Fungicide “Mancozeb (manganese ethylene bidithiocarbamate)” Impairs Embryo Implantation via Disrupting the Early Embryo Attachment and Decidualization

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Infertility and subfertility are becoming major problems in domestic animals, companion animals and human
Introduction


Asia

North America

Africa

Europe

United Nations, World Fertility Patterns, 2013
• 7.4 million women, or 11.9% of women (1 in 8 couples) have received any subfertility treatment in their lifetime.

• 1/3 of infertility is attributed to the female partner
  1/3 attributed to the male partner
  1/3 combination of problems in both partners or, is unexplained.

• 44% seek medical assistance
  Approximately 65% give birth.

Source: National Survey of Family Growth, CDC
Embryo Implantation: apposition, adhesion and invasion

- LIF signaling
- EGF signaling
- COX-2
- Regulation of prostaglandin production
- Hormones (Estrogen, Progesterone)
- ECM components
- Inner cell mass
- Liminal epithelium
- Syncytiotrophoblast
- Microvilli
- Pinapodes
- Growth factors and cytokines
- Regulation of the changes in the endometrium

Endocrine Disruptor (ED)

“An exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process.” (U.S. EPA)

e.g. TCDD, BPA, PFOA, PCB, pesticides, flame retardant …
Mancozeb

- A fungicide, Ethylene bidithiocarbamate (EBDC)
- Daily intake up to 50μg/day (Colocio et al., 2006)
- Usage of fungicide in Sri Lanka has doubled in last decade.
- Exposure:
  Ingestion, inhalation of air, skin absorption
  Agricultural workers, General public, Livestock
Endocrine disruptive effects of Mancozeb & ethylenethiourea (ETU)

- Ovarian function disruption
- Impair the oocyte maturation
- Low fertilization rates
- Embryo losses
- Gene toxicity / carcinogenesis
• Mancozeb impairs the embryo implantation in mice

- Vaginal plug
- Day 1
- Day 2
- Day 3
- Day 4
- Day 8
- Check implantation
- Mancozeb Treatment

a) Uterus of control pregnant female mice showing implantation sites at 9th gestation day. b) Uterus of pregnant female mice treated with 1mg/kg BW/d Mancozeb for 9th gestation days. c) Uterus of pregnant female mice treated with 16mg/kg BW/d Mancozeb for 9th gestation day. d) Uterus of female mice treated with 32mg/kg BW/d Mancozeb for 8 gestation days, showing implantation sites at 9th gestation day.
a) Control pregnant female mice showing implantation. b) 0.3mg/kg BW/d treated mice uterus. c) 3mg/kg BW/d treated mice uterus. d) 30mg/kg BW/d Mancozeb treated mice uterus.
• This concludes Mancozeb affects implantation by inhibiting the decidualization

• Proceeded to identify which pathways are affected in the process of decidualization by Mancozeb

• Prostaglandins are very important for decidualization of the endometrium for successful implantation
PGES Protein expression in the endometrium
Effect of Mancozeb on Human Embryo Implantation

In vitro trophoblastic spheroids attachment to endometrial epithelial cells monolayer

Kodithuwakku et al., Hum Reprod. 2011 Jan;26(1):167-75
JAr trophoblastic Spheroids Attachment Assay

Ishikawa cells (10% FBS + P/S + L-glut) up to monolayer in 12 well plate and treatment for 48hrs with or with out treatments plus hormones

Culture JAr in RPMI 1640 until monolayers and trypsinized

Shaker culture at 70 rpm for 24 hrs 37°C +5% CO₂ (2x10⁵ cells/ml)

Wash twice and refill with the new medium

Gently place 25-30 spheroids/well on RL 95-2 under microscope

Co-culture for 1hr 37°C +5% CO₂ and vigorous shaking for 10 min at 150 rpm after co-culture

Attached

Remove medium and re-fill. Count attached spheroids and the non-attached

Non-attached
A) 

Attachment %

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>MTX 0.01</th>
<th>MTX 0.1</th>
<th>MTX 1</th>
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<td></td>
<td>100</td>
<td>80</td>
<td>80</td>
<td>70</td>
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B) 

Mancozeb µg/ml

100µm spheroids

Attached spheroids

C)
• Mancozeb treatment at high dose down regulate key proteins in the Wnt signaling pathway

- Mucin-1
- Axin-2
- GSK3β
- Active β-Catenin
- β-Actin

<table>
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<tr>
<th>Mancozeb ug/ml</th>
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<tr>
<td>Control</td>
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<tr>
<td>0.01</td>
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<tr>
<td>0.10</td>
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In Summary,

- Mancozeb exposure at high dose can impair the embryo implantation

- The effect is critical during the decidualization period exposure

- The impairment is through altering the PG synthesis cascade and also changes in canonical Wnt Signaling pathway.

- Further investigations are warranted to find the receptor interactions and possible target identification for therapeutic approaches in the case of exposure.
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THANK YOU......