Dam and Fetal Analysis of Detoxification and Redox Pathways Altered by Vinclozolin Exposure During Pregnancy

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Introduction

- Endocrine disrupting compounds (EDCs) are widespread environmental pollutants.
- Vinclozolin is a well studied model EDC.
  - Vinclozolin and its metabolites M1 and M2 are antiandrogenic.
  - Vinclozolin causes hypospadias, a congenital genitalia defect, decreased adult fertility, and transgenerational health outcomes.
  - The effects of vinclozolin on hepatic physiology and whole-body metabolomics is unknown.
- We evaluated proteomics of liver tissues from mouse dams and fetuses and metabolomics of whole fetuses exposed to vinclozolin or corn oil.

Methods and Instrumentation

- Pregnant mice were gavaged with corn oil (Con) or vinclozolin (VCZ) (125 mg/kg) once a day on embryonic days (E)13.5-16.5.
- Proteomics analysis: pregnant dams were euthanized one hour after the final dose. Dam and fetal livers were removed, homogenized, and trypsin digested. Peptides were analyzed through liquid chromatography (LC) - mass spectrometry (MS) on a Thermo Qexactive orbitrap mass spectrometer. Data were analyzed using proteome discover, pathway analysis through gProfiler and Pathview, and network analysis via STRING.
- Metabolomics analysis: pregnant dams were euthanized one day after final dose on E17.5. Fetuses were removed and homogenized. Methanol was used to precipitate proteins and the extract was analyzed through LC-MS on a SciEx 5600+ quadrupole-Time-of-Flight mass spectrometer.

Dam Liver Proteomics

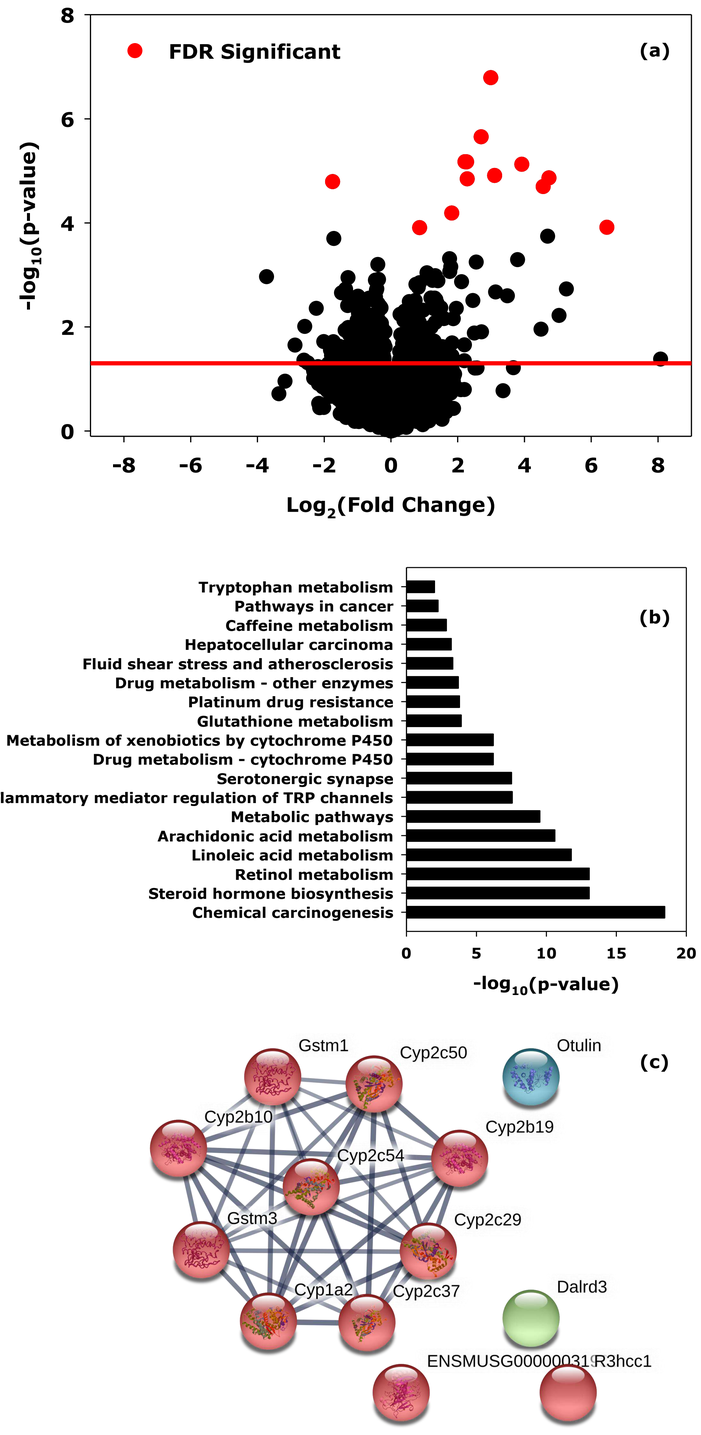

Figure 1. Volcano plot depicting proteins that were significantly different between vinclozolin and control dams, 13 proteins were significantly different after FDR correction (red points) (a). These 13 proteins influenced several pathways (b) and formed a network of detoxification proteins (c).

Fetal Liver Proteomics

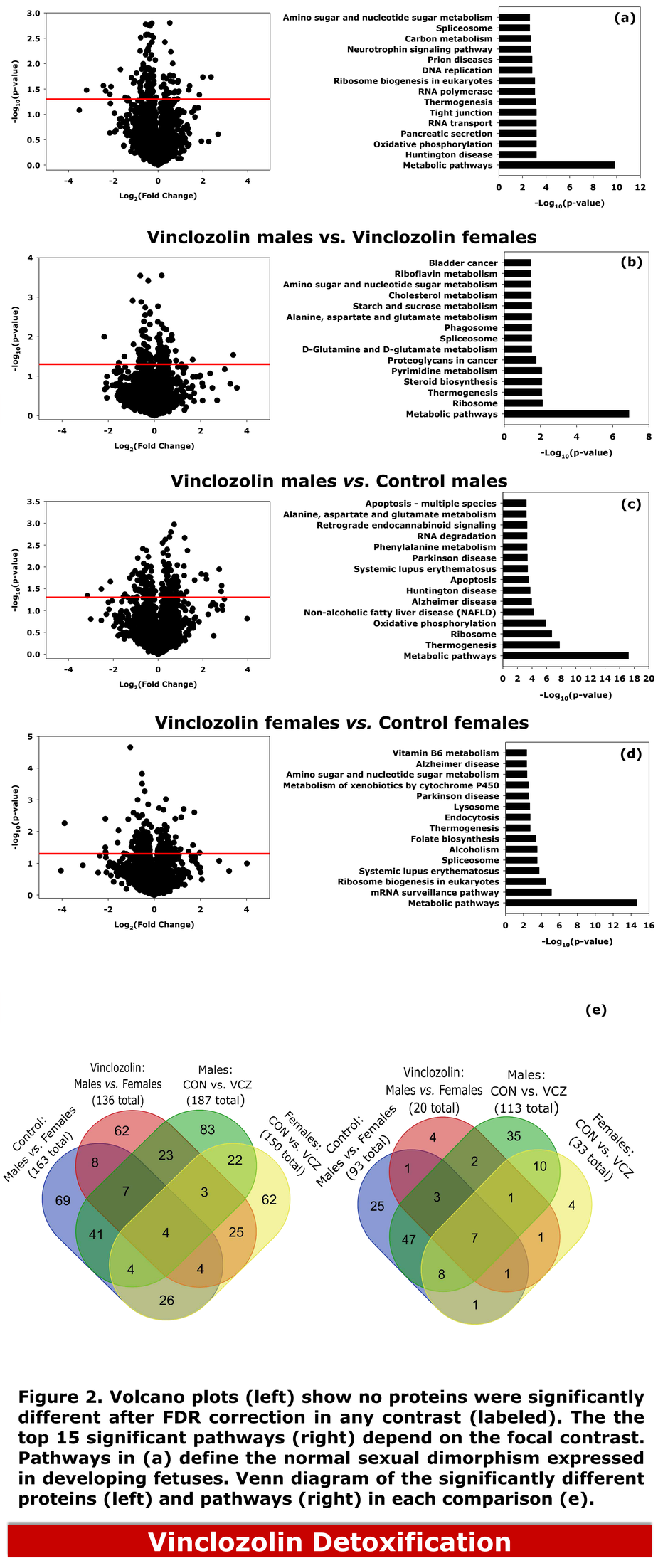

Figure 2. Volcano plots (left) show no proteins were significantly different after FDR correction in any contrast (labeled). The the top 15 significant pathways (right) depend on the focal contrast. Pathways in (a) define the normal sexual dimorphism expressed in developing fetuses. Venn diagram of the significantly different proteins (left) and pathways (right) in each comparison (e).

Vinclozolin Detoxification

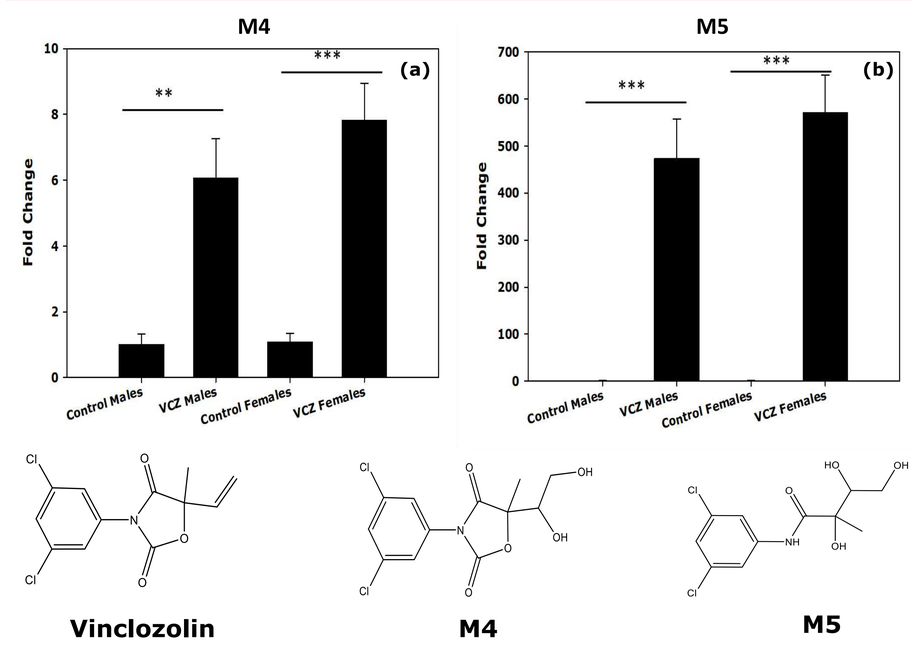

Figure 3. Fold change of vinclozolin metabolites M4 (a) and M5 (b) in control male and female mice and vinclozolin exposed (VCZ) male and female mice.

Glutathione Metabolomics

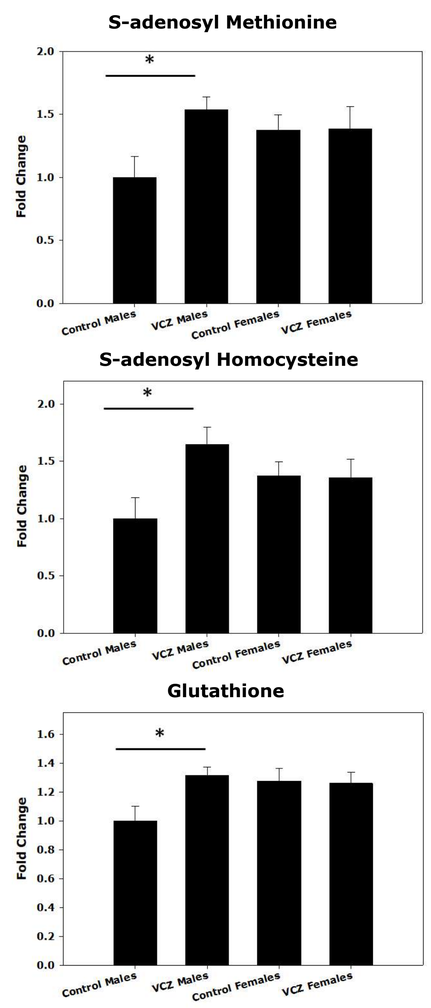

Figure 4. Fold change for S-adenosyl Methionine (a), S-adenosyl Homocysteine (b), and Glutathione (c). The diagram to the right shows the biosynthesis of Glutathione.

Fetal Liver Pathway Analysis

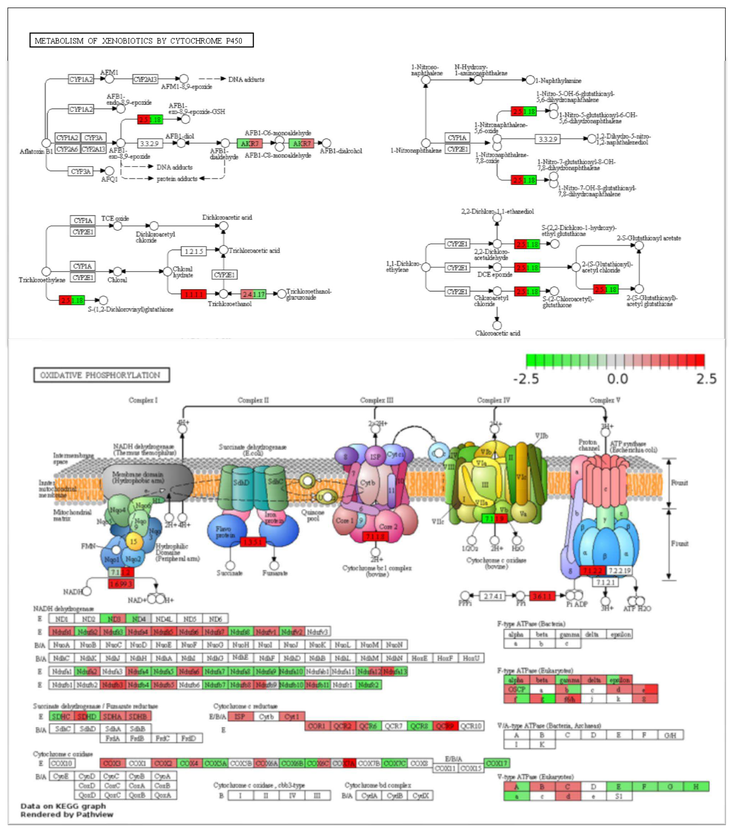

Figure 5. Selected pathway analyses. The left half of the box depicts fold change for females vinclozolin vs. control and the right half depicts fold change the male vinclozolin vs. control.

Conclusions

- Dam livers increase detoxification when exposed to vinclozolin (Figure 1).
- Dam and fetal responses to vinclozolin differ.
- Vinclozolin exposure changes sexually dimorphic pathways (Figure 2a & b).
- The effects of vinclozolin were sex specific (Figure 2c & d).
- Fewer pathways were sexually dimorphic after fetal vinclozolin exposure (Figure 2e).
- Vinclozolin exposure altered more pathways in males than females (Figure 2e).
- Vinclozolin elevated expression of detoxification proteins in females (Figures 2,3, & 5).
- Vinclozolin affects oxidative phosphorylation proteins differently in each sex (Figure 5).
- Female fetuses have elevated detoxified vinclozolin metabolites M4 and M5 (Figure 3).
- Male fetuses exposed to vinclozolin have increased S-adenosyl methionine, S-adenosyl homocysteine, and glutathione compared to male controls but not female fetuses (Figure 4).

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