



Published in final edited form as:

*Am J Reprod Immunol.* 2023 January ; 89(1): e13642. doi:10.1111/aji.13642.

## Matrix metalloproteinases in preterm prelabor rupture of membranes in the setting of chorioamnionitis: A scoping review

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### Abstract

Fetal or gestational membranes extend from the placenta to enclose the fetus and amniotic fluid. While the membranes spontaneously rupture at term in normal pregnancies, they can rupture prematurely before the onset of labor, termed preterm prelabor rupture of membranes (PPROM). PPRM can be triggered by bacterial infection or sterile inflammation in the membranes, known as chorioamnionitis (CAM). The membranes derive their tensile strength from a collagen-rich extracellular matrix (ECM); as such, understanding the enzymes and processes that can degrade the membrane ECM are of paramount importance. Matrix metalloproteinases (MMPs) are a class of enzymes capable of degrading collagen and other components of the ECM, and can be induced by inflammation. We used a scoping review to address the question of how MMP activity is associated with PPRM, particularly their induction due to sterile or nonsterile CAM. We have found that the most studied MMPs in PPRM were MMPs 2, 8, and 9. Additionally, some MMPs are constitutively active, while others are induced by inflammation. Mechanistic studies of the pathways that induce MMP activation are sparse, and this area is ripe for future studies. Targeting MMP activation could be a future strategy to delay or prevent PPRM.

### Keywords

chorioamnionitis; matrix metalloproteinase; preterm prelabor rupture of membranes; scoping review

## 1 | INTRODUCTION

The developing fetus and amniotic fluid are contained within gestational (“fetal”) membranes that extend from the placenta. These membranes are a deceptively simple component of the maternal-fetal interface, comprised of cells from the maternal decidua (outermost layer), adjacent to the fetal-derived chorion (middle layer) and (innermost) amnion. This interface provides important protection of the fetus from external threats

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### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

such as infection. As the membranes expand to accommodate fetal growth, they derive tensile strength from a dense, collagen-rich extracellular matrix (ECM) within the amnion. In normal pregnancies, at term, the membranes spontaneously rupture, a process associated with the onset of labor. This process of membrane rupture is associated with changes in the expression of extracellular enzymes that breakdown the ECM, though details of how this process is initiated remain an area of active investigation. When membranes rupture too soon the consequences can be severe to the viability of the fetus.

Bacterial infection or sterile inflammation of the fetal membranes, referred to as chorioamnionitis (CAM), can trigger the preterm, prelabor rupture of membranes (PPROM), which is associated with neonatal mortality as well as long- and short-term neonatal morbidity. Of those morbidities, respiratory distress is the most common complication reported, but PPRM can also be associated with neonatal sepsis, intraventricular hemorrhage, necrotizing enterocolitis, neurodevelopmental impairment, and neonatal white matter damage.<sup>1</sup> PPRM is implicated in 30%–40% of episodes of preterm labor and/or preterm delivery.<sup>2</sup> Similar to normal membrane rupture at term, the fundamental mechanisms of PPRM are incompletely detailed. There are likely several mechanisms acting in concert, including cytokine activation, oxidative stress and apoptosis of various cell types in the gestational membrane.<sup>3</sup> Ultimately, the degradation of membrane ECM follows the activation of matrix metalloproteinases (MMPs),<sup>4</sup> even though the cellular and molecular processes triggering MMP activation are not entirely known. Given the strong association of acute bacterial CAM with PPRM, MMP activation during nonsterile CAM likely depends upon the pathogen-induced proinflammatory state. The purpose of this review is to summarize the current literature regarding the known MMPs implicated in membrane rupture, with an overview of their expression and or activity in the setting of acute sterile or nonsterile CAM, including proposed signaling pathways governing their expression and activity.

## 2 | METHODS

We performed a *scoping review* based on methods described by Arksey and O'Mally.<sup>5</sup> The central research question was “What is known from the existing literature about MMPs in the setting of PPRM in the setting of chorioamnionitis?” To address this question, we systematically searched for, and reviewed, studies with the following inclusion criteria: English language primary studies that provided some evaluation of MMPs in the setting of CAM and/or PPRM.

Our initial search was conducted using PubMed with the search phrase ((matrix metalloproteinases) AND (chorioamnionitis) AND (preterm premature rupture of membranes)). To ensure that we got papers that also featured sterile CAM, we also used the search query ((matrix metalloproteinases) AND (preterm premature rupture of membranes) AND (DAMP) or (sterile)), with the last search for new manuscripts performed on September 9, 2022. The search is summarized in Figure 1. We identified additional studies through references of the reviewed articles. Further review to include/exclude identified articles consisted of reading article abstracts. If an article appeared relevant, but

the inclusion criteria could not be established, the full article was reviewed to determine if inclusion criteria were satisfied.

We have structured the results first by a review of individual MMPs known to be associated with the fetal membranes, their levels at steady state, the classes of MMPs activated during CAM, then a brief synopsis of what general classes of molecules or phenomena are linked to MMP activation (including pathogens, DAMPS, chemokines, and oxides), and finally the presence of MMP-related therapies in clinical use.

### 3 | RESULTS

#### 3.1 | MMPs

The MMPs are zinc-dependent matrix degrading enzymes with an ability to degrade collagenous and non-collagenous components within the fetal membrane ECM connecting the amnion and chorion together.<sup>2</sup> Currently, 25 MMPs and tissue inhibitors of MMP (TIMPs) have been identified, differing in their substrate specificity as well as mode of regulation. TIMPs are inhibitors of MMPs, among other proteins. Many are capable of promiscuous binding and inhibition of multiple MMPs. Several MMPs are produced in a pro-form (zymogen) and require activation through proteolytic cleavage,<sup>6</sup> an important detail for interpreting studies on MMP mRNA expression and protein levels, as many ELISA kits for assessing MMP protein concentrations recognize both the pro- and active forms of MMPs. Processing of the pro-forms of MMPs can be activated by numerous enzymes, including other MMPs such as membrane-type MMPs and TIMPs themselves.<sup>6</sup> MMPs can assemble into a multi-subunit complex in the fetal membranes during labor<sup>7</sup> to help facilitate the activation of zymogens. Additionally, MMPs have been shown to cleave multiple substrates not limited to collagens,<sup>8</sup> including insulin-like growth factor binding protein-1 in the amniotic fluid,<sup>9</sup> suggesting they are capable of acting on other proteins and substrates outside of the fetal membranes.

While the fetal membranes express many MMPs,<sup>2</sup> intensive study of MMPs in the membranes includes only a selection of the MMP family, and we will detail key aspects of those here. MMP1, also known as collagenase-1, preferentially degrades types I, II, and III collagen. These fibrillar collagens are essential components of the ECM providing the tensile strength of fetal membranes. MMP1 can also degrade basement membrane-associated collagens.<sup>10</sup> It is present in the fetal membranes as well as the amniotic fluid.<sup>11</sup> MMP2, a gelatinase, has a unique mode of activation, which is activation by binding to (and processing by) the membrane-bound MMP cell surface receptor (MT1-MMP), and is inhibited by TIMP2<sup>12</sup> while also being processed by TIMP2 for activation in a complex with MT1-MMP. MMP3 degrades an extremely broad array of ECM substrates, including proteoglycans, glycoproteins, fibronectin, laminin, and types II, IV and V collagen.<sup>10</sup> Additionally, the gelatin produced by MMP1's degradation of fibrillar collagen is further processed by MMP3.<sup>10</sup> It is known to form a complex with MMP9, MMP2, TIMP1, and TIMP2 that is associated with parturition in the rat chorioallantoic membranes.<sup>7</sup> MMP8, also known as neutrophil collagenase, primarily degrades type I collagen, but also has the ability to degrade non-matrix bioactive substances<sup>13</sup> such as insulin-like growth factor binding protein1 (IGFBP1) in the amniotic fluid.<sup>14</sup> Its capacity to cleave substrates such

as chemokines, cytokines, and growth factors allows it to modify immune reactions.<sup>13</sup> MMP8 is synthesized as a proenzyme within polymorphonuclear leukocytes, and unlike other MMPs, is stored in these cells rather than produced and released on demand.<sup>13</sup> Active MMP8 can be released from leukocytes by reactive oxygen species or by proteases.<sup>15</sup> MMP9, also known as gelatinase B, is considered the major MMP responsible for collagenase activity within the membranes and has been found in the decidua, chorion and amnion in the inactive precursor form attached to TIMP1.<sup>16</sup> When activated, its main action is on Type IV collagen, the main constituent of the basement membrane of the amnion.<sup>16,17</sup> MMP13 has both collagenolytic and gelatinolytic activity and has been localized to the fetal membrane and amniotic fluid.<sup>18</sup>

### 3.2 | MMPs in gestational tissues at steady state

Some MMPs are expressed in the membranes in the absence of infection or other pathologies, although they may be further upregulated (or suppressed) by inflammation. MMP1 was found to be expressed with advancing gestational age,<sup>11</sup> as is the matrilysin MMP7.<sup>19</sup> MMP8, another collagenase, was found in amniotic fluid in labor only<sup>13</sup>; the gelatinase MMP9 was also present with advanced labor and, additionally, rupture of membranes.<sup>20</sup> Gelatinase MMP2 can be encountered in the fetal membranes and has been proposed to be a physiologic constituent of amniotic fluid,<sup>12</sup> consistent with the demonstration that MMP2 is the predominant gelatinase in extra-embryonic celomic fluid and amniotic fluid in the second and third trimester of pregnancy.<sup>21</sup> Fortunato, et al.<sup>22</sup> found that membranes from laboring and nonlaboring pregnancies showed presence of the MT1-MMP receptor mRNA and peptide in the membranes. In situ hybridization and immunohistochemistry showed expression of MT1-MMP in amnion and chorion cells. Additional studies by Fortunato, et al.<sup>16</sup> showed TIMPs 1 and 2 expression in term amniochorion, and TIMPs 1 and 3 expression in amniochorion explant culture up to 9 days post-isolation. The TIMP 1 and 2 mRNA was localized to chorionic cytotrophoblasts, especially those bordering the decidual stromal cells. Both Riley, et al.<sup>12</sup> and Maymon, et al.<sup>21</sup> reported an increase in TIMP2 concentrations with advancing gestational age, suggesting the increase in TIMP2 concentration and activity in the amniotic cavity may be protective against the increase in gelatinolytic activity due to uterine distention with advancing gestational age.<sup>12</sup> Experiments in rat chorioallantoid membranes showed expression of TIMPs 1 and 2 at 12 h pre-labor.<sup>7</sup> Additionally, though TIMP2 primarily inhibits MMP2, it also inhibits MMP9.<sup>21</sup> TIMP2 concentrations decrease significantly with spontaneous preterm and term labor and rupture of membranes, both term and preterm.<sup>23,24</sup> MMP3 is a physiologic constituent of the amniotic fluid with constant levels despite advancing gestational age.<sup>25</sup> Work by Fortunato and Menon 2002<sup>2</sup> referenced expression of MMPs 1, 2, 3, 9, 10, 11, 13, 14, and TIMPs 1–4 in the human fetal membranes, and found expression of then-novel MMPs 15, 18, 19, and 23 in the amniochorion in pPROM, in term laboring, and term non-laboring amniochorion. They found MMP17 and 24 in term labor only, and MMP25 in term non-laboring amniochorion only.

### 3.3 | MMPs identified in CAM or PPROM

**3.3.1 | Collagenases (MMPs 1, 8, 13)**—Activation of many of the collagenases increases greatly with CAM. Maymon, et al.<sup>11</sup> found increasing levels of total MMP1

protein in amniotic fluid via ELISA with advancing gestational age and rupture of membranes, as mentioned before; however the concentration was greatest in the amniotic fluid in the setting of intra-amniotic infection. In a study by Oner, et al.<sup>10</sup> evaluating MMP1 in the decidua from uncomplicated term, idiopathic preterm, and CAM-complicated deliveries, increases in mRNA and both total and active protein expression of this collagenase were observed in the setting of CAM. Immunostaining demonstrated overexpression of MMP1 localized primarily to the cytoplasm of decidual cells in the setting of infection. Interestingly, a single nucleotide polymorphism in the MMP1 promoter is sufficient to increase MMP1 expression during stimulation; this mutation has been found in human fetal membranes and is associated with PPROM.<sup>26</sup>

MMP8 it is increased in the amniotic fluid of pregnancies with PPROM and has been hypothesized to contribute to chronic lung disease upon entering the fetal lung.<sup>27</sup> Maymon, et al.<sup>13</sup> performed a cross sectional study where they determined the amniotic fluid total MMP8 concentrations in pregnant women of in midtrimester, term nonlaboring, term laboring, preterm laboring, rupture of membranes, and MIAC. Amniotic fluid levels were increased with parturition at term compared to term not in labor, in preterm labor with preterm delivery compared to preterm labor with delivery at term, and preterm rupture of membranes compared to rupture at term; however, levels were most elevated with MIAC, more specifically MIAC in addition to PPROM. They also noted higher levels in the forebag compartment in comparison to the upper compartment of the amniotic sac. Furthermore, they explored the correlation between leukocytes and MMP8 levels in the amniotic fluid and observed a significant correlation between the two, which is consistent with MMP8 (human neutrophil collagenase) being largely produced by neutrophils. Several other studies also noted consistent findings with the most significant elevations of amniotic fluid MMP8 in the setting of MIAC,<sup>28-30</sup> supporting its relationship to CAM-induced leukocyte recruitment.

MMP8 concentration correlates with the severity of histologically-defined CAM, with higher concentrations found with more severe histological chorioamnionitis (HCA).<sup>31</sup> Higher levels of total MMP8 protein were observed when amnionitis, the final stage of extra-placental chorioamniotic inflammation, is present than with chorionitis alone.<sup>32</sup> Interestingly, amniotic fluid total MMP8 concentration in the setting of acute-chorioamnionitis was found to decrease throughout preterm-gestation.<sup>33</sup> Park, et al. arrived at this conclusion after evaluating MMP8 concentrations obtained by amniocentesis in pregnancies affected by chorioamnionitis. The investigators concluded the decrease suggests the inflammatory milieu within the amniotic fluid decreases in acute CAM with increasing gestational age.<sup>33</sup> Oh, et al.<sup>34</sup> also found similar results observing higher intensity of intra-amniotic inflammatory response, documented as higher levels of amniotic fluid total MMP8 and leukocytes, with earlier timing of PPROM and CAM caused specifically by *Ureaplasma* spp.

Our search only returned with one result that investigated MMP13, which is also known as collagenase-3. Soydinc, et al.<sup>35</sup> determined that patients affected by PPROM had elevated levels of total MMP13 protein in their vaginal lavage and those with PPROM and additionally complicated by CAM had even more significant elevation in MMP13 levels.

**3.3.2 | Gelatinases (MMPs 2, 9)**—There is discrepancy about the involvement of MMP2 in PPRM. Maymon, et al.<sup>12</sup> did not observe changes in the immunoreactive forms of MMP2 concentrations (total MMP2 protein) in amniotic fluid with advancing gestational age, labor, preterm labor, and rupture of membranes. An increase in amniotic fluid total MMP2 concentration was observed in patients with MIAC and PPRM; however, not in patients with MIAC and intact membranes. This implies that something in addition to the inflammatory response to pathogen exposure induces changes in MMP2 concentration. Other studies have consistently indicated that pro-inflammatory cytokines or GBS infection do not increase the expression of active MMP2 or 9<sup>36</sup> and, in some cases, may down-regulate its expression and activity in cytotrophoblast.<sup>37-39</sup>

Studies performed by Zaga-Clavelina, et al.<sup>40</sup> in transwell devices made from human fetal membranes found an increase in pro-MMP2 and pro-MMP9 during *E. coli* stimulation of both the amnion and choriodecidual compartments, although MMP2 was only produced from the choriodecidia. However, there were no increases in the secretion of active MMP2 under any stimulation, although the active forms of other MMPs did increase within the tissue.<sup>40</sup> Total MMP2 production from the choriodecidia specifically was further shown by Sharma, et al.<sup>41</sup> upon treatment of whole, mounted membrane punches treated on one or both sides of the membranes with granulocyte macrophage colony stimulating factor (GM-CSF). However, Garcia-Lopez, et al.<sup>42</sup> found increases of active MMP2 within the tissue upon LPS exposure, in contrast to Zaga-Clavelina.<sup>40</sup> This contrast may be attributed to differences in immune responses directed towards LPS versus a live gram-negative bacteria (*E. coli*). In support of MMP2 activation during CAM, TIMP2 concentrations decreased significantly with documented intraamniotic infection.<sup>23,24</sup>

Intra-amniotic infection with Group B *Streptococcus* (GBS) and *Ureaplasma spp.* both result in elevated levels of total and active MMP9 in the amniotic fluid.<sup>36,43</sup> In a study of women with PPRM, only those with positive amniotic fluid culture had elevated total MMP9 in the amniotic fluid, suggesting that MMP9 expression is linked to infection.<sup>44</sup> Further, in nonhuman primate studies of GBS, total MMP9 expression in amniotic fluid was induced by intra-amniotic infection while other MMPs were relatively constant.<sup>36</sup> Fortunato, et al.<sup>16</sup> compared expression of MMP9 in the amniochorionic membranes (fetal membranes stripped of decidua, clots, and fetal byproducts<sup>45</sup>) collected from women with CAM and from women with uncomplicated pregnancies, not in labor, delivered via elective cesarean section. MMP9 mRNA expression was seen in membranes from pregnancies affected by intraamniotic infection but not in membranes obtained from uncomplicated pregnancies delivered by cesarean section.<sup>16</sup> Within the amniotic fluid, total MMP9 was present with MIAC, advanced labor, and rupture of membranes.<sup>20</sup> Its inhibitor, TIMP1 has been noted to increase in the setting of infection.<sup>23</sup> In support of this, Athayade, et al.<sup>46</sup> showed that while TIMP1 was not induced upon MMP9 induction in amniotic fluid of women with PPRM with no MIAC, patients with MIAC did have increased TIMP1 concentrations, regardless of membrane status.

In transwell devices comprised of human fetal membranes treated with live *E. coli*, Zaga-Clavelina, et al.<sup>40</sup> found increases of pro-MMP9 from both choriodecidual and amniotic compartments, but no increase in secretion of active MMP9. However, tissue concentrations

of active MMP9 were increased dramatically, suggesting that looking only at secreted MMPs may not present the whole picture of MMP activity. This was also shown by Garcia-Lopez, et al.<sup>42</sup> wherein LPS exposure resulted in increased secretion of pro-MMP9 from the choriodecidua, no increase in secreted active MMP9, but greatly increased active MMP9 in association with the collagen IV-rich regions of the membrane (i.e. MMP9 still in the tissue and not secreted). In other studies, total and active MMP9 secretion from the choriodecidua (relative to the amnion) was further induced by GM-CSF<sup>41</sup> and by *U. parvum*.<sup>47</sup> Discrepancies between these studies can be attributed to quantification of secreted versus tissue-associated forms of the MMPs and by type of stimulation: bacteria, PAMP, and cytokine.

**3.3.3 | Stromelysins (MMPs 3, 10)**—MMP3's unique ability to activate secreted zymogenic forms of other MMPs, such as pro-MMP1 and pro-MMP9 suggests its important role in the proteolytic cascade of ECM degradation consequently leading to PPROM.<sup>10</sup> A cross-sectional study<sup>25</sup> included pregnant women who had an amniocentesis performed in the following categories: (1) in midtrimester (15–17 weeks) with a subsequent fetal loss after amniocentesis (2) preterm labor with intact membranes with term and preterm deliveries with and without a positive amniotic fluid culture (3) PPROM with and without MIAC (4) term without MIAC in and not-in-labor, with and without rupture of membranes regardless of membranes status. There was no increase observed in total MMP3 with just rupture of membranes (term or preterm). Increase in total MMP3 was most significant with MIAC with insignificant differences depending on membrane status. In their experiments, Oner, et al.,<sup>10</sup> observed upregulation of MMP3 expression in decidual cells in response to infection. Later, total MMP3 expression was increased in the amniotic cavity, in addition to the increase of MMP1 as stated earlier.

In studies performed on the placentas of pregnant rats exposed to GBS with evidence of CAM (as determined by neutrophil infiltration), MMP10 mRNA was upregulated in whole placenta in response to infection.<sup>48</sup> Protein localization studies then revealed MMP10 expression in the labyrinth of the placenta at 48 h (but not at earlier time points); MMP10 levels did not penetrate to the fetal circulation. These studies confined to the placenta and not including the fetal membranes suggest that MMP activation in placenta itself may contribute to the pathology associated with CAM.

**3.3.4 | Matrilysins (MMP 7)**—Our search returned with the one result for matrilysin, also known as MMP7. This MMP degrades fibronectin and proteoglycans. Similar to MMP2, amniotic fluid levels increase with advancing gestational age.<sup>19</sup> A study by Maymon, et al.<sup>19</sup> examined the amniotic fluid concentration of total MMP7 in preterm and term women under the following circumstances: labor and nonlaboring; with and without rupture of membranes; and with and without MIAC. They also included uncomplicated pregnancies at term not in labor. The concentration was increased in the setting of preterm labor with MIAC or PPROM with MIAC but was not found to be significantly elevated in any other setting, suggesting some specificity for MMP7 expression in CAM.

### 3.4 | Factors that influence MMP release

Our search query resulted in very few mechanistic papers detailing the cellular pathways implicated in MMP release. While the TIMPs inhibit the active form of the enzymes, inhibition of MMP release is affected by many factors.

**3.4.1 | Pathogens and PAMPs**—Multiple studies have demonstrated that bacterial infection induces MMP release from membranes; many of which are the gelatinases. For example, in Potts et al., the authors used a transwell system with the amnion facing the inside of a chamber and the choriondecidua facing outwards towards the surrounding well, with either the amnion or the choriondecidua receiving stimulation with *U. parvum*, a bacterium lacking a cell wall.<sup>47</sup> Infection induced secretion of the gelatinases MMP 2 and 9 (total protein), along with IL-8, primarily from the choriondecidual compartment and less so from the amnion.<sup>47</sup> Zymography confirmed the active forms of the MMPs. The activation of both gelatinases in response to infection, instead of the inducible MMP9 only, requires further investigation to tease apart what stimuli induce both gelatinases versus only one.

Feng, et al.<sup>49</sup> proposed that progesterone mediated through progesterone receptor membrane component 1 (PGRMC1) attenuates the inflammatory responses induced by *U. parvum* in amnion and chorion cells. Cells were transfected with PGRMC1 siRNA with and without treatment with progesterone and then infected with *U. parvum*. Results concluded that PGRMC1 and progesterone appeared to attenuate *U. parvum*-induced MMP9 mRNA in chorion cells and MMP9 activity in amnion cells. Similar experiments conducted with the bacterium GBS also showed the choriondecidua as the main source of total and active MMP2 and 9 rather than the amnion<sup>47,50</sup>; there was no difference in the levels of TIMPs 1 or 2.

Stimulation of transwells made with human membranes with gram negative bacterium *E. coli* applied to one or the other side also resulted in MMP2 and 9 release.<sup>40</sup> Experiments by Zaga-Clavellina, *et al.* examined both active and inactive forms of MMPs. With exposure to *E. coli*, secretion of the zymogen forms of both MMPs increased; however, unlike experiments with GBS and *U. parvum*, in *E. coli* studies the majority of pro-MMP2 and pro-MMP9 were induced from the choriondecidua,<sup>42</sup> suggesting differences in gestational membrane responses to distinct types of bacteria. There was no effect of *E. coli* stimulation on TIMPs 1 or 2. Pro-MMP2 induction from the amnion was not significantly increased during any conditions but pro-MMP9 was significantly induced from amnion when both sides of the membrane were stimulated with *E. coli*, suggesting that communication between the two regions is necessary for MMP9 induction. However, in contrast to the studies with *Ureaplasma* and GBS where MMP9 release was greater from the choriondecidua, total and active MMP9 release was greater from the amnion in response to *E. coli*. This could reflect a difference in stimulation by distinct species (GBS versus *E. coli*), or potentially by gram positive (i.e. GBS) versus gram negative (i.e. *E. coli*) bacteria. Of note, while there were no increases in secreted active forms of MMP2 or 9, active MMP9 was increased significantly in lysates prepared from the tissue itself, suggesting that much of the active form of these enzymes may be tissue-associated. They also completed this experiment with *Candida albicans* and noted increased total and active MMP9 release from mainly the choriondecidua side.<sup>51</sup>

Fortunato et al.<sup>16</sup> cultured membrane punches in media with bacterial LPS or peptidoglycan polysaccharide to the entire membrane that each stimulate inflammation via activation of  $\text{NF-}\kappa\text{B}$ . Both treatments induced the expression of MMP9, but not MMP2, from cultured membrane supernatants through zymography assays indicating that the MMP9 is both present and active. LPS induced both MMP2 and MMP9 activity in membrane punch explant cultures in experiments published by Lappas et al.<sup>52</sup> Lappas et al.<sup>52</sup> investigated natural and synthetic PPAR  $\gamma$  ligands given their ability to regulate LPS-stimulated pro-inflammatory cytokine release from human gestational tissues. They observed inhibition of total and active MMP9 release from human amnion cells with 15d-PGJ(2) and troglitazone treatment. Later studies<sup>42</sup> found increases in pro-MMP9 and pro-MMP2 secreted specifically from the choriodecidia in a transwell setup made from fetal membranes where one or both sides was stimulated. Amnion did not make appreciable amounts of MMPs in response to LPS stimulation. However, this study found no differences in the amount of active MMP2 or MMP9 in secreted media, despite having significant structural degradation in the tissue itself. To address this, Garcia-Lopez et al.<sup>42</sup> performed zymography on tissue extracts themselves treated with LPS on the amnion side and found that the active forms of the MMPs were significantly higher in LPS-treated sections. In these experiments, there were no change to TIMPs 1, 2, or 4. In contrast, Buhimschi et al.<sup>53</sup> found that in cultured membranes, LPS treatment alone induced neither MMP9 or MMP2 activity, also via zymography from cultured membrane punch supernatants where the whole punch was exposed to treatment. The reasons for discrepancy between these studies is unclear, as both Buhimschi et al.<sup>53</sup> Fortunato et al.<sup>16</sup> and Lappas et al.<sup>52</sup> used cultured media from fetal membrane punches from nonlaboring cesarean sections, although some experiments used the transwell setup, while others used the whole membrane punch method.

**3.4.2 | Damage-associated molecular patterns (DAMPs)**—Treatment with Serum amyloid A1 (SAA1), which is a DAMP induced early in response to tissue damage and inflammation, induced expression of MMPs 1, 8, and 13 in primary human amnion fibroblasts and whole amnion punches (treated on all sides with SAA1) at the mRNA, intracellular cellular protein, and secreted protein levels.<sup>54</sup> This was shown to be dependent upon TLR4 signaling (with little to no involvement of TLR2) through the  $\text{NF-}\kappa\text{B}$ , p38MAPK, JNK, and ERK1/2 pathways, with the exception that MMP13 expression did not require ERK1/2 signaling.<sup>54</sup> In follow up studies, Wang et al.<sup>55</sup> also found that MMP2 and 9 mRNA, total protein, and activity were induced by SAA1 in human amnion fibroblasts, although collagen degradation was affected both extracellularly by MMP activation and intracellularly through the induction of autophagy pathways, also by SAA1. MMP2 and 9 induction was shown to be dependent upon  $\text{NF-}\kappa\text{B}$ , as shown with MMPs 1, 3, 8, and 13 previously, and upon either TLR2 or 4 induction, but further specificity of receptor involved was not studied here. The group also found a positive correlation between SAA1 and MMP9, but not MMP2, total protein levels in amnion tissue of term laboring pregnancies but not term non-laboring, suggesting an involvement for SAA1 in parturition.

Fibronectin, a large protein capable of binding to many cell surface receptors and proteoglycans, along with itself, is found in ECM, including within the fetal membranes, termed fetal fibronectin (fFN). fFN in cervicovaginal fluid has been used as a biomarker of

preterm labor, and studies from Mogami et al.<sup>56</sup> showed that it can bind to TLR4 and act as a DAMP. Through this activation of TLR4 and the NF $\kappa$ B and ERK1/2 signaling, fFN was able to activate amniotic mesenchymal cell MMP1, 2, and 9 mRNA, and moreover using one domain of fFN, the EDA domain. fFN did not alter TIMP1 or 2 levels.

The alarmin S100A12 was recently investigated by Motomura et al.,<sup>57</sup> as it was previously found to be increased in women with preterm labor and sterile intra-amniotic inflammation.<sup>58</sup> Treatment of whole membrane punches on all sides with S100A12 increased MMP2 but not MMP9 activity, an interesting finding given that MMP2 is often the constitutive and MMP9 the inducible gelatinase.

Recent experiments from Saito Reis et al.<sup>59</sup> showed that the DAMP cell-free fetal DNA (cffDNA) applied to the amnion side of fetal membrane transwell-mounted explants induced pro-MMP2 secretion from the decidual side, slight but nonsignificant secretion from the apical side, and slight but nonsignificant activity of pro-MMP9 from either side. Interestingly, cffDNA treatment of membranes from pregnancies with male offspring induced higher levels of total MMP2 and 9 than pregnancies with female offspring, but only from cffDNA that was sonicated, not whole (resulting in fragments 100–1000 bp, rather than >1000 bp from whole cffDNA).

The DAMP HMGB1 has been associated with chorioamnionitis from many studies,<sup>60–64</sup> although studies specifically linking it to MMP expression are sparse. Playzo et al. did show that HMGB1 induced the mRNA and active form of MMP9 in supernatant from whole gestational membrane punches treated on all sides with HMGB1.<sup>65</sup>

**3.4.3 | Cytokines**—Pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) induce the production of cytokines from both structural cells and immune cells alike. In turn, these cytokines (of note, GM-CSF, IL-1 $\beta$ , TNF $\alpha$ , IL-10) induce protease expression from many cell types, even independent of the PAMP or DAMP themselves. As reviewed in Bowen et al., multiple cytokines directly induce MMP9 total protein secretion from first trimester trophoblasts, including TNF $\alpha$ , IL-1 $\beta$ , IL-6, and M-CSF,<sup>66</sup> while TGF $\beta$  treatment decreases this MMP9 secretion. TNF $\alpha$  is also shown to induce production of MMP1 and 3,<sup>67</sup> while IL-1 $\alpha$  increases MMP1 mRNA expression by cultured chorion cells.<sup>68</sup> Additionally, TNF $\alpha$  stimulation was sufficient to suppress TIMP expression in confluent chorionic cells.<sup>67</sup>

Sharma et al.<sup>41</sup> showed that treatment of the choriodecidua with GM-CSF induced active MMPs 2, 9, and 10, while decreasing TIMPs 1, 2, and 3. This regulation by GM-CSF was inhibited by stimulation with alpha-lipoic acid. Fetal membrane weakening occurred during GM-CSF treatment, but was rescued by alpha-lipoic acid treatment as assessed by an in vitro human explant model. This group previously showed that GM-CSF blockade inhibited fetal membrane weakening.<sup>69</sup>

Intra-amniotic infusion of IL-1 $\beta$  or TNF $\alpha$  in rhesus monkeys was associated with elevated amniotic fluid total MMP levels (specifically MMP9 levels).<sup>70</sup> In an in-vitro model, Oner et al.<sup>10</sup> found that IL-1 $\beta$  or TNF $\alpha$  induced total and active MMP1 and 3 secretion

from decidual cells. They then investigated mechanisms of inhibition of these activation pathways. In decidual cells treated with estrogen, the addition of TNF $\alpha$  or IL-1 $\beta$  enhanced MMP1 and 3 secretion. The addition of a progesterone analog, medroxyprogesterone acetate, to any of the combination of other stimuli suppressed much of the MMP induction, as did the addition of a MAPK pathway inhibitor, the compound SB203580. Identification of the MAPK pathway in decidual cell MMP activation, and the suppression of MMP secretion upon MAPK inhibition in decidual cells, suggests that administration of MAPK inhibitors could prevent premature delivery.

Another cytokine, activin-A, a pleiotropic mediator from the TGF $\beta$  superfamily involved in connective tissue remodeling, increases total MMP1 and 9 released by amniochorion.<sup>71</sup> MIAC induced expression of activin-A found in the amniotic fluid. Follistatin, a natural inhibitor of activin-A, effectively blunted the increase in amniotic fluid MMPs after activin-A treatment but not after LPS challenge, suggesting that LPS likely activates other pathways that modulate MMPs.<sup>71</sup>

In addition to cytokines that activate MMPs, a few studies have found cytokines that dampen MMP expression. Fortunato et al.<sup>72</sup> costimulated membranes with LPS and IL-10; the increase in mRNA and total protein of MMP2 and 9 from the amniochorion as described above was inhibited with the addition of IL-10. Izumi-Yoneda et al.<sup>73</sup> found oncostatin M, a member of the IL-6 cytokines, to be another modulator of MMP9, reducing total expression from amniotic epithelial cells. These findings suggest potential mechanisms of intervention to prevent rupture of membranes by gelatinases during CAM.

Further to cytokine-induced suppression of gelatinases, A20 is a nuclear factor-kappa beta (NF $\kappa$  $\beta$ ) responsive gene that acts as a negative regulator of NF $\kappa$  $\beta$ -induced inflammatory mediators. Amnion and myometrial cells were transfected with A20 siRNA. Transfected and control cells were exposed to pro-inflammatory mediators.<sup>74</sup> Transfected cells were noted to have augmented expression and/or secretion of pro-inflammatory cytokines, chemokines, adhesion molecules, contraction-associated proteins, total and active MMP9, and activation of NF $\kappa$  $\beta$ . Another study inhibiting downstream regulatory element antagonist modulator (DREAM), which regulates NF $\kappa$  $\beta$ , also utilized siRNA.<sup>75</sup> Silencing of DREAM in primary isolated myometrial or amnion cells results in significant decrease in expression of MMP9 mRNA expression and activity, along with other proinflammatory regulators. Thus DREAM and A20 may be other targets to prevent CAM-associated rupture of membranes.

**3.4.4 | Oxides**—In unique experiment on membranes collected from uncomplicated pregnancies delivered via cesarean section by Buhimschi et al.,<sup>53</sup> total and active MMP9 was induced directly by superoxide anions, which are a common product of macrophages and neutrophils, applied to culture medium of free-floating membrane punches. Additionally, these membranes were treated with N-acetylcysteine, which resulted in inhibition of intrinsic superoxide generation within the tissue as well as reduced basal activity of both MMP2 and 9.

### 3.5 | Clinical use

Several studies have described the rapid MMP8 bedside test as a method to assess for risk of preterm delivery.<sup>76–80</sup> In the study by Kim et al.,<sup>77</sup> the test was used on amniotic fluid obtained via transabdominal amniocentesis. A positive test was associated with a higher rate of intraamniotic infection/inflammation and adverse outcomes. Adverse outcomes included shorter interval to delivery, high rate of preterm delivery, histologic CAM, funisitis, low Apgar scores, and significant neonatal morbidity. Neonatal morbidity included neonatal sepsis, the presence of meningitis, urinary tract infection, pneumonia, respiratory distress syndrome, intraventricular hemorrhage. A positive test had a sensitivity of 90% and a specificity of 80%, a positive predictive value of 77% and a negative predictive value of 92% in the identification of intraamniotic infection/inflammation.

Chaemsaitong et al.<sup>76</sup> observed that the rapid MMP8 bedside test had better specificity for the detection of intra-amniotic infection than the rapid IL-6 assay. It has also been noted that patients who were not diagnosed with intra-amniotic infection or inflammation by standard cultivation technique or amniotic fluid WBC count, but had a positive MMP8 rapid test delivered preterm and had acute histologic CAM.<sup>76,81</sup>

The ability of protein microarrays to detect multiple biomarkers, which included MMPs, simultaneously in maternal serum for early diagnosis of intrauterine inflammation has been investigated.<sup>82,83</sup> Maternal plasma levels of MMP8 and 9 are elevated in the setting of HCA.<sup>84</sup> Park et al. (2019) suggested assessing these MMPs and other biomarkers known to be elevated in the setting of histologically-defined CAM in addition to conventional clinical factors to improve accurate diagnosis of HCA. Maternal plasma MMP9 as a solo measure for predicting intra-amniotic infection has proved to be ineffective.<sup>85</sup>

Given that MMP9 is induced in the amniotic fluid with intraamniotic infection, several investigators have assessed the potential role of using amniotic fluid levels of MMP9 in predicting intraamniotic infection in patients presenting with preterm labor and intact membranes.<sup>44,86,87</sup> Its use was found to have a sensitivity of 77–83%, specificity of 95%–100%, positive predictive value of 71%–100% and negative predictive value of 90%–97%.<sup>44,87</sup> Lastly, Soydisc et al.<sup>35</sup> investigated vaginal washing fluid levels of MMP13. They found it to be an important predictor for chorioamnionitis with a sensitivity of 93.33% and specificity of 65.52%.

## 4 | SUMMARY

We performed a systematic scoping review to address the fundamental question of “What is known from the existing literature about MMPs in the setting of PPRM in the setting of CAM?” As detailed in our findings above, bacterial infection or sterile inflammation in the gestational membranes can cause preterm prelabor rupture of membranes; this may be due in large part to various MMPs, which are induced by both pathogens and cytokines. The MMPs most studied in their association with pregnancy and PPRM are MMP2, MMP8, and MMP9; information on other MMPs such as MMP7 and 13 are limited. While some MMPs appear to have consistent levels throughout gestation (MMP3), others appear to be induced by infection (MMP2, MMP8, MMP9) or cytokines (MMP9). We have detailed expression,

induction, and distribution (where available) in Figure 2. In assessing MMP levels, care must be taken to investigate both secreted and tissue-associated MMPs, as secreted levels may not reflect the amount of active MMPs in the tissue itself. Mechanistic studies detailing the pathways through which the MMPs are produced are sparse, and while several studies have documented the localization of MMPs within specific layers of the membranes, the cell types responsible for MMP production often remain unclear.

#### 4.1 | Areas for future research

As this review has shown, there are many different factors which can modulate the activity of MMPs. Further studies are needed to understand the precise cellular pathways leading to MMP release and activation in the membranes. The identification of these pathways will elucidate possible manners in which to inhibit their activation and action on the ECM of the membranes.

## ACKNOWLEDGEMENTS

Funding for this work came from NIH grants 1F32HD100087-01A1 and R01HD102752 (AJE), and U01TR002398, R01AI134036, and the March of Dimes (D.M.A.). L.M.N. and D.M.A. came up with the scope of the manuscript. L.M.N. and A.J.E. performed the literature searches. LMN and AJE wrote the manuscript. D.M.A., A.J.E., and L.M.N. edited and revised the manuscript. A.J.E. would like to acknowledge Nadia Berlin for their assistance maintaining focus.

## DATA AVAILABILITY STATEMENT

The author has provided the required Data Availability Statement, and if applicable, included functional and accurate links to said data therein.

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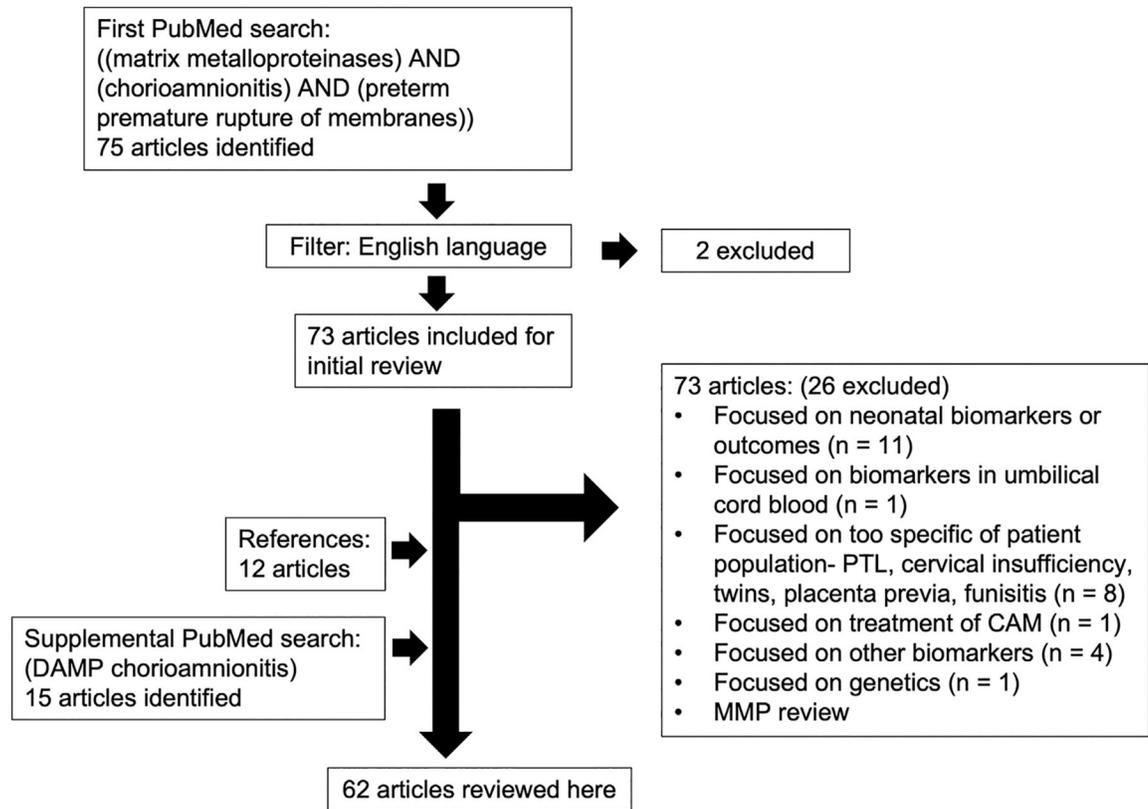
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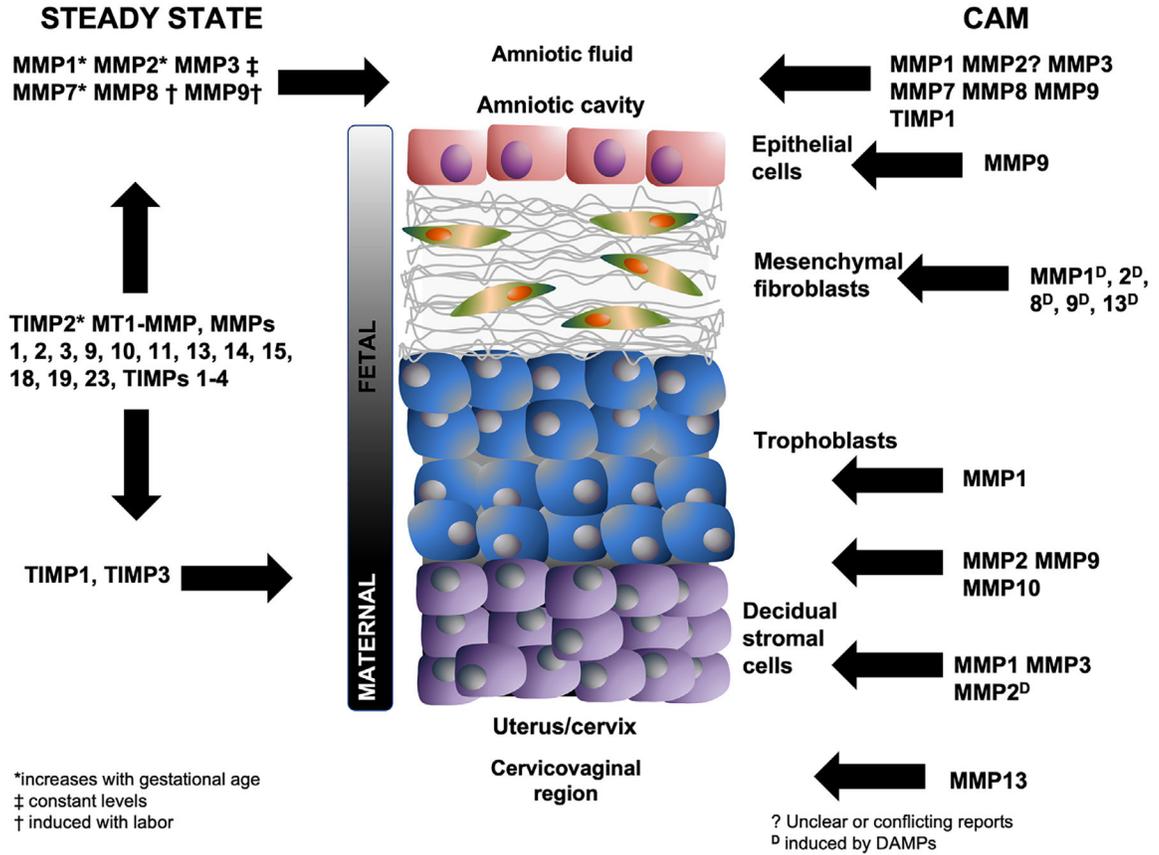
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**FIGURE 1.**  
Search and review flow



**FIGURE 2.** MMP and TIMP distribution throughout gestational fluids and tissues in nonpathological tissues and in tissues complicated by CAM. The major regions of the fetal membranes, from the amniotic fluid/cavity through the cervicovaginal region, are labeled. To the left of the diagram are the MMPs expressed at steady state. MMPs are followed by symbols as appropriate: \*increases with gestational age, ‡ constant levels, † induced with labor. Locations of MMP or TIMP localization are indicated by arrows towards the appropriate section of the gestational membranes or fluids. Arrows extending up and down signify expression found either throughout the membranes or which were localized to the membranes but to no particular region. To the right of the diagram are the MMPs expressed during CAM. Locations of MMP or TIMP localization are indicated by arrows towards the appropriate section of the gestational membranes or fluids. MMPs with arrows pointing at border regions between layers are expressed in both types of cells or in cells specifically at the border region. MMPs marked with ? are the subjects of unclear or conflicting reports, while those followed by a superscript D (<sup>D</sup>) are induced by DAMPs