


# Progesterone Inhibits Apoptosis in Fetal Membranes by Altering Expression of Both Pro- and Antiapoptotic Proteins

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## Abstract

**Objective:** Progesterone supplementation prevents preterm birth (PTB) in some high-risk women, but its mechanism of action is unknown. One-third of PTB is associated with preterm premature rupture of membranes (PPROMs). We have previously shown that progesterone inhibits basal and Tumor Necrosis Factor (TNF)  $\alpha$ -induced apoptosis in an explant model of human fetal membranes. This study investigates the molecular mechanisms responsible for progesterone-mediated inhibition of apoptosis in fetal membranes. **Methods:** Human fetal membranes were collected at elective cesarean at term (no labor, no infection;  $n = 6$ ), washed, and pretreated with/without progesterone (125 ng/mL) for 24 hours. Thereafter, membranes were treated with/without TNF $\alpha$  (50 ng/mL) and/or progesterone for 48 hours, harvested, and homogenized. Apoptosis was determined by evaluating caspase-3, -8, and -9 activities. Expression of pro- BH3 interacting domain death against, Bcl-2 associated X protein (BID, BAX) and antiapoptotic proteins (X-linked inhibitor of apoptosis protein [XIAP], Bcl-2, FLICE inhibitory protein [FLIP]) were measured by Western blot. **Results:** TNF $\alpha$  increased apoptosis (measured by caspase-3, -8, and -9 activities) in fetal membranes, and this effect was abrogated by progesterone. Under basal conditions, progesterone suppressed expression of the proapoptotic protein, BID, by 0.45 (0.14)-fold, and increased expression of the antiapoptotic proteins, Bcl-2 and XIAP; no change was seen in BAX or FLIP. In contrast, TNF $\alpha$  increased BID expression by 5.15 (2.92)-fold, which was prevented by pretreatment with progesterone. **Conclusions:** Progesterone inhibits apoptosis in fetal membranes by suppressing expression of the proapoptotic protein, BID (for both basal and TNF $\alpha$ -induced apoptosis), and upregulating expression of the antiapoptotic proteins, XIAP and Bcl-2 (under basal conditions only). These data provide a mechanism by which progesterone supplementation may prevent PPRM and PTB in some women at high risk.

## Keywords

apoptosis, progesterone, fetal membranes, PPRM, preterm birth

## Introduction

Preterm birth (PTB) complicates around 10% of all live births in the United States<sup>1</sup> and is a major cause of perinatal morbidity and mortality.<sup>2,3</sup> One-third of PTB is associated with preterm premature rupture of membranes (PPROMs),<sup>2,3</sup> defined as rupture of the membranes prior to 37 weeks' gestation. Preterm premature rupture of membranes is a serious obstetric complication that occurs in up to 3% to 4% of all pregnancies and has a high recurrence risk.<sup>4,5</sup> The fetal membranes act as a structural and functional barrier to ascending infection/inflammation. Once ruptured, the membranes rarely reseal and efforts to repair the membranes have thus far been unsuccessful. A better understanding of the molecular mechanisms underlying PPRM may allow for the development of interventions to prevent this complication and subsequent PPRM-related PTB.

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Although the precise etiology of PPRM cannot be established in most cases, accumulating evidence suggests that the primary pathways leading to weakening of the fetal membranes include intrauterine inflammation (with cytokine, matrix metalloproteinase, and prostaglandin activation), oxidative stress, and programmed cell death (apoptosis).<sup>4,6-12</sup> These biological processes can be triggered by multiple factors including intrauterine infection, decidual bleeding (placental abruption), and uterine overdistention.

Clinical trials have shown that progesterone supplementation can prevent PTB in some women at high risk by virtue of a prior unexplained spontaneous PTB<sup>13,14</sup> or a short cervix.<sup>15</sup> In February 2011, the food drug administration (FDA) approved the use of progesterone supplementation for the prevention of recurrent PTB in women at high risk due to a prior PTB,<sup>16</sup> although its mechanism of action is not known. We hypothesize that progesterone supplementation may prevent PTB, in part, by preventing PPRM. To this end, we have previously shown that progesterone inhibits basal and TNF $\alpha$ -induced apoptosis in fetal membranes.<sup>17</sup> This study investigates the molecular mechanisms responsible for this progesterone-mediated inhibition of apoptosis in the fetal membranes.

## Materials and Methods

### Tissue Source and Culture

Human fetal membranes were collected and cultured as previously described.<sup>17,18</sup> Briefly, fetal membranes were collected from women with uncomplicated pregnancies who had an elective repeat cesarean at term prior to the onset of labor ( $n = 6$ ). Samples were excluded if the women had any medical or obstetric complications or had received any medications in the preceding 24 hours. Infection was excluded using standard clinical criteria (absence of maternal fever, fundal tenderness, maternal and/or fetal tachycardia, foul vaginal discharge) and laboratory test (absence of elevated white cell count). Membranes were harvested under sterile conditions within 30 minutes of delivery, placed in sterile PBS containing 100 U/mL heparin, 100 U/mL penicillin, 100  $\mu$ g/mL streptomycin, and 0.25  $\mu$ g/mL fungizone, and transported to the laboratory. Membranes were washed 3 times with Hank balanced salt solution and adherent blood clots and decidual tissues removed. Membrane discs were cut using a 5-mm biopsy punch (Premier Medical Products, King of Prussia, Pennsylvania) and placed in 0.4- $\mu$ m cell culture inserts (Becton Dickinson Labware, Franklin Lakes, New Jersey) which, in turn, were placed in 24-well tissue culture plates (Becton Dickinson Labware). Membrane discs were cultured in Dulbecco Modified Eagle Medium (DMEM) with Ham F-12 nutrient mixture (1:1; Invitrogen, New York, New York) containing 15% heat inactivated fetal bovine serum and antibiotics, as above. In all, 0.5 mL DMEM/Ham's F-12 medium was placed in each of the culture inserts and an equal volume in each of the culture well. All cultures were carried out at 37°C in an atmosphere containing 95% air/5% CO<sub>2</sub>.

### In Vitro Treatment Conditions

After culture in DMEM/Ham's F-12 medium for 48 hours, membrane discs were washed twice with Opti-MEM medium (Invitrogen, Carlsbad, California) and continued in fresh Opti-MEM without antibiotics for subsequent stimulation studies. Based on prior dose- and time-course experiments,<sup>17</sup> membrane discs were pretreated with/without progesterone (125 ng/mL [Yale-New Haven Hospital Pharmacy, New Haven, Connecticut]) for 24 hours. Thereafter, medium was changed to fresh Opti-MEM (no antibiotics) containing progesterone alone or progesterone plus human recombinant TNF $\alpha$  (50 ng/mL [R&D Systems, Minneapolis, Minnesota]) for 48 hours. After stimulation, membrane discs and conditioned media were harvested, snap-frozen, and stored at -80°C until further analysis. Experiments were carried out in triplicate and repeated a minimum of 3 times.

### Protein Extraction and Caspase Activity Measurements

Total protein from amniochorionic tissues were isolated by homogenization with 20 mM Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.1% SDS, and protease inhibitors (Roche, Indianapolis, Indiana). Homogenization was followed by centrifugation at 12 000 rpm to precipitate tissue debris. Protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Benicia, California). Caspase activity was determined using the Caspase-Glo assay (Promega, Madison, Wisconsin) as previously described.<sup>17,19</sup> Briefly, 10  $\mu$ g of the tissue lysates were incubated in the dark at room temperature with either caspase-3, -8, or -9 specific substrates. Following 1-hour incubation, luminescence was measured using a TD-20/20 luminometer (Turner Designs, Sunnyvale, California) and specific caspase activity reported in relative light units (RLU). All samples were assayed in triplicate.

### Western Blot

Explants of human fetal membranes were isolated, stimulated, and total protein extracted and quantified as described. Western blot analysis was conducted using standard protocols as previously described.<sup>20</sup> Briefly, equal amounts of protein lysate (35  $\mu$ g) were separated on 4% to 15% Tris-HCl linear gradient gel (Bio-Rad Laboratories) and transferred to nitrocellulose membranes (Life Sciences, Boston, Massachusetts). After blocking, membranes were incubated overnight at 4°C with primary antibodies followed by 1-hour treatment at room temperature with the appropriate HRP-conjugated secondary antibody. Primary antibodies used included rabbit polyclonal antihuman BID (1:1000 dilution; Cell Signaling Technology, Beverly, Massachusetts), mouse monoclonal antihuman BAX (1:1000 dilution; BD Biosciences, San Jose, California), mouse monoclonal antihuman X-linked inhibitor of apoptosis protein (XIAP; 1:1000 dilution; BD Biosciences), rabbit monoclonal anti-human Bcl-2 (1:1000 dilution; Cell Signaling

Technology), and rabbit polyclonal antihuman FLICE inhibitory protein (FLIP, 1:1000 dilution; Cell Signaling Technology). Rabbit polyclonal antihuman heat shock protein 90 (HSP-90, 1:1000 dilution; Cell Signaling Technology) and/or rabbit polyclonal antihuman  $\alpha$ -tubulin (1:1000 dilution; Cell Signaling Technology) were used to confirm equal loading of protein. The blots were developed and the intensity of the bands analyzed using the enhanced chemiluminescence system according to the instructions of the manufacturer (PerkinElmer Life Sciences, Boston, Massachusetts). The density of the resulting bands was measured using NIH Image J software, version 1.51t, and the relative signals were quantified as the ratio normalized RLU (corrected for HSP-90 band intensity). As negative control, membranes were incubated with secondary antibody alone to validate the specificity of the signal.

### Statistical Analysis

All data were subjected to normality testing using the Kolmogorov-Smirnov method and reported as mean (standard deviation; for normally distributed data) or median and range (for nonparametric data). Comparisons between the groups were performed using analysis of variance and  $\chi^2$  tests. For Western blot quantification, statistical analysis was performed using MINITAB statistical software (Minitab, State College, Pennsylvania). Significance of differences for protein expression was determined using paired 2-tailed *t* tests. For all studies,  $P < .05$  was used to define statistical significance.

## Results

### *TNF $\alpha$ Induces Apoptosis in Fetal Membranes, Which Can Be Inhibited by Progesterone*

Consistent with our prior publication,<sup>17</sup> we confirmed that TNF $\alpha$ -induced apoptosis in fetal membranes *in vitro* as measured by an increase in caspase-3, -8, and -9 activities (data not shown). We further confirmed that progesterone pretreatment inhibited both basal and TNF $\alpha$ -induced caspase-3 activity (data not shown).

### *Effect of Progesterone on Mediators of Apoptosis in Human Fetal Membranes*

A number of proteins are known to regulate the apoptotic cascade within cells. Some of these proteins are known to promote apoptosis (BID, BAX), whereas others suppress programmed cell death (XIAP, Bcl-2, FLIP).<sup>21</sup> Under basal conditions in our experimental model, progesterone significantly decreased expression of the proapoptotic protein, BID, by 0.45 (0.14)-fold ( $P = .001$ ; Figure 1A). Progesterone also increased the expression of the antiapoptotic proteins, XIAP by 4.14 (2.06)-fold ( $P = .028$ ; Figure 1D) and Bcl-2 by 5.32 (2.94)-fold (although this failed to reach statistical significance with  $P = .061$ ; Figure 1C). Progesterone stimulation had no effect on the expression of BAX (Figure 1B) or FLIP (Figure 1E). These findings are summarized in Figure 2.

### *Effect of TNF $\alpha$ With/Without Progesterone on Mediators of Apoptosis in Fetal Membranes*

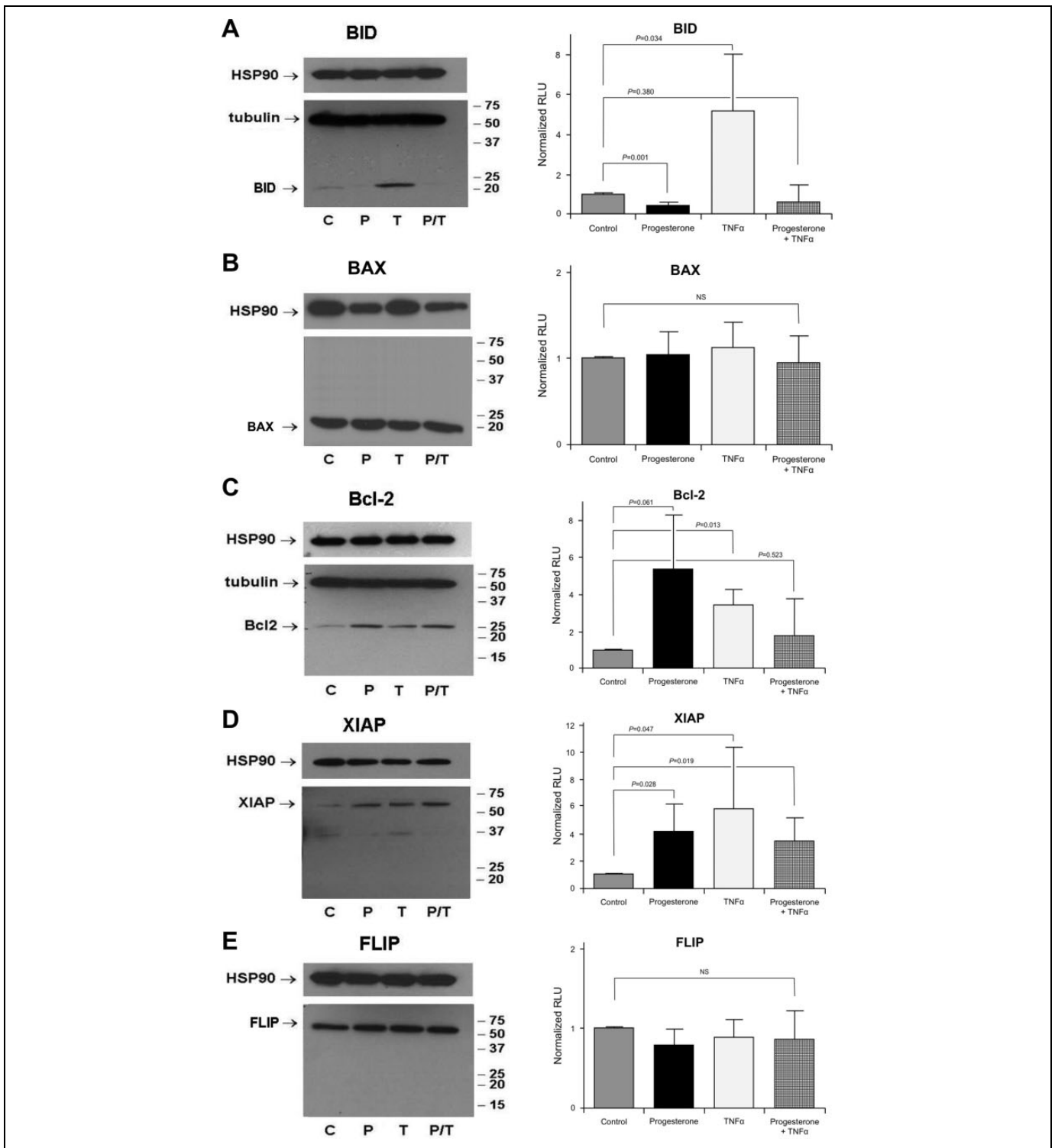
In contrast to progesterone, TNF $\alpha$  significantly increased expression of the proapoptotic protein, BID, in fetal membrane explants by 5.15 (2.92)-fold ( $P = .034$ ; Figure 1A). Interestingly, all of the other TNF $\alpha$ -mediated effects were similar to that seen with progesterone stimulation, including increased expression of the antiapoptotic proteins, Bcl-2 by 3.40 (0.89)-fold ( $P = .013$ ; Figure 1C) and XIAP by 5.85 (4.52)-fold ( $P = .019$ ; Figure 1D). Similarly, TNF $\alpha$  stimulation had no effect on the expression of BAX (Figure 1B) or FLIP (Figure 1E).

When looking at the effect of progesterone pretreatment, it is notable that progesterone completely abrogated the TNF $\alpha$ -mediated induction of the proapoptotic protein, BID ( $P = .031$ ), although not below the level of expression seen with progesterone treatment alone ( $P = .655$ ; Figure 1A). Progesterone pretreatment did not alter the response of BAX (Figure 1B), XIAP (Figure 1D), or FLIP (Figure 1E) to TNF $\alpha$  stimulation; it was neither additive nor synergistic. Interestingly, although progesterone alone increased the expression of Bcl-2, progesterone pretreatment blocked the increased expression of Bcl-2 induced by TNF $\alpha$  stimulation (Figure 1C).

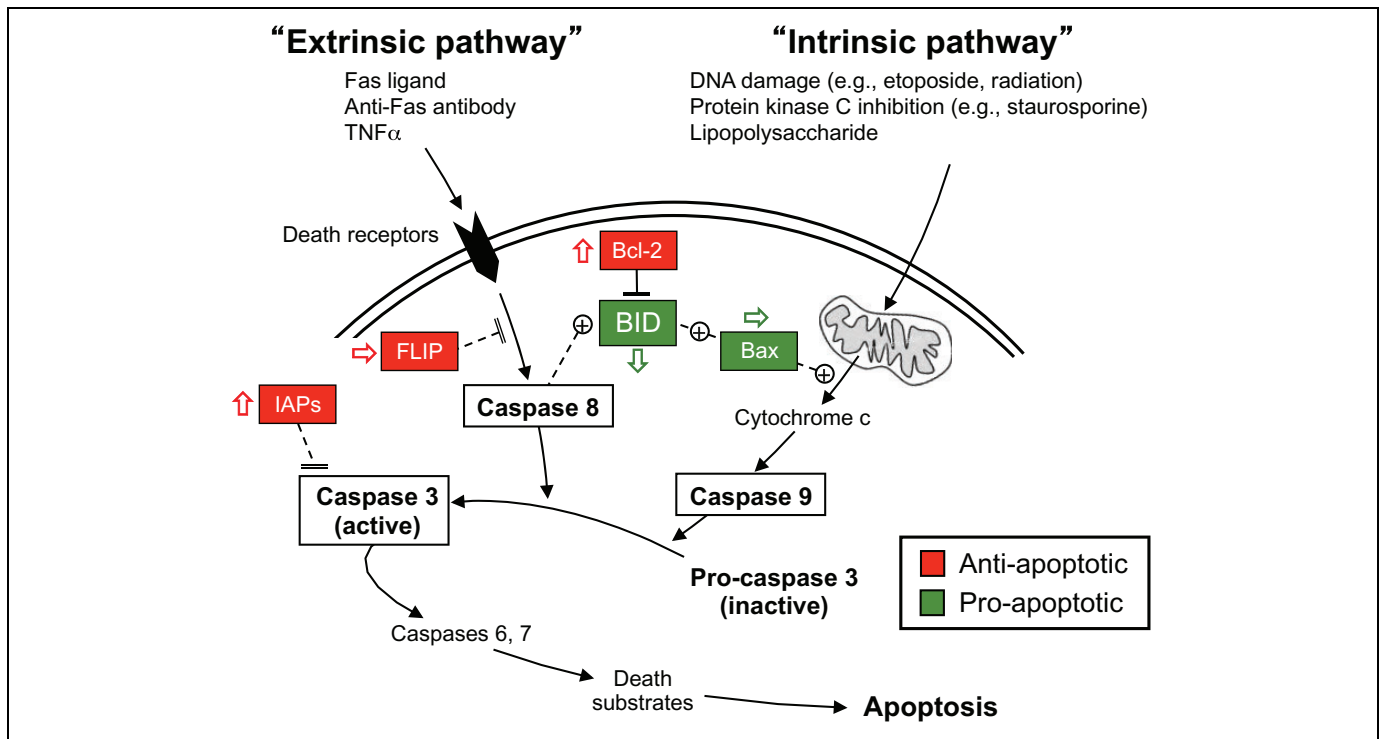
## Discussion

Progesterone supplementation starting at 16 to 20 weeks and continuing through 36 weeks of gestation can prevent PTB in some high-risk women.<sup>13-15</sup> This is the first medication that has been shown to decrease the rate of PTB in 30 years, and the first approved by the FDA<sup>16</sup> and by the American College of Obstetricians and Gynecologists<sup>22</sup> for this indication. Although a number of hypotheses have been put forward,<sup>23,24</sup> the mechanism by which progesterone exerts this protective effect is not known.

One-third of PTB occurs in the setting of PPRM.<sup>5</sup> While it is clear that initiation of progesterone therapy after PPRM has already occurred does not confer any benefit,<sup>25</sup> we hypothesize that progesterone supplementation starting at 16 to 20 weeks may prevent PTB, in part, by preventing PPRM. This hypothesis is consistent with the observations that fetal membranes are biologically active and likely play an important functional role in the timing of labor,<sup>26</sup> that many of the clinical trials that demonstrated a protective effect of progesterone supplementation included patients with PPRM,<sup>13,15</sup> and that patients on progesterone supplementation because of a history of a prior PTB due to PPRM are less likely to have a recurrent PTB than those on progesterone supplementation because of a prior spontaneous PTB.<sup>27</sup> This hypothesis is supported also by a number of experimental studies. We have previously shown that progesterone inhibits both basal and TNF $\alpha$ -induced apoptosis as measured by caspase activities in an explant model of human fetal membranes.<sup>17</sup> Similarly, Kumar et al<sup>28,29</sup> showed that progestogen pretreatment blocked TNF $\alpha$ - and thrombin-induced fetal membrane weakening as



**Figure 1.** Western blot analysis of pro- (BID, BAX) and antiapoptotic proteins (XIAP, Bcl-2, FLIP) expression in fetal membrane explants. Fetal membrane discs were prepared from term placenta (no labor) and cultured with/without progesterone and/or TNF $\alpha$  as described in the Materials and Methods section above. Total protein was extracted from the membrane discs, purified, and subjected to Western blot using specific antibodies for the proteins of interest. These included the proapoptotic proteins, BID (22 kDa) (A) and BAX (21 kDa) (B). They also include the antiapoptotic proteins, Bcl-2 (26 kDa) (C), XIAP (the antibody detects full-length 57-kDa protein and the degradation product at 38 kDa) (D), and FLIP (58 kDa) (E). Representative Western blot images are shown along with the quantitative analysis. Data are shown as mean (standard deviation) from a minimum of 3 separate experiments. HSP-90 (90 kDa) and  $\alpha$ -tubulin (52 kDa) were used as housekeeping proteins. FLIP indicates FLICE inhibitory protein; HSP-90 indicates heat shock protein 90; NS indicates nonsignificant; XIAP, X-linked inhibitor of apoptosis protein.



**Figure 2.** Effect of progesterone on the apoptotic cascade. The effects of progesterone stimulation on the expression of the proapoptotic (BID, BAX) and antiapoptotic proteins (XIAP, Bcl-2, FLIP) in human fetal membranes are shown.  $\uparrow$  increase;  $\downarrow$  decrease;  $\rightarrow$  no change. FLIP indicates FLICE inhibitory protein; XIAP, X-linked inhibitor of apoptosis protein.

measured by mechanical stretch and that it did so, in part, by inhibiting both the production and action of their upstream mediator, granulocyte-macrophage colony-stimulating factor. Taken together, these data are consistent with the hypothesis that prophylactic administration of progestogens may prevent PPRM.

The current study investigates the molecular mechanisms responsible for the previously documented progesterone-mediated inhibition of apoptosis in the fetal membranes.<sup>17</sup> In this study, we demonstrate: (1) that progesterone suppresses apoptosis under basal conditions, which appears to be mediated by a decrease in the expression of the proapoptotic factor, BID, and an increase in the expression of the antiapoptotic factors, Bcl-2 and XIAP, and (2) that progesterone blocks TNF $\alpha$ -mediated apoptosis, likely by eliminating the TNF $\alpha$ -induced increase in BID expression. These data suggest a molecular mechanism to explain how progesterone supplementation prevents amniochorion cell apoptosis, PPRM, and PPRM-related PTB.

Two major apoptotic pathways have been implicated in the pathogenesis of PPRM: an "extrinsic" TNF $\alpha$  receptor-Fas-mediated pathway, and an "intrinsic" p53-mediated pathway, which is normally initiated by DNA fragmentation.<sup>10,21</sup> The TNF $\alpha$  receptor-Fas-mediated pathway initiates signal transduction through various transmembrane death receptors, which in turn activate procaspase-8 to active caspase-8.<sup>21</sup> By contrast, the p53-mediated pathway proceeds via the release of proapoptotic messengers from the mitochondrial membrane

(including cytochrome c) and subsequent activation of procaspase-9 to active caspase-9. Both active caspase-8 and -9 can initiate a cascade of downstream caspase activation. Caspase-3, -7, and -6 are activated sequentially, leading to proteolysis of structural proteins, proteins of homeostasis, and several other target proteins resulting in apoptosis (Figure 2).

In this study, we identify BID as a putative essential target of progesterone-mediated inhibition of both basal level and TNF $\alpha$ -induced apoptosis. This finding is interesting as BID appears unique in its ability to play important roles in both the "extrinsic" TNF $\alpha$  pathway and the "intrinsic" p53 pathway. In the "extrinsic" pathway, BID interacts with caspase-8 and may be necessary for caspase-8-induced apoptosis.<sup>30,31</sup> In the "intrinsic" pathway, BID activates BAX, which, in turn, promotes the release of cytochrome c from mitochondria. These data suggest that BID may serve as a keystone protein, common to both apoptotic pathways and responsive to both progesterone and TNF $\alpha$  stimulation (Figure 2).

Both progesterone and TNF $\alpha$  treatments increased the expression of Bcl-2 and XIAP. Whereas these observations are consistent with the facility of progesterone to antagonize apoptosis, the finding that TNF $\alpha$  (an apoptosis-inducing signaling molecule) also increased the expression of these 2 antiapoptotic factors was unexpected. Equally surprising was the fact that cocubation with both progesterone and TNF $\alpha$  did not result in an additive or synergistic response compared with either progesterone or TNF $\alpha$  alone. Rather, for XIAP, there was no further increase (Figure 1D), suggesting that these 2 agonists may

increase protein expression through a shared signal transduction pathway that can be fully saturated. Progesterone pretreatment also appeared to block the increase in Bcl-2 expression induced by stimulation with TNF $\alpha$  (Figure 1C), although this inhibition did not reach statistical significance ( $P = .14$ ). Both progesterone and TNF $\alpha$  are known to regulate intracellular NF $\kappa$ B signaling, which, in turn, can affect the apoptotic cascade.<sup>32-34</sup> It is possible that the NF $\kappa$ B family of transcription factors serve as the common signaling pathway for the regulation of XIAP and Bcl-2 expression by both progesterone and TNF $\alpha$ . While the molecular mechanisms responsible for these observations remains to be fully elucidated, these data suggest that the release of TNF $\alpha$  as part of the intrauterine inflammatory response commonly associated with PTB may contribute to PPRM and PTB by changing the quality of progesterone signaling, which normally acts to promote uterine quiescence and maintain pregnancy.

The observation that progesterone suppresses basal levels of apoptosis in the fetal membranes suggests that this mechanism may be important also for normal labor. In most mammalian viviparous species, the onset of parturition at term is preceded by a decrease in circulating progesterone levels. Although humans do not exhibit a systemic decline in progesterone levels prior to labor, it is likely that a functional withdrawal of progesterone activity within the tissues of the uterus does indeed occur prior to the onset of labor.<sup>24,35,36</sup> Whether this is also true within the tissues of the fetal membranes is not known but could explain the progressive weakening of the fetal membranes that occurs in anticipation of labor at term.<sup>37,38</sup> Moreover, this weakening of the fetal membranes does not appear to be uniform throughout the uterus. A zone of maximal weakening is typically present directly over the cervix,<sup>37,38</sup> the precise location at which ascending inflammation (induced, in part, by TNF $\alpha$ ) would be expected to occur in concert with progressive cervical dilatation and loss of the protective mucus plug.

In summary, this study suggests a molecular mechanism involving the differential regulation of pro- and antiapoptotic proteins whereby progesterone is able to inhibit both basal level and TNF $\alpha$ -induced apoptosis in human fetal membranes. A functional withdrawal of progesterone activity at the level of the uterine tissues, which appears to occur in humans prior to the onset of labor at term, would result in increased apoptosis and weakening of the fetal membranes in anticipation of labor. This model also defines a mechanism by which progesterone supplementation may prevent PPRM and PTB in some high-risk women.

### Authors' Note

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### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### References

- Centers for Disease Control and Prevention. National Center for Health Statistics, Births in the United States, 2016. <https://www.cdc.gov/nchs/products/databriefs/db287.htm> Accessed November 11, 2017.
- Slattery MM, Morrison JJ. Preterm delivery. *Lancet*. 2002;360(9344):1489-1497.
- Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371(9606):75-84.
- Lannon SM, Vanderhoeven JP, Eschenbach DA, Gravett MG, Adams Waldorf KM. Synergy and interactions among biological pathways leading to preterm premature rupture of membranes. *Reprod Sci*. 2014;21(10):1215-1227.
- Menon R, Richardson LS. Preterm prelabor rupture of the membranes: a disease of the fetal membranes. *Semin Perinatol*. 2017;41(7):409-419.
- Menon R, Swan KF, Lyden TW, Rote NS, Fortunato S. Expression of inflammatory cytokines (interleukin-1 $\beta$  and interleukin-6) in amniochorionic membranes. *Am J Obstet Gynecol*. 1995;172(2 pt 1):493-500.
- Lei H, Furth EE, Kalluri R, et al. A program of cell death and extracellular matrix degradation is activated in the amnion before the onset of labor. *J Clin Invest*. 1996;98(9):1971-1978.
- Fortunato SJ, Menon R, Bryant C, Lombardi SJ. Programmed cell death (apoptosis) as a possible pathway to metalloproteinase activation and fetal membrane degradation in premature rupture of membranes. *Am J Obstet Gynecol*. 2000;182(6):1468-1476.
- Shobokshi A, Shaarawy M. Maternal serum and amniotic fluid cytokines in patients with preterm premature rupture of membranes with and without intrauterine infection. *Int J Gynecol Obstet*. 2002;79(3):209-215.
- Fortunato SJ, Menon R, Lombardi SJ. TNF- $\alpha$  promotes caspase activation and apoptosis in human fetal membranes. *J Assist Reprod Genet*. 2002;19(4):201-204.
- Jacobsson B, Mattsby-Baltzer I, Andersch B, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. *Acta Obstet Gynecol Scand*. 2003;82(5):423-431.
- George RB, Kalish J, Yonish B, Murtha AP. Apoptosis in the chorion of fetal membranes in preterm premature rupture of membranes. *Am J Perinatol*. 2008;25(1):29-32.
- Meis PJ, Klebanoff M, Thom E, et al; National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *N Engl J Med*. 2003;348(24):2379-2385.
- da Fonseca EB, Bittar RE, Carvalho MHB, Zugaib M. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomized placebo-controlled double-blind study. *Am J Obstet Gynecol*. 2003;188(2):419-424.

15. Fonseca EB, Celik E, Parra M, et al; Fetal Medicine Foundation Second Trimester Screening Group. Progesterone and the risk of preterm birth among women with a short cervix. *N Engl J Med*. 2007;357(5):462-469.
16. <https://www.nichd.nih.gov/news/releases/Pages/021611-FDA-approves-drug.aspx>. Accessed November 25, 2017.
17. Luo G, Abrahams VM, Tadesse S, et al. Progesterone inhibits basal and TNF-alpha-induced apoptosis in fetal membranes: a novel mechanism to explain progesterone-mediated prevention of preterm birth. *Reprod Sci*. 2010;17(6):532-539.
18. Fortunato SJ, Menon R, Swan KF, Lyden TW. Organ culture of amniochorionic membrane in vitro. *Am J Reprod Immunol*. 1994;32(3):184-187.
19. Abrahams VM, Straszewski SL, Kamsteeg M, et al. Epithelial ovarian cancer cells secrete functional Fas ligand. *Cancer Res*. 2003;63(17):5573-5581.
20. Parast MM, Yu H, Ciric A, Salata MW, Davis V, Milstone DS. PPARgamma regulates trophoblast proliferation and promotes labyrinthine trilineage differentiation. *PLoS One*. 2009;4(11):e8055.
21. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE. Cell death. *New Engl J Med*. 2009;361(16):1570-1583.
22. Committee on Practice Bulletins—Obstetrics, American College of Obstetricians and Gynecologists. Practice Bulletin No. 130: Prediction and prevention of preterm birth. *Obstet Gynecol*. 2012;120(4):964-973.
23. Sfakianaki AK, Norwitz ER. Mechanisms of progesterone action in preventing prematurity. *J Matern Fetal Neonatal Med*. 2006;19(12):763-772.
24. Mesiano S, Wang Y, Norwitz ER. Progesterone receptors in the human pregnancy uterus: do they hold the key to birth timing? *Reprod Sci*. 2011;18(1):6-19.
25. Briery CM, Veillon EW, Klauser CK, et al. Women with preterm premature rupture of the membranes do not benefit from weekly progesterone. *Am J Obstet Gynecol*. 2011;204(1):54.e1-e5.
26. Menon R, Mesiano S, Taylor RN. Programmed fetal membrane senescence and exosome-mediated signaling: a mechanism associated with timing of human parturition. *Front Endocrinol (Lausanne)*. 2017;8:196.
27. Coleman S, Wallace L, Alexander J, Istwan N. Recurrent preterm birth in women treated with 17 $\alpha$ -hydroxyprogesterone caproate: the contribution of risk factors in the penultimate pregnancy. *J Matern Fetal Neonatal Med*. 2012;25(7):1034-1038.
28. Kumar D, Springel E, Moore RM, et al. Progesterone inhibits in vitro fetal membrane weakening. *Am J Obstet Gynecol*. 2015;213(4):520.e1-e9.
29. Kumar D, Moore RM, Mercer BM, et al. In an in-vitro model using human fetal membranes, 17- $\alpha$  hydroxyprogesterone caproate is not an optimal progestogen for inhibition of fetal membrane weakening. *Am J Obstet Gynecol*. 2017;217(6):695.e1-695.e14.
30. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell*. 1998;94(4):491-501.
31. Huang K, Zhang J, O'Neill KL, Gurumurthy CB, Quadros RM, Luo X. Cleavage by caspase 8 and mitochondrial membrane association activate Bid during TRAIL-induced apoptosis. *J Biol Chem*. 2016;291(22):11843-11851.
32. Tamatani M, Che YH, Matsuzaki H, et al. Tumor necrosis factor induces Bcl-2 and Bcl-x expression through NFkappaB activation in primary hippocampal neurons. *J Biol Chem*. 1999;274(13):8531-8538.
33. Hsu SP, Yang HC, Kuo CT, et al. Progesterone receptor-NFk $\beta$  complex formation is required for progesterone-induced NFk $\beta$  nuclear translocation and binding onto the p53 promoter. *Endocrinology*. 2015;156(1):291-300.
34. Candanedo-Gonzalez F, Galindo-Hernandez O, Serna-Marquez N, et al. Progesterone induces NFk $\beta$  DNA binding activity through a PI3K/Akt-dependent pathway in MCF-7 breast cancer cells. *Cancer Res J*. 2014;2:63-69.
35. Zakar T, Hertelendy F. Progesterone withdrawal: key to parturition. *Am J Obstet Gynecol*. 2007;196(4):289-296.
36. Nadeem L, Shynlova O, Matysiak-Zablocki E, Mesiano S, Dong X, Lye S. Molecular evidence of functional progesterone withdrawal in human myometrium. *Nat Commun*. 2016;7:11565.
37. Moore RM, Mansour JM, Redline RW, Mercer BM, Moore JJ. The physiology of fetal membrane rupture: insight gained from the determination of physical properties. *Placenta*. 2006;27(11-12):1037-1051.
38. Kumar D, Moore RM, Mercer BM, Mansour JM, Redline RW, Moore JJ. The physiology of fetal membrane weakening and rupture: insights gained from the determination of physical properties revisited. *Placenta*. 2016;42:59-73.