HHS Public Access

Author manuscript

J Matern Fetal Neonatal Med. Author manuscript; available in PMC 2020 May 01.

Published in final edited form as:

J Matern Fetal Neonatal Med. 2019 May ; 32(10): 1703–1720. doi:10.1080/14767058.2017.1416083.

The Diagnostic Performance of the beta-Glucan Assay in the Detection of Intra-amniotic Infection with *Candida* species

Percy Pacora, MD[1,2], **Roberto Romero, MD, D.Med.Sci**[1,3,4,5], **Offer Erez, MD**[1,2], **Eli Maymon, MD**[1,2], **Bogdan Panaitescu, MD, PhD**[1,2], **Juan Pedro Kusanovic, MD**[6,7], **Adi L. Tarca, PhD**[1,2], **Chaur-Dong Hsu, MD, MPH**[2], and **Sonia S. Hassan, MD**[1,2]

[1]Perinatology Research Branch, Division of Obstetrics and Maternal-Fetal Medicine, Division of Intramural Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, Maryland, and Detroit, Michigan, USA

[2]Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

[3]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, USA

[4]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, Michigan, USA

[5]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan, USA

[6]Center for Research and Innovation in Maternal-Fetal Medicine (CIMAF), Department of Obstetrics and Gynecology, Sótero del Río Hospital, Santiago, Chile

[7]Division of Obstetrics and Gynecology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

Abstract

INTRODUCTION—A bioassay based on the detection of beta-glucan, a constituent of the cell wall of fungi, has been successfully used to diagnose fungal infections in a variety of biological fluids but not yet in the amniotic fluid.

OBJECTIVE—To determine the diagnostic performance of a beta-glucan bioassay in the detection of *Candida* species in the amniotic fluid.

METHODS—The study population comprised women who had a singleton pregnancy without congenital or chromosomal abnormalities, who experienced preterm labor or preterm prelabor rupture of the fetal membranes, and who underwent transabdominal amniocentesis for clinical indications. Samples of amniotic fluid were cultured for aerobic, anaerobic, genital mycoplasma, *Candida* species and assayed for beta-glucan using the (1→3)-beta-D-glucan-specific *Limulus* amebocyte lysate test (beta-glucan assay) in all cases. Amniotic fluid interleukin (IL)-6 assay results were also available for all cases. The beta-glucan assay takes about one hour to run: a

Correspondence: Roberto Romero, MD, D.Med.Sci., and Percy Pacora, MD, Perinatology Research Branch, NICHD/NIH/DHHS, Hutzel Women's Hospital, 3990 John R, 4 Brush, Detroit, Michigan 48201, Telephone: (313) 993-2700, Fax: (313) 993-2694, prbchiefstaff@med.wayne.edu; ppacora@med.wayne.edu.

concentration > 80 pg/mL was considered positive for fungi. Sterile intra-amniotic inflammation of the amniotic cavity was defined by the presence of an amniotic fluid IL-6 concentration ≥2.6 ng/mL and a negative amniotic fluid culture.

RESULTS—1) One hundred and ninety-seven (197) women met the study criteria, of whom 58 (29.4%) had an intrauterine contraceptive device (IUD) in place; 2) twenty (10.2%) women had a culture of proven intra-amniotic *Candida* species-related infection, 19 of whom had a positive beta-glucan assay [sensitivity, 95% (19/20; 95% confidence interval (CI): 75.1%–99.9%)]; and 3) the specificity of the beta-glucan assay was 75.1% [133/177; 95% CI: 68.1%–99.9%]. It was affected by the presence of non-fungal intra-amniotic infections and an IUD, but not by the presence of sterile intra-amniotic inflammation, and there was a significant interaction between the presence of an IUD and non-fungal intra-amniotic infections (estimated for the interaction effect = 2.1923, p value = 0.026). The assay's specificity was reduced when non-fungal intra-amniotic infections were diagnosed but only in women who did not have an IUD.

Among women without IUD, the assay's specificity was 91.4% (117/128); it was 93% (106/114) for those without intra-amniotic infection, and 78.6% (11/14) for those with a non-fungal intra-amniotic infection; the difference was not significant (p = 0.09). Among women with IUD, the assay's specificity was 32.7% (16/49); 42.9% (9/21) for those with a non-fungal intra-amniotic infection, and 25% (7/28) for those without intra-amniotic infection, and the difference was significant (p = 0.03).

CONCLUSIONS—The beta-glucan assay is a sensitive, rapid, point-of-care test used to diagnose intra-amniotic *Candida* species-related infection, and it has a high specificity in pregnant women who did not have an IUD in place.

Keywords

Amniotic fluid; *Candida albicans*; fetus; newborn; pregnancy

Introduction

Intra-amniotic fungal infection is often associated with early preterm birth (1–5), fetal death (6–11), and adverse neonatal outcome (12–20). The most common fungus isolated from the amniotic fluid of women with spontaneous preterm parturition [preterm labor with intact membranes or preterm prelabor rupture of the fetal membranes (preterm PROM)] is *Candida albicans* (3, 5, 21–26); additionally, *Candida parasilosis* (27), *Candida tropicalis* (7, 28–30), and *Candida glabrata* (11, 31, 32) have been isolated from the amniotic fluid and associated with fetal infection. In a meta-analysis (21), the overall frequency of *Candida* species-related infection found in the amniotic fluid of patients with preterm labor (22, 23, 32–42) and preterm PROM (24, 25, 28, 30, 43–46) was 0.6%.

The treatment of fungal intra-amniotic infections improves neonatal outcome (47–49), and it has been proposed that its efficacy can be expected to increase the earlier the infection is diagnosed and treatment begins (47–49). However, the results of traditional cultivation techniques may take from 48 hours up to 14 days to allow the recovery of slowly growing fungi (50, 51). Nevertheless, fungal infection can be diagnosed more rapidly with a bioassay (herein referred to as the beta-glucan assay) based on the identification of (1→3)-beta-D-

glucan, a component of the cell walls in many fungi. This bioassay has been shown to be effective in diagnosing fungal infections caused by *Candida* (52–54), *Aspergillus* (52, 53), *Cryptococcus* (52), *Trichosporon* (52, 53), *Pneumocystis carinii/Pneumocystis jirovecii* (55, 56), *Fusarium* (53, 54), *Trichosporon beigelii* (57), *Saccharomyces cerevisiae* (57), and *Acremonium* (57). The overall sensitivity and specificity of the beta-glucan assay for the detection of systemic fungal infection reported in a meta-analysis of 16 studies (2,979 subjects) were 76.8% and 85.3%, respectively (58).

The beta-glucan assay has been used to evaluate maternal serum (53, 54, 58), urine (59), cerebra-spinal (60–64) and peritoneal (65, 66) fluids, yet it has not been used to evaluate the amniotic fluid for the rapid diagnosis of fungal intrauterine infections. Therefore, the objective of this study was to determine the diagnostic performance of a beta-glucan bioassay for the detection of intra-amniotic fungal infection of women who had or did not have an intrauterine contraceptive device (IUD) in place during an episode of spontaneous preterm parturition.

Materials and Methods

Study population

The study population comprised pregnant women who met the following criteria: a singleton pregnancy without congenital or karyotypic abnormalities; preterm labor and/or preterm PROM evaluated with transabdominal amniocentesis; and a sufficient quantity of stored amniotic fluid (at least 0.5 mL) available to run a beta-glucan assay. Many of the samples had previously been used to study the biology of inflammation, hemostasis, angiogenesis, and growth-factor concentrations.

The study population comprised two separate groups culled from pregnant women with or without an IUD who had preterm parturition and who were enrolled between October 1990 and October 2000 at Hutzel Women's Hospital, Detroit, Michigan, USA, or at Sótero del Río Hospital, Puente Alto, Chile, in a prospective study protocol approved by the Institutional Review Boards of Wayne State University; the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS); and Sótero del Río Hospital. The patients provided written informed consent.

Clinical definitions

Gestational age was determined by the last menstrual period and confirmed by ultrasound examination or by ultrasound examination alone if the sonographic determination of gestational age was inconsistent with menstrual dating by more than one week.

Preterm parturition included spontaneous preterm labor and preterm PROM. Spontaneous preterm labor was defined as the presence of at least two regular uterine contractions occurring every 10 minutes, associated cervical effacement and shortening, and intact fetal membranes in patients with a gestational age between 20 and 36+ 6/7 weeks (67–69). Preterm prelabor rupture of the fetal membranes was defined as follows: rupture < 37 weeks of gestation occurring at least one hour before the presence of labor (uterine contractions

and cervical effacement and dilatation), diagnosed by the combination of a speculum examination confirming the pooling of amniotic fluid in the vagina, a positive nitrazine test, and a positive ferning test (68, 70–72).

Microbial invasion of the amniotic cavity (MIAC) or intra-amniotic infection was defined as a positive amniotic fluid culture for microorganisms using traditional cultivation techniques (22, 25, 73–81). Candida species-related intra-amniotic infection was defined as a positive amniotic fluid culture for *Candida* species using traditional cultivation techniques. Non-fungal intra-amniotic infection was defined as a positive amniotic fluid culture for microorganisms other than *Candida* species or other fungi. Intra-amniotic inflammation was defined as an amniotic fluid interleukin (IL)-6 concentration ≥2.6 ng/mL (74, 75, 82–91). Sterile intra-amniotic inflammation was defined as a negative amniotic fluid culture and an amniotic fluid IL-6 concentration ≥2.6 ng/mL (74, 75, 82, 83, 85–92).

Amniotic fluid collection and storage—Amniotic fluid specimens were collected by transabdominal amniocentesis for medical indications, such as karyotyping studies and fetal lung maturity, or to rule out intra-amniotic infection. These specimens were collected transabdominally under ultrasound guidance with a sterile aseptic technique using a 22-gauge needle. The first 1–2 mL of amniotic fluid were discarded to avoid maternal cell contamination. Amniotic fluid specimens were transported in a capped sterile syringe to the clinical laboratory at the respective hospitals.

Amniotic fluid specimens were cultured for aerobic and anaerobic bacteria, including *Mycoplasma* and *Ureaplasma*. Specifically, 1–2 drops of amniotic fluid were used to inoculate chocolate agar, trypticase soy agar with 5% sheep blood, and MacConkey agar culture media. Aerobic plates were incubated at 35° C in an 8% CO_2 chamber. Anaerobic plates were incubated at 35° C in an anaerobic chamber containing an atmosphere of 5% CO_2, 10% hydrogen, and 85% nitrogen. All plates were incubated for a total of four days.

Amniotic fluid not required for clinical purposes was centrifuged to remove cellular and particulate matter. Aliquots of amniotic fluid were stored at −70º C until analysis.

The beta-glucan assay test

The (1→3)-beta-D-glucan-specific *Limulus* amebocyte lysate (LAL) assay (beta-glucan assay; Fungitell®, Associates of Cape Cod, East Falmouth, MA, USA) is an aqueous extract, derived from the amoebocytes (blood cells) of the horseshoe crab (*Limulus polyphemus*), the most widely used method to measure (1→3)-beta-D-glucan in human serum (50, 53). The assay is based upon modification of the LAL pathway. This reagent, modified to eliminate Factor C, bypasses the activated factor B, and reacts only to (1→3)-beta-D-glucan; it does not react to other polysaccharides, including beta-glucans with different glycosidic linkages. When (1→3)-beta-D-glucan is present in a sample, it activates factor G, a serine protease zymogen. The activated Factor G converts the inactive pro-clotting enzyme to the active clotting enzyme (93) that, in turn, cleaves para-nitroaniline (pNA) from the chromogenic peptide substrate Boc-Leu-Gly-Arg-pNA, creating a chromophore that absorbs at 405 nm. The reagent is used in the beta-glucan kinetic assay to detect the rate of optical density increase produced by a sample. This rate is interpreted

against a standard curve to produce an estimate of (1→3)-beta-D-glucan concentration in the sample (93). Beta-glucan activity was calibrated with pure pachyman, a linear beta-glucan. All assays were performed in triplicate using a microtiter plate.

Amniotic fluid samples (5 μL) were pretreated for 10 minutes at 37° C with an alkaline reagent (20 μL; 0.125 M KOH/0.6 M KCl) to inactivate serine proteases as well as inhibitors in human serum and to enhance the reactivity to activated factor G (94, 95). After adding the beta-glucan assay reagent, the microtiter plate was inserted into a ThermoMax plate reader (Molecular Devices LLC, Sunnyvale, CA, USA), pre-incubated to 37° C, and a kinetic assay was run using SoftMax Pro software (Molecular Devices). An amniotic fluid beta-glucan concentration ≥80 pg/mL was interpreted as a positive result; an amniotic fluid beta-glucan concentration < 79 pg/mL was considered a negative test.

Statistical Analysis

The Kruskal-Wallis test was used to compare continuous variables (birthweight and gestational age at amniocentesis) as these were not normally distributed by the Shapiro-Wilk test. The Chi–square and Fisher's exact tests were used for comparison between categorical variables.

A log linear model was used to fit a multilayer contingency table of the data to determine the factors that affected assay specificity and the interactions between those factors. The statistical package used was SPSS Version 19 (IBM Corporation, Armonk, New York, USA). A p-value of <0.05 was considered statistically significant.

Results

Characteristics of the study population

One hundred and ninety-seven (197) women met the study criteria: the demographic characteristics of these women are shown in Table 1. Fifty-eight (29.4%) nulliparous women had an IUD in place: 57 (98.3%) were from Chile and one (1.7%) was from Detroit. All women who did not have an IUD in place were recruited from the Detroit area: 90.6% (126/139) of women were of African-American descent and 41% (57/139) were nulliparous (Table 1). Women with an IUD were more frequently Hispanic (p<0.001), and women without IUD were more frequently African-American (p<0.001). Women who had an IUD in place also underwent amniocentesis at an earlier gestational age and had a lower median birthweight than women who did not have an IUD in place (Table 1).

The frequency of intra-amniotic infection and fungal intra-amniotic infection

Fifty-five of 197 (27.9%) women had MIAC (Figure 1): 35 (17.8%) had a non-fungal intra-amniotic infection, 15 (7.6%) had *Candida* species alone -related intra-amniotic infections, and 5 (2.5%) had mixed *Candida species* and non-fungal intra-amniotic infections. Forty of 197 (20.3%) women had inflammation of the amniotic cavity alone without infection (sterile intra-amniotic inflammation). *Candida* species were the only fungi isolated.

Microbial invasion of the amniotic cavity was significantly more frequent in women with an IUD than in those without an IUD [51.7% (30/58) versus 18.0% (25/139); p < 0.001]. Non-

fungal intra-amniotic infections and intra-amniotic infection by *Candida* species only were significantly more frequent in women who had an IUD in place than in those who did not (Non-fungal intra-amniotic infection : with IUD, 36.2 % (21/58) versus without IUD, 10.1 % (14/139); $p < 0.001$); intra-amniotic-infection by Candida species only : with IUD, 13.8% (8/58) versus without IUD, 5.0% (7/139); p=0.04) (Figure 2).

Candida intra-amniotic infections and sterile inflammation of the amniotic cavity were also more frequent among women who had an IUD in place, but the differences were not statistically significant [Candida intra-amniotic infection: with IUD, 15.5% (9/58) versus without IUD, 7.9% (11/.139), $p = 0.12$; Sterile intra-amniotic inflammation: with IUD, 27.6% (16/58) versus without IUD, 17.3% (24/139), $p = 0.12$] (Figure 2). Only one in five women with an IUD in place had a sterile intra-amniotic cavity without inflammation, whereas the intra-amniotic cavity was sterile without inflammation in almost two-thirds of the women without an IUD in place ([20.7% (12/58) versus 64.7% (90/139), $p < 0.001$] (Table 1).

Diagnostic accuracy of the beta-glucan assay

The beta-glucan assay was positive in 19 of the 20 cases for which *Candida* species were cultured from the amniotic fluid specimens (sensitivity, 95%) (Tables 2 and 3). The overall specificity of the beta-glucan assay was 75.1% (133/177) (Tables 2 and 4).

The specificity of the assay was affected by the presence of an IUD and non-fungal intra-amniotic infection but not by the presence of sterile inflammation; there was an interaction between the presence of an IUD and non-fungal infection (estimated for the interaction effect = 2.1923, $p = 0.026$) (Tables 4 and 5).

Non-fungal intra-amniotic infection lowered the assay's specificity but only for women who did not have an IUD in place. Although non-fungal intra-amniotic infections were more common in women who had an IUD in place, non-fungal intra-amniotic infections did not lower the assay's specificity when compared to women with an IUD in place but who did not have a non-fungal intra-amniotic infection.

The specificity of the beta-glucan assay for women who did not have an IUD in place was 91.4% (117/128); it was 93% (106/114) for those who did not and 78.6% (11/14) for those who did have a non-fungal intra-amniotic infection; the difference was not statistically significant ($p = 0.09$) (Table 5). The assay's specificity for women who had an IUD in place was 32.7% (16/49); it was 42.9% (9/21) for those who did have and 25% (7/28) for those who did not have a non-fungal intra-amniotic infection; the difference was statistically significant ($p = 0.03$) (Table 5). The probability of culture-positive intra-amniotic *Candida* species-related infection in a woman without an IUD in place who had a positive beta-glucan assay was 47.6% (10/21) and 21.4% (9/42) in a woman with an IUD in place (Table 3).

Discussion

This is the first study to report the use of a beta-glucan assay to diagnose fungal intra-amniotic infection. The principal findings of the study are as follows: 1) the beta-glucan assay detected *Candida* species-related intra-amniotic infection with 95% sensitivity (95% CI, 75.1–99.9); 2) the assay's specificity was 75.1% (95% CI, 68.1%–81.3%) for the entire study population, 91.4% (95% CI, 85.1%–95.6%) for women without an IUD, and 32.7% (95% CI, 20.0%–47.5%) for those with an IUD; and 3) the assay's specificity was affected by the presence of non-fungal intra-amniotic infection and an IUD but not by sterile inflammation of the amniotic cavity; there was a significant interaction between the presence of an IUD and non-fungal intra-amniotic infection on the assay's specificity. Non-fungal infection of the amniotic cavity decreased the assay's specificity but only in women who did not have an IUD. The presence of an IUD significantly decreased the specificity of the assay.

Intra-amniotic *Candida* infection

Candida species colonize the vagina in at least 20% of all women (96), and the frequency rises to 30% in pregnant women (97). Candidiasis during pregnancy may be associated with an increased risk of pregnancy complications such as preterm PROM and poor pregnancy outcome (47, 98–100). Moreover, there is evidence that the eradication of *Candida* species in pregnancy may reduce the risk of preterm birth (101). Despite the clinical frequency of *Candida* species in the lower genital tract, they appear to be infrequent invaders of the amniotic cavity based on their low prevalence in the amniotic fluid (21).

In the current study, the rate of intra-amniotic *Candida* species-related infection was 7.9% (11/139) for pregnant women without an IUD and 15.5% (9/58) for pregnant women with an IUD in place. The association between the presence of an IUD and intra-amniotic infection with *Candida* species has been previously reported (1, 4, 102–106). Roque et al. (5) reported in a meta-analysis of 54 cases that 50% of patients who had an intra-amniotic infection with *Candida* species also had an IUD, and these factors are associated with severe infectious morbidity.

The IUD has the lowest failure rate (1%) of all contraception methods (107), and its use is recommended as a first-line contraceptive method for nearly all women, including adolescents and nulliparous women (108–110). Approximately 10% of North American women use an IUD as a contraceptive, and more than 60% of this group use the device for more than 48 months (111). However, pregnancy occurring with the presence of an IUD is associated with an increased rate of complications, e.g., intrauterine infection often due to fungi (1, 4, 102–105, 112), late spontaneous abortion (106, 113, 114), spontaneous preterm labor/birth (103, 113–120), histologic chorioamnionitis/funisitis (121), abruptio placentae (103), adverse neonatal outcome (5, 7, 8, 29, 104, 105, 112, 113, 115–120, 122–132), maternal sepsis (113, 116, 133), and even maternal death (134). In a large cohort study of 12,297 patients (103), 1.6% (196/12,297) conceived while using an IUD and 56.1% (110/196) of these pregnant women with an IUD delivered preterm. The prevalence of positive amniotic fluid cultures from patients who had an IUD in place was 45.9% (45/98), and *Candida* species-related intra-amniotic infection was present in 31% (14/45) of

pregnancies that occurred with an IUD, a rate five times higher compared to patients who conceived without an IUD (6.3%; 11/174) (103).

Antimicrobial properties of the amniotic fluid against fungi

Under normal circumstances, the amniotic fluid is sterile (135–139). Systematic investigations using cultivation and molecular microbiologic techniques have demonstrated that most women with a normal pregnancy outcome do not show evidence of bacteria (140, 141), viruses (142–144), or fungi (138) in the amniotic cavity. The absence of microorganisms is presumably accomplished by the presence of components of the innate immune system and antimicrobial peptides in the amniotic fluid (145).

The amniotic fluid concentration of antimicrobial peptides (e.g., bactericidal/permeability-increasing protein and calprotectin) increases with advancing gestational age, and their median concentration in the amniotic fluid at mid-trimester gestation is significantly reduced as compared to term gestation in normal pregnancies (145). In fact, amniotic fluid has anti-microbial properties against potential pathogenic microorganisms (146–152), and several studies demonstrated that human amniotic fluid from patients in their second and third trimesters of pregnancy can efficiently inhibit the growth of *Candida albicans in vitro* (146, 153, 154). Therefore, *Candida* species-related intra-amniotic infection in early pregnancy is more deleterious than during the third trimester of pregnancy (1–10, 100, 155, 156).

The current study reports that 25% (5/20) of the *Candida* species isolated in pregnancies with preterm labor were associated with other microorganisms. Additionally, our group previously reported that 4.8% (2/42) of the amniotic fluid samples from women with clinical chorioamnionitis at term was caused by *Candida* species (one identified as *Candida albicans* and the other as *Candida famata*) (75). Both cases indicated the presence of mixed flora: one was associated with Gram-negative bacilli and the other with *Staphylococcus epidermidis*, *Lactobacilllus species*, and *Micrococcus luteus* (75). These two studies suggest that *Candida* species-related intra-amniotic infection is more frequently associated with other microorganisms observed in early preterm gestation. The impaired anti-microbial property of the amniotic fluid found early in pregnancy may be a common pathogenic mechanism for the presence of a concurrent *Candida* species-related intra-amniotic infection. Thus, early pregnancies associated with cervical incompetence (2), cervical cerclage (1, 2, 102, 157), use of an IUD (1, 4, 102, 103), *in vitro* fertilization (9, 31, 158, 159), premature rupture of the fetal membranes (31), and immunocompromised individuals (160) are at increased risk of *Candida* species-related intra-amniotic infection as a result of the combination of an impaired anti-microbial property of the amniotic fluid early in pregnancy and an ascending infection of *Candida* species through the uterine cervical canal and fetal membranes (161, 162).

Congenital candidiasis and fetal response to Candida species-related intra-amniotic infection

Although congenital candidiasis has been reported to be 0.8% (4/492), based on *Candida* species-related placental infection in a series of 494 random consecutive singleton deliveries (163), congenital candidiasis is more commonly associated with the systemic spread and

worsened outcome for preterm neonates compared to term neonates (156, 164). Systemic neonatal candidiasis is estimated to occur in 2% to 4% of neonates with very low birthweight (13, 14, 102, 147, 165–167). The prevalence of *Candida* species-related infection in early preterm neonates weighing less than 1500 grams (very low birthweight) was 4.8% (7/146) during the first 24 hours of life (16). Additionally, there is an inverse correlation between the incidence of *Candida* species-related infection and neonatal birthweight (168) and gestational age (156, 169).

Candida species-related intra-amniotic infection is associated with severe fetal inflammatory response and adverse pregnancy outcomes that include early preterm birth (1–5, 20) and fetal death (6, 9, 10, 155, 165, 170). Several experiments utilizing sheep models demonstrated the pathogenicity of *Candida albicans* in the amniotic fluid: 1) *Candida albicans* colonization of the amniotic cavity rapidly resulted in a robust intrauterine inflammation characterized by an increase in IL-1β, IL-8 in the amniotic and lung fluids, fetal hypercortisolemia, leukopenia, and thrombocytopenia, and an elevated mRNA expression of IL-1β, IL-8, IL-6, tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein (MCP)-1 in the fetal lung, skin, and membranes (171); 2) a *Candida albicans* intrauterine infection in early pregnancy causes systemic fetal candidiasis, an infection associated with robust systemic inflammatory response and progressive cardiac dysfunction (172, 173); 3) intra-amniotic exposure to *Candida albicans* provokes acute systemic and neuro-inflammatory responses with concomitant white-matter injury (174); and 4) *Candida albicans* colonization of the amniotic cavity causes fetal intestinal infection, mucosal injury, and inflammation (175). In humans, the information regarding the effect of fetal candidiasis is mainly derived from case reports and case series (1–5) of late abortions (6, 114), preterm labor (21, 33), fetal death, and clinical chorioamnionitis (20, 176).

Intra-amniotic infection with candidiasis may be treatable with anti-fungal medication *in utero.* Treatment with fluconazole, administered intra-amniotically or intraperitoneally, in an animal model was associated with a significant reduction in the fetal IL-6 concentration on Day 3 in comparison to the untreated group. Intra-amniotic exposure to *Candida albicans* did not attenuate fetal neuro-inflammation and white-matter injury but rather reduced fetal mortality (174). Maneenil et al. (177) demonstrated that the severe fetal inflammatory response caused by intra-amniotic infection with *Candida albicans* in fetal sheep was transiently decreased with the use of fluconazole and that maternal anti-fungal therapy may prevent fetal injury associated with *Candida* species-related intrauterine infection. These results are in agreement with three clinical reports showing that early diagnosis and maternal as well as intra-amniotic administration of anti-fungal treatment improved the neonatal outcome in cases with intra-amniotic candidiasis (47–49).

To overcome the delay of a diagnosis of intra-amniotic fungal infection, which usually takes 2 to 14 days (50, 51), the use of a simple test providing a rapid, easy-to-interpret result is recommended; such a point-of-care test requires low maintenance and is cost-effective (69, 70, 178–182). Amniotic fluid matrix metalloproteinase (MMP)-8 (83, 178, 183–191) and IL-6 (82, 137, 180–182, 192–224) have diagnostic and prognostic value in the identification of intra-amniotic inflammation, imminent spontaneous preterm delivery, acute inflammatory lesions of the placenta, and adverse neonatal outcome. Additionally, concentrations of

MMP-8 and IL-6 perform better than the amniotic fluid white blood cell count, glucose concentration, and Gram stain for the identification of intra-amniotic inflammation/infection (83, 178, 184, 200–202, 205).

The rapid-assay kits evaluating IL-6 and MMP-8 in the amniotic fluid are currently being introduced for the timely identification of intra-amniotic infection and inflammation (within 15 to 20 minutes) without the need of sophisticated laboratory equipment (179); both assays show the same sensitivity for the detection of intra-amniotic inflammation (85.7%; 18/210); however, the specificity of the rapid MMP-8 test was higher than that of the rapid IL-6 with a cutoff of 745 pg/mL in the identification of intra-amniotic inflammation (179). Moreover, patients with a positive MMP-8 rapid test who did not have intra-amniotic infection or inflammation detected by either a standard cultivation technique or an amniotic fluid white blood cell count delivered preterm and showed evidence of acute histologic chorioamnionitis (179). Currently, the beta-glucan assay may yield results in less than one hour; and due to its high sensitivity (95%) and specificity (75%), it may identify patients with *Candida* species-related intra-amniotic infection who would benefit from prompt anti-fungal treatment (47–49).

Collectively, this evidence suggests that intra-amniotic infection with *Candida* species carries a high rate of fetal/neonatal morbidity and mortality that may be prevented with early diagnosis and *in utero* treatment. The introduction of point-of-care tests specifically intended for the identification of *Candida* species may improve the early detection and the prompt treatment of *Candida* species-related intra-amniotic infection.

Performance indices of the beta-glucan assay

The finding that the beta–glucan assay utilized in this study has a sensitivity of 95% and a specificity of 75.1% for the detection of *Candida* species-related intra-amniotic infection in women with preterm parturition is novel. The assay's values of sensitivity and specificity are similar to those reported in the cerebra-spinal fluid of children with fungal meningitis (60). The diagnostic indices of the beta-glucan assay for fungal intra-amniotic infection were as good as or better than those reported for other biological fluids (54, 58, 225).

Ostrosky-Zeichner et al. (54) reported that the beta-glucan assay, using a cutoff of 80 pg/mL in the serum of 163 patients with invasive fungal infection, had a sensitivity of 64.4% and a specificity of 92.4% as well as a positive predictive value of 89% and a negative predictive value of 73%. Of the 107 patients with proven candidiasis, 77.6% had a positive beta-glucan assay; among 118 patients with a positive culture for fungi who were treated with anti-fungal medication, 69.5% had a positive beta-glucan test (54). Karageorgopoulos et al. (58) conducted a meta-analysis in which 594 of 2,979 patients had proven fungal infection in their serum. The authors reported a pooled sensitivity and specificity of the beta-glucan assay as 76.8% and 85.3%, respectively, and an average false-positive rate of 1% among 310 beta-glucan assays performed for healthy individuals (58). In another meta-analysis that included 15 studies, Lu et al. (226) found a sensitivity of 76%, a specificity of 85%, a positive likelihood ratio of 5.05, and a negative likelihood ratio of 0.28 for the beta-glucan assay in serum. To date, the best diagnostic performance of a beta-glucan assay, reported by Malani et al. (60), showed a sensitivity of 96% and a specificity of 95% to identify and

diagnose proven fungal meningitis as well as a sensitivity of 84% and a specificity of 95% for the diagnosis of probable or proven spinal or para-spinal fungal infection among 233 patients.

Furthermore, a multi-center prospective study conducted in South Africa included 72 neonates with clinically suspected late-onset sepsis who were also at high risk for fungaemia; Mackay et al. (227), using a beta-glucan assay at a level of 80 pg/ml, found its sensitivity and specificity to be 70.7% and 77.4% as well as positive and negative predictive values of 80.6% and 66.7%, respectively, for the diagnosis of invasive fungal disease in neonates.

Goudjil et al. (228) found that the beta-glucan concentration was higher in 18 cases with neonatal invasive *Candida* species-related infection as compared to 43 non-infected neonates (364 pg/mL versus 89 pg/mL; $p < 0.001$). The authors concluded that the optimal cut-off for distinguishing between non-infected and infected patients was 125 pg/mL (sensitivity, 84%; specificity, 75%) (228). A cutoff >523 pg/ml for a beta-glucan assay of serum was reported for two low-birthweight neonates with a culture-proven *Candida parapsilosis* infection (229); another group of investigators utilized a cut-off > 200 pg/mL for a case series report on five preterm neonates with *Candida* species-related infections already proven by their positive blood cultures.(230). A retrospective study of 47 preterm neonates conducted by Cornu et al. (231) found that the median beta-glucan concentration was higher in 26 neonates with probable or proven invasive fungal infection versus 20 neonates without fungal colonization/infection [149 pg/ml (interquartile range (IQR): 85–364) versus 39 pg/ml (IQR: 20–94), $p < 0.001$]. A beta-glucan assay with a cutoff > 106 pg/ml had a sensitivity of 61.5% and a specificity of 81% for the diagnosis of invasive fungal infection; the beta-glucan assay concentration decreased with anti-fungal treatment. Therefore, the authors concluded that a beta-glucan assay may be useful for the early identification of invasive fungal infection and for monitoring the efficacy of anti-fungal therapy (231).

There are two likely explanations for why the assay's specificity was affected by non-fungal infections in the amniotic fluid and by the presence of an IUD. First, the beta-glucan test is very sensitive at detecting endotoxin in Gram-negative bacterial infections (232–235); therefore, a positive assay result for women who had a non-fungal infection was likely caused by the assay's cross-reactivity with other non-fungal microorganisms. The assay's specificity for women who did not have an IUD was 93% (106/114) in the absence of a non-fungal infection; that rate fell to 79% (11/14) if a non-fungal infection was present.

Second, IUDs are frequently contaminated with *Candida albicans* (236), and the longer an IUD has been in place, the more likely it is to be contaminated with *Candida* species (237, 238). *Candida* species, once attached to an IUD in the uterine cavity, become encapsulated within a protective extracellular matrix (biofilm form) that prevents detection of the flora when cultivation and molecular microbiological methods are used (239–246). However, the *Candida* species within the biofilm may secrete beta-glucan that then diffuses into the amniotic cavity and becomes detectable by the beta-glucan assay. This is the most likely explanation for the high "false-positive" rate of the beta-glucan assay for women who had an IUD as well as for the finding that the assay's specificity was lower in women who had a

non-fungal infection than in those who did not. As in women with an IUD, the frequency of occult *Candida* species infections may be higher in women who do not have a non-fungal infection than in those who do.

Candida species-related intra-amniotic infections were five times more common in the absence of a non-fungal infection in women who had an IUD in place [20% (8/36)] than in women who had a non-fungal intra-amniotic infection [4.5% (1/22)], although the difference did not reach statistical significance (p = 0.13, Fisher's exact test) (Tables 1 and 3). *Candida* species-related intra-amniotic infections were also significantly more common in the absence of a non-fungal intra-amniotic infection in women who had an IUD in place than in women who did not have an IUD [20% (8/36) versus 5.8% (7/121), p = 0.007]. Additional studies will be required to determine whether these associations are spurious or related to the protective biofilm that forms around an IUD, a factor that could decrease the probability of non-fungal intra-amniotic infections while at the same time causing occult *Candida* infections.

Strengths and limitations of the study

The strengths of this study are the large number of amniotic fluid samples that were assayed and the subsequent evaluation of factors affecting the assay's performance. The limitations of the study are the lack of information on the duration of each IUD's placement and the need for a biofilm study on the presence of *Candida* species inside of the patients' IUDs.

Conclusion

The beta-glucan assay, a sensitive, rapid method used to diagnose *Candida* species-related intra-amniotic infections, has high specificity and sensitivity rates for pregnant women who do not have an IUD in place.

Acknowledgments

Funding: This research was supported, in part, by the Perinatology Research Branch, Division of Obstetrics and Maternal-Fetal Medicine, Division of Intramural Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS); and, in part, with Federal funds from NICHD/NIH/DHHS under Contract No. HHSN275201300006C.

References

1. Whyte RK, Hussain Z, deSa D. Antenatal infections with Candida species. Arch Dis Child. 1982; 57(7):528–35. [PubMed: 7103544]
2. Romero R, Reece EA, Duff GW, Coultrip L, Hobbins JC. Prenatal diagnosis of Candida albicans chorioamnionitis. American journal of perinatology. 1985; 2(2):121–2. [PubMed: 3913429]
3. Bruner JP, Elliott JP, Kilbride HW, Garite TJ, Knox GE. Candida chorioamnionitis diagnosed by amniocentesis with subsequent fetal infection. American journal of perinatology. 1986; 3(3):213–8. [PubMed: 3718642]
4. Donders GG, Moerman P, Caudron J, Van Assche FA. Intra-uterine Candida infection: a report of four infected fetusses from two mothers. European journal of obstetrics, gynecology, and reproductive biology. 1991; 38(3):233–8.
5. Roque H, Abdelhak Y, Young BK. Intra amniotic candidiasis. Case report and meta-analysis of 54 cases. Journal of perinatal medicine. 1999; 27(4):253–62. [PubMed: 10560076]

6. Bittencourt AL, dos Santos WL, de Oliveira CH. Placental and fetal candidiasis. Presentation of a case of an abortus. Mycopathologia. 1984; 87(3):181–7. [PubMed: 6513996]
7. Nichols A, Khong TY, Crowther CA. Candida tropicalis chorioamnionitis. American journal of obstetrics and gynecology. 1995; 172(3):1045–7. [PubMed: 7892848]
8. Marelli G, Mariani A, Frigerio L, Leone E, Ferrari A. Fetal Candida infection associated with an intrauterine contraceptive device. European journal of obstetrics, gynecology, and reproductive biology. 1996; 68(1–2):209–12.
9. Asemota OA, Nyirjesy P, Fox R, Sobel JD. Candida glabrata complicating in vitro pregnancy: successful management of subsequent pregnancy. Fertility and sterility. 2011; 95(2):803e1–2.
10. Ozer E, Unlu M, Ersen A, Gulekli B. Intrauterine fetal loss associated with Candida glabrata chorioamnionitis: report of two cases. Turk patoloji dergisi. 2013; 29(1):77–9. [PubMed: 23354803]
11. Jackel D, Lai K. Candida glabrata sepsis associated with chorioamnionitis in an in vitro fertilization pregnancy: case report and review. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013; 56(4):555–8. [PubMed: 23143095]
12. Benirschke K, Raphael SI. Candida albicans infection of the amniotic sac. American journal of obstetrics and gynecology. 1958; 75(1):200–2. [PubMed: 13487703]
13. Lopez E, Aterman K. Intra-uterine infection by Candida. American journal of diseases of children. 1968; 115(6):663–70. [PubMed: 5654515]
14. Baley JE. Neonatal candidiasis: the current challenge. Clinics in perinatology. 1991; 18(2):263–80. [PubMed: 1879108]
15. Baley JE, Annable WL, Kliegman RM. Candida endophthalmitis in the premature infant. The Journal of pediatrics. 1981; 98(3):458–61. [PubMed: 7205461]
16. Baley JE, Kliegman RM, Boxerbaum B, Fanaroff AA. Fungal colonization in the very low birth weight infant. Pediatrics. 1986; 78(2):225–32. [PubMed: 3526268]
17. Baley JE, Kliegman RM, Fanaroff AA. Disseminated fungal infections in very low-birth-weight infants: clinical manifestations and epidemiology. Pediatrics. 1984; 73(2):144–52. [PubMed: 6420764]
18. Baley JE, Silverman RA. Systemic candidiasis: cutaneous manifestations in low birth weight infants. Pediatrics. 1988; 82(2):211–5. [PubMed: 3399294]
19. Aruna C, Seetharam K. Congenital candidiasis. Indian dermatology online journal. 2014; 5(Suppl 1):S44–7. [PubMed: 25506564]
20. Garcia-Flores J, Cruceyra M, Canamares M, Garicano A, Nieto O, Tamarit I. Candida chorioamnionitis: Report of two cases and review of literature. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology. 2016; 36(7):843–4. [PubMed: 27760473]
21. Chaim W, Mazor M, Wiznitzer A. The prevalence and clinical significance of intraamniotic infection with Candida species in women with preterm labor. Archives of gynecology and obstetrics. 1992; 251(1):9–15. [PubMed: 1550392]
22. Romero R, Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, et al. Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. American journal of obstetrics and gynecology. 1989; 161(3): 817–24. [PubMed: 2675611]
23. Romero R, Emamian M, Quintero R, Wan M, Hobbins JC, Mazor M, et al. The value and limitations of the Gram stain examination in the diagnosis of intraamniotic infection. American journal of obstetrics and gynecology. 1988; 159(1):114–9. [PubMed: 2456013]
24. Romero R, Quintero R, Oyarzun E, Wu YK, Sabo V, Mazor M, et al. Intraamniotic infection and the onset of labor in preterm premature rupture of the membranes. American journal of obstetrics and gynecology. 1988; 159(3):661–6. [PubMed: 3421266]
25. Romero R, Mazor M, Wu YK, Sirtori M, Oyarzun E, Mitchell MD, et al. Infection in the pathogenesis of preterm labor. Semin Perinatol. 1988; 12(4):262–79. [PubMed: 3065940]
26. Romero R, Mazor M. Infection and preterm labor. Clinical obstetrics and gynecology. 1988; 31(3): 553–84. [PubMed: 3066544]

27. Kellogg SG, Davis C, Benirschke K. Candida parapsilosis: previously unknown cause of fetal infection. A report of two cases. The Journal of reproductive medicine. 1974; 12(4):159–62. [PubMed: 4822891]
28. Romero R, Nores J, Hagay Z, Avila C, Hanaoka S, Sepulveda W, et al. Intraamniotic Infection with Candida tropicalis in Preterm Labor. Journal of Maternal-Fetal Medicine. 1992; 1(5):231–3.
29. Barth T, Broscheit J, Bussen S, Dietl J. Maternal sepsis and intrauterine fetal death resulting from Candida tropicalis chorioamnionitis in a woman with a retained intrauterine contraceptive device. Acta obstetricia et gynecologica Scandinavica. 2002; 81(10):981–2. [PubMed: 12366491]
30. Canpolat FE, Cekmez F, Tezer H. Chorioamnionitis and neonatal sepsis due to Candida tropicalis. Archives of gynecology and obstetrics. 2011; 283(4):919–20. [PubMed: 20844885]
31. Alfei A, Rizzo A, Cavanna C, Lallitto F, Spinillo A. Candida glabrata and pre-term premature rupture of membrane complicating in vitro pregnancy: case report and confirmation of mother to neonate transmission. Archives of gynecology and obstetrics. 2014; 290(2):211–4. [PubMed: 24691825]
32. Ganer Herman H, Mevorach Zussman N, Krajden Haratz K, Bar J, Sagiv R. Candida glabrata Chorioamnionitis following in vitro Fertilization: Review of the Literature. Gynecologic and obstetric investigation. 2015; 80(3):145–7. [PubMed: 26087702]
33. Bobitt JR, Hayslip CC, Damato JD. Amniotic fluid infection as determined by transabdominal amniocentesis in patients with intact membranes in premature labor. American journal of obstetrics and gynecology. 1981; 140(8):947–52. [PubMed: 7270607]
34. Wallace RL, Herrick CN. Amniocentesis in the evaluation of premature labor. Obstetrics and gynecology. 1981; 57(4):483–6. [PubMed: 7243098]
35. Hameed C, Tejani N, Verma UL, Archbald F. Silent chorioamnionitis as a cause of preterm labor refractory to tocolytic therapy. American journal of obstetrics and gynecology. 1984; 149(7):726–30. [PubMed: 6465222]
36. Wahbeh CJ, Hill GB, Eden RD, Gall SA. Intra-amniotic bacterial colonization in premature labor. American journal of obstetrics and gynecology. 1984; 148(6):739–43. [PubMed: 6702942]
37. Iams JD, Clapp DH, Contos DA, Whitehurst R, Ayers LW, O'Shaughnessy RW. Does extra-amniotic infection cause preterm labor? Gas-liquid chromatography studies of amniotic fluid in amnionitis, preterm labor, and normal controls. Obstetrics and gynecology. 1987; 70(3 Pt 1):365–8. [PubMed: 3627582]
38. Duff P, Kopelman JN. Subclinical intra-amniotic infection in asymptomatic patients with refractory preterm labor. Obstetrics and gynecology. 1987; 69(5):756–9. [PubMed: 3574802]
39. Skoll MA, Moretti ML, Sibai BM. The incidence of positive amniotic fluid cultures in patients preterm labor with intact membranes. American journal of obstetrics and gynecology. 1989; 161(3):813–6. [PubMed: 2782366]
40. Harger JH, Meyer MP, Amortegui A, Macpherson TA, Kaplan L, Mueller-Heubach E. Low incidence of positive amnionic fluid cultures in preterm labor at 27–32 weeks in the absence of clinical evidence of chorioamnionitis. Obstetrics and gynecology. 1991; 77(2):228–34. [PubMed: 1988886]
41. Garite TJ, Freeman RK, Linzey EM, Braly P. The use of amniocentesis in patients with premature rupture of membranes. Obstetrics and gynecology. 1979; 54(2):226–30. [PubMed: 460758]
42. Garite TJ, Freeman RK. Chorioamnionitis in the preterm gestation. Obstetrics and gynecology. 1982; 59(5):539–45. [PubMed: 7070724]
43. Cotton DB, Hill LM, Strassner HT, Platt LD, Ledger WJ. Use of amniocentesis in preterm gestation with ruptured membranes. Obstetrics and gynecology. 1984; 63(1):38–43. [PubMed: 6691016]
44. Broekhuizen FF, Gilman M, Hamilton PR. Amniocentesis for gram stain and culture in preterm premature rupture of the membranes. Obstetrics and gynecology. 1985; 66(3):316–21. [PubMed: 2410839]
45. Vintzileos AM, Campbell WA, Nochimson DJ, Weinbaum PJ, Escoto DT, Mirochnick MH. Qualitative amniotic fluid volume versus amniocentesis in predicting infection in preterm premature rupture of the membranes. Obstetrics and gynecology. 1986; 67(4):579–83. [PubMed: 3515258]

46. Feinstein SJ, Vintzileos AM, Lodeiro JG, Campbell WA, Weinbaum PJ, Nochimson DJ. Amniocentesis with premature rupture of membranes. Obstetrics and gynecology. 1986; 68(2): 147–52. [PubMed: 3737033]
47. Mazor M, Chaim W, Shinwell ES, Glezerman M. Asymptomatic amniotic fluid invasion with Candida albicans in preterm premature rupture of membranes. Implications for obstetric and neonatal management. Acta obstetricia et gynecologica Scandinavica. 1993; 72(1):52–4. [PubMed: 8382435]
48. Shalev E, Battino S, Romano S, Blondhaim O, Ben-Ami M. Intraamniotic infection with Candida albicans successfully treated with transcervical amnioinfusion of amphotericin. American journal of obstetrics and gynecology. 1994; 170(5 Pt 1):1271–2. [PubMed: 8178851]
49. Bean LM, Jackson JR, Dobak WJ, Beiswenger TR, Thorp JA. Intra-amniotic fluconazole therapy for Candida albicans intra-amniotic infection. Obstetrics and gynecology. 2013; 121(2 Pt 2) Suppl 1:452–4. [PubMed: 23344406]
50. Murray PR, Masur H. Current approaches to the diagnosis of bacterial and fungal bloodstream infections in the intensive care unit. Critical care medicine. 2012; 40(12):3277–82. [PubMed: 23034460]
51. Bosshard PP. Incubation of fungal cultures: how long is long enough? Mycoses. 2011; 54(5):e539–45. [PubMed: 21605185]
52. Obayashi T, Yoshida M, Mori T, Goto H, Yasuoka A, Iwasaki H, et al. Plasma (1-->3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet. 1995; 345(8941):17–20. [PubMed: 7799700]
53. Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge RJ, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004; 39(2):199–205. [PubMed: 15307029]
54. Ostrosky-Zeichner L, Alexander BD, Kett DH, Vazquez J, Pappas PG, Saeki F, et al. Multicenter clinical evaluation of the (1-->3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005; 41(5):654–9. [PubMed: 16080087]
55. Yasuoka A, Tachikawa N, Shimada K, Kimura S, Oka S. (1-->3) beta-D-glucan as a quantitative serological marker for Pneumocystis carinii pneumonia. Clin Diagn Lab Immunol. 1996; 3(2): 197–9. [PubMed: 8991635]
56. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, et al. Diagnostic accuracy of serum 1,3-beta-D-glucan for pneumocystis jiroveci pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. Journal of clinical microbiology. 2012; 50(1): 7–15. [PubMed: 22075593]
57. Yoshida M, Obayashi T, Iwama A, Ito M, Tsunoda S, Suzuki T, et al. Detection of plasma (1 --> 3)-beta-D-glucan in patients with Fusarium, Trichosporon, Saccharomyces and Acremonium fungaemias. J Med Vet Mycol. 1997; 35(5):371–4. [PubMed: 9402532]
58. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011; 52(6):750–70. [PubMed: 21367728]
59. Raggam RB, Fischbach LM, Prattes J, Duettmann W, Eigl S, Reischies F, et al. Detection of (1-->3)-beta-D-glucan in same-day urine and serum samples obtained from patients with haematological malignancies. Mycoses. 2015; 58(7):394–8. [PubMed: 25959065]
60. Malani AN, Singal B, Wheat LJ, Al Sous O, Summons TA, Durkin MM, et al. (1,3)-beta-d-glucan in cerebrospinal fluid for diagnosis of fungal meningitis associated with contaminated methylprednisolone injections. Journal of clinical microbiology. 2015; 53(3):799–803. [PubMed: 25540391]
61. Salvatore CM, Chen TK, Toussi SS, DeLaMora P, Petraitiene R, Finkelman MA, et al. (1-->3)-beta-d-Glucan in Cerebrospinal Fluid as a Biomarker for Candida and Aspergillus Infections of the Central Nervous System in Pediatric Patients. Journal of the Pediatric Infectious Diseases Society. 2016; 5(3):277–86. [PubMed: 26407252]

62. Stevens DA, Zhang Y, Finkelman MA, Pappagianis D, Clemons KV, Martinez M. Cerebrospinal Fluid (1,3)-Beta-d-Glucan Testing Is Useful in Diagnosis of Coccidioidal Meningitis. Journal of clinical microbiology. 2016; 54(11):2707–10. [PubMed: 27558179]

63. Litvintseva AP, Lindsley MD, Gade L, Smith R, Chiller T, Lyons JL, et al. Utility of (1–3)-beta-D-glucan testing for diagnostics and monitoring response to treatment during the multistate outbreak of fungal meningitis and other infections. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014; 58(5):622–30. [PubMed: 24336827]

64. Lyons JL, Thakur KT, Lee R, Watkins T, Pardo CA, Carson KA, et al. Utility of measuring (1,3)-beta-d-glucan in cerebrospinal fluid for diagnosis of fungal central nervous system infection. Journal of clinical microbiology. 2015; 53(1):319–22. [PubMed: 25378578]

65. Worasilchai N, Leelahavanichkul A, Kanjanabuch T, Thongbor N, Lorvinitnun P, Sukhontasing K, et al. (1-->3)-beta-D-glucan and galactomannan testing for the diagnosis of fungal peritonitis in peritoneal dialysis patients, a pilot study. Medical mycology. 2015; 53(4):338–46. [PubMed: 25851260]

66. Ginocchio F, Verrina E, Furfaro E, Cannavo R, Bandettini R, Castagnola E. Case Report of the Reliability 1,3-beta-D-Glucan Monitoring during Treatment of Peritoneal Candidiasis in a Child Receiving Continuous Peritoneal Dialysis. Clinical and Vaccine Immunology. 2012; 19(4):626–7. [PubMed: 22357650]

67. Rizzo G, Capponi A, Vlachopoulou A, Angelini E, Grassi C, Romanini C. The diagnostic value of interleukin-8 and fetal fibronectin concentrations in cervical secretions in patients with preterm labor and intact membranes. Journal of perinatal medicine. 1997; 25(6):461–8. [PubMed: 9494917]

68. Lam-Rachlin J, Romero R, Korzeniewski SJ, Schwartz AG, Chaemsaithong P, Hernandez-Andrade E, et al. Infection and smoking are associated with decreased plasma concentration of the anti-aging protein, alpha-klotho. Journal of perinatal medicine. 2013; 41(5):581–94. [PubMed: 23770558]

69. Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, et al. A rapid interleukin-6 bedside test for the identification of intra-amniotic inflammation in preterm labor with intact membranes. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016; 29(3):349–59.

70. Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, et al. A point of care test for interleukin-6 in amniotic fluid in preterm prelabor rupture of membranes: a step toward the early treatment of acute intra-amniotic inflammation/infection. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016; 29(3):360–7.

71. Maymon E, Romero R, Pacora P, Gomez R, Mazor M, Edwin S, et al. A role for the 72 kDa gelatinase (MMP-2) and its inhibitor (TIMP-2) in human parturition, premature rupture of membranes and intraamniotic infection. Journal of perinatal medicine. 2001; 29(4):308–16. [PubMed: 11565199]

72. Park KH, Chaiworapongsa T, Kim YM, Espinoza J, Yoshimatsu J, Edwin S, et al. Matrix metalloproteinase 3 in parturition, premature rupture of the membranes, and microbial invasion of the amniotic cavity. Journal of perinatal medicine. 2003; 31(1):12–22. [PubMed: 12661139]

73. Berger A, Witt A, Haiden N, Kretzer V, Heinze G, Kohlhauser C. Microbial invasion of the amniotic cavity at birth is associated with adverse short-term outcome of preterm infants. Journal of perinatal medicine. 2003; 31(2):115–21. [PubMed: 12747227]

74. Romero R, Miranda J, Chaiworapongsa T, Chaemsaithong P, Gotsch F, Dong Z, et al. A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes. American journal of reproductive immunology. 2014; 71(4):330–58. [PubMed: 24417618]

75. Romero R, Miranda J, Kusanovic JP, Chaiworapongsa T, Chaemsaithong P, Martinez A, et al. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. Journal of perinatal medicine. 2015; 43(1):19–36. [PubMed: 25720095]

76. Romero R, Chaemsaithong P, Korzeniewski SJ, Tarca AL, Bhatti G, Xu Z, et al. Clinical chorioamnionitis at term II: the intra-amniotic inflammatory response. Journal of perinatal medicine. 2016; 44(1):5–22. [PubMed: 25938217]
77. Romero R, Chaemsaithong P, Korzeniewski SJ, Kusanovic JP, Docheva N, Martinez-Varea A, et al. Clinical chorioamnionitis at term III: how well do clinical criteria perform in the identification of proven intra-amniotic infection? Journal of perinatal medicine. 2016; 44(1):23–32. [PubMed: 25918914]
78. Romero R, Chaemsaithong P, Docheva N, Korzeniewski SJ, Tarca AL, Bhatti G, et al. Clinical chorioamnionitis at term IV: the maternal plasma cytokine profile. Journal of perinatal medicine. 2016; 44(1):77–98. [PubMed: 26352068]
79. Romero R, Chaemsaithong P, Docheva N, Korzeniewski SJ, Kusanovic JP, Yoon BH, et al. Clinical chorioamnionitis at term VI: acute chorioamnionitis and funisitis according to the presence or absence of microorganisms and inflammation in the amniotic cavity. Journal of perinatal medicine. 2016; 44(1):33–51. [PubMed: 26352071]
80. Martinez-Varea A, Romero R, Xu Y, Miller D, Ahmed AI, Chaemsaithong P, et al. Clinical chorioamnionitis at term VII: the amniotic fluid cellular immune response. Journal of perinatal medicine. 2017; 45(5):523–38. [PubMed: 27763883]
81. Chaiyasit N, Romero R, Chaemsaithong P, Docheva N, Bhatti G, Kusanovic JP, et al. Clinical chorioamnionitis at term VIII: a rapid MMP-8 test for the identification of intra-amniotic inflammation. Journal of perinatal medicine. 2017; 45(5):539–50. [PubMed: 28672752]
82. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. American journal of obstetrics and gynecology. 2001; 185(5):1130–6. [PubMed: 11717646]
83. Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, et al. A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. American journal of obstetrics and gynecology. 2007; 197(3):292e1–5. [PubMed: 17826425]
84. Park CW, Lee SM, Park JS, Jun JK, Romero R, Yoon BH. The antenatal identification of funisitis with a rapid MMP-8 bedside test. Journal of perinatal medicine. 2008; 36(6):497–502. [PubMed: 19127606]
85. Pacora P, Romero R, Chaiworapongsa T, Kusanovic JP, Erez O, Vaisbuch E, et al. Amniotic fluid angiopoietin-2 in term and preterm parturition, and intra-amniotic infection/inflammation. Journal of perinatal medicine. 2009; 37(5):503–11. [PubMed: 19435449]
86. Madan I, Romero R, Kusanovic JP, Mittal P, Chaiworapongsa T, Dong Z, et al. The frequency and clinical significance of intra-amniotic infection and/or inflammation in women with placenta previa and vaginal bleeding: an unexpected observation. Journal of perinatal medicine. 2010; 38(3):275–9. [PubMed: 20146660]
87. Romero R, Chaiworapongsa T, Alpay Savasan Z, Xu Y, Hussein Y, Dong Z, et al. Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2011; 24(12): 1444–55.
88. Park KH, Kim SN, Oh KJ, Lee SY, Jeong EH, Ryu A. Noninvasive prediction of intra-amniotic infection and/or inflammation in preterm premature rupture of membranes. Reproductive sciences. 2012; 19(6):658–65. [PubMed: 22457430]
89. Romero R, Miranda J, Chaiworapongsa T, Chaemsaithong P, Gotsch F, Dong Z, et al. Sterile intra-amniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2014:1–17.
90. Romero R, Miranda J, Chaemsaithong P, Chaiworapongsa T, Kusanovic JP, Dong Z, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. The journal of maternal-fetal & neonatal medicine : the official journal of the European

Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2015; 28(12):1394–409.

91. Romero R, Chaemsaithong P, Chaiyasit N, Docheva N, Dong Z, Kim CJ, et al. CXCL10 and IL-6: Markers of two different forms of intra-amniotic inflammation in preterm labor. American journal of reproductive immunology. 2017; 78(1)
92. Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, et al. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. American journal of reproductive immunology. 2014; 72(5):458–74. [PubMed: 25078709]
93. Kedzierska A, Kochan P, Pietrzyk A, Kedzierska J. Current status of fungal cell wall components in the immunodiagnostics of invasive fungal infections in humans: galactomannan, mannan and (1-->3)-beta-D-glucan antigens. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2007; 26(11):755–66.
94. Tamura H, Arimoto Y, Tanaka S, Yoshida M, Obayashi T, Kawai T. Automated kinetic assay for endotoxin and (1-->3)-beta-D-glucan in human blood. Clin Chim Acta. 1994; 226(1):109–12. [PubMed: 8070129]
95. Aketagawa J, Tanaka S, Tamura H, Shibata Y, Saito H. Activation of limulus coagulation factor G by several (1-->3)-beta-D-glucans: comparison of the potency of glucans with identical degree of polymerization but different conformations. J Biochem. 1993; 113(6):683–6. [PubMed: 8370664]
96. Sobel JD. Recurrent vulvovaginal candidiasis. American journal of obstetrics and gynecology. 2016; 214(1):15–21. [PubMed: 26164695]
97. Aguin TJ, Sobel JD. Vulvovaginal candidiasis in pregnancy. Current infectious disease reports. 2015; 17(6):462. [PubMed: 25916994]
98. Ilkit M, Guzel AB. The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: a mycological perspective. Critical reviews in microbiology. 2011; 37(3):250–61. [PubMed: 21599498]
99. Farr A, Kiss H, Holzer I, Husslein P, Hagmann M, Petricevic L. Effect of asymptomatic vaginal colonization with Candida albicans on pregnancy outcome. Acta obstetricia et gynecologica Scandinavica. 2015; 94(9):989–96. [PubMed: 26084843]
100. Holzer I, Farr A, Kiss H, Hagmann M, Petricevic L. The colonization with Candida species is more harmful in the second trimester of pregnancy. Archives of gynecology and obstetrics. 2017; 295(4):891–5. [PubMed: 28255766]
101. Roberts CL, Rickard K, Kotsiou G, Morris JM. Treatment of asymptomatic vaginal candidiasis in pregnancy to prevent preterm birth: an open-label pilot randomized controlled trial. BMC pregnancy and childbirth. 2011; 11:18. [PubMed: 21396090]
102. Friebe-Hoffmann U, Bender DP, Sims CJ, Rauk PN. Candida albicans chorioamnionitis associated with preterm labor and sudden intrauterine demise of one twin. A case report. The Journal of reproductive medicine. 2000; 45(4):354–6. [PubMed: 10804496]
103. Kim SK, Romero R, Kusanovic JP, Erez O, Vaisbuch E, Mazaki-Tovi S, et al. The prognosis of pregnancy conceived despite the presence of an intrauterine device (IUD). Journal of perinatal medicine. 2010; 38(1):45–53. [PubMed: 19650756]
104. Smith CV, Horenstein J, Platt LD. Intraamniotic infection with Candida albicans associated with a retained intrauterine contraceptive device: a case report. American journal of obstetrics and gynecology. 1988; 159(1):123–4. [PubMed: 3394730]
105. Spaun E, Klunder K. Candida chorioamnionitis and intra-uterine contraceptive device. Acta obstetricia et gynecologica Scandinavica. 1986; 65(2):183–4. [PubMed: 3727943]
106. Kusanovic JP, Romero R, Martinovic C, Silva K, Erez O, Maymon E, et al. Transabdominal collection of amniotic fluid “sludge” and identification of Candida albicans intra-amniotic infection. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2017:1–6.
107. Sundaram A, Vaughan B, Kost K, Bankole A, Finer L, Singh S, et al. Contraceptive Failure in the United States: Estimates from the 2006–2010 National Survey of Family Growth. Perspectives on sexual and reproductive health. 2017; 49(1):7–16. [PubMed: 28245088]

108. Teal SB, Romer SE, Goldthwaite LM, Peters MG, Kaplan DW, Sheeder J. Insertion characteristics of intrauterine devices in adolescents and young women: success, ancillary measures, and complications. American journal of obstetrics and gynecology. 2015; 213(4): 515e1–5. [PubMed: 26116873]

109. American College of O, Gynecologists Committee on Gynecologic P, Long-Acting Reversible Contraception Working G. ACOG Committee Opinion no. 450: Increasing use of contraceptive implants and intrauterine devices to reduce unintended pregnancy. Obstetrics and gynecology. 2009; 114(6):1434–8. [PubMed: 20134301]

110. Gynecologists ACoOa. Committee Opinion No. 539: Adolescents and Long-Acting Reversible Contraception: Implants and Intrauterine Devices. Obstetrics & Gynecology. 2012; 120(4):983–8. [PubMed: 22996129]

111. Diedrich JT, Madden T, Zhao Q, Peipert JF. Long-term utilization and continuation of intrauterine devices. American journal of obstetrics and gynecology. 2015; 213(6):822e1–6. [PubMed: 26409157]

112. Michaud P, Lemaire B, Tescher M. Spontaneous abortion with an IUD and Candida chorioamnionitis. Rev Fr Gynecol Obstet. 1989; 84(1):45–6. [PubMed: 2648545]

113. Brahmi D, Steenland MW, Renner RM, Gaffield ME, Curtis KM. Pregnancy outcomes with an IUD in situ: a systematic review. Contraception. 2012; 85(2):131–9. [PubMed: 22067777]

114. Chaim W, Mazor M, Meril T, Peleg R, Maor E. Late miscarriage and intraamniotic candidiasis in a woman with a retained intrauterine contraceptive device. Archives of gynecology and obstetrics. 1993; 253(3):157–60. [PubMed: 8250605]

115. Chaim W, Mazor M. Pregnancy with an intrauterine device in situ and preterm delivery. Archives of gynecology and obstetrics. 1992; 252(1):21–4. [PubMed: 1417085]

116. Dreishpoon IH. Complications of pregnancy with an intrauterine contraceptive device in situ. American journal of obstetrics and gynecology. 1975; 121(3):412–3. [PubMed: 1115156]

117. Ganer H, Levy A, Ohel I, Sheiner E. Pregnancy outcome in women with an intrauterine contraceptive device. American journal of obstetrics and gynecology. 2009; 201(4):381e1–5. [PubMed: 19716537]

118. Ozgu-Erdinc AS, Tasdemir UG, Uygur D, Aktulay A, Tasdemir N, Gulerman HC. Outcome of intrauterine pregnancies with intrauterine device in place and effects of device location on prognosis. Contraception. 2014; 89(5):426–30. [PubMed: 24508123]

119. Tatum HJ, Schmidt FH, Jain AK. Management and outcome of pregnancies associated with the Copper T intrauterine contraceptive device. American journal of obstetrics and gynecology. 1976; 126(7):869–79. [PubMed: 1033668]

120. Waites KB, Bobo RA, Davis RO, Brookings ES, Cassell GH. Clinically silent polymicrobial amnionitis and intrauterine fetal death associated with a Cu-7 intrauterine contraceptive device. American journal of obstetrics and gynecology. 1984; 150(8):998–9.

121. Qureshi F, Jacques SM, Bendon RW, Faye-Peterson OM, Heifetz SA, Redline R, et al. Candida funisitis: A clinicopathologic study of 32 cases. Pediatric and developmental pathology : the official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society. 1998; 1(2):118–24.

122. Brandsma MA, Braaksma JT, van der Harten JJ. Immature delivery after intrauterine Candida albicans infection. European journal of obstetrics, gynecology, and reproductive biology. 1975; 5(6):331–5.

123. Delprado WJ, Baird PJ, Russell P. Placental candidiasis: report of three cases with a review of the literature. Pathology. 1982; 14(2):191–5. [PubMed: 7099725]

124. Honore LH. Placental candidiasis: report of two cases, one associated with an IUCD in situ. Contraception. 1984; 30(6):555–60. [PubMed: 6529912]

125. Bruner JP, Elliott JP, Kilbride HW, Garite TJ, Knox GE. Candida chorioamnionitis diagnosed by amniocentesis with subsequent fetal infection. Am J Perinatol. 1986; 3(3):213–8. [PubMed: 3718642]

126. Rios R, Aguilar R, Zaror L. Chorioamnionitis caused by candida associated with intrauterine devices. Rev Chil Obstet Ginecol. 1988; 53(5):297–8. [PubMed: 3153092]

127. Bider D, Ben-Rafael Z, Barkai G, Mashiach S. Intrauterine fetal death apparently due to Candida chorioamnionitis. Archives of gynecology and obstetrics. 1989; 244(3):175–7. [PubMed: 2735775]

128. Donders GG, Moerman P, Caudron J, Van Assche FA. Intra-uterine Candida infection: a report of four infected fetusses from two mothers. Eur J Obstet Gynecol Reprod Biol. 1991; 38(3):233–8. [PubMed: 2007451]

129. Mazor M, Chaim W, Pak I, Goldstein D. Intraamniotic infection with Candida albicans associated with a retained intrauterine device. A case report. J Reprod Med. 1992; 37(11):950–2. [PubMed: 1460616]

130. Chaim W, Mazor M, Meril T, Peleg R, Maor E. Late miscarriage and intraamniotic candidiasis in a woman with a retained intrauterine contraceptive device. Arch Gynecol Obstet. 1993; 253(3): 157–60. [PubMed: 8250605]

131. Horn LC, Nenoff P, Ziegert M, Hockel M. Missed abortion complicated by Candida infection in a woman with rested IUD. Archives of gynecology and obstetrics. 2001; 264(4):215–7. [PubMed: 11205713]

132. Deveer R, Engin-Ustun Y, Sarikaya E, Aydogan P, Doganay M, Mollamahmutoglu L. Comparison of C-reactive protein levels in pregnancies with retained and removed intrauterine device. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2011; 24(9):1152–4.

133. Mermet J, Bolcato C, Rudigoz RC, Dargent D. Outcome of pregnancies with an intrauterine devices and their management. Rev Fr Gynecol Obstet. 1986; 81(4):233–5. [PubMed: 3715307]

134. Potasman I, Leibovitz Z, Sharf M. Candida sepsis in pregnancy and the postpartum period. Reviews of infectious diseases. 1991; 13(1):146–9. [PubMed: 2017614]

135. Romero, R, Lockwood, CJ. Pathogenesis of Spontaneous Preterm Labor. In: Creasy, RK, Resnik, R, Iams, JD, editorsMaternal-fetal medicine : principles and practice. 6. Philadelphia, Pa: Saunders; 2009. 521–43.

136. Gray DJ, Robinson HB, Malone J, Thomson RB Jr. Adverse outcome in pregnancy following amniotic fluid isolation of Ureaplasma urealyticum. Prenat Diagn. 1992; 12(2):111–7. [PubMed: 1553356]

137. Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. Journal of perinatal medicine. 2012; 40(4):329–43. [PubMed: 22752762]

138. Rowlands S, Danielewski JA, Tabrizi SN, Walker SP, Garland SM. Microbial invasion of the amniotic cavity in midtrimester pregnancies using molecular microbiology. American journal of obstetrics and gynecology. 2017; 217(1):71e1–e5. [PubMed: 28268197]

139. Perez-Munoz ME, Arrieta MC, Ramer-Tait AE, Walter J. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. Microbiome. 2017; 5(1):48. [PubMed: 28454555]

140. Romero R, Nores J, Mazor M, Sepulveda W, Oyarzun E, Parra M, et al. Microbial invasion of the amniotic cavity during term labor. Prevalence and clinical significance. The Journal of reproductive medicine. 1993; 38(7):543–8. [PubMed: 8410850]

141. Lee SE, Romero R, Kim CJ, Shim SS, Yoon BH. Funisitis in term pregnancy is associated with microbial invasion of the amniotic cavity and intra-amniotic inflammation. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2006; 19(11):693–7.

142. McLean LK, Chehab FF, Goldberg JD. Detection of viral deoxyribonucleic acid in the amniotic fluid of low-risk pregnancies by polymerase chain reaction. American journal of obstetrics and gynecology. 1995; 173(4):1282–6. [PubMed: 7485338]

143. Baschat AA, Towbin J, Bowles NE, Harman CR, Weiner CP. Prevalence of viral DNA in amniotic fluid of low-risk pregnancies in the second trimester. The journal of maternal-fetal & neonatal

medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2003; 13(6):381–4.

144. Gervasi MT, Romero R, Bracalente G, Chaiworapongsa T, Erez O, Dong Z, et al. Viral invasion of the amniotic cavity (VIAC) in the midtrimester of pregnancy. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2012; 25(10):2002–13.
145. Espinoza J, Chaiworapongsa T, Romero R, Edwin S, Rathnasabapathy C, Gomez R, et al. Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2003; 13(1):2–21.
146. Miller J, Michel J, Bercovici B, Argaman M, Sacks T. Studies on the antimicrobial activity of amniotic fluid. American journal of obstetrics and gynecology. 1976; 125(2):212–4. [PubMed: 817602]
147. Saiman L, Ludington E, Dawson JD, Patterson JE, Rangel-Frausto S, Wiblin RT, et al. Risk factors for Candida species colonization of neonatal intensive care unit patients. The Pediatric infectious disease journal. 2001; 20(12):1119–24. [PubMed: 11740316]
148. Jarvis WR. The epidemiology of colonization. Infection control and hospital epidemiology. 1996; 17(1):47–52. [PubMed: 8789688]
149. Uko S, Soghier LM, Vega M, Marsh J, Reinersman GT, Herring L, et al. Targeted short-term fluconazole prophylaxis among very low birth weight and extremely low birth weight infants. Pediatrics. 2006; 117(4):1243–52. [PubMed: 16585321]
150. Healy CM, Baker CJ, Zaccaria E, Campbell JR. Impact of fluconazole prophylaxis on incidence and outcome of invasive candidiasis in a neonatal intensive care unit. The Journal of pediatrics. 2005; 147(2):166–71. [PubMed: 16126043]
151. Kaufman D, Boyle R, Hazen KC, Patrie JT, Robinson M, Donowitz LG. Fluconazole prophylaxis against fungal colonization and infection in preterm infants. The New England journal of medicine. 2001; 345(23):1660–6. [PubMed: 11759644]
152. Manzoni P, Stolfi I, Pugni L, Decembrino L, Magnani C, Vetrano G, et al. A multicenter, randomized trial of prophylactic fluconazole in preterm neonates. The New England journal of medicine. 2007; 356(24):2483–95. [PubMed: 17568029]
153. Appelbaum PC, Holloway Y, Ross SM, Dhupelia I. The effect of amniotic fluid on bacterial growth in three population groups. American journal of obstetrics and gynecology. 1977; 128(8): 868–71. [PubMed: 888865]
154. Jankowski RP, Aikins HE, Vahrson H, Gupta KG. Antibacterial activity of amniotic fluid against Staphylococcus aureus, Candida albicans and Brucella abortus. Archiv fur Gynakologie. 1977; 222(3):275–8. [PubMed: 407884]
155. Jackel D, Lai K. Candida glabrata sepsis associated with chorioamnionitis in an in vitro fertilization pregnancy: case report and review. Clin Infect Dis. 2013; 56(4):555–8. [PubMed: 23143095]
156. Johnsson H, Ewald U. The rate of candidaemia in preterm infants born at a gestational age of 23–28 weeks is inversely correlated to gestational age. Acta paediatrica. 2004; 93(7):954–8. [PubMed: 15303812]
157. Poliquin V, Al-Sulmi E, Menticoglou S. Intra-amniotic infection involving Candida albicans subsequent to emergency cerclage: A case series. Can J Infect Dis Med Microbiol. 2015; 26(5): 245–6. [PubMed: 26600809]
158. Sfameni SF, Talbot JM, Chow SL, Brenton LA, Scurry JP. Candida glabrata chorioamnionitis following in vitro fertilization and embryo transfer. The Australian & New Zealand journal of obstetrics & gynaecology. 1997; 37(1):88–91. [PubMed: 9075555]

159. Ibara AS, Marcorelles P, Le Martelot MT, Touffet N, Moalic E, Hery-Arnaud G, et al. Two cases of systemic Candida glabrata infection following in vitro fertilization and embryo transfer. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2004; 23(1):53–6.

160. Akhanoba F, MacDougall J, Mathur R, Hassan W. Severe systemic candidiasis following immunomodulation therapy in in vitro fertilisation-embryo transfer (IVF-ET). BMJ case reports. 2014; 2014

161. Hayashi S, Mochizuki T, Watanabe S. Infection of human fetal membranes in vitro with Candida albicans. Mycoses. 1989; 32(3):119–22. [PubMed: 2659984]

162. Gurgan T, Diker KS, Haziroglu R, Urman B, Akan M. In vitro infection of human fetal membranes with Candida species. Gynecologic and obstetric investigation. 1994; 37(3):164–7. [PubMed: 8005544]

163. Maudsley RF, Brix GA, Hinton NA, Robertson EM, Bryans AM, Haust MD. Placental inflammation and infection. A prospective bacteriologic and histologic study. American journal of obstetrics and gynecology. 1966; 95(5):648–59. [PubMed: 5949478]

164. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clinical microbiology reviews. 2004; 17(3):638–80. [PubMed: 15258097]

165. Marelli G, Mariani A, Frigerio L, Leone E, Ferrari A. Fetal Candida infection associated with an intrauterine contraceptive device. Eur J Obstet Gynecol Reprod Biol. 1996; 68(1–2):209–12. [PubMed: 8886709]

166. Engelhart CM, van de Vijver NM, Nienhuis SJ, Hasaart TH. Fetal Candida sepsis at midgestation: a case report. European journal of obstetrics, gynecology, and reproductive biology. 1998; 77(1): 107–9.

167. Manzoni P, Farina D, Galletto P, Leonessa M, Priolo C, Arisio R, et al. Type and number of sites colonized by fungi and risk of progression to invasive fungal infection in preterm neonates in neonatal intensive care unit. Journal of perinatal medicine. 2007; 35(3):220–6. [PubMed: 17378718]

168. Barton M, O'Brien K, Robinson JL, Davies DH, Simpson K, Asztalos E, et al. Invasive candidiasis in low birth weight preterm infants: risk factors, clinical course and outcome in a prospective multicenter study of cases and their matched controls. BMC infectious diseases. 2014; 14:327. [PubMed: 24924877]

169. Manzoni P, Arisio R, Mostert M, Leonessa M, Farina D, Latino MA, et al. Prophylactic fluconazole is effective in preventing fungal colonization and fungal systemic infections in preterm neonates: a single-center, 6-year, retrospective cohort study. Pediatrics. 2006; 117(1):e22–32. [PubMed: 16326690]

170. Nichols A, Khong TY, Crowther CA. Candida tropicalis chorioamnionitis. Am J Obstet Gynecol. 1995; 172(3):1045–7. [PubMed: 7892848]

171. Payne MS, Kemp MW, Kallapur SG, Kannan PS, Saito M, Miura Y, et al. Intrauterine Candida albicans infection elicits severe inflammation in fetal sheep. Pediatric research. 2014; 75(6):716–22. [PubMed: 24632681]

172. Stock SJ, Patey O, Thilaganathan B, White S, Furfaro LL, Payne MS, et al. Intrauterine Candida albicans Infection Causes Systemic Fetal Candidiasis With Progressive Cardiac Dysfunction in a Sheep Model of Early Pregnancy. Reproductive sciences. 2016

173. Di Naro E, Cromi A, Ghezzi F, Giocolano A, Caringella A, Loverro G. Myocardial dysfunction in fetuses exposed to intraamniotic infection: new insights from tissue Doppler and strain imaging. American journal of obstetrics and gynecology. 2010; 203(5):459e1–7. [PubMed: 20691411]

174. Ophelders DR, Gussenhoven R, Lammens M, Kusters B, Kemp MW, Newnham JP, et al. Neuroinflammation and structural injury of the fetal ovine brain following intra-amniotic Candida albicans exposure. Journal of neuroinflammation. 2016; 13:29. [PubMed: 26842664]

175. Nikiforou M, Jacobs EMR, Kemp MW, Hornef MW, Payne MS, Saito M, et al. Intra-amniotic Candida albicans infection induces mucosal injury and inflammation in the ovine fetal intestine. Scientific Reports. 2016; 6:29806. [PubMed: 27411776]

176. Berry DL, Olson GL, Wen TS, Belfort MA, Moise KJ Jr. Candida chorioamnionitis: a report of two cases. J Matern Fetal Med. 1997; 6(3):151–4. [PubMed: 9172056]

177. Maneenil G, Payne MS, Senthamarai Kannan P, Kallapur SG, Kramer BW, Newnham JP, et al. Fluconazole treatment of intrauterine Candida albicans infection in fetal sheep. Pediatric research. 2015; 77(6):740–8. [PubMed: 25760552]

178. Nien JK, Yoon BH, Espinoza J, Kusanovic JP, Erez O, Soto E, et al. A rapid MMP-8 bedside test for the detection of intra-amniotic inflammation identifies patients at risk for imminent preterm delivery. American journal of obstetrics and gynecology. 2006; 195(4):1025–30. [PubMed: 17000236]

179. Chaemsaithong P, Romero R, Docheva N, Chaiyasit N, Bhatti G, Pacora P, et al. Comparison of rapid MMP-8 and interleukin-6 point-of-care tests to identify intra-amniotic inflammation/infection and impending preterm delivery in patients with preterm labor and intact membranes. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2017:1–17.

180. Chaemsaithong P, Romero R, Korzeniewski SJ, Dong Z, Yeo L, Hassan SS, et al. A point of care test for the determination of amniotic fluid interleukin-6 and the chemokine CXCL-10/IP-10. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2015; 28(13):1510–9.

181. Kacerovsky M, Musilova I, Stepan M, Andrys C, Drahosova M, Jacobsson B. Detection of intraamniotic inflammation in fresh and processed amniotic fluid samples with the interleukin-6 point of care test. American journal of obstetrics and gynecology. 2015; 213(3):435–6. [PubMed: 26003057]

182. Kacerovsky M, Musilova I, Hornychova H, Kutova R, Pliskova L, Kostal M, et al. Bedside assessment of amniotic fluid interleukin-6 in preterm prelabor rupture of membranes. American journal of obstetrics and gynecology. 2014; 211(4):385e1–9. [PubMed: 24705131]

183. Park CW, Yoon BH, Kim SM, Park JS, Jun JK. The frequency and clinical significance of intra-amniotic inflammation defined as an elevated amniotic fluid matrix metalloproteinase-8 in patients with preterm labor and low amniotic fluid white blood cell counts. Obstetrics & gynecology science. 2013; 56(3):167–75. [PubMed: 24327997]

184. Maymon E, Romero R, Chaiworapongsa T, Berman S, Conoscenti G, Gomez R, et al. Amniotic fluid matrix metalloproteinase-8 in preterm labor with intact membranes. American journal of obstetrics and gynecology. 2001; 185(5):1149–55. [PubMed: 11717649]

185. Maymon E, Romero R, Chaiworapongsa T, Kim JC, Berman S, Gomez R, et al. Value of amniotic fluid neutrophil collagenase concentrations in preterm premature rupture of membranes. American journal of obstetrics and gynecology. 2001; 185(5):1143–8. [PubMed: 11717648]

186. Maymon E, Romero R, Pacora P, Gomez R, Athayde N, Edwin S, et al. Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. American journal of obstetrics and gynecology. 2000; 183(1):94–9. [PubMed: 10920315]

187. Yoon BH, Oh SY, Romero R, Shim SS, Han SY, Park JS, et al. An elevated amniotic fluid matrix metalloproteinase-8 level at the time of mid-trimester genetic amniocentesis is a risk factor for spontaneous preterm delivery. American journal of obstetrics and gynecology. 2001; 185(5):1162–7. [PubMed: 11717651]

188. Park JS, Romero R, Yoon BH, Moon JB, Oh SY, Han SY, et al. The relationship between amniotic fluid matrix metalloproteinase-8 and funisitis. American journal of obstetrics and gynecology. 2001; 185(5):1156–61. [PubMed: 11717650]

189. Angus SR, Segel SY, Hsu CD, Locksmith GJ, Clark P, Sammel MD, et al. Amniotic fluid matrix metalloproteinase-8 indicates intra-amniotic infection. American journal of obstetrics and gynecology. 2001; 185(5):1232–8. [PubMed: 11717662]

190. Moon JB, Kim JC, Yoon BH, Romero R, Kim G, Oh SY, et al. Amniotic fluid matrix metalloproteinase-8 and the development of cerebral palsy. Journal of perinatal medicine. 2002; 30(4):301–6. [PubMed: 12235718]

191. Biggio JR Jr, Ramsey PS, Cliver SP, Lyon MD, Goldenberg RL, Wenstrom KD. Midtrimester amniotic fluid matrix metalloproteinase-8 (MMP-8) levels above the 90th percentile are a marker

for subsequent preterm premature rupture of membranes. American journal of obstetrics and gynecology. 2005; 192(1):109–13. [PubMed: 15672011]

192. Combs CA, Gravett M, Garite TJ, Hickok DE, Lapidus J, Porreco R, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. American journal of obstetrics and gynecology. 2014; 210(2):125e1–e15. [PubMed: 24274987]
193. Kacerovsky M, Musilova I, Andrys C, Hornychova H, Pliskova L, Kostal M, et al. Prelabor rupture of membranes between 34 and 37 weeks: the intraamniotic inflammatory response and neonatal outcomes. American journal of obstetrics and gynecology. 2014; 210(4):325e1–e10. [PubMed: 24184182]
194. Cobo T, Palacio M, Navarro-Sastre A, Ribes A, Bosch J, Filella X, et al. Predictive value of combined amniotic fluid proteomic biomarkers and interleukin-6 in preterm labor with intact membranes. American journal of obstetrics and gynecology. 2009; 200(5):499e1–6. [PubMed: 19375569]
195. Yoon BH, Romero R, Park JS, Kim CJ, Kim SH, Choi JH, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. American journal of obstetrics and gynecology. 2000; 182(3):675–81. [PubMed: 10739529]
196. Combs CA, Gravett M, Garite TJ. Reply: To PMID 24274987. American journal of obstetrics and gynecology. 2014; 211(6):708–9.
197. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. The Journal of clinical investigation. 1990; 85(5):1392–400. [PubMed: 2332497]
198. Santhanam U, Avila C, Romero R, Viguet H, Ida N, Sakurai S, et al. Cytokines in normal and abnormal parturition: elevated amniotic fluid interleukin-6 levels in women with premature rupture of membranes associated with intrauterine infection. Cytokine. 1991; 3(2):155–63. [PubMed: 1888885]
199. Romero R, Sepulveda W, Kenney JS, Archer LE, Allison AC, Sehgal PB. Interleukin 6 determination in the detection of microbial invasion of the amniotic cavity. Ciba Foundation symposium. 1992; 167:205–20. [PubMed: 1425014]
200. Romero R, Yoon BH, Mazor M, Gomez R, Diamond MP, Kenney JS, et al. The diagnostic and prognostic value of amniotic fluid white blood cell count, glucose, interleukin-6, and gram stain in patients with preterm labor and intact membranes. American journal of obstetrics and gynecology. 1993; 169(4):805–16. [PubMed: 7694461]
201. Romero R, Yoon BH, Mazor M, Gomez R, Gonzalez R, Diamond MP, et al. A comparative study of the diagnostic performance of amniotic fluid glucose, white blood cell count, interleukin-6, and gram stain in the detection of microbial invasion in patients with preterm premature rupture of membranes. American journal of obstetrics and gynecology. 1993; 169(4):839–51. [PubMed: 7694463]
202. Romero R, Yoon BH, Kenney JS, Gomez R, Allison AC, Sehgal PB. Amniotic fluid interleukin-6 determinations are of diagnostic and prognostic value in preterm labor. American journal of reproductive immunology. 1993; 30(2–3):167–83. [PubMed: 8311926]
203. Greig PC, Ernest JM, Teot L, Erikson M, Talley R. Amniotic fluid interleukin-6 levels correlate with histologic chorioamnionitis and amniotic fluid cultures in patients in premature labor with intact membranes. American journal of obstetrics and gynecology. 1993; 169(4):1035–44. [PubMed: 8238116]
204. Gomez R, Romero R, Galasso M, Behnke E, Insunza A, Cotton DB. THE VALUE OF AMNIOTIC-FLUID INTERLEUKIN-6, WHITE BLOOD-CELL COUNT, AND GRAM STAIN IN THE DIAGNOSIS OF MICROBIAL INVASION OF THE AMNIOTIC CAVITY IN PATIENTS AT TERM. American journal of reproductive immunology. 1994; 32(3):200–10. [PubMed: 7533501]
205. Coultrip LL, Lien JM, Gomez R, Kapernick P, Khoury A, Grossman JH. The value of amniotic fluid interleukin-6 determination in patients with preterm labor and intact membranes in the detection of microbial invasion of the amniotic cavity. American journal of obstetrics and gynecology. 1994; 171(4):901–11. [PubMed: 7943100]

206. Dudley DJ, Hunter C, Mitchell MD, Varner MW. Clinical value of amniotic fluid interleukin-6 determinations in the management of preterm labour. British journal of obstetrics and gynaecology. 1994; 101(7):592–7. [PubMed: 8043537]

207. Yoon BH, Romero R, Kim CJ, Jun JK, Gomez R, Choi JH, et al. Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. American journal of obstetrics and gynecology. 1995; 172(3): 960–70. [PubMed: 7892891]

208. Negishi H, Yamada H, Mikuni M, Kishida T, Okuyama K, Sagawa T, et al. Correlation between cytokine levels of amniotic fluid and histological chorioamnionitis in preterm delivery. Journal of perinatal medicine. 1996; 24(6):633–9. [PubMed: 9120746]

209. Yoon BH, Romero R, Jun JK, Park KH, Park JD, Ghezzi F, et al. Amniotic fluid cytokines (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8) and the risk for the development of bronchopulmonary dysplasia. American journal of obstetrics and gynecology. 1997; 177(4):825–30. [PubMed: 9369827]

210. Wenstrom KD, Andrews WW, Hauth JC, Goldenberg RL, DuBard MB, Cliver SP. Elevated second-trimester amniotic fluid interleukin-6 levels predict preterm delivery. American journal of obstetrics and gynecology. 1998; 178(3):546–50. [PubMed: 9539524]

211. Baud O, Emilie D, Pelletier E, Lacaze-Masmonteil T, Zupan V, Fernandez H, et al. Amniotic fluid concentrations of interleukin-1beta, interleukin-6 and TNF-alpha in chorioamnionitis before 32 weeks of gestation: histological associations and neonatal outcome. British journal of obstetrics and gynaecology. 1999; 106(1):72–7. [PubMed: 10426263]

212. Yoon BH, Park CW, Chaiworapongsa T. Intrauterine infection and the development of cerebral palsy. Bjog. 2003; 110:124–7. [PubMed: 12763129]

213. Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Nikolaitchouk N, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. Acta obstetricia et gynecologica Scandinavica. 2003; 82(5):423–31. [PubMed: 12752072]

214. Jacobsson B, Mattsby-Baltzer I, Hagberg H. Interleukin-6 and interleukin-8 in cervical and amniotic fluid: relationship to microbial invasion of the chorioamniotic membranes. Bjog. 2005; 112(6):719–24. [PubMed: 15924526]

215. Holst RM, Laurini R, Jacobsson B, Samuelsson E, Savman K, Doverhag C, et al. Expression of cytokines and chemokines in cervical and amniotic fluid: Relationship to histological chorioamnionitis. J Matern-Fetal Neonatal Med. 2007; 20(12):885–93. [PubMed: 18050018]

216. Menon R, Camargo C, Thorsen P, Lombardi SJ, Fortunato SJ. Amniotic fluid interleukin-6 increase is an indicator of spontaneous preterm birth in white but not black Americans. American journal of obstetrics and gynecology. 2008; 198(1)

217. Massaro G, Scaravilli G, Simeone S, Capuano S, Pastore E, Forte A, et al. Interleukin-6 and Mycoplasma hominis as markers of preterm birth and related brain damage: Our experience. J Matern-Fetal Neonatal Med. 2009; 22(11):1063–7. [PubMed: 19900045]

218. Thomakos N, Daskalakis G, Papapanagiotou A, Papantoniou N, Mesogitis S, Antsaklis A. Amniotic fluid interleukin-6 and tumor necrosis factor-alpha at mid-trimester genetic amniocentesis: Relationship to intra-amniotic microbial invasion and preterm delivery. Eur J Obstet Gynecol Reprod Biol. 2010; 148(2):147–51. [PubMed: 19945777]

219. Marconi C, Ramos BRD, Peracoli JC, Donders GGG, da Silva MG. Amniotic Fluid Interleukin-1 Beta and Interleukin-6, but not Interleukin-8 Correlate with Microbial Invasion of the Amniotic Cavity in Preterm Labor. American journal of reproductive immunology. 2011; 65(6):549–56. [PubMed: 21214658]

220. Cobo T, Kacerovsky M, Palacio M, Hornychova H, Hougaard DM, Skogstrand K, et al. A prediction model of histological chorioamnionitis and funisitis in preterm prelabor rupture of membranes: analyses of multiple proteins in the amniotic fluid. J Matern-Fetal Neonatal Med. 2012; 25(10):1995–2001. [PubMed: 22372866]

221. Cobo T, Kacerovsky M, Holst RM, Hougaard DM, Skogstrand K, Wennerholm UB, et al. Intra-amniotic inflammation predicts microbial invasion of the amniotic cavity but not spontaneous preterm delivery in preterm prelabor membrane rupture. Acta obstetricia et gynecologica Scandinavica. 2012; 91(8):930–5. [PubMed: 22524241]

222. Bogavac M, Brkic S, Simin N, Celic D. Mid-pregnancy interleukins levels in serum and amniotic fluid as predictors of preterm delivery. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2012

223. Romero R, Kadar N, Miranda J, Korzeniewski SJ, Schwartz AG, Chaemsaithong P, et al. The diagnostic performance of the Mass Restricted (MR) score in the identification of microbial invasion of the amniotic cavity or intra-amniotic inflammation is not superior to amniotic fluid interleukin-6. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2014; 27(8):757–69.

224. Cobo T, Jacobsson B, Kacerovsky M, Hougaard DM, Skogstrand K, Gratacos E, et al. Systemic and Local Inflammatory Response in Women with Preterm Prelabor Rupture of Membranes. PloS one. 2014; 9(1)

225. White PL, Price JS, Posso RB, Barnes RA. An evaluation of the performance of the Dynamiker(R) Fungus (1–3)-beta-D-Glucan Assay to assist in the diagnosis of invasive aspergillosis, invasive candidiasis and Pneumocystis pneumonia. Medical mycology. 2017

226. Lu Y, Chen YQ, Guo YL, Qin SM, Wu C, Wang K. Diagnosis of invasive fungal disease using serum (1-->3)-beta-D-glucan: a bivariate meta-analysis. Intern Med. 2011; 50(22):2783–91. [PubMed: 22082890]

227. Mackay CA, Ballot DE, Perovic O. Serum 1,3-betaD-Glucan assay in the diagnosis of invasive fungal disease in neonates. Pediatric reports. 2011; 3(2):e14.

228. Goudjil S, Kongolo G, Dusol L, Imestouren F, Cornu M, Leke A, et al. (1–3)-beta-D-glucan levels in candidiasis infections in the critically ill neonate. J Matern-Fetal Neonatal Med. 2013; 26(1): 44–8.

229. Mularoni A, Furfaro E, Faraci M, Franceschi A, Mezzano P, Bandettini R, et al. High Levels of beta-D-glucan in immunocompromised children with proven invasive fungal disease. Clinical and vaccine immunology : CVI. 2010; 17(5):882–3. [PubMed: 20335432]

230. Montagna MT, Coretti C, Lovero G, De Giglio O, Montagna O, Laforgia N, et al. Diagnostic performance of 1-->3-beta-d-glucan in neonatal and pediatric patients with Candidemia. International journal of molecular sciences. 2011; 12(9):5871–7. [PubMed: 22016633]

231. Cornu M, Goudjil S, Kongolo G, Leke A, Poulain D, Chouaki T, et al. Evaluation of the (1,3)-beta-D-glucan assay for the diagnosis of neonatal invasive yeast infections. Medical mycology. 2017

232. Romero R, Kadar N, Hobbins JC, Duff GW. Infection and labor: the detection of endotoxin in amniotic fluid. American journal of obstetrics and gynecology. 1987; 157(4 Pt 1):815–9. [PubMed: 2445204]

233. Romero R, Kadar N, Lafreniere D, Durum S, Hobbins JC, Duff GW. Do blood and meconium affect the detection of endotoxin in amniotic fluid with the limulus amebocyte gel clot assay? American journal of perinatology. 1987; 4(4):356–9. [PubMed: 3307803]

234. Romero R, Roslansky P, Oyarzun E, Wan M, Emamian M, Novitsky TJ, et al. Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. American journal of obstetrics and gynecology. 1988; 158(5):1044–9. [PubMed: 3369483]

235. Romero R, Yoon BH, Chaemsaithong P, Cortez J, Park CW, Gonzalez R, et al. Bacteria and endotoxin in meconium-stained amniotic fluid at term: could intra-amniotic infection cause meconium passage? The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2014; 27(8):775–88.

236. Ramage G, Vandewalle K, Wickes BL, Lopez-Ribot JL. Characteristics of biofilm formation by Candida albicans. Revista iberoamericana de micologia. 2001; 18(4):163–70. [PubMed: 15496122]

237. Pal Z, Urban E, Dosa E, Pal A, Nagy E. Biofilm formation on intrauterine devices in relation to duration of use. Journal of medical microbiology. 2005; 54(Pt 12):1199–203. [PubMed: 16278434]

238. Zahran KM, Agban MN, Ahmed SH, Hassan EA, Sabet MA. Patterns of Candida biofilm on intrauterine devices. Journal of medical microbiology. 2015; 64(Pt 4):375–81. [PubMed: 25681320]

239. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284(5418):1318–22. [PubMed: 10334980]

240. Marrie TJ, Costerton JW. A scanning and transmission electron microscopic study of the surfaces of intrauterine contraceptive devices. American journal of obstetrics and gynecology. 1983; 146(4):384–94. [PubMed: 6859159]

241. Jacques M, Marrie TJ, Costerton JW. Review: Microbial colonization of prosthetic devices. Microbial ecology. 1987; 13(3):173–91. [PubMed: 24213294]

242. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, et al. Bacterial biofilms in nature and disease. Annual review of microbiology. 1987; 41:435–64.

243. Espinoza J, Goncalves LF, Romero R, Nien JK, Stites S, Kim YM, et al. The prevalence and clinical significance of amniotic fluid 'sludge' in patients with preterm labor and intact membranes. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2005; 25(4):346–52.

244. Kusanovic JP, Espinoza J, Romero R, Goncalves LF, Nien JK, Soto E, et al. Clinical significance of the presence of amniotic fluid 'sludge' in asymptomatic patients at high risk for spontaneous preterm delivery. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2007; 30(5):706–14.

245. Romero R, Kusanovic JP, Espinoza J, Gotsch F, Nhan-Chang CL, Erez O, et al. What is amniotic fluid 'sludge'? Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2007; 30(5):793–8.

246. Romero R, Schaudinn C, Kusanovic JP, Gorur A, Gotsch F, Webster P, et al. Detection of a microbial biofilm in intraamniotic infection. American journal of obstetrics and gynecology. 2008; 198(1):135e1–5. [PubMed: 18166328]

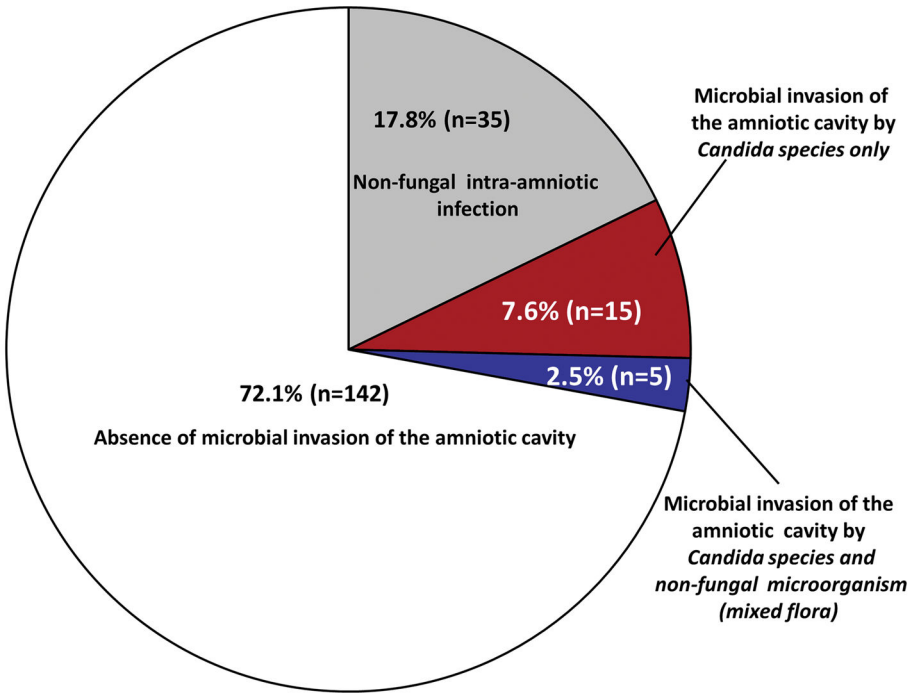

Figure 1.
The frequency of microbial invasion of the amniotic cavity (MIAC) in the study population.

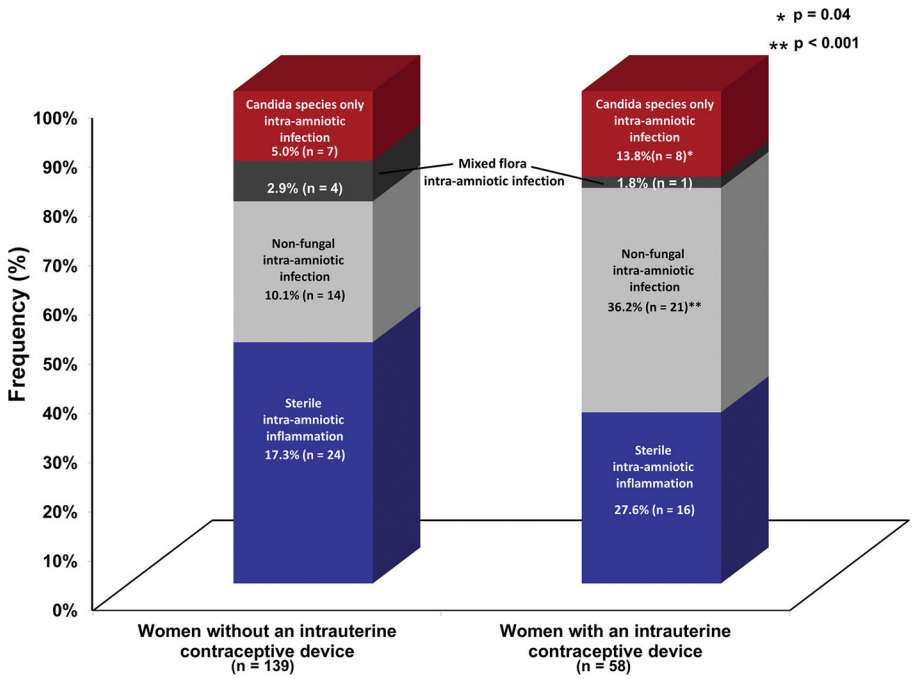

Figure 2.

The frequency of sterile intra-amniotic inflammation and intra-amniotic infection by *Candida* species only, mixed flora (*Candida* species and non-fungal microorganisms), and non-fungal microorganisms in pregnant women with and without an intrauterine contraceptive device (IUD).

Table 1

Demographic and clinical characteristics of the study population.

	Pregnant women with an IUD (n=58)	Pregnant women without an IUD (n=139)	p value
Age (years)	29.0 (24.5–34.0)	23.0 (20.0–27.0)	<0.001
Body Mass Index (kg/m2)	26.2 (23.7–29.8)	24.7 (21.1–32.4)	0.26
Gravidity	2.0 (1.0–3.0)	3.0 (2.0–4.0)	<0.001
Nulliparity	0 (0%)	57 (41.0%)	<0.001
Ethnicity			
African-American	1 (1.7%)	126 (90.6%)	<0.001
Caucasian	0 (0%)	10 (7.2%)	0.03
Hispanic	57 (98.3%)	1 (0.7%)	<0.001
Other	0 (0%)	2 ((1.4%)	1.00
Gestational age at amniocentesis (weeks)	24.9 (21.9–29.5)	29.4 (24.6–32.0)	<0.001
Gestational age at delivery (weeks)	28.9 (24.9–33.2)	34.4 (28.4–37.9)	<0.001
Birthweight (kg)	1.51 (0.827–2.21)	2.29 (1.15–2.85)	0.01
Sterile intra-amniotic inflammation	16 (27.6%)	24 (17.3%)	0.12
Without intra-amniotic infection/inflammation	12 (20.7%)	90 (64.7%)	<0.001

IUD: Intrauterine contraceptive device; IL: interleukin.

Values are expressed as median (interquartile range) or number (percentage).

Candida intra-amniotic infection: Identification of *Candida* species in the amniotic fluid by culture.

Mixed infection intra-amniotic infection: Identification of *Candida* species and non-fungal microorganism(s) by culture.

Sterile inflammation: Amniotic fluid IL-6 ≥2.6 ng/mL and negative amniotic fluid culture result.

Sterile without inflammation: Amniotic fluid IL-6 <2.6 ng/mL and negative amniotic fluid culture result.

The Mann Whitney *U* test was used for continuous variables; the chi-square and Fisher's exact tests for categorical variables.

Table 2

The diagnostic performance of a beta-glucan test for identifying intra-amniotic *Candida* infection

	Study population (n=197)		Women with IUD (n=58)		Women without IUD (n=139)	
	% (n)	95% CI	% (n)	95% CI	%(n)	95% CI
Frequency of *Candida* intra-amniotic infection	10.2% (20/197)	6.3%–15.2%	15.5% (9/58)	7.4%–27.4%	7.9% (11/139)	4.0%–13.7%
Sensitivity	95.0% (19/20)	75.1%–99.9%	100.0% (9/9)	63.4%–100.0%	90.9% (10/11)	58.7%–99.7%
Specificity	75.1% (133/177)	68.1%–81.3%	32.7% (16/49)	20.0%–47.5%	91.4% (117/128)	85.1%–95.6%
Positive likelihood ratio	3.8	2.9–5.0	1.5	1.2–1.8	10.6	5.8–19.2
Negative likelihood ratio	0.1	0.01–0.5	0.0		0.1	0.02–0.7
Positive predictive value	30.2% (19/63)	24.7%–36.3%	21.4% (9/42)	18.3%–24.9%	47.6% (10/21)	33.4%–62.2%
Negative predictive value	99.3% (133/134)	95.2%–99.9%	100.0% (16/16)		99.2% (117/118)	94.7%–99.9%

LEGEND. CI: confidence interval; IUD: intrauterine contraceptive device.

Table 3

Beta-glucan assay results stratified by infection-inflammation status of the amniotic cavity, intra-amniotic infection, and the presence of an intrauterine contraceptive device (IUD).

		Women with an IUD		Women without an IUD		Overall		
		Beta-glucan assay		Beta-glucan assay		Beta-glucan assay		
		Positive	Negative	Positive	Negative	Positive	Negative	Total
Infection-inflammation status of the amniotic cavity	**-Intra-amniotic infection with inflammation**	20	9	10	7	30	16	46
	-Intra-amniotic infection without inflammation	1	0	3	5	4	5	9
	-Absence of intra-amniotic infection and inflammation	8	4	5	85	13	89	102
	-Sterile inflammation	13	3	3	21	16	24	40
	Total	**42**	**16**	**21**	**118**	**63**	**134**	**197**
Intra-amniotic infection	***Candida* spp.**	9	0	10	1	19	1	20
	-*Candida* species only	8	0	7	0	15	0	15
	-*Candida* spp. and non-fungal microorganism(s)	1	0	3	1	4	1	5
	Non-fungal microorganism(s)	12	9	3	11	15	20	35
	Absence of intra-amniotic infection	21	7	8	106	29	113	142
	Total	**42**	**16**	**21**	**118**	**63**	**134**	**197**

Table 4

Beta-glucan assay results for 177 women without intra-amniotic *Candida* infection stratified by infection-inflammatory status of the amniotic cavity and the presence of an intrauterine contraceptive device (IUD).

Infection and inflammation status of the amniotic cavity			Women with an IUD Beta-glucan assay		Women without an IUD Beta-glucan assay		Overall Beta-glucan assay		
			Positive	Negative	Positive	Negative	Positive	Negative	Total
Intra-amniotic inflammation	Present	Intra-amniotic infection	12	9	2	6	14	15	29
		Absence of intra-amniotic infection	13	3	3	21	16	24	40
	Absent	Intra-amniotic infection	0	0	1	5	1	5	6
		Absence of intra-amniotic infection	8	4	5	85	13	89	102
Total			**33**	**16**	**11**	**117**	**44**	**133**	**177**

Table 5

Effects of non-fungal intra-amniotic infection, intra-amniotic inflammation, and an intrauterine contraceptive device (IUD) on the specificity of the beta- glucan assay.

Patients	Total (n)	Negative assay (n)	Specificity
Overall	177	133	75.1%
Women with an IUD	49	16	32.7%
Women without an IUD	128	117	91.4%
No IUD, no intra-amniotic infection	114	106	93.0%
No IUD, non-fungal intra-amniotic infection	14	11	78.6%
No IUD, no intra-amniotic infection, no intra-amniotic inflammation	90	85	94.4%
No IUD, no inflammation, non-fungal intra-amniotic infection	6	5	83.3%
No IUD, no intra-amniotic infection, no intra-amniotic inflammation	24	21	87.5%
No IUD, non-fungal-intra-amniotic infection, intra-amniotic inflammation	8	6	75.0%
IUD, no intra-amniotic infection	28	7	25.0%
IUD, non-fungal intra-amniotic infection	21	9	42.9%
IUD, no intra-amniotic infection, no intra-amniotic inflammation	12	4	33.3%
IUD, no intra-amniotic infection, intra-amniotic inflammation	16	3	18.8%
IUD, non-fungal intra-amniotic infection, intra-amniotic inflammation	21	9	42.9%

LEGEND. n: number.