Acrosome integrity

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Introduction

The effect of DMSO and ethanol is evaluated at concentrations from 0 up to 2% on acrosome integrity of spermatozoa.

DMSO

```
ai_dmso <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "acrosome integrity_DMSO")
ai_dmso$donor <- as.factor(ai_dmso$donor)
names(ai_dmso) <- c("donor", "conc", "acrointact", "total")
ai_dmso$acrointact_frac <- ai_dmso$acrointact / ai_dmso$total
skimr::skim(ai_dmso)
```

Table 1: Data summary

Name	ai_dmso
Number of rows	20
Number of columns	5
_____	
Column type frequency:	
factor	1
numeric	4
_____	
Group variables	None

Variable type: factor

skim_variable	n_missing	complete_rate	ordered	n_unique	top_counts
donor	0	1	FALSE	4	6: 5, 7: 5, 8: 5, 9: 5

Variable type: numeric

skim_variable	n_missing	complete_rate	mean	sd	p0	p25	p50	p75	p100	hist
conc	0	1	0.72	0.75	0.00	0.10	0.50	1.00	2.00	
acrointact	0	1	184.85	5.80	168.00	183.00	186.00	189.00	191.00	
total	0	1	200.45	2.01	200.00	200.00	200.00	200.00	209.00	
acrointact_frac	0	1	0.92	0.03	0.84	0.91	0.92	0.94	0.96	

There are four donors, no missing data.

```
table(ai_dmso$donor, as.factor(ai_dmso$conc))
```

```
  0 0.1 0.5 1 2
6 1   1   1 1 1
7 1   1   1 1 1
8 1   1   1 1 1
9 1   1   1 1 1
```

The data are balanced with one observation for each concentration and each donor and no missing data.

```
ai_dmso_m1 <- glmer(cbind(acrointact, total - acrointact) ~ conc + (conc | donor),
  data = ai_dmso, family = binomial(link = "logit"))
summary(ai_dmso_m1)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(acrointact, total - acrointact) ~ conc + (conc | donor)
   Data: ai_dmso

     AIC      BIC   logLik deviance df.resid
   115.9    120.9    -53.0    105.9       15
```

```
Scaled residuals:
     Min       1Q   Median       3Q      Max
-1.04554 -0.48370 -0.02401  0.56732  0.96683

Random effects:
 Groups Name        Variance Std.Dev. Corr
 donor  (Intercept) 0.004637 0.0681
        conc        0.026459 0.1627   1.00
Number of obs: 20, groups:  donor, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.69266    0.09485  28.390   <2e-16 ***
conc        -0.25370    0.11347  -2.236   0.0254 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.219
```

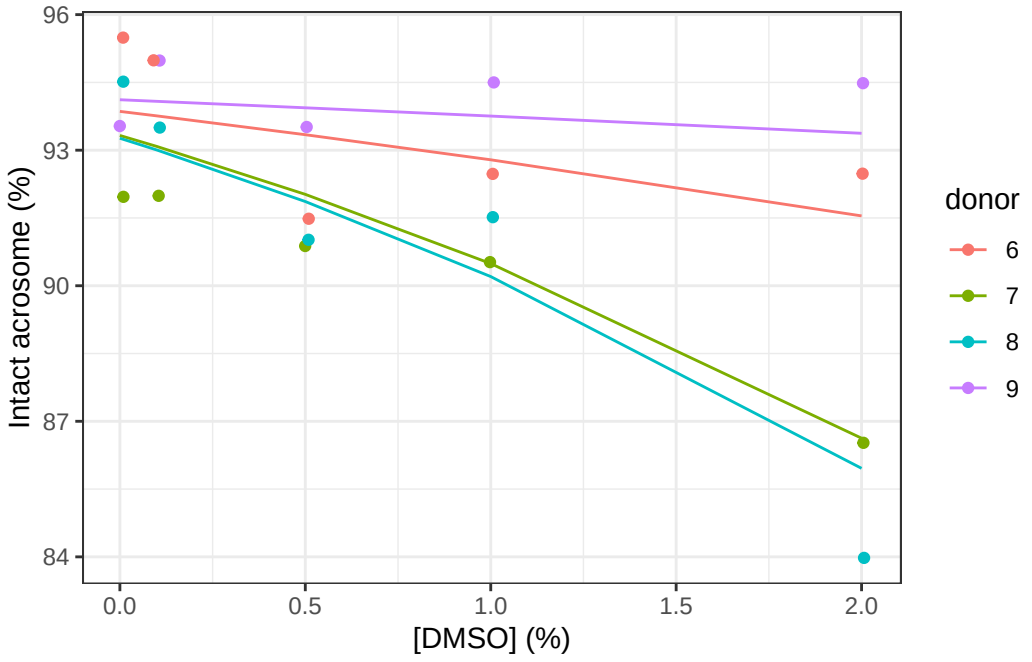
The model is:

,

$$
\begin{aligned}
&\text{acrointact}_i \sim \text{Binomial}(n = 1, \text{prob}_{\text{acrointact}=1} = \widehat{P}) \\
&, \log\left[\frac{\hat{P}}{1-\hat{P}}\right] = \alpha_{j[i]} + \beta_{1j[i]}(\text{conc}) \\
&\begin{pmatrix} \alpha_j \\ \beta_{1j} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu_{\alpha_j} \\ \mu_{\beta_{1j}} \end{pmatrix}, \begin{pmatrix} \sigma^2_{\alpha_j} & \rho_{\alpha_j\beta_{1j}} \\ \rho_{\beta_{1j}\alpha_j} & \sigma^2_{\beta_{1j}} \end{pmatrix}\right), \text{for donor j} = 1, \ldots, \text{J}
\end{aligned}
\quad , \qquad (1)
$$

Here is a plot of this model:

```
ggplot(data = ai_dmso) +
  geom_jitter(aes(x = conc, y = acrointact_frac * 100, col = donor),
    width = 0.01) +
  geom_line(aes(x = conc, y = fitted(ai_dmso_m1) * 100, col = donor)) +
  labs(x = "[DMSO] (%)", y = "Intact acrosome (%)")
```



Generally, slopes are all negative, suggesting a negative concentration effect. Data are rather widespread. Shifts in the intercept per donor is not obvious here, but change in slope is much more marked. We may try simplifying the model so that only slopes vary between donors. Let's check it with a likelihood ratio test:

```
ai_dmso_m2 <- glmer(cbind(acrointact, total - acrointact) ~ conc  + (0 + conc:donor | dono
  data = ai_dmso, family = binomial(link = "logit"))
```

```
boundary (singular) fit: see help('isSingular')
```

```
anova(ai_dmso_m1, ai_dmso_m2, refit = FALSE) # Despite the name, it is indeed a LR test
```

```
Data: ai_dmso
Models:
ai_dmso_m1: cbind(acrointact, total - acrointact) ~ conc + (conc | donor)
ai_dmso_m2: cbind(acrointact, total - acrointact) ~ conc + (0 + conc:donor | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
ai_dmso_m1    5 115.92 120.89 -52.958   105.92
ai_dmso_m2   12 129.20 141.15 -52.601   105.20 0.7123  7     0.9982
```

The likelihood ratio test does not detects significant differences between the full and simplified models at $\alpha = 5\%$. But... the model had a problem because we had a singularity. Let's try the simplification where the random effect `donor` only accounts for the intercept:

```
ai_dmso_m3 <- glmer(cbind(acrointact, total - acrointact) ~ conc  + (1 | donor),
  data = ai_dmso, family = binomial(link = "logit"))
anova(ai_dmso_m1, ai_dmso_m3, refit = FALSE) # Despite the name, it is indeed a LR test
```

```
Data: ai_dmso
Models:
ai_dmso_m3: cbind(acrointact, total - acrointact) ~ conc + (1 | donor)
ai_dmso_m1: cbind(acrointact, total - acrointact) ~ conc + (conc | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
ai_dmso_m3    3 115.50 118.49 -54.752   109.50
ai_dmso_m1    5 115.92 120.89 -52.958   105.92 3.5899  2     0.1661
```

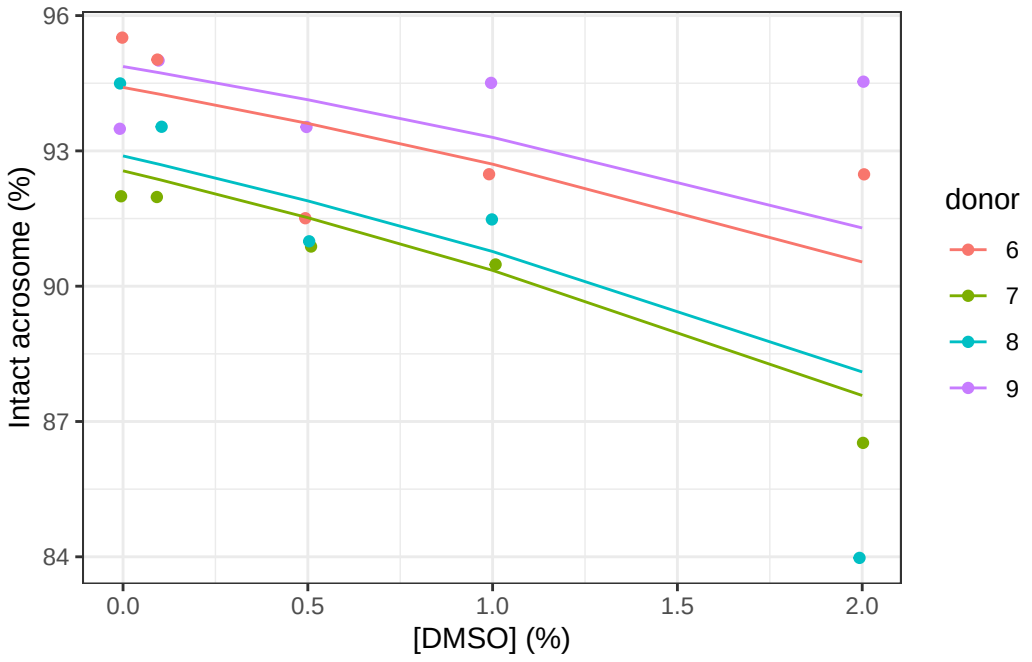
Here we draw the same conclusion, but this time our model fits without any problems. Let's continue our analysis with this simpler model `ai_dmso_m3`. This model is:

,

$$
\begin{aligned}
\text{acrointact}_i &\sim \text{Binomial}(n = 1, \text{prob}_{\text{acrointact}=1} = \widehat{P}) \\
, \log\left[\frac{\hat{P}}{1-\hat{P}}\right] &= \alpha_{j[i]} + \beta_1(\text{conc}) \qquad , \qquad (2) \\
\alpha_j &\sim N\left(\mu_{\alpha_j}, \sigma^2_{\alpha_j}\right), \text{ for donor j} = 1, \ldots, \text{J}
\end{aligned}
$$

Here is a plot of this model:

```
ggplot(data = ai_dmso) +
  geom_jitter(aes(x = conc, y = acrointact_frac * 100, col = donor),
    width = 0.01) +
  geom_line(aes(x = conc, y = fitted(ai_dmso_m3) * 100, col = donor)) +
  labs(x = "[DMSO] (%)", y = "Intact acrosome (%)")
```



Visually, it seems not too bad, but it seems we have the two last points for donor 9 suggesting a smaller slope and last point for donor 8 in favour of a larger slope. Here, we have too few data points to really decide what is the best model. However, considering all the other variables studied here, a model with intercept depending on the donor is not to be rejected (it is clearly the best model wherever more data are available).

```
summary(ai_dmso_m3)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(acrointact, total - acrointact) ~ conc + (1 | donor)
   Data: ai_dmso

     AIC      BIC   logLik deviance df.resid
   115.5    118.5    -54.8    109.5       17

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.7904 -0.4015 -0.0199  0.5077  1.6093

Random effects:
```

```
 Groups Name        Variance Std.Dev.
 donor  (Intercept) 0.03849  0.1962
Number of obs: 20, groups:  donor, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.71161    0.13232  20.493  < 2e-16 ***
conc        -0.28386    0.07669  -3.701 0.000214 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.494
```

The Z test indicates that `conc` is significantly different from zero at $\alpha = 5\%$. However, it is not the best test in the case of a mixed model like here. We prefer to rely on the 95% confidence interval calculated either on the profile, or via parametric bootstrap (and especially the later one):

```
confint(ai_dmso_m3, level = 0.95) # 95% CI based on profile
```

```
Computing profile confidence intervals ...
```

```
                  2.5 %     97.5 %
.sig01       0.05229171  0.5531516
(Intercept)  2.40826337  3.0303645
conc        -0.43348853 -0.1322598
```

```
set.seed(96347)
# 1000x parameter bootstrap
(ai_dmso_m3_conf <- confint(ai_dmso_m3, level = 0.95, method = "boot", nsim  = 1000L))
```

```
Computing bootstrap confidence intervals ...
```

```
194 message(s): boundary (singular) fit: see help('isSingular')
6 warning(s): Model failed to converge with max|grad| = 0.00227339 (tol = 0.002, component 1)
```

```
                 2.5 %     97.5 %
.sig01       0.0000000  0.3531096
(Intercept)  2.4379061  2.9935609
conc        -0.4339087 -0.1238000
```

1/5 of bootstrapped models present singularities. However, 95%CI from profiles and for parametric bootstrap are very close. So, we can trust them. Slope for `conc` is significantly different from zero at $\alpha = 5\%$ because the 95% CI does not contain zero.

Additional verifications

We could double-check the significance of the slope `conc` by looking at a likelihood ratio test when dropping `conc` from the model:

```
#drop1(ai_dmso_m3, scope = "conc")
ai_dmso_m4 <- glmer(cbind(acrointact, total - acrointact) ~ 1 + (1 | donor),
  data = ai_dmso, family = binomial(link = "logit"))
anova(ai_dmso_m3, ai_dmso_m4, refit = TRUE)
```

```
Data: ai_dmso
Models:
ai_dmso_m4: cbind(acrointact, total - acrointact) ~ 1 + (1 | donor)
ai_dmso_m3: cbind(acrointact, total - acrointact) ~ conc + (1 | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
ai_dmso_m4    2 126.77 128.76 -61.386   122.77
ai_dmso_m3    3 115.50 118.49 -54.752   109.50 13.268  1    0.00027 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The model with `conc` is significantly different at α level 5% from a reference model that sets the slope `conc` $= 0$. There is thus a significant effect of DMSO concentration (confirmation of results obtained from 95% CI).

We also double-check convergence of the model by trying different optimisation engines (just to make sure). First, is there a singularity in the model?

```
isSingular(ai_dmso_m3)
```

```
[1] FALSE
```

… then, a report about the model convergence:

```
ai_dmso_m3_all <- allFit(ai_dmso_m3)
```

```
Loading required namespace: dfoptim
```

```
Loading required namespace: optimx
```

```
bobyqa : [OK]
Nelder_Mead : [OK]
nlminbwrap : [OK]
nmkbw : [OK]
optimx.L-BFGS-B : [OK]
nloptwrap.NLOPT_LN_NELDERMEAD : [OK]
nloptwrap.NLOPT_LN_BOBYQA : [OK]
```

```
summary(ai_dmso_m3_all)
```

```
$which.OK
                         bobyqa                     Nelder_Mead
                           TRUE                            TRUE
                      nlminbwrap                          nmkbw
                           TRUE                            TRUE
               optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                           TRUE                            TRUE
    nloptwrap.NLOPT_LN_BOBYQA
                           TRUE

$msgs
$msgs$bobyqa
NULL

$msgs$Nelder_Mead
NULL

$msgs$nlminbwrap
NULL

$msgs$nmkbw
NULL
```

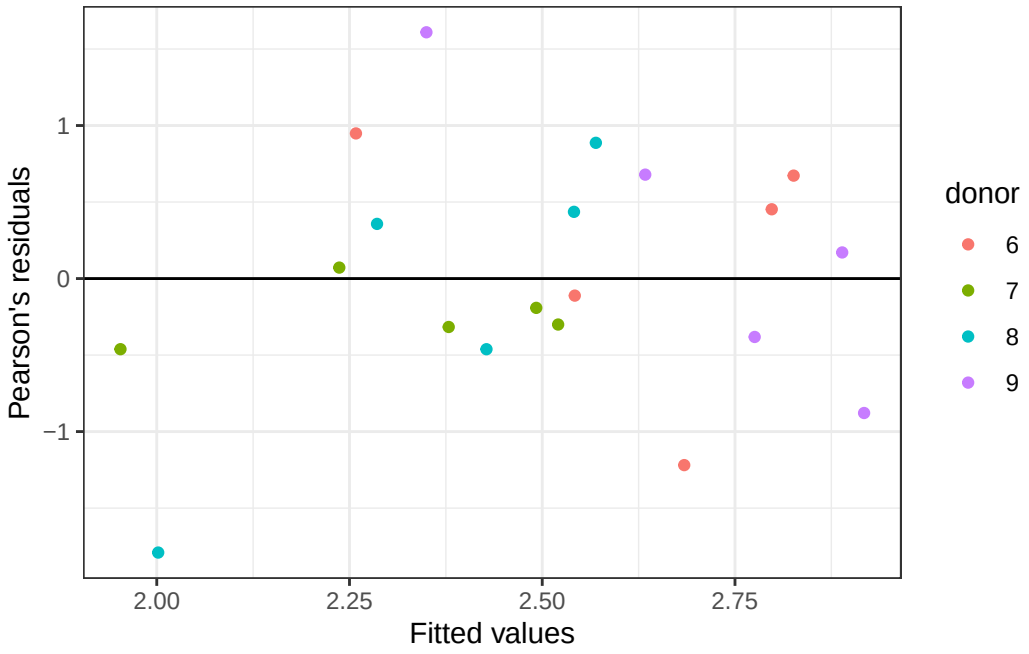
```
$msgs$`optimx.L-BFGS-B`
NULL

$msgs$nloptwrap.NLOPT_LN_NELDERMEAD
NULL

$msgs$nloptwrap.NLOPT_LN_BOBYQA
NULL


$fixef
                              (Intercept)       conc
bobyqa                           2.711615 -0.2838597
Nelder_Mead                      2.711619 -0.2838580
nlminbwrap                       2.711617 -0.2838619
nmkbw                            2.711609 -0.2838352
optimx.L-BFGS-B                  2.711615 -0.2838595
nloptwrap.NLOPT_LN_NELDERMEAD    2.711510 -0.2838224
nloptwrap.NLOPT_LN_BOBYQA        2.711618 -0.2838643

$llik
                        bobyqa                     Nelder_Mead
                     -54.75245                       -54.75245
                    nlminbwrap                            nmkbw
                     -54.75245                       -54.75245
               optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                     -54.75245                       -54.75245
    nloptwrap.NLOPT_LN_BOBYQA
                     -54.75245

$sdcor
                              donor.(Intercept)
bobyqa                                0.1961798
Nelder_Mead                           0.1961796
nlminbwrap                            0.1961816
nmkbw                                 0.1961364
optimx.L-BFGS-B                       0.1961824
nloptwrap.NLOPT_LN_NELDERMEAD         0.1962364
nloptwrap.NLOPT_LN_BOBYQA             0.1961850

$theta
                              donor.(Intercept)
bobyqa                                0.1961798
```

```
Nelder_Mead                             0.1961796
nlminbwrap                              0.1961816
nmkbw                                   0.1961364
optimx.L-BFGS-B                         0.1961824
nloptwrap.NLOPT_LN_NELDERMEAD           0.1962364
nloptwrap.NLOPT_LN_BOBYQA               0.1961850

$times
                              user.self sys.self elapsed user.child sys.child
bobyqa                            0.040    0.000   0.041          0         0
Nelder_Mead                       0.057    0.000   0.057          0         0
nlminbwrap                        0.042    0.000   0.041          0         0
nmkbw                             0.061    0.000   0.063          0         0
optimx.L-BFGS-B                   0.335    0.001   0.337          0         0
nloptwrap.NLOPT_LN_NELDERMEAD     0.052    0.000   0.051          0         0
nloptwrap.NLOPT_LN_BOBYQA         0.040    0.000   0.042          0         0

$feval
                          bobyqa                       Nelder_Mead
                              54                                93
                       nlminbwrap                             nmkbw
                              NA                               111
                 optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                              16                                84
      nloptwrap.NLOPT_LN_BOBYQA
                              36

attr(,"class")
[1] "summary.allFit"
```

Analysis of the residuals

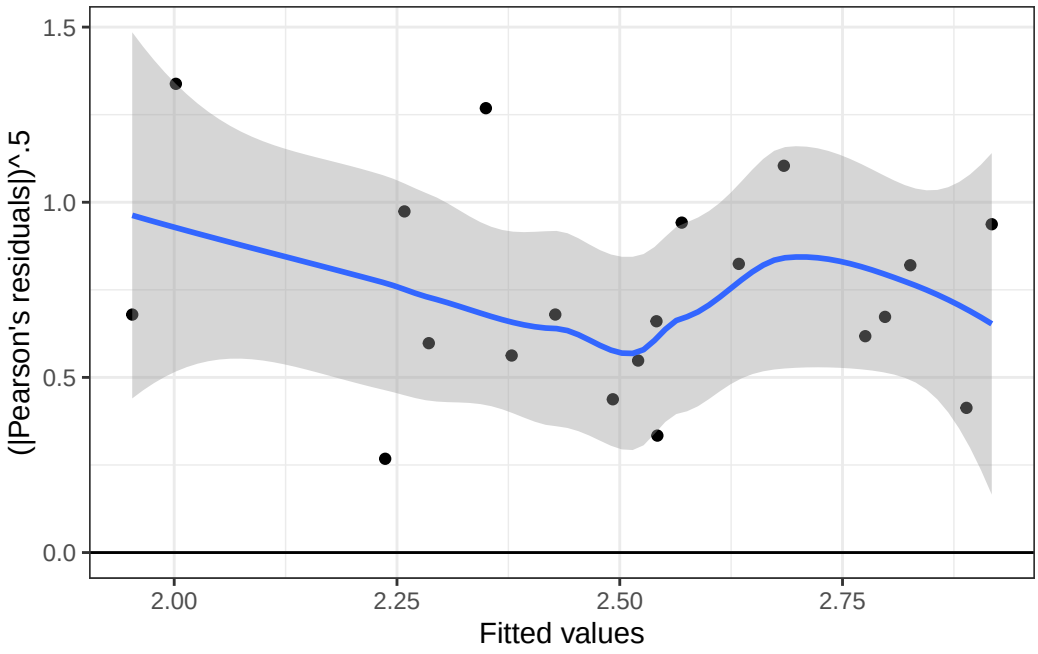
Let's check how the Pearson's residuals distribute and if there is homoscedasticity.

```
ai_dmso <- fortify.merMod(ai_dmso_m3)
ggplot(data = ai_dmso, aes(x = .fitted, y = .scresid, col = donor)) +
  geom_point() +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "Pearson's residuals")
```



Residuals do not seem weird, given the scarcity of the data.

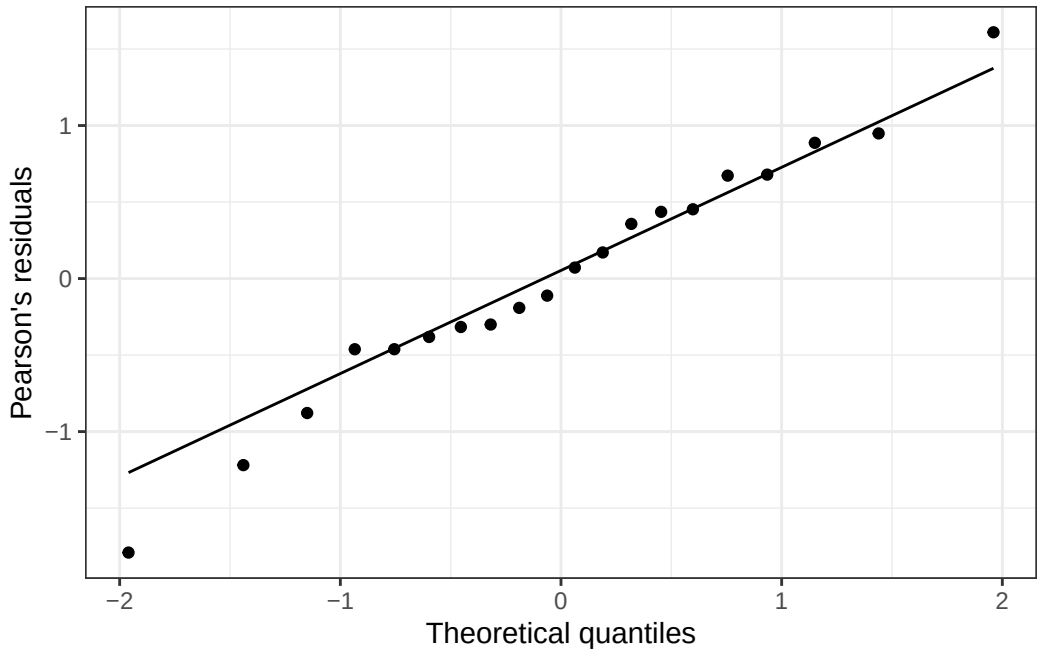
```
ggplot(data = ai_dmso, aes(x = .fitted, y = sqrt(abs(.scresid)))) +
  geom_point() +
  geom_smooth(method = "loess", formula = y ~ x) +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "(|Pearson's residuals|)^.5")
```



Homoscedasticity of the residuals seems acceptable (the blue curve that is a loess smoothing in the data is relatively horizontal).

Note: according to Gauss-Markov theorem that indicates that linearity, random sample, non-collinearity between predictors, non-correlation between predictors and error term and homoscedasticity are the only requirements for our GLMM, we do not have to check it. Also the model is robust to departure of Normality, and none of the tests we made depend on a Normal distribution of the residuals (we said we don't trust z/t tests and replace them by likelihood ratio tests and parameterized bootstrapped confidence intervals). However, for completeness, here is the quantile-quantile plot of the residuals:

```
ggplot(data = ai_dmso, aes(sample = .scresid)) +
  geom_qq() +
  geom_qq_line() +
  labs(x = "Theoretical quantiles", y = "Pearson's residuals")
```



It appears to be good. A Shapiro-Wilk test does not confirm Normality, but we are pretty sure it is caused by the extreme value:

```
shapiro.test(ai_dmso$.scresid)
```

```
    Shapiro-Wilk normality test

data:  ai_dmso$.scresid
W = 0.98424, p-value = 0.9765
```

Predictions

The model allows to calculate the drop in acrosome integrity according to DMSO concentration from 0 to 2%. Note that an inverse logit transformation is required. Here is the calculations:

```
ai_dmso_slope <- c(
  ci95_min   = min(ai_dmso_m3_conf["conc", ]),
  estimate = fixef(ai_dmso_m3)[["conc"]],
  ci95_max  = max(ai_dmso_m3_conf["conc", ]))
ai_dmso_slope
```

```
  ci95_min   estimate   ci95_max
-0.4339087 -0.2838577 -0.1238000
```

```
#saveRDS(ai_dmso_slope, "../data/acrosome_integrity_DMSO_slope.rds")
```

Let's say we want to calculate the drop in acrosome integrity for various DMSO concentrations between 0 and 2% if the acrosome integrity of a sample without DMSO is 94%. The calculation is:

```
predict_logit <- function(conc, intercept = 1, slopes) {
  slopes_mat <- matrix(slopes, nrow = 1,
    dimnames = list(NULL, names(slopes)))
  data.frame(conc = conc, -intercept +
      boot::inv.logit(boot::logit(intercept) +
      conc %*% slopes_mat))
}
dmso_conc <- (0:20) / 10
ai_dmso_lost <- predict_logit(dmso_conc, 0.94, ai_dmso_slope)
ai_dmso_lost
```

```
   conc      ci95_min      estimate      ci95_max
1   0.0  1.110223e-16  1.110223e-16  1.110223e-16
2   0.1 -2.494478e-03 -1.621096e-03 -7.020474e-04
3   0.2 -5.085482e-03 -3.283042e-03 -1.411773e-03
4   0.3 -7.776143e-03 -4.986707e-03 -2.129247e-03
5   0.4 -1.056964e-02 -6.732969e-03 -2.854542e-03
6   0.5 -1.346920e-02 -8.522717e-03 -3.587731e-03
7   0.6 -1.647810e-02 -1.035685e-02 -4.328885e-03
8   0.7 -1.959964e-02 -1.223626e-02 -5.078077e-03
9   0.8 -2.283715e-02 -1.416188e-02 -5.835380e-03
10  0.9 -2.619402e-02 -1.613461e-02 -6.600868e-03
11  1.0 -2.967361e-02 -1.815539e-02 -7.374615e-03
12  1.1 -3.327934e-02 -2.022515e-02 -8.156695e-03
13  1.2 -3.701460e-02 -2.234482e-02 -8.947182e-03
14  1.3 -4.088279e-02 -2.451536e-02 -9.746151e-03
15  1.4 -4.488730e-02 -2.673770e-02 -1.055368e-02
16  1.5 -4.903149e-02 -2.901279e-02 -1.136983e-02
17  1.6 -5.331870e-02 -3.134159e-02 -1.219470e-02
18  1.7 -5.775220e-02 -3.372505e-02 -1.302835e-02
19  1.8 -6.233522e-02 -3.616412e-02 -1.387086e-02
20  1.9 -6.707093e-02 -3.865975e-02 -1.472231e-02
```

```
21  2.0 -7.196240e-02 -4.121290e-02 -1.558276e-02
```

```
#saveRDS(ai_dmso_lost, "../data/acrosome_integrity_DMSO_lost.rds")
```

This is the lost in acrosome integrity that the model predicts. At 2% DMSO, we lose roughly 4%, and the 95%CI gives us a maximum lost of 7%.

Ethanol

```
ai_etoh <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "acrosome integrity_EtOH")
ai_etoh$donor <- as.factor(ai_etoh$donor)
names(ai_etoh) <- c("donor", "conc", "acrointact", "total")
ai_etoh$acrointact_frac <- ai_etoh$acrointact / ai_etoh$total
skimr::skim(ai_etoh)
```

Table 4: Data summary

Name	ai_etoh
Number of rows	20
Number of columns	5
_____	
Column type frequency:	
factor	1
numeric	4
_____	
Group variables	None

Variable type: factor

skim_variable	n_missing	complete_rate	ordered	n_unique	top_counts
donor	0	1	FALSE	4	6: 5, 7: 5, 8: 5, 9: 5

Variable type: numeric

skim_variable	n_missing	complete_rate	mean	sd	p0	p25	p50	p75	p100	hist
conc	0	1	0.72	0.75	0.00	0.10	0.50	1.00	2.00	
acrointact	0	1	187.15	7.34	174.00	183.75	187.00	189.00	213.00	

skim_variable	n_missing	complete_rate	mean	sd	p0	p25	p50	p75	p100	hist
total	0	1	202.05	6.30	200.00	200.00	200.00	200.25	228.00	
acrointact_frac	0	1	0.93	0.02	0.87	0.91	0.93	0.94	0.96	

There are four donors, no missing data.

```
table(ai_etoh$donor, as.factor(ai_etoh$conc))
```

```
  0 0.1 0.5 1 2
6 1   1   1 1 1
7 1   1   1 1 1
8 1   1   1 1 1
9 1   1   1 1 1
```

The data are balanced with one observation for each concentration and each donor and no missing data.

```
ai_etoh_m1 <- glmer(cbind(acrointact, total - acrointact) ~ conc + (conc | donor),
  data = ai_etoh, family = binomial(link = "logit"))
```

```
boundary (singular) fit: see help('isSingular')
```

```
summary(ai_etoh_m1)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(acrointact, total - acrointact) ~ conc + (conc | donor)
   Data: ai_etoh

     AIC      BIC   logLik deviance df.resid
   116.6    121.6    -53.3    106.6       15

Scaled residuals:
     Min       1Q   Median       3Q      Max
-1.04177 -0.54026  0.04142  0.43251  1.51864
```

```
Random effects:
 Groups Name        Variance Std.Dev. Corr
 donor  (Intercept) 0.00000  0.0000
        conc        0.03567  0.1889    NaN
Number of obs: 20, groups:  donor, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.63558    0.08723  30.212   <2e-16 ***
conc        -0.11817    0.12533  -0.943    0.346
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.469
optimizer (Nelder_Mead) convergence code: 0 (OK)
boundary (singular) fit: see help('isSingular')
```

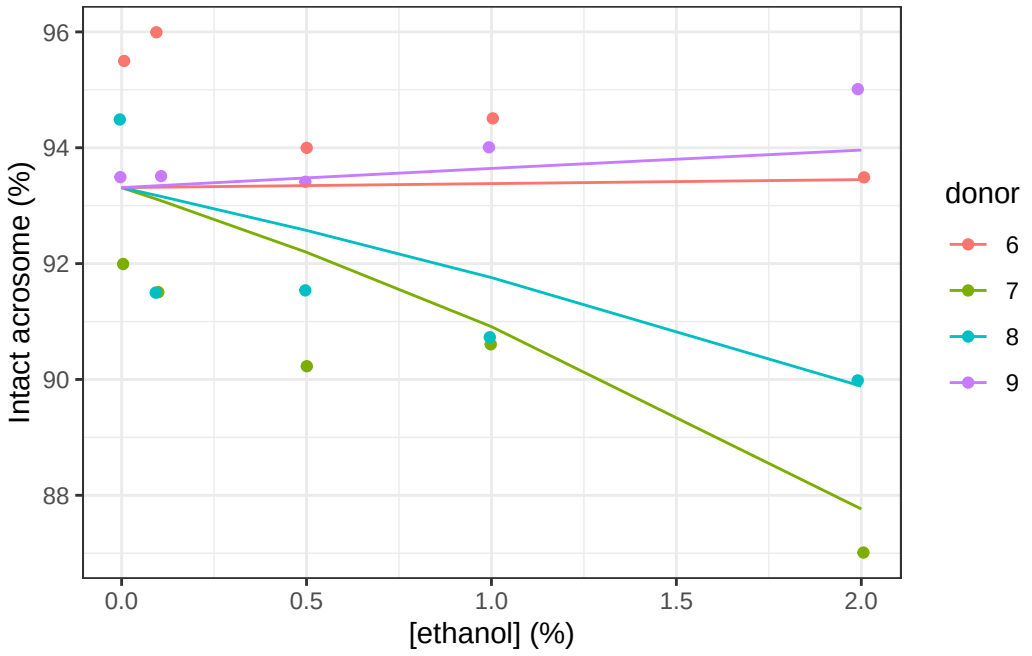
We have a singularity here. The model is:

,

$$
\begin{aligned}
&\text{acrointact}_i \sim \text{Binomial}(n = 1, \text{prob}_{\text{acrointact}=1} = \widehat{P}) \\
&, \log\left[\frac{\hat{P}}{1-\hat{P}}\right] = \alpha_{j[i]} + \beta_{1j[i]}(\text{conc}) \\
&\begin{pmatrix} \alpha_j \\ \beta_{1j} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu_{\alpha_j} \\ \mu_{\beta_{1j}} \end{pmatrix}, \begin{pmatrix} \sigma^2_{\alpha_j} & \rho_{\alpha_j\beta_{1j}} \\ \rho_{\beta_{1j}\alpha_j} & \sigma^2_{\beta_{1j}} \end{pmatrix}\right), \text{for donor j} = 1, \ldots, \text{J}
\end{aligned}
\quad , \qquad (3)
$$

Here is a plot of this model:

```
ggplot(data = ai_etoh) +
  geom_jitter(aes(x = conc, y = acrointact_frac * 100, col = donor),
    width = 0.01) +
  geom_line(aes(x = conc, y = fitted(ai_etoh_m1) * 100, col = donor)) +
  labs(x = "[ethanol] (%)", y = "Intact acrosome (%)")
```



Here the complete model was not able to estimate the variation of intercept per donor (so, it used the same one). However, data at concentration zero are more widespread. It is not clear if the model could be simplified for the intercept or the slope for the random effect `donor`. Let's look at both options…

```
ai_etoh_m2 <- glmer(cbind(acrointact, total - acrointact) ~ conc  + (1 | donor),
  data = ai_etoh, family = binomial(link = "logit"))
anova(ai_etoh_m1, ai_etoh_m2) # Despite the name, it is indeed a LR test
```

```
Data: ai_etoh
Models:
ai_etoh_m2: cbind(acrointact, total - acrointact) ~ conc + (1 | donor)
ai_etoh_m1: cbind(acrointact, total - acrointact) ~ conc + (conc | donor)
           npar    AIC    BIC  logLik deviance Chisq Df Pr(>Chisq)
ai_etoh_m2    3 110.37 113.36 -52.185   104.37
ai_etoh_m1    5 116.63 121.60 -53.313   106.63     0  2          1
```

Another model, with same intercept but different slopes:

```
ai_etoh_m3 <- glmer(cbind(acrointact, total - acrointact) ~ conc  + (0 + conc:donor | dono
  data = ai_etoh, family = binomial(link = "logit"))
```

```
boundary (singular) fit: see help('isSingular')
```

```
anova(ai_etoh_m1, ai_etoh_m3) # Despite the name, it is indeed a LR test
```

```
Data: ai_etoh
Models:
ai_etoh_m1: cbind(acrointact, total - acrointact) ~ conc + (conc | donor)
ai_etoh_m3: cbind(acrointact, total - acrointact) ~ conc + (0 + conc:donor | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
ai_etoh_m1    5 116.63 121.60 -53.313   106.63
ai_etoh_m3   12 128.98 140.93 -52.492   104.98 1.6426  7      0.977
```

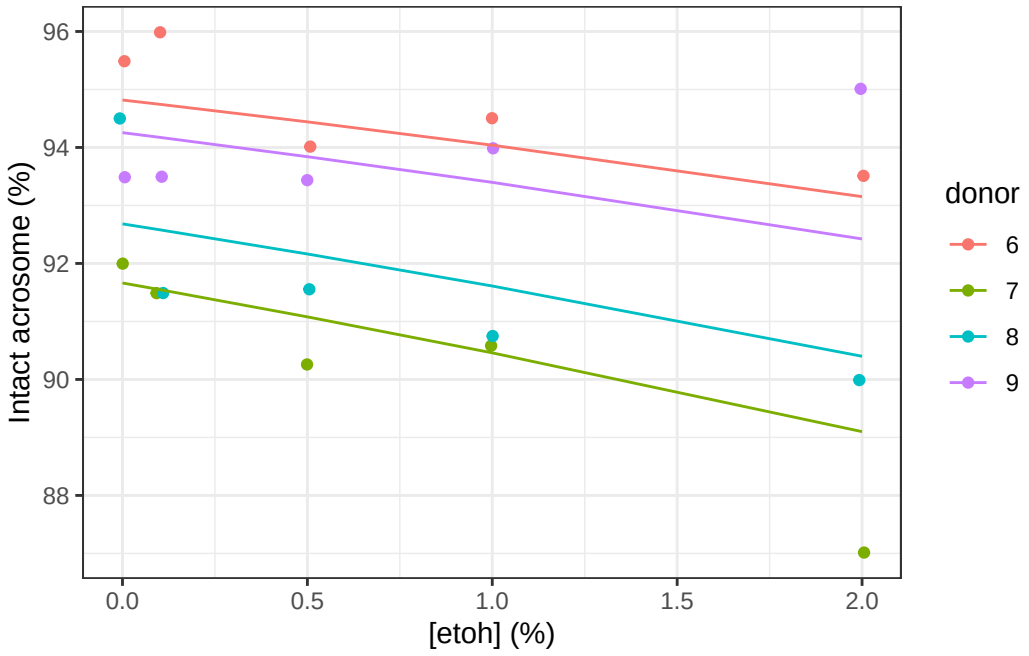
The likelihood ratio test does not detects significant differences between the full and both simplified models at $\alpha = 5\%$, but the second model has singularities too. Using different slopes produces singular gradient. Here is the model with only intercept depending on the donor, which is fitted without problems:

,

$$\begin{aligned} \text{acrointact}_i &\sim \text{Binomial}(n = 1, \text{prob}_{\text{acrointact}=1} = \widehat{P}) \\ \text{, log}\left[\frac{\hat{P}}{1-\hat{P}}\right] &= \alpha_{j[i]} + \beta_1(\text{conc}) \qquad \text{,} \\ \alpha_j &\sim N\left(\mu_{\alpha_j}, \sigma^2_{\alpha_j}\right), \text{ for donor j} = 1, \ldots, \text{J} \end{aligned} \tag{4}$$

Here is a plot of this model:

```
ggplot(data = ai_etoh) +
  geom_jitter(aes(x = conc, y = acrointact_frac * 100, col = donor),
    width = 0.01) +
  geom_line(aes(x = conc, y = fitted(ai_etoh_m2) * 100, col = donor)) +
  labs(x = "[etoh] (%)", y = "Intact acrosome (%)")
```




```
summary(ai_etoh_m2)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(acrointact, total - acrointact) ~ conc + (1 | donor)
   Data: ai_etoh

     AIC      BIC   logLik deviance df.resid
   110.4    113.4    -52.2    104.4       17

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.9529 -0.4104 -0.1084  0.2921  1.3771

Random effects:
 Groups Name        Variance Std.Dev.
 donor  (Intercept) 0.05266  0.2295
Number of obs: 20, groups:  donor, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
```

```
(Intercept)  2.66406    0.14470  18.411   <2e-16 ***
conc        -0.14824    0.08022  -1.848   0.0646 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.436
```

The Z test indicates that `conc` is not significantly different from zero at $\alpha = 5\%$. However, it is not the best test in the case of a mixed model like here. We prefer to rely on the 95% confidence interval calculated either on the profile, or via parametric bootstrap (and especially the later one):

```r
confint(ai_etoh_m2, level = 0.95) # 95% CI based on profile
```

```
Computing profile confidence intervals ...
```

```
                  2.5 %     97.5 %
.sig01       0.08352725 0.62762234
(Intercept)  2.32574073 3.01787271
conc        -0.30431936 0.01089323
```

```r
set.seed(7431)
# 1000x parameter bootstrap
(ai_etoh_m2_conf <- confint(ai_etoh_m2, level = 0.95, method = "boot", nsim  = 1000L))
```

```
Computing bootstrap confidence intervals ...
```

```
160 message(s): boundary (singular) fit: see help('isSingular')
7 warning(s): Model failed to converge with max|grad| = 0.00254552 (tol = 0.002, component 1)
```

```
                 2.5 %     97.5 %
.sig01       0.0000000 0.37669024
(Intercept)  2.4006581 2.95902373
conc        -0.3153885 0.02246623
```

We had 160 bootstrapped model with singularity among the 1000. Lower bound for the bootstrapped 95%CI is rather different to the one from profiles. This is not surprising since we have rather few data here. Slope for `conc` is not significantly different from zero at $\alpha = 5\%$ because the 95% CI contains zero. However, it could be due to the scarcity of the data. Yet, the effect appears weak with a lost of a few percents for a concentration of 2% ethanol. We conclude here that the effect is either weak, or inexistent. Using upper bound 95%CI, we would have a variation of:

```
# Let's consider a value of 0.94 at conc = 0, with a slope of -0.32
# (most negative slope from C95%I), we lose:
-0.94 + boot::inv.logit(boot::logit(0.94) - 0.32 * 2)
```

```
[1] -0.0479807
```

That is, we have less than 5% variation in acrosome integrity at worst at ethanol concentration of 2%.

Additional verifications

We also double-check convergence of the model by trying different optimisation engines (just to make sure). First, is there a singularity in the model?

```
isSingular(ai_etoh_m2)
```

```
[1] FALSE
```

… then, a report about the model convergence:

```
ai_etoh_m2_all <- allFit(ai_etoh_m2)
```

```
bobyqa : [OK]
Nelder_Mead : [OK]
nlminbwrap : [OK]
nmkbw : [OK]
optimx.L-BFGS-B : [OK]
nloptwrap.NLOPT_LN_NELDERMEAD : [OK]
nloptwrap.NLOPT_LN_BOBYQA : [OK]
```

```
summary(ai_etoh_m2_all)
```

```
$which.OK
                        bobyqa                     Nelder_Mead
                          TRUE                            TRUE
                    nlminbwrap                           nmkbw
                          TRUE                            TRUE
              optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                          TRUE                            TRUE
    nloptwrap.NLOPT_LN_BOBYQA
                          TRUE

$msgs
$msgs$bobyqa
NULL

$msgs$Nelder_Mead
NULL

$msgs$nlminbwrap
NULL

$msgs$nmkbw
NULL

$msgs$`optimx.L-BFGS-B`
NULL

$msgs$nloptwrap.NLOPT_LN_NELDERMEAD
NULL

$msgs$nloptwrap.NLOPT_LN_BOBYQA
NULL


$fixef
                             (Intercept)       conc
bobyqa                         2.664067 -0.1482448
Nelder_Mead                    2.664057 -0.1482393
nlminbwrap                     2.664066 -0.1482438
nmkbw                          2.663973 -0.1481829
```

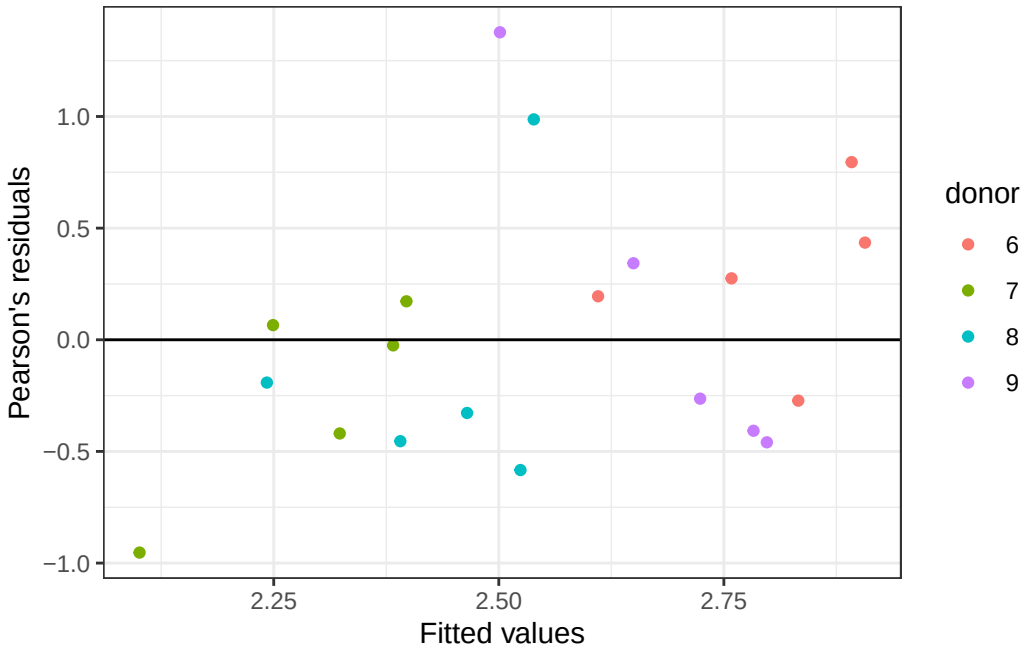
```
optimx.L-BFGS-B                  2.664067 -0.1482447
nloptwrap.NLOPT_LN_NELDERMEAD    2.664063 -0.1482756
nloptwrap.NLOPT_LN_BOBYQA        2.664068 -0.1482449

$llik
                        bobyqa                     Nelder_Mead
                     -52.18489                       -52.18489
                    nlminbwrap                           nmkbw
                     -52.18489                       -52.18489
               optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                     -52.18489                       -52.18489
    nloptwrap.NLOPT_LN_BOBYQA
                     -52.18489

$sdcor
                               donor.(Intercept)
bobyqa                                 0.2294803
Nelder_Mead                            0.2294782
nlminbwrap                             0.2294793
nmkbw                                  0.2294284
optimx.L-BFGS-B                        0.2294825
nloptwrap.NLOPT_LN_NELDERMEAD          0.2294788
nloptwrap.NLOPT_LN_BOBYQA              0.2294829

$theta
                               donor.(Intercept)
bobyqa                                 0.2294803
Nelder_Mead                            0.2294782
nlminbwrap                             0.2294793
nmkbw                                  0.2294284
optimx.L-BFGS-B                        0.2294825
nloptwrap.NLOPT_LN_NELDERMEAD          0.2294788
nloptwrap.NLOPT_LN_BOBYQA              0.2294829

$times
                               user.self sys.self elapsed user.child sys.child
bobyqa                             0.041    0.001   0.042          0         0
Nelder_Mead                        0.053    0.000   0.053          0         0
nlminbwrap                         0.045    0.000   0.046          0         0
nmkbw                              0.055    0.000   0.055          0         0
optimx.L-BFGS-B                    0.344    0.001   0.345          0         0
nloptwrap.NLOPT_LN_NELDERMEAD      0.049    0.001   0.049          0         0
nloptwrap.NLOPT_LN_BOBYQA          0.037    0.000   0.038          0         0
```

```
$feval
                         bobyqa                     Nelder_Mead
                             59                              83
                      nlminbwrap                           nmkbw
                             NA                             101
              optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                             16                              87
    nloptwrap.NLOPT_LN_BOBYQA
                             32

attr(,"class")
[1] "summary.allFit"
```

Analysis of the residuals

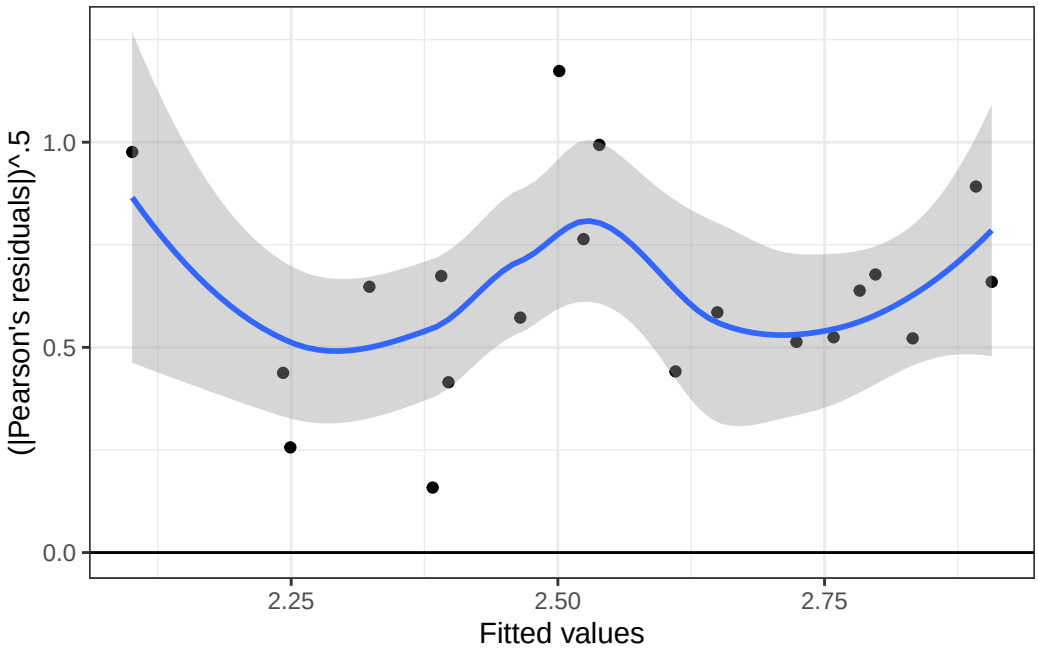
Let's check how the residuals distribute and if there is homoscedasticity.

```
ai_etoh <- fortify.merMod(ai_etoh_m2)
ggplot(data = ai_etoh, aes(x = .fitted, y = .scresid, col = donor)) +
  geom_point() +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "Pearson's residuals")
```



Given the scarcity of the data, residuals do not seem abnormal.

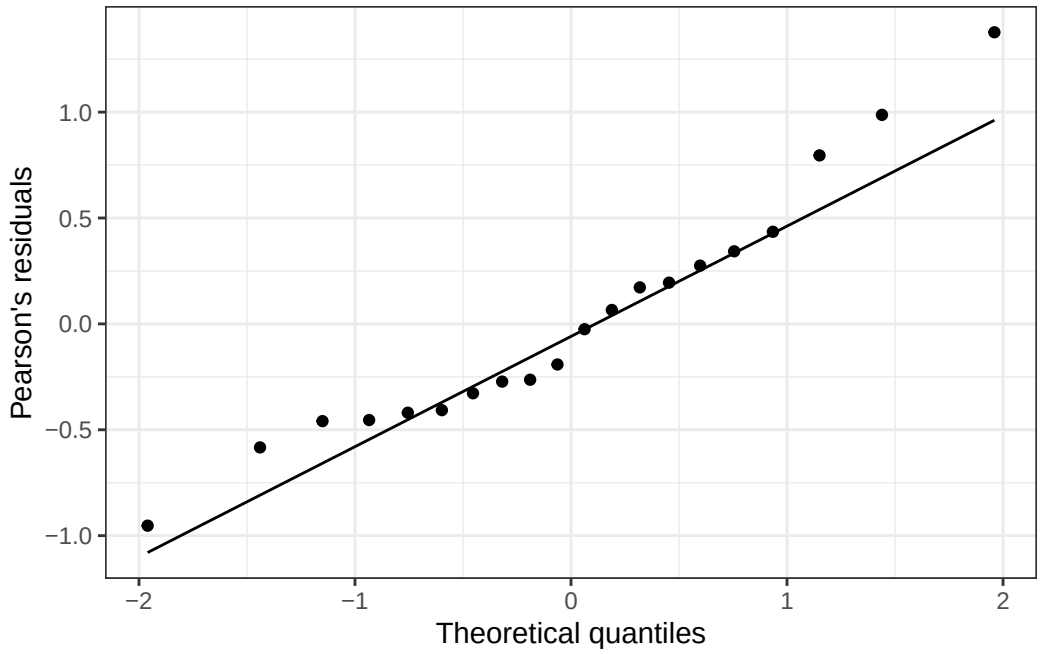
```
ggplot(data = ai_etoh, aes(x = .fitted, y = sqrt(abs(.scresid)))) +
  geom_point() +
  geom_smooth(method = "loess", formula = y ~ x) +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "(|Pearson's residuals|)^.5")
```



Homoscedasticity of the residuals seems here acceptable (the blue curve that is a loess smoothing in the data is relatively horizontal).

With the same remark as for DMSO, here is the quantile-quantile plot of the residuals:

```
ggplot(data = ai_etoh, aes(sample = .scresid)) +
  geom_qq() +
  geom_qq_line() +
  labs(x = "Theoretical quantiles", y = "Pearson's residuals")
```



It appears not particularly bad. A Shapiro-Wilk test confirms Normality (with caution because this test tends to be conservative):

```
shapiro.test(ai_etoh$.scresid)
```

```
    Shapiro-Wilk normality test

data:  ai_etoh$.scresid
W = 0.94869, p-value = 0.3476
```

General informations

```
sessionInfo()
```

```
R version 4.1.3 (2022-03-10)
Platform: x86_64-apple-darwin17.0 (64-bit)
Running under: macOS Big Sur/Monterey 10.16

Matrix products: default
```

```
LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] ggplot2_3.3.5 lme4_1.1-29   Matrix_1.4-1

loaded via a namespace (and not attached):
 [1] tidyr_1.2.0         jsonlite_1.8.0      splines_4.1.3
 [4] equatiomatic_0.3.1  shiny_1.7.1         assertthat_0.2.1
 [7] highr_0.9           broom.mixed_0.2.9.4 cellranger_1.1.0
[10] yaml_2.3.5          globals_0.14.0      numDeriv_2016.8-1.1
[13] pillar_1.7.0        backports_1.4.1     lattice_0.20-45
[16] glue_1.6.2          digest_0.6.29       promises_1.2.0.1
[19] minqa_1.2.4         colorspace_2.0-3    dfoptim_2020.10-1
[22] htmltools_0.5.2     httpuv_1.6.5        pkgconfig_2.0.3
[25] broom_0.8.0         listenv_0.8.0       purrr_0.3.4
[28] xtable_1.8-4        scales_1.2.0        later_1.3.0
[31] tibble_3.1.6        mgcv_1.8-40         generics_0.1.2
[34] farver_2.1.0        ellipsis_0.3.2      withr_2.5.0
[37] furrr_0.2.3         repr_1.1.4          skimr_2.1.4
[40] cli_3.2.0           magrittr_2.0.3      crayon_1.5.1
[43] readxl_1.4.0        mime_0.12           evaluate_0.15
[46] fansi_1.0.3         future_1.24.0       parallelly_1.31.0
[49] nlme_3.1-157        MASS_7.3-56         forcats_0.5.1
[52] tools_4.1.3         lifecycle_1.0.1     stringr_1.4.0
[55] munsell_0.5.0       compiler_4.1.3      rlang_1.0.2
[58] grid_4.1.3          nloptr_2.0.0        rstudioapi_0.13
[61] base64enc_0.1-3     labeling_0.4.2      rmarkdown_2.13
[64] boot_1.3-28         gtable_0.3.0        codetools_0.2-18
[67] DBI_1.1.2           R6_2.5.1            knitr_1.38
[70] dplyr_1.0.8         optimx_2021-10.12   fastmap_1.1.0
[73] utf8_1.2.2          stringi_1.7.6       parallel_4.1.3
[76] Rcpp_1.0.8.3        vctrs_0.4.1         tidyselect_1.1.2
[79] xfun_0.30
```