Experiment 2 – Red Eggs

## Experiment 2 Red Eggs–Overview

As mentioned in the previous document, the red egg stage is the second of three developmental stages at which egg viability was assessed. In normal development, there is a near total correlation between egg viability and eggs turning from white to red. Irradiation, however, can cause fatal developmenal problems aftere this color shift which reduced the reliablity of egg color as an indicator of fertility. As previously, the sexes are separated because of expected differences in radiosensitivy and the proportion of red eggs are by x-ray treatment (0, 100, 200, 300, or 400 Gy)

library(readxl)  
# Load data and display first few rows  
df\_red <- read\_excel("Dataset.xlsx", sheet = "Development\_400Gy")  
  
# Set x-ray dose as a factor (categorical)  
df\_red$dose <- as.factor(df\_red$dose)

Separate sexes

library(dplyr)  
# Isolate data for females and display the first few rows  
females <- df\_red %>%   
 filter(sex == "female")  
females

## # A tibble: 37 × 5  
## rep FemID sex dose sum\_red  
## <chr> <chr> <chr> <fct> <dbl>  
## 1 c C2 female 0 175  
## 2 c C5 female 0 145  
## 3 e E1 female 0 107  
## 4 e E2 female 0 14  
## 5 e E3 female 0 0  
## 6 e E4 female 0 0  
## 7 e E5 female 0 0  
## 8 c C7 female 100 110  
## 9 c C9 female 100 4  
## 10 e E10 female 100 96  
## # ℹ 27 more rows

# Isolate data for males and display the first few rows  
males <- df\_red %>%   
 filter(sex == "male")  
males

## # A tibble: 37 × 5  
## rep FemID sex dose sum\_red  
## <chr> <chr> <chr> <fct> <dbl>  
## 1 b B21 male 0 198  
## 2 b B22 male 0 208  
## 3 b B23 male 0 180  
## 4 b B24 male 0 240  
## 5 b B25 male 0 217  
## 6 d D1 male 0 25  
## 7 d D3 male 0 4  
## 8 d D4 male 0 179  
## 9 d D5 male 0 19  
## 10 b B1 male 100 160  
## # ℹ 27 more rows

## Summary and statistical analysis of red eggs from irradiated females

library(FSA) # for SE  
# n, mean, and SE by dose for females  
females %>%   
 group\_by(dose) %>%  
 summarise(nObs = sum(!is.na(sum\_red)),  
 mn = mean(sum\_red, na.rm = TRUE),  
 sem = se(sum\_red),  
 pct\_sterile = 100\*(1-(sum(sum\_red > 0)/nObs)))

## # A tibble: 5 × 5  
## dose nObs mn sem pct\_sterile  
## <fct> <int> <dbl> <dbl> <dbl>  
## 1 0 7 63 29.1 42.9  
## 2 100 4 52.5 29.3 25   
## 3 200 9 12.3 5.38 11.1  
## 4 300 8 7.62 4.56 50   
## 5 400 9 2.11 1.14 55.6

The mean and SE are consistent with overdispersed count data. The negative binomial is appropriate for such data. The percent sterility calculation reminds us that some females are laying at least some red eggs.

# Analysis of Deviance using GLM with negative binomial--females  
library(MASS)  
m1 <- MASS::glm.nb(sum\_red ~ dose, data = females)  
summary(m1)

##   
## Call:  
## MASS::glm.nb(formula = sum\_red ~ dose, data = females, init.theta = 0.2966718039,   
## link = log)  
##   
## Coefficients:  
## Estimate Std. Error z value Pr(>|z|)   
## (Intercept) 4.1431 0.6956 5.957 2.58e-09 \*\*\*  
## dose100 -0.1823 1.1538 -0.158 0.874442   
## dose200 -1.6308 0.9313 -1.751 0.079926 .   
## dose300 -2.1117 0.9600 -2.200 0.027823 \*   
## dose400 -3.3959 0.9544 -3.558 0.000374 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## (Dispersion parameter for Negative Binomial(0.2967) family taken to be 1)  
##   
## Null deviance: 53.093 on 36 degrees of freedom  
## Residual deviance: 38.536 on 32 degrees of freedom  
## AIC: 246.58  
##   
## Number of Fisher Scoring iterations: 1  
##   
##   
## Theta: 0.2967   
## Std. Err.: 0.0784   
##   
## 2 x log-likelihood: -234.5770

Examine model fit for females

# Calculate p-value under the chi-squared distribution  
dev <- deviance(m1)  
df <- df.residual(m1)  
p\_value <- pchisq(dev, df, lower.tail = FALSE)  
cat("Chi-square goodness-of-fit p-value:", p\_value, "\n")

## Chi-square goodness-of-fit p-value: 0.1978609

The residual deviance is not significantly different (P > 0.05) from the expected value for the residual degrees of freedom.

library(car)  
# Model parameters with type II degrees of freedom  
Anova(m1)

## Analysis of Deviance Table (Type II tests)  
##   
## Response: sum\_red  
## LR Chisq Df Pr(>Chisq)   
## dose 14.557 4 0.005713 \*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Overall, there were significant differences. Proceeding to comparisons of multiple means.

library(emmeans)  
library(multcomp)  
# Means separation  
emm\_fem <- emmeans(m1, ~ dose, type = "response")  
multcomp::cld(emm\_fem, Letters = LETTERS, decreasing = TRUE)

## dose response SE df asymp.LCL asymp.UCL .group  
## 0 63.00 43.80 Inf 16.117 246.3 A   
## 100 52.50 48.30 Inf 8.641 319.0 A   
## 200 12.33 7.64 Inf 3.664 41.5 AB   
## 300 7.62 5.04 Inf 2.085 27.9 AB   
## 400 2.11 1.38 Inf 0.586 7.6 B   
##   
## Confidence level used: 0.95   
## Intervals are back-transformed from the log scale   
## P value adjustment: tukey method for comparing a family of 5 estimates   
## Tests are performed on the log scale   
## significance level used: alpha = 0.05   
## NOTE: If two or more means share the same grouping symbol,  
## then we cannot show them to be different.  
## But we also did not show them to be the same.

## Summary and statistical analysis of red eggs from females mated with irradiated males

# n, mean, and SE by dose for females  
males %>%   
 group\_by(dose) %>%  
 summarise(nObs = sum(!is.na(sum\_red)),  
 mn = mean(sum\_red, na.rm = TRUE),  
 sem = se(sum\_red),  
 pct\_sterile = 100\*(1-(sum(sum\_red > 0)/nObs)))

## # A tibble: 5 × 5  
## dose nObs mn sem pct\_sterile  
## <fct> <int> <dbl> <dbl> <dbl>  
## 1 0 9 141. 31.9 0   
## 2 100 7 151 47.5 14.3  
## 3 200 5 109. 37.6 0   
## 4 300 7 39.3 23.4 14.3  
## 5 400 9 17.3 12.3 33.3

# Analysis of Deviance using GLM with negative binomial--males  
m2 <- MASS::glm.nb(sum\_red ~ dose, data = males)  
summary(m2)

##   
## Call:  
## MASS::glm.nb(formula = sum\_red ~ dose, data = males, init.theta = 0.527194438,   
## link = log)  
##   
## Coefficients:  
## Estimate Std. Error z value Pr(>|z|)   
## (Intercept) 4.94955 0.45994 10.761 < 2e-16 \*\*\*  
## dose100 0.06773 0.69532 0.097 0.92240   
## dose200 -0.25454 0.76990 -0.331 0.74094   
## dose300 -1.27869 0.69725 -1.834 0.06667 .   
## dose400 -2.09692 0.65476 -3.203 0.00136 \*\*   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## (Dispersion parameter for Negative Binomial(0.5272) family taken to be 1)  
##   
## Null deviance: 56.944 on 36 degrees of freedom  
## Residual deviance: 44.726 on 32 degrees of freedom  
## AIC: 386.47  
##   
## Number of Fisher Scoring iterations: 1  
##   
##   
## Theta: 0.527   
## Std. Err.: 0.118   
##   
## 2 x log-likelihood: -374.469

Examine model fit for males

# Calculate p-value under the chi-squared distribution  
dev <- deviance(m2)  
df <- df.residual(m2)  
p\_value <- pchisq(dev, df, lower.tail = FALSE)  
cat("Chi-square goodness-of-fit p-value:", p\_value, "\n")

## Chi-square goodness-of-fit p-value: 0.06688904

The residual deviance is not significantly different (P > 0.05) from the expected value for the residual degrees of freedom.

# Model parameters with type II degrees of freedom  
Anova(m2)

## Analysis of Deviance Table (Type II tests)  
##   
## Response: sum\_red  
## LR Chisq Df Pr(>Chisq)   
## dose 12.218 4 0.0158 \*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Overall, there were significant differences. Proceeding to comparisons of multiple means.

# Model parameters with type II degrees of freedom  
emm\_males <- emmeans(m2, ~ dose, type = "response")  
multcomp::cld(emm\_males, Letters = LETTERS, decreasing = TRUE)

## dose response SE df asymp.LCL asymp.UCL .group  
## 100 151.0 78.70 Inf 54.34 419.6 A   
## 0 141.1 64.90 Inf 57.29 347.6 A   
## 200 109.4 67.50 Inf 32.62 366.9 AB   
## 300 39.3 20.60 Inf 14.07 109.7 AB   
## 400 17.3 8.08 Inf 6.95 43.2 B   
##   
## Confidence level used: 0.95   
## Intervals are back-transformed from the log scale   
## P value adjustment: tukey method for comparing a family of 5 estimates   
## Tests are performed on the log scale   
## significance level used: alpha = 0.05   
## NOTE: If two or more means share the same grouping symbol,  
## then we cannot show them to be different.  
## But we also did not show them to be the same.