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Acute Chorioamnionitis and Funisitis: Definition, Pathologic Features, and Clinical Significance

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Abstract

Acute inflammatory lesions of the placenta consist of diffuse infiltration of neutrophils at different sites in the organ. These lesions include acute chorioamnionitis, funisitis, and chorionic vasculitis, and represent a host response (maternal or fetal) to a chemotactic gradient in the amniotic cavity. While acute chorioamnionitis is evidence of a maternal host response, funisitis and chorionic vasculitis represent fetal inflammatory responses. Intra-amniotic infection has been generally considered to be the cause of acute histologic chorioamnionitis and funisitis; however, recent evidence indicates that “sterile” intra-amniotic inflammation, which occurs in the absence of demonstrable microorganisms but can be induced by “danger signals”, is frequently associated with these lesions. In the context of intra-amniotic infection, chemokines (such as interleukin-8 and granulocyte chemotactic protein) establish a gradient favoring the migration of neutrophils from maternal or fetal circulation into the chorioamniotic membranes or umbilical cord,

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respectively. Danger signals released during the course of cellular stress or cell death can also induce the release of neutrophil chemokines. The prevalence of chorioamnionitis is a function of gestational age at birth, and is present in 3-5% of placentas delivered at term, but in 94% of placentas delivered between 21-24 weeks of gestation. The frequency is higher in patients with spontaneous labor, preterm labor, clinical chorioamnionitis (preterm or term), or ruptured membranes. Funisitis and chorionic vasculitis are the hallmarks for the fetal inflammatory response syndrome, a condition characterized by an elevation in fetal plasma concentrations of interleukin-6, associated with the impending onset of preterm labor, a higher rate of neonatal morbidity (after adjustment for gestational age), and multi-organ fetal involvement. This syndrome is the counterpart of the systemic inflammatory response syndrome in adults; however, in fetuses, it is a risk factor for short- and long-term complications (i.e. neonatal sepsis, bronchopulmonary dysplasia, periventricular leukomalacia, and cerebral palsy). This article reviews the definition, pathogenesis, grading and staging, and clinical significance of the most common lesions in placental pathology. Illustrations of the lesions and diagrams of the mechanisms of disease are provided.

Keywords

acute villitis; ascending intra-amniotic infection; chorionic vasculitis; clinical chorioamnionitis; CXCL6; fetal inflammatory response syndrome; granulocyte chemotactic protein; interleukin (IL)-8; microbial invasion of the amniotic cavity; nosology; pathologic grading; placental pathology; pregnancy; prematurity; staging; sterile inflammation

1. Introduction

Acute chorioamnionitis is the most frequent diagnosis in placental pathology reports, and is generally considered to represent the presence of intra-amniotic infection or “amniotic fluid infection syndrome”[1-10]. Yet, acute chorioamnionitis can occur with “sterile intra-amniotic inflammation”, which occurs in the absence of demonstrable microorganisms, but can be induced by “danger signals” released under conditions of cellular stress, injury or death [11-15]. Therefore, acute chorioamnionitis is evidence of intra-amniotic inflammation, and not intra-amniotic infection. The characteristic feature of acute chorioamnionitis is diffuse infiltration of neutrophils into the chorioamniotic membranes [9]. Since obstericians use the term “chorioamnionitis” to refer to a clinical syndrome (the combination of fever, maternal-fetal tachycardia, uterine tenderness, foul-smelling amniotic fluid, etc.) frequently associated with “acute chorioamnionitis” on microscopic examination of the placenta, the word “histologic” has been introduced into the medical lexicon to specify the differences between the clinical syndrome, *clinical chorioamnionitis*, and the pathologic diagnosis of *acute chorioamnionitis*. These terms are not synonymous, and confusion is caused when they are used interchangeably. Herein the term “acute chorioamnionitis” will refer to *acute histologic chorioamnionitis* because the focus of this article is the pathologic condition rather than the clinical syndrome. We will review the acute inflammatory responses deployed by the mother and fetus in response to inflammatory stimuli within the amniotic cavity.

2. Definition

The placenta is composed of three major structures: the placental disc, the chorioamniotic membranes, and the umbilical cord (Figure 1). Acute inflammatory lesions of the placenta are characterized by the infiltration of neutrophils in each of these structures.[9] Specifically, when the inflammatory process affects the chorion and amnion, this is termed acute chorioamnionitis[9]; if it affects the villous tree, this represents acute villitis[9]. If the inflammatory process involves the umbilical cord (umbilical vein, umbilical artery, and the Wharton's jelly), this is referred to as funisitis, the histological counterpart of the fetal inflammatory response syndrome[16] (Figure 1).

3. Prevalence of acute histologic chorioamnionitis

Table 1 shows the frequency of acute chorioamnionitis as a function of gestational age at delivery in a study of 7,505 placentas from singleton pregnancies delivered after 20 weeks of gestation[2]. It is noteworthy that the frequency of acute chorioamnionitis in patients who delivered between 21 to 24 weeks of gestation was 94.4% (17/18)[2]. This is consistent with extensive studies subsequently reported by our group[17, 18] and others[19, 20], and emphasizes the importance of acute inflammation in early preterm deliveries and midtrimester spontaneous abortions.

Acute chorioamnionitis is more frequently observed in the placentas of women who delivered after spontaneous labor at term than in the absence of labor[21, 22] [early labor with cervical dilatation < 4 cm =11.6% (10/86) vs. no labor=4.4% (34/775); $p<0.01$][22]. Moreover, the frequency of histologic chorioamnionitis is higher with longer the duration of labor and cervical dilatation ≥4 cm [active labor=30.4% (7/23) vs. early labor=11.6% (10/86); $p<0.05$][23]. Two explanations can be invoked from this observation: first, the frequency of microbial invasion of the amniotic cavity is higher in women in spontaneous labor at term with intact membranes than in those without labor (17% *vs* 1.5%)[24]. Alternatively, labor *per se* is an inflammatory state, as demonstrated by the the study of the gene expression profile of the chorioamniotic membranes[25]. The chorioamniotic membranes obtained from women who experienced labor (even in the absence of any detectable histologic chorioamnionitis) overexpressed neutrophil-specific chemokines [Chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL2, and Interleukin (IL)-8], and monocyte-specific chemokines (C-C motif) ligand 3 (CCL3; Macrophage inflammatory protein (MIP)-1α), CCL4 (MIP-1β), and CCL20 (MIP-3α)[25] (Figure 2). This is consistent with reports that the amniotic fluid concentrations of chemokines such as IL-8[26], Monocyte chemoattractant protein (MCP)-1[27], Growth-regulated oncogene (GRO)-α [28], MIP-1α [29] and cytokines such as IL-1[30-32], IL-6[33, 34] are higher in women in spontaneous labor at term than in those not in labor at term.

4. Pathology

The placenta can be considered as the apposition or fusion of the fetal membranes/placental disc to the uterine mucosa (decidua) for physiologic exchange[35]. The decidua is of maternal origin, while the chorioamniotic membranes and villous tree are of fetal origin.Thus, the

precise origin of the inflammatory process (maternal vs. fetal) can be determined by whether infiltrating neutrophils are of maternal or fetal origin.

Neutrophils are not normally present in the chorioamniotic membranes, and are thought to migrate from the decidua into the membranes in cases of acute chorioamnionitis[36, 37] (Figure 3). On the other hand, neutrophils in the maternal circulation are normally present in the intervillous space (Figure 1). When there is a chemotactic gradient attracting neutrophils toward the amniotic cavity, neutrophils in the intervillous space migrate into the chorionic plate of the placenta, which is also normally devoid of these cells. Thus, inflammation of the chorionic plate is also a maternal inflammatory response.

Neutrophils in acute chorioamnionitis are of maternal origin. Fluorescence *in situ* hybridization (FISH) with probes for X and Y chromosomes performed in cytospin slides of placentas from male fetuses showed that approximately 90% of neutrophils derived from the membranes were of maternal origin[36]. Subsequently, FISH combined with immunohistochemistry for CD45 (to identify leukocytes) demonstrated that cells staining for CD45 in the chorioamniotic membranes were of maternal origin[37]. In contrast, inflammation of the umbilical cord and the chorionic vessels on the chorionic plate of the placenta is of fetal origin[38]. This conclusion is largely based on the understanding of the anatomy of these tissues, as neutrophils invading the walls of the umbilical vein and arteries must migrate from the fetal circulation to enter the walls of these vessels (Figure 4). Insofar as the origin of white blood cells in the amniotic fluid in cases of intra-amniotic inflammation, the only study reported to date in cases of clinical chorioamnionitis with intact membranes suggested that 99% of neutrophils are of fetal origin [39].

Inflammation of the umbilical vessels begins in the vein (phlebitis) and is followed by involvement of the arteries (arteritis), then infiltration of neutrophils into the Wharton's jelly [40]. The molecular pathogenesis of funisitis has been studied using microarray analysis followed by quantitative real-time PCR of RNA obtained from micro-dissected umbilical arteries and veins. The expression of IL-8 mRNA (the prototypic neutrophil chemokine) is higher in the umbilical vein than in the umbilical artery [40]. Moreover, there are substantial differences in the genes expressed by the walls of the umbilical artery and vein. The pattern of gene expression suggests that the wall of the umbilical vein is more prone to a pro-inflammatory response than the umbilical arteries [40]. This explains why the umbilical vein is the first vessel to show inflammatory changes, and the presence of arteritis is evidence of a more advanced fetal inflammatory process [40]. Indeed, the umbilical cord plasma concentrations of IL-6 (a cytokine used to define systemic inflammation) and the frequency of neonatal complications are higher in cases with umbilical cord arteritis than in those with phlebitis only [41].

Systematic studies of the umbilical cord suggest that acute funisitis begins as multiple, discrete foci, along the umbilical cord, which then merge with the progression of the inflammatory process [40]. Figure 4 illustrates the topography of the inflammatory process in several umbilical cords serially sectioned at 1 mm intervals. The chemotactic gradient attracting neutrophils from the lumen of the umbilical vessels into the Wharton's jelly is thought to be dependent on elevated concentrations of chemokines in the amniotic fluid. The

severity of funisitis correlates with fetal plasma IL-6 concentrations (an indicator of the severity of the systemic fetal inflammatory response) and amniotic fluid IL-6 – the latter reflects the intensity of the intra-amniotic inflammatory response [41].

5. Histological Grading and Staging of Acute Chorioamnionitis

Several grading and staging systems have been proposed to describe the severity of acute chorioamnionitis [9, 18, 19, 42-47]. The most widely used is that recommended by the Amniotic Fluid Infection Nosology Committee of Perinatal Section, the Society for Pediatric Pathology, and reported by Redline *et al*. in 2003 [9]. Although that article contains the term "amniotic fluid infection syndrome", it is now clear that these lesions do not always represent intra-amniotic infection.

Redline *et al*. classified acute inflammatory lesions of the placenta into two categories: "maternal inflammatory response" and "fetal inflammatory response" [9]. The term "stage" refers to the progression of disease based on the anatomical regions infiltrated by neutrophils, while the term "grade" refers to the intensity of the acute inflammatory process at a particular site [9]. In the context of a maternal inflammatory response, a stage 1 lesion is characterized by the presence of neutrophils in the chorion or subchorionic space; stage 2 refers to neutrophilic infiltration of the chorionic connective tissue and/or amnion, or the chorionic plate; and stage 3 is necrotizing chorioamnionitis with degenerating neutrophils (karyorrhexis) [9].

Grade 1 (mild to moderate) refers to individual or small clusters of maternal neutrophils, diffusely infiltrating the chorion laeve, chorionic plate, subchorionic fibrin or amnion. Grade 2 (severe) consists of the presence of three or more chorionic microabscesses, which are defined as confluence of neutrophils measuring at least 10×20 cells [9]. Microabscesses are typically located between the chorion and decidua, and/or under the chorionic plate [9]. Grade 2 is also applied in the presence of a continuous band of confluent neutrophils in the chorion of more than 10 cells in width, occupying more than half of the subchorionic fibrin, or one revolution of the membrane roll. Other staging and grading systems have been used and subsequently modified [18, 19, 42-47].

Staging and grading are also applicable to the fetal inflammatory response [9]. Staging (which refers to the location of neutrophil infiltration) is more important and reproducible than grading in the assessment of the severity of the inflammatory process [48]. For example, involvement of the amnion (amnionitis) is associated with more intense fetal and intra-amniotic inflammation, measured by the concentration of cytokines, than involvement of the chorion alone [49]. The rates of funisitis and positive amniotic fluid culture for microorganisms, as well as the median umbilical cord plasma C-reactive protein, median amniotic fluid Matrix metalloproteinase (MMP)-8 concentration and amniotic fluid white blood cell count are higher when the inflammatory process of the membranes involves amnion and chorion than when neutrophil infiltration is restricted to the chorion/decidua [49]. (Figure 5 stage and grade of acute chorioamnionitis, Figure 6 acute funisitis). Moreover, amniotic fluid MMP-8 concentration is correlated with the severity of acute histologic chorioamnionitis (grading) [50].

The reproducibility of the grading and staging of maternal and fetal inflammation has been subject of a rigorous study by Redline et al. [9] in which 20 cases were reviewed by six pathologists who were asked to identify 12 inflammatory lesions. The kappa coefficient was used to measure agreement among observers. In general, the presence or absence of inflammation had a very high kappa value (0.93 for acute chorioamnionitis, and 0.90 for acute chorioamnionitis/fetal inflammatory response). A kappa value between 0.81 and 1 is considered to represent almost perfect agreement. In contrast, the value of kappa was lower for grading and staging. The authors concluded that there is a greater degree of agreement among pathologists in identifying the presence or absence of inflammation, rather than in quantifying grading and staging [9].

6. Pathways of microbial invasion of the amniotic cavity

Under normal conditions, the amniotic cavity is sterile for microorganisms using cultivation [51] and molecular microbiologic techniques, based on the detection of the 16S rRNA gene (present in all bacteria, but not in mammalian cells). Four pathways have been proposed whereby microorganisms reach the amniotic cavity [52-56]: 1) ascending from the lower genital tract [1, 7, 57, 58]; 2) hematogenous [59-61]; 3) accidental introduction at the time of amniocentesis, percutaneous umbilical cord blood sampling, fetoscopy, or another invasive procedure [62-68]; and 4) retrograde seeding from the peritoneal cavity from the fallopian tubes [57] . However, there is limited evidence in support of the latter pathway.

Ascending microbial invasion from the lower genital tract appears to be the most frequent pathway for intra-amniotic infection (Figure 7 and Figure 8) [53]. While all pregnant women have microorganisms in the lower genital tract, most do not have intra-amniotic infection. The mucus plug represents an anatomical and functional barrier to ascending infection during pregnancy [69-75]. In the non-pregnant state, the endometrial cavity is not sterile [76-78], but the decidua is thought to be sterile during pregnancy.

A hematogenous pathway can operate during the course of blood-born maternal infections [59-61]. Microorganisms such as *Listeria monocytogenes* [79-81], *Treponema pallidum, Yersinia pestis*, Cytomegalovirus, *Plasmodium species*, and others can gain access through the maternal circulation to the intervillous space, from where they invade the villi and the fetal circulation [53]. Bacteria involved in periodontal disease may use this pathway to reach the amniotic cavity [82-88].

Intra-amniotic infection has been documented in patients with preterm labor with intact membranes [11, 89-114], prelabor rupture of membranes [13, 115-130], cervical insufficiency [131-135], an asymptomatic short cervix [14, 136-138], idiopathic vaginal bleeding [139], placenta previa [140], and clinical chorioamnionitis at term [15]. Rupture of membranes is not necessary for bacteria to reach the amniotic cavity – indeed, there is experimental evidence that bacteria can cross intact membranes [141]. Most of these infections are subclinical in nature, and therefore, they occur in the absence of clinical chorioamnionitis [90, 142, 143]. Hence, most of these infections are undetected unless the amniotic fluid is analyzed. The most frequent microorganisms found in the amniotic cavity are genital mycoplasmas [93, 103, 122, 142, 144-147], and in particular, *Ureaplasma*

species [135, 148-155], *Gardnerella vaginalis* [15, 90, 127, 156-158], *Fusobacteria* species, etc. [11, 110, 127]. Fungi can also be found – women who became pregnant with intrauterine contraceptive devices are at high risk for intra-amniotic infection with *Candida albicans* [159-168]. Polymicrobial invasion of the amniotic cavity is present in approximately 30% of cases [11, 13, 93, 110, 127, 169]. Table 2 describes the frequency of microbial invasion of the amniotic cavity in different obstetrical syndromes. Table 3 demonstrates the microorganisms detected in amniotic cavity in patients with preterm labor with intact membranes [110] and clinical chorioamnionitis at term [15]

Microorganisms gaining access to the uterine cavity from the lower genital tract are first localized in the decidua of the supracervical region. Subsequent propagation and chorioamniotic passage of the microorganisms can lead to the establishment of microbial invasion of the amniotic cavity [170, 171]. Although some investigators believe that there is a stage in which the bacteria are diffusely located in the choriodecidual layer, our studies, using FISH with a bacterial 16S rRNA probe, indicate that there is not extensive involvement of the chorion-decidua in cases with microbial invasion of the amniotic cavity [172]. Indeed, bacteria are primarily found in the amnion in cases of intra-amniotic infection, indicating that microbial invasion of the amniotic cavity is a prerequisite for substantial invasion of the amnion and chorion [172]. Specifically, bacteria are more frequently detected in the amniotic fluid than in the chorioamniotic membranes of patients with positive amniotic fluid culture (100% vs. 33%; $P<0.0001$) [172] (Figure 9).

In the past, investigators have reported that the space between the chorioamniotic membranes could contain bacteria, even though such bacteria may not be detectable in the amniotic fluid [4, 173]. The frequency with which this phenomenon occurs remains to be determined. Studies using a combination of cultivation and molecular microbiologic techniques have not yet been conducted to determine the frequency with which this phenomenon occurs. This question is important for the understanding of the pathogenesis of intra-amniotic infection. Experimental models in nonhuman primates have been generated by the inoculation of bacteria in either the decidua or amniotic cavity. Preterm labor occurs more frequently when bacteria are introduced into the amniotic cavity, rather than between the decidua and chorion [171, 174]. Therefore, it seems that intra-amniotic inoculation of bacteria more closely resembles microbial invasion of the amniotic cavity with preterm labor than decidual inoculation [171, 174].

Microbial invasion of the amniotic cavity has been traditionally attributed to planktonic or free-floating bacteria. However, recent evidence suggests that amniotic fluid bacteria can form biofilms [175-182]. Biofilms are defined as communities of sessile organisms that attach to a substratum or to each other. The presence of biofilms can be clinically suspected when sludge is detected as particulate matter in the amniotic fluid using ultrasound [175-182] (Figure 10). Bacteria in biofilms are embedded in a hydrated matrix of extracellular polymeric substances, and exhibit an altered phenotype with respect to growth rate and gene transcription in comparison to planktonic (free floating) cells. In addition, more than 99% of the bacteria in biofilms are capable of growing on a wide variety of surfaces [183]. Biofilms play a major role in human infections, such as periodontitis otitis media and endocarditis , and are important because bacteria in biofilms are resistant to antibiotic treatment . The

formation of biofilms in the amniotic cavity may explain the difficulty in the treatment of intra-amniotic infection. Biofilms are also more common in infections associated with a device (e.g., intra-uterine contraceptive device, prosthetic valves, catheters). Notably, the eradication of intra-amniotic infection diagnosed by amniocentesis in patients with preterm prelabor rupture of membranes (PROM) [184, 185] and those with an asymptomatic short cervix [137] is possible with the administration of intravenous antibiotics to the mother. Success has been documented by demonstrating the absence of microorganisms at the time of a second amniocentesis [137, 184]. We believe that the success of this treatment is due to the fact that the infections had been detected early, before the onset of a substantial intra-amniotic inflammatory (see next section). Once microbial invasion of the amniotic cavity leads to an intra-amniotic cytokine storm clinically manifested by preterm labor, parturition is largely irreversible, and eradication of such infection has not been possible with antibiotic treatment.

7. Inflammatory response to microbial invasion of the amniotic cavity

Microbial invasion of the amniotic cavity induces a robust local inflammatory response, and this is accompanied by a dramatic increase in the concentrations of pro-inflammatory cytokines such as IL-1 [31, 32, 34, 106, 186-192], tumor necrosis factor-alpha (TNF-α) [188-190, 193-196], IL-6 [12, 34, 94, 129, 188, 197-205], IL-8 (CXCL8) [26, 187-189, 196, 199, 200, 202, 206-211], and CXCL6 [212], as well as a cellular response (e.g. increased neutrophil count). Table 4 describes amniotic fluid cytokine/chemokine response to microbial invasion of the amniotic cavity.

Neutrophils express chemokine (C-X-C motif) receptor 2 (CXCR2), which is the receptor for both IL-8 and CXCL6 – potent chemokines for these leukocytes [213-217]. The primary cells and tissues responsible for the intra-amniotic inflammatory response include fetal skin, cells comprising the chorioamniotic membranes, and the umbilical cord. The amnion and chorion-decidua respond to bacterial products by increasing the expression of IL-1β [218-220], and TNF-α [221, 222]. Amnion cells also synthesize IL-8. [223-225]

The temporal relationship between infection or the introduction of inflammatory stimuli (i.e. endotoxin, IL-1, TNFα, IL-6) in the amniotic cavity and the production of cytokines and prostaglandins has been extensively studied using non-human primate models [174, 190, 226-237], sheep [238-245] and other species (rabbits [246-252] and mice [253-261]). The works of Gravett and Novy's laboratories, in which maternal blood, amniotic fluid, and fetal blood have been serially sampled, have provided unique information about the relationship between inflammation, prostaglandin production, and myometrial contractility [226, 234]. Similar studies have been conducted by the groups of Newham and Jobe using sheep [237-245]. Such studies have characterized the complex nature of the fetal immune response after exposure to live bacteria, bacterial products (endotoxin), or inflammatory cytokines (IL-1β) [237-245, 262-265].

The gradient of chemokine concentrations established across the chorioamniotic membranes and the decidua is responsible for diffuse amniotropic infiltration of neutrophils into the chorioamniotic membranes [53]. A systematic proteomic analysis of the amniotic fluid in

cases of intra-amniotic infection and inflammation reveals dramatic changes in the protein composition, and shows increased availability of matrix-degrading enzymes and other proteins involved in the mechanisms of membrane rupture (i.e. neutrophil elastase) and host defense, such as lactoferrin (an anti-microbial protein), calgranulins, and alarmins such as heat shock protein and S100 proteins [266, 267].

The concentrations of cytokines, matrix-degrading enzymes, and other products released during the course of inflammation have been extensively studied to determine if they have diagnostic and prognostic value in cases of suspected intra-amniotic inflammation/infection. Thus far, amniotic fluid concentrations of MMP-8 [268, 269] and IL-6 [101, 111, 124, 198, 270-272] appear to be the best predictors of pregnancy outcome and neonatal complications in patients with preterm labor and intact membranes [11, 12, 109, 112, 273, 274], preterm PROM [13, 275], as well as in those undergoing genetic amniocentesis for standard clinical indications [276-282]. Originally tested as researched methods, rapid analysis with point-of-care tests to identify intra-amniotic inflammation with cytokines [113, 130, 205, 283, 284] and MMP-8 is now possible [285-293].

Detection of microorganisms has traditionally relied on cultivation methods. However, novel approaches allow identifications of genes and species within 8 hours [11]. Increased amniotic fluid IL-6 [195, 294, 295] and MMP-8 in [269, 295] patients at risk for preterm delivery is a risk factor for neonatal brain white matter lesions and the subsequent risk of cerebral palsy.

8. Pathogenesis: Chemotactic signals in the amniotic cavity are responsible for chorioamnionitis and funisitis

Chemotactic stimuli are required for neutrophils to migrate into tissue (Figure 11) [215, 216]. Such stimuli are provided by neutrophil chemokines (e.g. IL-8, also known as neutrophil activating peptide, and CXCL6 – granulocyte chemotactic protein) [215, 216, 296]. Intra-amniotic inflammation due to microorganisms or “danger signals” can result in the production of the following chemokines: IL-8 [26, 187-189, 196, 199, 200, 202, 206-210], Macrophage inhibitory cytokine [297, 298], MCP [27, 299-302], MCP-2, MCP-3 [303], MIP-1α [29, 196, 302, 304], CXCL6 [212], CXCL10 [281], CXCL13 [305], ENA-78 [306], RANTES [307] and GRO-α [28, 208]. Therefore, amniotic fluid chemokine concentrations are elevated and establish a chemotactic gradient favoring migration of neutrophils. In the absence of microorganisms, danger signals released by cells under stress conditions or cell death can induce intra-amniotic inflammation (“sterile inflammation”)[308-319]. The diagnosis of this condition is one of exclusion and requires examination of the amniotic fluid with both cultivation and molecular microbiologic techniques[11-15].

9. Acute chorioamnionitis should not be equated with intra-amniotic infection

Acute inflammatory lesions of the placenta have been traditionally considered as reflective of amniotic fluid infection[1-10, 149, 320-322]. In 1987, Dong *et al* reported that acute histologic chorioamnionitis was present in 97% (32/33) of patients with intra-amniotic infection,

defined as the presence of microorganisms using cultivation techniques[323]. However, amniotic fluid samples for microbiologic studies in that study were obtained by transcervical collection[323]. Indeed, acute chorioamnionitis was found in 37% (18/49) of patients with negative amniotic fluid cultures, suggesting that contamination of samples retrieved by a transcervical route is difficult, if not impossible[323].

The most rigorous evidence that intra-amniotic infection is associated with acute chorioamnionitis is derived from studies in which a trans-abdominal amniocentesis was performed in patients with preterm labor and intact membranes, and the placenta was examined within 48 hours of the procedure[7]. Placentas with acute chorioamnionitis and acute funisitis were from mothers who had intra-amniotic infection proven by culture in 71.1% and 78.7% of cases respectively[7]. The prevalence of microbial invasion of the amniotic cavity was 38%. The negative predictive values of acute chorioamnionitis and funisitis for intra-amniotic infection were 87% and 82%, respectively[7].

Recently, we reported a new type of intra-amniotic inflammation termed “sterile inflammation”, which is more frequent than intra-amniotic infection (microbial-associated intra-amniotic inflammation) in patients with preterm labor with intact membranes[12], preterm PROM[13] and an asymptomatic short cervix[14]. Interestingly, sterile intra-amniotic inflammation is associated with acute histologic chorioamnionitis (40-60% of cases)[11-15]. Moreover, acute inflammatory lesions of the placenta are present in a small subset of patients without intraamniotic inflammation in the context of preterm labor[11, 13], preterm PROM[13], short cervix[14], and clinical chorioamnionitis[15]. Potential explanations are: 1) inflammation of chorioamniotic membranes is a non-specific mechanism of host defense against “danger signals” of non-microbial origin; 2) extra-amniotic infection, which is probably rare; 3) non-viable microorganisms which may release chemotactic factors leading to inflammation[7]. These organisms may have invaded the amniotic cavity and been cleared by the immune system.

The observation that acute histologic chorioamnionitis is present without demonstrable intra-amniotic infection is now well-established[11-15, 324]. Roberts *et al* reported, using both cultivation and molecular microbiologic techniques, that only 4% of patients with acute histologic chorioamnionitis at term have microorganisms in the placenta[324]. Therefore, acute histologic chorioamnionitis should not be considered synonymous with amniotic fluid infection. The characterization of any biological fluid as “sterile” is dependent on the sensitivity of the assays used to detect microorganisms. Cultivation can be very sensitive, and even one microorganism can grow into a colony under optimal conditions; however, such conditions are rarely provided in clinical laboratories. Molecular microbiologic techniques are considered more sensitive; yet, sufficient microbial DNA must be present for this methodology to provide a positive result. PCR assays with specific primers for a microorganism are considered superior to broad range PCR assays based on conserved regions of the bacterial genome (e.g. 16S gene). The use of deep sequencing can change what is known about the microbiologic landscape of biological fluids. Extreme caution must be used when interpreting the results of sequencing studies, as contamination during metagenomics can occur.

10. The host response to microbial invasion of the amniotic cavity is stronger in preterm than in term gestations

The frequency of microbial invasion of the amniotic cavity is similar in patients with spontaneous labor at term and those with preterm labor and intact membranes who subsequently deliver a preterm neonate (17% vs. 22%, respectively) [24, 93]. Yet, preterm neonates born to mothers with microbial invasion of the amniotic cavity have a higher frequency of neonatal sepsis, a systemic inflammatory response (defined as an elevated umbilical cord IL-6 concentration), and funisitis than those born to mothers at term with microbial invasion of the amniotic cavity. Why? Microbial invasion of the amniotic cavity in women in spontaneous labor at term is of shorter duration and can occur after the initiation of parturition [201]. For example, bacteria can be introduced when the chorioamniotic membranes are exposed to the vaginal microbiota during the course of digital examinations performed during labor to determine cervical dilatation and effacement. Such microbial invasion typically has a low inoculum size which elicits a mild intra-amniotic inflammatory response and rarely leads to fetal microbial invasion (hence, the low frequency of funisitis and neonatal sepsis).

On the other hand, in preterm labor with intact membranes or preterm PROM, microbial invasion is established before the initiation of preterm labor. Such infections have a higher microbial burden than those observed in most women in spontaneous labor at term, have probably lasted longer, and therefore, result in a more intensive intra-amniotic inflammatory response [201].Given the longer duration of infection, the likelihood of a fetal attack is higher, and thus, not surprisingly, the rate of congenital neonatal sepsis is greater in preterm than in term neonates (2.27-5.14/1000 in preterm neonates versus 0.04-0.89/1000 term neonates) [325].

11. The Fetal Inflammatory Response Syndrome (FIRS)

Microbial invasion of the amniotic cavity can progress to fetal invasion. The ports of entry for bacteria into the fetus include the respiratory tract (fetal breathing), gastrointestinal tract (swallowing), skin, and ear. Amniotic fluid fills the external auditory canal and bacteria can invade the tympanic membrane and middle ear. Similarly, depending upon the gestational age, microorganisms may gain access to the conjunctiva.

Once microorganisms gain access to the fetal mucosa, they are recognized by pattern recognition receptors such as Toll-like receptors (TLRs), and ligation of such receptors can induce the production of transcription factors such as NFκB and elicit a localized (and subsequently systemic) inflammatory response [326]. For example, fetuses exposed to bacteria can develop severe dermatitis or pneumonitis. Subsequently, microorganisms reaching the fetal circulation could lead to a systemic inflammatory response.

The frequency with which microorganisms invade the human fetus is difficult to ascertain; however, studies in which amniocentesis and cordocentesis have been performed in patients with preterm PROM indicate that 30% of patients with microbial invasion of the amniotic cavity have positive fetal blood cultures for microorganisms (i.e. bacteremia) [327, 328].

Similar findings have been reported when cultures for genital mycoplasmas have been performed in umbilical cord blood at the time of birth [144, 329]. Therefore, the frequency of congenital microbial invasion of the fetus is likely to be higher than that reported in the pediatric literature – the reasons for this are multiple (e.g. bacteremia may not be continuous in the neonatal period; the inoculum size may be small, leading to a high rate of negative blood cultures; and the lack of detection of the most common microorganisms, genital mycoplasmas, may reflect that cultures for these organisms require special media, and such cultures are not routinely performed in neonatal intensive care units) [330-332].

We have defined a fetal systemic inflammatory response syndrome (FIRS) using the fetal plasma concentrations of IL-6 [16, 327, 333-343]. This cytokine is a major mediator of the acute phase response, and its concentration can be readily determined using immunoassays. It is noteworthy that the systemic inflammatory response syndrome (SIRS, in adults) was originally defined using clinical criteria such as fever, tachycardia, respiratory rate, and white blood cell count [344-346]. However, this definition cannot be used in the human fetus, because the vital signs (with the exception of heart rate) cannot readily be determined before birth or during the intrapartum period [347]. Our definition of FIRS was based on the concentration of fetal plasma IL-6 associated with adverse outcome (in samples obtained by cordocentesis) [327], and was introduced in 1997 [348]. Subsequently, in 2001, the American College of Chest Physicians and the Society of Critical Care Medicine noted that an elevated plasma concentration of IL-6 was associated with the likelihood of SIRS, and proposed that the concentrations of this cytokine may be useful in its diagnosis [349].

Despite the similarities between FIRS and SIRS, the unique circumstances of the patient [330] and its environment (uterus) pose challenges which are *sui generis* for the diagnosis, management, and treatment of FIRS [56, 143, 350, 351]. Importantly, FIRS and SIRS share an important feature – both can be caused by non-microbial-related insults. Although the consequences of microbial invasion/proliferation in adult and neonatal medicine are well-known, and the evolution of FIRS/SIRS to sepsis, septic shock and death has been well-characterized, SIRS can occur in cases of sterile inflammation (e.g. pancreatitis or burns) [346, 352]. Since our original report of FIRS, we identified that some cases of this syndrome are observed without demonstrable microbial invasion of the amniotic cavity, and have proposed that such cases represent the response to danger signals which cause cellular stress in the amniotic cavity and fetus [11-13]. The precise nature of the danger signals in sterile intra-amniotic inflammation and corresponding cases of FIRS has not been elucidated; yet, we have proposed that this may result from insults that trigger cell death (necrosis, pyroptosis, etc.) [308, 310, 311, 314, 316, 318].

The presence of FIRS was originally described in fetuses presenting with preterm labor and preterm PROM [327], and was associated with three major consequences: 1) a shorter interval-to-delivery [327]; 2) higher neonatal morbidity after adjustment for gestational age at birth [327]; and 3) multi- organ involvement [351], including hematopoietic system [336, 338, 339, 353], immune system [336, 353-356] thymus [357-361], heart [362], adrenal glands (e.g. alteration in cortisol) [363], skin [335], lung [188, 333], brain [195, 294, 364-366], kidney [367] and gut [46, 368, 369] (Figure 12). Although these observations were originally made in humans, subsequent experimental studies in non-human primates as well as sheep have demonstrated the

involvement of multiple organ systems when the fetus is exposed to inflammatory stimuli [242]. A full description of fetal immune response to chorioamnionitis/intra-amniotic infection in animal model is available in a review by Kallapur and Jobe et al [242].

12. Conclusions

Acute chorioamnionitis, acute funisitis and chorionic vasculitis are acute inflammatory lesions with important short- and long-term clinical significance. Substantial progress has been made in the understanding of the mechanisms responsible for maternal and fetal inflammation in the context of infection. The causes of sterile intra-amniotic inflammation are unknown, and represent important clinical and scientific challenges.

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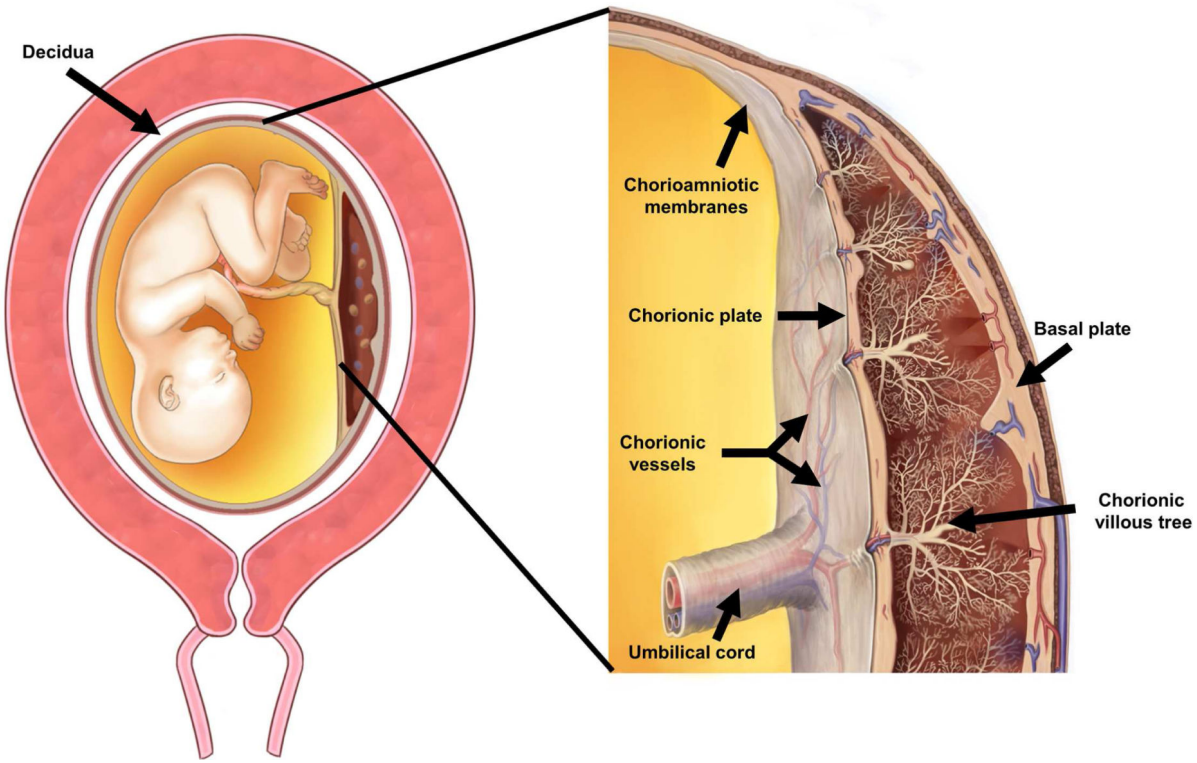
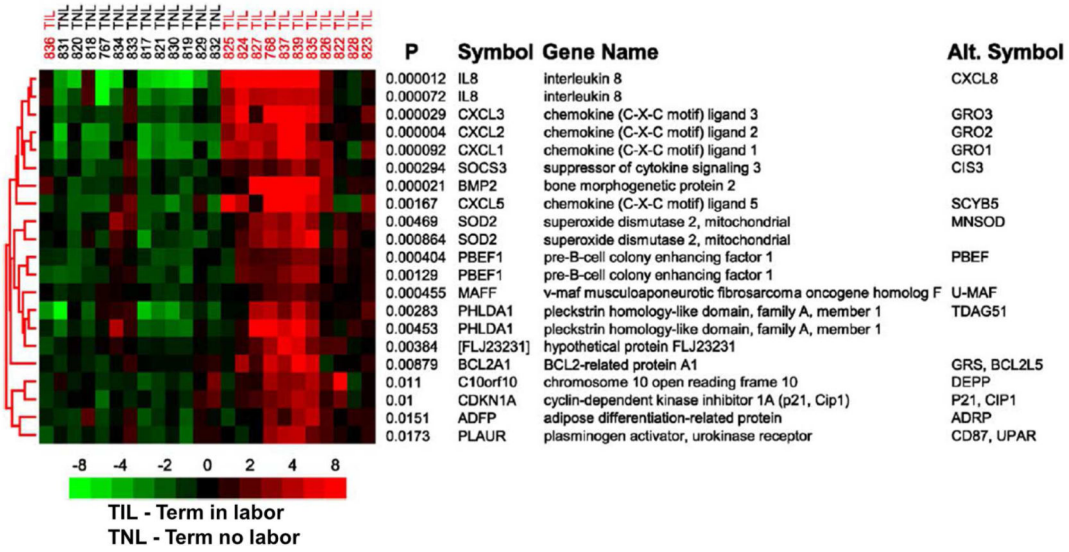


Figure 1.

The anatomy of the pregnant uterus with an emphasis on the placenta. The upper part of the figure illustrates the fetus, umbilical cord and placenta. The chorioamniotic membranes include the amnion and chorion. Decidua is of maternal origin (secretory endometrium) and is adjacent to the myometrium. The lower part of the figure represents a cross-section of the human placenta, including the chorionic plate, chorioamniotic membranes, umbilical cord, and the intervillous space. The basal plate of the placenta is formed of decidua, and is traversed by the spiral arteries, which bring maternal blood into the intervillous space. The villous circulation (fetal) is illustrated in a cross-section of the stem villi. The fetal vessels on the surface of the chorionic plate include arteries and veins, which coalesce to form the umbilical vein and umbilical arteries. Modified from Benirschke K, Burton GJ, Baergen RN. Infectious Diseases. Pathology of the Human Placenta. Sixth ed. Berlin Heidelberg: Springer; 2012. p. 33.



P	Symbol	Gene Name	Alt. Symbol
0.000012	IL8	interleukin 8	CXCL8
0.000072	IL8	interleukin 8	
0.000029	CXCL3	chemokine (C-X-C motif) ligand 3	GRO3
0.000004	CXCL2	chemokine (C-X-C motif) ligand 2	GRO2
0.000092	CXCL1	chemokine (C-X-C motif) ligand 1	GRO1
0.000294	SOCS3	suppressor of cytokine signaling 3	CIS3
0.000021	BMP2	bone morphogenetic protein 2	
0.00167	CXCL5	chemokine (C-X-C motif) ligand 5	SCYB5
0.00469	SOD2	superoxide dismutase 2, mitochondrial	MNSOD
0.000864	SOD2	superoxide dismutase 2, mitochondrial	
0.000404	PBEF1	pre-B-cell colony enhancing factor 1	PBEF
0.00129	PBEF1	pre-B-cell colony enhancing factor 1	
0.000455	MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F	U-MAF
0.00283	PHLDA1	pleckstrin homology-like domain, family A, member 1	TDAG51
0.00453	PHLDA1	pleckstrin homology-like domain, family A, member 1	
0.00384	[FLJ23231]	hypothetical protein FLJ23231	
0.00879	BCL2A1	BCL2-related protein A1	GRS, BCL2L5
0.011	C10orf10	chromosome 10 open reading frame 10	DEPP
0.01	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	P21, CIP1
0.0151	ADFP	adipose differentiation-related protein	ADRP
0.0173	PLAUR	plasminogen activator, urokinase receptor	CD87, UPAR

Figure 2.

The subcluster of genes with the smallest discriminant *P* values included many genes known to be involved in the inflammatory response. Row labels correspond to the permuted t test P value followed by the HUGO Gene Nomenclature Committee (HGNC) official gene symbol, and include the most commonly used alternative gene symbol. Gene expression levels were median-centered and pseudocolored such that red indicates an increased, green indicates a decreased, and black represents the median expression levels, as indicated by the color bar whose numbers indicate fold change. Labels at the top indicate individual patient samples and their clinical designation. Modified from Figure 2 Haddad R, Tromp G, Kuivaniemi H, Chaiworapongsa T, Kim YM, Mazor M, Romero R., Am J Obstet Gynecol. 2006 Aug;195(2):394.e1-24.

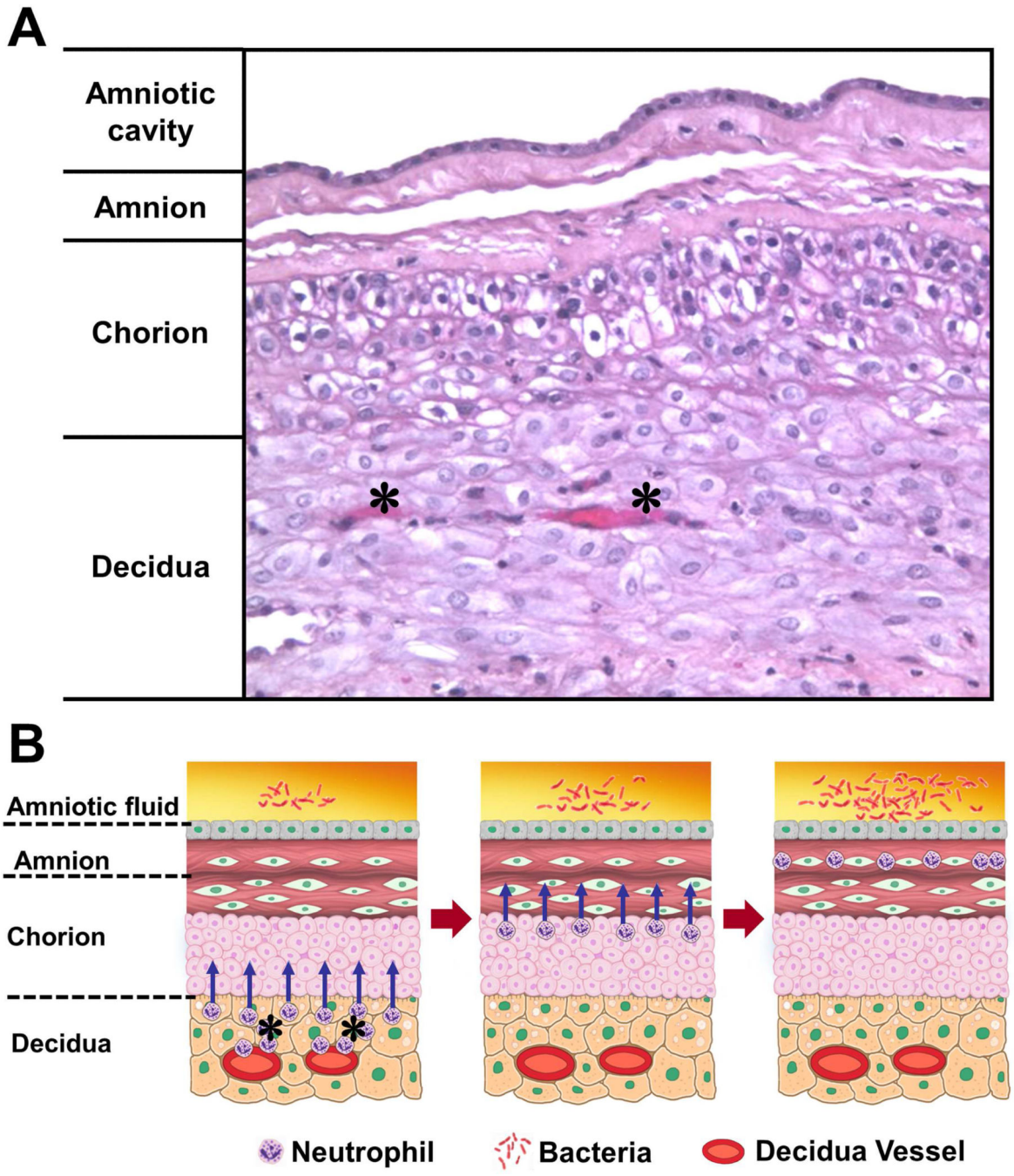


Figure 3.

Migration of the neutrophils from decidual vessels into the chorioamniotic membranes. (A) Normal histology of the chorioamniotic membranes, which are composed of amnion and chorion laeve. The decidua is adjacent to the chorion and contains maternal capillaries (black asterisk). Neutrophils migrate from the maternal circulation in the presence of chemotactic gradient (increased amniotic fluid neutrophil chemokine concentrations). (B) Progression of neutrophils from the decidual vessels (in red) towards the amnion. The location of bacteria is within the amniotic cavity. Initially, neutrophils accumulate in the

choriodecidual interface (B; left); however, in subsequent stages, invade the chorion (B, center) and amnion (B, right).

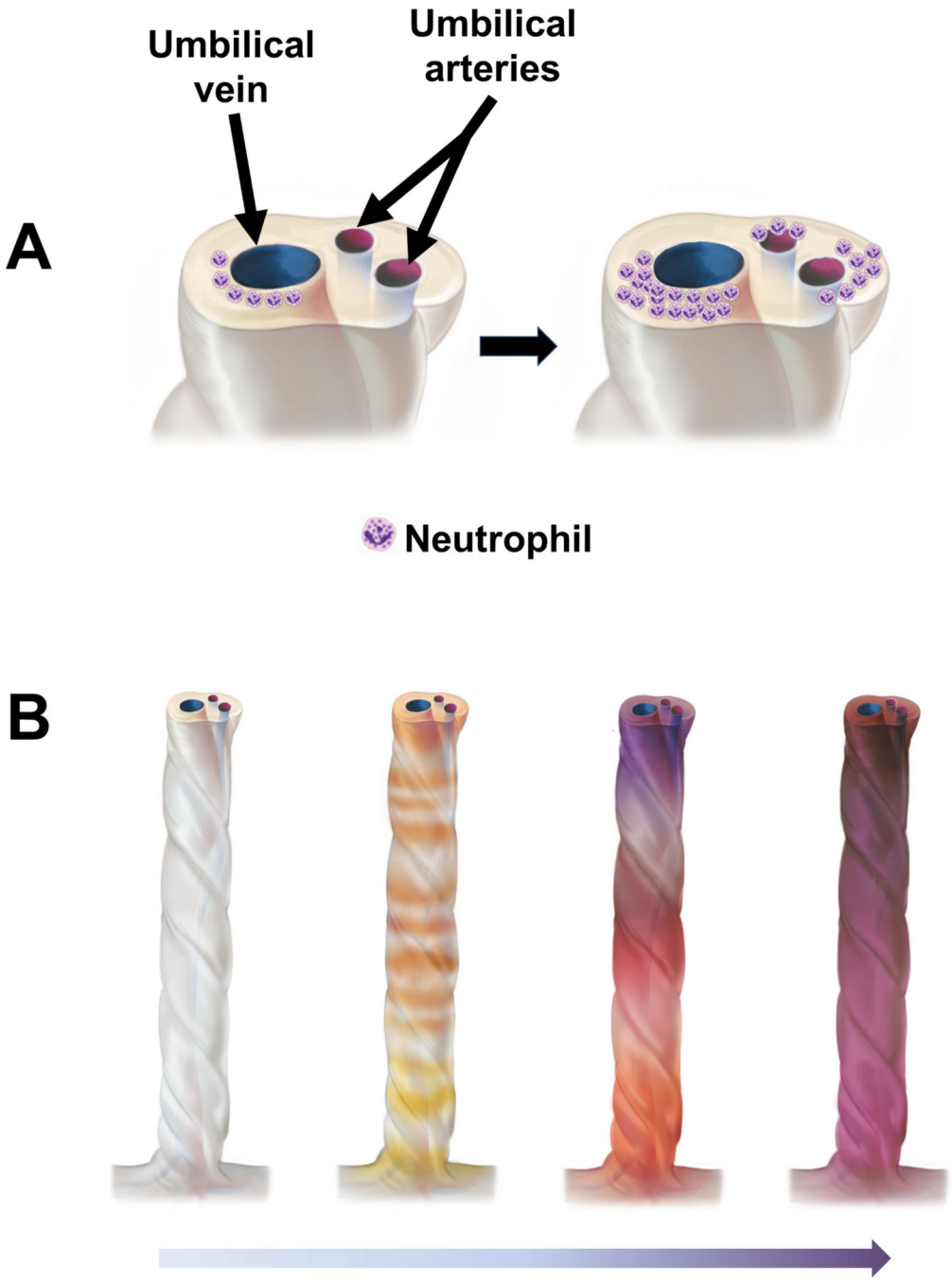


Figure 4.

Topography of the inflammatory process in the umbilical cord. (A) Typically, acute funisitis begins as inflammation of the umbilical vein (umbilical phlebitis; the red vessel represents the umbilical vein), followed by umbilical arteritis involving the umbilical arteries (blue). (B) Progression of inflammation along the length of the umbilical cord. The initial phase is multi-focal, as demonstrated by the yellow/orange rings in the second umbilical cord from left to right in figure 3B. Subsequently, the areas of inflammation coalesce, and funisitis affects the entire umbilical cord.

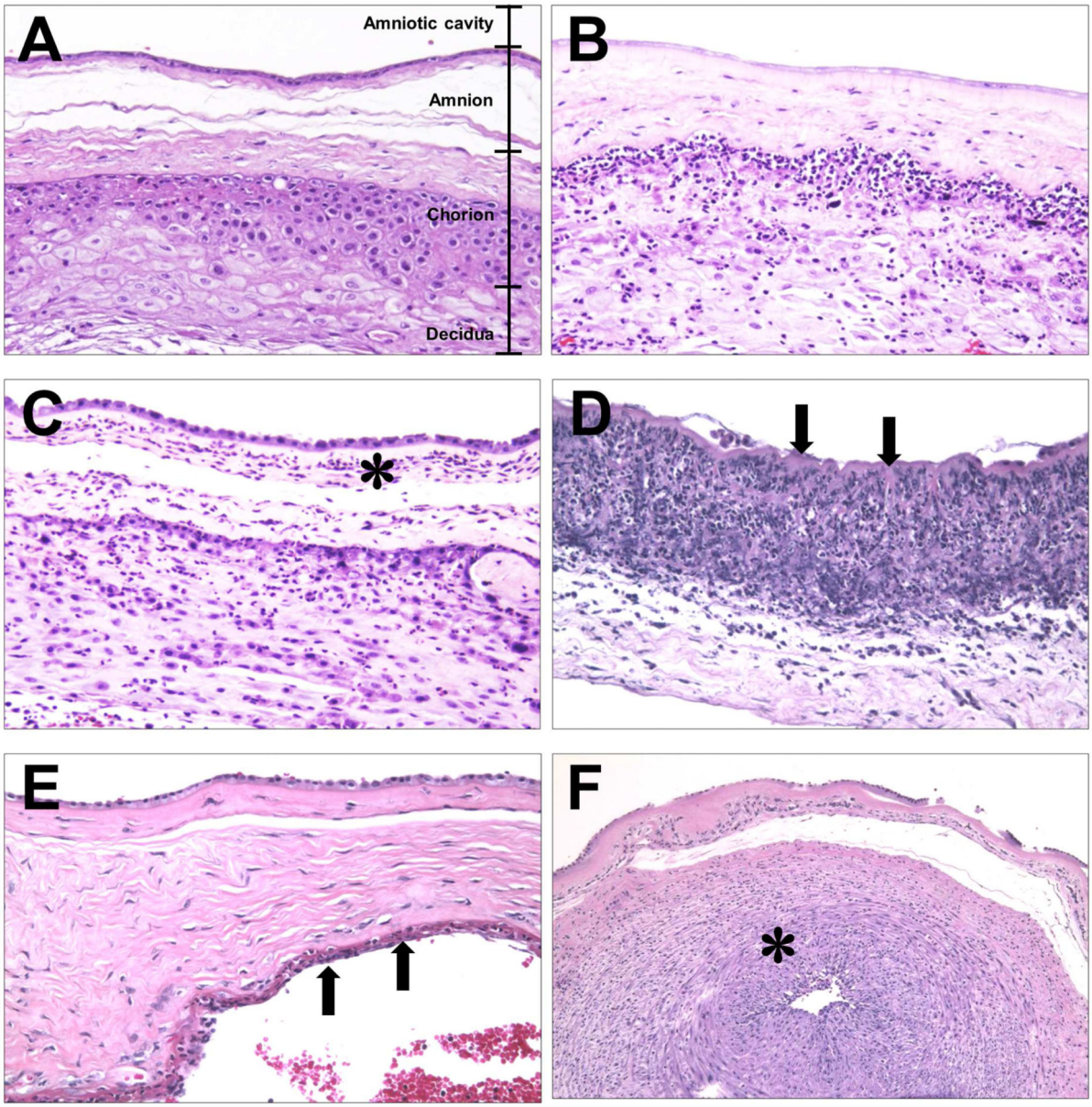


Figure 5.

Staging of acute chorioamnionitis. (A-D) Acute chorioamnionitis of the extraplacental chorioamniotic membranes. (A) Normal chorioamniotic membranes showing the absence of neutrophils. (B) Acute chorionitis is stage 1 acute inflammation of the chorioamniotic membranes, in which neutrophilic infiltration is limited to the chorion. (C) Acute chorioamnionitis is stage 2 acute inflammation of the chorioamniotic membranes, showing neutrophilic migration into the amniotic connective tissue (asterisk). (D) Necrotizing chorioamnionitis is stage 3 acute inflammation of the chorioamniotic membranes, whose characteristic is the amnion epithelial necrosis (arrows). (E, F) Acute inflammation of the chorionic plate. (E) Acute subchorionitis, stage 1 acute inflammation shows neutrophils in the subchorionic fibrin in the chorionic plate (arrows). The area immediately below the arrows represents the intervillous space. (F) Acute chorionic vasculitis (asterisk) is a stage 1

fetal inflammatory response, while acute inflammation of the chorioamniotic membranes (A-F) represents a maternal inflammatory response. Chorionic vasculitis is inflammation on the surface of the fetal vessels within the chorionic plate (see Figure 1 for anatomical location).

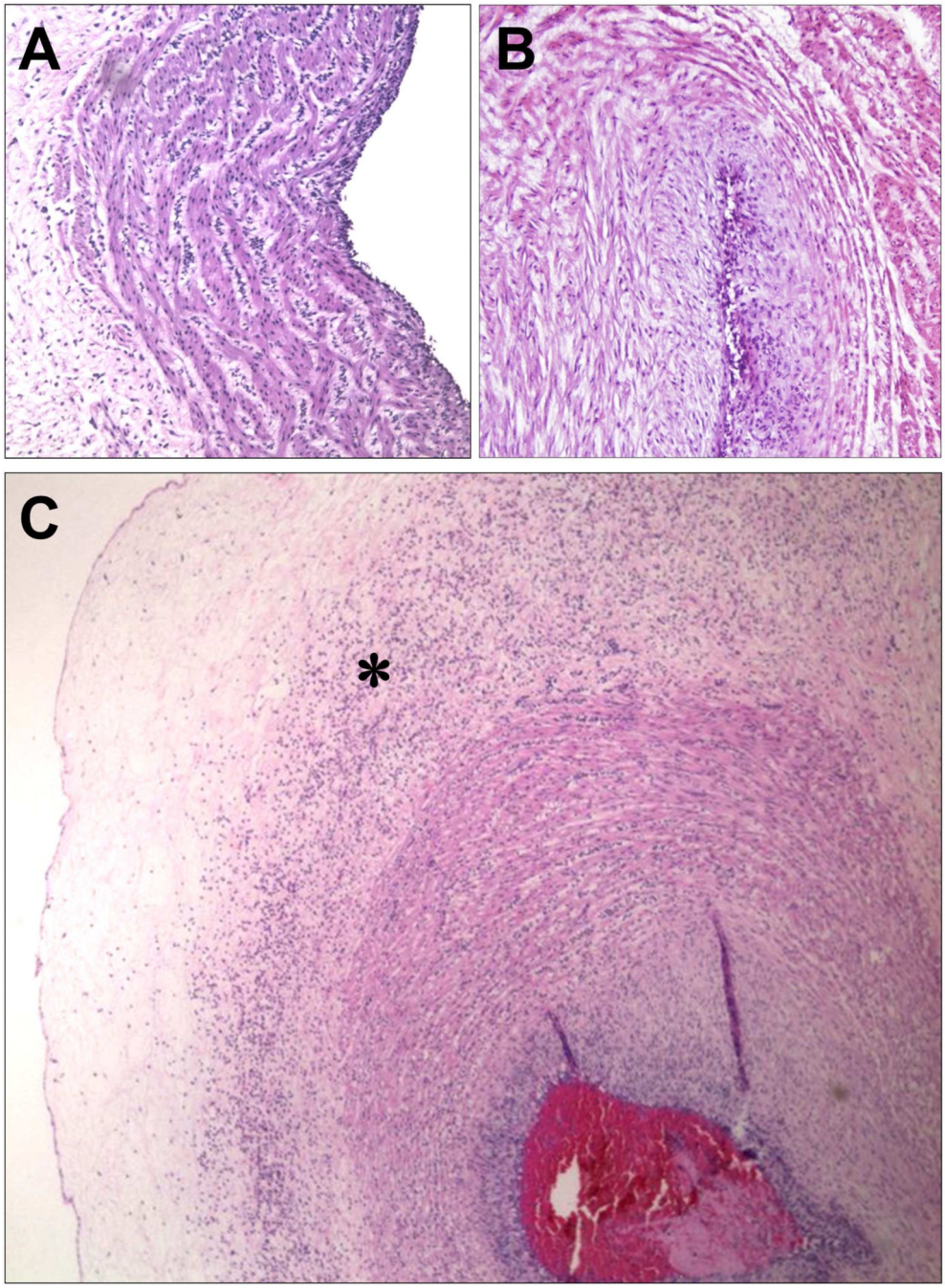


Figure 6.

Staging of acute funisitis. (A) Umbilical phlebitis showing amniotropic migration of fetal neutrophils into the muscle layer of the umbilical vein. Umbilical phlebitis represents stage 1 fetal inflammation. (B) Umbilical arteritis is a stage 2 fetal inflammatory response. (C) Necrotizing funisitis is considered stage 3 fetal inflammatory response. Its characteristic feature is concentric, perivascular distribution of degenerated neutrophils (asterisk). The presence of a thrombus should be considered as a severe fetal inflammatory response.

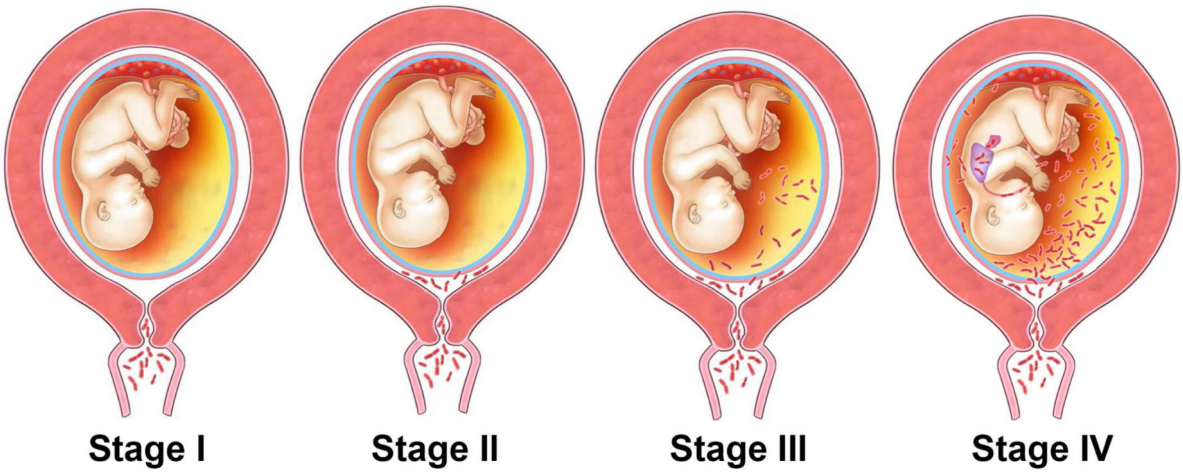


Figure 7.

The stages of ascending infection in preterm labor. Stage I in the process of ascending infection is corresponding to a change in the vaginal/cervical microbial flora or the presence of pathologic organisms in the cervix. Once microorganisms gain access to the amniotic cavity, they reside in the lower pole of the uterus between the membranes and the chorion (Stage II). The microorganisms may invade the fetal vessels (choriovasculitis) or proceed through the amnion (amnionitis) into the amniotic cavity leading to an intra-amniotic infection (Stage III). The microorganisms may invade the fetus by different ports of entry (Stage IV). Modified from Figure 1 in Romero R, Mazor M, Infection and Preterm Labor, Clinical Obstetrics and Gynecology;31:1988:553-584.

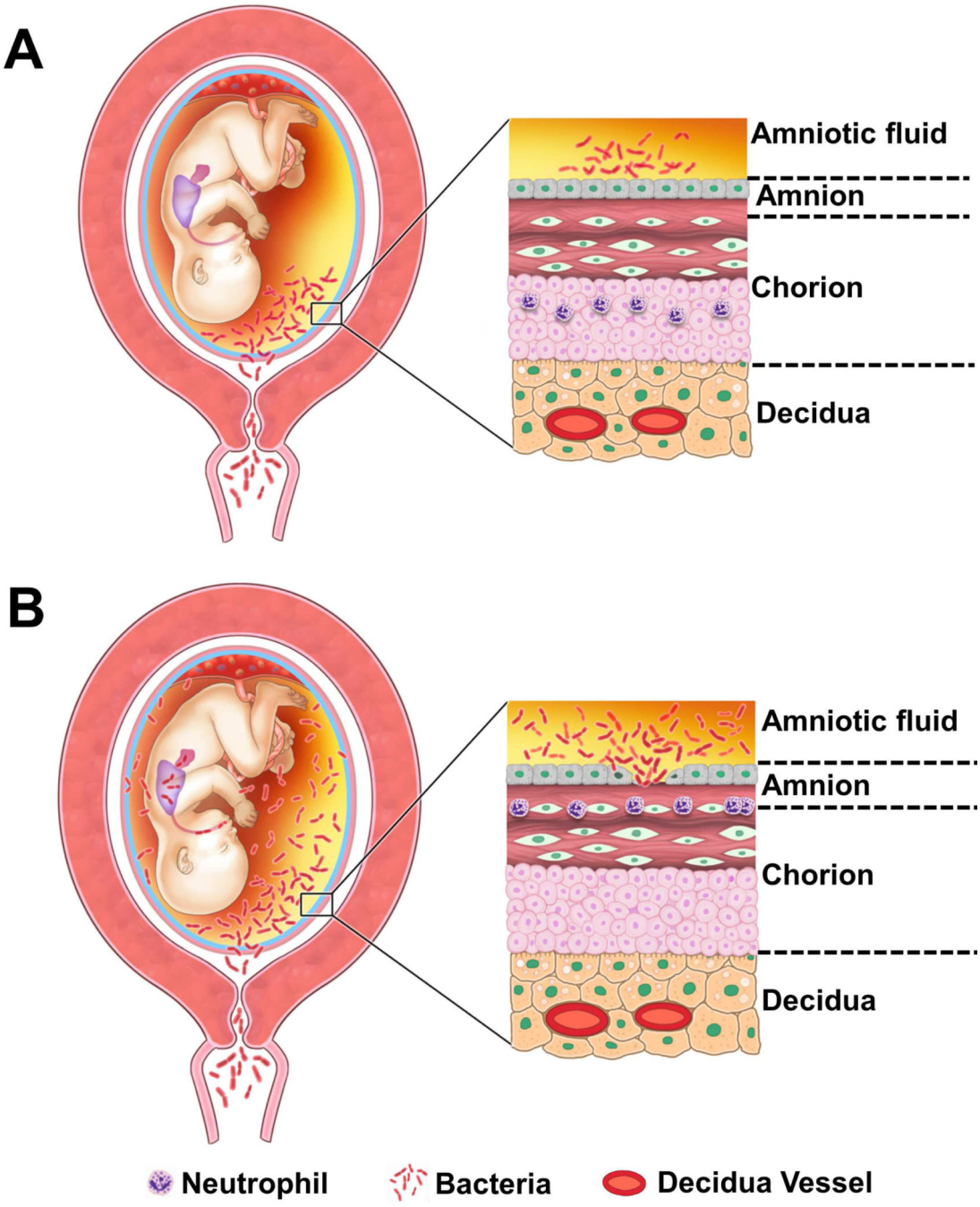


Figure 8.

Pathways of intra-amniotic infection. (A) Most cases of microbial invasion of the amniotic cavity are the result of ascending infection from the vagina and cervix. (B) Extensive microbial invasion of the amniotic cavity can result in fetal infection (bacteria are located in the fetal lung) and damaged chorioamniotic membranes (i.e. necrotizing chorioamnionitis). The destruction of the amnion epithelium is a cardinal feature of necrotizing chorioamnionitis. Modified from Figure 5 Kim MJ, Romero R, Gervasi MT, Kim JS, Yoo

W, Lee DC, Mittal P, Erez O, Kusanovic JP, Hassan SS, Kim CJ. Lab Invest. 2009 Aug; 89(8):924-36.

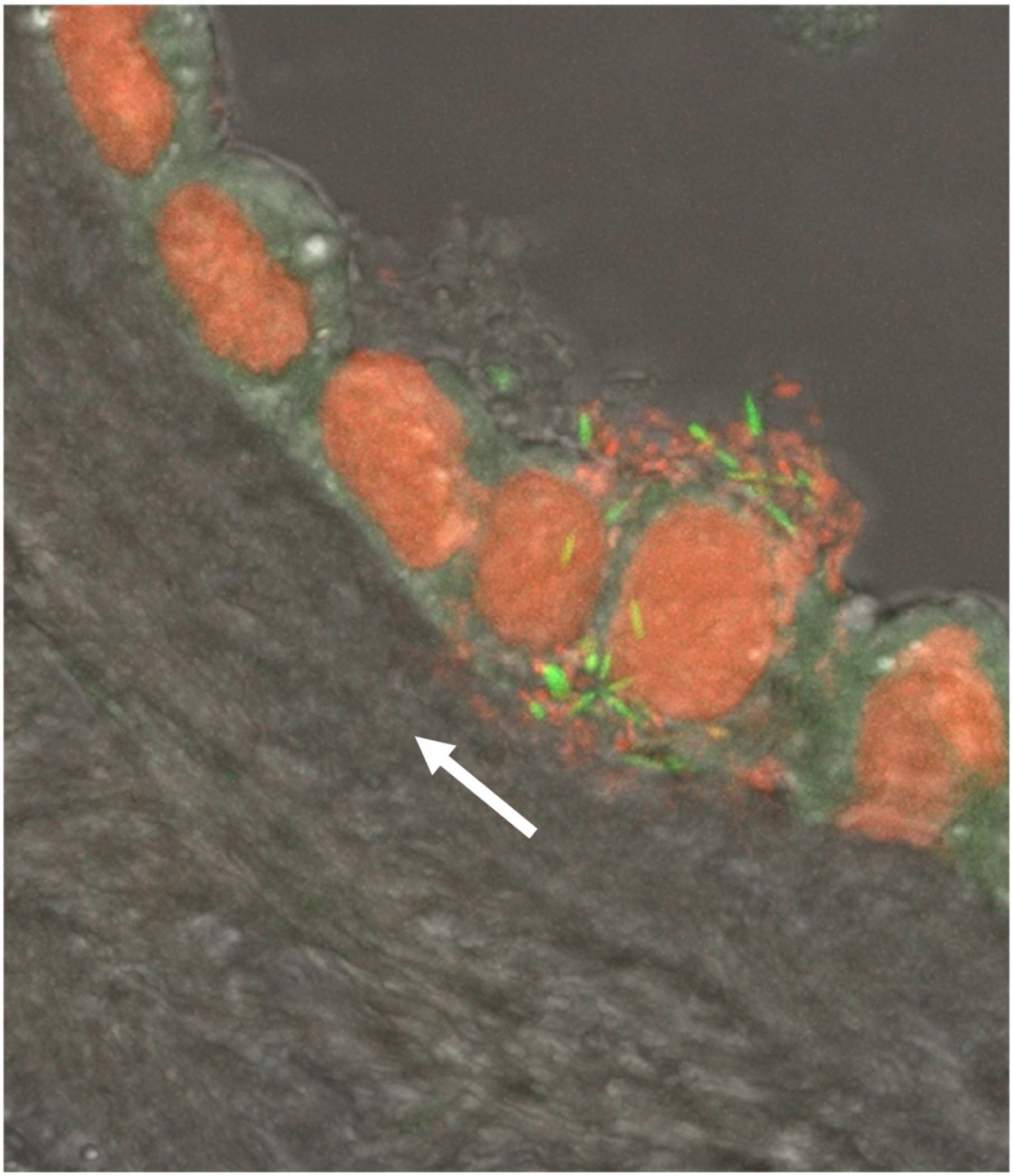


Figure 9.

A cluster of bacteria in amniotic fluid and bacterial invasion of amniotic epithelial cells demonstrated by fluorescent staining. Live bacteria were stained with SYTO 9 (green fluorescence), and dead bacteria were stained with propidium iodide (red fluorescence). Note the lack of bacteria in the chorioamniotic connective tissue indicating bacterial propagation from the amniotic cavity (white arrow). Modified from Figure 3C Kim MJ, Romero R, Gervasi MT, Kim JS, Yoo W, Lee DC, Mittal P, Erez O, Kusanovic JP, Hassan SS, Kim CJ. Lab Invest. 2009 Aug;89(8):924-36.

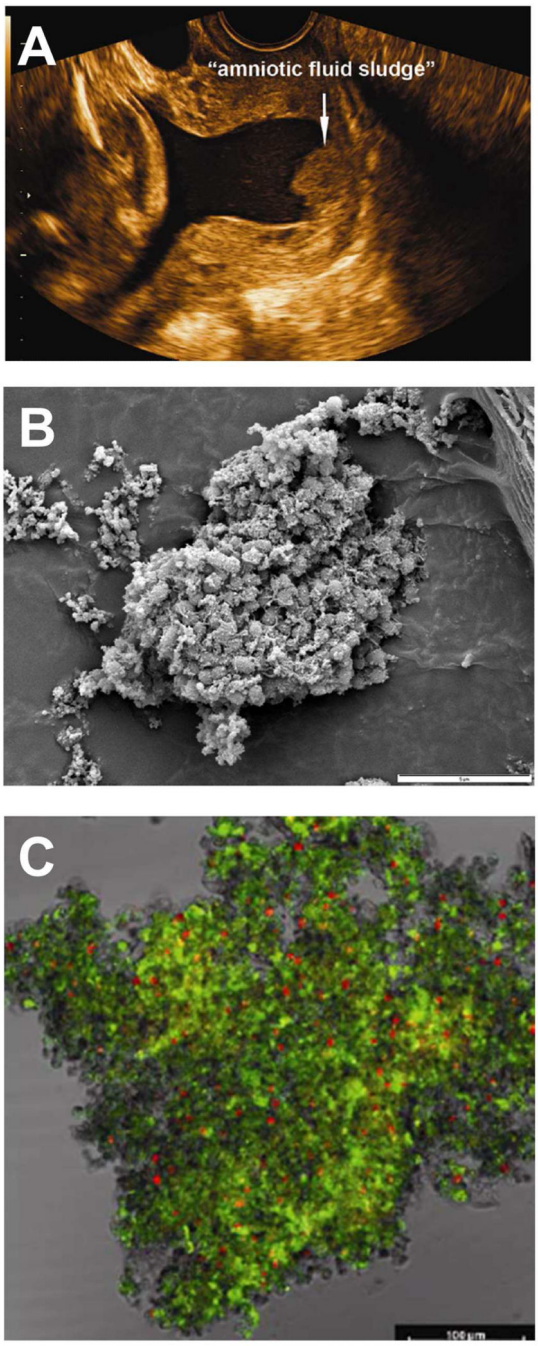


Figure 10.

Microbial biofilms in the amniotic cavity. (A) Two-dimensional transvaginal ultrasound image showing the presence of "amniotic fluid sludge". (B) Scanning electron micrograph of a floc of "amniotic fluid sludge" showing the bacterial cells and the exopolymeric matrix material which constitute a biofilm. In the center of the image, cocci are resolved amongst a fibrous mass of matrix material. (C) Confocal laser scanning microscopy displays bacteria (red dots), matrix material (green), and some unstained material which is likely to represent host components trapped by the biofilm. The bar represents 100 microns. Bacteria (red dots)

are stained with the EUB338-Cy3probe which reacts with the 16S rRNA (component of bacteria). The matrix material has been stained with wheat germ agglutinin, which reacts with the N-acetylglucosamine of the component of the matrix material that forms the structural framework of the biofilm. Modified from Figure 1,3 and 4 Romero R, Schaudinn C, Kusanovic JP, Gorur A, Gotsch F, Webster P, Nhan-Chang CL, Erez O, Kim CJ, Espinoza J, Gonçalves LF, Vaisbuch E, Mazaki-Tovi S, Hassan SS, Costerton JW., Am J Obstet Gynecol. 2008 Jan;198(1):135.e1-5.

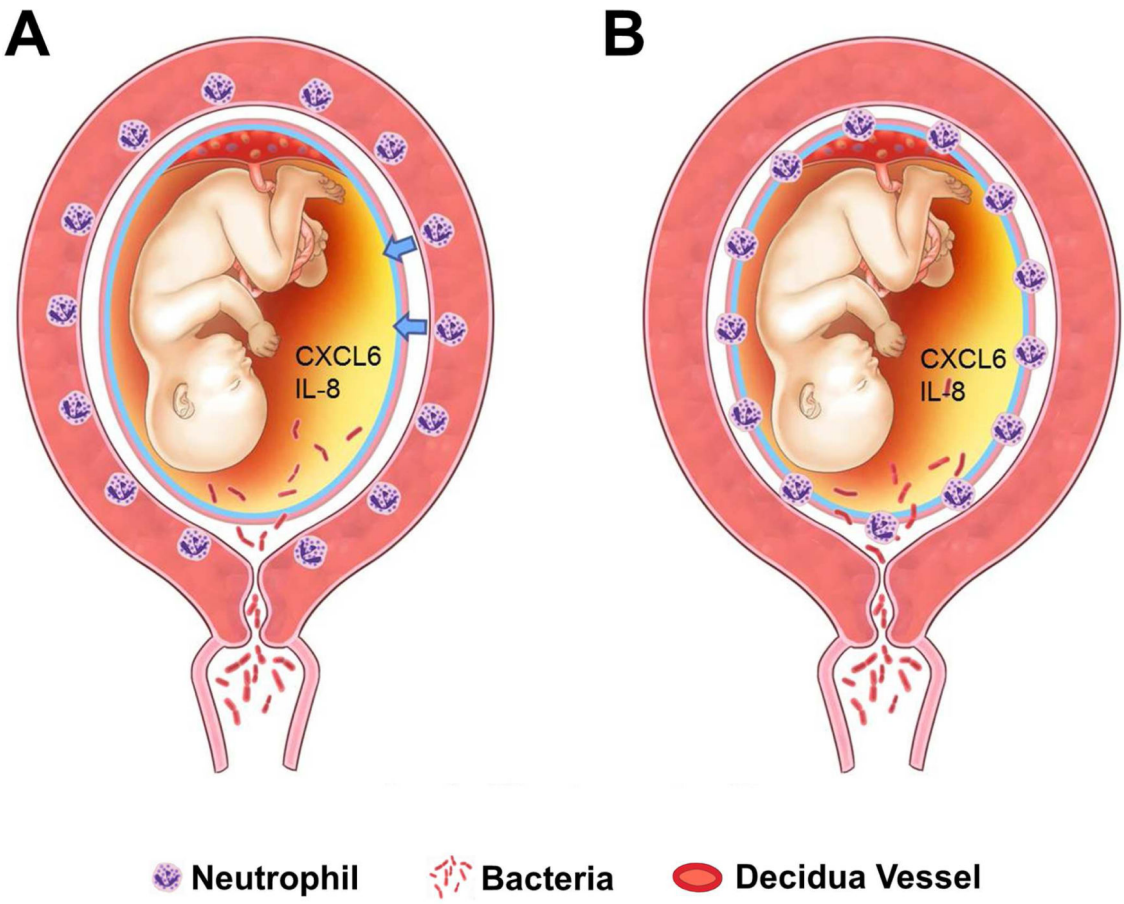


Figure 11.

Chemotactic stimuli for neutrophil migrate into tissue. (A) With the increase in the amniotic fluid concentrations of chemokines such as CXCL6 and IL-8, CXCR2 positive neutrophils show amniotropic migration (arrows). (B) As a consequence maternal neutrophils show infiltration into the chorioamniotic membranes from the decidual vessels.

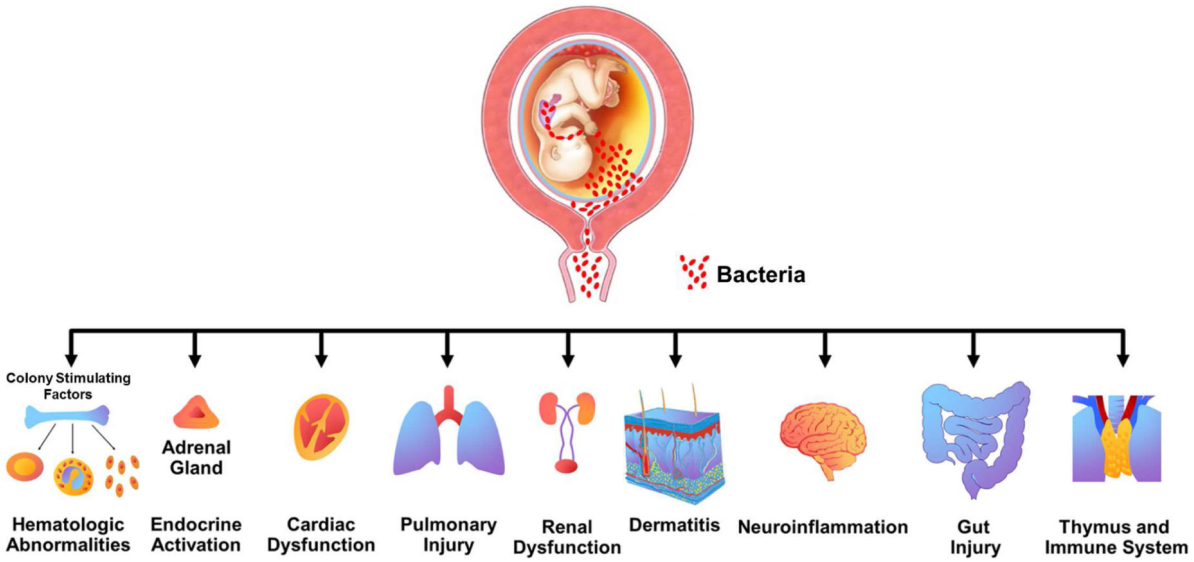


Figure 12.

Fetal target organs during the fetal inflammatory response syndrome. Modified from Figure 2 in Gotsch F, Romero R, Kusanovic JP et al, The fetal inflammatory response syndrome, Clinical Obstetrics and Gynecology; 50: 2007: 652-683

Table 1

The frequency of microbial invasion of the amniotic cavity (MIAC) in obstetrical disorders as determined by amniotic fluid studies obtained by transabdominal amniocentesis using cultivation techniques

Obstetrical disorders	**Prevalence of MIAC (%)**
Spontaneous labor at term with intact membranes	6.3-18.8 [21, 24, 33, 201]
Preterm labor with intact membranes	8.7-34 [11, 89-104, 106-114, 327]
Prelabor premature rupture of membranes without labor	17-57.7 [13, 97, 98, 115-130, 327]
Clinical chorioamnionitis at term	61 [15]
Prelabor premature rupture of membranes in labor	75 [122]
Spontaneous rupture of membranes at term	34.3 [370]
Sonographic short cervix	2.2-9 [14, 136-138]
Cervical insufficiency	8-51.5 [131-135]
Twin gestations with preterm labor and intact membranes	11.9-35 [371-373]
Meconium stained amniotic fluid in preterm gestations	33 [374]
Meconium stained amniotic fluid in term gestations	19.6 [375]
Placenta previa	5.7 [140]
Idiopathic vaginal bleeding	14 [139]
Pregnancy with intra-uterine device	45.9 [168]
Preeclampsia	1.6 [376]
Small for gestational age fetuses	6 [377]
Stillbirth	2.3-13.3 [378, 379]

MIAC: microbial invasion of the amniotic cavity

Table 2

Frequency of chorioamnionitis according to gestational age at delivery

Weeks of gestation	Chorioamnionitis (n)	Total number of patients	Percent (%)
21–24	17	18	94.4
25–28	19	48	39.6
29–32	34	96	35.4
33–36	53	497	10.7
37–40	233	6139	3.8
41–44	36	707	5.1
Total	392	7505	5.2

Modified from Russell P, Inflammatory lesions of the human placenta I, The American journal of diagnostic gynecology and obstetrics 1979; 1: 127-137

Table 3

Microorganisms detected in the amniotic fluid of patients with spontaneous preterm labor with intact membranes and patients with clinical chorioamnionitis at term using cultivation and molecular microbiologic technique

Patients with spontaneous preterm labor with intact membranes [110]	**Patients with clinical chorioamnionitis at term** [15 1515]
Fusobacterium nucleatum	*Ureaplasma* species
Sneathia sanguinegens	*Gardnerella vaginalis*
Ureaplasma species	*Mycoplasma hominis*
Streptococcus mitis	*Streptococcus agalactiae*
Gardnerella vaginalis	*Lactobacillus* species
Peptostreptococcus species	*Bacteroides* species
Leptotrichia amnionii	*Acinetobacter* species
Mycoplasma hominis	*Sneathia*
Streptococcus agalactiae	*Streptococcus viridans*
Lactobacillus species	*Porphyromonas* species
Bacillus species	*Veillonella* species
Coagulase-negative *Staphylococcus* species	*Peptostreptococcus* species
Prevotella species	*Escherichia coli*
Others: Uncultivated *Bacteroidetes, Delftia acidovorans, Neisseria cinerea*	*Pseudomonas aeruginosa*
	Staphylococcus aureus
	Eubacterium species
	Gram (–) *bacilli*
	Enterococcus species
	Others: *Fusobacterium* species, *Candida* species, *Abiotrophia defective, Micrococcus luteus, Staphylococcus epidermidis, Firmicute, Propionibacterium acnes*

Table 4

Cytokines implicated in the pathogenesis of intra-amniotic inflammation/infection

Pro- and anti-inflammatory cytokines	Functions
IL-1α (IL1F1) [32]	Alarmin (endogenous molecules that signal tissue and cell damage) Proinflammatory effects by inducing production of cytokines and chemokines Mediates neutrophil recruitment
IL-1β (IL1F2) [32]	Proinflammatory cytokine and a major mediator of the inflammatory response
IL-6 [94, 380]	Key mediator of the acute phase response to infection and tissue injury Activates Tcells and natural killer (NK) cells Stimulates proliferation and immunoglobulin production by B cells
Tumor necrosis factor-alpha (TNF-α) [381]	Proinflammatory cytokine and a major mediator of sepsis
IL-4 [382]	Inhibits production of IL-1β Induces differentiation of helper T cells Stimulates IgG and IgE production
IL-10 [383]	Inhibits the production of pro-inflammatory cytokines (cytokine inhibitory factor) Downregulates T-cell functions Potent suppressor of the effector functions of macrophages and natural killer (NK) cells

Chemokines	Functions
IL-8 (Neutrophil activating peptide, CXCL8) [26]	Recruitment and activation of acute inflammatory cells, primarily neutrophils Promote angiogenesis
CXCL6 (Granulocyte chemotactic protein-2) [212]	Potent pro-inflammatory chemokine Neutrophil activator
CXCL10 (IP-10) [281, 283]	T cell chemotactic cytokine Recruits and potentiates helper T cell responses and pathogenesis of allograft rejection Pro-inflammatory and anti-angiogenic properties
CXCL13 (BCA-1) [305]	Induces migration of B and T lymphocytes to areas of infection and inflammation
CCL3 (MIP-1α) [29]	Chemotactic cytokine, activates human granulocytes (neutrophils, eosinophils and basophils) in response to inflammation and infection
CCL4 (MIP-1β) [196]	Chemotactic cytokine, activates human granulocytes (neutrophils, eosinophils and basophils) in response to inflammation and infection
CCL20 (MIP-3α) [384]	Chemotactic activity for immature dendritic cells, effector or memory CD4(+) T lymphocytes, and B lymphocytes
Macrophage inhibitory cytokine [298]	Regulates the adaptive immune response, induces cell proliferation, and angiogenesis Inhibits the migration of macrophages and stimulates TNF-α and nitric oxide from macrophages and IL-2 production
MCP-1 (CCL2) [300]	Recruits monocytes/macrophages into sites of inflammation Stimulates the respiratory burst required for macrophage activation
MCP-2 (CCL8) [303]	Role in the inflammatory response Activates immune cells (including mast cells, eosinophils and basophils, monocytes, T cells, and natural killer (NK) cells)
MCP-3 (CCL7) [303]	Monocyte chemoattractant

Chemokines	Functions
	Regulates macrophage function
ENA-78 (CXCL5) [306]	Potent neutrophil chemoattractant and activator Ligand for CXCR2 (IL-8 receptor; chemokine receptor that is activated by IL-8)
GRO-α (CXCL1) [28]	Recruits and activates neutrophils, lymphocytes and monocytes in host defense Role in wound healing, growth regulation, angiogenesis, tumorigenesis and apoptosis
RANTES [307]	Chemoattractant of monocytes, lymphocytes, basophils and eosinophils Regulates the inflammatory response and recruitment of macrophages to the implantation site in early pregnancy Regulates the host response to intrauterine infection

IL = Interleukin; CXCL = Chemokine (C-X-C motif) ligand; IP = Interferon-gamma-inducible protein; BCA = B cell-attracting chemokine; CCL = chemokine (C-C motif) ligand; MIP = Macrophage inflammatory protein; MCP = Monocyte chemoattractant protein; ENA = Epithelial cell-derived neutrophil-activating peptide; GRO = Growth-regulated oncogene; RANTES = Regulated on activation, normal T cell expressed and secreted