Estrogen Bioassay

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Executive Summary

- After controlling for predictors and random effects, uterus weight indeed showed an increasing dose response trend for EE and a decreasing dose response trend for ZM.
- Dose responses varied across labs, and there are approximately 8 outlier labs.
- Protocols differed in their sensitivity to detecting EE and ZM effects, protocol B is recommended for ZM, and protocol A is recommended for EE.

Introduction

To test the effect of estrogen agonists and antagonists on a particular hormonal response, researchers varied the amount of the agonist or antagonist given to the rats. The response is the weight of the uterus, and it is expected that uterus weight shows an increasing trend as the dosage of estrogen agonists increase, and a decreasing trend as the dosage of estrogen antagonists increase. In this article, we will try to explore the experiment results by leveraging the multilevel regression model to examine the above hypothesis. Specifically, We will use the logged uterus weight as our response variable and include EE, ZM, protocol, and two interaction terms between EE & protocol and ZM & protocol. Here are the questions we want to answer in this report:

- Is the uterotrophic bioassay successful at identifying the estrogenic effects of EE and anti-estrogenic effects of ZM? That is, after controlling for predictors and random effects, does uterus weight exhibit an increasing dose-response trend for EE and a decreasing dose-response trend for ZM?
- Does the dose-response vary across labs? If so, are there certain labs that appear to be outliers?
- Do the protocols differ in their sensitivity to detecting EE and ZM effects? If so, is there one protocol that can be recommended?

The article is organized into the following sections: Data and EDA, Model Selection, Results, and Conclusion. We will outline key predictors and trends in the EDA section, identify the best model in the Model Selection section, and answer our key inference questions in the Results and Conclusion sections.

Data and EDA

The dataset includes bioassay experimental results from 19 different laboratories across the world. Most laboratories performed all 4 different prototypes of the experiment. Each laboratory carried out multiple groups of experiments that varied in the protocol, estrogen agonist (EE), and antagonist dosage (ZM). In particular, groups 1 and 2 are control groups where mice were not given any estrogen treatment. The effect of estrogen is quantified by measuring the weight of the rat's uterus.

The final dataset has 2677 experimental samples. For each sample, the weight of the mouse and its uterus, estrogen (agonist and antagonist) dosage, protocol types, group number, and the lab was recorded. There were 4 rows with missing data and we decided to omit them since the portion of missing data is small. Moreover, we decided to conduct a log transformation for our response variable: uterus weight, because the original distribution is not normally distributed.

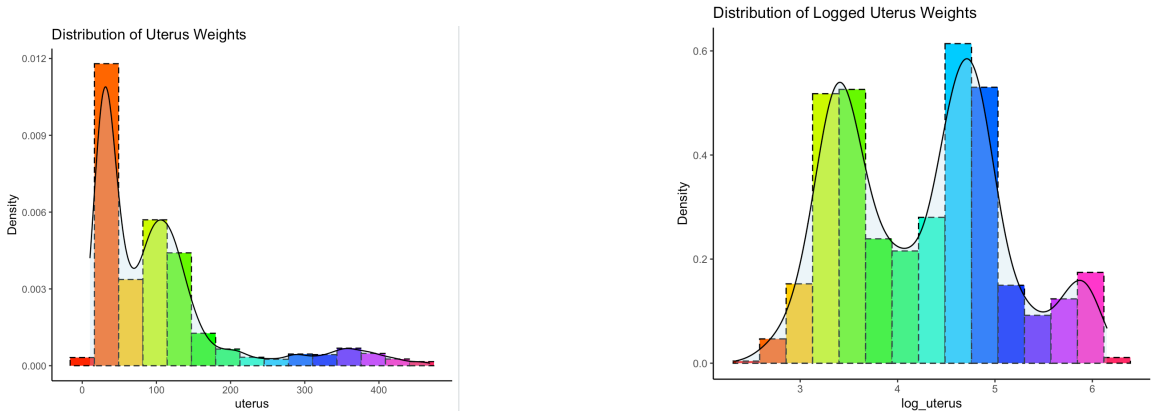

Figure 1. Distribution of Response Variable before and after transformation

We noticed, even after the transformation, there are two peaks in the distribution. We discovered that the two peaks were likely caused by the protocols. Protocol A and B use immature rats that naturally have a smaller, lighter uterus, and protocol C and D use mature rats that have larger, heavier uterus. We were convinced that this is the nature of the dataset and no other transformation method can fix the issue.

For EDA, we explored the effect of each predictor against the response variable. The most notable finding was that EE has a positive correlation and ZM has a negative correlation with the response variable. We also saw weight and protocol has an association with the response variable. Since half of the protocol uses immature rats and a half uses mature rats, this suggests that there is a strong correlation between protocol and mouse weight, hinting that we should only choose one predictor from the two in our model. This was further confirmed by the plot below:

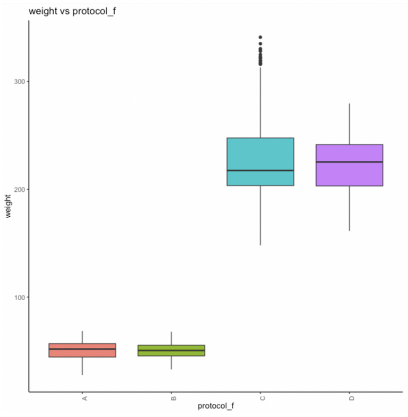

Figure 2. Boxplot of Weight vs Protocol

We then looked at how uterus weight varies across each lab. Although there is a big discrepancy across the medians, the ranges overlap. This suggests that we should group the labs when modeling. Finally, we checked for interaction terms and did not notice any significant relationships. (See Figure 1 in Appendix)

Model Selection

We began our modeling process with a baseline model utilizing log(uterus weight) as the response variable, and having predictors EE, ZM, Weight, Protocol, and individual intercepts for each lab. We used ANOVA tests to consider predictors and interactions individually, and determine if they improved our model. With confirmation from our ANOVA tests, we included interactions between EE & Protocol and ZM & Protocol. We also looked at the confidence intervals of our coefficients and took note of coefficients with confidence intervals that included zero. We removed the predictor "rat weight" because its confidence interval contained zero, and it correlated heavily with the protocol. After this model selection process, we arrived at our final model below.

$$\text{Log}(Uterus\ Wt_{ij}) = (\beta_0+\gamma_{0j}) + \beta_1 EE_{ij} + \beta_2 ZM_{ij} + \beta_3 Protocol_{ij} + \beta_4 EE{:}\,Protocol_{ij} + \beta_5 ZM{:}\,Protocol_{ij}$$
$$i = 1, \dots n_j \;;\quad j = 1, \dots J$$
$$\varepsilon_{ij} \sim N(0, \sigma^2)$$
$$\gamma_{0j} \sim N(0, \tau_0^2)$$

After creating our final model we used our covariance matrix to check our model for multicollinearity and redundant variables. We were satisfied with our low covariance values and went on to check our model assumptions. We created graphs to check Linearity, Independence and Equal Variance, and Normality. Due to the categorical nature of our EE and ZM dosage, our linearity plots did not offer useful results. However, our Independence, Equal Variance, and Normality plots are below. We found our Independence and Equal Variance assumption to be satisfied, though slightly clustered. We also found our Normality plot to be satisfied, though it wavers slightly due to the 2 clusters in weight. All of our modeling assumptions improved with our logarithmic response variable as opposed to a non-logarithmic response variable.

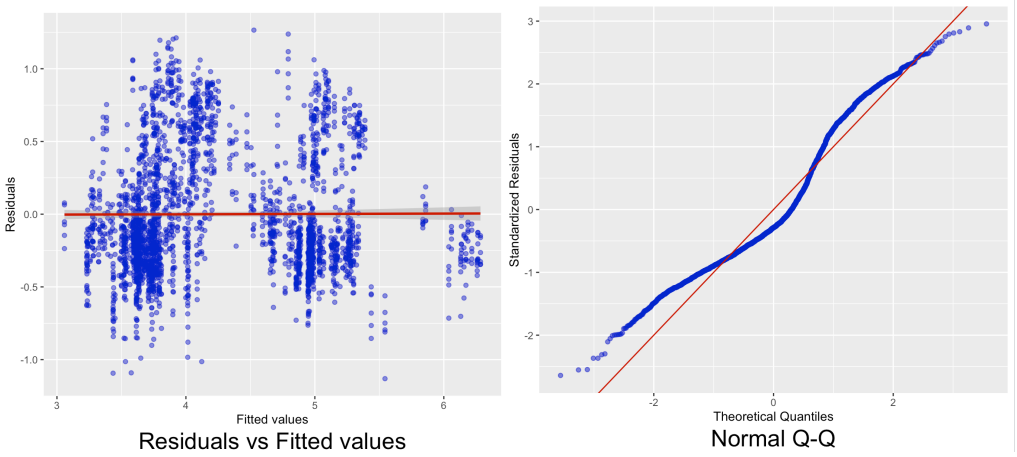

Figure 3. Plots of Model Validation

Results

	log_uterus		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	3.56	3.47 – 3.65	**<0.001**
EE	0.15	0.14 – 0.16	**<0.001**
ZM	-0.20	-0.30 – -0.11	**<0.001**
protocol_f [B]	0.12	0.06 – 0.17	**<0.001**
protocol_f [C]	1.36	1.30 – 1.42	**<0.001**
protocol_f [D]	1.36	1.28 – 1.44	**<0.001**
ZM * protocol_f [B]	-0.63	-0.77 – -0.49	**<0.001**
ZM * protocol_f [C]	-0.30	-0.45 – -0.15	**<0.001**
ZM * protocol_f [D]	-0.54	-0.75 – -0.34	**<0.001**
EE * protocol_f [B]	-0.00	-0.01 – 0.01	0.899
EE * protocol_f [C]	-0.04	-0.05 – -0.02	**<0.001**
EE * protocol_f [D]	-0.02	-0.04 – -0.00	**0.029**
Random Effects			
σ^2	0.19		
$\tau_{00\ lab\_f}$	0.03		
ICC	0.15		
$N_{\ lab\_f}$	19		
Observations	2677		
Marginal R^2 / Conditional R^2	0.703 / 0.747		

Table 1. Regression Results

According to the above regression table, our analysis sought to answer 3 inference questions about the effects of estrogenic agonist and antagonist on uterus weight. Specifically, we were asked to assess the effects of EE (agonist) and ZM (antagonist) on uterus weight. We found that EE was associated with a significant positive effect on uterus weight. EE has a positive coefficient of 0.15, resulting in an exponentiated point estimate of 1.16 and a 95% confidence interval of (1.16, 1.18). Thus, with a 1 unit increase in EE the uterus weight increased on average by 16%. Similarly, ZM was associated with a significant negative effect on uterus weight. ZM has a negative coefficient of -0.20, resulting in an exponentiated point estimate of 0.81 and a 95% confidence interval of (0.74, 0.89). Thus, with a 1 unit increase in EE, the uterus weight decreases on average by 18.5%.

Furthermore, we found that dose responses vary across labs. Looking at the dot-plot below, there are around 4 labs on two ends of the plot that do not contain 0 in the 95% confidence interval. These labs distinctively vary from the mean labs towards the center. As a result, this shows that the dose response results measured, does vary across the labs.

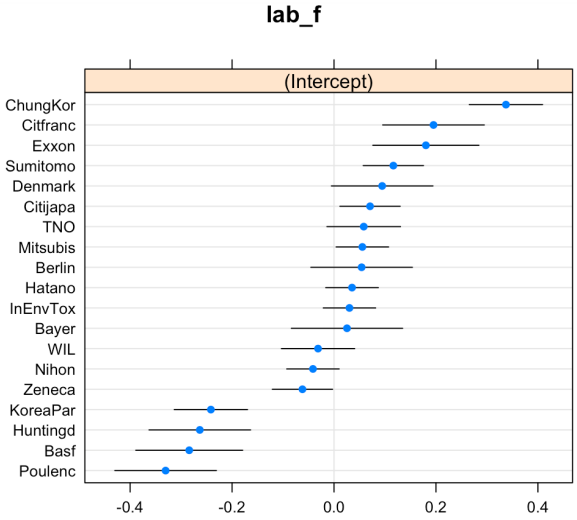

Figure 4. Dotplot of Lab Variations

Besides, we found that protocols differ in their sensitivity to detecting EE and ZM effects. Looking at the interaction between EE & protocol, we identified Protocol A, the baseline, to be the most sensitive. Protocol B, C, and D, all have a negative effect on uterus weight compared to the baseline Protocol A. Similarly, we looked at the interaction between ZM & protocol, we identified Protocol B to be the most sensitive. Protocol B has the largest negative slope compared to the other protocols. As a result, Protocol B is the most sensitive protocol for ZM, compared to the baseline Protocol A.

Conclusion

This report tested the hypothesis made by estrogen agonists and antagonists researchers by using a multilevel regression model. The empirical results echoed the expectation of the researchers showing that logged uterus weight indicated an increasing dose response trend for EE, and a decreasing dose response trend for ZM. However, we would argue that there are limitations that would affect our study. First, the response variable was not completely normally distributed which would inevitably affect the model explanatory power. Secondly, checking for model assumptions raised concerns regarding normality and linearity. However, there was insufficient data to further improve the model. To conclude, this analysis only serves as a preliminary insight into the questions and there remains room for improvement.