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Colonization of second trimester placenta parenchyma

Andrew B. Onderdonk, PhD[a], **Jonathan L. Hecht, MD, PhD**[b], **Thomas F. McElrath, MD, PhD**[c], **Mary L. Delaney, MS**[a], **Elizabeth N. Allred, MS**[d], and **Alan Leviton, MD**[e] **for the ELGAN study Investigators**

[a]Brigham and Women's Hospital, Department of Pathology, Harvard Medical School, Boston, Massachusetts

[b]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

[c]Brigham and Women's Hospital, Department of Medicine, Boston, Massachusetts

[d]Children's Hospital, Harvard Medical School, Harvard School of Public Health, Boston, Massachusetts

[e]Children's Hospital, Harvard Medical School, Boston, Massachusetts

Abstract

Objective—The overtly healthy, non-pregnant uterus harbors bacteria, *Mycoplasma* and *Ureaplasma*. The extent of colonization remains elusive, as are relationships between isolated microorganisms, preterm labor and fetal inflammation.

Study Design—Biopsies of chorion parenchyma from 1083 placentas delivered before the beginning of the 28th week of gestation were cultured, and the placenta was examined histologically. The frequencies of individual microorganisms and groups of microorganisms were evaluated in strata of processes leading to preterm delivery, routes of delivery, gestational age, and placenta morphology

Results—Placentas delivered by cesarean section with preeclampsia had the lowest bacterial recovery rate (25%). Preterm labor had the highest rates, which decreased with increasing gestational age from 79% at 23 weeks to 43% at 27 weeks. The presence of microorganisms in placenta

Corresponding author: Andrew B. Onderdonk, Channing Laboratory, 181 Longwood Avenue, Boston, Ma 02115, Telephone: 617-525-2506, Fax: 617-731-1541, aonderdonk@partners.org.
Reprints not available from author

Participating institutions (site principal investigator, pathologist and perinatologist)
Baystate Medical Center, Springfield MA (Bhavesh Shah, Solveg Pflueger, Glen Markenson)
Beth Israel Deaconess Medical Center, Boston MA (Camilia R. Martin, Jonathon L. Hecht, Bruce Cohen)
Brigham & Women's Hospital, Boston MA (Linda J. Van Marter, Thomas McElrath)
Children's Hospital, Boston MA (Alan Leviton)
Massachusetts General Hospital, Boston MA (Robert Insoft, Drucilla Roberts, Laura Riley)
New England Medical Center, Boston MA (Cynthia Cole/John Fiascone, Ina Bhan, Sabrina Craigo/Theresa Marino)
U Mass Memorial Health Center, Worcester, MA (Francis Bednarek, Gamze Ayata, Ellen Delpapa)
Yale-New Haven Hospital, New Haven CT (Richard Ehrenkranz, Miguel Reyes-Múgica/Eduardo Zambrano, Keith P. Williams)
Forsyth Hospital, Baptist Medical Center, Winston-Salem NC (T. Michael O'Shea, Dennis W Ross, Maggie Harper)
University Health Systems of Eastern Carolina, Greenville NC (Stephen Engelke, John Christie, Hamid Hadi)
North Carolina Children's Hospital, Chapel Hill NC (Carl Bose, Chad Livasy, Kim Boggess)
DeVos Children's Hospital, Grand Rapids MI (Mariel Portenga, Barbara. Doss, Curtis Cook)
Sparrow Hospital, Lansing MI (Padmani Karna, Gabriel Chamyan, Steve Roth)
University of Chicago Hospital, Chicago IL (Michael D. Schreiber, Aliya Husain, Mahmoud Ismail)
William Beaumont Hospital, Royal Oak MI (Daniel Batton, Chung-ho Chang, Robert Lorenz)
Pathologists who contributed beyond their home institution (Harvey Kliman and Pat Senagore)

Condensation: Bacteria found in the placenta parenchyma appear to influence the placenta and the pregnancy.

parenchyma was associated with the presence of neutrophils in the fetal stem vessels of the chorion and the vessels of the umbilical cord.

Conclusions—The high rate of colonization appears to coincide with phenomena associated with preterm delivery and gestational age. The presence of microorganisms within placenta parenchyma is biologically important.

Keywords

Placenta parenchyma; bacterial colonization; microorganisms and pregnancy outcome

Introduction

The endometrium of non-pregnant women frequently harbors bacteria.[1-5] This phenomenon is also observed for the endometrium of women between pregnancies, [6-7] raising the possibility that gravid deciduas also frequently harbor bacteria.

Trans-cervical sampling techniques result in more frequent recovery of microorganisms than samples obtained by transabdominal endometrial aspirations [6, 8] or from surgically removed specimens[3, 4]. This has led to the impression that many of the microorganisms recovered represent contamination by the vaginal microflora.

We report observations in a sample of placentas delivered before the 28th postmenstrual week. The frequency of recovery of microorganisms from biopsies of chorion parenchyma from these placentas varies with gestational age in multiple strata, defined by delivery-indication and by placenta morphology, in a way that could not be explained by contamination of the specimens. These findings support two hypotheses, the placenta delivered in the second trimester is not a sterile organ and microorganisms harbored within placental tissue influence the placenta and the outcome of the pregnancy.

Material and Methods

Sample

The ELGAN (the acronym for **E**xtremely **L**ow **G**estational **A**ge **N**ewborn) Study enrolled infants born before the beginning of the 28th week of gestation at 14 sites in 5 states between the years 2002-2004. This project was reviewed and approved by the Partners Healthcare Institutional Review Board acting on behalf of the Brigham and Women's Hospital and the Massachusetts General Hospital. To be included in this sample, the newborn had to survive long enough to have one cranial ultrasound scan. Although the ELGAN was the focus, the mother was asked to contribute her placenta, along with data about herself and her pregnancy. This set of analyses is limited to the 605 placentas delivered when the delivery indication was preterm labor, 297 for membrane rupture and 181 for preeclampsia. Of the total 1083 placentas, 414 placentas were delivered vaginally and 669 delivered by Cesarean section (CS) (Table I). The indication for CS was either malpresentation or non-reassuring fetal status. The 282 placentas delivered because of abruption, cervical insufficiency or fetal indications are not included in these analyses.

Placentas

Delivered placentas were placed in a sterile exam basin and transported to a sampling room. Eighty-two percent of the samples were obtained within 1 hour of delivery. Because the fetal membranes are more likely to be exposed to sources of bacterial contamination during delivery than placenta parenchyma, which is located beneath the tightly attached membranes, we sampled placenta parenchyma to minimize contamination.

The samples were obtained from the area at the midpoint of the longest distance between the cord insertion and the edge of the placental disk as described previously [13]. When multiple fetuses were delivered, the corresponding umbilical cord was marked by the delivery team. The placenta was laid out as per the protocol for singleton placentas, and the 'equator' of the dividing membrane was noted. The sampling area was identified based on the side of the placental equator corresponding to each fetus. Once the area was identified, the overlying amnion was lifted with a set of sterile forceps and cut with sterile scissors. Using forceps, the amnion was gently pulled away from the underlying chorion. The amnion was then opened with the sterile scissors, and peeled away from the initial site of entry, thus exposing the chorion. With a second set of sterile forceps and scissors, traction was put on the chorion and underlying trophoblast tissue by gently pulling on it. A piece of tissue was excised by cutting at the base of the section with the sterile scissors. The tissue sample was placed in a sterile 2 ml cryovial. The specimen was immediately frozen in liquid nitrogen and stored in an -80°C freezer until shipped. Frozen samples from the 14 study sites were express shipped in the frozen state using dry ice to the central microbiology laboratory located in Boston, Massachusetts on a regular basis. Each sample was assigned an identification number for processing purposes, without any demographic or medical data included, to blind those performing the actual analytic processes, such as microbiology culture and histopathology interpretations.

Microbiologic procedures

Samples were kept frozen at -80°C until processed. Prior to bacterial culture samples were removed from the freezer and allowed to thaw at room temperature. The thawed samples were placed into a sterile Petri dish. With sterile forceps and a disposable sterile scalpel, a portion approximately 1 cm square of each tissue specimen was removed, placed into a pre-weighed vial and weighed again. The difference between the two measurements was recorded as the sample weight. Sterile phosphate buffered saline (PBS) was added to the vial containing the sample to achieve a 10 fold w/v dilution. The sample, in PBS, was then homogenized using a hand held Pro-200 homogenizer (PRO Scientific, Inc., Oxford, CT) until the placental tissue was completely dispersed. Serial 10 fold dilutions of the tissue homogenate were made in PBS and aliquots of the original tissue homogenate and dilutions plated onto selective and nonselective bacteriologic media.

The culture media for recovering obligate anaerobes was pre-reduced brucella-base agar with 5% sheep blood enriched with hemin and vitamin K_1. For facultative anaerobes, tryptic soy agar with 5% sheep blood was used (PML, Microbiologicals, Mississauga, Ontario, Canada). Chocolate agar was employed for the recovery of fastidious microorganisms and A-7 agar (Northeast Laboratory, Waterville, ME) was inoculated for the detection of *Mycoplasma* and *Ureaplasma*. Within ten minutes of processing, anaerobic culture plates and A-7 plates were incubated in an anaerobic chamber with an atmosphere of 10% carbon dioxide, 10% hydrogen and 80% nitrogen for a minimum of 120 hours at 35°C. Tryptic soy agar plates were incubated in air and chocolate agar plates in 5% carbon dioxide for 48 hours at 37 °C. After incubation, the various colony types were enumerated, isolated and identified by established criteria. [9-13]

Gram-positive, catalase-negative microaerophilic or anaerobic bacilli that produced a large amount of lactic acid, as determined by gas-liquid chromatography, were classified as *Lactobacillus* sp. Obligate anaerobes were classified by Gram stain and by gas-liquid chromatographic analysis of glucose fermentation products, and the final identification was determined using the Microbial Identification System for LCCFA analysis (MIDI, Inc., Newark, NJ) or the Rapid ANA II system (Remel Inc., Lenexa, KS). *Mycoplasma* and *Ureaplasma* were identified based on their differentiating colony morphologic characteristics following growth on A-7 agar. [9] All bacterial concentrations were expressed as $\log_{10}$ colony forming units (CFU) per gram of placenta tissue. By using the weight of the sample as the

common denominator, quantitative comparisons between specimens could be made where applicable.

Histologic examination of the placenta

After the creation of a reference manual with definitions and illustrations [14] and completion of procedures to minimize observer variability, a pathologist at each site examined the slides for histologic characteristics listed on the data form. For multiple births, separate forms were filled out for each newborn. Twins with fused placentas also had multiple forms filled out. Following the guidelines of the CAP Conference[15], representative sections were taken from all abnormal areas as well as routine sections of the umbilical cord, a membrane roll, and full thickness sections from the center and paracentral zone of the placental disc. Fetal vasculitis was defined as the presence of neutrophils in the fetal stem vessels in the chorionic plate, or in the perivascular Wharton's jelly of the umbilical cord.

Pregnancy variables

Preterm labor was defined as the presence of cervical change in the setting of regular uterine activity with intact membranes. Preterm premature rupture of fetal membranes (pPROM) was defined as amniorrhexis prior to regular uterine activity. Preeclampsia was defined as new onset hypertension and proteinuria. We understand that, given the early gestational age range, this would inevitably involve severe preeclampsia.

Data analysis

The major task of data analysis for this study was to help separate the signal from background noise. The signal in this case is the biologic information represented by the microorganisms recovered from placenta parenchyma. The noise is potential bacterial contamination of the specimen that may occur during delivery.

One goal of the analytic strategy was to determine whether bacteria present in the placenta tissue were part of a colonization process. If isolated microorganisms did not represent true colonization, then we would expect that:

- Recovery rates would be higher for vaginal than for CS deliveries (for the same delivery indication).
- Recovery of microorganisms that are usually the dominant members of normal skin microflora (*Propionibacterium* sp., coagulase negative *Staphylococcus* sp) from CS deliveries and vaginal microflora from vaginal deliveries (*Lactobacillus* sp., coagulase negative *Staphylococcus* sp).
- A high rate of polymicrobial cultures.
- A lack of association with inflammatory phenomena (preterm labor, vasculitis of the chorionic plate or umbilical cord).

An assessment of each of these indicators was made. For example, we compared situations where contamination is least likely (deliveries for maternal indications and delivery by CS) to situations where it is most likely (preterm labor/pPROM with vaginal delivery).

The second goal of the analytic strategy was to determine the significance of the biologic signal. If microorganisms are involved in phenomena related to preterm delivery and placenta inflammation, then recovery rates should vary with:

- Gestational age (regardless of route of delivery),

- The cause of preterm delivery (preterm labor, pPROM, or preeclampsia), especially among CS-delivered placentas, and
- Histologic findings.

As above, the data were evaluated for each of these possibilities, especially for placentas least likely to be contaminated (*e.g.*, CS deliveries, single microorganism cultures).

The generalized form of the null hypothesis is that the rate of microorganism recovery does not vary among placentas obtained from pregnancies with different characteristics. Variations on this theme are that recovery rates are similar whether the delivery was vaginal or by CS, and whether the reason for the preterm delivery was preterm labor, pPROM or preeclampsia. In most cases, comparing the rates of any microorganism recovery from the different strata of the data set assessed this hypothesis.

To avoid distortion due to confounding, many of the analyses were stratified by potentially confounding variables, or restricted to one subgroup of these potential confounding variables. These analyses focus on three potential confounders, each of which is known to be associated with placenta inflammation and which can be initiated by bacteria.

Route of delivery is perhaps the primary potential confounder. Sampling the chorion after the amnion is pulled back exposes what should be sterile tissue. Nevertheless, the possibility that surface contamination might occur as the placenta passes through the birth canal cannot be discounted. Consequently, most analyses stratify by route of delivery and compare findings among placentas delivered vaginally to findings among placentas delivered by CS.

Gestational age is another important potential confounder. Both systemic signs of inflammation in the mother and local inflammation in the placenta tend to decrease with advancing gestation.[16, 17] Consequently, all analyses were stratified by gestational age.

To avoid confounding associated with the reason for preterm birth, we also stratified some analyses by the attributed cause of the preterm delivery.

Because specific comparisons were not planned prior to data analysis, the most appropriate p values are those for row by column arrays. The Chi-square statistic then tests that the observed occurrence in any cell is unlikely to differ from that expected by chance. With most displays comparing two rows by week of gestation (23, 24, 25, 26, 27), a “significant p value” does not inform us about which of the many comparisons within this 2×5 array is what makes the p value significant.

We did use nonparametric tests for trend for many displays. These are not comparisons between rows. Rather they inform us that the values increase or decrease with increasing gestational age in a statistically significant manner.

The only quantitative data comparisons are for median colony-forming units (CFU) per gram of placental tissue. These were tested for significance using the Kruskal- Wallis test.

Those who favor strong adjustments for multiple comparisons can be considered classicists.[18] We were more concerned that these adjustments increase the likelihood of a beta or type II error, especially when the multiple comparisons are not independent, and have treated the data accordingly.

Results

Slightly more than half (321/605=53%) the placentas from pregnancies that were complicated by preterm labor were delivered by CS (Table I). In contrast, 96% (174/181) of placentas from pregnancies delivered because of severe preeclampsia were delivered by CS.

The increase in numbers of placentas with increasing gestational age was much more prominent among placentas delivered by CS than among those delivered vaginally. Fifty-eight percent of all placentas delivered by CS were delivered during the final two weeks of the five-week eligibility period.

The rate of organism recovery decreases with increasing gestational age for both vaginal and CS delivery routes (Table II). The rates are prominently higher for those delivered vaginally, but even for placentas delivered abdominally, more than half the placentas delivered before the end of the 24th week, and 1/3 of those delivered before the end of the 27th week harbored one or more microbial species.

The most prominent decline in microorganism recovery rates with increasing gestational age is seen for placentas from pregnancies that ended with spontaneous labor (Table II). The decline in microorganism recovery with increasing gestational age was also seen when the placenta samples were stratified by route of delivery, duration of membrane rupture, and number of fetuses (all p values < .001). However, the probability of recovering a microorganism did not vary with gestational age among placentas from pregnancies that ended because of pre-labor rupture of membranes, or because of severe preeclampsia. Placentas from pregnancies that were delivered in the setting of severe preeclampsia tended to have relatively low, but nevertheless sizable rates of microorganism recovery. Approximately half of placentas delivered by CS because of preeclampsia and unaccompanied by labor harbored vaginal microorganisms (any or a combination or Lactobacillus species, Prevotella bivia, Peptostreptococcus magnus or Gardnerella vaginalis), a frequency very similar to the rate of skin microorganisms (any or a combination of Corynebacterium, Propionibacterium or Staphylococcus species) (52% data not shown).

Because some women may have had labor initiated after they presented for another reason, our sample was dichotomized by the presence/absence of labor. The findings for any labor are very similar to those for placentas from women who presented with preterm labor. On the other hand, the “no labor” group, an amalgam of placentas from the pPROM and severe preeclampsia, shows lower recovery rates at every week of gestation. The rate of microorganism recovery from placentas delivered more than one hour after membrane rupture tend to be higher than from placentas delivered within one hour of membrane rupture. An analysis of the data examining the types of organisms recovered as a function of the hours in labor preceding a vaginal delivery or CS showed that the placentas of women who had longer labors were not more likely to be infected than the placentas of women with shorter labor durations (data not shown). Furthermore there was no association between vaginal or skin microflora and the duration of labor.

In light of the findings in Table II, we examined gestational age trends in subgroups of placentas restricted to one cause of preterm delivery and one route of delivery (Table III). The rate of microorganism recovery declined with increasing gestational age among placentas delivered after labor, whether by the vaginal route ($p \leq .0005$) or by CS ($p < .001$). At 23, 25, and 27 weeks, placentas delivered vaginally had recovery rates 24% or higher than rates among placentas delivered by CS. The microorganism recovery rates from preeclamptic placentas delivered by CS did not decline with increasing gestational age (p=0.80). Duration of labor did not vary appreciably with gestational age (data not shown).

Among pregnancies with preterm labor, the recovery rate for multiple microorganisms is much higher from vaginally delivered placentas (47%) than from CS-delivered placentas (16%) (Table IV). The recovery rate of multiple microorganisms is lowest (4%) among CS-delivered placentas from pregnancies that ended because of the maternal indication of severe preeclampsia. The rates of multiple microorganism cultures did not vary appreciably among the 13 institutions that provided substantial numbers of placentas (data not shown).

Among placentas delivered by CS, anaerobes, aerobes and *Mycoplasma* were recovered much more commonly following labor than when preeclampsia was the reason for delivery (Table V). Vaginal, but not skin colonizers were among the microorganisms that were recovered more frequently following labor than preeclampsia.

Only three microorganisms occurred in pure culture in at least 3% of CS-delivered placentas after labor, *Lactobacillus sp. Propionibacterium sp*, and coagulase negative *Staphylococcus* sp (data not shown). When single- and multi-microorganism cultures are considered together, however, among the other microorganisms that achieve this 3% criterion are *Prevotella bivia*, Group D *Streptococcus*, alpha hemolytic *Streptococcus*, anaerobic *Streptococcus*, *Gardnerella vaginalis, Ureaplasma urealyticum*, and *Mycoplasma* sp. other than *Ureaplasma urealyticum*. In contrast, among CS-delivered placentas from preeclamptic pregnancies, only *Propionibacterium* sp., and coagulase negative *Staphylococcus* sp. satisfy this 3% criterion.

Among placentas delivered by CS, aerobes and *Mycoplasma* were recovered much more commonly in those with fetal vasculitis than without (Table VI). Anaerobes were recovered equally from both groups. Neither vaginal nor skin colonizers as groups contributed to the observed differences.

The microorganisms recovered in pure culture most frequently from placentas with fetal vasculitis included; *E. coli*, Group B *Streptococcus*, alpha hemolytic *Streptococcus*, *Ureaplasma urealyticum, and Mycoplasma* sp. other than *Ureaplasma urealyticum* (data not shown). Microorganisms that were components of polymicrobial cultures that distinguished between placentas with and without fetal vasculitis included *Actinomyces sp.*, *Prevotella bivia*, *Corynebacterium sp.*, *Peptostreptococcus magnus*, and anaerobic *Streptococcus* sp.

Among CS delivered placentas from pregnancies with preterm labor, *Prevotella sp.*, a genus usually found as part of vaginal microflora, but also recovered as an invasive pathogen from a variety of infections, tended to occur in association with *E. coli*, and anaerobic streptococci, and perhaps with Groups B and D *Streptococcus sp.* and alpha hemolytic *Streptococcus sp.* (Table VII). *Prevotella* sp. tended not to occur with other vaginal microorganisms, nor did other vaginal microorganisms show much of a tendency to occur together. On the other hand, *Actinomyces* sp. tended to occur with *Gardnerella vaginalis, Ureaplasma urealyticum* and other *Mycoplasma* species.

The median bacterial counts are high for all groups of microorganisms and at all gestational ages, and tend not to vary with gestational age (Table VIII), except when considering single microorganism samples. The median bacterial counts tend to be higher among multi-microorganism cultures than among single microorganism cultures. This is expected given that two microorganisms at similar concentrations should give higher median bacterial counts than single microorganism cultures.

Comment

The rate of organism recovery in this study is so high that we wondered how much reflects colonization and how much can be attributed to contamination. We offer findings from this

and other studies to support the view that many of the microorganisms isolated during this study represent colonization of the placenta parenchyma.

The methods used during this study were designed to recover both obligately anaerobic and facultative bacterial species. Previous studies have shown that both obligate anaerobes and facultative species are readily recovered from frozen tissues and that anaerobes that are part of normal vaginal microflora can survive 4 or more hours of exposure to room air. [19-22] Since tissues removed from a systemic supply of oxygen have a much lower oxygen tension than room air (20% O_2), the tissue itself serves as a protective barrier for presumed oxygen sensitive bacteria. We believe that the methods employed were adequate for recovering microorganisms when present. Moreover, the use of PCR on a subset of specimens with both universal and specific primers did not identify microbial DNA in culture-negative or culture-positive specimens.[13]

Placentas from pregnancies that ended with preterm labor had recovery rates that declined with increasing gestational age. If the presence of microorganisms within the placenta were simply a reflection of contamination, the rate of recovery should be the same regardless of gestational age. Because the duration of labor did not vary appreciably with gestational age, the observed gestational age-organism recovery relationships are unlikely to be confounded by duration of labor. In this study, the chorion of placentas from pregnancies that ended with preterm labor had much higher rates of microorganism recovery than placentas from pregnancies that ended early because of increasingly severe preeclampsia. The data suggest that recovery of microorganisms from placental tissue convey biologically important information. Indeed, our findings are consistent with other evidence that infectious/inflammatory phenomena contribute to the onset of labor before the third trimester. [23, 24]

Placentas that had neutrophils in fetal stem vessels within the chorion or in the vessels of the cord (identified as fetal vasculitis) had higher rates of microorganism recovery than placentas without fetal vasculitis. These histologic characteristics are considered manifestations of a fetal inflammatory response, a recognized antecedent of preterm labor. [24]

The numbers of colony forming units/gram of tissue also exceed the levels generally associated with contamination. Contamination of tissue specimens obtained after antiseptic preparation usually occurs with recovery of very low levels of microorganisms (*e.g.* fewer than 10^2 colony forming units (cfu)/gram of tissue). The uniformly high median counts of organisms we recovered suggest that most of the microorganisms isolated were not contaminants.

The rate of microorganism isolation was higher in placentas delivered vaginally than in those delivered by CS. Because some of the difference in isolation rate might be due to contamination as the placenta passed through the vagina, we restricted our attention to placentas delivered by CS. Nevertheless, some of our inferences might also apply to vaginally delivered placentas.

The sterile technique to obtain sterile tissue included lifting and cutting open the amnion with sterile instruments, and peeling away this protective cover to expose the chorion. A second set of sterile instruments was then used to sample the chorion and underlying trophoblast. These efforts should have kept contamination to a minimum. No single species or small group of species consistent with the distribution of species found on skin or on the vaginal epithelium accounted for a sizable proportion of microorganisms isolated from placentas following preterm labor, or from placentas characterized by low gestational age or fetal vasculitis. This is what would be expected with low-virulence organisms, and what has been seen with cultures of amniotic fluid and membranes from preterm pregnancies. [23, 24]

In a separate study of term placentas, we used the same sample collection and culture methods for CS delivered placentas and found that only 17% (2/12) of specimens yielded a

microorganism when cultured. This is similar to our recovering organisms from approximately 20% of preeclamptic placentas delivered before the 28th post-menstrual week. We also found in our small study of term placentas that organisms were not recovered in the absence of labor (unpublished data).

Because bacteria-initiated inflammation has not been associated with severe preeclampsia, CS delivered placentas of preeclampsia pregnancies are considered least likely to yield a microorganism. Yet, a microorganism was recovered from almost a quarter of these placentas. In 83% of these cases, the isolate was a single microorganism, such as *Propionibacterium*, *E. coli*, coagulase negative staphylococci, Group D streptococci, and alpha hemolytic streptococci. While *Propionebacterium* sp., and coagulase negative staphylococci may be considered as possible contaminants from skin, *E. coli*, Group D streptococci and alpha hemolytic streptococci are not commonly members of the cutaneous microflora, nor would any of these species be expected to survive the routine antiseptic preparation before a Caesarian delivery. Consequently, we cannot estimate accurately how much contamination as opposed to true colonization occurs in CS-delivered placentas.

We acknowledge that it is provocative to propose that the presence of microorganisms in these specimens represents colonization and that those organisms in the immediate environment are most likely to colonize the placenta. On the other hand, antibiotics can promote endometrial resolution, but not the eradication, of gram-variable rods such as *G. vaginalis*, as well as new bacterial acquisitions. [25]

More than 80% of 820 endometrium cultures obtained three months after delivery yielded a microorganism. [5] The authors of that study argued that contamination could not account for all that they saw because half of these specimens had plasma cell endometritis, and because the microorganisms recovered did not match the distribution of species in the vagina, the most probable source of the putative contamination. In light of our study, and the others that have recovered organisms from the endometrium [1-5, 6-7], we feel the time has come to recognize that the uterus is not a sterile organ.

Until recently, it was thought that microorganisms from the vagina passed the barrier created by the fetal membranes thereby gaining access to the amniotic sac, [26-28] and that maternal immunosuppression that allowed a tolerance of the fetal allograft also prevented an intense immunologic response to these bacteria. [29] What are these microorganisms doing in the amniotic sac and placenta? The deleterious effects of colonization appear to provoke an inflammatory response that results in preterm labor and delivery. Such an undesirable result might occur if the placenta fails at its filtering/removal function and lets microorganisms survive and invade feta tissues. [30] Indeed, one could argue that the higher levels of bacterial isolation from vaginally delivered placentas simply indicate that the fetus senses a hostile microbiologic environment and signals the host in such a way as to escape from this environment. [31]

Because the normal route of delivery is through the birth canal, the delivered fetus requires some rudimentary protection in place as it passes through an area containing large numbers of microorganisms. [32] Bacterial colonization of the placenta might promote normal development of the fetal immune system without harm to fetus or mother. For example, *Lactobacillus* sp., and coagulase negative *Staphylococcus* sp. are among the least pathogenic, most commonly isolated and most numerous of vaginal microflora microorganisms. Both groups of microorganisms have cell wall constituents, such as peptidoglycan and lipoteichoic acid that are considered highly immunogenic.[33-35] Small amounts of these cell wall constituents have the potential to promote maturation of the fetal immune system during pregnancy. Since much

of the developing neonatal immune system is provoked by GI microflora following birth,[36] might placenta bacteria play a role in immune development before birth?

Placenta bacteria might also be beneficial in other ways. One group of commentators went so far as to suggest, "The emerging picture is that microbial-host interactions in the endometrial cavity are important for reproductive success".[37] In addition to those microorganisms considered to be non-pathogens, other species including *Prevotella* sp., *Gardnerella vaginalis*, *Ureaplasma urealyticum* and *Mycoplasma* sp., may be both important immunomodulators, and capable of escaping the placental/maternal defense mechanisms to cause an outright inflammatory process.

The axiom that single microorganism cultures are more likely to be invasive pathogens is not born out by the findings of Table V. Fifty-six percent of all cultures that yielded coagulase negative staphylococci from labor-initiated deliveries were single-microorganism cultures (Table V). This is the exact same percent of all *E. coli* cultures that were unaccompanied by another microorganism. Polymicrobial infections do occur, particularly adjacent to surfaces with complex microflora [38] and often include commensal microbial species. [39,40]

Perhaps the most attractive feature of this study is the large number of placentas cultured. This has allowed stratification of the data by potential confounding variables. An added attraction, and one that makes this study unique, is the use of placenta parenchyma for culture and not the more commonly cultured amniotic and chorionic membranes or amniotic fluid. If one assumes that placenta parenchyma is sterile, then finding microorganisms within the tissue specimens is an important observation.

Previous reports of the association of bacteria with preterm labor have relied on the recovery of microorganisms from amniotic fluid or the membranes.[17, 41-44] To our knowledge, this is the first report of an analysis of bacteria within placenta parenchyma at the time of preterm delivery.

In summary, the increased rates of microorganism recovery from placenta parenchyma associated with low gestational age, preterm labor and fetal vasculitis suggest that an appreciable proportion of the microorganisms recovered contribute to preterm labor and the fetal inflammatory response, which might be an intermediary between the microorganism and preterm labor. In addition, the finding of presumed non-pathogenic microorganisms within the placenta parenchyma is an important new observation.

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Table I

Sample description. The numbers in each cell are the numbers of placentas with each set of characteristics defined by the route of delivery, gestational age, and the presumed cause of the preterm delivery.

a. Route of delivery: vaginal						
Presumed cause of preterm delivery	**Gestational age (weeks)**					
	23	**24**	**25**	**26**	**27**	**Total**
Labor	47	62	63	52	60	284
Rupture of membranes	13	18	29	24	39	123
Preeclampsia	1	0	1	3	2	7
Total	61	80	93	79	101	414
b. Route of delivery: Cesarean section (CS)						
Presumed cause of preterm delivery	Gestational age (weeks)					
	23	24	25	26	27	Total
Labor	16	69	66	61	109	321
Rupture of membranes	12	34	33	42	53	174
Preeclampsia	3	21	24	57	69	174
Total	31	124	123	160	231	669

Table II

The percent of all infants born during each week of gestation that had a microorganism recovered from the chorion parenchyma of their placenta. Groups of newborns are stratified by different delivery characteristics. These are cell-specific percents. p-value as determined by Chi-square for linear trend.

	Gestational age (weeks)					
Stratification variables	**23**	**24**	**25**	**26**	**27**	**p-value**
Route of delivery						
Vaginal	85	46	69	51	60	≤.0005
CS	58	52	41	41	35	.001
Initiator of delivery						
Labor	79	68	55	46	43	≤.0005
Rupture of membranes	76	60	61	56	60	.29
Preeclampsia	25	24	24	27	23	.87
Labor						
Yes	79	67	57	46	48	≤.0005
No	43	36	34	38	27	.27
Membrane rupture						
1+ hours	77	66	58	56	56	.009
< 1 hour	76	57	47	36	34	≤.0005
Number of fetuses						
Single	73	61	53	47	48	≤.0005
More than one	83	61	53	35	35	≤.0005

Table III

Percent of placentas delivered because of preterm labor or preeclampsia that harbored a microorganism. Groups are stratified by the week of gestation and whether or not the placenta traversed the birth canal. These are cell-specific percents. p-values as determined by Chi-square for linear trend.

Initiator of delivery	Route of delivery	Gestational age					
		23	24	25	26	27	p-value
Labor	Vaginal	87	74	68	48	58	≤.0005
Labor	CS	56	62	42	44	34	.001
Preeclampsia	CS	33	24	21	28	22	.80

Table IV

Percent of cultures that yielded each number of microorganisms. These are row percents. p-values as determined by Chi-square for linear trend. N is the total number of samples for each row.

Initiator of delivery	Route of delivery	Number microorganisms/placenta					
		0	1	2	3+	N	p-value
Labor	Vaginal	33	20	14	33	284	≤.0005
Labor	CS	55	29	9	7	321	≤.0005
Preeclampsia	CS	76	20	3	1	174	≤.0005

Table V

The percent of all CS delivered placentas delivered after labor or preeclampsia that had a representative of each group of microorganisms, either alone or with another microorganism.

Initiator of delivery →	**Labor (n=321)**			**Preeclampsia (n=174)**		
Number of microorganisms →	**1**	**2+**	**Any**	**1**	**2+**	**Any**
Any anaerobe	12	14	26	13	4	17
Any aerobe	12	10	22	7	4	11
Any Mycoplasma	5	4	9	0	0	0
Skin microorganisms* Corynebacterium sp Propionebacterium sp Staphylococcus sp	7	6	13	13	3	16
Vaginal microorganisms* Prevotella bivia Lactobacillus sp Peptostreptococcus magnus Gardnerella vaginalis	7	8	16	0	1	1

* Skin and vaginal microorganisms represent a subgroup of the total number of microorganisms isolated. Skin microorganisms included any or a combination of Corynebacterium, Propionibacterium and Staphylococcus species. Vaginal microorganisms included any or a combination of Lactobacillus species, Prevotella bivia, Peptostreptococcus magnus and Gardnerella vaginalis

Table VI

The percent of all CS delivered placentas delivered with, or without, fetal vasculitis (neutrophils in fetal stem vessels or in umbilical cord vessels) that also had a representative of each group of microorganisms, either alone or with another microorganism.

Fetal vasculitis →	**yes (n=156)**			**no (n=440)**		
Number of microorganisms →	**1**	**2+**	**Any**	**1**	**2+**	**Any**
Any anaerobe	9	21	29	13	8	21
Any aerobe	18	24	42	10	5	16
Any Mycoplasma	11	9	20	2	2	3
Skin microorganisms*	5	10	15	10	4	14
Corynebacterium sp						
Propionebacterium sp						
Staphylococcus sp						
Vaginal microorganisms*	3	8	11	5	4	9
Prevotella bivia						
Lactobacillus sp						
Peptostreptococcus magnus						
Gardnerella vaginalis						

*Skin and vaginal microorganisms represent a subgroup of the total number of microorganisms isolated. Skin microorganisms included any or a combination of Corynebacterium, Propionibacterium and Staphylococcus species. Vaginal microorganisms included any or a combination of Lactobacillus species, Prevotella bivia, Peptostreptococcus magnus and Gardnerella vaginalis

Table VII

Quantitative data for CS delivered placenta. Percent of cultures positive for the microorganism or microorganism group on the left that are positive or negative for the microorganism or microorganism group heading the columns (numbers are column percents except the column N, which is displayed just beneath "+" and "-"). Columns are displayed only if there were at least 10 placentas with that microorganism. These are based on cultures of CS delivered placentas.

	Prevotella		**Lactobacillus**		**Propionibact**		**Staph**		**Gp B Strep**		**α Strep**		**Anaer Strep**		**Gardnerella**		**Mycoplasma**		**Uu**	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
N	**11**	**310**	**26**	**295**	**22**	**299**	**18**	**303**	**12**	**309**	**12**	**309**	**14**	**307**	**13**	**308**	**18**	**303**	**13**	**308**
Actinomyces sp.	9	2	8	2	0	3	0	2	0	3	0	3	7	2	15	2	11	2	15	2
Prevotella bivia			0	4	0	4	0	4	17	3	17	3	21	3	0	4	0	4	8	3
Corynebacterium sp.	9	1	4	1	0	1	11	1	0	1	8	1	7	1	0	1	0	1	0	1
Escherichia coli	27	2	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3
Lactobacillus sp	0	8			5	8	11	8	0	8	8	8	14	8	15	8	17	8	0	8
Peptostretococcus magnus	9	1	4	1	5	1	0	2	0	2	0	2	0	2	0	2	0	2	0	2
Propionibacterium sp.	0	7	4	7			0	7	8	7	0	7	14	7	8	7	11	7	15	6
Staphylococcus sp.	0	6	8	5	0	6			8	6	17	5	14	5	0	6	0	6	8	6
Group B Streptococcus	18	3	0	4	5	4	6	4			17	3	7	4	0	4	6	4	0	4
Group D Streptococcus	18	2	4	3	5	3	0	3	0	3	0	3	7	3	8	3	0	3	0	3
alpha hemolytic Strep	18	3	4	4	0	4	11	3	17	3			14	3	0	4	6	4	0	4
anaerobic Streptococcus	27	4	8	4	9	4	11	4	8	4	17	4			8	4	0	5	0	5
Gardnerella vaginalis	0	4	8	4	5	4	0	4	0	4	0	4	7	4			11	4	0	4
Mycoplasma sp.	0	6	12	5	9	6	0	6	8	6	8	6	0	6	15	5			8	6
Ureaplasma urealyticum	9	4	0	4	9	4	6	4	0	4	0	4	0	4	0	4	6	4		

Table VIII

The median colony-forming unit (CFU) count per gram for C/S delivered placentas in each gestational age category that also had the characteristic listed on the left. These are cell specific medians

Microorganism	**Gestational age (completed weeks)**					
	23	**24**	**25**	**26**	**27**	**p-value**
Any anaerobe	3.2	3.1	3.1	2.6	3.0	.36
Any aerobe	3.9	3.3	3.5	3.2	2.8	.35
Any Mycoplasma	3.4	4.0	3.9	4.1	4.5	.50
# microorganisms isolated: 1	3.7	2.3	2.9	2.6	3.0	.02
2+	3.3	3.8	3.6	4.0	3.6	.92
Skin microorganisms Corynebacterium sp Propionebacterium sp Staphylococcus sp	3.0	2.3	2.6	2.6	2.5	.80
Vaginal microorganisms Prevotella bivia Lactobacillus sp Peptostreptococcus magnus Gardnerella vaginalis	3.1	3.3	3.6	2.9	3.4	.65
Total number placentas	18	64	51	65	82	