Do Progestational Agents Target the Cervix?

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Initial studies investigating progestational agents and preterm birth

- **17-OHP**
- Delalutin
- P4
- MPA

**NEJM Fonesca et al**

**Meis, NEJM DaFonesca; AJOG**

**PREGNANT Trial**

**STOPPIT**

- **O’Brien et al**
- Grobman et al

**Meta-analysis; Keirse**

Timeline:
- 1950s
- 1970s
- 1990s
- 2003
- 2008
- 2011
## Progesterone & PTB: clinical evidence

### Progesterone prevents PTB
- Singletons with prior PTB
  - Meis et al. NEJM 2003
  - DaFonseca et al. AJOG 2003
- Short cervix in mid-trimester
  - Fonseca et al. NEJM 2008
- Short cervix in mid-trimester
  - Hassan et al. 2011
- Short cervix + prior PTB
  - DeFranco et al. US OBGYN, 2007

### Progesterone Did NOT prevent PTB
- Singletons with prior PTB
  - O’Brien et al. US OBGYN, 2011
- Twins
  - Several RCTs
- Singletons with short cervix
  - Grobman et al. 2012
How do progestational agents prevent preterm birth?
Genes turned on or off leading to activation (or inhibition) of specific signal transduction pathways.
Does it really matter if it is 17P or vaginal progesterone used in these studies?

Are all progesterones created equal?
• Natural progesterone, but not medroxyprogesterone acetate, enhances the beneficial effect of estrogen on exercise-induced myocardial ischemia in postmenopausal women.
  – 2000, J Am Coll Cardiol 36:2154-9

• Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling.
  – 2003 Proc Natl Acad Sci U S A 100:10506-11

• Progesterone, but not medroxyprogesterone, inhibits vascular cell adhesion molecule-1 expression in human vascular endothelial cells.

• Chronic treatment with progesterone but not medroxyprogesterone acetate restores the endothelial control of vascular tone in the mesenteric artery of ovariectomized rats.
  – 2004 Menopause 11:255-63

• Medroxyprogesterone acetate, but not progesterone, protects against inflammation-induced parturition and intrauterine fetal demise. 2
Is it biologically plausible that progestational agents can act directly on the cervix?
The Evidence:

- **In vitro evidence**
  - Ectocervical cells express PR, GR, and AR but only GR and AR are transcriptionally active
    - Africander et al, 2011

- **In vivo evidence: animal**
  - AR present and active. Androgens appear to regulate cervical resistance by altering proteoglycan content
    - Ji et al, AJOG 2008

- **In vivo evidence: human**
  - Cervical biopsies obtained at term before labor. PR-A and PR-B present by IHC.
    - Stjernholm-Vladic et al, Gynecol Endocrinol 2004
  - GR, PR and AR present in term cervical tissues
Is there any evidence regarding molecular mechanisms by which 17P or progesterone can prevent PTB?
Fact: Many cases of spontaneous preterm birth have evidence of inflammation.

Question: Do progestational agents prevent preterm birth by modifying the immune response?
Mouse Model of Localized Intrauterine Inflammation

Inhaled Isoflurane anesthesia

Mini-laparotomy with isolation of right lower uterine horn

Infusion of saline or LPS between lower two gestational sacs

Routine closure
**Table 1:** Preterm delivery (<24 hrs) after intrauterine LPS

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>Preterm Delivery at 24 hours* (%)</th>
<th>P values (compared to LPS alone)</th>
<th>N</th>
<th>Complete Preterm Delivery** (%)</th>
<th>P values (compared to LPS alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>26</td>
<td>0</td>
<td>&lt;0.00001</td>
<td>26</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LPS</td>
<td>36</td>
<td>94</td>
<td>***</td>
<td>32</td>
<td>88</td>
<td>***</td>
</tr>
<tr>
<td>LPS + 17-P</td>
<td>34</td>
<td>76</td>
<td>0.04</td>
<td>21</td>
<td>42</td>
<td>0.008</td>
</tr>
<tr>
<td>LPS + MPA</td>
<td>47</td>
<td>36</td>
<td>&lt;0.000001</td>
<td>35</td>
<td>11</td>
<td>&lt;0.00001†</td>
</tr>
</tbody>
</table>

* Preterm delivery rate 24 hours is defined as observing the pups in the cage prior to this time point.

** Complete preterm delivery is defined as empty uterine horns at the time of sacrifice 24-28 hours after intrauterine LPS.

† Fisher Exact of Chi square used for analysis.

MPA inhibited preterm birth (P=0.008, Fisher Exact Test) and complete preterm birth (P=0.01, Fisher Exact Test) to a greater extent that 17-P.

Elovitz et al, AJOG 2004 & 2006
What are the mechanisms by which progestational agents inhibit inflammation-induced preterm birth?
Effects of MPA in the Uterus and Cervix

• MPA inhibited inflammation-induced increase in contraction associated proteins (COX-2, CX-43)
• MPA modified the inflammation-induced rise of pro-inflammatory cytokines
• Unexpectedly, MPA also modified the immune response in the cervix
Effect of MPA on LPS-induced chemokine expression in the cervix

Target mRNA/18S rRNA

- MCP-1
- MIP-alpha
- Eotaxin
- Rantes
- IL-8

Legend:
- Saline
- LPS
- LPS + MPA

* indicates statistical significance.
MPA and LPS-induced Cervical Ripening

- Control/E15
- LPS
- LPS+MPA
• In the setting of inflammation, progestational agents appear to modulate the immune response (MPA >> P>17P)
• Whether these agents are acting via PR, GR, AR or other mechanism (non-genomic, mPR) is not known
• Whether modulation of cervical remodeling is secondary to inhibition of the immune response is not yet clear
But, is a model of acute inflammation accurately mimic what we are doing clinically? What do these agents do in normal pregnancy?
Assess
1) Collagen content
2) Pathways involved in uterine activity/quiescence
3) Immune cells in the cervix
4) Mucosal immunity
Serum Progesterone

**P<0.005**

*P<0.05
### Results of qRT-PCR on effect of vaginal progesterone and 17 alpha-hydroxyprogesterone caproate on pathways associated with myometrial contractility

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Replens</th>
<th>Vaginal progesterone</th>
<th>P value</th>
<th>Castor oil</th>
<th>17P</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraction-associated proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connexin-43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxytocin receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cyclooxygenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone-mediated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stat 5b</td>
<td>1.33 ± 0.26</td>
<td>1.15 ± 0.18</td>
<td>.58</td>
<td>1.05 ± 0.05</td>
<td>0.94 ± 0.08</td>
<td>.28</td>
</tr>
<tr>
<td>Zeb 1</td>
<td>1.53 ± 0.34</td>
<td>1.05 ± 0.09</td>
<td>.22</td>
<td>1.07 ± 0.10</td>
<td>0.77 ± 0.05</td>
<td>.06</td>
</tr>
<tr>
<td>Zeb 2</td>
<td>1.58 ± 0.37</td>
<td>1.13 ± 0.13</td>
<td>.30</td>
<td>1.15 ± 0.28</td>
<td>1.00 ± 0.11</td>
<td>.63</td>
</tr>
<tr>
<td>20-alpha-hydroxysteroid dehydrogenase</td>
<td>1.45 ± 0.35</td>
<td>0.97 ± 0.24</td>
<td>.30</td>
<td>1.07 ± 0.07</td>
<td>1.00 ± 0.29</td>
<td>.81</td>
</tr>
<tr>
<td>Micro-RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-200a</td>
<td>0.96 ± 0.21</td>
<td>0.99 ± 0.25</td>
<td>.97</td>
<td>1.16 ± 0.20</td>
<td>0.94 ± 0.06</td>
<td>.33</td>
</tr>
<tr>
<td>miR-429</td>
<td>1.22 ± 0.30</td>
<td>1.29 ± 0.23</td>
<td>.87</td>
<td>1.25 ± 0.13</td>
<td>1.12 ± 0.23</td>
<td>.63</td>
</tr>
</tbody>
</table>

Replens, Lily Drug Store Products, Inc., Cedar Rapids, IA.
PCR, polymerase chain reaction; 17P, 17 alpha-hydroxyprogesterone caproate.

FIGURE 2
Effect of vaginal progesterone and 17 alpha-hydroxyprogesterone caproate on collagen content and macrophage presence

No difference in any of the outcome measures

Mean and SEM (n = 3 per treatment group) of number of cell nuclei per area, optical density of birefringence of transmitted polarized light, and number of macrophages in cervix from mice in 4 treatment groups, unpaired Student t test.

Replens, Lith Drug Store Products, Inc., Cedar Rapids, IA.
17P, 17 alpha-hydroxyprogesterone caproate; VP, vaginal progesterone.

Mean and SEM (n = 4 per treatment group) for effect of vaginal progesterone (VP) compared to Replens (Lil’ Drug Store Products, Inc., Cedar Rapids, IA) and 17 alpha-hydroxyprogesterone caproate (17P) compared to castor oil (CO) on expression of defensin 1 in cervix. Exposure to VP significantly increased expression of defensin 1 compared to Replens (*P < .01), unpaired Student t test.

In normal murine pregnancy, exogenous administration of progestational agents has minimal effects on pathways known to be involved in uterine activity and/or cervical remodeling.
Some progestational agents appear to prevent preterm birth in some clinical trials – Singletons with prior PTB (17P) – Singletons with a short cervix defined as <20 (Vaginal progesterone) defined as <30 not work (17P). Remains unclear if 17P and vaginal progesterone are having the same or divergent molecular effects in their ability to prevent some cases of PTB.

So, what do we know
What is the answer?

• Progestational agents may have no effect on the cervix in normal pregnancy
• Progestational agents *may be modifying* the immune response in the cervico-vaginal space thus delaying premature cervical remodeling..in the setting of an inflammatory state
• Much remains unknown and more research is needed to elucidate the precise mechanisms by which preterm birth occurs
• Once pathways are understood, than progesterone—or more directed therapeutics—can be given to those at greatest risk
Acknowledgements

- Society of Maternal Fetal Medicine Award
- March of Dimes Research Award
- Burroughs Wellcome Prematurity Pilot Award
- Maternal and Child Health Research Fund at the University of Pennsylvania

- Steve Yellon, PhD
- Chris Nold, MD
- Juan Gonzalez, MD
- Hua Xu, PhD
- Amy Brown, PhD
Cervical remodeling

- CV microbiota
- Host immune response
- Genetic differences
- Environmental modifiers

Changes in mRNA
Changes in miRNA
Alterations in protein expression
Biochemical basis for the observed divergent effects between progestational agents

- **Bamberger et al J Clin Endo Metab 1999**
  - MPA has anti-inflammatory properties, in vitro
  - These properties are mediated by the affinity of MPA for the glucocorticoid receptor

- **Simoncini T, et al Endocrinology, Sept 9, 2004**
  - MPA activates GR to much greater extent than progesterone
  - MPA and P4, even when both are bound to PR, can activated divergent signaling pathways (SPRM)
  - MPA and P4 action are most likely cell and tissue specific
Figure 2. Left: Photomicrographs of cervix with macrophages stained dark brown with BM8 antibody. Right: Number of macrophage in the cervix of mice 6 hours after treatment. Data are the mean macrophage numbers normalized to cell nuclei density (± SE; evaluation of 10-19 nonoverlapping vertical and horizontal grid placements in 2 sections/mouse in 3 mice/group) to adjust for variability in hypertrophy of cervix. Scale bar in top left panel equals 50 μm and applies to all photomicrographs. Sal = saline; LPS = lipopolysaccharide; MPA = medroxyprogesterone acetate.
MPA inhibits CAPs and Immune Response

A  COX-2 mRNA Expression

B  Connexin-43 mRNA Expression

Elovitz et al AJOG 2004
## Adaptive immune response

<table>
<thead>
<tr>
<th>TH1/TH2 LUS</th>
<th>Fold Increase in Gene Expression</th>
<th>TH1/TH2 Cervix</th>
<th>Fold Increase in mRNA Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPS/NS</td>
<td>LPS/LPS+MPA</td>
<td>LPS/NS</td>
</tr>
<tr>
<td>CD28/TP44</td>
<td>31.7</td>
<td>1.0</td>
<td>26.3</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>3.2</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>37.0</td>
<td>-5.3</td>
<td>10.3</td>
</tr>
<tr>
<td>Stat1</td>
<td>4.0</td>
<td>13.0</td>
<td>-1.5</td>
</tr>
<tr>
<td>Stat4</td>
<td>8.2</td>
<td>66.0</td>
<td>17.0</td>
</tr>
<tr>
<td>T-bet</td>
<td>-1.5</td>
<td>6.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Tcrr</td>
<td>4.5</td>
<td>-3.7</td>
<td>6.0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>20.0</td>
<td>-2.7</td>
<td>12.0</td>
</tr>
<tr>
<td>IL-1β</td>
<td>12.3</td>
<td>-3.2</td>
<td>15.7</td>
</tr>
<tr>
<td>IL-6</td>
<td>59.0</td>
<td>5.2</td>
<td>68.0</td>
</tr>
<tr>
<td>IL-15</td>
<td>11.9</td>
<td>-3.2</td>
<td>15.7</td>
</tr>
<tr>
<td>IL-2</td>
<td>5.3</td>
<td>-1.7</td>
<td>64.3</td>
</tr>
<tr>
<td>IL-4</td>
<td>20.8</td>
<td>-7.8</td>
<td>60.0</td>
</tr>
<tr>
<td>IL-13</td>
<td>6.8</td>
<td>-4.1</td>
<td>9.3</td>
</tr>
<tr>
<td>IL-10</td>
<td>11.6</td>
<td>1.5</td>
<td>24.0</td>
</tr>
</tbody>
</table>
Preventing cervical ripening: the primary mechanism by which progestational agents prevent preterm birth?

Hua Xu, PhD; Juan M. Gonzalez, MD; Ella Ofori; Michal A. Elovitz, MD

OBJECTIVE: Recent clinical trials suggest that progestational agents may prevent preterm birth, specifically in women with short cervices. These studies sought to assess novel pathways by which progestational agents (PAs) may modulate signal transduction pathways that are involved in cervical ripening.

STUDY DESIGN: A microarray analysis was performed on pregnant mouse cervix that was exposed to a MPA. Appropriate microarray and cluster analyses were performed. Target genes of interest were investigated in both PA- and inflammation-exposed cervixes by quantitative polymerase chain reaction and immunohistochemistry.

RESULTS: Microarray analysis identified both the previously recognized and novel pathways that are involved in cervical ripening. PAs differentially regulate expression of claudin-2, hyaluronan synthase 2, and lipocalin 2. Claudin expression is significantly decreased by inflammation, which is prevented by PAs.

CONCLUSION: PAs significantly modulate gene expression in the cervix in the presence and absence of inflammation. The regulation of these pathways, specifically claudin proteins, may be a critical mechanism by which PAs prevent preterm birth, especially in women with premature cervical shortening.

Key words: cervical ripening, medroxyprogesterone acetate, microarray, preterm birth, progesterone

Of 226 selected modulated genes, 79 genes were classified. The number of classified genes in each pathway is indicated next to the pathway. Some genes may be represented in >1 functional category.

### TABLE 3
Validation of microarray findings by QPCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Microarray</th>
<th>QPCR (48 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fold change</td>
<td>Fold change</td>
</tr>
<tr>
<td>Claudin2</td>
<td>5.79</td>
<td>2.01</td>
</tr>
<tr>
<td>Hyaluronan synthase 2</td>
<td>-4.41</td>
<td>-3.3</td>
</tr>
<tr>
<td>Lipocalin 2</td>
<td>3.01</td>
<td>2.51</td>
</tr>
<tr>
<td>Intercellular adhesion molecule 1 (Icam 1)</td>
<td>1.53</td>
<td>1.55</td>
</tr>
<tr>
<td>Chemokine ligand 4 (Cxcl 4)</td>
<td>1.61</td>
<td>1.24</td>
</tr>
<tr>
<td>Retinoic acid induced 14 (Rai14)</td>
<td>-1.45</td>
<td>-1.33</td>
</tr>
<tr>
<td>FK506 binding protein 5 (Fkbp5)</td>
<td>1.8</td>
<td>1.46</td>
</tr>
<tr>
<td>Epoxide hydrolase 1 (Ephx1)</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Synccollin</td>
<td>8.33</td>
<td>3.71</td>
</tr>
</tbody>
</table>

*P value calculated by T-test or Mann Whitney Rank Sum

### Table 4

**Effect of progestational agents on gene expression at 24 hours**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Multiple Comparison (4 treatment groups)*</th>
<th>MPA</th>
<th>Progesterone</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin2</td>
<td>P = 0.001</td>
<td>7.8</td>
<td>4.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Hyaluronan synthase 2</td>
<td>P = 0.06</td>
<td>-1.9</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>Lipocalin 2</td>
<td>P = 0.17</td>
<td>1.7</td>
<td>-2.6</td>
<td>-2.3</td>
</tr>
<tr>
<td>Intercellular adhesion molecule 1 (icam 1)</td>
<td>P = 0.03</td>
<td>3.1</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>Chemokine (C-X-C motif) ligand 4 (Cxcl 4)</td>
<td>P = 0.07</td>
<td>1.5</td>
<td>1.2</td>
<td>-1.1</td>
</tr>
<tr>
<td>Retinoic acid induced 14</td>
<td>P = 0.07</td>
<td>1.1</td>
<td>-1.1</td>
<td>-1.3</td>
</tr>
<tr>
<td>FK506 binding protein 5</td>
<td>P = 0.005</td>
<td>2.3</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Epoxide hydrolase 1</td>
<td>P = 0.6</td>
<td>1.4</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td>Synccollin</td>
<td>P = 0.64</td>
<td>1.9</td>
<td>2.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

MPA = medroxyprogesterone acetate; DEX = dexamethasone; NP = not performed; NS = not significant

*4 treatment groups: MPA, DEX, Progesterone and Vehicle exposed cervixes. Multiple comparisons by One-way ANOVA or ANOVA on Ranks. Pair-wise comparisons were performed only if statistical significance was reached (P < 0.05).