SESSION 1

THE MATERNAL AND FETAL POLYMORPHONUCLEAR CELL CONTRIBUTIONS IN THE PLACENTA AND UMBILICAL CORD OF PATIENTS WITH CLINICAL CHORIOAMNIONITIS AND PRETERM DELIVERY. M. Mohammady D.O, T. Walls, B.S, S. Jacques, M.D, F. Qureshi, M.D, B. Gonick, M.D. Dept. of Obstetrics and Gynecology and Pathology, Hutzel Hosp, Wayne State University, Detroit, MI.

Objective: To describe the maternal and fetal polymorphonuclear cell (PMN) contributions in the umbilical cord and placenta in preterm deliveries complicated by clinical chorioamnionitis.

Study Design: Five cases of preterm deliveries complicated by clinical chorioamnionitis were prospectively identified. Histologically severe chorioamnionitis and funiculitis were confirmed by hematoxylin and eosin staining. Male fetuses were studied. Thick sections of tissue were obtained from representative paraffin tissue blocks. Cell suspensions were obtained and cytospin slides were generated. The slides were then prepared for X and Y chromosome probe labeling and fluorescent in-situ hybridization (FISH). One hundred polymorphonuclear cells were identified morphologically and the presence of two XX signals or a single X and Y pair were noted under fluorescent microscopy. The ratio of maternal to fetal PMNs were noted and compared from the placenta and the umbilical cord.

Results: Maternal cells accounted for a mean of 89.4% of the total PMNs identified in the placenta. Fetal PMNs accounted for a mean of 99.9% of the PMNs seen in the umbilical cord specimens.

Conclusion: In preterm clinical chorioamnionitis, the PMN response observed in the placental mass is predominantly fetal. Conversely, the accompanying funiculitis seen on histologic examination is predominantly fetal in origin.

INTERLEUKIN-1, TUMOR NECROSIS FACTOR-α, OR LIPOPOLYSACCHARIDE DOES NOT INDUCE APOPTOSIS OF AMNIOTIC CELLS IN VITRO. B.T. Other, R. M. Silver, S.S. Edwen, M. Monga, K. Ward. Dept. of Obstetrics and Gynecology and Human Genetics, University of Utah, Salt Lake City, UT and Dept. of Obstetrics and Gynecology and Reproductive Sciences, UT-HSC, Houston, TX.

Objective: Tumor necrosis factor-α (TNF) and other inflammatory mediators are increased in the gestational tissues of women with infection related premature rupture of membranes (PROM) and preterm labor. Also, apoptosis has been reported in fetal membranes isolated from pregnancies complicated by PROM and has been proposed as a mechanism of membrane rupture.

Study Design: Amnion cells isolated and cultured in Ham'sF12-Dulbecco's modified medium were treated with various concentrations of TNF (1-100 ng/ml) for 24 hours. The cells then lysed and the radioactivity associated with DNA fragmentation was measured. One hundred amnion cells were counted and the apoptosis index was calculated as: (1 - total optical density of treated cells/total optical density of untreated cells) * 100.

Results: Inflammation, sloughing and necrosis were positively associated with higher PMN counts. In cases of preterm delivery, lower PMN counts were noted. In similar logistic model, inflammation was also positively associated with API (Adjusted OR 9.1, 95% CI 1.3-73.7).

Conclusion: Intra-amniotic infection is associated with elevation of PMN counts in amniotic fluid. Lower PMN counts are noted in cases of preterm delivery.

ASSOCIATION BETWEEN EASILY OBSERVED MEMBRANE ROLL CHARACTERISTICS AND HIGH AMNIOTIC FLUID INTERLEUKIN-6 LEVELS AMONG PATIENTS DELIVERING PREMATURELY. R. Patton, J. H. Hill, M. Krohn, S. Hiller, D. A. Eschenbach, Department of Obstetrics and Gynecology and Medicine, University of Washington, Seattle, and Department of Obstetrics and Gynecology, University of Pittsburgh, Pittsburgh. INTRODUCTION: Amniotic fluid infection (AIF) and elevated cytokine levels have been associated with prematurity. However, amniocentesis is not routinely performed in the assessment of patients in preterm labor.

OBJECTIVE: To determine whether easily observed characteristics of membrane rolls are associated with increased amniotic fluid (AF) interleukin-6 (IL-6) and AIF among patients delivering at ≤34 weeks.

STUDY DESIGN: The study population consisted of 102 women who presented in preterm labor with intact membranes and delivered at ≤34 weeks gestation. AF was collected by amniocentesis for culture and IL-6 enzyme immunoassay. Formalin fixed, paraffin embedded, membrane rolls were assessed by light microscopy for the presence of any inflammation, chorioamnion inflammation, and necrosis. Logistic regression was used to calculate adjusted OR for the associations between membrane characteristics and an elevated level of IL-6 or a positive culture.

RESULTS: Inflammation, sloughing and necrosis were positively associated with elevated IL-6 levels. Membrane Roll "Inflammation" (n=97) (n=95) Adjusted 95% CI

Characteristic Characteristic

Inflammation 33/36 (92%) 31/63 (49%) 7.6 1.8-29.7
Sloughing 12/27 (32%) 14/65 (22%) 3.2 1.1-9.9
Necrosis 13/37 (35%) 4/65 (6%) 5.2 1.2-23.2

*Adjusted for other factors in tables and gestational age at delivery. In a similar logistic model, inflammation was also positively associated with AIF (Adjusted OR 9.1, 95% CI 1.3-73.7).

CONCLUSION: Easily observed membrane roll characteristics are positively associated with elevated levels of IL-6 and AIF. In cases of preterm delivery, light microscopic evaluation of the membrane roll is a simple and inexpensive method for estimating whether AIF has occurred, and may be of prognostic value to the clinician.

AMNIOTIC FLUID DEFENSIN LEVELS ARE ELEVATED IN PRETERM LABOR PATIENTS WITH SUBCLINICAL INTRA-AMNIOTIC INFECTION. R. Phillips, H. Lo, M.D., K. Miller, M.D., Leo Mortimer, M.S., Phillip Gries, M.D.* University of Pittsburgh, Magee-Womens Hospital, Department of Obstetrics, Gynecology and Reproductive Sciences, Pittsburgh, PA.* Greeneville Hospital System, Maternal Fetal Medicine Division, Greeneville, TN.

OBJECTIVE: Defensins are neutrophil granule proteins released from activated neutrophils in the setting of infection. The purpose of this study was to determine if amniotic fluid defensin levels are elevated in preterm labor patients with subclinical intra-amniotic infection.

STUDY DESIGN: Amniotic fluid samples were obtained from 186 pregnant patients with the following clinical characteristics: Group I term, no labor (N=50). Group 2 preterm, no labor (N=81). Group 3, preterm labor with intra-amniotic infection (N=50). Group 4, preterm labor without intra-amniotic infection (N=55). Defensin levels were measured by ELISA. Patient groups were compared utilizing the Mann-Whitney U test.

RESULTS: Median amniotic fluid defensin levels for the varying patient groups are presented in the table. Patients with intra-amniotic infection had a significant increase in amniotic fluid defensins when compared to other groups (p < .0001). An amniotic fluid defensin level of > 400 ng/ml was highly suggestive of infection with a sensitivity of 83% and a specificity of 97%. CONCLUSION: Amniotic fluid defensin levels are elevated in patients with PTI and subclinical intra-amniotic infection. Amniotic fluid defensin levels may be a useful clinical tool for selecting preterm labor patients who might benefit from antimicrobial therapy, closer observation, or early delivery.

BROAD-SPECTRUM BACTERIAL RIBOSOMAL RNA POLYMERASE CHAIN REACTION FOR THE DETECTION OF AMNIOTIC FLUID INFECTION AMONG WOMEN IN PRETERM LABOR. J. D. Riley, MA Krohn, BL Hiller, K. A. Prewitt, J. C. Kroger, DA Eshenbach, Departments of Obstetrics/Gynecology and Urology, University of Washington.

OBJECTIVE: To determine the rate of amniotic fluid (AF) infection in preterm labor patients with negative AF cultures, using bacterial ribosomal RNA polymerase chain reaction (rPCR) DNA.

STUDY DESIGN: 70 preterm labor patients with intact membranes at 22-34 weeks gestation had AF collected by amniocentesis for culture and interleukin-6 (IL-6) enzyme immunoassay. AF bacterial (rPCR)-PCR for 17 patients with positive cultures were compared with 14 patients with negative cultures and IL-6>2000 pg/ml and 39 patients with negative cultures and low AF IL-6. Kruskal-Wallis ANOVA and paired Mann-Whitney test with Bonferroni correction were used to examine differences between +culture, -culture+rPCR, and -culture+PCR groups.

RESULTS: Bacterial rPCR was positive in 16(88%) of 17 patients with positive cultures, 5(36%) of 14 patients with negative cultures and high IL-6, and 1(3%) of 39 patients with negative cultures and low IL-6. Bacterial rPCR was negative in 2(12%) of 17 patients with positive cultures, 5(36%) of 14 patients with negative cultures and high IL-6, and 37(94%) of 39 patients with negative cultures and low IL-6. Bacterial rPCR was positive in 3(21%) of 14 patients with negative cultures and low IL-6, and 37(94%) of 39 patients with negative cultures and low IL-6.
negative cultures and low IL-6 (p=0.0001 vs. 1). Median (95% CI) cytokine levels, days to delivery and birthweights are summarized:

| Culture | Total | PCR+ | PCR- |
|---------|-------|------|------|
| +       | 28(34%) | 18(10%) | 10(17%) |
| -       | 54(66%) | 6(7%) | 48(83%) |

**CONCLUSIONS:** PCR detects AF bacteria in 39% of patients with negative cultures and IL-6 levels > 2000 pg/ml. Cytokine levels and pregnancy outcomes are similar in the culture- and culture-PCR groups. The association between AF infection and preterm labor may be underestimated by culture alone.

**CERVICAL ANTIBODIES TO HERPES SIMPLEX VIRUS DURING THE THIRD TRIMESTER OF PREGNANCY.** K.A. The study was conducted between 1989 and 1993, involving 9,667 deliveries. Of the 9 HSV seropositive women (8 HSV-2 only and 1 HSV-1 and HSV-2), 6 (56%) had a history of symptomatic herpetic infections and 4 (44%) had no history of genital symptoms. Cervical anti-HSV IgA was detected in 6 (35.7%) of 84 sampled women. Anti-HSV IgA was detected in significantly more women in a history of symptomatic genital infections (21 of 37, 56.8%) than those with a history of genital HSV (> 49, 19.9%) (P<0.001, Fisher's exact test). No anti-HSV IgA was detected beyond 37 weeks. Anti-HSV IgA positive days were more likely to be PCR negative (20 of 82, 24%), than PCR positive (8 of 82, 10%) (P=0.001). McNemar's test:

**RESULTS:** Out of 9,667 total deliveries between 1989 and 1993, 6,338 women and 7,285 neonates had cultures obtained. Fifty-seven women had positive cultures, of whom 47 were HSV-1 positive and 10 were HSV-2 positive. Of the 9 HSV seropositive women, 5 (56%) women had a history of symptomatiches and 4 (44%) had no history of genital symptoms. Cervical anti-HSV IgA was detected in 6 (35.7%) of 84 sampled women. Anti-HSV IgA was detected in significantly more women in a history of symptomatic genital infections (21 of 37, 56.8%) than those with a history of genital HSV (> 49, 19.9%) (P<0.001, Fisher's exact test). No anti-HSV IgA was detected beyond 37 weeks. Anti-HSV IgA positive days were more likely to be PCR negative (20 of 82, 24%), than PCR positive (8 of 82, 10%) (P=0.001). McNemar's test:

**CONCLUSION:** ATP bioluminescence is an effective screening test for ASB in pregnancy. Implementation of ATP testing prior to culture, thereby selecting women with the greatest likelihood of ASB, would result in a 50% reduction in urine cultures. Future work will focus on optimizing differential cell lysis in order to improve specificity and further reduce the need for routine culture.
During the study period, 4 infants were infected with neonatal HSV. Two of the infants were culture negative and were born to culture positive mothers. The other two infected infants were born to culture negative mothers, but did not receive culture in the delivery room.

Conclusion: The addition of culturing the infant for HSV at the time of delivery did not appear to enhance the sensitivity for detecting infants at risk for developing neonatal HSV.

**VAGINAL DEFENSINS AS LESS-INVASIVE MARKERS FOR SEXUALLY TRANSMITTED DISEASES (STD)**

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**OBJECTIVES:** Less-invasive STD testing strategies using inexpensive diagnostic tests are urgently needed. We evaluated the levels of defensins, neutrophil granule products, from the distal vagina as markers for the presence of STD's. 2500 patients attending STD clinic were enrolled.

All patients were tested for *G. vaginalis* (GC), *C. trachomatis* (CT), and/or culture. Swabs from the distal vagina were collected, and defensins levels were measured by an ELISA assay.

**RESULTS:** Overall, (16%) women were infected with STD. Thirty-four women were infected with GC. 31 with CT, and 0 with TV. Median vaginal defensin levels are presented in the graph. Levels associated with each organism were significantly greater than those in uninfected women (p<0.001).

**CONCLUSION:** Defensins are elevated in the distal vagina in women infected with GC, CT, or TV. The use of vaginal defensins as sensitive markers for the presence of STD's may be a less-invasive strategy for STD testing. Expensive diagnostic tests may then be restricted to those women at greatest likelihood of having an STD, as determined by elevated vaginal defensin levels.

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**DETECTION OF CHLAMYDIA TRACHOMATIS IN PREGNANT WOMEN BY POLYMERASE CHAIN REACTION (PCR)**

Cheryl A. Larios, M.D., Sarah M. Bongiovanni, SRInglis, Depts. of Ob/Gyn, Cornell University Medical College, New York, NY and New Jersey City Medical Center, Jersey City, NJ.

**Objectives:** To compare the prevalence of cervical *C. trachomatis* in pregnant women as detected by PCR, IGA antibodies and antigen detection and ascertain their relation to pregnancy outcome.

**Study Design:** A total of 211 endocervical samples from 167 women were evaluated. All sample aliquots were tested for *C. trachomatis* by PCR (Amplicor, Roche Diagnostics) and for IGA antibodies to *C. trachomatis* by a 6 minute assay (Chlamydia IGA, Rapid Serotest, Savon Diagnostics). A third sample was analyzed by the hospital clinical laboratory for *C. trachomatis* antigen (Chlamydiazyme, Abbott). Pregnancy outcomes were obtained. Laboratory results were not available to the clinician prior to delivery.

**Results:** *C. trachomatis* was detected by PCR in 27 (12.8%) samples from 23 (13.8%) women, antiChlamydia IGA was present in 37 (17.5%) samples from 32 women (19.2%) while 34 (14.6%) samples from 20 (12.0%) women were positive for chlamydial antigen. Antibody positive, PCR negative samples were tested by a second antibody test and a repeat PCR using samples spiked with *C. trachomatis*.

**Conclusion:** Compared to PCR, the IGA Rapid Serotest had a sensitivity of 99.3% and a specificity of 93.5%; the antigen assay had a sensitivity relative to PCR of 74.1% and a specificity of 97.6%. The pregnancy outcomes were: 96 (51.8%) patients with term labor (TL), 41 (24.8%) with preterm labor (PTL) and 38 (22.0%) with premature rupture of membranes (PROM). The proportions of 11 (43.8%) of the 23 PCR positive women were opposed to 30 (21.1%) of 143 PCR negative women ended in PTL (P=0.000). Only hene women positive in the IGA or antigen assay who were also PCR positive had PTL. There was no relation between Chlamydia detection by any assay and PROM.

**Conclusions:** PCR-detectable *C. trachomatis* in the endocervix of pregnant women correlates with PTL. In settings where PCR is not available the IGA Rapid Serotest, which can be performed in 6 minutes without any special equipment, is an accurate and sensitive procedure for detection of cervical *C. trachomatis* in pregnant women.
Study Design: We studied ten women diagnosed with postpartum endomyometritis. All women received 4.5 mg/kg actual body weight of gentamicin intravenously once daily as part of their antibiotic regimen. Serum peak and trough levels were obtained after the first two doses, as well as an eight hour level after the second dose. Serum creatinine levels were monitored, and ototoxicity was assessed clinically.

Results: The doses ranged from 255-600 mg (mean=360 mg), and the duration of therapy was 2-5 days. The mean elimination constant (Ke) was 0.015 (SD +/- 0.008), and the volume of distribution (V0) was 0.45 kg (SD +/- 0.07). The mean peak level after the first and second doses were 11.6 μg/ml (SD +/- 2.3) and 13.0 μg/ml (SD +/- 2.5), respectively. Both trough levels were < 0.3 μg/ml. The mean half-life was 6.6 hrs. There was no nephrotoxicity or ototoxicity observed. Clinical response was seen in 90% of patients; one patient responded with the addition of heparin for presumed septic pelvic thrombophlebitis.

Conclusions: Once daily dosing of gentamicin results in pharmacokinetic parameters similar to those observed in non-pregnant patients. No significant drug accumulation or toxicity occurred. Therefore, single daily dose gentamicin in the post partum patient appears safe to use at 4.5 mg/kg actual body weight. Further studies are necessary to confirm the safety and efficacy demonstrated in this report.

FETAL MEMBRANE MICROBIAL FLORA AT TERM: NO ASSOCIATION WITH CHORIOAMNIONITIS. Pål Halvorsen, Kristin H. Halvorsen, PhD, Thomas Halvorsen, MD, PhD, Maria A. Høiby, MD, PhD, and S. Nielsen, MD, PhD. Department of Obstetrics and Gynecology, University of Bergen, Bergen, Norway.

Objectives: To study relationships between microbial flora of fetal membranes, histopathologic findings of the membranes/placenta/umbilical cord, and clinical symptoms among women delivering at term.

Methods: We studied consecutive women with singleton pregnancy delivering at term during a 3-month period. Immediately after delivery of the placenta, the nurse-midwife separated the fetal membranes. Specimens for culture were collected from the membranes, close to the placental edge with cotton-tipped wooden swabs treated with charcoal. The specimens were kept at room temperature in Stuart's medium and transported to the laboratory within 24 hours (within 48 hours during week-ends). Formalin-fixed membrane, and placental and umbilical tissue specimens were studied by a pathologist who was blinded as to clinical and micrologic findings.

Results: We obtained culture specimens from the fetal membranes of 312 (96.9%) of the 330 eligible women who delivered during the study period. At least one bacterial species was recovered in 218 cases (69.9%). Pathogenic microorganisms were recovered from 106 (34%) of the 312 membranes. Culture specimens from the membranes of eight women delivered by elective cesarean section showed no growth. In total, 25% of the 173 consecutively delivered women had histopathologic evidence of chorioamnionitis. We recovered pathogens from the fetal membranes of 35 (57.2%) women who had 21 (30.6%) cases of histopathologic evidence of chorioamnionitis in the membranes (OR = 1.4, p < 0.04). Women with fever during labor (n = 7) were significantly more likely than those not having fever to have chorioamnionitis (100% vs. 32.1%, p = 0.001). The diagnosis of chorioamnionitis was made on the basis of the presence of maternal leukocytosis and foul-smelling amniotic fluid.

Conclusions: Growth of pathogens between the fetal membranes after vaginal delivery do not predict chorioamnionitis.
COMPARISON OF CLINICAL MANIFESTATION WITH LAPAROSCOPIC FINDINGS IN ACUTE SALPINGITIS

Eschenbach DA, Wolter-Hansen F, Hawes SE, Pavletic A, Pavonen J, Holmes KK. Department of Obstetrics and Gynecology and Medicine, University of Washington, Seattle.

OBJECTIVE: In this report we compared clinical manifestations and tubal abnormalities observed at laparoscopy among women with acute salpingitis.

STUDY DESIGN: We compared the type, and when applicable, the severity of clinical manifestations in 82 women with definite laparoscopic evidence of acute salpingitis. Women with findings other than salpingitis (n=22), normal findings (n=9) or possible, but not definite salpingitis (n=42) were excluded from these analyses.

RESULTS: Two general categories of laparoscopic findings were present: 1) tubal occlusion and moderate to severe adhesions tended to occur together, in 30 patients and 2) pelvic/abdominal exudate tended to occur separately, in 27 patients. Tubal occlusion was positively associated with older age, palpable adnexal mass and negatively associated with rebound tenderness, the tenderness score and the isolation of Neisseria gonorrhoeae and Chlamydia trachomatis. Moderate to severe adhesions were positively associated with duration of abdominal pain and negatively associated with the tenderness score. Exudate in the pelvic/abdominal peritoneum was positively associated with the tenderness score, the white blood count and N. gonorrhoeae and negatively associated with the duration of pain, oral contraceptive use and palpable adnexal mass. Reanalyses among patients with no prior history of PID did not change the findings.

CONCLUSION: Clinical manifestation traditionally used to judge the clinical severity of PID partially predict the laparoscopic findings, tend to distinguish those with tubal occlusion and/or moderate to severe adhesion from those with peritonitis, and provide insight into the pathophysiology. However, the predictive value of clinical manifestations are low and not reliable for the individual patient.

Session 4

IDENTIFICATION OF acc-4 MUTATIONS THAT EFFECT VIRULENCE OF GONOCOCCAL PID STRAINS

Stella Nowicki, D.D.S., L. Prashanth Ram, M.D., D. Andrzej Skarpetowski, M.D., Tuan Pham, M.D. and Bogdan Nowicki, M.D., Ph.D., D.D.S. 1,2, Dept. of Ob/Gyn & Microbiology, The University of Texas Medical Branch, Galveston, Texas.

OBJECTIVES: Gonococcal PID is a most serious and costly complication of gonorrhea. Pathogenesis of PID is poorly understood. Two major factors contribute to the virulence of gonococci, 1) resistance to the bactericidal effect of normal human serum and 2) attachment to human tissues. Clq-mediated virulence is the first significant difference found between the PID and local strains (Perkin Elmer Award to S. Nowicki's laboratory for the best research in STD in 1995). The acc-4 DNA region of N. gonorrhoeae is known to confer partial serum resistance (serR). We have recently identified a 344 bp base pair segment in the 3' end of the acc-4 region which upon transformation with plasmid pRP350 conferred Clq dependent virulence to laboratory strain F62. Recently we found two unique local isolates of N. gonorrhoeae which carried 344 bp fragment but were avirulent in our psp model. We hypothesize that the 344 bp fragment from local strains carry a point mutation(s) resulting in abolishing Clq dependent virulence.

STUDY DESIGN: N. gonorrhoeae strains 1655 and 1653 were used to PCR amplify 344 bp fragments. PCR products were subcloned to appropriate vector. Resultant plasmids pRP1659, pRP1665 were sequenced, and transformed to laboratory strain F62. Virulence of these constructs was examined on psp model.

RESULTS: A 344 bp fragments from local isolates did not confer Clq mediated virulence to N. gonorrhoeae F62 upon transformation. In control plasmid pRP350 from PID isolate transmuted strain F62 to a Clq dependent virulent strain. Sequencing of plasmids pRP1659 and pRP1665 from local strains and pRP350 from PID strain has been carried out and two deletions and three substitutions were identified. Deletion of the 3' segment of pRP350 that overlap identified mutations abolished Clq dependent virulence.

CONCLUSIONS: These results are consistent with the hypothesis that mutations in the 344 bp segment of acc-4 may effect virulence of gonococcal isolates.
**AZITHROMYCIN POWDER VERSUS ERYTHROMYCIN IN THE TREATMENT OF CHLAMYDIAL CERVICITIS IN PREGNANCY**

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Objectives: Determine if a single one gram dose of azithromycin (Pfizer Labs, New York, NY) has fewer side effects and better compliance rate than multiple doses of erythromycin due to gastrointestinal side effects.

Study Design: This was an ongoing randomized clinical trial. All women attending their first and 36 week prenatal visit had cervical swabs taken and analyzed with the Gen-Probe PACE 2 (Gen-Probe, Inc., San Diego, CA) DNA probe for Chlamydia trachomatis. All patients were randomized to one group or the other. Patients were assigned to receive either a one gram oral dose of azithromycin powder or seven days of erythromycin base, 500 mg q.i.d.

Results: Twenty-seven percent of patient treated with erythromycin were unable to tolerate the medication due to gastrointestinal side effects and required crossover to azithromycin. No patient in the azithromycin group required crossover.

Conclusions: Azithromycin powder is better tolerated and has a higher compliance rate than erythromycin in the treatment of chlamydial cervicitis in pregnancy.
THE EFFECT OF CHLAMYDIA TRACHOMATIS AND INFLAMMATORY LEUKOCYTES ON HIV-1 REPLICATION IN VITRO

Daniel V. Landers, M.D., John P. Mills, R.N., Br., MT(ASCP), Faith DiBiasi, BS, M.A.S.C.P., Julia Schachter, Ph.D.

University of Pittsburgh and Magee-Womens Research Institute and University of California, San Francisco

Objectives: Epidemiological studies have shown that Chlamydia trachomatis (CT) infection is a risk factor for HIV acquisition. Polymorphonuclear leukocytes (PMNs) and mononuclear cells (PBMCs) are found in female genital tract response to CT infection. We studied the effects of CT extracts and inflammatory leukocytes on HIV-1 replication in vitro.

Study Design: Chlamydia-infected mononuclear cells (UC) were incubated with CT (human E strain) extract, PMNs, or PBMCs. HIV replication was determined by p24 antigen measurement at 24, 48, 72, and 96 hours.

Results: After 96 hours of incubation HIV replication was increased 23 fold with PBMC coinoculation (p=0.01), 48 fold with PMN coinoculation (p=0.01), 27 fold with CT extract and PBMC coinoculation (p=0.01), and 33 fold with CT extract and PMN coinoculation. Table 1 shows data for 24, 48, 72, and 96 hours.

| Time (h) | CONTROL | CT | PMNs | PMNs + CT | PMNs + CT |
|---------|---------|----|------|-----------|-----------|
| 24h     | 0.00    | 2.48| 4.00 | 5.00      | 9.00      |
| 48h     | 0.00    | 4.00| 6.00 | 8.00      | 13.00     |
| 72h     | 0.00    | 6.00| 9.00 | 12.00     | 19.00     |
| 96h     | 0.00    | 9.00| 13.00| 19.00     | 33.00     |

Conclusion: Inflammatory cells found in the female genital tract, in association with CT infection, significantly enhance HIV replication in vitro. CT alone or in combination with inflammatory cells did not further enhance HIV replication. This data suggests that the inflammatory response to CT infection is not responsible for increased risk of HIV acquisition. Further studies are needed to elucidate this complex interaction.

VAGINAL INFECTIONS IN HIV

Helton, A.M., Erickson, M., M.D., Myers, G., CNM, Lorimer, R., Ph.D., Blanco, J., M.D.

Objectives: In some reports, HIV-infected women have an increased incidence of recurrent vaginal infections. The purpose of this prospective study was to identify the rate of vaginal infection and treatment response in a cohort of HIV-infected women in Houston, Texas.

54 • INFECTIOUS DISEASES IN OBSTETRICS AND GYNECOLOGY
TNF-α AND IL-6 PRODUCTION IN THE CHORIOAMNION AT TERM BY MACROPHAGES OF MATERNAL AND FETAL ORIGIN AS DETERMINED IN VIVO BY mRNA IN SITU HYBRIDIZATION AND IMMUNOHISTOLOGY. L. Brooks, M.D., S. Myerson, M.D., S.L. Hillier, Ph.D., G. Cadle, Ph.D., A. Brown, M.D., G. Firestein, M.D., D.A. Ensenbach, M.D. 

OBJECTIVES: The origin of macrophages associated elevated amniotic fluid (AF) cytokine (CK) levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) was examined in term pregnancies.

StUDY DESIGN: 33 women undergoing cesarean section had specimen of AF, chorion (CA), decidua (D) and placenta (Plac) collected at delivery. AF, CA and Plac were cultured. AF TNF-α and IL-6 concentrations were determined by ELISA. Immunohistologic differentiation was performed on all tissues for T- and B-lymphocytes and macrophages using antibody markers and [gamma]-glutamyl transpeptidase (GGT) identified by morphology. TNF-α and IL-6 producing cells were identified by mRNA in situ hybridization using 5'-labeled probes. Positive macrophages were identified by combined in situ immunohistochemistry and hybridization using macrophage specific antibody HAM56 and a DNA probe for Y-chromatin.

RESULTS: Both AF infection and labor were associated with elevated AF levels of TNF-α and IL-6. Macrophages and PMN's were the predominant cell types in the CA. IL-6 mRNA signal in the decidua was related to labor while IL-6 mRNA signal within macrophages in the amnion and chorion was related to a positive AF and CA culture. Macrophages in the amnion were related to positive AF culture. Using the double stain technique, Y-chromatin-positive macrophages were present in all 19 pregnancies with a male fetus and in none of 14 pregnancies with a female fetus (p<0.001).

CONCLUSIONS: Fetal amnion and chorion macrophages synthesize CK in response to infection. Most of these macrophages in the amnion and chorion appear to be of fetal origin.

RISK OF HEPATITIS B TRANSMISSION AFTER AMNIOCENTESIS IN CHRONIC HEPATITIS B CARRIERS. James Alexander, M.D., Ronald Rams, M.D., Greg Jackson, M.D., Barbara Soccerly, R.N., George Wendel, M.D. The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas

OBJECTIVES: The risk of transmission of hepatitis B virus (HBV) from amniocentesis in HBV carriers has not been thoroughly investigated. In one report from Taiwan, the risk of perinatal HBV transmission in HBV carriers was not higher after amniocentesis compared to a control group of HBV carrier. Immunophenotypes failures occur in 1/8 of infants in both groups. Our objective was to assess the risk of transmission of HBV in chronic carriers who undergo amniocentesis at our hospital.

Study Design: This was a prospective, longitudinal study, and data were collected about women who were HBV carriers and underwent amniocentesis. The infants of these women were followed in a special clinic from birth to one year of age. Maternal data examined included HBV antigen and antibody status, liver function tests (LFT), and the amniocentesis procedure report. Pediatric data was obtained from clinic records including the neonatal and 12 month HBsAg and vaccine record.

Results. 25 women were identified. Two of 25 neonates were stillborn unrelated to hepatitis, and 5 infants were sent to follow-up, leaving 18 mother-child pairs to evaluate. All 18 women were chronic HBV carriers at the time of amniocentesis and delivery. No mother had abnormal LFTs, and only 1 of 18 women was positive for HBsAg. 10 amniocenteses were for advanced maternal age, and 3 were for abnormal MSAFP screening. None of the amniocenteses were recorded as bloody, and the placenta was exterior in 5 of 18 procedures. None of the 18 infants (95% CI:0.04-5.1%) were positive for HBsAg during the first month of life or at 12 months of age. All infants received HBV vaccine and 100% immunoprophylaxis.

Conclusion. The risk of transmission of HBV to the fetus after amniocentesis in women who are HBV carriers is low. Immunophenotypes in these infants was successful.

DETECTION OF TOXOPLASMA GONDII IN AMNIOTIC FLUID BY A POLYMERASE CHAIN REACTION/ENZYME-LINKED IMMUNOSORBENT ASSAY (PCR/ELISA). J. Jermias, W. Ledger, and S. Wiltse, Dept. of Obst/Gyn, Cornell University Medical College, New York, NY

Objectives: Maternal exposure to Toxoplasma gondii during pregnancy may result in congenital infection and serious sequelae in the neonatal period or years after birth. However, there is little consensus about screening during pregnancy, and the tests used to establish a fetal diagnosis of toxoplasmosis are complex and time consuming. Preterm diagnosis of congenital toxoplasmosis is based on ultrasonography, fetal blood sampling or inoculation of cultured cells or mice with amniotic fluid. Given the risk associated with fetal-blood sampling and the delay in obtaining definitive results with conventional parasitologic methods or ultrasound, better methods are needed.

T. gondii has been diagnosed by PCR but detection of the amplified product by ELISA has, to our knowledge, not been reported. ELISA detection has increased sensitivity over other methods.

Study Design. Using human amniotic fluids that had been spiked with T. gondii, we established parameters for the amplification and detection of a fragment of the T. gondii repetitive B1 gene. The 115-base pair product, digoxigenin-labeled, was hybridized to a biotinylated internal probe and detected by ELISA. The hybridization validated the specificity of the amplified PCR product. The possible presence of PCR inhibitors in individual amniotic fluids was evaluated by amplifying a portion of the human beta actin gene.

Results. T. gondii DNA was readily detected in amniotic fluids by our PCR/ELISA. PCR analysis of DNA extracted from various bacteria, yeast, and viruses using the T. gondii primer pairs did not result in amplification of any contaminating DNA. Similarly, amniotic fluids obtained for genetic analysis were uniformly negative for T. gondii.

Conclusion. The PCR/ELISA will allow for the definitive diagnosis of congenital T. gondii infection in one day. This will reduce the incidence of unnecessary pregnancy terminations based on uncertainty of fetal infection as well as allow the prompt initiation of antibiotic treatment to infected fetuses.
SEROLOGICAL RESPONSE TO THE CHLAMYDIAL 60 kD HEAT SHOCK PROTEIN IN WOMEN WITH TUBAL FACTOR INFERTILITY by, B

Serological Response to the Chlamydial 60kDa Heat Shock Protein: An In Vitro Model, SM Mou, MD; S. Faro, MD, PhD. University of Missouri-Kansas City, Kansas City, MO 64108

Objectives: Studies documenting the harmful effects of phage infection in dairy lactobacilli are common; however, few studies have been conducted to determine if phage infections can have similar harmful effects on vaginal lactobacilli. The objectives of this study were to isolate phages from vaginal lactobacilli and establish an in vitro model to show that phages released from one woman's vaginal strains can infect lactobacilli from other women.

Study Design: Vaginal samples were obtained from 39 reproductive-aged women (1 Native American, 5 Asians, 6 Blacks, and 27 Caucasians). The selective Rogosa agar was used to isolate lactobacilli, from which phages were isolated by using an agar overlay method. In this study, 37 of 39 isolates were identified as L. acidophilus, L. casei, and L. crispatus. The selective Rogosa agar was used to isolate lactobacilli, from which phages were isolated by using an agar overlay method. In this study, 37 of 39 isolates were identified as L. acidophilus, L. casei, and L. crispatus. The selective Rogosa agar was used to isolate lactobacilli, from which phages were isolated by using an agar overlay method. In this study, 37 of 39 isolates were identified as L. acidophilus, L. casei, and L. crispatus. The selective Rogosa agar was used to isolate lactobacilli, from which phages were isolated by using an agar overlay method. In this study, 37 of 39 isolates were identified as L. acidophilus, L. casei, and L. crispatus.

Results: Of 19 samples from women with vaginal infections, 12 did not have lactobacilli. From the remaining 7 infection samples and the 20 samples from healthy women, 40 Lactobacillus strains were isolated, from which 6 Lactobacillus species were identified and 7 phages were isolated. One phage, kco39, was characterized as follows: plaque morphology, small and clear, 1-2 mm in diameter; burst size, 300/cell; spontaneous induction rate, 10^-3/cell; and DNA, double stranded and linear, 41 kb, and a papillomavirus head and a non-contractile tail. This phage attacked in vitro 8 out of 39 Lactobacillus strains from other women. One strain, kco313, was completely lysed by kco39.

Conclusions: Bacteriophages were isolated from human vaginal lactobacilli for the first time, and an in vitro phage-infection model was established. Further studies are needed to determine if phage infection in vaginal lactobacilli occurs in vivo in humans and if it is clinically significant. (Supported by the Concerned Parents for AIDS Research/AmFAR and the University of Missouri Research Board.)

SUSCEPTIBILITY TESTING OF VAGINAL YEAST ISOLATES USING A BROTH MICRODILUTION BIOPASSAGE J. Gunter M.D.; D. Dober B.Sc., S. Faro M.D., Ph.D.

Objectives: To determine the MIC's of fluconazole, miconazole, and amphotericin B for vaginal yeast isolates using the dilution method. Study Design: The organisms (C. albicans, T. glabrata, and C. tropicalis) were isolated from the posterior vaginal fornix on Sabouraud dextrose agar, identified by standard methods, and subcultured twice to ensure purity. The broth microdilution was performed according to the NCCLS standard guidelines with modification for the Alamar blue colorimetric end point.
PROPHYLACTIC ANTIBIOTICS ARE NOT NECESSARY FOR MANUAL EXTRACTION OF PLACENTA FOLLOWING VAGINAL DELIVERY.

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OBJECTIVE: Retained placenta is a common intrapartum complication following vaginal delivery which requires manual extraction (ME). Because of concerns related to bacterial contamination and subsequent infection, many clinicians administer antibiotic prophylaxis with this procedure. There are no data to support the need for antibiotic prophylaxis with ME.

STUDY DESIGN: This retrospective study examined 190 patients who underwent ME following vaginal delivery. Seventy one patients received prophylactic antibiotics and 119 patients did not. Indications for ME were prolonged third stage (123), retained products (16), bleeding (14), avulsed cord (14), and other (23). None of these patients received intrapartum antibiotics or were suspected of infection during their labor. Indications for ME, risk factors for infection and concomitant curettage were similar for both study groups. The decision to administer antibiotic prophylaxis was based on individual clinician preference. If antibiotics were given, they were administered from the time of ME up to 48 hours post procedure. Patients receiving antibiotic prophylaxis were compared to those not receiving prophylaxis for post partum infectious morbidity.

RESULTS: Three patients in the prophylaxis group developed post partum endometritis (4.2%). Of these three patients, one also had uterine curettage. One subject was admitted on post partum day 10 for suspected endometritis. All three had 24 hours of prophylactic antibiotics. No patient in the group not receiving antibiotics developed post partum endometritis.

CONCLUSION: These data suggest that ME following vaginal delivery does not place the patient at increased risk for post partum endometritis. Therefore, prophylactic antibiotics are not necessary.

OVER-THE-COUNTER (OTC) AND ALTERNATIVE MEDICINES (AM) IN THE TREATMENT OF CHRONIC VAGINAL SYMPTOMS. Paul Nutner, MD, M. Velma Weitz, MSN; M.H. Terry Grody, MD; Bennett Lorber, MD. Temple University School of Medicine, Philadelphia, PA.

Objective. To investigate the use of OTCs and AMs in patients with chronic vaginal symptoms.

Study Design. A prospective cohort study of 109 patients referred by their gynecologists for evaluation of chronic vaginal symptoms. Patients were interviewed about their use of OTCs and AMs over the preceding year, the amount of money spent on each, and whether their physicians had been informed of these treatments.

Results. The mean age was 35 ± 9.2 years; 50.9% had finished college. The age 35+9.2 years; 50.9% had finished college. Symptom duration was 3.4 ± 3.5 years. 79 (72.5%) patients had self-treated with OTCs such as miconazole (75.6% of OTC users), clotrimazole (38.5%), or Betadine (14.1%). The average estimated expenditure for OTCs was $88 ± $139. 42 (38.5%) had used AMs, most frequently acidophilus pills orally (37.1%) or vaginally (11.5%), yogurt orally (21.4%) or vaginally (19.0%), garlic pills (9.6%) and acupuncture (8.4%). Estimated expenditure for AMs was $171 ± $284. Fewer physicians (75%) were aware of the use of AMs than of OTCs (96%) (p<0.01).

Although all patients had received at least one prescribed course of antimycotics for their conditions, the most common diagnoses at initial presentation were vulvovaginal candidiasis (VVC) in 28 (26.7%), irritant dermatis in 18 (17%), and bacterial vaginosis in 12 (11.4%). Women who actually had VVC were more likely to have used AMs (RR 1.8, 95%CI 1.12-2.77) than other patients.

Conclusions. Women with chronic vaginal symptoms often use OTCs and AMs which significantly add to health care costs and are unlikely to be of benefit.