Effects of Maternal Oxycodone Exposure on Mouse Placental Development

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Background

Opioid Crisis

- Correlation between maternal opioid use disorder and detrimental fetal development effects have been observed (2, 6).
- Maternal exposure to oxycodone is correlated with specific changes in hormone levels that are important in the development of the conceptus; it also causes changes in placental structure, namely to the trophoblast giant cell layer(1).
- Maternal exposure to opioids is also correlated with postnatal health impacts in offspring (6).

The Role of the Placenta in Fetal Development

- The placenta allows for the exchange of gases, nutrients, and waste (3). Gross and microanatomical structure of the mouse placenta is shown below (**Figure 1**).
- The placenta possesses an opioid response system that is sensitive to opioids present in the maternal blood stream (4).

The Role of Trophoblast Stem Cells in the Placenta

- Trophoblast cells form the layer of placenta most proximal to the maternal uterine tissue and blood supply (5).
- The differentiation of trophoblast stem cells into specialized trophoblast cell types promotes necessary changes in maternal physiology (5).

Hypothesis

Changes in placental structure will be correlated with alteration in expression of key genes in the affected regions of the placenta.

Methods

Dosing

Twelve CF1 mice were randomly assigned to either an oxycodone treatment or saline control group. The mice received daily doses of 5 mg oxycodone/ kg body weight or saline control via intraperitoneal injection beginning two weeks prior to breeding.

Analysis of Functional Changes

Placenta samples were collected at embryonic age 12.5, and the corresponding fetal sex was determined. RNA-seq revealed gender specific changes in gene expression between the oxycodone and control groups. Quantitative PCR trials were conducted to validate changes in the regulation of *Ceacam11, Ceacam12, Ceacam13, Ceacam14, Prl2bl, Prl7bl, Tpbpa*, and *Tpbpb*. These genes were differentially expressed in oxycodone female vs control female placenta samples. *GAPDH* served as an internal reference gene for these trials.

Analysis of Gene Expression in Mouse Trophoblast Stem Cells

Quantitative PCR analyses were conducted to analyze the expression of *Ceacam11, Ceacam12, Ceacam 13, Ceacam14, Prl2bl, Prl7bl, Tpbpa,* and *Tpbpb* in differentiated mouse trophoblast stem cells. *GAPDH* served as the internal reference gene for these trials. The expression of these genes in undifferentiated mouse trophoblast stem cells served as controls for these analyses.

Statistical Analysis

Quantitative PCR results were analyzed using the ΔΔCt method. Fold changes were calculated to compare gene expression between the OXY and CTL placenta. Fold changes were also used to compare gene expression between undifferentiated and differentiated mouse trophoblast stem cells. All graphs depict the mean ± SEM.

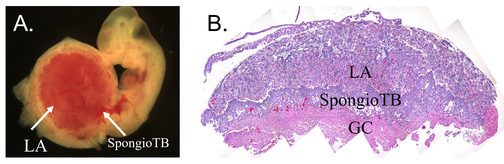

Figure 1: SpongioTB Location in the mouse placenta. A.) A sub-gross view of the placenta is shown. B.) A histological view of the placenta is shown. The labyrinth region (LB), spongiotrophoblast (spongioTB), and giant cell region (GC) are shown.

Results

qPCR Validates RNA-Seq Results

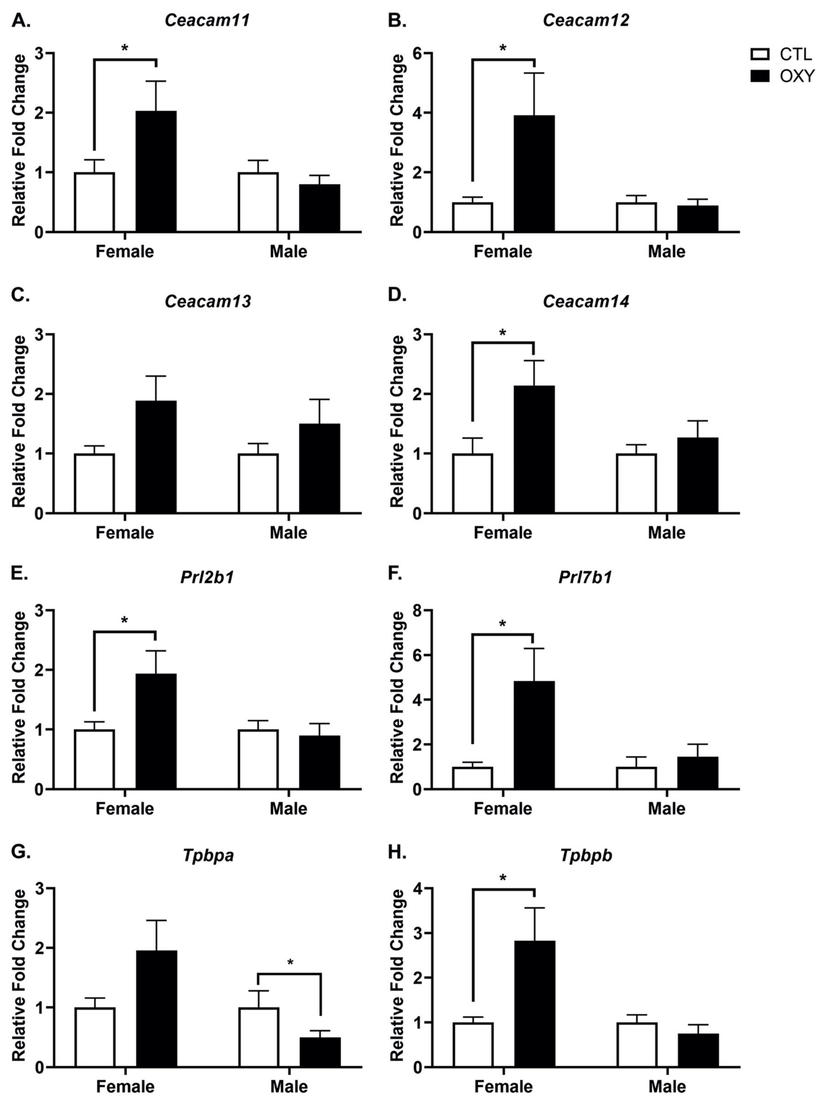

Figure 2: Comparison of gene expression in CTL vs OXY placenta. The data are presented as mean ± SEM. The graphs depict the relative expression of *Ceacam11, Ceacam12, Ceacam14, Prl2b1, Prl7b1, Tpbpa,* and *Tpbpb* in both OXY and CTL placenta. Results were further broken down according to sex to facilitate the analysis of sex dependent gene expression in the samples. All significant differences are denoted by an asterisk. *GAPDH* served as the internal reference gene for these analyses.

Mouse Trophoblast Stem Cells Express Validated Genes

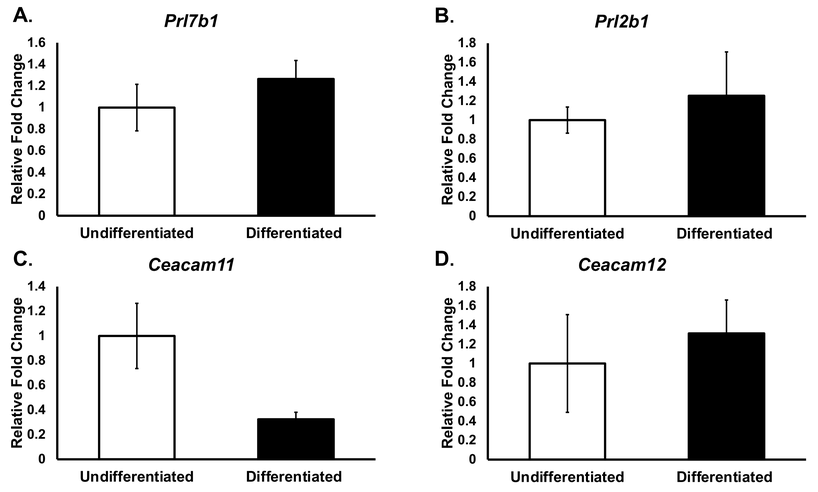

Figure 3: Comparison of gene expression in undifferentiated vs differentiated mouse trophoblast stem cells. The data are presented as mean ± SEM. The graphs depict the relative expression of *Prl2b1, Prl7b1, Ceacam11, and Ceacam 12* in both undifferentiated and differentiated mouse trophoblast stem cells. *GAPDH* served as the internal reference gene for these analyses.

Conclusions & Future Aims

- Sex dependent changes in gene expression were confirmed in placenta samples exposed to oxycodone.
- Upregulation of *Ceacam11, Ceacam12, Ceacam14, Prl2bl, Prl7bl,* and *Tpbpb* was confirmed in the female OXY placenta samples.
- Downregulation of *Tpbpa* was confirmed in male OXY placenta samples.
- All eight genes of interest are expressed by mouse trophoblast stem cells prior to and following differentiation.
- *Prl7b1, Prl2b1, and Ceacam12* showed a trend toward upregulation in differentiated mouse trophoblast stem cells while *Ceacam12* showed a trend towards downregulation.

Acknowledgements

We would like to thank Robert Schmidt, Sarabjit Kaur, Camryn Long, Tess Willemse, and Alaina Baumgart for assisting with animal husbandry throughout this experiment.

References

1. Čemerikić, B., Genbačev, O., Šulović, V., Beaconsfield, R., (1988). Life Sciences, 42(18), 1773-1779. Retrieved from https://www.sciencedirect.com/science/article/abs/pii/0024320588900446?via%3Dihub#!
2. Centers for Disease Control and Prevention. (2019). Centers for Disease Control and Prevention. Retrieved April, 28, 2020.
3. Mao, J., Jain, A., Denslow, N. D., Nouri, M.-Z., Chen, S., Wang, T., . . . Rosenfeld, C. S.(2020). Proceedings of the National Academy of Sciences,201919563. doi:10.1073/pnas.19195631172.
4. Rosenfeld C. S. (2015). Endocrinology, 156(10), 3422–3434. https://doi.org/10.1210/en.2015-1227
5. Simmons, D. G., & Cross, J. C. (2005). *Developmental Biology, 284*(1), 12-24. doi:https://doi.org/10.1016/j.ydbio.2005.05.010
6. Yazdy, M. M., Desai, R. J., & Brogly, S. B. (2015). Journal of pediatric genetics, 4(2), 56–70. https://doi.org/10.1055/s-0035-1556740