Elucidating the Molecular Effects of Di-n-butyl Phthalate Exposure on the Testis

Jill Patel[1,4], Jason Ramirez[1,4], Hovhaness M. Saribekyan[2,3,4], Zelieann R. Craig[5,6], and Estela J. Jauregui[2,3,4]
Arizona College of Osteopathic Medicine[1], College of Graduate Studies[2], Department of Physiology[3], Midwestern University[4],
School of Animal & Comparative Biomedical Sciences[5], University of Arizona[6]

Introduction

- Di-n-butyl phthalate (DBP) is a widely used plasticizer found in cosmetics, food packaging, clothing, and medical devices, leading to continuous human exposure through ingestion, inhalation, and dermal absorption[1].
- Environmentally relevant exposure levels are estimated at ~7–10 µg/kg/day, raising concern about DBP as a potential endocrine-disrupting chemical.
- Infertility rates are increasing worldwide, and environmental contaminants such as phthalates may contribute to reproductive dysfunction[2].
- Previous studies show that DBP exposure impairs steroidogenesis and male reproductive function, highlighting the need to better understand its molecular mechanisms and impact on fertility[3].

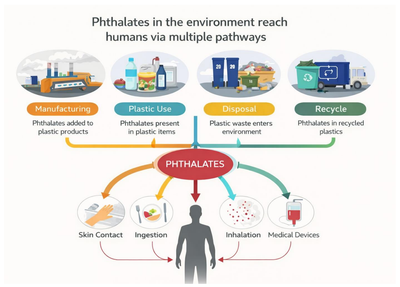

Figure 1: Phthalates can leach from plastics into consumer products, leading to human exposure through multiple routes. These endocrine-disrupting chemicals may adversely impact human health[4].

Objective

To investigate the effects of DBP exposure on the testicular transcriptome and identify molecular pathways disrupted in the whole testis, while validating the RiboTag$^+$/*Cyp17*iCre$^+$ mouse model for Leydig cell–specific transcriptomic analyses.

Methods

- Male RiboTag$^+$/*Cyp17*iCre$^+$ mice (60 days old) were orally treated with DBP (100 µg/kg/day) or corn oil (control) for 10 days.
- Whole testes were collected and RNA was extracted for gene expression analysis.
- RNA sequencing (RNA-seq) was performed to identify differentially expressed genes in DBP-treated mice (n = 3) compared with controls (n = 3).
- Gene Ontology and Reactome pathway enrichment analyses were conducted using DAVID.
- Immunohistochemistry (IHC) using an anti-hemagglutinin (HA) antibody was conducted to confirm HA-tag expression and validate effective RiboTag labeling of Leydig cells.

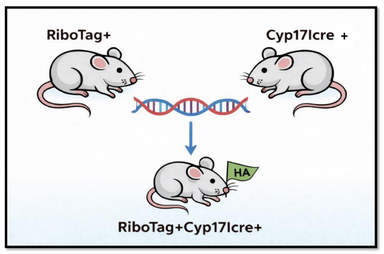

Figure 2: Illustrates the genetic combination leading to the mouse model used in this study.

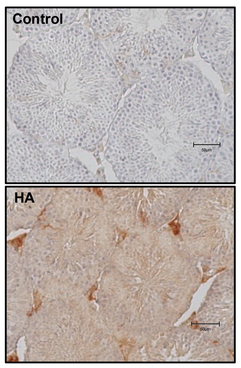

Figure 3: IHC for HA in testicular cross-sections showing control and HA-stained sections.

Results

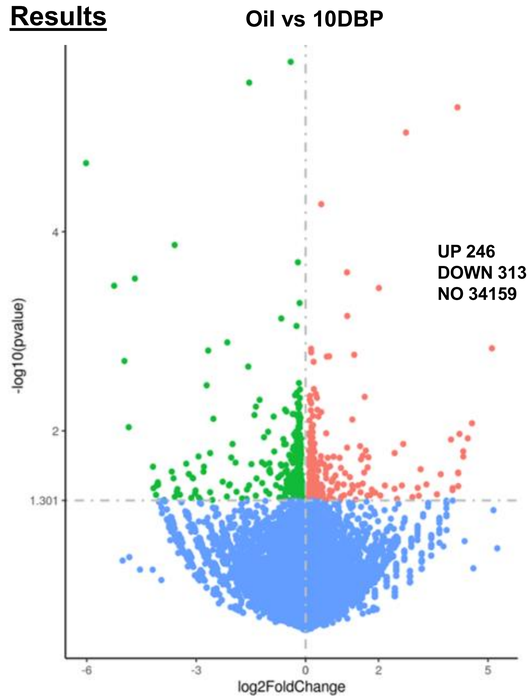

Figure 4: Heat Map of Gene Expression Changes. It highlights 246 genes that are significantly upregulated and 313 genes that are significantly downregulated (p-value < 0.05).

Gene Alterations Associated with Biological Processes, Cellular Components, and Reactome Pathways

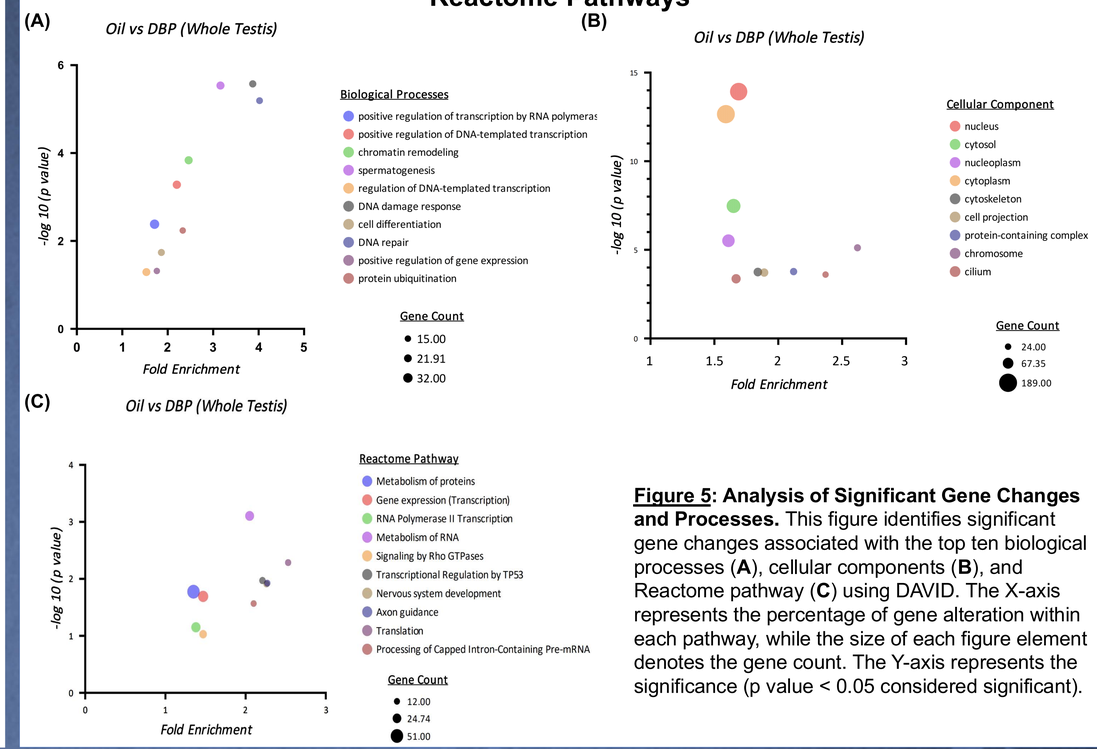

Figure 5: Analysis of Significant Gene Changes and Processes. This figure identifies significant gene changes associated with the top ten biological processes (**A**), cellular components (**B**), and Reactome pathway (**C**) using DAVID. The X-axis represents the percentage of gene alteration within each pathway, while the size of each figure element denotes the gene count. The Y-axis represents the significance (p value < 0.05 considered significant).

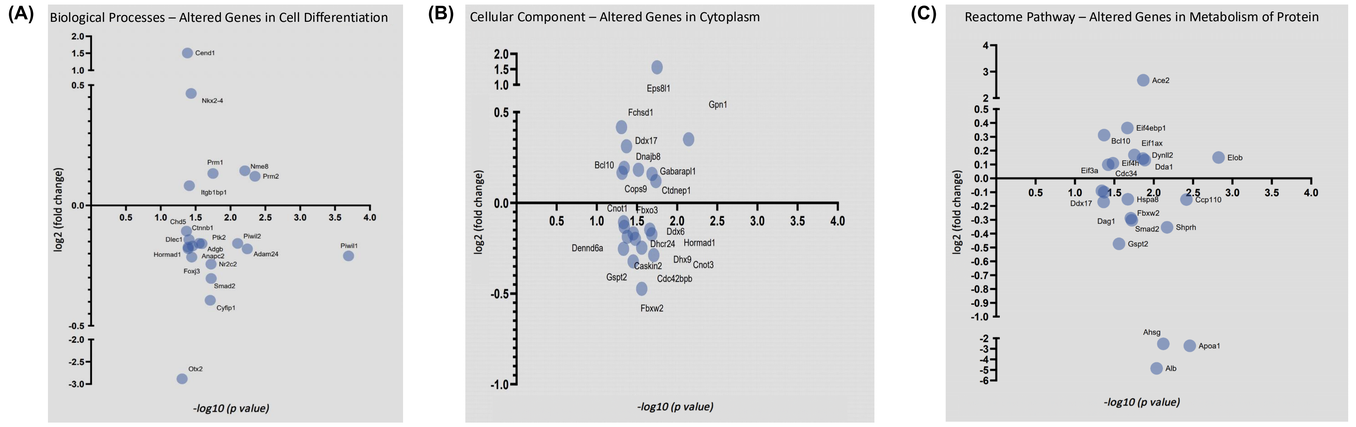

Figure 6: Differentially Expressed Genes Associated with Enriched Pathways following DBP Exposure. Scatter plots showing genes linked to Biological Processes (cell differentiation) (**A**), Cellular Components (cytoplasm) (**B**), and Reactome pathways (protein metabolism) (**C**). The x-axis represents −log10 (p value) and the y-axis represents log2 (fold change).

Conclusion

- HA immunostaining confirmed RiboTag labeling of Leydig cells, validating the model for future Leydig cell–specific transcriptomic studies.
- RNA-seq identified 246 upregulated and 313 downregulated genes in DBP-treated testes ($p < 0.05$).
- Enriched pathways included transcriptional regulation, spermatogenesis, cell differentiation, DNA damage response, and protein metabolism.
- Protein synthesis and degradation pathways (e.g., translation initiation and ubiquitin–proteasome pathways) were significantly affected.
- Overall, DBP exposure alters the testicular transcriptome, providing insight into molecular mechanisms of phthalate-induced reproductive toxicity.

References

1. Wang Y, Qian H. Phthalates and their impacts on human health. *Healthcare (Basel).* 2021;9(5):603. https://doi.org/10.3390/healthcare9050603
2. Kramp ME. Combined manual therapy techniques for the treatment of women with infertility: a case series. *J Am Osteopath Assoc.* 2012;112(10):680–684.
3. Källsten L, Almamoun R, Pierozan P, et al. Adult exposure to di-n-butyl phthalate (DBP) induces persistent effects on testicular cell markers and testosterone biosynthesis in mice. *Int J Mol Sci.* 2022;23(15):8718. https://doi.org/10.3390/ijms23158718
4. Katsikantami I, Sifakis S, Tzatzarakis MN, et al. A global assessment of phthalates burden and related links to health effects. *Environment International.* 2016;97:212–236.

Acknowledgements:

This work was supported by the Physiology Department Start-up Research Funds (EJ), and the Midwestern University Kenneth A. Suarez Research Fellowship 2025 (JP).