Endometrial-peritoneal cross-talk and development of endometriosis

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Commercial disclosure

- Astellas
- Bayer Healthcare
- Roche
- TEVA Pharmaceutical
- ValiRx
Outline

- Background
- Endometriosis
- Peritoneum
- Stem/progenitor cells
- Mesothelial damage
- Conclusions
Endometriosis
<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of studies</th>
<th>Number of patients</th>
<th>Number with disease</th>
<th>% with disease (range)</th>
<th>% with Stage I-II disease (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic pain</td>
<td>15</td>
<td>2,400</td>
<td>688</td>
<td>24.5 (4.5 – 62.0)</td>
<td>69.9 (61.0 – 100)</td>
</tr>
<tr>
<td>Infertility</td>
<td>32</td>
<td>14,971</td>
<td>2,812</td>
<td>19.6 (2.1 – 78.0)</td>
<td>65.6 (16.3 – 95.0)</td>
</tr>
<tr>
<td>Sterilisation</td>
<td>13</td>
<td>10,634</td>
<td>499</td>
<td>4.1 (0.7 – 43.0)</td>
<td>91.7 (20.0 – 100)</td>
</tr>
</tbody>
</table>

Why care?

Patients

Society
Patients

Infertility

Pain

- Dysmenorrhea
- Non-menstrual pain
- Deep dyspareunia
- Dysuria
- Dyschezia
## Delay in diagnosis (years)

<table>
<thead>
<tr>
<th>Country</th>
<th>Symptom onset</th>
<th>Surgical diagnosis</th>
<th>Delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil ¹</td>
<td>20.5 *</td>
<td>33.0 *</td>
<td>7.4 *</td>
</tr>
<tr>
<td>USA ²</td>
<td>18.9</td>
<td>28.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Australia</td>
<td>17.1</td>
<td>29.6</td>
<td>12.5</td>
</tr>
<tr>
<td>UK ³</td>
<td>22.0</td>
<td>30.5</td>
<td>8.5</td>
</tr>
<tr>
<td>USA</td>
<td>19.2</td>
<td>30.9</td>
<td>11.7</td>
</tr>
<tr>
<td>UK ⁴</td>
<td>24.4</td>
<td>32.6</td>
<td>8.0</td>
</tr>
<tr>
<td>GER/AUT ⁵</td>
<td>21.2</td>
<td>32.0</td>
<td>10.4</td>
</tr>
</tbody>
</table>

* median values
otherwise all means

¹ Arruda et al., Hum Reprod 2003
² Sinaii et al., Hum Reprod 2002
³ Treloar et al., Fertil Steril 2002
⁴ Hadfield et al., Hum Reprod 1996
⁵ Hudelist et al., Hum Reprod 2012
RESULTS (n=905)

Average annual cost = €9,579 (95% CI €8,559-€10,599)

- Average cost of direct healthcare costs = €3,113
  - surgery (29%)
  - monitoring tests (19%)
  - hospitalisation (18%)
  - medication (10%)

- Average cost of productivity loss = €6,298

Cost of loss of productivity is twice that of healthcare costs!

Simoens S et al., Hum Reprod 2012
Definition

The presence of endometrial LIKE tissue in sites outside the uterine cavity

Kennedy SH et al., Hum Reprod 2005
Histology

Glandular epithelial cells

Stromal cells
Endometrium
Sampson theory? - 1927
Higher incidence:

- short menstrual cycles
- increased duration of bleeding
- decreased parity
- obstructed outflow (Müllerian anomalies)
The anatomical distribution of the disease is predominantly in dependent areas of the pelvis.

90% of women (with patent tubes) have blood in peritoneal fluid at time of menstruation.

Problem – although most women have retrograde menstruation most do not have endometriosis.

Supporting Sampson (2)
Clinical presentation

Giudice LC, NEJM 2010
Coelomic metaplasia

- Starts with invagination of mesothelium
- Mesothelial inclusions
- Metaplastic process
Possible mechanisms

- Reduced apoptosis
- Increased proliferation
- Stem cells
- Increased adhesiveness/invasiveness
- Inheritability
- Angiogenesis
- Impaired immune response
- Hormonal imbalance
Disease progression

Always progressive – self curing?

Second look (24 wks) laparoscopy (gestrinone)

<table>
<thead>
<tr>
<th>Placebo group</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease improved</td>
<td>29%</td>
</tr>
<tr>
<td>Disease eliminated</td>
<td>24%</td>
</tr>
</tbody>
</table>

Thomas EJ & Cooke ID, Br Med J 1987
Disease progression

Second look (6 months) laparoscopy (surgery)

<table>
<thead>
<tr>
<th>Diagnostic laparoscopy group</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease progressed</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>Disease unchanged</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Disease improved</td>
<td>4</td>
<td>22</td>
</tr>
</tbody>
</table>

Abbott J et al., Fertil Steril 2004
Minimal endometriosis

- All ectopic endometrium = disease?
- Only physiological variant?
- Transient phenomenon?

What is the definition of a normal pelvis?
## Microscopic endometriosis

<table>
<thead>
<tr>
<th>Macroscopic endometriosis</th>
<th>No evidence of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>11%</td>
<td>6%</td>
</tr>
<tr>
<td>13%</td>
<td>6%</td>
</tr>
</tbody>
</table>
One disease?

Peritoneal endometriosis

Ovarian endometriosis

Recto-vaginal endometriosis
Distribution of aromatase in endometriosis tissue

Heilier JF et al., Fert Steril 2006
Peritoneum
Peritoneum

- *Peritonaion* = stretch around (gr.)
- Serous, semi-permeable membrane 1 - 2 m²
- 100 ml serous transudate daily
- 80% of lymphatic drainage through subdiaphragmatic lymph vessels
- Cavity closed in males, open in females (Fallopian tubes)
- Mesothelium
- Cubical epithelium (ovaries)
- Columnar ciliated epithelium (fimbriae)
Peritoneum

Parietal peritoneum:

- Embryologically derived from the somato-pleural layer of the lateral plate of mesoderm
- Blood supply and innervation same as overlying body wall
- Pain sensitive (somatic innervation)

Visceral peritoneum:

- Embryologically derived from the splanchno-pleural layer of the lateral plate of mesoderm
- Blood supply and innervation same as underlying viscera
- Pain insensitive (autonomic innervation)
Peritoneal function

- Contains phagocytic cells
- Contains lymphocytes for humoral and cellular immune reaction
- Movements of viscera
- Absorption
- Storage of fat
- Transformation of mesothelial cells for wound healing
Origin of endometriotic cells

• Locally through transformation/differentiation
• Endometrium (Retrograde menstruation)
• Bone marrow
• Combination
Endometrial stem cells

Necessary growth factors:

Epithelial colony-forming units:
- EGF
- TGF-α
- PDGF-BB

Stromal colony-forming units:
- EGF
- TGF-α
- PDGF-BB
- bFGF

Side populations

Gargett CE et al., Mol Cell Endocrinol 2008
Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells

A

Cumulative population doubling level

EN12
EN17
EN18
EN19

Days

B

Growth Rate (cpd/day)

Stromal cells
EN-MSCs
Stromal cells
EN-MSCs

Eutopic
Ectopic

C

Colony forming efficiency (%)

Eutopic EN-MSCs
Ectopic EN-MSCs

D

Average of invaded cells

Eutopic stromal cells
Eutopic EN-MSCs
Ectopic stromal cells
Ectopic EN-MSCs

Migration
Invasion
Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells
Possible role of endometrial stem/progenitor cells in the pathogenesis of endometriosis.

Bone marrow origin

Male-to-female BM transplantation model

Red = Y chromosome
Green = CD45
Blue = Nuclei
Yellow = Cytokeratin

Du H and Taylor HS., Stem Cells 2007
Bone marrow origin

- LacZ endometriosis transplantation model
- Endometriosis auto-transplantation
  
  Incorporation of BM-derived stem cells into endometriosis-like lesions

Du H and Taylor HS., Stem Cells 2007
Angiogenesis
Endothelial progenitor cells
GFP +/- bone marrow

Wild type + GFP +/- bone marrow + Wild type -> WT GFP +/- bone marrow

Wild type + WT GFP +/- bone marrow + WT endo -> WT GFP +/- bone marrow

WT endo
CEP in endometriosis model (C57BL/6 mice)

1 week post surgery

Control  Sham  Endometriosis

**P = 0.049**

Becker CM et al., Am J Pathol 2011
Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis: Implications for cellular surrogate marker analysis of antiangiogenesis

Yuval Shaked,1 Francesco Bertolini,2 Shan Man,1 Michael S. Rogers,3 Dave Cervi,1,4 Thomas Foutz,2 Kimberley Rawn,1 Daniel Voskas,1,4 Daniel J. Dumont,1,4 Yaakov Ben-David,1,4 Jack Lawler,5 Jack Henkin,6 Jim Huber,7 Daniel J. Hicklin,7 Robert J. D’Amato,3 and Robert S. Kerbel1,4*
CEP in endometriosis model (6 lesions)

C57BL/6 mice

129SvJ mice

Becker CM et al., Am J Pathol 2011
Bone marrow origin

Becker CM et al., Am J Pathol 2011
CAC levels in women with and without endometriosis

Webster K at al., Hum Reprod 2013
CAC levels in healthy women throughout the menstrual cycle

Webster K et al., Hum Reprod 2013
Mesothelial-epithelial interaction

Laschke MW, Menger MD, Hum Reprod Update 2007
## Impact of tissue integrity on infiltration and endometriosis-like lesion formation in the chorioallantoic membrane (CAM)

<table>
<thead>
<tr>
<th>Endometrium</th>
<th>No. of CAM</th>
<th>Infiltration</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsied cyclic endometrium</td>
<td>19</td>
<td>14 (74)</td>
<td>13 (68)</td>
</tr>
<tr>
<td>Biopsied menstrual endometrium</td>
<td>18</td>
<td>14 (78)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Endometrial cells isolated from menstrual effluent collected in</td>
<td>50</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>Keeper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue fragments in menstrual effluent collected in Keeper</td>
<td>16</td>
<td>10 (63)</td>
<td>7 (44)**</td>
</tr>
<tr>
<td>Collagenase digested biopsied cyclic endometrium</td>
<td>45</td>
<td>24 (53)**</td>
<td>1 (2)*</td>
</tr>
<tr>
<td>Biopsied menstrual endometrium, stored in Keeper</td>
<td>13</td>
<td>10 (77)</td>
<td>9 (69)</td>
</tr>
<tr>
<td>Biopsied menstrual endometrium, stored in Keeper, processed as</td>
<td>11</td>
<td>6 (55)</td>
<td>4 (36)**</td>
</tr>
<tr>
<td>menstrual effluent</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nap AW et al., Hum Reprod 2003
Mesothelial-epithelial interaction

Groothuis PG et al., Fertil Steril 1999
Mesothelial-epithelial interaction

Hull ML et al., Am J Pathol 2010
Light micrographs of HOMEC after overnight co-incubation with conditioned medium.

Demir Weusten AY et al., Hum. Reprod. 2000
Epithelial mesenchymal transformation (EMT)

Omental mesenchymal cells incubated with normal or conditioned media (from antegrade menstruation blood)
Differential expression and phosphorylation of mesothelial proteins.

Expression of 35 proteins was altered:
- organization of the cytoskeleton
- signal transduction
- regulation of the redox state
- production of ATP
Urinary MMPs in endometriosis patients

<table>
<thead>
<tr>
<th>MMP Biomarker</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9/NGAL</td>
<td>6.3</td>
<td>1.7 – 22.8</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>MMP-9</td>
<td>7.8</td>
<td>2.5 – 25.1</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>MMP-2</td>
<td>4.8</td>
<td>1.8 – 13.2</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Any MMP above</td>
<td>8.3</td>
<td>3.0 – 22.7</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

Becker CM et al., Fertil Steril 2010
Factors of mesothelial damage

- Mechanical trauma
- Toxic insult
- Hypoxia
- Hyperoxia
- Desiccation

Inflammation
HIF-1α expression is regulated at the protein level

- Prolyl hydroxylation, ubiquitination and degradation

- Stabilization and nuclear localization

- Hypoxia, Stress

- Normoxia

HIF-1α

ARNT (HIF-1β)

Hypoxia Response Element (HRE)

Glycolytic enzymes
Angiogenic factors
Apoptosis control proteins
EPO
Hypoxia in uterine and endometriosis tissue (Hypoxyprobe HRP immunohistochemistry)
HIF-1α expression in endometriotic tissue (HRP immunohistochemistry)
Adhesions after 10 min pneumoperitoneum

Adhesions after 60 min pneumoperitoneum

Molinas et al., Fertil Steril 2003
Conclusion

• Endometriosis remains an enigmatic disease.

• Stem cells or retrogradely menstruated endometrium or transformed mesothelial cells or a combination of all are likely to be involved in lesion formation.

• Mesothelial-endometrial interaction appears to be crucial for the development of endometriosis.