Session: Fish model species in human and environmental toxicology (I)
Platform Presentation
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Proteomics based screening tool to detect molecular responses following aromatase inhibition

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Chemical exposure to endocrine disruptors can have adverse outcomes on organism health and function; however, the current reliance on end-points such as egg number, plasma VTG content and morphological changes to determine effects of endocrine disrupting chemicals has given rise to series of questions related to chemicals exhibiting similar effects but different mode-ofaction (MoA). Mechanistic identification of biological responses preceding to apical endpoints has become crucial for analyzing, accessing and determining chemical effects. Proteomics, therefore, show appreciable promise as a molecular screening tool for identifying specific alterations between exposures and controls, which is therefore imperative in discriminating endocrine disruptors from substances with a non-endocrine MoA. Such tool waives the need for elongated highertier testing.

The main aim of this study is to identify alteration in molecular-toxicity pathways that are specific to chemical-induced apical responses in zebrafish. The study focused on fadrozole, a known inhibitor of cytochrome P450 aromatase. Thus an excellent model substance to evaluate and validate proteomic methods with the integration of organ-specific effects. Spawning adult zebrafish groups (5 males, 5 females) maintained at 25-26°C on a 16:8 h light/dark cycle; were exposed for 21 days to fadrozole (0, 0.1, 1, 10 µg/L) and analysed for plasma vitellogenin content, egg numbers and organ histopathology. Livers and gonads were isolated for shotgun proteomics and qPCR to characterize substance induced specific molecular toxicity pathways. Proteins involved in steroid hormone secretion and estrogen stimulus such as vtg1, vtg3, vtg6 and lman1, were significantly deregulated. Several of the prominently affected pathways involved regulation of xenobiotic stimulus, lipid metabolism, metabolic processes, TCA metabolism and calcium signalling.

Our study demonstrated that the downstream induced-estrogen receptor suppression by aromatase inhibition triggered the downregulation of estrogen synthesis, which was assumed to induce the observed decrease in egg numbers and oocyte atresia with membrane folding in the ovary. We anticipate that this improvement leads to the identification of reliable biomarkers to determine chemical-induced adverse outcomes of ecological relevance in order to avoid unnecessary extensive testing.

PROTEOMICS BASED SCREENING TOOL TO DETECT MOLECULAR RESPONSES FOLLOWING AROMATASE INHIBITION

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Setac Europe
Rome, Italy
13. – 17. May 2018

BACKGROUND

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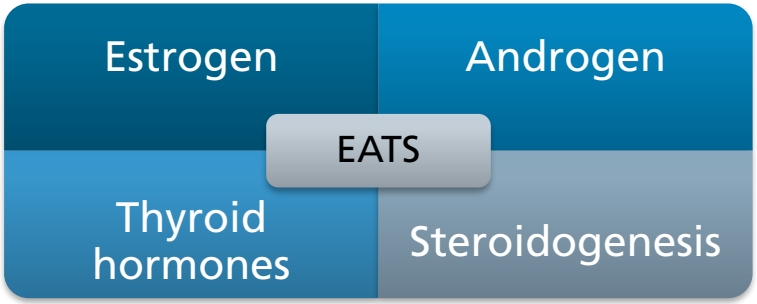
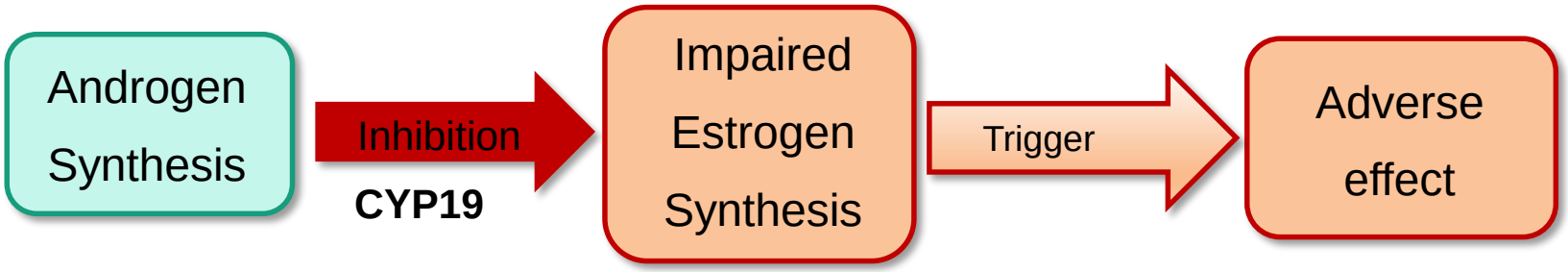
Endocrine Disruptors - are chemicals that interfere with the endocrine system and trigger an adverse developmental, reproductive and immune effects to exposed organism or its descendant population[1].

OECD Testing and Assessment framework for Endocrine Disruptors[2]



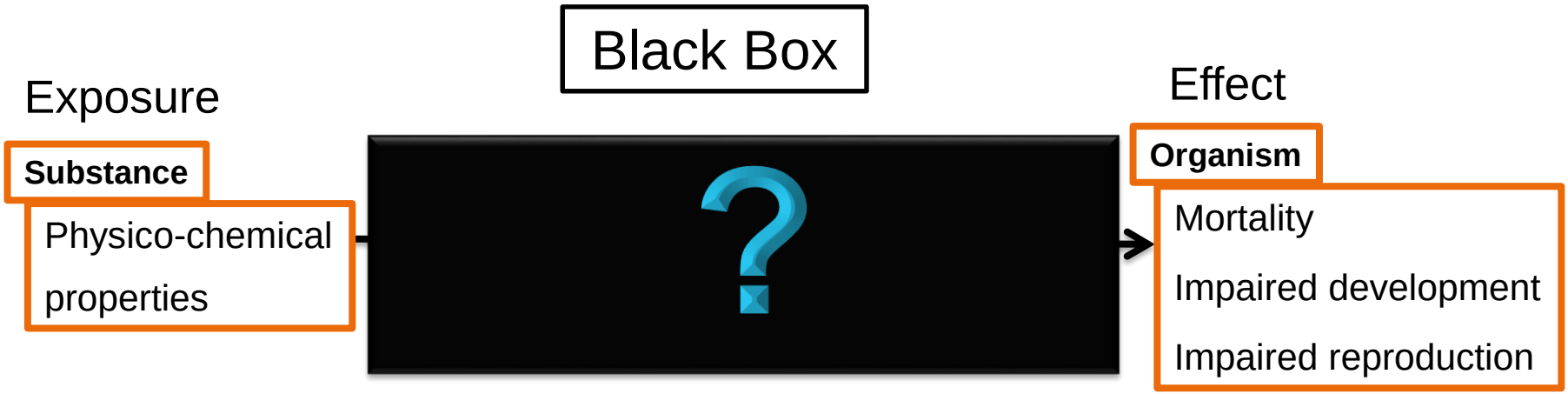
Aromatase inhibition



[1]WHO-IPCS, International Program on Chemical Safety, 2002.

[2]OECD, Conceptual Framework for Testing and Assessment of Endocrine Disruptors, 2012a

THE BLACK BOX BETWEEN EXPOSURE AND EFFECT



For some substances, similar apical endpoints in standard tests but different MoAs result in **false interpretation and inconsistency in chemical labelling**.

Criteria for endocrine disruptors in a regulatory context:

- Adversity, mode-of-action, causality, relevance, specificity, potency

Is the effect really mediated by an **endocrine mode-of-action or non endocrine MoA**

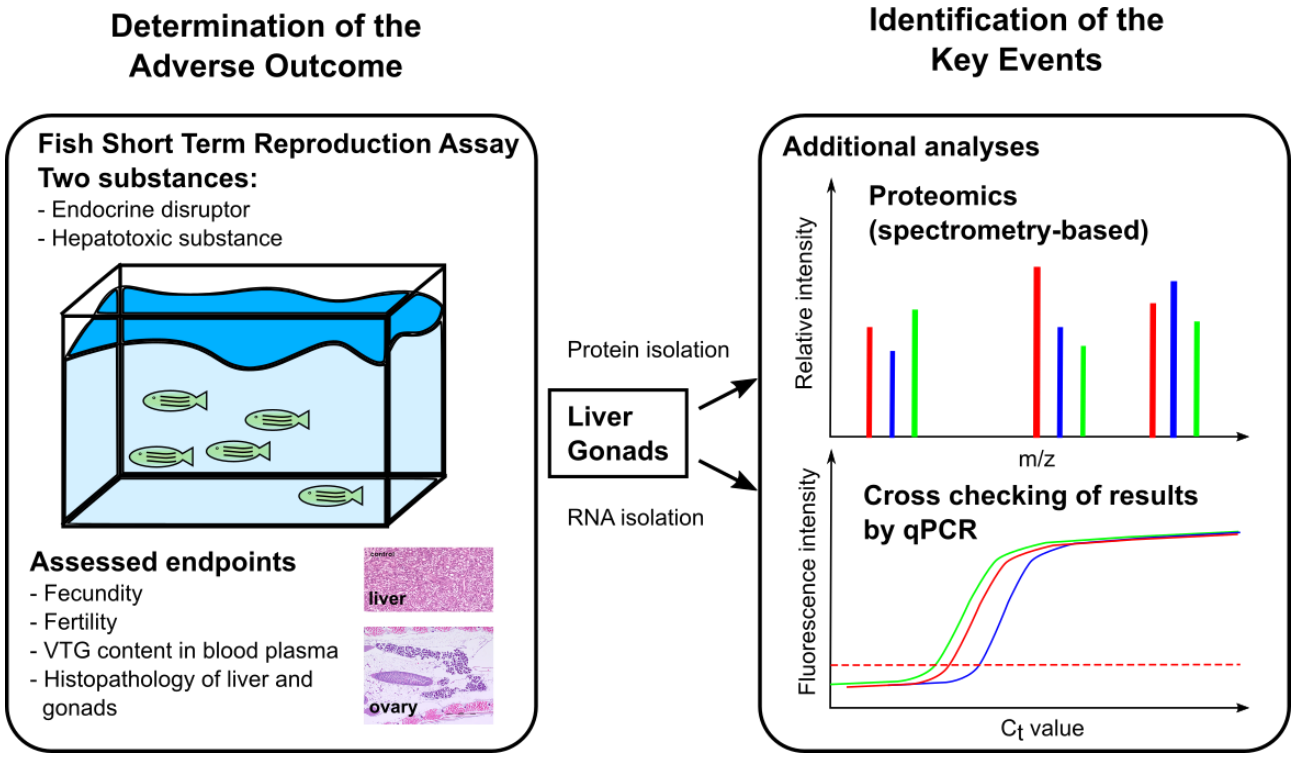
TEST STRATEGY

TEST STRATEGY

Reproduction Study: Fish short term reproduction assay (FSTRA)[3]

Objective: To identify alterations in molecular-toxicity pathways that are specific to chemical-induced apical responses.

- 21 days of exposure
- 5 males and females
- 3 treatment conditions and control
- Daily eggs count



[3]OECD, Test No. 229: Fish short term reproduction assay. 2009.

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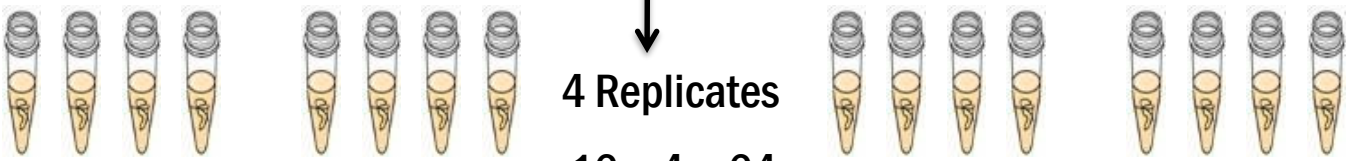
PROTEOMICS TECHNIQUE

Exposure

Protein Extraction



Male Liver | Testis | Female Liver | Ovary

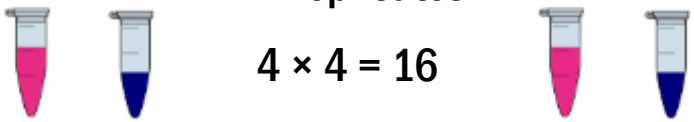
4 Replicates

16 × 4 = 64 samples

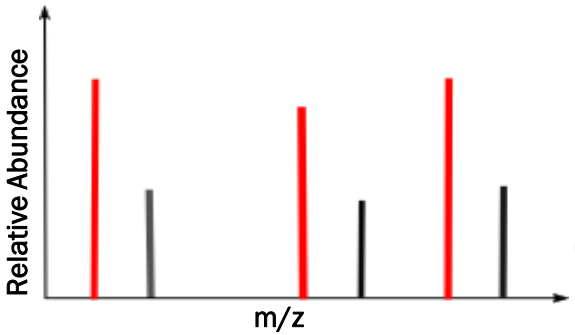
Protein Digestion & TMT6plex Labelling

4 Replicates

4 × 4 = 16 samples



Dried and re-solubilized in 0.1% FA 2% ACN



LC-MS/MS Analysis

RESULTS FROM FSTRA STANDARD ENDPOINTS

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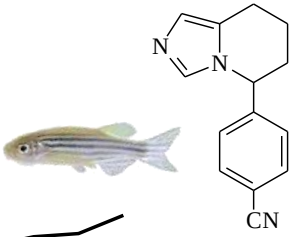
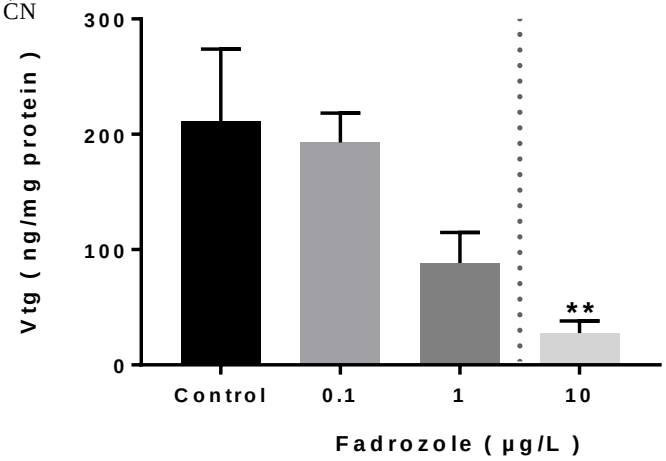
RESULTS – FSTRA ENDPOINTS

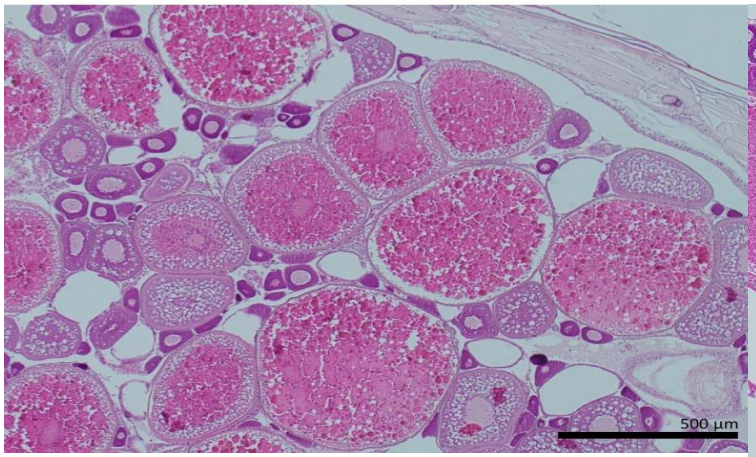
Reproduction

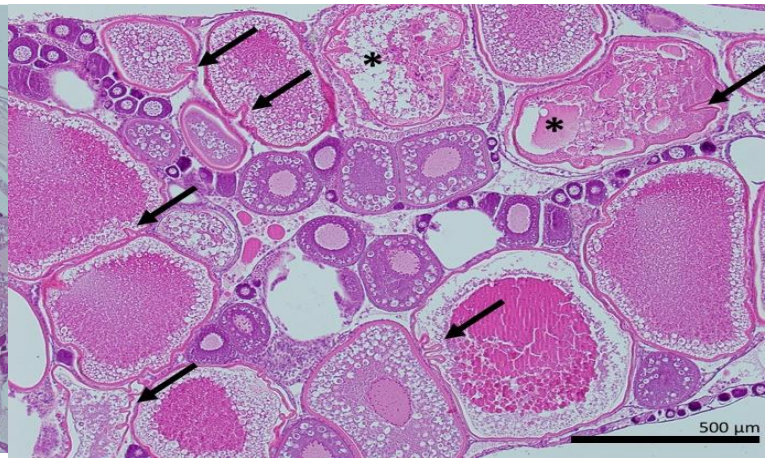


VTG content in plasma



Histopathology - Ovary





Fadrozole (10µg/L)

- Arrows: membrane folding
- Asterisks: Atretic oocytes

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PROTEOMICS RESULTS

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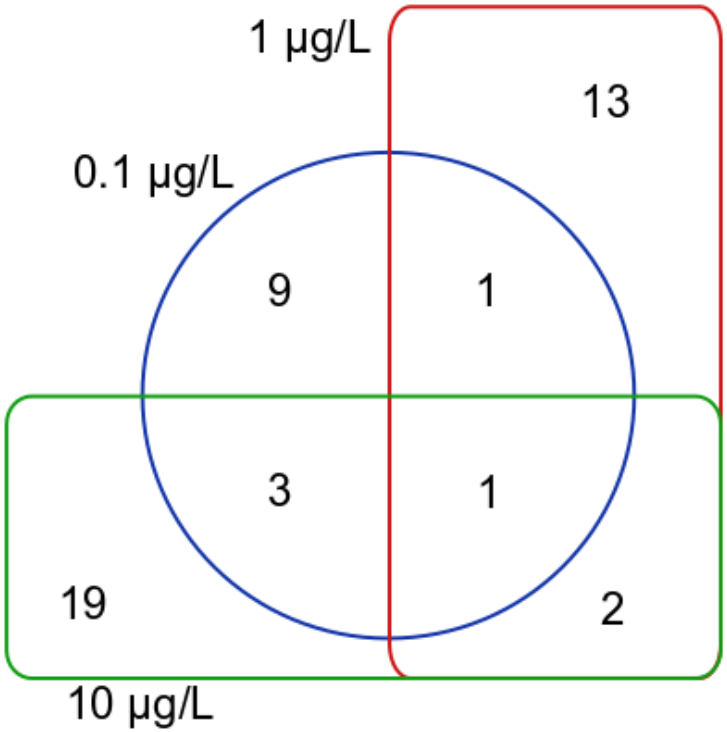
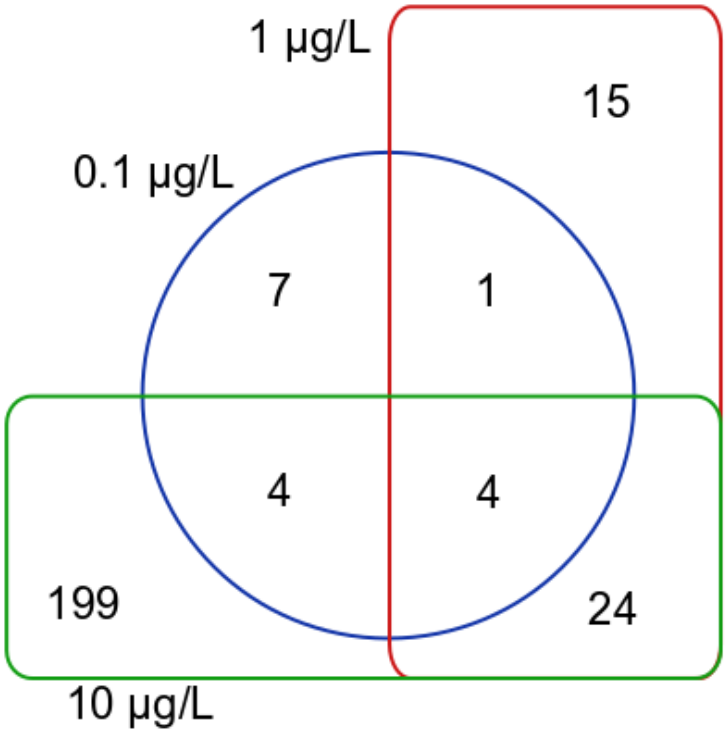
PROTEOMIC ANALYSIS OF FEMALE LIVER & OVARY

Venn diagram – Female Liver



Venn diagram – Female Ovary



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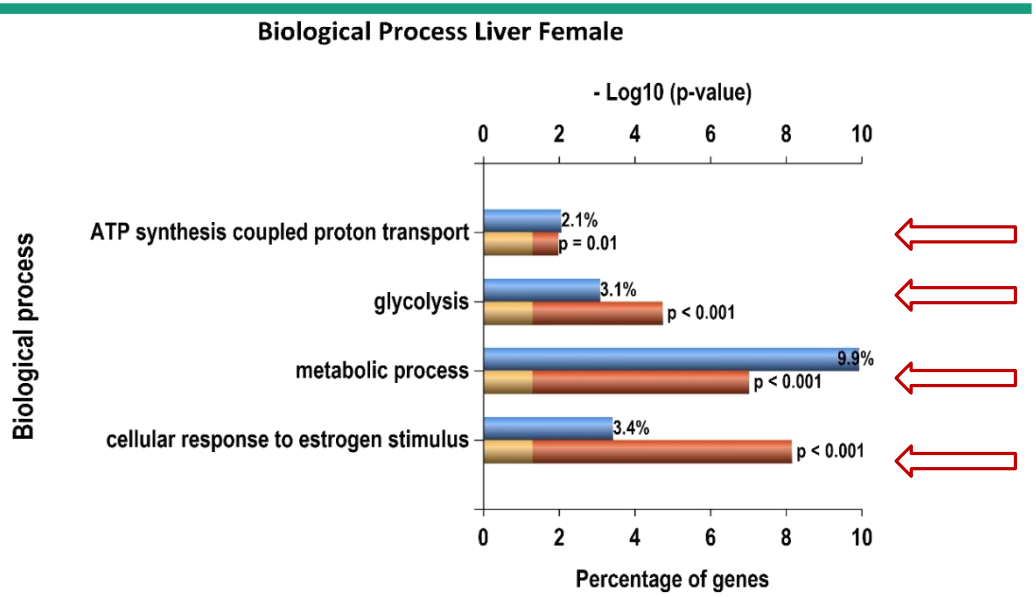
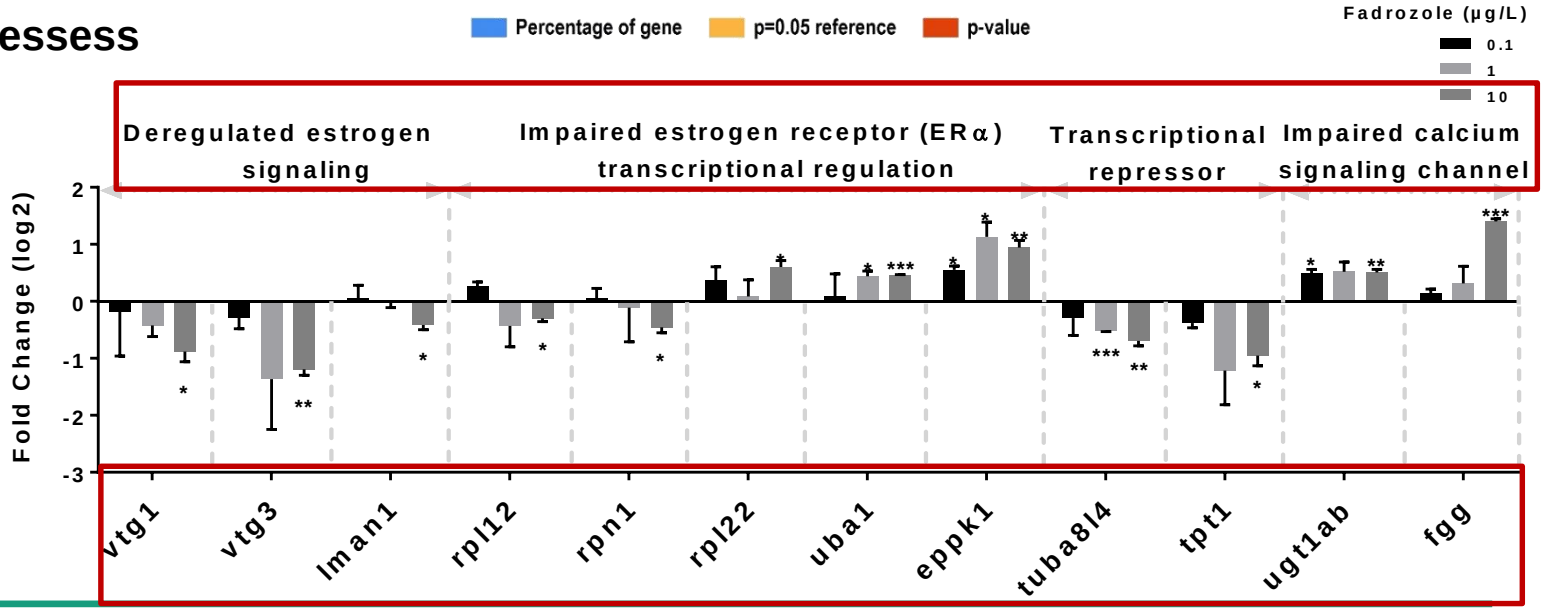
PROTEOMIC ANALYSIS OF FEMALE LIVER



Potential molecular biomaker

Regulated Processess



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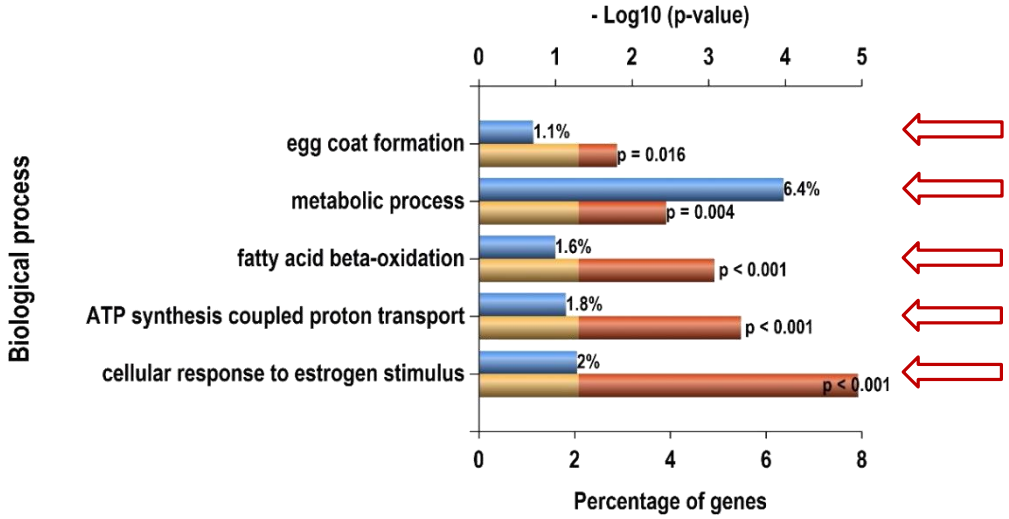
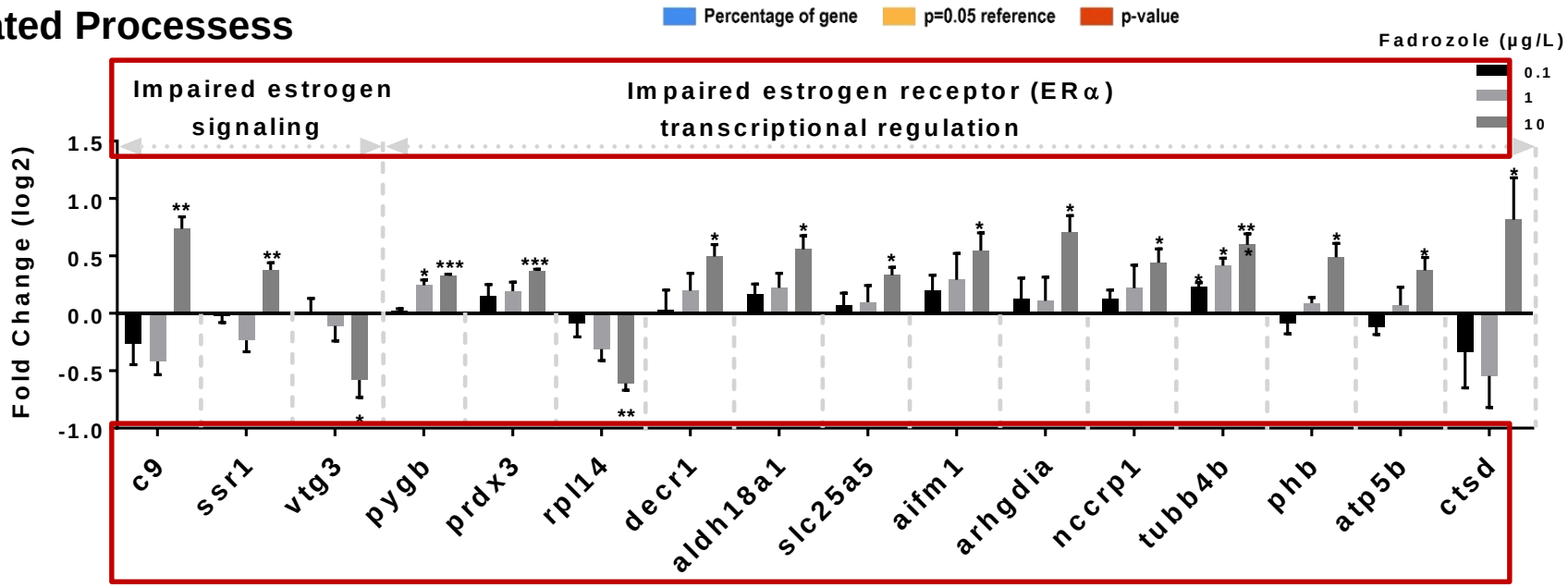
PROTEOMIC ANALYSIS OF OVARY



Potential molecular biomaker

Regulated Processess



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TAKE HOME MESSAGE

TAKE HOME MESSAGE

- Proteomics results fit to FSTRA apical endponts and can provide important data for adverse outcome pathway.
- Proteomics-based analysis of sex-specific responses revealed primary impairment in estrogen synthesis following aromatase inhibition.
- Comparing treatment to normal conditions would help to determine candidate molecular toxicty biomarkers for early prediction of apical endpoints.
- The obtained result further support the need for the integration of –omics approach into new test strategies to provide a link between apical effects and specific chemical-induced MoAs.

Thank you very much for your attention!

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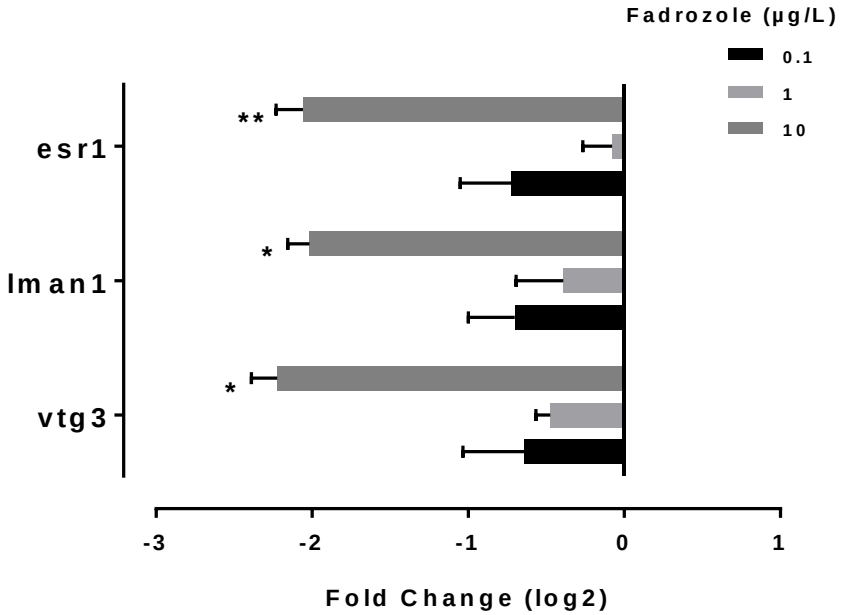
Real-time PCR Verifications

Female Liver – Validation of expression changes of proteins involved in steroid biosynthesis



The direction of expression changes of validated genes were consistent with proteomic analysis.

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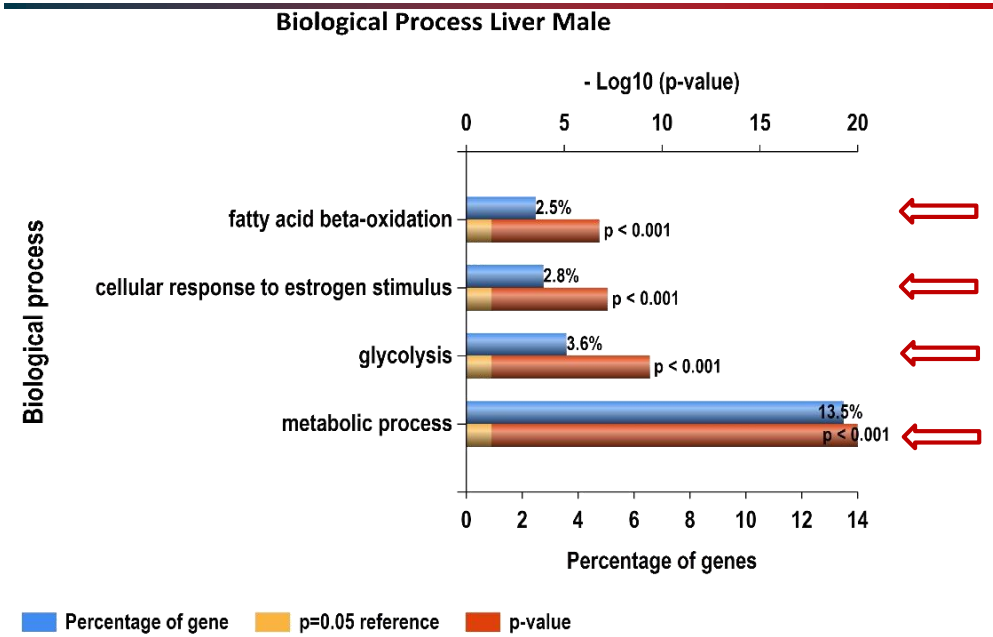
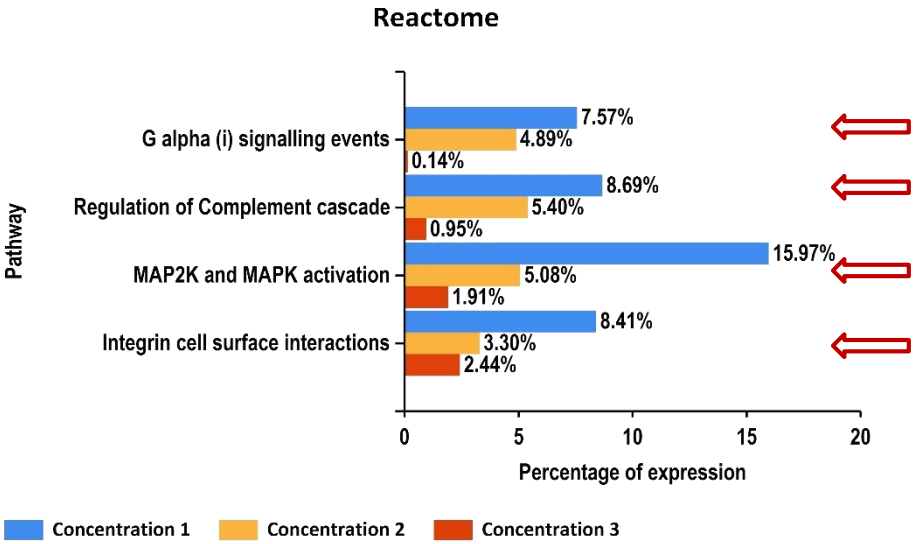
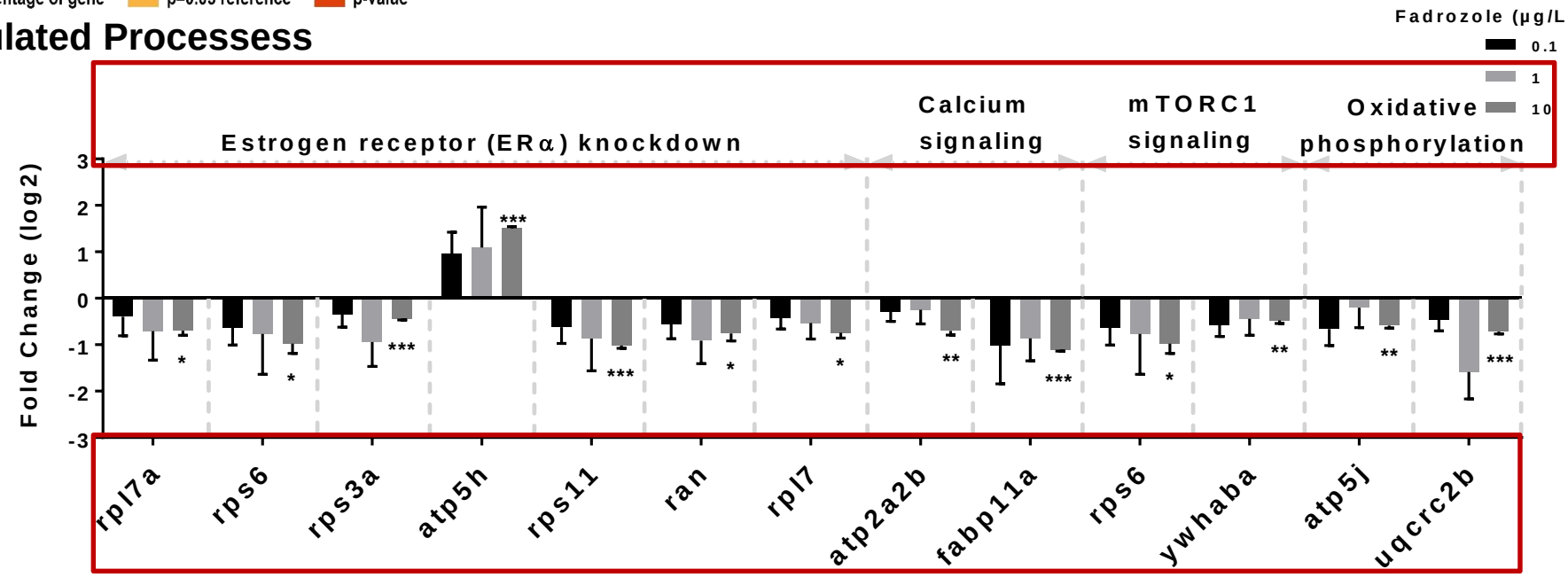
PROTEOMIC ANALYSIS OF MALE LIVER





Regulated Processess



Potential molecular biomaker

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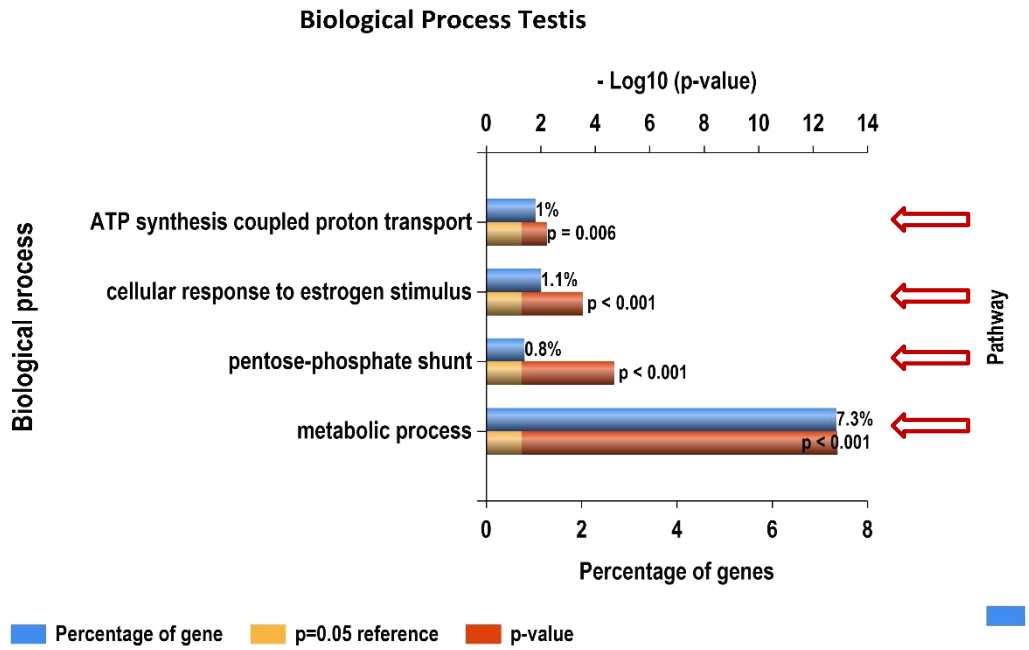
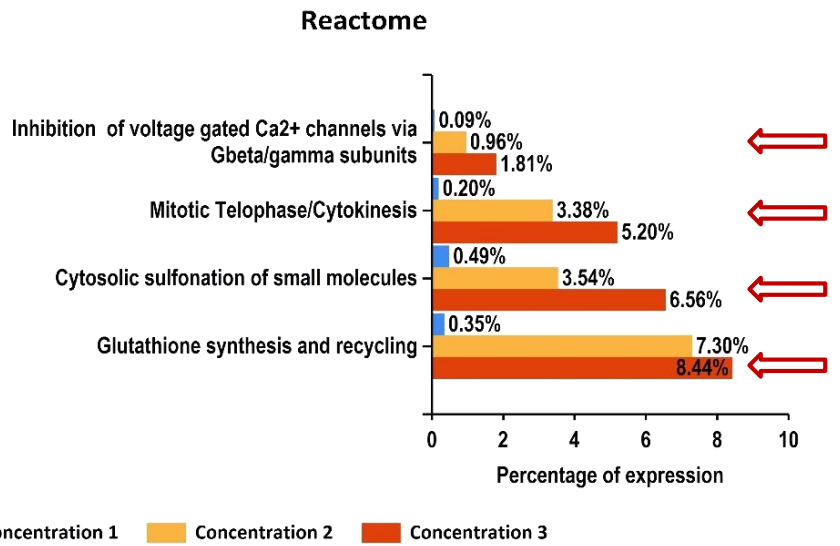
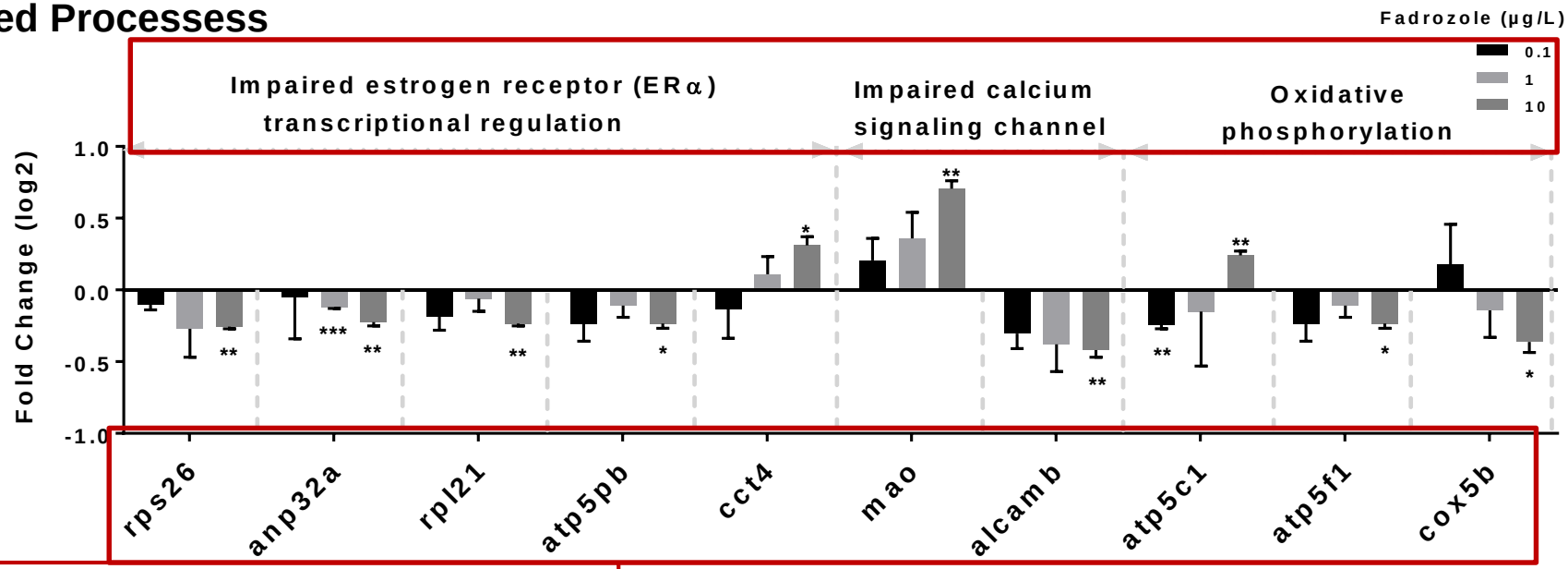
PROTEOMIC ANALYSIS OF TESTIS





Regulated Processess



Potential molecular biomaker

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