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Inflammasome Assembly in the Chorioamniotic Membranes during Spontaneous Labor at Term

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Acute chorioamnionitis, apoptosis-associated speck-like protein containing a CARD (ASC), ASC speck, caspase-1, cytokine, interleukin-1 beta, intra-amniotic inflammation, intra-amniotic infection, neutrophil, NOD-like receptor (NLR), pattern recognition receptor (PRR), parturition, pregnancy, PYCARD**ABSTRACT**

Problem: Inflammasome activation requires two steps: priming and assembly of the multimeric complex. The second step includes assembly of the sensor molecule and adaptor protein ASC (an

apoptosis-associated speck-like protein), which results in ASC speck formation and the recruitment of caspase (CASP)-1. Herein, we investigated whether there is inflammasome assembly in the chorioamniotic membranes and choriodecidual leukocytes from women who underwent spontaneous labor at term.

Method of Study: Using *in situ* proximity ligation assays, ASC/CASP-1 complexes were determined in the chorioamniotic membranes from women who delivered at term without labor or underwent spontaneous labor at term with or without acute histologic chorioamnionitis (n=10-11 each). Also, ASC speck formation was determined by flow cytometry in the choriodecidual leukocytes isolated from women who delivered at term with or without spontaneous labor (n=9-12 each).

Results: 1) ASC/CASP-1 complexes were detected in the chorioamniotic membranes; 2) ASC/CASP-1 complexes were greater in the chorioamniotic membranes from women who underwent spontaneous labor at term than in those without labor; 3) ASC/CASP-1 complexes were even more abundant in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis than in those without this placental lesion; 4) ASC speck formation was detected in the choriodecidual leukocytes; and 5) ASC speck formation was greater in the choriodecidual leukocytes isolated from women who underwent spontaneous labor at term than in those without labor.

Conclusion: There is inflammasome assembly in the chorioamniotic membranes and choriodecidual leukocytes during spontaneous labor at term.

INTRODUCTION

Inflammasomes are multimeric protein complexes located in the cytoplasm capable of detecting microorganisms, damage-derived signals, and other cellular stressors[1-14]. The inflammasome structure includes: 1) a sensor molecule or pattern recognition receptor (PRR), 2) pro-caspase 1 (pro-CASP-1), and 3) the adaptor protein ASC (an apoptosis-associated speck-like protein containing a CARD)[1-14]. The ASC protein is encoded by *PYCARD* and includes two death-fold domains: one pyrin domain and one caspase activation and recruitment domain (CARD)[15, 16]. The pyrin domain interacts with the sensor molecule of the inflammasome, triggering the assembly of the ASC protein into a large complex termed “speck” that consists of multimers of ASC dimers[17, 18]. The CARD domain of the ASC protein recruits monomers of pro-CASP-1 (ASC/CASP-1 complexes), initiating the self-cleavage of this enzyme and the formation

of its active subunits (p10 and p20)[19]. These subunits can then assemble to form active CASP-1 hetero-tetramers, which are able to convert pro-IL-1β and pro-IL-18 into their mature and secreted forms[20-27]. The secretion of large amounts of pro-inflammatory cytokines along with the activation of CASP-1 induces a programmed cell death pathway termed pyroptosis[28].

Most of the inflammasomes contain sensor molecules from the NOD-like receptor (NLR) family, namely NLRP1[1], NLRP3 (also known as cryopyrin or NALP3)[2], NLRP6[29, 30], NLRP7[31, 32], and NLRP12 or NLRC4 (NLR family CARD domain-containing protein 4 or IPAF)[33]. Yet, there are inflammasomes that do not contain an NLR (e.g., interferon gamma-inducible protein 16 (IFI16)[34], absent in melanoma 2 (AIM2)[35-39], and pyrin[40-42]). The best-characterized inflammasome is the NLRP3 and its activation includes a two-step process[43]. The first is a priming step that includes the activation of the nuclear factor kappa B (NF-κB) pathway, which induces the upregulation of pro-IL-1β and the NLR protein to a functional level[43, 44]. The second step includes the assembly and activation of the inflammasome complex, which culminates in the activation of CASP-1 and the consequent processing of pro-IL-1β into its mature form[43]. Priming and assembly of the NLRP3 inflammasome can be triggered by both exogenous and endogenous molecules; therefore, such signaling platforms are implicated in the mechanisms that lead to sterile inflammation and microbial-derived inflammation[43].

Recently, we provided evidence supporting a role for the NLRP3 inflammasome in the mechanisms responsible for sterile[45] and microbial-associated (e.g., acute histologic chorioamnionitis[46, 47]) inflammation in the chorioamniotic membranes. Specifically, we demonstrated that there is priming of the inflammasome in the chorioamniotic membranes during spontaneous labor at term with[46] or without[45] acute histologic chorioamnionitis. This concept is supported by the fact that the chorioamniotic membranes from women who underwent spontaneous labor at term express increased mRNA and protein levels of IL-1β and NLRP3[45]. All of which are even greater in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis[46]. However, whether there is inflammasome assembly in the chorioamniotic membranes from women who underwent spontaneous labor at term with or without acute histologic chorioamnionitis is unknown.

Inflammasome assembly can be assessed by identifying ASC/CASP-1 complexes using *in situ* proximity ligation assays[47] or by evaluating ASC speck formation using flow cytometry[48]. Herein, we determined ASC/CASP-1 complexes in the chorioamniotic membranes and ASC

speck formation in the choriodecidual leukocytes in order to investigate whether there is inflammasome assembly during spontaneous labor at term.

MATERIALS AND METHODS

Human Subjects, Clinical Specimens, and Definitions

Chorioamniotic membrane samples were obtained from the Bank of Biological Specimens of the Detroit Medical Center, Wayne State University, and the Perinatology Research Branch (Detroit, MI, USA), an intramural program of 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). The collection and utilization of biological materials for research purposes were approved by the Institutional Review Boards of Wayne State University and NICHD. All participating women provided written informed consent. The following three study groups were included: 1) women who delivered at term without labor; 2) women who underwent spontaneous labor at term without acute histologic chorioamnionitis; and 3) women who underwent spontaneous labor at term with acute histologic chorioamnionitis. Table 1 contains the demographic and clinical characteristics of the three study populations utilized for *in situ* proximity ligation assays**.** Table 2 contains the demographic and clinical characteristics of the two study populations utilized for the determination of ASC speck formation**.** Multiparous women or women with neonates having congenital or chromosomal abnormalities were excluded. Labor at term was defined by the presence of regular uterine contractions occurring at a frequency of at least two contractions every 10 minutes with cervical changes resulting in delivery.

Placental Histopathological Examinations

Five-µm-thick sections of formalin-fixed, paraffin-embedded tissue specimens were cut and mounted on SuperFrost™ Plus microscope slides (Erie Scientific LLC, Portsmouth, NH, USA). In each case, several tissue sections of the chorioamniotic membranes, umbilical cord, and placental disc were examined. After deparaffinization, slides were rehydrated, stained with hematoxylin-eosin, and evaluated by pathologists who had been blinded to the clinical outcome, according to published criteria[49, 50]. The diagnosis of acute histologic chorioamnionitis was made when the infiltration of neutrophils into the chorionic trophoblast layer or chorioamniotic connective tissue was observed[49, 50].

***In Situ* Proximity Ligation Assay**

ASC/CASP-1 complexes were detected by co-localizing ASC and CASP-1 using a Duolink® *in situ* proximity ligation assay kit (Olink Bioscience, Uppsala, Sweden), following the manufacturer's instructions. Briefly, chorioamniotic membrane tissues were frozen in Tissue-Plus O.C.T. compound (Fisher HealthCare, Houston, TX, USA) immediately after collection. Cryogenic sections were cut to 10 µm and placed onto glass microscope slides (Fisherbrand Superfrost Plus slides; Thermo Scientific, Waltham, MA, USA). The sections were fixed using 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) for 20 min at room temperature, rinsed with 1X PBS, and permeabilized using 0.25% Triton X-100 (Promega, Madison, WI, USA) for 5 min at room temperature. Prior to staining, non-specific antibody interactions were blocked using serum-free protein blocker (Cat#X09090; DAKO North America, Carpinteria, CA, USA) for 30 min at room temperature. The sections were then stained with the following antibodies at 4°C overnight: rabbit anti-human ASC (Cat#AG-25B-0006-C100; Adipogen, San Diego, CA, USA) and mouse anti-human CASP-1 (Cat#MAB6251, clone 661228; R&D Systems, Minneapolis, MN, USA). Rabbit IgG and mouse IgG2A were used as negative controls, respectively. Following staining, slides were briefly washed with 1X Wash Buffer A from the Duolink® kit and incubated with the provided proximity ligation assay probes for 1 h at 37°C, followed by a second wash with 1X Wash Buffer A. The slides were then incubated with the Duolink® ligase solution for 30 min at 37°C, washed with 1X Wash Buffer A, and incubated for 100 min with the Duolink® polymerase solution. Finally, the slides were washed with 1X Wash Buffer B and mounted with Duolink® mounting media with DAPI. Immunofluorescence was visualized using an Olympus BX60 fluorescence microscope (Olympus, Tokyo, Japan) at 400X magnification. Images were acquired using an Olympus DP71 camera and DP Controller software (Olympus). Semi-quantification was performed using the Duolink® image analysis software. Images and videos were acquired using a Zeiss LSM 800 laser scanning confocal microscope (Carl Zeiss Microscopy, Jena, Germany) at the Microscopy, Imaging, and Cytometry Resources Core at the Wayne State University School of Medicine (http://micr.med.wayne.edu/). ASC/CASP-1 complexes were calculated by dividing the number of signals over the area of the tissue. The area of the tissue is expressed in pixels.

ASC Speck Formation

ASC speck formation in inflammasome-competent cells can be assessed by flow cytometry[48]. Choriodecidual leukocytes were isolated, as previously described[51] [purity >85% CD45+ cells within the forward scatter (FSC) vs. size scatter (SSC) gate for leukocytes]. Isolated choriodecidual leukocytes were incubated with 20 µL of human FcR blocking reagent (Miltenyi Biotec) in 80 µL of stain buffer (catalog number 554656; BD Biosciences, San Jose, CA) for 10 min at 4°C. Choriodecidual leukocytes were then washed with 1 mL of stain buffer, re-suspended in 250 µL of BD Cytofix/Cytoperm Fixation and Permeabilization Solution (BD Biosciences), and incubated for 30 min at 4°C in the dark. After fixation, choriodecidual leukocytes were washed with 1 mL of 1X BD Perm/Wash Buffer (BD Biosciences) and re-suspended in 50 µL of the 1X BD Perm/Wash Buffer. Choriodecidual leukocytes were then stained with anti-ASC-PE (clone HASC-71; catalog number 653903; BioLegend, San Diego, CA) or the IgG isotype control (catalog number 400139; BioLegend) for 30 min at 4°C in the dark. Finally, the stained cells were washed with 1 mL of 1X BD Perm/Wash Buffer, re-suspended in 0.5 mL of stain buffer, and acquired using the BD LSR Fortessa Flow Cytometer and BD FACSDiva 6.0 software. The analysis and figures were performed using FlowJo software version 10 (FlowJo, Ashland, OR, USA).

As a positive control for ASC speck formation, THP-1 cells (monocyte cell line; American Type Culture Collection; ATCC; Manassas, VA; 2.5×10^6 cells/mL) were incubated with 1 µg/mL of lipopolysaccharide (LPS; Escherichia coli 0111:B4; Sigma-Aldrich, 2 h) and 20µM of nigericin (catalog number N7142, Sigma-Aldrich, St. Louis, MO, 1 h). Non-treated THP-1 cells were used as a negative control.

Statistical Analyses

The SPSS v.19.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the demographic and clinical characteristics of the study populations. Normality of the data was tested using the Shapiro-Wilk test. Comparisons among groups were performed using the Kruskal–Wallis test and/or the Mann-Whitney U test. Comparisons of proportions were made using the Fisher's exact test. A p-value of ≤0.05 was used to determine statistical significance.

RESULTS

ASC/CASP-1 complex formation is increased in the chorioamniotic membranes from women who underwent spontaneous labor at term

As a read-out for inflammasome assembly, we first performed *in situ* proximity ligation assays to identify ASC/CASP-1 complex formation[47]. Figure 1 shows representative images of ASC/CASP-1 complexes in the chorioamniotic membranes. ASC/CASP-1 complexes were rarely found in the chorioamniotic membranes from women who delivered at term without labor (Figure 1A). A 3-D reconstruction shows that ASC/CASP-1 complexes were scarce in the chorioamniotic membranes from women who delivered at term without labor (Video 1). ASC/CASP-1 complex formation was evident in the chorioamniotic membranes from women who underwent spontaneous labor at term (Figure 1B), and a 3-D reconstruction shows that such complexes are mostly localized in the compact layer of the amnion. Yet, ASC/CASP-1 complexes were more abundant in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis than in those without this placental lesion (Figure 1C vs. 1B). A 3-D reconstruction shows that ASC/CASP-1 complexes were abundant in both the amnion and chorion from women who underwent spontaneous labor at term with acute histologic chorioamnionitis (Video 3). The isotype controls did not show any non-specific background signal (Figure 1D-F). Semi-quantification of the fluorescence signal revealed that, indeed, ASC/CASP-1 complexes were greater in the chorioamniotic membranes from women who underwent spontaneous labor at term than in those who delivered at term without labor (Figure 2). ASC/CASP-1 complexes were even greater in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis than in those without this placental lesion (Figure 2). Collectively, these results provide evidence that there is inflammasome assembly in the chorioamniotic membranes during spontaneous labor at term and that such an event is enriched in cases with acute histologic chorioamnionitis.

ASC speck formation is increased in the choriodecidual leukocytes isolated from women who underwent spontaneous labor at term

The ASC adaptor protein is diffusely located throughout the nucleus and cytoplasm; yet, upon inflammasome activation, it forms a large perinuclear agglutination termed "speck"[15, 18]. Therefore, the presence of ASC specks represents inflammasome activation[18]. Recently, a novel

flow cytometry method was developed to determine ASC speck formation in inflammasome-competent cells[48]. Using this method, we evaluated whether there is ASC speck formation, as a read-out of inflammasome activation, in the choriodecidual leukocytes isolated from women who underwent spontaneous labor at term. The gating strategy used to evaluate ASC speck formation is shown in Figure 3A. Briefly, choriodecidual leukocytes were first gated within the FSC-area vs. SSC-area gate to exclude cellular debris and stromal cells. Next, doublets were excluded using FSC-area vs. FSC-width profile characteristics. Choriodecidual leukocytes stained for ASC were then gated, and the detection of ASC specks was analyzed within the ASC-area vs. ASC-width gate. As negative and positive controls, we used untreated (Figure 3B) or treated (LPS + nigericin; Figure 3C) THP-1 cells, respectively. Isotype controls for THP-1 cells are shown in Figure 3E and 3F. Detection of ASC specks in choriodecidual leukocytes isolated from women who underwent spontaneous labor at term is shown in Figure 3D along with its isotype control (Figure 3G). Flow cytometric quantification revealed that ASC speck formation is greater in the choriodecidual leukocytes isolated from women who underwent spontaneous labor at term than in those who delivered without labor. These results indicate that there is inflammasome activation in choriodecidual leukocytes isolated from women who underwent spontaneous labor at term.

DISCUSSION

Principal findings of the study: 1) ASC/CASP-1 complexes were detected in the chorioamniotic membranes; 2) ASC/CASP-1 complexes were greater in the chorioamniotic membranes from women who underwent spontaneous labor at term than in those without labor; 3) ASC/CASP-1 complexes were even more abundant in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis than in those without this placental lesion; 4) ASC speck formation was detected in the choriodecidual leukocytes; and 5) ASC speck formation was greater in the choriodecidual leukocytes isolated from women who underwent spontaneous labor at term than in those without labor. Collectively, these results provide evidence that there is assembly of the inflammasome in the chorioamniotic membranes and choriodecidual leukocytes during spontaneous labor at term.

Spontaneous labor at term is a state of physiological inflammation[52-57] that, in most women, is considered sterile because it occurs in the absence of intra-amniotic infection[58, 59]. This concept is supported by evidence demonstrating that there is an increased bioavailability of

pro-inflammatory mediators, such as cytokines, in the amniotic fluid of women who underwent spontaneous labor at term[60-79]. Of all these cytokines, interleukin (IL)-1β seems to be a central mediator in the process of parturition since its concentrations are increased in the amniotic fluid[61, 62] and chorioamniotic membranes[45, 80, 81] from women who underwent spontaneous labor at term. Indeed, the systemic[63, 82] or intra-amniotic[83, 84] administration of IL-1β induces preterm labor and birth. The function of this cytokine during the process of labor includes, but is not limited to, the induction of i) the biosynthesis of prostaglandin E2 by the human amnion[85] and myometrial cells[86, 87]; ii) the expression of cyclooxygenase-2 in human myometrial cells[88]; and iii) the expression of matrix-metabolizing enzymes (MMP-1, -3, -9, and cathepsin S) in human cervical smooth muscle cells[89]. However, the mechanisms that lead to the processing of the bioactive and mature form of IL-1β during labor were poorly understood. Recently, we provided evidence that there is priming of the inflammasome (i.e., upregulation of pro-IL-1β and the NLRP3 protein) in the chorioamniotic membranes of women who underwent spontaneous labor at term, which may be implicated in the processing of mature IL-1β[45]. In the study herein, we found that there is assembly of the inflammasome (i.e., the ASC protein forms complexes with CASP-1) in the chorioamniotic membranes during spontaneous labor at term. These findings along with the fact that there is activation of CASP-1 and processing of mature IL-1β in the chorioamniotic membranes[45] support the concept that the inflammasome is involved in the processing of IL-1β by the chorioamniotic membranes during spontaneous labor at term[45, 90, 91].

In the current study, we also observed that there is increased assembly of the inflammasome in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis. This finding is in line with our previous observations demonstrating that the chorioamniotic membranes from women who underwent spontaneous labor at term without acute histologic chorioamnionitis, when compared to those with this placental lesion: 1) express higher mRNA and protein levels of IL-1β and NLRP3 (i.e., priming of the inflammasome); 2) contain increased quantities of the active forms of CASP-1; and 3) release greater concentrations of mature IL-1β[46]. Together, these data suggest that, in the pathological process of acute histologic chorioamnionitis, there is an exacerbated activation of the inflammasome in the chorioamniotic membranes.

Choriodecidual leukocytes express pro-inflammatory mediators implicated in the processes of term and preterm labor[57, 81, 92-99]. Indeed, choriodecidual leukocytes isolated from

women who underwent spontaneous labor at term exhibit an increased expression of IL-1β[81], which likely contributes to the amount of this cytokine released by the chorioamniotic membranes[45] and decidual cells[100]. These findings prompted us to investigate whether choriodecidual leukocytes could express inflammasome components. Herein, we observed that choriodecidual leukocytes display increased ASC speck formation (i.e., inflammasome assembly/activation) during spontaneous labor at term. Although most of the isolated cells were leukocytes (purity > 85%), we do not overlook the possibility that, in some decidual cells, there is inflammasome activation during parturition. Together, these data suggest that there is inflammasome assembly, and most likely activation, in the choriodecidual leukocytes during spontaneous labor at term.

In summary, the study herein provides further evidence that there is inflammasome activation (assembly of the ASC protein with CASP-1 and ASC speck formation) in the chorioamniotic membranes and choriodecidual leukocytes from women who underwent spontaneous labor at term. These data also support the hypothesis stating that the inflammasome mediates the activation of CASP-1 and the consequent release of mature IL-1β during physiological and pathological inflammation of the chorioamniotic membranes in spontaneous labor at term[45, 46, 90, 91].

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Disclosure/Conflict of Interest

The authors disclose no conflicts of interest.

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FIGURE LEGENDS

Figure 1. ASC/CASP-1 complex formation in the chorioamniotic membranes. *In situ* proximity ligation assays for ASC and CASP-1 in the chorioamniotic membranes from women who delivered at term without labor or underwent spontaneous labor at term with or without acute histologic chorioamnionitis. Representative images of ASC/CASP-1 complexes (red signal) in each group (A-C). Representative images of isotype controls in each group (D-F). The blue signal is DAPI (nuclei). 400× magnification. CASP-1, caspase 1; ASC, apoptosis-associated speck-like protein containing a CARD

Figure 2. Semi-quantification of ASC/CASP-1 complexes in the chorioamniotic membranes. ASC/CASP-1 complexes were calculated by dividing the number of signals over the area of the tissue. The area of the tissue is expressed in pixels (N=10-11 each).

Figure 3. ASC speck formation in the choriodecidual leukocytes. (A) Flow cytometry gating strategy used to determine ASC speck formation in the choriodecidual leukocytes. THP-1 cells were untreated (negative control, B) or treated with lipopolysaccharide (LPS) plus nigericin (positive control, C). A representative image of ASC speck formation in the choriodecidual leukocytes isolated from women who underwent spontaneous labor at term (D). All experiments included isotype controls.

Figure 4. Flow cytometric quantification of ASC speck formation in the choriodecidual leukocytes. ASC speck formation in the choriodecidual leukocytes isolated from women who delivered at term without labor or underwent spontaneous labor at term (N=9-12 each).

VIDEO LEGENDS

Video 1. A 3-D reconstruction of ASC/CASP-1 complexes in the chorioamniotic membranes from women who delivered at term without labor. *In situ* proximity ligation assays for ASC and CASP-1 in the chorioamniotic membranes from women who delivered at term without labor. ASC/CASP-1 complexes are shown in red and the nuclei (DAPI) are shown in blue. 400× magnification.

Video 2. A 3-D reconstruction of ASC/CASP-1 complexes in the chorioamniotic membranes from women who underwent spontaneous labor at term. *In situ* proximity ligation assays for ASC and CASP-1 in the chorioamniotic membranes from women who

underwent spontaneous labor at term. ASC/CASP-1 complexes are shown in red and the nuclei (DAPI) are shown in blue. 400× magnification.

Video 3. A 3-D reconstruction of ASC/CASP-1 complexes in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis. *In situ* proximity ligation assays for ASC and CASP-1 in the chorioamniotic membranes from women who underwent spontaneous labor at term with acute histologic chorioamnionitis. ASC/CASP-1 complexes are shown in red and the nuclei (DAPI) are shown in blue. 400× magnification.

Table 1. Demographic and clinical characteristics of the study populations utilized for in situ proximity ligation assays

	TNL (n=10)	**TIL (n=10)**	**TIL-ACA (n=11)**	**p value**
Age (y; median [IQR])[a]	29.0 (25.5-30.8)	23.5 (19.3-31.0)	23.0 (21.0-26.5)	NS
Body mass index (kg/m^2; median [IQR])[a]	32.3 (29.4-36.6)	24.1 (22.5-31.0)	28.2 (23.9-33.6)	p=0.028
Gestational age at delivery (wk; median [IQR])[a]	39.3 (39.0-39.4)	39.3 (39.0-39.9)	39.1 (38.8-39.9)	NS
Birth weight (g; median [IQR])[a]	3705.0 (3272.5-3842.5)	3220.0 (3011.3-3304.0)	3270.0 (3072.5-3585.0)	NS
Race (n[%])[b]				
African-American	7 (70%)	10 (100%)	11 (100%)	NS
Caucasian	1 (10%)	0 (0%)	0 (0%)	
Other	2 (20%)	0 (0%)	0 (0%)	
Primiparity (n[%])[b]	0 (0%)	4 (40%)	1 (9.1%)	NS
C-section (n[%])[b]	10 (100%)	1 (10%)	2 (18.2%)	NS
Acute fetal inflammatory response (n[%])[b]				
No fetal inflammatory response	10 (100%)	10 (100%)	0 (0%)	p<0.001
Stage 1 (acute phlebitis/chorionic vasculitis)	0 (0%)	0 (0%)	3 (27.3%)	NS
Stage 2 (acute arteritis)	0 (0%)	0 (0%)	8 (72.7%)	NS
Stage 3 (necrotizing funisitis)	0 (0%)	0 (0%)	0 (0%)	NS

[a]Kruskal-Wallis test

[b]Fisher's exact test

IQR = interquartile range

Table 2. Demographic and clinical characteristics of the study populations utilized for the determination of ASC speck formation

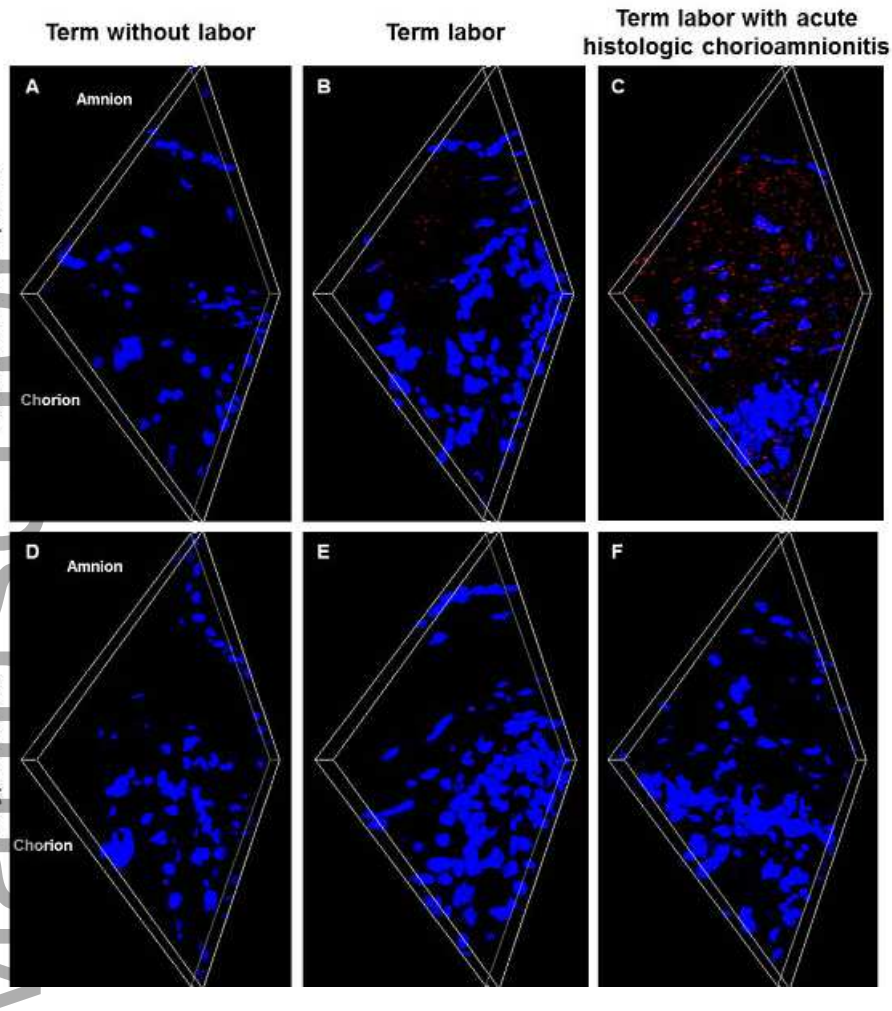
	TNL (n=12)	**TIL (n=9)**	**p value**
Age (y; median [IQR])[a]	28.5 (27.0-35.0)	24.0 (22.0-27.0)	p=0.032
Body mass index (kg/m^2; median [IQR])[a]	32.4 (29.3-45.4)	32.3 (26.6-37.0)	NS
Gestational age at delivery (wk; median [IQR])[a]	39.0 (38.8-39..9)	38.0 (38.0-39.0)	NS
Birth weight (g; median [IQR])[a]	3287.5 (2820.0-3438.8)	3140.0 (2950.0-3275.0)	NS
Race (n[%])[b]			NS
African-American	10 (83.3%)	7 (77.8%)	
Caucasian	2 (16.7%)	1 (11.1%)	
Hispanic	0 (0%)	1 (11.1%)	
Primiparity (n[%])[b]	0 (0%)	1 (11.1%)	NS
C-section (n[%])[b]	12 (100%)	0 (0%)	p<0.001

[a]Kruskal-Wallis test

[b]Fisher's exact test

IQR = interquartile range

None of the samples showed signs of a fetal inflammatory response



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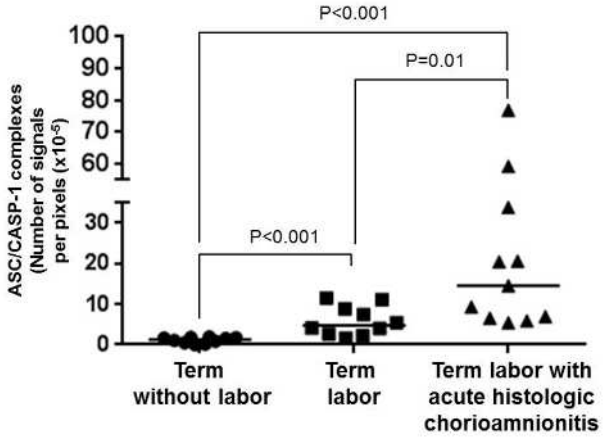

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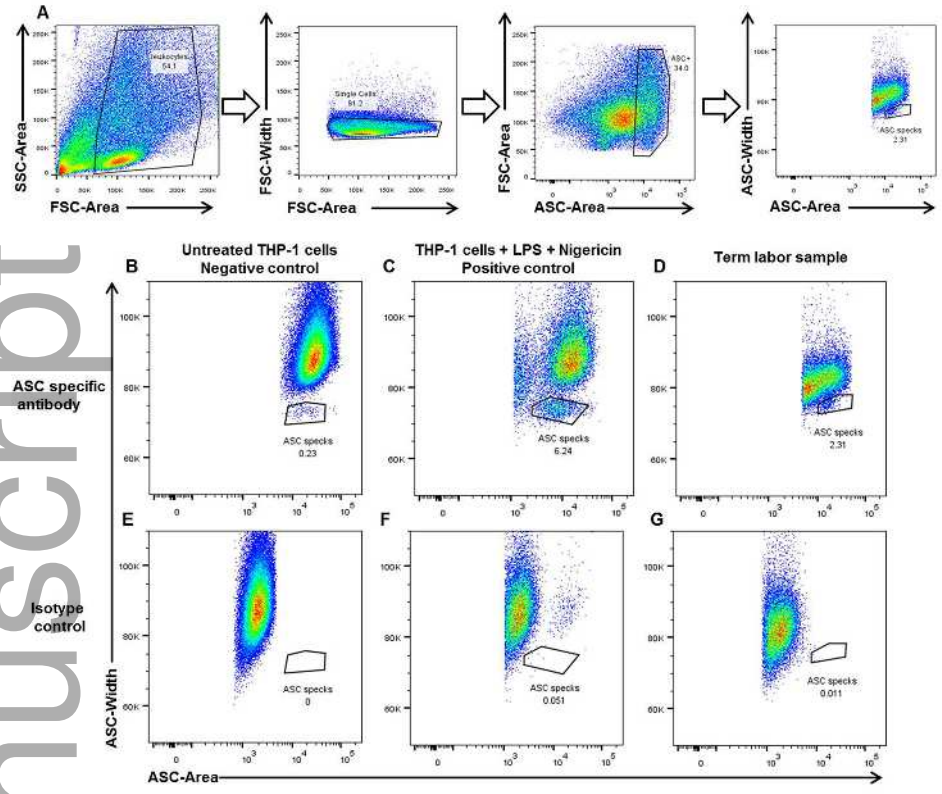

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