. These cells are gradually eliminated by apoptosis and spontaneous migration out of the placenta, and disappeared from the endometrium at the term of pregnancy. The present study aimed to establish histologically how differentiation and elimination of murine uNK cells could be affected by the delayed implantation (DI) and delayed parturition (DP).

At day 8 of pregnancy (D8) in DI mice, both uNK cells and their granules showed higher number and smaller size than these of the control mice. In DI mice, when the period of high level progesterone (P4) in serum became longer, the size of uNK cells and their granules did larger. The granules were quite small compared to the control. This indicated that differentiation of uNK cells in DI mice was delayed compared to the control.

The cell density of uNK cells at D17 in DP mice was similar to that in the control. However, even at D19 when all uNK cells disappeared from the endometrium in normal pregnant mice, these cells survived in the metrial gland of DP mice. The metrial gland was well maintained. Abundant uNK cells were present even at D21, though their granules decreased in number. Although uNK cells of both DP and the control decreased in number from D15 to D17, the decreasing rate was milder in the DP mice than the control.

This study established that implantation is not directly related to differentiation of uNK cells, but parturition is closely involved in elimination of these cells. Since differentiation of uNK cells in DI mice was delayed compared to the control, other factors than P4 should be involved in differentiation of uNK cells. On the other hand, parturition occurs following the fall of serum P4 level. The uNK cells could survive in the metrial gland even after D19, due to high P4 level in DP mice.

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XIAP CONFERS TROPHOBLAST CELL RESISTANCE TO FAS-MEDIATED APOPTOSIS. Shawn L. Straszewski, Vikki M. Abrahams, Gil Mor Yale University, New Haven, CT.

BACKGROUND: Apoptosis occurs in the placenta throughout gestation, with a higher frequency at term compared to first trimester trophoblast cells. The Fas/FasL system represents one of the main apoptotic pathways controlling placental apoptosis. Although first trimester trophoblast cells express both Fas and FasL, they are resistant to Fas-induced apoptosis. Therefore, trophoblast resistance to Fas-mediated apoptosis may be due to inhibition of the pathway downstream of Fas stimulation. X-linked inhibitor of apoptosis (XIAP) immunoreactivity was recently identified in the trophoblast layer of first trimester placentas, but not in placentas at term. As a potent caspase inhibitor, XIAP prevents the activation of caspase-9 through its BIR3 domain and caspase-3 activation via the linker-BIR2 domain. Using an XIAP inhibitor, Phenoxodiol, we demonstrate that XIAP protects first trimester trophoblast cells from Fas-mediated apoptosis.
METHODS: Trophoblast cells were isolated from first trimester and term placentas of clinically normal pregnancies. Trophoblast cell viability was assessed by flow cytometry and a cell viability assay. The correlation between the expression and activation of XIAP and the intracellular components of the Fas pathway was evaluated by Western Blot analysis and a caspase-3 activity assay.
RESULTS: High levels of XIAP expression were detected in first trimester trophoblast cells, but not in trophoblast cells isolated from term placentas. Upon XIAP inactivation following Phenoxodiol treatment, first trimester trophoblast cells became sensitive to Fas-induced apoptosis, as evidenced by a synergetic decrease in the viability of trophoblast cells treated with both Phenoxodiol and an anti-Fas mAb. This was confirmed at the molecular level by the anti-Fas mAb

dose-dependent increase in the activation of caspase-8, caspase-9 and caspase-3. In addition, an increase in caspase-3 activity could also be observed in the trophoblast cells treated with both Phenoxodiol and the anti-Fas mAb compared to Phenoxodiol alone.
CONCLUSION: We demonstrate that Phenoxodiol-induced XIAP inactivation renders first trimester trophoblast cells sensitive to Fas-mediated apoptosis. This suggests a functional role for XIAP in the regulation of Fas-induced apoptosis of trophoblast cells. Since apoptosis is important for normal placental development, alterations in the regulation of placental apoptosis may lead to pathological conditions such as recurrent abortion and preeclampsia.

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REGULATION OF APOPTOSIS BY PLACENTA GROWTH FACTOR (PLGF) IN HUMAN TROPHOBLAST. Juan A. Arroyo, Donlad S. Torry Southern Illinois University School of Medicine Medical Microbiology and Immunology, Springfield, IL.

BACKGROUND: Abnormal regulation of apoptosis has been implicated in the onset and progression of a broad range of diseases. A higher degree of apoptosis is found in placentae from pregnancies complicated by fetal growth restriction and preeclampsia. The increase in trophoblast apoptosis has been shown to correlate with the decrease in PlGF expression found in preeclamtpic placentae. Human trophoblast express PlGF, and its receptor, flt-1. Exogenous PlGF induces the stress activated protein kinase (SAPK) signal transduction pathways in normal term trophoblast and protects the trophoblast from serum withdrawal induced apoptosis. The mechanism of how PlGF protects trophoblast from apoptosis is not known. Our goal is to elucidate the anti-apoptotic molecules induced by PlGF in trophoblast.
METHODS: Trophoblast were isolated from normal term placentae. RNA was extracted from the trophoblast at 24, 48 and 72 hours of serum deprivation cultures in the presence or absence of PlGF. RNase protection assay was preformed to determine what genes of the bcl2 and IAP families could be involved in the protection exerted by PlGF in these cells. Western blot analysis was used to confirm changes observed in RNA levels.
RESULTS: Annexin V studies showed an increase of 60% of apoptotic cells in trophoblast during culture in the absence of serum. This apoptosis was significantly reduced (90%) in the presence of PlGF. Trophoblast RNA and protein expression of bcl-2, Mcl-1, bfl-1, XIAP, and TRPM-2 anti-apoptotic molecules were increased by PlGF.
CONCLUSIONS: PlGF mediated bcl-2, MCl-1, bfl-1, XIAP and TRPM-2 production suggests an involvement for these molecules in the PlGF-induced protection from apoptosis in trophoblast. Furthermore, these results suggest that the relative lack of PlGF expression previously noted in preeclamptic trophoblast may increase their susceptibility to undergo apoptosis. (supported by NIH HD36830).

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CD4 INDEPENDENT INTEGRATION OF HIV INTO HUMAN DECIDUAL CD56 BRIGHT LYMPHOCYTES. Satoshi Hayakawa, Kenji Sugita, Tatsuo Yamamoto, Mitsuo Honda Nihon University, School of Medicine Department of Obstetrics and Gynaecology, Tokyo, Japan.

OBJECTIVE: Though positive staining of HIV related antigens is observed in decidual-placental tissues of HIV infected women, most infants are not infected. In order to analyze local immune system and kinetics of HIV, we examined possible infection of decidual CD56 bright large granular lymphocytes.
MATERIALS AND METHODS: Under informed consents, the first trimester decidual samples were obtained from 2 patients with HIV infection and 8 women undergoing induced abortion for socio-economical reasons. CD56 bright LGL were prepared by magnetic beads separation.

1 Integration of HIV provirus DNA in CD56 bright cells collected from HIV positive patients were examined by genomic PCR. 2 Expression of CD4, CCR1-11,CXCR1-6 was examined by RT-PCR. 3 Ninety six hours after challenge of 10 TCID50 of M-tropic and T-tropic HIV, provirus DNA was estimated by genomic PCR. 3 Effects of anti CD4, anti CCR5 and anti CXCR-4 monoclonal antibodies were examined.
RESULTS: 1 CD56 bright cells collected from HIV infected patients integrated HIV provirus DNA. 2 CD56 bright were expressed CCR5,CCR7,CXCR-3 and 4.3The cells were susceptible to both M-tropic and T-tropic HIV 3 Infection was not affected by anti CD4 but strongly suppressed by both anti CCR5 and antiCXCR-4 monoclonal antibodies and chemokines.
CONCLUSION: Decidual CD56 bright cells are susceptible to HIV through chemokine receptors. These cells can be reservoirs of vertical HIV infection.

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EFFECT OF SEX HORMONES ON SUSCEPTIBILITY TO VIRAL SEXUALLY TRANSMITTED DISEASES. Amy Gillgrass, Sherie Fernandez, Kenneth L. Rosenthal, Charu Kaushic McMaster University Pathology Hamilton, Ontario, Canada.

OBJECTIVE: We have previously demonstrated that sex hormones have a profound effect on susceptibility to bacterial STDs. Using a mouse model for HSV-2 we examined if viral STDs were also influenced by the hormonal environment.
METHODS: Inbred C57BL/6 female mice were ovariectomized (OVX). Two weeks later they were administered either saline (control) or estradiol or progesterone or a combination of both for 3 days. The mice were then infected intravaginally (IVAG) with HSV-2. The vaginal pathology was examined daily. Viral titers were measured in genital secretions. Histopathology was also examined.
RESULTS: In the absence of any hormones, OVX mice were found to be susceptible to genital HSV-2 infection. Progesterone treated animals were found to have maximum infection as determined by viral shedding in vaginal washes as well as vaginal pathology. Estradiol treated animals did not show any pathology or viral shedding. Histopathology showed maximum damage to the epithelium in progesterone treated mice. Estradiol treated animals did not have significant damage to the epithelium. Immunohistological examination showed that progesterone treated mice had maximum infection and infiltration by polymorphonuclear cells 24 hours post-infection. Most of the infiltrating cells were neutrophils. By Day 3 post-infection the combination hormone treated mice had extensive infection in their genital tract. Interestingly, uninfected estradiol treated mice also had neutrophils infiltration by D3 post-hormone treatment. We also examined the chemokine expression in the hormone-treated, infected animals. Uninfected, hormone treated mice did not have detectable levels of chemokines, except after estradiol treatment. However infection led to significant induction of chemokines. Maximum expression was seen in progesterone and combination treated groups.
CONCLUSIONS: Susceptibility to viral STDs is profoundly influenced by sex hormones. Hormonal influences have to be taken into consideration for both prophylactic as well as therapeutic strategies against viral STDs.

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DO INTRADUCTAL MACROPHAGES PLAY A ROLE IN EARLY BREAST CANCER PROGRESSION?. Nevine D. Ali, Jennifer Trinh, Susan A. Higgins, David L. Rimm, Bonnie L. King Yale School of Medicine Therapeutic Radiology New Haven CT.

AIM: Ductal lavage is a new procedure for collecting fluids from the breast ducts for early cancer detection and risk assessment. In preliminary studies to define the cell populations harvested by this method we observed extraordinarily high numbers of macrophages in specimens collected from breast ducts classified for normal or atypical epithelial cell cytology (1). These observations suggest that macrophages home to the mammary gland and populate the intraductal compartment of the breast during the earliest stages of breast cancer progression. Previous studies have shown that estrogen, associated with the development of breast cancer, is concentrated within breast fluids relative to serum (2). Others have shown that macrophages express the enzyme aromatase, which catalyzes the synthesis of estrogen (3). We are examining the hypothesis that infiltration of macrophages to the intraductal compartment of the breast constitutes one of the earliest stages of breast cancer progression by increasing the estrogen levels within the ductal fluids bathing the breast epithelial cells.
MATERIALS AND METHODS: Intraductal breast fluids are collected by ductal lavage from high-risk asymptomatic women, and from women scheduled to undergo breast surgery. Cells are isolated by centrifugation, aliquoted onto glass slides, and processed for immunocytochemistry using a monoclonal antibody for human aromatase.
RESULTS: Aromatase-positive macrophages have been detected in 11/14 (79%) of the ductal lavage specimens. Seven of the ducts with aromatase-positive macrophages were classified as having benign epithelial cell cytology, whereas three were classified as atypical, and one as malignant.
CONCLUSIONS: Our observations indicate that intraductal macrophages express aromatase, suggesting a possible mechanism for the concentration of estrogen within the fluids bathing the breast ductal epithelium, from which most breast cancers originate.

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HUMAN IMMATURE DENDRITIC CELLS EXPRESS A FUNCTIONAL MEMBRANE ESTROGEN RECEPTOR. David C. Gondek, Paul M. Guyre, Charles R. Wira, John E. Connolly Dartmouth Medical School, Lebanon, NH.

Dendritic cells (DC) are the most potent antigen-presenting cells (APC) and play a pivotal role in development and maintenance of the induction, effector and memory phases of the immune response. The influence of estrogen on cells of the immune system can be generally broken down into genomic and non-genomic effects. The genomic effects of estrogen are mediated by interaction with its nuclear hormone receptors subunits, alpha and beta (ERa and ERb).

Non-genomic effects are far more rapid, demonstrating calcium flux and mitogen activated protein kinase (MAPK) activation within minutes to seconds after estrogen exposure. We demonstrate that monocyte derived human iDC are able to specifically bind membrane impermeable E2 conjugates. This binding is lost with upon maturation of the dendritic cell. Membrane ER engagement on immature dendritic cells leads to an activation of cellular signaling pathways including a rapid dose dependent calcium flux in response to treatment with both E2 as well as membrane impermeable E2 conjugates. These observations indicate that immature human dendritic cells express a functional membrane estrogen receptor and provide a mechanism by which estrogen may modulate dendritic cell maturation and function.

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TOWARDS DESIGNING AN IMPROVED HCG CONTRACEPTIVE VACCINE. Om Singh, Rahul Pal, Sarita Ahlawat, Poonam Tewari, Shafiuddin, Sarita Singh National Institute of Immunology Immunoendocrinology New Delhi, Delhi.

A prototype vaccine inducing antibodies against human chorionic gonadotropin (hCG) has previously undergone clinical trials that demonstrated the feasibility of the approach in preventing pregnancy in women without any notable side effects. hCG is considered essential for the establishment of early pregnancy, and is also a potential target for tumor immunotherapy. Protective levels were however not attained in some recipients. We demonstrated that a large protein carrier such as tetanus or diphtheria toxoid may not be universally effective carriers in a genetically diverse population. This problem can be overcome by use of a combination of pathogen derived broadly reactive non-B Th peptides, employing a permissible adjuvant. Antibodies have a better hCG neutralization index than those generated in the protein carrier group. No anti-peptide antibodies were detected, eliminating the possibility of carrier mediated suppression of the immune response. Prior natural exposure to the infectious agents from which peptides are derived may in fact prove be beneficial rather than detrimental. There may thus be an advantage of choosing peptides from infectious agents since a significant percentage of the population may be naturally pre-sensitized to them. Inclusion of a novel adjuvant consistently enhanced the anti-hCG antibody response in outbred as well as inbred mice of different haplotypes, which included both high as well low responder strains. Significantly, increase in response was not only observed in the IgG1 subclass but also in the IgG2a and IgG2b subclasses. Antibodies were of 10 M-1) and capable of neutralizing the hormonal≈ high affinity for hCG (Ka bioactivity as a function of the antibody titers. In conclusion, the improved formulation consisting of Th-cell peptides as carriers and a novel adjuvant can elicit and maintain antibody levels adequate for immuno-contraception, and also raises the prospect of effective immunotherapy of cancers that utilize hCG as a growth factor.

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A ROLE OF COMPLEMENT IN MALE FERTILITY: TARGETED DELETION OF THE COMPLEMENT REGULATORY PROTEIN CD59B RESULTS IN PROGRESSIVE LOSS OF MALE FERTILITY. Xuebin Qin, Martin Dobarro, Sylvia J. Bedford-Guaus, Sean P. Ferris, Jose A. Halperin Harvard Medical School Lab. for Translational Research Cambridge, MA.

The complement system is a main effector of the immune system that mediates both acquired and innate responses against foreign antigens. Activation of complement leads to formation of the membrane attack complex (MAC), which is the major mediator of complement-induced cell damage and lysis. The presence of foreign antigens on sperm exposes them to "self" immune/complement attack and also poses a threat to successful fertilization and fetal development due to the risk of targeting by the female immune/complement systems. To protect the sperm from a complement attack, a variety of non-specific and specific complement inhibitors present at high concentrations in the seminal fluid literally "bath" the sperm, and sperm cells express multiple proteins that inhibit complement activation. Of particular importance is CD59, the complement regulatory membrane protein that specifically inhibits formation of the MAC. The uniform high-density expression of CD59 at all over the sperm surface and in the seminal fluid has fueled the notion that CD59 may play a key role in sperm protection and survival. We have recently reported the targeted deletion of the mouse CD59 gene, termed mCd59b (Immunity, Vol. 18, 217-227, February, 2003). mCd59b-/- mice show 1) spontaneous hemolytic anemia, as expected from the loss of protection of red blood cells against complement-mediated attack; and 2) progressive loss of male fertility. We present here a detailed analysis of sperm at different time points after birth. The data shows a dramatic decrease in sperm number and mobility, abnormal sperm morphology and a significant increase in the number of dead sperm

cells in the mCd59b-/- mice as compared to its age-matched wild type counterpart. These studies provide a novel animal model to study the long suspected role of complement modulation in male fertility.

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IL-10 FUNCTIONS AS A TROPHOBLAST SURVIVAL FACTOR AND POSSESES ANTI-APOPTOTIC ACTIVITY AGAINST SERUM FROM PRE-ECLAMPTIC PATIENTS. Michael Plevyak, Shaun Patrick Murphy, Nazeeh Hanna, James Padbury, Surendra Sharma Women and Infants Hospital/ Brown University Departments of Pediatrics and Ob/Gyn Providence, RI

Preeclampsia (PE) is a complicated pregnancy condition with a multi-factorial etiology. Common findings in PE include hypertension, proteinuria, vasospasm, small placenta, endothelial and epithelial cell injury, and fetal growth restriction. This disease is associated with an increased incidence of maternal and perinatal morbidity and mortality. The mechanisms underlying degeneration of trophoblast cells in PE and their survival in normotensive pregnancy are unknown. Inflammatory (cytotoxic) factors which cause placental injury have been implicated in the pathogenesis of PE. Identification of such cytotoxic factors in PE serum may serve as predictive markers for this condition. We provide evidence that a subgroup of PE serum samples induced apoptotic cell death in cultures of an extravillous cytotrophoblast cell line, TCL1. To measure the apoptotic index, we used a flow cytometry detection method based on identification of apoptotic cells by an antibody, M30, raised against a novel neoepitope of cytokeratin 18. This neoepitope is generated by cleavage of cytokeratin 18 by caspase 3 in apoptotic cells. TCL1 cells were grown as a monolayer, trypsinized, washed and cultured at 2×105 cells/ml in the absence or presence of 20% serum from either PE or normotensive patients. For active or apoptotic growth, cells were also cultured in 10% or 0.5% FCS, respectively. After 24 hour culture period, cells were analyzed for positive staining with M30. Our results demonstrate that 6 of 9 PE serum samples induced appearance of M30 positive cells, albeit with varying potency, suggesting heterogeneity of the disease. In contrast, serum samples ($n = 7$) from normotensive patients did not induce any detectable cell death in TCL1 cells. Importantly, IL-10 (20 ng/ml) significantly inhibited PE serum induced apoptosis. These data suggest the presence of cytotoxic factors in PE serum which can be directly identified using the method of M30 staining of cells. Furthermore, IL-10 appears to play an important role in the survival of trophoblast cells. Our data will provide a basis for establishing novel in vitro assays for PE.

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ACTIONS OF TUMOR NECROSIS FACTOR-A ON OOCYTE MATURATION, EMBRYONIC DEVELOPMENT AND APOPTOSIS IN BOVINE PREIMPLANTATION EMBRYOS. Paolete Soto, Roger P. Natzke, Peter J. Hansen University of Florida Dept. of Animal Sciences Gainesville, FL.

Infertility can accompany mastitis in cattle. Involvement of tumor necrosis factor-α (TNF-α) in this phenomenon is suggested by observations that circulating concentrations of TNF-α are elevated after intramammary infection or infusion of endotoxin. Here, it was hypothesized that 1) TNF-α acts on the oocyte during maturation to decrease the percent of oocytes that cleave and develop following fertilization; 2) exposure of embryos to TNF-α after fertilization reduces development to the blastocyst stage; and 3) TNF-α increases the proportion of blastomeres that undergoes apoptosis in a stage-of-development dependent manner. In experiment 1, oocytes were matured with various concentrations of TNF-α and then fertilized and cultured without TNF-α. Addition of TNF-α to maturation medium did not affect the proportion of oocytes that cleaved. However, the percent of oocytes that developed to the blastocyst stage at day 8 after insemination was reduced ($P = 0.05$) at all TNF-α concentrations (20, 13, 12, 15 and 13% for 0, 0.1, 1, 10, and 100 ng/ml TNF-α). In experiment 2, embryos were cultured with TNF-α for 8 days beginning after fertilization. There was no significant effect of TNF-α on the proportion of oocytes that became blastocysts. In experiment 3, embryos were collected at the 2 or 4-cell stage (at 28–30 hours after insemination) or when >8 cells (at day 4 after insemination) and cultured $+/-$ TNF-α for 24 h. For 2- and 4-cell embryos, there was no effect of TNF-α on the percentage of cells labeled with the TUNEL procedure. For embryos >8 cells, 10 and 100 ng/ml TNF-α increased ($P < 0.05$) the percent of blastomeres labeling as TUNEL-positive (12, 12, 11, 27, and 30% for 0, 0.1, 1, 10 and 100 ng/ml TNF-α). In conclusion, TNF-α can have deleterious actions on oocyte maturation that compromise development of the resultant embryo. While exposure of fertilized embryos to TNF-α did not inhibit development to the blastocyst stage, TNF-α increased the percentage of blastomeres undergoing apoptosis when exposure occurred for embryos >8 cells. The implications of increased blastomere apoptosis for embryo survival in utero needs to be determined. (USDA 2002-35203-12664).

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ACUTE VERSUS CHRONIC INFLAMMATION: WHAT MAKES THE INTRA-UTERINE ENVIRONMENT "UNFRIENDLY" TO THE FETUS? FROM FREE RADICALS TO PROTEOMICS. Irina A. Buhimschi, Catalin S. Buhimschi, Carl P. Weiner Wayne State University Ob./Gyn. Detroit, MI.

AIM OF THE STUDY: Intra-amniotic inflammation is associated with poor neonatal outcome independent of prematurity. Whether the inflammatory process responsible for the long-term sequelae is acute or chronic is difficult to evaluate due to limitations in assessing the intrauterine environment directly and repeatedly. First, we aimed to assess the acute inflammatory response by using proteomic technology (SELDI: surface enhanced laser desorption ionization). Second, we reasoned that the presence of oxidized or carbonylated proteins will provide additional information about the intensity and/or duration of the insult and thus may be an indicator of chronicity.
METHODS: Assessment of the acute intra-amniotic inflammation was provided by examination of SELDI tracings of patients admitted with PPROM or preterm labor contractions. We extracted the inflammatory profile using a novel, step-wise logical approach. The biomarkers composing the inflammatory profile were further identified by mass, isoelectric point determinations and peptide mapping using the PBS II instrument and a variety of ProteinChip arrays. Assessment of chronic intra-amniotic inflammation was provided by a semiquantitative analysis of carbonylated/oxidized proteins after in-vitro and in-vivo exposure to various oxidative insults.
RESULTS: Intra-amniotic inflammation which leads to preterm delivery is characterized by the presence in amniotic fluid of a distinct proteomic profile of 3 or 4 of the following proteins: neutrophil defensins-1 and 2, and calgranulins A and C. Based on the presence or absence of these biomarkers, we devised a score: the MR (mass restricted) score ranging from 0 (all biomarker peaks absent) to 4 (all biomarker peaks present). Oxidative modifications of amniotic fluid proteins are associated with intense protein fragmentation which are both dependent on the time and dose of oxidant suggesting that protein carbonylation may be a valuable indicator of chronic intra-amniotic inflammation
CONCLUSION: Proteomic analysis of amniotic fluid reveals the presence of biomarkers characteristic of intrauterine inflammation. A concomitant evaluation of oxidative post-translational modifications provides additional information on the time or extent the fetus has been exposed to the "unfriendly" intrauterine environment. Assessment of the acute or chronic nature of the inflammatory process is essential as different therapeutical approaches are needed to prevent the extension of fetal damage in-utero.

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IMMUNOMODULATION BY CD200 AS A BASIS FOR PREVENTION OF CYTOKINE-DEPENDENT FETAL LOSS SYNDROME. David A. Clark, Gerard Chaouat McMaster University Medicine Hamilton, Ontario, Canada.

The CD200 (OX-2) tolerance-signalling molecule, expressed in decidua and on trophoblast of both mouse and human origin, is also expressed on a subpopulation of CD11c+ blood mononuclear dendritic cells (DC). CD200+ DC stimulate expression of IDO in small-sized f4/80+ macrophages, and IL-10 and TGF-beta-producing antigen-specific gamma-delta T suppressor cells (a Th1 to Th2,3 shift). Fresh allogenic BALB/c splenocytes can prevent spontaneous cytokine-dependent abortions in the CBA x DBA/2 model, and activity is blocked by monoclonal anti-CD200. Activity is also lost if the cells are stored at 4C overnight before use. A similar loss of activity has been reported in human clinical trials of allogeneic peripheral blood mononuclear cells (PBL). The aim of the study was to test possible loss of surface CD200 into culture medium during storage. Materials included CBA/J, DBA/2J, and BALB/cJ mice, monoclonal anti-mouse and anti-human CD200 +/− PE-label, and FITC-anti-CD11c, isopaque-ficoll-purified PBL, 51Cr-labelled NK target cells, standard media, and IVIG. Methods included mouse breeding and resorption counting on gestation day 13.5, flow cyometry, and 51Cr-release assays.
RESULTS: Soluble recombinant murine CD200Fc prevents abortions in the CBA x DBA/2 model, and medium with 10% FBS conditioned by BALB/c splenocytes at 4C overnight aquired similar protective activity. Conditioned supernatants also blocked binding of PE-anti-CD200 mAb to freshly-isolated CD200+ splenocytes. Supernatants made in PBS or medium with 10% FBS using human PBL acquired a similar blocking activity, but not if the cells had been incubated in plasma. Nevertheless, cells incubated in plasma lost surface CD200. As Heine et al reported mIVIG could prevent spontaneous abortions in the CBA x DBA/2 model (Res Exp Med 1992;192:49–52), to test whether shed CD200 might bind to plasma IgG, anti-CD200 was added to cultures in which the NK lysis of K562 target cells by PBL was being inhibited by IVIG. Anti-CD200 mAb reversed the inhibition.
CONCLUSIONS: Both cell-associated and soluble shed CD200 molecules may play an important role in immunomodulation necessary for pregnancy success in a Th-1 dominant uterine environment. (Supported by

CIHR, Bayer-Blood Partnership, and INSERM/CNRS.)

POSTER PRESENTATIONS

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FAILURE OF ANTI-TUMOR IMMUNITY IN MAMMALS - EVOLUTION OF THE HYPOTHESIS. Ivan Vladimir Bubanovic, Stevo Najman Medica Center Ob/Gyn Nis, Yugoslavia.

Observation at morphological and functional similarity between embryonic or trophoblast tissues and tumors are very old. In time, many investigators were created different hypotheses for origin of cancerogenesis or tumors efficiency in relationship with host immune system. Some of these ideas were rejected, but many of them are still current. Presumption for inefficiency of anti-tumor immunity in mammals because trophoblast cells are very similar against tumor cells is very real. Mechanisms for the escape of tumors from immune response are very similar alongside mechanisms for the escape of fetoplacental unit from maternal immune response. Similarity between these two mechanisms is so significant that any randomness is banished. At the same time, incidence of malignant tumors and type of most frequently tumors in non-mammalians vertebrates is significantly different than in mammals. At last, mechanisms of anti-tumor immunity in mammals are substantially different as against mechanisms of anti-tumor immunity in other classes of ver-tebrates. These facts indicate that immune system of mammals during anti-tumor immune response is tricked with similarity between tumor cells and trophoblast or other placental cells.

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REGULATION OF TROPHOBLAST INVASION OF DECIDUA IN THE FIRST TRIMESTER BY FAS/FAS-LIGAND SYSTEM. Marco Sbracia, Fabio Scarpellini, Gabriele Rossi, Roberta Poverini Cerm via carlo Porta Rome, Italy.

AIM OF THE STUDY: Recently it has been reported that human endometrium expresses FAS-Ligand during secretory phase and in the decidua. Furthermore, trophoblast expresses Fas and Fas Ligand. Apoptosis has been found in the syncithyum trophoblast during all pregnancy. We studied in the same pregnant decidual tissues of first trimester, the expression of FAS, FAS-Ligand, bcl2 and the presence of apoptosis both in trophoblast and endometrial component.
DESIGN: Apoptosis by TUNEL and the expression of FAS, Fas Ligand and bcl2 by immunohistochemestry in decidual tissue and trophoblast in first trimester pregnancy were studied.
MATERIALS AND METHODS: We evaluated tissue specimens of first trimester decidua and trophoblast obtained from 15 pregnant women underwent voluntary abortion. The specimens of decidual tissue were fixed in formalin overnight and paraffin embedded. The indirect avidin-biotin complex (Vectastin ABC-peroxidase kit) immunoperoxidase assay and m thick sectionsthe TUNEL assay were performed on de-waxed and re-hydrated 5 of 10%. Anti-Fas-Receptor monoclonal antibodies and anti-FAS-Ligand polyclonal primary rabbit antibody with a 1:50 dilution phosphate-buffered solution were used. The anti-mouse and anti-rabbit serum were used as second antibody. The Vectastain ABC-peroxidase (Vector USA) was used for immunocytochemical localization. Staining was developed using 1.7μM 3.3 diaminobenzidine and 0.05% hydrogen peroxidase.
RESULTS: In the trophoblast villis Fas is expressed by syncithyum trophoblast whereas inside the villis FAS-L was expressed by cytotrophoblast. Apoptosis was observed almost exclusively in the syncithyum trophoblast especially in syncithial knots, in the same cells showing the expression of FAS. The apoptosis ratio was $0.018 + 0.0008$ in syncithyum trophoblast. In the epithelial cells of decidua FAS-L was strongly expressed especially in the regions close to villis, whereas FAS was expressed mostly by stromal cells and in some glands. Also apoptosis was found mostly in the stromal cells of decidual cells.
CONCLUSIONS: The presence of apoptosis in syncithyum trophoblast with the concomitant expression of Fas in the same cells whereas the strong expression of Fas-Ligand in the epithelial cells of decidua seem to show that Fas/Fas-Ligand system regulates and limits the invasion of trophoblast by inducing apoptosis.

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GRANULOCYTE COLONY STIMULATION FACTOR(G-CSF) SUPPRESSES AUTOLOGOUS TUMOUR KILLING(ATK) ACTIVITY OF THE PERIPHERAL BLOOD LYMPHOCYTES IN THE PATIENTS WITH OVARIAN CARCINOMA. Yoshiaki Ohta, Satoshi Hayakawa, Fumihisa Chishima, Tatsuo Yamamoto Nihon Universuty School of Medicine Department of Obstetrics and Gynaecology Tokyo, Japan.

PURPOSE AND BACKGROUNDS: Reported by us and others, Granulocyte colony stimulation factor (G-CSF) suppress NK cell activity. We have proposed

possible suppression of materanal immune response by locally produced G-CSF in fetomaternal interface. G-CSF is often administered for the patients with chemotherapy induced leukocytopenia. However its effects on cancer immunology have not been reported. In this study, we examined effects of G-CSF on IL-2/IL-12 induced IFN-gamma production of peripheral blood mononuclear cells and autologous tumour killing activity against primary culture ovarian carcinoma cells.

MATERIALS AND METHODS: Eight cases of the patients with advanced primary epithelial ovarian carcinoma (FIGO Stage Ic<) were examined. Informed consent was obtained before sampling. Primary culture of carcinoma cells was established from ascites obtained during surgery. Peripheral blood mononuclear cells were collected from cancer bearing patients and 6 age matched healthy female controls by venopuncture. We separated CD4+, CD8+ and CD16+ cells by magnetic columns. Cells were cultured for 48 hours under stimulation of IL-2(100 IU/ml) and IL-12(10 IU/ml) and various concentrations of G-CSF. Frequencies of IFN-γ producing cells was examined by ELISPOT assay.

ATK activity of PBMC was examined by LDH release assay.

RESULT: (1) CD8+T cells and CD4+T cells and CD16+ NK cells produced IFN-γ under stimulation of IL-2 and IL-12. (2) Patients with advanced ovarian carcinoma showed less frequent IFN-γ production compared with healthy controls. (3) G-CSF suppressed IFN-γ production of patients and control subjects. (4) Stimulation of peripheral blood mononuclear cells by combination of IL-2 and IL-12 induced strong ATK activity. (5) G-CSF completely suppressed ATK activity over 100 pg/ml..

CONCLUSION: In addition to its well known trophic effects on hematopoiesis, our results suggest possible suppressive role of G-CSF on tumour immunity.

Routine administration of G-CSF on cancer bearing patients is not recommended except for febrile neutropenia.

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ROLE OF PKR AND PROMOTER METHYLATION IN THE REGULATION OF IFN^{g} IN NAIVE T CELLS. Anurag Kumar Gupta, Corrine Rusterholz, Wolfgang Holzgreve, Sinuhe Hahn University Women's Hospital Obstetrics and Gynaecology Basel, Switzerland.

The inability of cord blood (CB) na T cells to produce IFNg is poorly understood. Contradictory reports, stating that either CB T cells require a high threshold of activation or that low level of IFNg production is due to some intrinsic defect, have left the issue unresolved. The intrinsic defect theories favour regulation either at transcriptional level or translational level.

In order to give better insight in this issue, we quantified IFNg mRNA, by using Real Time PCR, and IFNg protein production, either by FACS or ELISA, in PMA and Ionomycin stimulated cord and adult blood T cells. We found that IFNg RNA accumulation in na CD4 and CD8 T was much lower than in adult T cells. We checked whether this low IFNg mRNA triggered PKR activation (IFNg dependent kinase regulating translation). Since protein production was not enhanced by treatment with the PKR inhibitor drug 2-Amino-purine (2-AP), this indicates that PKR is not involved. Low mRNA and low protein in na T cells indicates that IFNg expression is not regulated at post-transcriptional or translational levels. We next examined possible role of promoter hypermethylation by examining IFNg mRNA accumulation following 5-aza-2'-deoxycytidine treatment. This treatment did not lead to any significant increase in mRNA accumulation either. Therefore, we conclude that regulation of IFNg expression in na T cells occurs at the transcriptional level, and that induction of IFNg expression in these cells is hindered by an intrinsic program which is lost as they become activated mature cells. The nature of this block is still unclear.

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ACCUMULATION OF CD68+ AND CD14+ CELLS IN THE ENDOMETRIUM OF PREGNANT SHEEP: REGULATION BY SYSTEMIC EFFECTS OF PREGNANCY, LOCAL PRESENCE OF THE CONCEPTUS, AND PROGESTERONE. Saban Tekin, Peter J. Hansen University of Florida Animal Sciences Gainesville, FL.

Regulation of macrophage function during pregnancy has been proposed to be important for survival of the conceptus. Here, an experiment was conducted using sheep to test whether macrophages accumulate in uterine endometrium during pregnancy and to evaluate the regulatory basis for this phenomenon. The approach was to immunolocalize cells expressing CD68 (a marker of macrophage/monocytes, dendritic cells and granulocytes) in endometrium from ewes in the luteal phase of the estrous cycle (n = 4), ovariectomized (ovx) ewes (n = 4), ovx ewes treated for 44 days with 50 mg/day progesterone (n = 4), and from the non-pregnant and pregnant uterine horns of unilaterally-pregnant ewes at day 140 of pregnancy (n = 6). Unilateral pregnancies were established by

removing one ovary and ligating the ipsilateral uterine horn before breeding. Selected tissues were also analyzed for CD14 (macrophage/monocyte marker) and CD45R (marks CD8+ T cells in the sheep uterus). In cyclic ewes and ovx ewes ± progesterone treatment, CD68+ cells were scattered throughout the endometrium and were generally not abundant. In both uterine horns of unilaterally-pregnant ewes, in contrast, there was a large increase in the number of CD68+ cells in the stratum compactum region of the endometrial stroma. The abundance of CD68+ cells was determined by a subjective scoring system from 0–5. The average score was 1.6 ± 0.3 (cyclic), 1.0 ± 0.3 (ovx), 1.0 ± 0.3 (ovx + progesterone), 2.8 ± 0.3 (non-pregnant horn of pregnant ewes) and 4.7 ± 0.3 (pregnant horn of pregnant ewes). Staining score was higher for pregnant ewes than other groups ($P < 0.001$) and, among pregnant ewes, higher for the pregnant horn than non-pregnant horn ($P < 0.001$). Cells positive for CD14 were also more abundant during pregnancy and were located in similar regions of endometrium as for CD68+ cells. In conclusion, macrophages accumulate in the stratum compactum region of the endometrium during pregnancy in sheep. This accumulation is caused in part by a systemic signal associated with pregnancy that does not appear to be progesterone and in part by the local presence of the conceptus. These observations imply that macrophages play an important role in the uterus by the end of pregnancy. (Support: USDA NRICGP 2001-35204-10797)

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IGG BUT NOT IGM ANTIPHOSPHOLIPID ANTIBODIES IN FOLLICULAR FLUIDS OF IVF-ET PATIENTS. Hidehiko Matsubayashi, Toshitaka Sugi, Tadashi Arai, Masako Shida, Akane Kondo, Takahiro Suzuki, Shun-ichiro Izumi, Tsunehisa Makino Tokai University School of Medicine Department of OB/GYN Kanagawa Japan.

PROBLEM: Patients having in-vitro fertilization and embryo transfer (IVF-ET)-failures show an increased incidence of antiphospholipid antibodies (aPL) in the sera, but controversy remains. Since the direct effect of aPLs is found on embryos recently, we asked if some aPL might be observed in follicular fliids (FF).
METHOD OF STUDY: Patients with more than 2 failures of IVF-ET were tested to detect IgG, IgA and IgM isotypes of aPL in the sera. Of these, 26 aPL-positive patients and 12 aPL-negative patients gave us informed consent to collect FF and to detect aPL at the succeeding IVF-ET attempt. aPL in FF was measured by ELISA with 1/10 dilution of FF instead of 1/100 dilution of sera.
RESULTS: aPL-negative patients did not show any aPL in FF. FF from aPL-positive patients showed only IgG isotype of aPL detected in the sera, but they did not show IgM isotype of aPL which was detected in the sera. No IgA isotype was detected in these patients' sera and FF.
CONCLUSIONS: We need further research, but IgG isotype of aPL in FF may be responsible for some IVF-ET failures.

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GONADOTROPIN MODULATION OF CYTOKINE PRODUCTION IN BLOOD AND FOLLICULAR FLUID OF PATIENTS UNDERGIONG CONTROLLED OVARIEN HYPERSTIMULATION (COH) AND THEIR EFFECT ON ICSI OUTCOME. Mohamad Eid Hammadeh, Heidron Hoffmeister, Peter Rosenbaum, Werner Schmidt University of Saarland Obstetrics & Gynecology Homburg/Saar, Germany.

PURPOSE: To determine the influence of controlled ovarian hyperstimulation on soluble intracellular adhesion molecule (sICAM-1) and fibroblast growth factor (hFGF) production in serum and Follicular fluid (FF) and their effect on ICSI outcome.
MATERIALS AND METHODS: 75 Patients undergoing (COH) for ICSI therapy were included in this study. They subdivided into three groups according to ovarian stimulation protocol after pituitary down regulation with GnRH-anologa (GnRH-a): Group 1: Human menopausal gonadotropin (hMG, $n = 11$), Group2: rec. follicle stimulating hormone Gonal-F® (rFSH, $n = 38$), Group 3: HMG plus rFSH ($n = 26$).

The concentrations of sICAM-1 and SCF- in serum and (FF) were assessed with appropriate enzyme-linked immunosorbent assay kits.
RESULTS: The mean concentration of s-ICAM (ng/ml) in serum of all three investigated group was (215.5 ± 45.22; 225.4 ± 75.9 and 247.9 ± 70.7) and in FF (197,9 ± 30.3; 188.4 ± 40.6 and 231.24 ± 66.5 respectively). Group 3 showed a significantly higher concentration in FF in comparison to Group1 ($p = 0.023$) or group 2 ($p = 0.0001$). The concentration of hFGF in serum was significantly lower than the value in FF of all investigated groups 1 (6.3 ± 2.92; 6.11 ± 3.3 and 8.8 ± 10.3pg/ml Vs. 123.7 ± 60.0; 107.4 ± 61.1 and 121.5 pg/ml respectively). Besides, no significant difference was found between the groups either in serum ($p = 0.804$) or FF ($p = 0.404$). However, the mean number of retrieved oocyte, fertilization rate and pregnancy rate were higher in G.2. (9.7 ± 3.8%; 49,5 ± 29.7% and 39.0%) in comparison to these clinical parameter in G.1 (8.8 ± 4.1%;

50.9 ± 26.7% and 18.2%) or in G.3 (8.1 ± 3.7%; 47.3 ± 29.9% and 24,1%).
IN CONCLUSION: Gondatropins influence the production and secretion of sICAM-1 at the periovulatory period which in turn may influence the oocyte quality and ability for fertilization and implantation.

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NEW INSIGHT INTO THE ETIOLOGY OF PCOD. Soo Hong Min, John C. Chapman, Sandra D. Michael Binghamton University Biological Sciences Binghamton, NY.

AIM: The etiology of polycystic ovarian disease (PCOD) remains an enigma. Forty years ago, the steroid injected, female rat and mouse were characterized as animal models for the study of PCOD. Theories to account for anovulation were subsequently postulated without considering the action of steroids on the immune system. The present study examined whether or not the thymus gland is involved in estrogen-induced anovulation in the female mouse.
MATERIALS AND METHODS: (C57BL/6J × A/J)F1 B6A female mice were injected on days 5, 6, and 7 with vehicle (group 1), or with vehicle containing 20 mg estradiol-17b (E2) (group 2). Other females were thymectomized on day 3 (Tx-3), and infused on day 7 with thymocytes from either 7-day-old female donors (group 3), or from adult female donors (group 4). Other Tx-3 females were injected with E2 on days 5, 6, and 7, and at 20 days of age infused with thymocytes from adult female donors (group 5).

A number of animals from groups 1 and 2 were killed at 12 days of age, thymuses removed and weighed, and thymocytes counted. All others were killed at 100 days of age, ovaries removed and examined for the presence of corpora lutea (CLs) and ovarian cysts.
RESULTS: Vehicle-injected females had ovaries with CLs. E2-injected females had ovaries with cysts. At 12 days of age, the thymuses of E2-injected animals contained 30 million fewer thymocytes. Tx-3 females infused with thymocytes from 7-day-old female donors had ovaries with cysts; whereas, Tx-3 females infused with thymocytes from adult female donors had ovaries with CLs. Tx-3 females injected with E2, and infused with thymocytes from adult female donors, had ovaries with CLs.
CONCLUSION: Estrogen-induced anovulation in female B6A mice is the result of E2 affecting the premature release of thymocytes. Thymocytes released on day 7 are the actual cause of anovulation. Whether this is because these thymocytes are normally deleted in the intact thymus, or because of their incomplete maturation, will require further research.

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AUTOANTIBODIES TO FACTOR XII IN FACTOR XII DEFICIENT PATIENTS WITH RECURRENT PREGNANCY LOSSES. Toshitaka Sugi, Dan Lu, Tsunehisa Makino Tokai Univ. Sch. of Med. Ob/Gyn Isehara, Kanagawa.

Antiphospholipid antibodies (aPL) have been described in patients with thrombosis and recurrent pregnancy loss (RPL). We recently reported that a strong association between antiphosphatidylethanolamine antibodies (aPE) and RPL (Fertil Steril, 71, 1060, 1999). We also reported that certain aPE are not specific for phosphatidylethanolamine (PE) per se but are directed to PE-binding plasma proteins, such as kininogens (Blood, 86, 3083, 1995). Coagulation factor XII, prekallikrein and high molecular weight kininogen are known as plasma contact proteins in the intrinsic pathway of blood coagulation. However, deficiencies of these proteins are not associated with clinical bleeding despite marked prolongation of in vitro surface-activated coagulation time. Paradoxically, studies suggest that these proteins have anticoagulant, profibrinolytic functions in a physiologic milieu, on endothelial cells. Recently, surprisingly high prevalence of factor XII deficiency among patients with thrombosis and RPL have been reported. Since the presence of antibodies to factor XII in patients with lupus anticoagulant has been reported, we have now tested plasma samples from RPL patients for the factor XII activity and for the presence of autoantibodies to factor XII. One hundred ninety one patients with RPL were screened for factor XII activity. We found 34 (17.8%) patients with factor XII deficiency (activity < 60%). Our data show that RPL are associated with factor XII deficiency ($p = 0.0056$, OR 11.7). Eleven (32.4%) of these 34 patients were positive for aPE. We hypothesized that factor XII deficiency may be partly due to presence of antibodies to factor XII and tested plasma samples from patients with factor XII deficiency for the presence of antibodies to factor XII by immunoblot and ELISA. We found that 53.5% of factor XII deficient patients with RPL were positive for IgG anti-factor XII antibodies ($p < 0.001$, OR36.8). All anti-FXII antibodies recognized the heavy chain of FXII in immunoblot. Since contact proteins may play an important role in fetoplacental unit, deficiencies of these proteins and/or autoantibodies to these proteins may be associated with pregnancy losses.

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EFFECTS OF OVARIAN STEROIDS ON IMMUNOGLOBULIN-SECRETING CELLS FUNCTION IN HEALTHY WOMEN. Fabien Lü, Zhongmin Ma, Susie Moser, Thomas G. Evans, Chris J. Miller California National Primate Research Center, University of California, Davis, CA.

To determine the effect of menstrual cycle on the humoral immune function of normal women, the frequency of immunoglobulin-secreting cells (ISC), complete blood counts and lymphocyte subsets were examined in blood of six healthy female volunteers with normal menstrual cycles over 3 months. We found that the frequency of ISC in the PBMC of women was significantly higher during periovulatory stage of the menstrual cycle compared to the luteal and follicular stages. The observed changes in ISC frequency were not due to changes in T cell subsets, B cells or CD56+ cells in PBMC, as the frequency of these cells did not change during the menstrual cycle. In addition, IgA-secreting cells (IgA-ISC) were 4 fold more frequent than IgG-secreting cells (IgG-ISC) in PBMC. These results demonstrate that the cyclic changes of the endogenous ovarian steroid hormone cycle regulate ISC frequency in PBMC of women. The observed, relatively high, number of ISC in the periovulatory period provides a possible explanation for the fact that women have higher serum Ig levels, are more resistant to viral infections and tend to have more immune-mediated diseases than men.

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OVARIAN LEUKOCYTES: SUBTYPE AND THEIR POTENTIAL ROLES. Cindy Q. Zhou, Jason Borillo, Yahuan Lou University of Texas at Houston Basic Science, Houston, TX.

In recent years, increasing recognition has been given to interactions between the immune and endocrine systems. Leukocytes and some of their products are believed to play important roles in various ovarian functions. We investigated the subpopulation and lineages of the ovarian leukocyte populations with different cell surface markers by immunohistochemistry and flow cytometry. In additional to F4/80+ + α macrophages, which have been recognized for many years, we discovered a CD8 population. In contrast to F4/80+ macrophages, which are mainly located on the + cells are concentrated in the theca layer of α interstitial tissue, the CD8 developing follicles. More importantly, a dramatic change in the number as well + cell during ovulation cycle was observed. The number of α as shape of CD8+ cell peaked at 9 and 2 hrs prior ovulation, coincident with α ovarian CD8 +α reduction in F4/80+ macrophage number. Infiltration of granulose layers by CD8 + cells rapidly decrease in number post α cells was seen during ovulation. The CD8 ovulation. Release of chemokines during ovulation, which was demonstrated by in vitro leukocyte invasion assays, was coincident with the change in the number of + may be involved in the ovulation α+. These observations suggest that CD8α CD8 process. On the other hand, F4/80+ cells are probably related with the repairing and cleaning process.

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METHOD FOR MEASUREMENT OF PERISTALTIC TRANSPORT OF VAGINAL FLUIDS TO THE UTERINE CAVITY: IMPLICATIONS FOR SUB-CLINICAL INFECTION AND ANTI-MICROBIAL INTERVENTION. Anna K. Parsons, Jeanne L. Becker, Jorge L. Londano, Thomas R. Moench, Richard A. Cone University of South Florida, College of Medicine Obstetrics and Gynecology, Tampa, FL.

During vaginal intercourse, STDs are known to be transmitted to women across cervicovaginal epithelium, but not the upper reproductive tract. Evidence suggests, however, that potentially infectious agents within vaginal fluids may be transported to the uterine cavity and fallopian tubes via peristalsis. We sought to (a) confirm that the upper tract is chronically exposed to vaginal fluids, (b) monitor the ascent of these fluids and (c) investigate empirical anti-microbial therapy for potential pathogens in such fluid, in women with mild pelvic pain. We developed a technique for direct visualization of fluid movement, using high frequency vaginal ultrasound (VU). With this method, vaginal fluids, semen and microbicides are labeled with a sonographic contrast agent (Optison) and their presence and movement upon entry into the upper genital tract followed. Using this technique, we observed peristaltic uterine uptake of vaginal fluids, with maximal contractions at midcycle. Visualization of Optison-labeled semen was also observed. We conclude, therefore, that the upper reproductive tract is chronically exposed to small inocula of vaginal fluids, including semen and associated potential pathogens, and that this may limit the interaction of pathogens with a microbicide within the vagina. These findings led us to postulate that such a mechanism could be associated with "sub-clinical" infection in women presenting with pelvic pain. In a series of 102 patients with documented proximal tubal pain on VU assisted palpation, and negative for Chlamydia trachomatis and Neisseria gonorrhoeae, 14-day doxycycline

treatment yielded an overall response rate of 81%, with complete resolution of pain symptoms in 77% and partial improvement in 4% of patients. The highest response rates were observed in the 40-59 year old patient group, considerably outside the traditional "at risk group" for pelvic inflammatory disease associated with recognized STD agents. We further speculate that hormonal influences may affect transport of pathogens to upper tract mucosal sites, since this age group may be relatively hyperestrogenic secondary to ovarian dysfunction occurring during the climacteric. Based upon these collective results, we propose the term vaginally transmitted disease, "VTD", to account for delivery of infectious agents via uterine peristaltic transport of vaginal fluids.

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CD4+CD25+ REGULATORY T CELLS ARE INCREASED IN THE HUMAN EARLY PREGNANCY DECIDUA AND HAVE IMMUNOSUPPRESSIVE ACTIVITY. Yasushi Sasaki, Satomi Miyazaki, Masatoshi Sakai, Shigeru Saito Toyama Medical and Pharmaceutical University Obstetrics and Gynecology, Toyama-shi, Toyama-ken, Japan.

OBJECTIVE: Immunosuppression is important to maintenance for pregnancy. Control of self-reactive T cells by regulatory T (Tr) cells has been proposed to mediate anergy and peripheral tolerance. Recent data showed that regulatory CD4+ T cells express interleukin-2 receptor alpha (CD25) on their surface and suppress the immune responses of T cells through CTLA-4 molecule. In this study, we investigated whether Tr cells are present in the early human decidua and these Tr cells induce peripheral tolerance.
METHODS: The ratio of CD4+CD25+ regulatory T cells per CD4+ T cells (CD4+CD25+ / CD4+) in early pregnancy decidua and peripheral blood were measured by flow cytometory. Furthermore, we purified CD4+ T cells from early pregnancy decidua and separated CD4+CD25+ regulatory T cells from the other CD4+CD25- T cells using magnetic beads and VarioMACS-negative and -positive selection columns. Then, to evaluate suppressive effect of decidual CD4+CD25+ T cells, we cultured CD4+CD25- T cells alone and co-cultured CD4+CD25- T cells with CD4+CD25+ regulatory T cells and stimulated with anti-CD3 antibody and irradiated antigen presenting cells. The proliferative response was measured by [3H] thymidine uptake.
RESULTS AND DISCUSSION: CD4+CD25+/CD4+ in the decidua was significantly higher than that in peripheral blood ($28.0 \pm 1.2\%$ vs. $12.2 \pm 1.7\%$, $p < 0.001$, $n = 19$). Moreover, more than 90% of decidual CD4+CD25+ cells expressed CTLA-4 in their cytoplasm demonstrating that these cells are regulatory T cells. The proliferative response of the CD4+CD25- T cells was significantly suppressed by the addition of not only peripheral blood CD4+CD25+ T cells but also decidual CD4+CD25+ T cells. Decidual CD4+CD25+ regulatory T cells are activated because they expressed CTLA-4 molecules on their surface. On the other hand peripheral blood CD4+CD25+ T cells did not express CTLA-4 on their surface. These results suggest that the increased regulatory T cells in the decidua regulate alloreactive T cell responses and play some important roles for maintenance of pregnancy.

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THE CLINICAL ASSESSMENT OF MATERNAL HIV TESTING IN EARLY PREGNANCY TO PREVENT MOTHER TO CHILD TRANSMISSION IN JAPAN. Kimikazu Hayashi, Tuneo Kita, Yasuyuki Hasuo, Ryouzou Totani, Naoto Yoshino, Yuuichi Wada, Satoshi Hayakawa, Yuuki Tukahara, Masashi Takano, Haruki Taniguchi, Shigeki Minoura, Noriyuki Inaba National Shimonoseki Hospital Obstetrics and Gynecology, Shimonoseki, Japan.

INTRODUCTION: Mother to child transmission (MTCT) of HIV can occur at every stage of pregnancy(antepartum,intrapartum,postpartum).Therefore, Early diagnosis of HIV infection in pregnant women is the best way to prevent MTCT. Maternal HIV testing can be used to find HIV infection in pregnant women in early pregnancy. But all of pregnant women don't have maternal HIV testing during pregnancy in Japan. The goals of this study are to investigate the screening rate of maternal HIV testing in early pregnancy and evaluate its regional difference.
MATERIAL AND METHOD: The questionnaire about maternal HIV testing in early pregnancy was sent to 1,700 hospitals providing obstetric department during 1999-2001. 64.3% 81.6% of these hospitals responded the questionnaire.
RESULT: 248 cases of HIV positive pregnant women were reported from 1987 to 2002 in Japan. 62 cases were recognized in 1999,but 28 cases in 2001. Therefore, 7.9 HIV positive cases were admitted to 100,000 pregnant women in 2001 in Japan. The national average rate of maternal HIV testing was 73.2% in 1999,and it rose to 82.6% in 2001. But the district average rate jn 2001 was as follows. Hokkaido/Tohoku district: 75.0%, Kanto/Koushinetu district: 96.6%, Toukai/Hokuriku district: 90.8%, Kinki district: 79.0%, Tyugoku/Shikoku district: 64.1%, Kyusyu/

Okinawa district : 51.5%. Except for Kanto/Koushinetu and Toukai/Hokuriku district, maternal HIV testing rates in other districts were below the national average.
CONCLUSION: The high average rate (more than 80%) was recognized in Japan about the screening for maternal HIV testing. But district difference of maternal HIV testing rate was accepted remarkably in some districts of the west side in Japan (.Tyugoku/Shikoku district, Kyusyu/Okinawa district). The reason that district difference was caused is because no pregnant woman in these districts really knows her real risk for HIV infection. If all pregnant women take maternal HIV testing during her pregnancy, we can completely reduce MTCT of HIV.

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GRANULYSIN CONCENTRATION IN MATERNAL SERUM IN PREECLAMPTIC PATIENTS. Masatoshi Sakai, Arihiro Shiozaki, Satoshi Yoneda, Yasushi Sasaki, Kazuyuki Ogawa, Tetsuya Nagata, Shigeru Saito Toyama Medical and Pharmaceutical University Obstetrics and Gynecology Toyama, Japan.

OBJECTIVES: Th1 immunity became dominant in preeclamptic patients, when Th1/Th2 ratios in peripheral blood mononuclear cells were determined by flow cytometory. Because the evaluation of Th1/Th2 immunity by flow cytometry is difficult, more easy methods were necessary. Granulysin is known to be a new marker for the Th1 immunity, and it can be easily measured by ELISA. In this study, we examined the granulysin concentration in maternal serum in preeclamptic patients.
MATERIALS AND METHODS: We measured the granulysin concentrations of maternal serum in 17 non-pregnant subjects, 50 normal pregnancy (later than 20 weeks), 21 preeclampsia and 8 pregnancies with proteinuria but not with hypertension. The concentration of granulysin was measured by ELISA method. We compared the granulysin concentrations in each groups, and examined the correlation between the concentration of granulysin and Th1/Th2 cells ratios. In addition, we examined the correlation between the concentration of granulysin and maternal mean blood pressure.
RESULTS: Granulysin concentration (ng/ml) in normal pregnant women (1.9 ± 0.8) was significantly lower than that in non-pregnant subjects (3.0 ± 1.6), ($p = 0.038$). Granulysin concentration in preeclamptic patients (3.3 ± 1.5) was significantly higher than that in normal pregnant women ($p = 0.006$). A significant positive correlation was observed between the concentration of granulysin and Th1/Th2 cells ratios ($r = 0.701$, $p = 0.001$). A significant positive correlation was observed between the concentration of granulysin and maternal mean blood pressure ($r = 0.556$, $p = 0.002$). We found upward tendency in granulysin concentration in severe preeclamptic patients (3.8 ± 1.8) compared with that in mild preeclamptic patients (2.7 ± 0.6), ($p = 0.091$). Both of them were significantly higher than that in normal pregnant women. While, no significant change was found between granulysin concentration in patients with only proteinuria (2.0 ± 0.9) and that in normal pregnant women.
CONCLUSIONS: Granulysin concentration in maternal serum may be a easy and useful marker for detecting Th1/Th2 balance. Elevated granulysin levels in preeclampsia suggests that Th1 type immunity is present in preeclampsia.

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EFFECT OF RECOMBINANT AND ENDOGENOUS MACROPHAGE MIGRATION INHIBITORY FACTOR ON UTERINE NK CELLS ACTIVITY. Felice Arcuri, Paola Viganò, Antonietta Carducci, Stefania Papa, Fabiana Pompei, Roberta Romagnoli, Marcella Cintorino University of Siena Human Pathology and Oncology Siena, Italy.

AIM OF THE STUDY: Macrophage migration inhibitory factor (MIF) is a cytokine highly expressed in human reproductive tissues. We have previously demonstrated MIF expression in the cycling endometrium and in the cytotrophoblasts of both the inner layer of villi and trophoblastic cell islands of first trimester human placenta. Although recent studies have demonstrated an inhibitory action of MIF on circulating NK (CD56+ CD16+) cell-mediated cytolysis, there are not data available on the effect of this cytokine on uterine NK (CD56+ CD16-; uNK) cells. This study was therefore aimed to evaluate the capability of MIF to affect uNK cell cytolytic activity.
METHODS: First trimester human decidua was obtained from elective abortion at 6-12 weeks of gestation. Tissues were minced and digested by collagenase. The cell suspension was overlaid on Lymphoprep and the NK cell layer collected and washed. CD56+ uNK cells were then further purified by magnetic separation using anti-CD56 microbeads. Recombinant human MIF (rhMIF) was produced in E. coli. Cytolytic activity of uNK cells was measured by a standard 4-h 51Cr-release assay. MIF expression in uNK cells was evaluated by RT-PCR, western blot and immunohistochemistry.

RESULTS: Uterine NK cytolytic activity was only partially affected by rhMIF, with a maximum inhibition achieved at the concentration of 10 μg/ml (14.62% ± 11 Vs 18.5% ± 10; n = 10). The synthesis of MIF by purified uNK cells was assessed by RT-PCR analysis, which detected the presence of a band corresponding in size to the MIF product in all the preparations tested. These results were supported by western blot analysis of uNK extract using an anti-MIF antibody, that identified a single band comigrating with rhMIF, and by a double immunostaining procedure that showed intense MIF immunoreactivity in CD56+ cells. Neutralization of the endogenous MIF using a polyclonal antibody resulted in a sharp increase of the cytolytic activity of uNK cells of 36% (30.0 % ± 14.3 Vs 22.1 % ± 15; n = 3).
CONCLUSIONS: These results suggest the existence of a novel paracrine/autocrine mechanism of action of MIF on uNK cells, indicating a previously unrevealed role for this cytokine in the maternal-fetal interactions.

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TRANSCUTANEOUS IMMUNISATION PREVENTS CHLAMYDIA MURIDARUM GENITAL TRACT INFECTION. Linda Berry, Danica Hickey, Katheryn Skelding, Shisan Bao, Phil Hansbro, Kenneth Beagley The University of Newcastle Immunology and Microbiology Newcastle, NSW.

AIM: To determine if transcutaneous immunisation (TCI) with Chlamydial Major Outer Membrane Protein (MOMP) admixed with combination cholera toxin (CT) and CpG adjuvant can protect mice against genital tract challenge with Chlamydia muridarum.
MATERIALS AND METHODS: Female BALB/c mice were immunised 3X at weekly intervals with MOMP (200 ug) admixed with CT (10 ug) and CpG oligonucleotides (10 ug) by direct application to shaved skin. Following immunisation, MOMP-specific antibody (IgG, IgG1, IgG2a, IgA) in serum and vaginal lavage (VL) was determined by ELISA. Antibody in VL was also assayed for inhibition of in vitro chlamydial infection. MOMP-specific antibody-secreting cells in female reproductive tract (FRT) were determined by immunohistochemistry. Cytokine secretion by T cells in FRT-draining caudal and lumbar lymph nodes (CLN) was determined by real time PCR. Mice were treated with progesterone and challenged by intravaginal instillation of C. muridarum. Bacterial clearance was monitored by PCR.
RESULTS: TCI with combination adjuvant induced MOMP-specific IgG and IgA in serum. Both IgG1 and IgG2a were detected indicative of a combination Th1/Th2 response profile. MOMP-specific IgA and IgG (IgG1 > IgG2a) were detected in vaginal lavage and MOMP-specific ASC were abundant in vaginal tissues. In vitro stimulation of CLN T cells from mice immunised with combination adjuvant resulted in a 25-fold increase in MOMP-specific IFN? production compared to T cells from mice immunised with MOMP alone. C. muridarum levels in vaginal swabs from immunised mice at day 12 post-infection were reduced by > 105 compared to controls.
CONCLUSIONS: TCI targets protective anti-chlamydial immunity to the FRT. Using combination CT and CpG adjuvants induces a mixed Th1: Th2 immune response that should provide optimum protection against chlamydial infection. IgA and IgG in vaginal lavage can target extracellular elementary bodies (EB) whilst local Th1 T cell immunity can target intracellular chlamydial infection. Immunostimulatory human CpG motifs have been identified and non-toxic mutant CT is available for human use, therefore TCI may provide an effective method for preventing chlamydial genital tract infection. Supported by grants from The Arnott Foundation, Hunter Medical Research Institute and The University of Newcastle

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THE EFFECT OF HNE IN PREMATURE LABOR WITH CHORIOAMNIONITIS. Kumiko Temma, Koichiro Shimoya, Qing Zhang, Yukinobu Ota, Shoji Kamiura, Fumitaka Saji, Tomoko Tsujie, Toru Kanzaki, Tadashi Kimura, Masayasu Koyama, Yuji Murata Osaka University Graduate School of Medicine Obstetrics and Gynecology Osaka, Japan.

OBJECTIVE: Chorioamnionitis (CAM) is one of the causes of preterm labor. Recent study has indicated NADPH oxidase, reactive oxygen species (ROS)-producing enzyme, is activated in CAM. CAM is thought to be closely associated with oxidative stress. The purpose of this study is to examine the effect of 4-hydroxy-2-nonenal (HNE), which is marker of oxidative stress, on human placenta during preterm labor.
METHODS: HNE-modified proteins in human placentas were investigated by immunoblotting and immunohistochemistry using anti-HNE antibody. To examine the effect of HNE on uterine contraction, we stimulated human placental tissue with HNE. The expression of cyclooxygenase-2 (COX-2) mRNA and protein were observed by RT-PCR and Western blotting, respectively. To determine COX-2 enzyme activity, prostaglangin E2 (PGE2) in the supernatants of placental tissue was determined by ELISA on time-dependent manner.

RESULTS: The levels of HNE-modified proteins were increased in the placenta with CAM, compared to the normal placenta. HNE induced the expression of COX-2 mRNA and protein in the placental tissue. The concentration of PGE2 in the supernatants of placental tissue culture stimulated with HNE was increased on time dependent fashion.
CONCLUSIONS: HNE-modified proteins, which were increased in the placenta with CAM, play an important role in preterm labor.

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DIFFERENTIAL EXPRESSION AND LOCALIZATION OF DECORIN IN HUMAN CHORIODECIDUAL MEMBRANE AT THE THIRD TRIMESTER OF PREGNANCY. Kazuhide Ogita, Hitomi Nakamura, Shinsuke Koyama, Yoko Matsumura, Koichirou Shimoya, Tomoko Tsujie, Mari Tomiie, Masayasu Koyama, Yuji Murata Osaka University Graduate School of Medicine Obstetrics and Gynecology Osaka Japan.

We attempted to characterize the genes that are dynamically regulated at the feto-maternal interface around parturition. Suppression subtractive hybridization (SSH) method was used to compare gene expression patterns of human choriodecidual membranes from elective Cesarean delivery and vaginal delivery at term. Among the subtracted clones, we have selected decorin for further investigation. Northern blotting revealed that the highest decorin expression was observed in 2 of 7 tissues from term elective Cesarean delivery before onset of labor and in 2 of 3 from Cesarean birth in labor. In the tissues from term elective Cesarean birth, the expression levels of decorin varied among samples although the mean level of decorin expression was the same as in the samples after vaginal birth. Low level of decorin mRNA was detected in the choriodecidual membranes from preterm birth. In situ hybridization revealed that decorin transcripts were mainly expressed in the decidual cells in the membrane. Decorin gene expression was also upregulated in the term myometrium. In order to elucidate physiological role of decorin on parturition, we administrated the recombinant human decorin to pregnant mice intravenously. We found neither alteration of the timing of labor nor dystocia. Although the function of decorin during pregnancy in vivo should further be elucidated, the present results suggest that decorin plays a role as an uterotropic factor in choriodecidual membrane at term parturition rather than triggering factor for uterine contraction

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HIV MOTHER-TO-CHILD VERTICAL INFECTION IN JAPAN: A NATIONAL CO-OPERATIVE STUDY REPORT. Totani Rzyozo, Hayashi Kimikazu, Hasuo Yasuyuki, Hayakawa Satoshi, Kita Tsunekazu, Takahashi Shoko, Yoshino Naoto, Wada Yuichi, Tsukahara Yuki, Kasai takeo, Oba Satoru, Togawa Masao obstetrics and gynecology Nagoya National Hospital Nagoya, Aichi.

PURPOSE: This study reports cumulative data concerning the number of HIV infected pregnant females, the achievability of preventative procedures, and the number of HIV infected infants delivered in Japan.
METHOD: A nationwide @survey was conducted by a well-funded research team consisting of obstetrics and gynecologists, pediatricians, epidemiologists, immunologists and virologists.
PROCEDURE: The occurrence in pregnancy of HIV infected females was surveyed nationwide via questionnaire at all hospitals other than private institutions. Of the 1670 surveys sent, responses were received from 1080, indicating a recovery ratio of 64.3%.
RESULT: The first case of a HIV infected pregnant female in Japan was reported in 1987. The survey revealed that, to date (March, 2002) a total of 248 cases of HIV infection occurred nationwide at the respondent hospitals. Of this number, 66 cases of artificial abortion (D & C) were performed, and 159 pregnancies were carried to term. Data on 23 cases could not be ascertained. Of the 159, 130 pregnancies were delivered by caesarean section, and 29 by vaginal delivery. Of the 130, only 2 cases of mother-to-child vertical infection occurred, while of the 29 vaginal deliveries, 19 cases of vertical infection were reported. The survey also revealed that, if, during the antenatal period, a HIV antibody test wasn't performed, the infant was delivered vaginally, and a resulting high incidence of birth of HIV positive infants occurred.
CONCLUSIONS: The results highlight the importance of the administration of a HIV antibody test. However, as the numbers of HIV positive pregnant women in Japan are considered to be extremely few (less than 1 positive test per 10,000 conducted for a total result of 248 positive results), the administration of the HIV antibody test antenatally is coming to be considered of minor importance, in light of the financial cost of its administration and the benefits gained. From 1987 to 2002, approximately 18,000,000 births occurred at an average of 1,200,000 annually.

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INFLUENCE OF HUMAN IMMUNODEFICIENCY VIRUS ON MOTHER-TO-CHILD TRANSMISSION IN JAPAN. Tsunekazu Kita, Kimikazu Hayashi, Noriyuki Inaba, Ryozo Totani, Satoshi Hayakawa, Yuki Tukahara, Yuichi Wada, Masato Togawa, Satoru Ohba, Takeo Kasai, Shigeki Minoura, Haruki Taniguchi National Kyushu Medical Center and National Cooprative Study Group Obstetrics & Gynecology Fukuoka, Japan.

OBJECTIVES: The purpose of this study was to reveal the prevalence of the vertical transmission of HIV from mother to infant in Japan.
METHODS: A questionnaire about pregnant women infected with HIV was sent to 1670 hospitals providing obstetric department. Sixty-four percent of these hospitals responded to this questionnaire and we obtained the details of the perinatal information of the HIV infected pregnant women defined from 1987 to 2002 in Japan.
RESULTS: Totally 248 cases of HIV infected pregnancies were reported. Eighty-three percent of pregnant women were screened by HIV antibody test before their delivery. One hundred and thirty cases were resulted in cesarean delivery and 29 cases were in vaginal delivery. Seventy-one percent of total cases were defined in Kanto-Kosinetsu district around Tokyo metropolitan followed by Hokuriku-Tokai district around Nagoya (10%) and Kinki district around Osaka (10%). Ninety-six (38%) cases were Japanese and 90 (36%) were Thailander. The number of HIV infected pregnancy was increasing through twelve years from 1987, resulting in 40 cases in 1999. The number of Japanese pregnant women was larger than that of Thailander in recent 4 years. Zidovudine or other agents was administered by 61.5% of pregnant women in cesarean delivery group and by on 6.9% of those in vaginal delivery group. Antenatal level of virus RNA was measured in 67 cases from 130 cases of cesarean delivery group. These levels were more than 100,000 copies/ml in 6 (9%) cases and more than 10,000 copies/ml in 20 (30%) cases of 67 cases measured. The vertical transmission was avoided in all cases except ten whose babies were not diagnosed finally for HIV infection. RNA viral loads declined to less than one per cent of the highest levels in 10 (48%) cases and to less than one tenth in 14 (67%) cases by the combination chemotherapy (HAART). On the other hand, RNA viral loads elevated during pregnancy in 4 (57%) cases without anti-retrovirus agents and 5 (29%) cases administered with zidovudine alone.
CONCLUSION: There were signicant differeces of the prevalence between each districts in Japan.

The anti-HIV agents could be effective to avoid the vertical transmission.

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ANDROGEN AFFECTS THE EXPRESSION OF TRANSITION PROTEIN 1 (TP1). Hideaki Sawai, Hiroyuki Kasumi, Nozomi kanazawa, Koji Koyama Hyogo College of Medicine, Dept of OB/GYN Nishinomiya, Hyogo.

AIM: Transition protein1(TP1) plays an important role in condensing the chromosome during spermatogenesis. In the experiment with knockout mice of TP1, a spermatogenic failure was pointed out, suggesting that the expression of TP1 is important for spermatogenesis. On the other hand, androgen (A) is an important factor in development of testis and plays a crucial role in spermatogenesis. Therefore, the androgenic effects via androgen receptor (AR) for expression of TP1 were analyzed in this study.
METHODS: On the basis of a DNA database, the 5' region of TP1 was searched and isolated by using PCR. The isolated 5' region of TP1was cloned into a reporter plasmid with luciferase gene. Human AR genes with various CAG repeats (12, 15 or 22 repeats) were also cloned into expression vectors. These plasmids were cotransfected into COS7 cells by electroporation. Luciferase activities were measured with or without dihydrotestosterone(DHT).
RESULTS: The 5′ region of TP1 gene (920bp) was successfully isolated.) In vitro expression experiments, the increase of luciferase activity was identified in COS 7 cells transfected AR with 22 CAG repeats(wild type). On the other hand, any increase of luciferase activities were not identified in COS 7 cells transfected AR with short CAG repeats of 12 or 15.
CONCLUSION: In this study, we demonstrated first time that both A and the length of CAG repeats of AR affected the expression of TP1. According to DNA sequencing, any responsive elements which reported previously were not present in the 5' region of TP1. However, a DNA region similar to steroid responsive elements was identified. This region seems to be related to the interaction of A-AR complex. In summary, androgen seems to affect spermatogenesis via the expression of TP1.

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STAGE-SPECIFIC EXPRESSION OF IL-18 AND IL-18 RECEPTOR MRNA IN GERM CELLS DURING SPERMATOGENESIS, AND DETERIORATION OF SPERM QUALITY IN IL-18 GENE-DEFICIENT MICE. Yoshiyuki Tsuji, Nozomi Kanazawa, Akiko Hasegawa, Haruki Okamura, Tomoko Hashimoto Tamaoki, Koji Koyama Hyogo college of medicine Department of Obstetrics and Gynecology Nishinomiya, Hyogo.

The reproductive and immune systems are closely related through various cytokines. Inflammation is believed to cause impairments of the male as well as the female reproductive system. IL-18, an interferon-inducing Th1 cytokine, is strongly involved in various inflammations. Recently we reported IL-18 plays an important role in the maturation of the ovum. Here we investigated whether IL-18 is also involved in spermatogenesis. Using a highly sensitive in situ hybridization method, we found that both IL-18 and IL-18 receptor mRNAs were expressed in testicular germ cells. IL-18 mRNA was clearly detected in primary spermatocytes, but its levels were much reduced in spermatids. On the other hand, IL-18 receptor mRNA was found in spermatids but not in primary spermatocytes. Caspase-1 which converts 24-kDa precursor IL-18 to the 18-kDa active mature form was coexpressed with IL-18 receptor mRNA in spermatids. This suggests that expression and activation of IL-18 is closely related to spermatid maturation process. Histopathologic examination of testes of IL-18 knockout mice showed a decrease in the number of spermatids, and the number, motility and insemination ability of sperm in IL-18 knockout mice were generally lower than those in wild-type mice. These results suggest that, in the absence of IL-18, spermatogenesis may become unstable, resulting in impairment of sperm production and deterioration of sperm quality.

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DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO TROPHOBLAST-LIKE CELLS. Ido Solt, Hanna Segev, Joseph Eldor Itskovitz Rambam Medical Center Obstetrics and Gynecology Haifa, Israel.

AIM: Trophectoderm, the precursor of the trophoblast is the first lineage to differentiate in the mammalian preimplantation embryo. It plays a crucial role in implantation and placentation. However, our knowledge of this early and crucial process in human pregnancy is limited. Our aim was to study the human embryonic stem cell differentiation to trophoblast-like syncytium.
MATERIALS AND METHODS: In order to keep the human embryonic stem cells in undifferentiated mode, the human embryonic stem cells were grown on a mouse embryonic fibroblast feeder layer. To allow spontaneous differentiation, the human embryonic stem cells were removed from the feeder layer and were plated on gelatin substratum for 30 days. The expression of beta-hCG was measured using microparticle enzyme immunoassay in a conditioned medium from the cells, one day after plating on gelatin substratum (1.1 ± 0.1 mIU/ml) and after 30 days of differentiation on substratum (8.8 ± 0.6 mIU/ml). One-way analysis of variance test showed significant difference in protein expression between the two groups [$F = 18.17$, $P = 0.001$, $n = 9$]. High level of beta-hCG indicates continuing trophoblast differentiation. Using Reverse-transcription PCR, we compared the expression of GnRH, GnRH receptor and chorionic gonadotropin-alpha in 1-week-old embryoid bodies compared to 1-month-old embryoid bodies and in undifferentiated human embryonic stem cells. Another batch of human embryonic stem cells were grown for a week with or without fibroblast growth factor 4 and then another week without it. The cells were stained by immuno-fluorescence with antibodies against human chorionic gonadotropin and human placental lactogen as well as with dapi nuclear staining.
RESULTS: GnRH and GnRH-receptors were noticed in week-old and increased in month-old embryoid bodies while chorionic gonadotropin-alpha was only noticed in month-old embryoid bodies. These genes were not expressed in undifferentiated human embryonic stem cells. Immuno-fluorescence and dapi nuclear stains showed fusion of cells to syncytium.
CONCLUSION: This study examined the potential of human embryonic stem cells to differentiate to trophoblast-like syncytium in vitro. Embryoid bodies expressed GnRH, GnRH-receptors and chorionic gonadotropin-alpha. These genes were not expressed in undifferentiated human embryonic stem cells. Human embryonic stem cells grown with fibroblast growth factor 4 demonstrated syncytium formation with immuno-fluorescence and dapi stains.

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SYSTEMIC CYTOKINE RESPONSES TO PATERNAL AND VACCINATION ANTIGENS IN PREECLAMPSIA: NO DIFFERENCES COMPARED WITH NORMAL PREGNANCY. Yvonne Jonsson, Christina Ekerfelt, Göran Berg, Katri Nieminen, Surendra Sharma, Jan Ernerudh, Leif Matthiesen Linköping University Department of Molecular and Clinical Medicine, Division of Clinical Immunology Linköping, Sweden.

AIM: We have previously shown with ELISPOT that the production of IL-4 from PBMCs in response to paternal antigens is increased, while the production of IFN-γ is unaltered in women during normal pregnancy compared with non-pregnant controls. Furthermore, we have shown that the secretion in blood of both IL-4 and IFN-γ in general is increased during normal pregnancy. The aim of this study was to examine if

there are differences in the cytokine pattern (IFN-γ, IL-4, IL-10 and IL-12) in women with preeclampsia compared to women with normal pregnancies.
MATERIALS AND METHODS: Freshly isolated mononuclear cells were analyzed by ELISPOT. The secretion of IFN-γ, IL-4, IL-10 and IL-12 was measured spontaneously and with one-way cytokine-MLC, utilizing inactivated paternal PBMCs to detect "fetus-specific" cytokine secretion. Furthermore, PPD and TT were used as Th1 and Th2 inducing antigens, respectively, in order to measure IFN?γ, IL-4 and IL-10. LPS was used to stimulate IL-12 secretion. Samples were collected in the third trimester from 15 women with preeclampsia and 15 women with normal pregnancies. Preeclampsia was diagnosed according to WHO criteria.
RESULTS: Neither spontaneous, "fetus-specific" nor antigen-specific secretion of IFN-γ, IL-4, IL?10 and IL-12 differed between women with preeclampsia and normal pregnancies.
CONCLUSION: Our data indicate that no significant differences are obvious in the systemic cytokine profile from women with preeclampsia compared with normal pregnancies. However, this does not exclude the possibility of disease-associated changes in the local immune responses and cytokine balance.

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MONOCYTE CHEMOTACTIC PROTEIN-3 DOWNREGULATION IN EARLY MOUSE PREGNANCY AND MIFEPRISTONE(RU486) INDUCED UPSURGE – A PLAUSIBLE PATHWAY FOR RU486 INDUCED ABORTION. Jaya Nautiyal, Pradeep G. Kumar, Malini Lalorayap Molecular Reproduction Unit Indore.

The survival of an embryo bearing the paternal antigens within the immunocompetent environment of the maternal uterus renders 'pregnancy' to be a state of immunological paradox. MCP3, a pro-inflammatory, CC chemokine elicits significant anti-tumoral immune response because of its ability to recruit a wide range of lymphoid cells. The idea behind the following study was to investigate the expression of MCP3 in the murine uterine tissue and embryo at different days of initial pregnancy and its regulation by ovarian steroids. SDS-PAGE and Western Blotting were performed using cellular protein lysates from uteri of different days of pregnancy as well as hormone treated uteri using immature & delayed implantation models. The separated proteins were electroblotted onto PVDF membrane and probed with the MCP3 (monoclonal) antibody, later incubated with Biotinylated Goat Anti Mouse IgG/Streptavidin-Alkaline Phosphatase conjugate followed by colour development using BCIP/NBT. Paraffin sections of pregnancy and hormone primed uteri and flushed embryos were used for immunocytochemistry studies using MCP3 and FITC conjugated antibodies subsequently. The results were documented using Nikon Epi-fluorescence microscope, Cool Snap camera and Documentation System from the Alpha InnCorp., USA. All experiments had 5 replicates & the results were subjected to a Skewed, 1-tailed t-test. Our results show that an elevated MCP3 expression is maintained at the preimplantation Day 3 (10am) and Day 4 (10am) stages. There is a down-regulation in MCP3 expression at the peri-implantation Day 5 (5am) stage. A mellowed down profile is maintained at the post-implantation Day 5 (10am) and Day 6 (10am) stages. The implanting embryo also exhibits lowered MCP3 expression. Progesterone and Estrogen maintain low levels of MCP3 expression while Ru486 which is an effective progesterone antagonist and a potent contraceptive when given in competitive mode with progesterone led to drastic surge in MCP3 expression in both immature mice and delayed implantation models. We suggest that down-regulation of MCP3 is important for the success of pregnancy, rendering immune tolerance during the implantation process. We propose that the crosstalk between Ru486 and amplified MCP3 expression may be one of the mechanisms by way of which RU486 performs its abortificient and anti tumor role.

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PROLACTIN (PRL) UP-REGULATES IDO (INDOLEAMIN-2,3-DIOXYGENASE) EXPRESSION OF PERIPHERAL CD14 + MONOCYTES. Satoshi Hayakawa, Miki Karasaki-Suzuki, Yoshiaki Ohta, Yukie Matsumoto, Maho Kondo-Ozawa, Tatsuo Yamamoto, Tadao Tanaka Jikei University of Medicine Department of Obstetrics and Gynecology Tokyo, Japan.

PURPOSE AND BACKGROUNDS: IDO is one of the enzymes in Tryptophan metabolism. Recently its roles for successful pregnancy have been reported by several groups. IDO protects developing fetuses from maternal immune responses by suppression of allo-mediated T cell responses. In humans, IDO is expressed in both early pregnancy deciduas and term placentae, but the mechanisms of its expression is not well clarified. IDO is induced by type 1 cytokines i.e. IFN-g and TNF-a. In this study we examined effects of PRL on IDO expression of peripheral CD14 + monocytes because PRL receptor shares common structure with type-1 cytokine receptors and common signal transduction.

METHODS: Peripheral blood sample were obtained from 6 non-pregnant women. Informed consent was obtained before sampling. Mononuclear cells were prepared by gradient centrifugation and cultured for 2 days in the presence or absence of stimulations (INF-γ 5-100 U/ml, M-CSF and/or PRL 10–500 ng/ml). Intracellular expression of IDO was estimated by flow cytometry in order to specify which cell type is the source of IDO.
RESULTS: IDO was mainly produced by peripheral CD14+ cells. INF-γ induced IDO expressions in peripheral blood mononuclear cells in a dose dependent manner This induction was independent of M-CSF. Of interest, supra-physiological concentrations (>100 ng) PRL up-regulated IFN-g induced IDO expression while PRL alone showed weak IDO induction. Effective concentrations of PRL were comparable to serum levels of normal pregnancy.
CONCLUSION: We discovered novel roles of PRL on IDO expression. Increased level of peripheral blood PRL during pregnancy may protect fetal allograft by suppression of type-1 cytokine activated CTLs.

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CYTOKINE PROFILES IN HUMAN DECIDUA BEFORE AND AFTER LABOUR. Pernilla Hummerdal, Charlotte Lidström, Leif Matthiesen, Christina Ekerfelt, Göran Berg, Surendra Sharma, Jan Ernerudh Univesity Hospital Molecular and Clinical Medicine, Clinical immunology Linköping, Sweden.

PROBLEM: Inflammatory cytokines stimulate prostaglandin production, which leads to myometrial contractility and thereby triggering the onset of labour. In order to understand the role of the maternal immune response in parturition it is relevant to study the local responses in the feto-placental unit. The aim of this study was to investigate if there was a difference in the secretion of IL-4, IFN-Y, IL-10, TGF-b, and TNF-a by decidual mononuclear cells before and after labour.
METHODS OF STUDY: Decidual tissue is collected from healthy women undergoing elective cesarean section before the onset of labour and from women undergoing normal vaginal deliveries. Decidual tissue is removed from placenta and mononuclear cells are isolated by gradient centrifugation and enriched by a immunomagnetic cell separation. Cytokine secretion is detected by a sensitive enzyme-linked immunosorbent spot-forming cell (ELISPOT) assay.
PRELIMINARY RESULTS: The decidual preparation showed a high purity of leukocytes (median proportion 97%). Cells secreting IL-4 (median 18, range 11–74.5), IFN-g (median 49, range 11–116), IL-10 (median 138, range 18–385), TGF-b (median 11, range 3–22), and TNF-a (median 740, range 97–991) were detected in all decidua samples from women undergoing elective cesarean section (n = 5). The leukocytes present in the decidua from women undergoing elective cesarean section were T-cells (range 36–47%), NK-cells (range 55–66%), macrophages (range 1.5–15%), and NKT-cells (range 10–12%). So far only one sample from vaginal delivery has been investigated.
CONCLUSION: So far data are not conclusive. The method proves ability to achieve a pure population of decidual mononuclear cells that show measurable levels of spontaneously secreted cytokines.

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EFFECTS OF SOLUBLE HLA-G1 ON HUMAN DECIDUAL NATURAL KILLER CELLS. Andreas Schaumann, Justine S. Fitzgerald, Maryse Aguerre-Girr, Philippe Le Bouteiller, Udo R. Markert Friedrich-Schiller-University Dept. Obstetrics, Placenta-Lab Jena, Germany.

OBJECTIVES: HLA-G1s is produced by fetal trophoblast cells in the placenta. Aim of this investigation was to analyze the capacities of HLA-G1s to regulate IL-2-induced stimulation of natural killer (NK) cells isolated from the human decidua.
METHODS: HLA-G1s was produced by the cell line 221-G1s and isolated from supernatants. NK cells were isolated from the decidual layer of human placenta and cultivated for 24 h with or without IL-2 stimulation. Cultures were supplemented with various concentrations of HLA-G1s. By using flow cytometry, NK cells were analyzed for expression of CD25 and CD71. For investigation of cytotoxity of NK cells K562 cells were used as targets and stained with CFSE and DAPI for flow cytometry. Additionally, NK cells were analyzed for expression of signal transducer and activator of transcription 3 (Stat3) by polyacrylamid-gel electrophoresis and Western blotting.
RESULTS: In NK cells, IL-2 increases CD25, CD71 and Stat3 expression as well as cytotoxitys. The expression of CD71, cytotoxity and the expression of STAT3 decreased dose dependently when HLA-G1s was added to stimulated cultures in concentrations between 1.6 μg/ml–0.16 ng/ml.
CONCLUSION: HLA-G1s is involved in the regulation of NK cells in the decidua. Stat3 activation is reported to be correlated with perforin expression. Regulation of Stat3 may be a major mechanism responsible for reduced cytotoxicity of decidual NK cells.

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A BIODEGRADABLE MICROSPHERE FORMULATION FOR TARGETING MUCOSAL VACCINES TO M CELLS. Mark E. Keegan, Thomas Leung, W Mark Saltzman Yale University Biomedical Engineering New Haven, CT.

Stimulation of immune responses at mucosal sites is a desirable property for vaccines, particularly those for sexually transmitted diseases. Recent animal studies have demonstrated that oral administration of antigen encapsulated within biodegradable microspheres produces disseminated mucosal immune responses. A limitation of oral immunization with biodegradable microspheres is low efficiency; the vaccine dose required to induce an immune response to orally administered microspheres is typically much higher than that required when the microspheres are administered by injection. The ability to target vaccine-carrying microspheres to the relevant cells for uptake in the intestine (M cells) could potentially increase the efficiency of these orally administered vaccines. It is hypothesized that by coating microspheres with ligands that show M cell-specific binding, the microspheres will have an increased probability of uptake by the M cells and exposure to the appropriate cells of the immune system. We have developed a poly(lactic-co-glycolic acid) (PLGA) microsphere formulation that allows for direct chemical conjugation of amine-containing molecules (such as proteins) to the microsphere surface. The primary-amine-bearing fluorescein analogue 5-(aminoacetamido)fluorescein was conjugated to PLGA microspheres of approximately 1 micron in size. The extent of fluorophore conjugation to the microspheres was measured by flow cytometry, and found to be similar to that obtained by fluorophore conjugation to the surface of carboxylated polystyrene microspheres of similar size. Human IgA and the lectin Ulex europaeus agglutinin 1 were also conjugated to the PLGA microspheres, and protein binding was verified by immunocytometry. The durability of the covalent linkage between ligands and the PLGA microsphere surface was determined by incubating the fluorophore-conjugated microspheres in phosphate buffered saline at 37°C for a period of 40 days. Observed fluorescence loss from the surface of the microspheres was less than 10 percent during this period. These experiments demonstrate that this PLGA microsphere formulation is a biodegradable candidate vaccine vehicle with the capability to stably bind ligands to the microsphere surface for an extended period of time.

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SUCCESSFUL INHIBITION OF HLA-G PRODUCTION IN JEG-3 CHORIOCARCINOMA CELLS BY RNA INTERFERENCE. Tobias Wengenmayer, Tobias Pöhlmann, Udo R. Markert Friedrich-Schiller-University Dept. Obstetrics, Placenta-Lab Jena, Germany.

OBJECTIVES: HLA-G plays a major role in escape of trophoblast cells from maternal cytotoxicity. Unless its malignant transformation, Jeg-3 choriocarcinoma cell line maintained the capacity of HLA-G production. For the analysis of function and mechanisms of HLA-G induced immune regulation, a human cellular model with suppressed HLA-G would be very helpful. The RNA interference (RNAi) method potentially provides this possibility.
METHODS: At 50% confluence, Jeg-3 cells were treated with RNAi oligonucleotides. Oligonucleotides were designed to interfere exclusively with HLA-G mRNA as confirmed by a PubMed blast. Scrambled oligonucleotides were designed to not interfere with any known human protein. Oligonucleotides were applied at different concentrations. After 36 hours, the HLA-G content in Jeg-3 cells was analyzed by Western blots.
RESULTS: Applying the described methods the cellular content of HLA-G was oligonucleotide dose dependently reduced as assessed in several independent Western Blots.
CONCLUSION: A slight remaining cellular content of HLA-G may be due to a long time turnover of HLA-G. By using the RNAi method HLA-G production in Jeg-3 cells can be almost completely blocked. To our knowledge, these cells represent the first possibility to investigate and compare HLA-G competent cells with their deficient analogue. Such cells may be used for any further investigation on HLA-G effects.

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PREGNANCY OUTOCOME: ASSOCIATION WITH ANTI-LAMININ-1 ANTIBODY IN HUMAN AND IN IMMUNIZED MICE. Akane Kondo, Shelly Tartakover Matalon, Miri Blank, Junko Inagaki, Kazuko Kobayashi, Tatsuji Yasuda, Kouji Aoki, Takao Koike, Yehuda Shoenfeld, Eiji Matsuura, Tsunehisa Makino Tokai University School of Medicine Department of Obstetrics and Gynecology Kanagawa, Japan.

OBJECTIVES: Laminin-1, a glycoprotein that provides an integral part of the structural scaffolding of basement membranes, has an important role during embryonic development. Recently, we have reported

that there is a significant association of anti-lamiinin-1 autoantiboides and recurrent abortion. Following the observation, we also found some infertility patients with endometriosis show high titers of IgG anti-laminin-1 autoantibodies. In the present study, we demonstrate that IgG anti-laminin-1 autoantibodies may affect on fetal development in anti-laminin-1 immunized mice. Further, monoclonal anti-laminin-1 antibodies were established from those mice.
MATERIALS AND METHODS: We immunized BALB/c mice with laminin-1 to establish mice model having anti-laminin-1 autoantibodies. Immunized mice was evaluated on their pregnancy outcome and their placentas. ELISA and Western blot was performed to analyze specificity of IgG monoclonal anti-laminin-1 autoantibodies, which were established from those immunized mice.
RESULTS: High titers of anti-laminin-1 antibodies were detected in mice immunized with laminin-1 and those mice were less prone to have a successful pregnancy after mating, as compared with those not having high antibody titers. Further, mice having high titer of anti-laminin-1 antibodies had a higher incidence of fetal resorption and reduced mean weight of their placentas and embryos, as compared with those of the control (non-immunized) mice. IgG monoclonal anti-laminin-1 autoantibodies were established from the immunized mice and it was confirmed that monoclonal antibody is definitely specific to a purified Laminn-1 molecule, by ELISA and Western blot analysis.
CONCLUSION: All of these results suggest that of anti-laminin-1 antibodies have an in vivo pathogenic role on pregnancy outcome. Besides, we are further characterizing epitopic structures recognized by those antibodides, using a phage library.

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EARLY PREGNANCY AND IFN-TAU STIMULATE THE EXPRESSION OF CYCLOOXYGENASE-2 AND GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IN THE BOVINE UTERUS. Vincent Emond, Joe A. Arosh, Sarah Kimmins, Michel A. Fortier, Leslie A. MacLaren, Raymond D. Lambert Université Laval Centre de Recherche en Biologie Ste-Foy, QC.

AIM: We have previously reported that the expression of cyclooxygenase(COX)-2 and/or granulocyte-macrophage colony-stimulating factor (GM-CSF) are stimulated in bovine immune and non-immune cells in vitro in response to interferon-tau (IFN-tau) and prostaglandin(PG) E2. The goal of the present study was to verify in vivo the effect of the estrous cycle, pregnancy and IFN-tau on the expression of COX-1, COX-2 and GM-CSF in the bovine uterus.
METHODS: In the first experiment (XP#1), uteri from heifers (n = 3 per day) were collected at days (d) 0, 7, 16 and 18 of the estrous cycle (C), and at d7, 16, 18, 21, 24 and 30 of pregnancy (P). In the second experiment (XP#2), cyclic cows were treated (intra-uterine injections) with either vehicle (n = 3) or IFN-tau (n = 3) before slaughter at d16. Immunohistochemistry was performed on cryosections from XP#1 and on paraffin-embedded samples from XP#2. Image analysis was used to evaluate the optical density of the red staining in the luminal epithelium (LE), subepithelial stroma (S) and the conceptus.
RESULTS: COX-2 and GM-CSF were localized in the conceptus, LE, glandular epithelium (GE), S and in blood vessels, whereas COX-1 was only expressed in leucocyte-like cells of the subepithelial S. Staining for COX-2 and GM-CSF is increased during pregnancy in both the LE and S. However, in response to intra-uterine injections of IFN-tau, COX-2 was upregulated only in the LE of the ipsilateral horn and GM-CSF was moderately enhanced in the LE of both uterine horns.
CONCLUSION: These results suggest that the conceptus, in part through IFN-tau synthesis, stimulates the expression of COX-2 and secretion of GM-CSF in the endometrium, thus orchestrating accommodation mechanisms during the elongation/attachment period in the cow. Indeed, PGE2, produced downstream of COX-2, is proposed to play important roles during the establishment of pregnancy, delaying luteolysis, exerting immunomodulation, and preparing the endometrium for attachment, whereas GM-CSF promotes the survival and development of the conceptus.

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POLARIZED RAT UTERINE EPITHELIAL CELLS RELEASE THE CHEMOKINE MIP3-ALPHA AND TNF-ALPHA IN RESPONSE TO *E. COLI* AND PATHOGEN ASSOCIATED MOLECULAR PATTERNS. Mardi A. Crane-Godreau, Charles R. Wira Dartmouth Medical School, Lebanon, NH.

PROBLEM: We have previously shown that MIP3-alpha, chemotactic to bone marrow derived immature dendritic cells, B cells and memory T cells, is produced by polarized rat uterine epithelial cells in culture. Here we ask if MIP3-alpha and TNF-alpha are released by uterine epithelial cells in response to both live and heat killed E. coli as well as pathogen associated molecular patterns (PAMP) which are known TLR2 and TLR4 agonists.

METHOD OF STUDY: Uterine epithelial cells from adult Lewis rats were cultured in NUNC cell culture inserts in F12K plus 10% fetal bovine serum (complete medium) and grown to confluence as determined by transepithelial resistance. Polarized cell cultures were treated apically with live or heat killed E. coli (10(3) to 10(5) CFU) in saline as well as with lipoteichoic acid (LTA), lipopolysaccharide (LPS), Pam3Cys or ultra-pure LPS in complete medium (1–1000 ng/ml). Basolateral media were collected after 24 hr incubation and assayed by ELISA for the presence of MIP3-alpha and TNF-alpha.
RESULTS: Live and heat killed E. coli placed in the apical chamber of polarized epithelial cells stimulate the release of TNF-alpha and MIP3-alpha into the basolateral media. To more fully define the nature of this stimulatory effect, known PAMP were placed in the apical chamber for 24 hr prior to cytokine measurement of basolateral chamber media. LTA, LPS, Pam3Cys and ultra-pure LPS, placed in the apical chamber for 24 hr, significantly increased the basolateral release of TNF-alpha (1–2 fold). When media were assayed for MIP3-alpha, increases of up to 10 fold were seen. In contrast, E. coli and PAMP had no effect on viability/integrity, as measured by transepithelial resistance.
CONCLUSIONS: These studies demonstrate that E. coli and PAMP have direct effects on uterine epithelial MIP3-alpha and TNF-alpha production without affecting epithelial cell integrity. Further, these findings suggest up regulation of MIP3-alpha and TNF-alpha is mediated through TLR2 and TLR4 on rat uterine epithelial cells. A better understanding of bacteria and epithelial interactions in the uterus should provide incite into both the innate and adaptive immune systems present in the female reproductive tract. Supported by NIH Grant AI-13541.

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MECHANISM OF HYPOXIC DOWN REGULATION AND EFFECT OF PRO-INFLAMMATORY CYTOKINES ON HUMAN PLACENTA GROWTH FACTOR (PLGF) GENE EXPRESSION. Debbie Mukherjea, Donald S. Torry Southern Illinois Unviersity School of Medicine Medical Microbiology and Immunology Springfield, IL.

BACKGROUND: PlGF is an angiogenic growth factor predominantly expressed by trophoblast during normal pregnancy. PlGF may be an important regulator of angiogenesis and trophoblast function during pregnancy. Local placental bed hypoxia, increased pro-inflammatory cytokines and increased endothelial nitric oxide synthase (NOS) activity have been implicated in preeclampsia, one of the leading causes of maternal and fetal morbidity and mortality. PlGF protein and mRNA levels are significantly decreased in preeclampsia and hypoxia down regulates trophoblast PlGF expression in vitro. However, little is known regarding the regulatory mechanisms of PlGF expression in trophoblast. Our long term goals are to study the regulation of PlGF expression by proinflammatory cytokines and to elucidate the molecular mechanisms of hypoxia-mediated down regulation of PlGF expression in trophoblast.
METHOD: JEG-3 choriocarcinoma cells were treated with the proinflammatory cytokines TNF-α and IFN-γ in dose and time dependent manners. Syncytiotrophoblast were treated with inhibitors of NOS and hypoxia inducible factor-1 (HIF-1alpha) individually and in combination during hypoxic culture conditions for 24 hours. PlGF mRNA expression was determined by northern blot and semi-quantitative reverse transcriptase-PCR.
RESULTS: Proinflammatory cytokines TNF-α and IFN-γ transiently down regulate PlGF expression by approximately 60% at 1 hour and expression returns to normal by 6 hours. Hypoxia increased NOS expression in the trophoblast and inhibition of NOS activity during hypoxia blocked the decrease of PlGF expression. Inhibition of NOS activity in combination with inhibiting HIF-1alpha activity led to down regulation of PlGF mRNA.
CONCLUSION: We show that TNF-α and IFN-γ down regulate trophoblast PlGF expression. Hypoxic down regulation of PlGF expression is mediated by increased nitric oxide production which acts possibly by inhibiting the binding capacity of HIF-1alpha to its responsive elements in the PlGF promoter. These studies help clarify the regulation of PlGF gene expression in trophoblast and may contribute to understanding trophoblast and endothelial cell dysfunction in perfusion compromised pregnancies like preeclampsia. (Supported by NIH HD36830, Excellence in Academic Medicine Grant, SIUSOM, 2002).

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HUMAN IMMATURE DENDRITIC CELLS EXPRESS A FUNCTIONAL MEMBRANE ESTROGEN RECEPTOR. David C. Gondek, Paul M. Guyre, Charles R. Wira, John E. Connolly Dartmouth Medical School, Immunology and Microbiology Lebanon, NH.

Dendritic cells (DC) are the most potent antigen-presenting cells (APC) and play a pivotal role in development and maintenance of the induction, effector and memory phases of the immune response. The influence of estrogen on cells of the immune system can

be generally broken down into genomic and non-genomic effects. The genomic effects of estrogen are mediated by interaction with its nuclear hormone receptors subunits, alpha and beta (ERa and ERb). Non-genomic effects are far more rapid, demonstrating calcium flux and mitogen activated protein kinase (MAPK) activation within minutes to seconds after estrogen exposure. We demonstrate that monocyte derived human iDC are able to specifically bind membrane impermeable E2 conjugates. This binding is lost with upon maturation of the dendritic cell. Membrane ER engagement on immature dendritic cells leads to an activation of cellular signaling pathways including a rapid dose dependent calcium flux in response to treatment with both E2 as well as membrane impermeable E2 conjugates. These observations indicate that immature human dendritic cells express a functional membrane estrogen receptor and provide a mechanism by which estrogen may modulate dendritic cell maturation and function.

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ENDOGENOUS UTERINE MAST CELL DEGRANULATION MODIFIES CONTRACTILITY OF ISOLATED MYOMETRIUM FROM PREGNANT WOMEN. Egle Bytautiene, Yuri P. Vedernikov, George R. Saade, Roberto Romero, Robert E. Garfield University of Texas Medical Branch, Galveston OB/GYN, Galveston, TX.

AIM OF THE STUDY: Mast cells (MC) are the tissue-based effectors in type I hypersensitivity reactions. Preformed and newly synthesized mediators released upon mast cell degranulation have been shown to induce smooth muscle, including myometrial, contraction. This study was designed to test the hypothesis that endogenous mast cell degranulation could alter contractility of myometrial tissues from pregnant women in vitro.

MATERIALS AND METHODS: Longitudinal myometrial strips (10mm x 3mm) from the lower uterine segment of preterm and term laboring and nonlaboring women undergoing elective cesarean section were hung in an organ bath for isometric tension recording. Responses to compound 48/80 (MC degranulating agent) were compared in the absence or presence of cromolyn (inhibitor of MC degranulation), S(+)-chlorpheniramine maleate (H1-receptor antagonist), cimetidine (H2-receptor antagonists), nordihydroguaretic acid (cyclooxygenase and lipoxygenase inhibitor), ibuprofen (cyclooxygenase inhibitor), linoleyl hydroxamic acid (lipoxygenase inhibitor), or solvent. Changes in the contractile activity over baseline were expressed in percentage. Student's t tests were used for paired and unpaired comparisons, as appropriate (significance: $P < 0.05$).

RESULTS: Endogenous mast cell degranulation by compound 48/80 increased spontaneous contractile activity in myometrial strips from pregnant human uterine tissue. The mast cell stabilizer (cromolyn) inhibited this effect in all preterm and term laboring tissues. H1-receptor antagonist suppressed the contractile effect induced by mast cell degranulation only in term laboring tissues, while cyclooxygenase inhibitor did so in preterm nonlaboring tissues. Lipoxygenase inhibitor significantly reduced responses in term labor tissues and augmented the responses in preterm tissue. The H2-receptor antagonist, as well as a combined cyclooxygenase and lipoxygenase inhibitor, did not influence uterine strips' responses to compound 48/80. There was no statistical difference in the responses to endogenous mast cell degranulation between preterm and term, laboring and nonlaboring tissues.

CONCLUSIONS: Endogenous mast cell degranulation stimulated preterm and term human myometrial tissue contractility that was abolished by cromolyn. To this challenge, human myometrium responded similarly throughout the pregnancy. Inhibition of mast cell degranulation, or the effects of their mediators, could be important in preventing increased uterine contractility in pregnancy due to type I hypersensitivity reactions.

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ANTIGEN CHALLENGE INCREASES CONTRACTILITY OF UTERINE AND CERVICAL STRIPS FROM SENSITIZED TERM PREGNANT GUINEA PIGS. Egle Bytautiene, Yuri P. Vedernikov, George R. Saade, Roberto Romero, Robert E. Garfield University of Texas Medical Branch, Galveston, OB/GYN, Galveston, TX.

AIM OF THE STUDY: Since labor has been likened to a type I hypersensitivity reaction, our objective was to study the effect of ovalbumin challenge on uterine and cervical contractility.

MATERIALS AND METHODS: Hartley strain pregnant (day 41-43) guinea pigs were sensitized by the injection of an ovalbumin - aluminum hydroxyde suspension intraperitoneally and subcutaneously into lumbar and neck areas. Control animals were injected with the aluminum hydroxyde suspension only. Two weeks after sensitization, acute cutaneous anaphylaxis on the shaved back of each guinea pig was performed with a minimal amount of antigen (10 microgram of ovalbumin) to test animal sensitivity. On day 55–57 of pregnancy, longitudinal uterine and cervical strips

(10mm × 3mm) from sensitized and nonsensitized guinea pigs were hung in organ baths for isometric tension recording. Responses to ovalbumin (150 microgram/ml) were compared in the absence or presence of cromolyn (inhibitor of mast cell degranulation), S(+)-chlorpheniramine maleate (H1-receptor antagonist), nordihydroguaretic acid (cyclooxygenase and lipoxygenase inhibitor), ibuprofen (cyclooxygenase inhibitor), BW-B 70C (lipoxygenase inhibitor). Changes in integral activity over 10 min after application of the ovalbumin were expressed as percentage of the basal activity. Student's t-test and one way ANOVA were used for statistical analysis (significance: $P < 0.05$).

RESULTS: Intracutaneous antigen challenge produced an edema and erythema in all sensitized animals. There was no reaction in the skin of nonsensitized animals. Ovalbumin significantly increased contractility of uterine and cervical strips from sensitized versus nonsensitized animals. H1-receptor antagonist abolished ovalbumin-induced increase in contractile activity in uterine and cervical strips from sensitized guinea pigs. Ovalbumin-induced increase in contractile activity was abolished by cromolyn only in cervical strips from sensitized animals. None of the other inhibitors had a significant effect on the response to ovalbumin.

CONCLUSIONS: Challenge with specific antigen, ovalbumin, stimulates contractility of uterine and cervical tissue via histaminergic H1-receptors from sensitized, but not control nonsensitized animals. A type I hypersensitivity reaction may result in preterm labor and delivery. Inhibition of mast cell degranulation may be a potentially useful tocolytic strategy in such cases.

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EPITOPE ANALYSIS FOR HUMAN SPERM-IMMOBILIZING MONOCLONAL ANTIBODIES, MAB H6-3C4, 1G12 AND CAMPATH-1. Shinji Komori, Akiko Hasegawa, Hideaki Sawai, Koji Koyama Hyogo College of Medicine, Department of Obstetrics and Gynecology Nishinomiya, Japan.

AIM OF STUDY: Previously, we produced a human monoclonal antibody, MAb H6-3C4, possessing strong sperm-immobilizing activity from an infertile woman. MAb H6-3C4 has been suggested to react with a carbohydrate moiety of the male reproductive tract CD52 (mrtCD52) by several research groups. CD52 is a kind of glycosylphosphatidylinositol (GPI) anchor protein occurring in lymphocytes and the male reproductive tract including sperm and seminal plasma. Male reproductive tract CD52 (mrtCD52) has been shown to contain a unique N-linked carbohydrate that does not cross-react with other tissues. In the present study, we analyzed the epitope on mrtCD52 using several monoclonal antibodies.

MATERIALS AND METHODS: mrtCD52 was extracted by a mixture of chloroform, methanol and water from washed sperm of healthy donors. The extracted material was solubilized in a buffer (PBS), and subjected to SDS-PAGE or 2-dimensional PAGE. Western blot analysis probed with monoclonal antibodies (MAb H6-3C4, 1G12 and campath-1) was carried out to examine for a reactive antigen. For carbohydrate analysis of mrtCD52, N-linked and O-linked carbohydrate was removed by Endoglycosidase F and mild alkaline treatment, respectively.

RESULTS: MAb H6-3C4 reacted to mrtCD52 with a polymorphic reaction pattern in Western blotting and the reactivity disappeared after removal of the N-linked carbohydrate moiety. Two other monoclonal antibodies (1G12, campath-1) having sperm-immobilizing activity recognized mrtCD52 in a polymorphic manner similar to MAb H6-3C4. The reactivities of 1G12 and campath-1 remained after removal of the N-linked and O-linked carbohydrates, suggesting that 1G12 recognize a structure formed by the peptide and/or a glycosylphosphatidylinositol (GPI) anchor portion as does campath-1.

CONCLUSION: We confirmed that MAb H6-3C4 recognizes an N-linked carbohydrate moiety of mrtCD52 and indicated that the epitope of MAb H6-3C4 is similar to but distinct from those of 1G12 and campath-1. In conclusion, mrtCD52 contains different antigenic epitopes.

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UNIQUE SIGNAL TRANSDUCTION REGULATION BY PLACENTA GROWTH FACTOR (PLGF) IN TERM HUMAN TROPHOBLAST. Juan A. Arroyo, Donald S. Torry Southern Illinois University School of Medicine, Department of Medical Microbiology and Immunology and Obstetrics and Gynecology Springfield, Illinois.

BACKGROUND: The human placenta is rich in angiogenic growth factors and their receptors. Placenta growth factor (PlGF) is an angiogenic factor that is abundantly expressed through out gestation by normal trophoblast. Receptors for PlGF include the fms-like tyrosine kinase (flt-1) receptor, which is expressed on endothelial cells as well as trophoblast. We have previously shown that exogenous PlGF induces activation of the stress activated protein kinase (SAPK) signal transduction pathways, JNK and p38, with minor induction of the ERK1/2 pathways in trophoblast. In contrast, PlGF induces strong ERK1/2

activation, but no JNK or p38 responses in human umbilical vein endothelial cells (HUVEC). These results suggest that there might be unique trophoblast upstream regulatory molecules responsible for the differences in signal transduction between the two cell types. Our goal is to elucidate the regulatory signal transduction proteins responsible for the PlGF induced signal transduction differences between trophoblast and HUVEC.
METHODS: Trophoblast and HUVEC were isolated from normal term placentae. Trophoblast and HUVEC cultures were deprived of fetal calf serum (FCS) for 24 hrs. Cultures were treated for ten minutes with 10 ng/ml of rhEGF or rhPlGF. Cell lysates were immunoprecipitated with antibodies to SHP-2, Gab-2, PLC-gamma and Nck followed by western blotting with anti-phosphotyrosine to determine activation status of each molecule.
RESULTS: Treatment with exogenous PlGF induced similar activation of Nck and PLC-gamma in both HUVEC and trophoblast cells. In contrast, both SHP-2 and Gab-2 were strongly activated by PlGF in the endothelial cells but PlGF produced no activation of either SHP-2 or Gab2 in trophoblast. Exogenous EGF, which activates ERK1/2 in trophoblast, also activated SHP-2 and Gab2 in the trophoblast thereby confirming their ligand specific functional capabilities.
CONCLUSIONS: SHP-2 and Gab-2, but not PLC-gamma or Nck, represent two important signal transduction regulatory proteins that contribute to the different PlGF signal transduction responses in trophoblast and HUVEC. These cell type specific biochemical responses help elucidate the different biological functions of PlGF that have been noted in different cell types. (Supported by NIH HD36830).

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PREDOMINANCE OF TH2-PROMOTING DENDRITIC CELLS IN EARLY HUMAN PREGNANCY DECIDUA. Satomi Miyazaki, Shinichi Hori, Masatoshi Sakai, Sigeru Saito Toyama Medical and Pharmaceutical University Obstetrics and Gynecology, Toyama, Toyama.

THE AIM OF THE STUDY: Cytokines produced by Th2 cells seem to be important for the maintenance of pregnancy. A distinct shift towards Th2-type reactions occurs, especially at the fetomaternal interface. Dendritic cells (DCs) are specialized antigen presenting cell required for the priming and activation of T cells, and promote the differentiation of native CD4+ T cells toward either Th1 or Th2 phenotype. In this study we examined the characterization of DCs in human early pregnancy decidua, such as producing capacity of IL-12, TARC, and MDC, and the ability of DCs to promote Th1 or Th2 cells.
MATERIALS AND METHODS: The mononuclear cells were separated from decidua and peripheral blood. Surface markers and intracellular cytokines were examined by flow cytometry. DCs were isolated from peripheral blood mononuclear cells and decidual mononuclear cells by magnetic cell sorting. DCs were stimulated with LPS, SAC and CD40 ligand, and IL-12 in the supernatant was assayed by EIA. Mitomycin C treated DCs were added to naive CD4 T cells at 1:100 ratio. After 7-10 days of IL-2 expansion, Th1/Th2 ratios were determined by flow cytometry.
RESULTS AND CONCLUSION: 1. The percentage of DCs (CD45+ lineage markers- HLA-DR++) to mononuclear cells in decidua was $1.11 \pm 0.26\%$ and these were significantly higher than that in peripheral blood. 2. The ratio of myeloid DCs in decidua was significantly higher than that in peripheral blood. 3. The percentages lymphoid DCs in decidua were significantly lower than those in peripheral blood. 4. IL-12 producing cells in myeloid DCs in the decidua were significantly lower than those in peripheral blood. 4. IL-12 secretion by activated decidual myeloid DCs was significantly lower than that by peripheral DCs. 5. Naive CD4+T cells primed with decidual myeloid DCs led to higher percentage of Th2 cells in comparison with that with peripheral blood. 6. The percentage of TARC and MDC producing DCs in the decidua was similar in peripheral blood. We firstly demonstrated that decidual DCs promote the differentiation of naive T cells toward Th2 phenotype in vitro. These data suggest that DCs in the decidua regulate Th1/Th2 balance to Th2 dominant state, leading to maintenance of pregnancy.

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A STUDY OF UTERINE NK CELLS IN THE PREGNANT UTERUS OF MICE WITH THE DELAYED IMPLANTATION OR DELAYED PATURITION. Keiji Kokubu, Noriko Sakaguchi, Eiichi Hondo, Yasuo Kiso Yamaguchi University, The United Graduate School of Veterinary Science Yamaguchi Prefecture, Japan.

Murine uterine NK (uNK) cells, which emerge in the metrial gland and the decidua basalis, differentiate at each implantation site during early pregnancy. The number of uNK cells drastically increases from implantation to placentation period. These cells are gradually eliminated by apoptosis and spontaneous migration out of the placenta, and disappeared from the endometrium at the term of pregnancy. The present study aimed to establish histologically how differenti-

ation and elimination of murine uNK cells could be affected by the delayed implantation (DI) and delayed parturition (DP). At day 8 of pregnancy (D8) in DI mice, both uNK cells and their granules showed higher number and smaller size than these of the control mice. In DI mice, when the period of high level progesterone (P4) in serum became longer, the size of uNK cells and their granules did larger. The granules were quite small compared to the control. This indicated that differentiation of uNK cells in DI mice was delayed compared to the control. The cell density of uNK cells at D17 in DP mice was similar to that in the control. However, even at D19 when all uNK cells disappeared from the endometrium in normal pregnant mice, these cells survived in the metrial gland of DP mice. The metrial gland was well maintained. Abundant uNK cells were present even at D21, though their granules decreased in number. Although uNK cells of both DP and the control decreased in number from D15 to D17, the decreasing rate was milder in the DP mice than the control. This study established that implantation is not directly related to differentiation of uNK cells, but parturition is closely involved in elimination of these cells. Since differentiation of uNK cells in DI mice was delayed compared to the control, other factors than P4 should be involved in differentiation of uNK cells. On the other hand, parturition occurs following the fall of serum P4 level. The uNK cells could survive in the metrial gland even after D19, due to high P4 level in DP mice.

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CYTOKINE PRODUCTION BY HUMAN UTERINE NK CELLS. Mikael Eriksson, Sarah K. Meadows, Charles R. Wira, Charles L. Sentman Dartmouth Medical School Microbiology & Immunology Lebanon, NH.

PROBLEM: CD56+ NK cells account for a major population of lymphocytes in the human endometrium, and NK cells can be a significant source of cytokines that have the potential to alter local immune responses. Blood NK cells have been shown to produce an array of cytokines in response to specific monokine activation in vitro. The aim of this study was to characterize the cytokine production of human uterine NK cells.
METHODS: We examined cytokine production by NK cells and NK cell clones derived from human endometrium. These uterine NK cells were prepared from hysterectomy tissues prior to stimulation with IL-12, IL15 and/or TGF-β1.
RESULTS: Stimulation with IL-12 and IL-15 induced IFN-γ and IL-10 production by uterine NK cells. IFN-γ production by CD56br uterine NK cell clones was completely inhibited by 2ng/ml TGF-β1. The inhibition occured in a concentration dependent manner with an IC50 value of 0.02 ng/ml TGF-β1. A similar inhibition of blood NK cells by TGF-β1 was found. IL-10 secretion by uterine NK cell clones was also inhibited by TGF-β1 at similar concentrations. We found that both IL-12 and IL-15 can be expressed in human endometrium.
CONCLUSIONS: These data indicate that uterine NK cells can produce immunoregulatory cytokines and suggest that locally produced IL-12, IL-15, and TGF-β1 play a role in regulating NK cell function in the endometrium. This work was supported by grant AI/NS 51877 from the NIH.

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CHARACTERIZATION OF CHEMOKINES AND RECEPTORS THAT MAY RECRUIT CD56+ NK CELLS TO THE HUMAN ENDOMETRIUM. Charles L. Sentman, Sarah K. Meadows, Charles R. Wira, Mikael Eriksson Dartmouth Medical School Microbiology & Immunology Lebanon NH.

PROBLEM: CD56+ NK cells account for a major population of lymphocytes in the human endometrium. The number of NK cells increases in the secretory phase of the menstrual cycle, so sex hormones may regulate recruitment to and/or expansion of NK cells within the endometrium. Chemokines are an important part of the recruitment of specific cell subsets into distinct tissue locations. The aim of these studies was to characterize the chemokines and chemokine receptors that may be involved in the recruitment of NK cells to the endometrium.
METHODS: We examined the expression and function of chemokine receptors on human uterine NK cells and chemokines isolated from uterine tissues of hysterectomy patients.
RESULTS: NK cell clones derived from human uterine NK cells were found to express high levels of CXCR3, variable levels of CCR5, and no or low levels of CCR7 and CXCR4. Real-time PCR analysis determined that several chemokines that are known to induce chemotaxis of blood NK cells were expressed in human endometrium and that the expression of these chemokines may be regulated by sex hormones.
CONCLUSIONS: These data indicate that uterine NK cells express receptors for specific chemokines that are expressed in the human uterine endometrium. These findings suggest that these chemokines may play a role in the recruitment of NK cells to uterine endometrium. This work was supported by grant AI/NS 51877 from the NIH.

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EFFECTS OF SOLUBLE HLA-G1 ON HUMAN DECIDUAL NATURAL KILLER CELLS. Tobias Pöhlmann, Andreas Schaumann, Justine S Fitzgerald, Maryse Aguerre-Girr, Philippe Le Bouteiller, Udo R Markert Friedrich-Schiller-University Dept Obstetrics, Placenta-Lab Jena, Germany.

OBJECTIVES: HLA-G1s is produced by fetal trophoblast cells in the placenta. Aim of this investigation was to analyze the capacities of HLA-G1s to regulate IL-2-induced stimulation of natural killer (NK) cells isolated from the human decidua.
METHODS: HLA-G1s was produced by the cell line 221-G1s and isolated from supernatants. NK cells were isolated from the decidual layer of human placenta and cultivated for 24 h with or without IL-2 stimulation. Cultures were supplemented with various concentrations of HLA-G1s. By using flow cytometry, NK cells were analyzed for expression of CD25 and CD71. For investigation of cytotoxity of NK cells K562 cells were used as targets and stained with CFSE and DAPI for flow cytometry. Additionally, NK cells were analyzed for expression of signal transducer and activator of transcription 3 (Stat3) by polyacrylamid-gel electrophoresis and Western blotting.
RESULTS: In NK cells, IL-2 increases CD25, CD71 and Stat3 expression as well as cytotoxitys. The expression of CD71, cytotoxity and the expression of STAT3 decreased dose dependently when HLA-G1s was added to stimulated cultures in concentrations between 1.6 μg/ml–0.16 ng/ml.
CONCLUSION: HLA-G1s is involved in the regulation of NK cells in the decidua. Stat3 activation is reported to be correlated with perforin expression. Regulation of Stat3 may be a major mechanism responsible for reduced cytotoxicity of decidual NK cells.

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HUMAN ENDOMETRIUM EXPRESSES CANNABINOID RECEPTOR OF TYPE-2 IN THE SECRETORY PHASE AND IT IS ASSOCIATED WITH APOPTOSIS. Roberta Poverini, Marco Sbracia, Gabriele Rossi, Piero Alo', Beata Szabolcs Centro di Fisiopatologia della Riproduzione Villa Mafalda Rome, Italy.

AIM OF THE STUDY: Recently the receptors for cannabinoids, psychotropic substances found in marijuana, have been identified as a guanine nucleotide-binding-protein (G-protein)-coupled receptor. CB1 receptor was defined as a central cannabinoid receptor, whereas CB2 receptor has been defined as peripheral receptor type and it has been described in human macrophage population. In humans there are no data about the expression of CB receptors in endometrium. We studied the expression of mRNA and protein of CB receptors in human endometrium in vivo and in vitro by immunohistochemistry and PCR during the menstrual phases.
MATERIAL AND METHODS: Tissue specimens of eutopic endometrium obtained from 15 healthy women in different phases of the menstrual cycle were used to establish "in vitro" cell culture of stromal and epithelial cells. From which, cultured cells proteins and mRNA were extracted and used to perform immunoblot and RT-PCR. Fragments of the tissue specimens were fixed and used for immunohistochemestry and TUNEL staining with polyclonal antibodies for CB-1 and CB-2 receptors and TUNEL kit assay.
RESULTS: RT-PCR showed the presence of both RNA for CB1 and CB2 receptors in the epithelial cells and stromal cells. An immunohistochemistry study did not show any staining for CB1 receptor in endometrium and decidua whereas CB2 receptor was expressed in epithelial cells of the secretory phase and was negative during the proliferative phase. The immunoblot, with CB2 receptor anti-serum, confirmed its expression in the epithelial cells. TUNEL assay showed the presence of apoptotic cells in stromal and epithelial cells in the secretory phase endometria and the positivity to CB-2 receptor was positively correlated with the levels of apoptotic cells.
CONCLUSION: We have shown that human endometrium expresses CB2 receptor during the secretory phase and in the human decidua. The CB2 receptor-ligand system may be one the mechanisms regulating the apoptosis in the endometrium during the menstrual cycle.

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THROMBOPHILIC EFFECT OF THE PAI-1 4G/4G AND THE ACE D/D GENOTYPE MAY NOT ONLY BE A RISK FOR EARLY MISCARRIAGES. Nina Rogenhofer, Peter Lohse, Christian J. Thaler, Tina Buchholz Klinikum Grosshadern, LMU Muenchen, Germany.

We have shown, that patients with unexplained recurrent spontaneous abortions (RSA) have a significantly increased prevalence of the D/D genotype of the plasminogen activator inhibitor – 1 (PAI-1) gene combined with the 4G/4G genotype of the angiotensin converting enzyme (ACE) gene compared to a control group. This specific combination leads to an amplified PAI-1 expression, resulting in reduced fibrinolysis and

thrombophilia. In contrast, RSA patients are significantly less frequent in carrying the wildtype combination D/I together with 4G/5G than controls. Thrombophilia in pregnancies may cause early and late miscarriages, stillbirth or pregnancy complications mainly by decreasing placental perfusion. We have analysed, how the duration of the pregnancy relates to both RSA patient groups. Twenty-five RSA patients with the D/D + 4G/4G allels were compared to 32 RSA patients with the D/I + 4G/5G allels. The results show, that exclusively first trimester miscarriages occured less frequently in the D/D + 4G/4G group (72% vs. 88%, $p = 0.14$). Primary RSA happened also less frequently in that group (56% vs. 78%, $p = 0.09$). From our data we conclude, that the thrombophilic effect of the D/D + 4G/4G genotype combination may affect not exclusively first trimester miscarriages, but may also be related to pregnancy complications during later gestation.

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PHENOXODIOL REMOVES THE BLOCKERS OF APOPTOSIS SENSITIZING OVARIAN CANCER TO CHEMOTHERAPEUTIC AGENTS. David O'Malley, Marijka Kamsteeg, Wei Chen, Peter Schwartz, Thomas Rutherford, Gil Mor Yale University School of Medicine OB/GYN New Haven, CT.

OBJECTIVE: Ovarian cancer cells are resistant to apoptosis, however, in our current understanding these cells contain all of the components of the apoptotic pathway. Phenoxodiol (Ph), a synthetic analogue of the isoflavone, genistein, induces apoptosis in ovarian cancer cells by removing the blockers of apoptosis. In the present in vitro study, we demonstrated Ph removes the blockers of apoptosis which sensitizes the epithelial ovarian cancer cells to therapies that were previously resistant.

METHODS: The in vitro studies were done using ovarian epithelial cancer cells isolated from ascites using an immunomagnetic assay and established ovarian cancer cell lines (CP70 and A2780). Cell viability was determined using CellTiter. The cell lines were checked independently by western blot analysis for activation of caspase 3, 8, 9 and inactivation of XIAP in response to drug treatment.

RESULTS: Ph treatment for 48 hours (h) induces 60–90% decrease in cell viability in carboplatin and paclitaxel resistant cells. Pre-treatment with Ph alone for 2 h decreased cell viability by 40–90%. Furthermore, pre-treatment (2 h) with Ph in carboplatin resistant cells reduced the carboplatin IC50 by 20–100 fold. Western blot analysis demonstrated XIAP inactivation only in those cells that were chemosensitive while no XIAP inactivation was seen in resistant cells. Ph inactivated XIAP after 2 h of treatment in Taxol and carboplatin resistant cells. Pre-treatment with Ph in carboplatin-resistant cells resulted in a marked increase in XIAP inactivation after treatment with carboplatin.

CONCLUSION: This data suggests that Ph may restore the sensitivity to standard chemotherapies in resistant ovarian cancer cells by the removal of XIAP. When Ph is given in combination with standard chemotherapies the IC50 of these agents are significantly lowered. We have previously reported our in vivo results that showed a significant reduction in tumor volume when Ph was given in combination with cisplatin. In the future we will attempt to identify the most effective in vitro combination to maximize tumor cytotoxicity and to continue to perform clinical trials utilizing these combinations.