Schistosoma mansoni infection associated infertility

Mónica Botelho
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OUTLINE

1. Schistosomes
   • Facts of figures

2. Infertility-associated schistosomiasis
   • Pathway of hormonal imbalance induced by schistosomes

3. Development of functional tools for schistosomes
   • Animal models of *S. mansoni* induced impaired reproduction
Schistosomes: Life cycle
Infertility-associated schistosomiasis

The role of estrogens and estrogen receptor signaling pathways in cancer and infertility: the case of schistosomes

Mónica C. Botelho, Helena Alves, Alberto Barros, Gabriel Rinaldi, Paul J. Brindley, and Mário Sousa

• Homonal imbalance caused by estrogen-like molecules produced by schistosomes
Schistosomes estrogen-like molecules and down-regulation of estrogen receptor

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>E2 Range</th>
<th>Testosterone Range</th>
<th>LH Range</th>
<th>Sex</th>
<th>Age (years)</th>
<th>E2 Range</th>
<th>Testosterone Range</th>
<th>LH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>4</td>
<td>62,8</td>
<td>0-22</td>
<td>&lt;15,0</td>
<td>2-10</td>
<td>0,114</td>
<td>&lt;2,5</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>30,8</td>
<td>0-25</td>
<td>77,5</td>
<td>5-500</td>
<td>1,79</td>
<td>0,2-8,0</td>
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<td></td>
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<tr>
<td>Male</td>
<td>14</td>
<td>79,8</td>
<td>0-25</td>
<td>363</td>
<td>5-500</td>
<td>1,89</td>
<td>0,2-8,0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>45,7</td>
<td>0-25</td>
<td>724</td>
<td>&gt;200</td>
<td>5,89</td>
<td>1,4-7,7</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>17</td>
<td>31,9</td>
<td>0-25</td>
<td>535</td>
<td>&gt;200</td>
<td>7,65</td>
<td>1,4-7,7</td>
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<tr>
<td>Male</td>
<td>20</td>
<td>68,3</td>
<td>&lt;56,0</td>
<td>982</td>
<td>262-1593</td>
<td>2,87</td>
<td>1,4-7,7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gene Expression

![Gene Expression Graph]

pERE-Luc transfection

![pERE-Luc transfection Graph]

MS identified molecules extracted from *S. haematobium*
CATECHOL-OESTROGENS (oxidative metabolites derived from estrogens)

m/z 716

m/z 803

m/z 813

m/z 817
Infertility-associated *Schistosomiasis haematobia* in women

**Urinary Estrogen Metabolites and Self-Reported Infertility in Women Infected with *Schistosoma haematobium***

Júlio Santos¹, Maria João Gouveia², Nuno Vale², Maria de Lurdes Delgado³, Ana Gonçalves⁴, José M. Teixeira da Silva⁴, Cristiano Oliveira⁴, Pedro Xavier⁴, Paula Gomes², Lúcio L. Santos¹,⁵, Carlos Lopes¹,⁶, Alberto Barros⁴,⁷, Gabriel Rinaldi⁸,⁹, Paul J. Brindley⁸, José M. Correia da Costa³,¹⁰, Mário Sousa¹¹, Mónica C. Botelho³,¹⁰

*Plos One 9 (2014) e96774*

<table>
<thead>
<tr>
<th>Category</th>
<th>$E^+$ (n=25)</th>
<th>$E^-$ (n=21)</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertile women (ages)</td>
<td>2 (29–63)</td>
<td>6 (28–94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2+3 (ages)</td>
<td>15 (19–41)</td>
<td>2 (21–34)</td>
<td>4.33</td>
<td>1.13–16.70</td>
<td>0.03</td>
</tr>
<tr>
<td>Group 2 (ages)</td>
<td>9 (18–20)</td>
<td>1 (21)</td>
<td>2.67</td>
<td>0.60–11.80</td>
<td>n.a.</td>
</tr>
<tr>
<td>Group 3 (ages)</td>
<td>6 (27–41)</td>
<td>1 (34)</td>
<td>4.75</td>
<td>0.51–44.50</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12 years</td>
<td>8</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Women unable to become pregnant after one year of trial (*Self-reported primary infertility - Group 2*) and those who had borne fewer children than desired (*Self-reported secondary infertility - Group 3*). OR, odds ratio; CI, confidence interval.
Infertility associated *Schistosomiasis mansoni*

*Schistosoma mansoni* infection impairs reproduction in mice (In Preparation)

- Mating (1 year)
- Gestational period
- Synchronization
- Number pups
**Infertility associated Schistosomiasis mansoni**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Gestational length (days)</th>
<th>Synchronicity (days)</th>
<th>Number of pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>2FCx1MC</td>
<td>25</td>
<td>0-1</td>
<td>15.1</td>
</tr>
<tr>
<td>2FCX1MI</td>
<td>25.6</td>
<td>0-2</td>
<td>14.5</td>
</tr>
<tr>
<td>2FIX1MC</td>
<td>22.8</td>
<td>1-6</td>
<td>13.8</td>
</tr>
<tr>
<td>2FIX1MI</td>
<td>21.8</td>
<td>3-8</td>
<td>11.9</td>
</tr>
</tbody>
</table>

**Ovary**

- **Control**
- **S. mansoni**

**Tube**

- **Control**
- **S. mansoni**

**Testes**

- **Control**
- **S. mansoni**
Pathway for Schistosomiasis Bladder Cancer

Catechol

Quinone

Estrone/Estradiol [E₁(E₂)]

CYP1B1

4-OHE₁(E₂)

CYP450 or peroxiases

E₁(E₂)-3,4-Q

DNA

4-OHE₁(E₂)-1-N₇Gua

4-OHE₁(E₂)-1-N₃Ade

Depurinating Adducts

Bladder carcinoma with squamous differentiation

Cancer ← Mutations ← Error-prone base excision repair ← DNA with apurinic sites

Botelho et al, 2015
Pathway for Female Fertility

Female fertility

LH FSH

Follicle maturation

Ovulation

LH surge

Estrogen Receptor (pituitary)

E2

Ovocyte with follicular cells

A – Polar body
B – Zona pellucida
Mechanism for Schistosomiasis Female Infertility

- Female fertility
- LH surge
- Follicle maturation
- Estrogen Receptor (pituitary)
- Ovulation

A – Polar body
B – Zona pellucida

Ovocite with follicular cells
E2
Two different complementary pathways probably contribute to estrogen imbalance leading to:

- Initiation and promotion of cancer progression
- Infertility
Conclusions

1. *S. mansoni* induced impaired reproduction in animal models

2. Novel catechol-oestrogen molecules derived from the eggs could be involved in infertility
1. Synthesize and/or purify and/or isolate reactive catechol-estrogens.

2. Evaluate impact of catechol estrogens on culture cells in vitro, at the phenotypic and gene expression levels.

3. Evaluate impact of catechol estrogens in an informative mouse model.

4. Investigate schistosome catechol estrogen–DNA adducts in informative human cases from a schistosomiasis haematobia endemic regions. (Potential for Biomarkers screening)