Influence of two widely used solvents, ethanol and dimethyl sulfoxide, on human sperm parameters - plots

Marie Bisconti, Philippe Grosjean & Elise Hennebert

Total motility with DMSO

```
mt_dmso <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "total motility_DMSO")
names(mt_dmso) <- tolower(names(mt_dmso))
mt_dmso$donor <- as.factor(mt_dmso$donor)
names(mt_dmso) <- c("donor", "conc", "motile", "total")
mt_dmso$motile_frac <- mt_dmso$motile / mt_dmso$total
mt_dmso$motile_perc <- mt_dmso$motile_frac * 100
mt_dmso$conc_f <- as.factor(mt_dmso$conc)
# Selected model
mt_dmso_m2 <- glmer(cbind(motile, total - motile) ~ conc  + (1 | donor),
  data = mt_dmso, family = binomial(link = "logit"))
#summary(mt_dmso_m2)
```

Plot of the model with lines per donor (the **correct** plot according to the model). Black dots + error bars= mean ± standard deviation, black line = concentration effect, excluding random donor effect = average effect across all donors.

```
mt_conc_effect_dmso <- as.data.frame(effects::predictorEffect(mt_dmso_m2,
  predictor = "conc")[c("x", "fit")])
mob_c <- ggplot(data = mt_dmso) +
  geom_jitter(aes(x = conc, y = motile_perc, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(mt_dmso_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = motile_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
```

```
    stat_summary(aes(x = conc, y = motile_perc),
      geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
      alpha = 0.7, width = 0.05) +
    geom_line(data = mt_conc_effect_dmso, aes(x = conc,
      y = boot::inv.logit(fit) * 100), size = 0.8) +
    ylim(25, 100) +
    labs(x = "DMSO (%)", y = "Motile sperm (%)") +
    scale_color_manual(values = hex9[1:8]) +
    guides(colour = guide_legend(nrow = 1))
```

Total motility with ethanol

```
mt_etoh <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "total motility_EtOH")
mt_etoh$donor <- as.factor(mt_etoh$donor)
names(mt_etoh) <- c("donor", "conc", "motile", "total")
mt_etoh$motile_frac <- mt_etoh$motile / mt_etoh$total
mt_etoh$motile_perc <- mt_etoh$motile_frac * 100
mt_etoh$conc_f <- as.factor(mt_etoh$conc)
# Selected model
mt_etoh_m2 <- glmer(cbind(motile, total - motile) ~ conc  + (1 | donor),
  data = mt_etoh, family = binomial(link = "logit"))
#summary(mt_etoh_m2)
```

```
mt_conc_effect_etoh <- as.data.frame(effects::predictorEffect(mt_etoh_m2,
  predictor = "conc")[c("x", "fit")])
mob_a <- ggplot(data = mt_etoh) +
  geom_jitter(aes(x = conc, y = motile_perc, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(mt_etoh_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = motile_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = motile_perc),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = mt_conc_effect_etoh, aes(x = conc,
    y = boot::inv.logit(fit) * 100), size = 0.8) +
  ylim(25, 100) +
  labs(x = "EtOH (%)", y = "Motile sperm (%)") +
```

```r
  scale_color_manual(values = hex9[1:8]) +
  guides(colour = guide_legend(nrow = 1))
```

Progressive motility with DMSO

```r
mp_dmso <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "progressive motility_DMSO")
mp_dmso$donor <- as.factor(mp_dmso$donor)
names(mp_dmso) <- c("donor", "conc", "prog", "total")
mp_dmso$prog_frac <- mp_dmso$prog / mp_dmso$total
mp_dmso$prog_perc <- mp_dmso$prog_frac * 100
mp_dmso$conc_f <- as.factor(mp_dmso$conc)
# Selected model
mp_dmso_m2 <- glmer(cbind(prog, total - prog) ~ conc  + (1 | donor),
  data = mp_dmso, family = binomial(link = "logit"))
#summary(mp_dmso_m2)
```

```r
mp_conc_effect_dmso <- as.data.frame(effects::predictorEffect(mp_dmso_m2,
  predictor = "conc")[c("x", "fit")])
mob_d <- ggplot(data = mp_dmso) +
  geom_jitter(aes(x = conc, y = prog_perc, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(mp_dmso_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = prog_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = prog_perc),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = mp_conc_effect_dmso, aes(x = conc,
    y = boot::inv.logit(fit) * 100), size = 0.8) +
  ylim(25, 100) +
  labs(x = "DMSO (%)", y = "Progressive motile sperm (%)") +
  scale_color_manual(values = hex9[1:8]) +
  guides(colour = guide_legend(nrow = 1))
```

Progressive motility with ethanol

```r
mp_etoh <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "progressive motility_EtOH")
mp_etoh$donor <- as.factor(mp_etoh$donor)
names(mp_etoh) <- c("donor", "conc", "prog", "total")
mp_etoh$prog_frac <- mp_etoh$prog / mp_etoh$total
mp_etoh$prog_perc <- mp_etoh$prog_frac * 100
mp_etoh$conc_f <- as.factor(mp_etoh$conc)
# Selected model
mp_etoh_m2 <- glmer(cbind(prog, total - prog) ~ conc  + (1 | donor),
  data = mp_etoh, family = binomial(link = "logit"))
#summary(mp_etoh_m2)
```

```r
mp_conc_effect_etoh <- as.data.frame(effects::predictorEffect(mp_etoh_m2,
  predictor = "conc")[c("x", "fit")])
mob_b <- ggplot(data = mp_etoh) +
  geom_jitter(aes(x = conc, y = prog_perc, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(mp_etoh_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = prog_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = prog_perc),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = mp_conc_effect_etoh, aes(x = conc,
    y = boot::inv.logit(fit) * 100), size = 0.8) +
  ylim(25, 100) +
  labs(x = "EtOH (%)", y = "Progressive motile sperm (%)") +
  scale_color_manual(values = hex9[1:8]) +
  guides(colour = guide_legend(nrow = 1))
```

Combined plots DMSO + ethanol

```
mob <- ggpubr::ggarrange(mob_a, mob_b, mob_c, mob_d, labels = "AUTO",
   common.legend = TRUE, legend = "bottom", hjust = -0.5, vjust = 1.5,
   font.label = list(size = 14, color = "black", face = "plain", family = NULL))
```
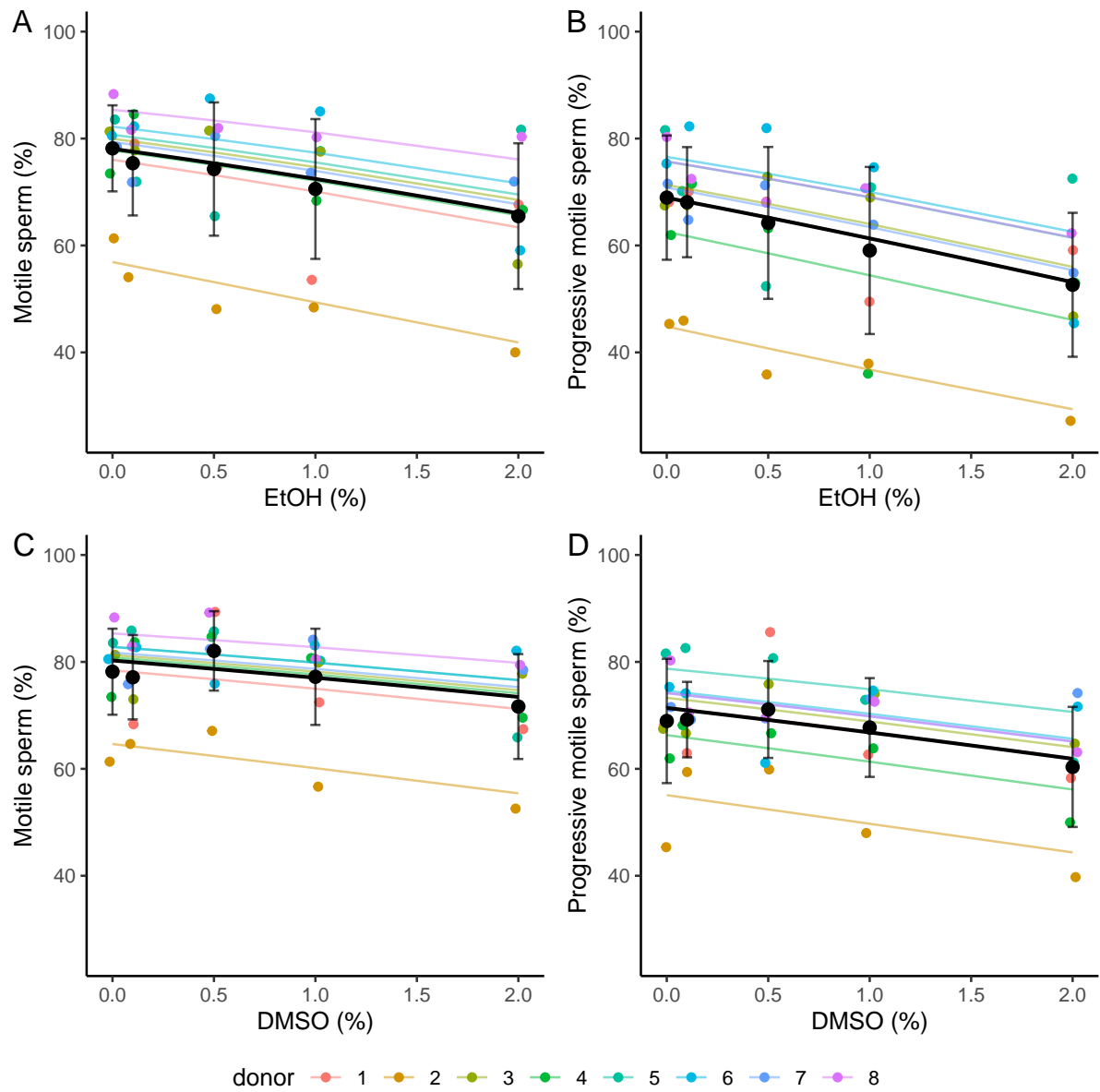
```
Warning: Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).
```

```
mob
```

```
ggsave("../figures/motility.pdf", plot = mob)
```

Saving 7 x 7 in image

Acrosome integrity with DMSO

```
ai_conc_effect_dmso <- as.data.frame(effects::predictorEffect(ai_dmso_m2,
  predictor = "conc")[c("x", "fit")])
ac_b <- ggplot(data = ai_dmso) +
  geom_jitter(aes(x = conc, y = acrointact_perc, col = donor),
    width = 0.025) +
  geom_line(aes(x = conc, y = fitted(ai_dmso_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = acrointact_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = acrointact_perc),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = ai_conc_effect_dmso, aes(x = conc,
    y = boot::inv.logit(fit) * 100), size = 0.8) +
  ylim(83, 100) +
  labs(x = "DMSO (%)", y = "Acrosome intact sperm (%)") +
  scale_color_manual(values = hex9[6:9]) +
  guides(colour = guide_legend(nrow = 1))
```

Acrosomal reaction with ethanol

```
ai_conc_effect_etoh <- as.data.frame(effects::predictorEffect(ai_etoh_m2,
  predictor = "conc")[c("x", "fit")])
ac_a <- ggplot(data = ai_etoh) +
  geom_jitter(aes(x = conc, y = acrointact_perc, col = donor),
    width = 0.025) +
  geom_line(aes(x = conc, y = fitted(ai_etoh_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = acrointact_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = acrointact_perc),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = ai_conc_effect_etoh, aes(x = conc,
    y = boot::inv.logit(fit) * 100), size = 0.8) +
  ylim(83, 100) +
  labs(x = "EtOH (%)", y = "Acrosome intact sperm (%)") +
  scale_color_manual(values = hex9[6:9]) +
```

```
  guides(colour = guide_legend(nrow = 1))


acro <- ggpubr::ggarrange(ac_a, ac_b, labels = "AUTO",
  common.legend = TRUE, legend = "bottom", hjust = -0.5, vjust = 1.5,
  font.label = list(size = 14, color = "black", face = "plain", family = NULL))
```

Warning: Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).

```
acro
```
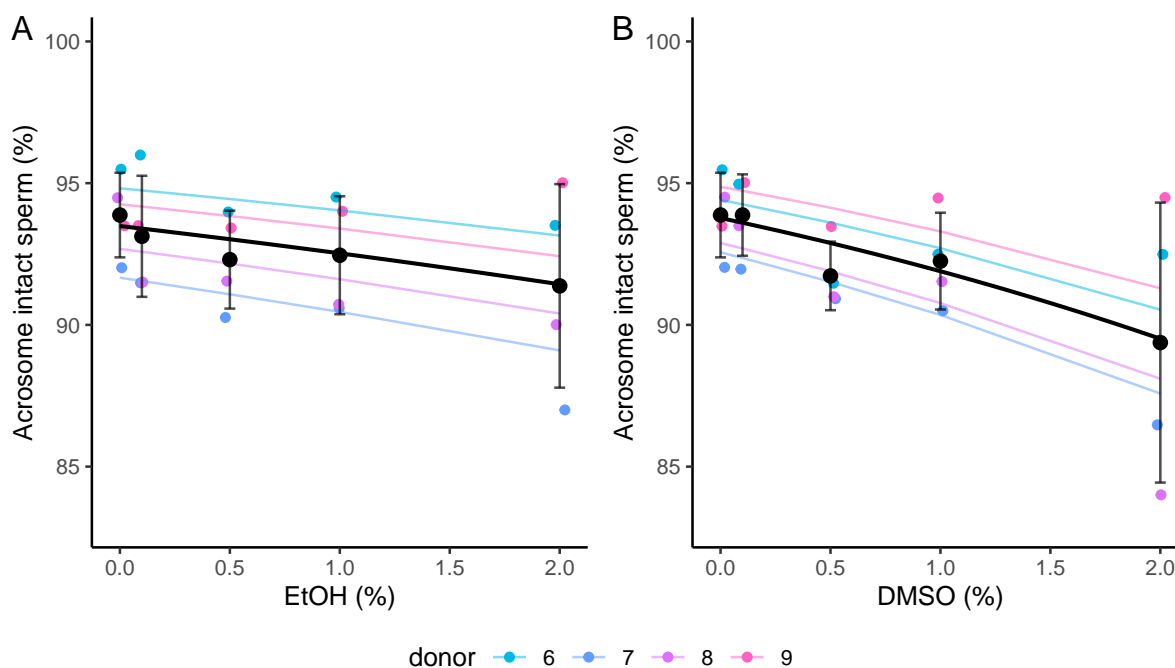


```
ggsave("../figures/acrosome.pdf", plot = acro)
```

Saving 7 x 4 in image

Vitality with DMSO

```r
vit_conc_effect_dmso <- as.data.frame(effects::predictorEffect(vit_dmso_m2,
  predictor = "conc")[c("x", "fit")])
vit_b <- ggplot(data = vit_dmso) +
  geom_jitter(aes(x = conc, y = live_perc, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(vit_dmso_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = live_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = live_perc),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = vit_conc_effect_dmso, aes(x = conc,
    y = boot::inv.logit(fit) * 100), size = 0.8) +
  ylim(83, 100) +
  labs(x = "DMSO (%)", y = "Live sperm (%)") +
  scale_color_manual(values = hex9[1:5]) +
  guides(colour = guide_legend(nrow = 1))
```

Vitality with ethanol

```r
vit_conc_effect_etoh <- as.data.frame(effects::predictorEffect(vit_etoh_m2,
  predictor = "conc")[c("x", "fit")])
vit_a <- ggplot(data = vit_etoh) +
  geom_jitter(aes(x = conc, y = live_perc, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(vit_etoh_m2) * 100, col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = live_perc), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = live_perc),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = vit_conc_effect_etoh, aes(x = conc,
    y = boot::inv.logit(fit) * 100), size = 0.8) +
  ylim(83, 100) +
  labs(x = "EtOH (%)", y = "Live sperm (%)") +
  scale_color_manual(values = hex9[1:5]) +
  guides(colour = guide_legend(nrow = 1))
```
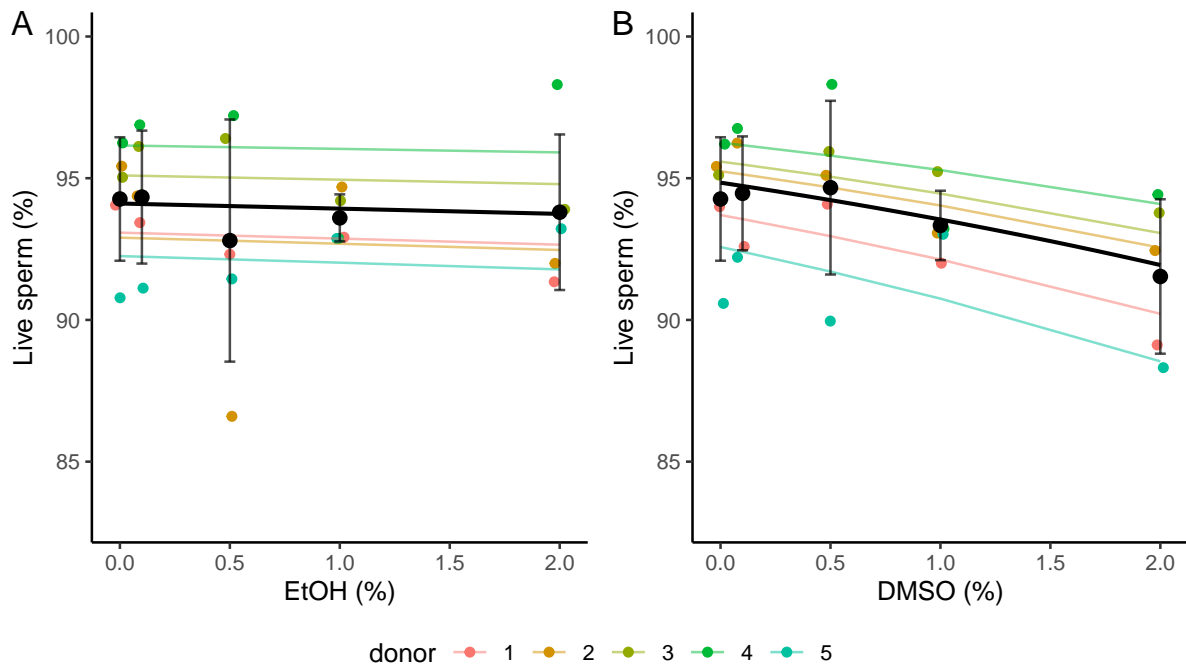
```r
vital <- ggpubr::ggarrange(vit_a, vit_b, labels = "AUTO",
    common.legend = TRUE, legend = "bottom", hjust = -0.5, vjust = 1.5,
    font.label = list(size = 14, color = "black", face = "plain", family = NULL))
```

```
Warning: Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).
Removed 5 rows containing missing values (geom_segment).
```

```r
vital
```



```r
ggsave("../figures/vitality.pdf", plot = vital)
```

```
Saving 7 x 4 in image
```

Capacitation with DMSO

```r
cap_conc_effect_dmso <- as.data.frame(effects::predictorEffect(cap_dmso_m2,
  predictor = "conc")[c("x", "fit")])
cap_b <- ggplot(data = cap_dmso) +
  geom_jitter(aes(x = conc, y = capa, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(cap_dmso_m2), col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = capa), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = capa),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = cap_conc_effect_dmso, aes(x = conc,
    y = fit), size = 0.8) +
  ylim(0, 1.5) +
  labs(x = "DMSO (%)", y = "Relative densitometry\nphosphotyrosines/ß-tubulin") +
  scale_color_manual(values = hex9[1:5]) +
  guides(colour = guide_legend(nrow = 1))
```

Capacitation with ethanol

```r
cap_conc_effect_etoh <- as.data.frame(effects::predictorEffect(cap_etoh_m2,
  predictor = "conc")[c("x", "fit")])
cap_a <- ggplot(data = cap_etoh) +
  geom_jitter(aes(x = conc, y = capa, col = donor), width = 0.025) +
  geom_line(aes(x = conc, y = fitted(cap_etoh_m2), col = donor),
    alpha = 0.5) +
  stat_summary(aes(x = conc, y = capa), geom = "pointrange",
    fun = "mean", shape = 16, size = 0.6) +
  stat_summary(aes(x = conc, y = capa),
    geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
    alpha = 0.7, width = 0.05) +
  geom_line(data = cap_conc_effect_etoh, aes(x = conc,
    y = fit), size = 0.8) +
  ylim(0, 1.5) +
  labs(x = "EtOH (%)", y = "Relative densitometry\nphosphotyrosines/ß-tubulin") +
  scale_color_manual(values = hex9[1:5]) +
  guides(colour = guide_legend(nrow = 1))
```

```r
cap_c <- cap_d <- ggplot() + theme_minimal()
capa <- ggpubr::ggarrange(cap_c, cap_d, cap_a, cap_b, labels = "AUTO",
  common.legend = TRUE, legend = "bottom", hjust = -0.5, vjust = 1.5,
  font.label = list(size = 14, color = "black", face = "plain", family = NULL))
```

Warning: Removed 2 rows containing non-finite values (stat_summary).
Removed 2 rows containing non-finite values (stat_summary).

Warning: Removed 2 rows containing missing values (geom_point).

Warning: Removed 5 rows containing missing values (geom_segment).

Warning: Removed 2 rows containing non-finite values (stat_summary).
Removed 2 rows containing non-finite values (stat_summary).

Warning: Removed 2 rows containing missing values (geom_point).

Warning: Removed 5 rows containing missing values (geom_segment).

Warning: Removed 1 rows containing non-finite values (stat_summary).
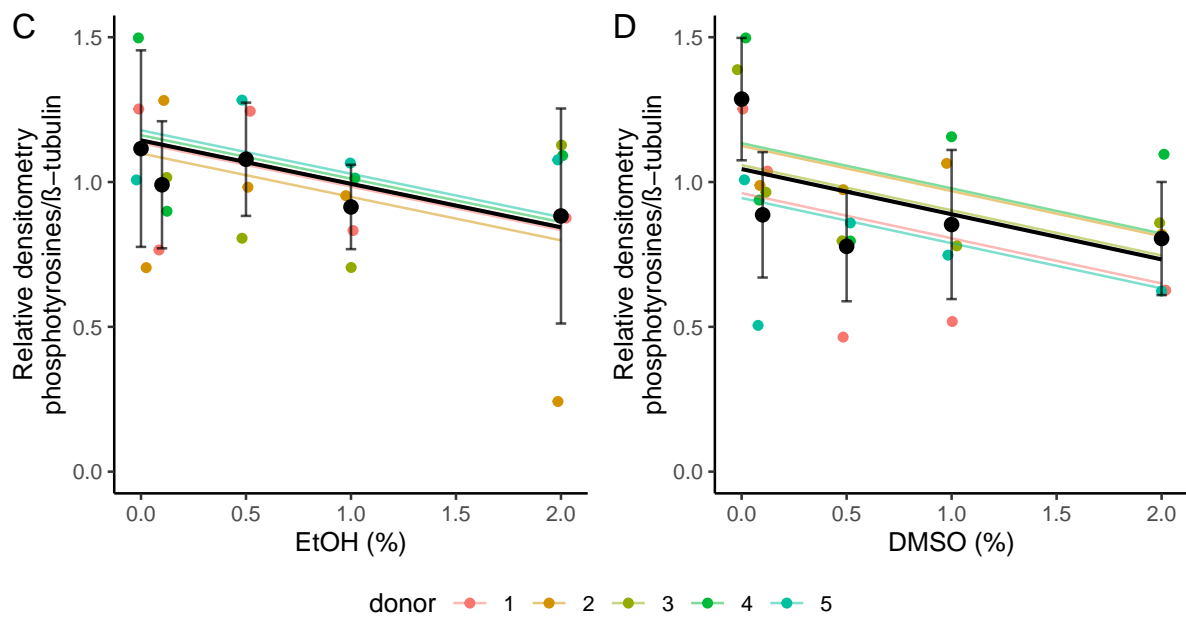Removed 1 rows containing non-finite values (stat_summary).

Warning: Removed 1 rows containing missing values (geom_point).

Warning: Removed 5 rows containing missing values (geom_segment).

```r
capa
```

A

B

C

D

donor ─●─ 1 ─●─ 2 ─●─ 3 ─●─ 4 ─●─ 5

```
ggsave("../figures/capacitation.pdf", plot = capa)
```

Saving 7 x 7 in image

Effect of time on total motility

```r
mtt_cont <- readxl::read_excel("../data/Table S3.xlsx", sheet = "total motility_control")
mtt_cont$treat <- "control"
mtt_dmso <- readxl::read_excel("../data/Table S3.xlsx", sheet = "total motility_2%DMSO")
mtt_dmso$treat <- "DMSO 2%"
mtt_etoh1 <- readxl::read_excel("../data/Table S3.xlsx", sheet = "total motility_1%EtOH")
mtt_etoh1$treat <- "EtOH 1%"
mtt_etoh2 <- readxl::read_excel("../data/Table S3.xlsx", sheet = "total motility_2%EtOH")
mtt_etoh2$treat <- "EtOH 2%"
mtt <- rbind(mtt_cont, mtt_dmso, mtt_etoh1, mtt_etoh2)
mtt$donor <- as.factor(mtt$donor)
mtt$treat <- as.factor(mtt$treat)
names(mtt) <- c("donor", "time", "motile", "total", "treat")
mtt$motile_frac <- mtt$motile / mtt$total
mtt$motile_perc <- mtt$motile_frac * 100
# Selected model
mtt_m2 <- glmer(cbind(motile, total - motile) ~ treat * time + (1 | donor),
  data = mtt, family = binomial(link = "logit"))
```

```r
mtt_conc_effect <- as.data.frame(effects::predictorEffect(mtt_m2,
  predictor = "time")[c("x", "fit")])
time_a <- ggplot(data = mtt) +
  geom_jitter(aes(x = time, y = motile_perc, col = treat),
    width = 0.035, alpha = 0.5, size = 1.2) +
  #geom_line(aes(x = time, y = fitted(mtt_m2) * 100, col = treat),
  #  alpha = 0.5) +
  geom_line(data = mtt_conc_effect, aes(x = x.time,
    y = boot::inv.logit(fit) * 100, col = x.treat), size = 0.8) +
  stat_summary(aes(x = time, y = motile_perc, col = treat), geom = "point",
    fun = "mean", shape = 19, size = 2.5) +
  #stat_summary(aes(x = time, y = motile_perc, col = treat),
  #  geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
  #  alpha = 0.7, width = 0.15, position = "dodge") +
  ylim(25, 100) +
  scale_color_manual(values = c("darkgray", "red", "darkblue", "royalblue1")) +
  labs(x = "Time (h)", y = "Motile sperm (%)") #+
  #guides(colour = guide_legend(title = "Treatment", nrow = 1))
```

Effect of time on progressive motility

```r
mpt_cont <- readxl::read_excel("../data/Table S3.xlsx", sheet = "progressive motility_cont
mpt_cont$treat <- "control"
mpt_dmso <- readxl::read_excel("../data/Table S3.xlsx", sheet = "progressive motility_2%DM
mpt_dmso$treat <- "DMSO 2%"
mpt_etoh1 <- readxl::read_excel("../data/Table S3.xlsx", sheet = "progressive motility_1%E
mpt_etoh1$treat <- "EtOH 1%"
mpt_etoh2 <- readxl::read_excel("../data/Table S3.xlsx", sheet = "progressive motility_2%E
mpt_etoh2$treat <- "EtOH 2%"
mpt <- rbind(mpt_cont, mpt_dmso, mpt_etoh1, mpt_etoh2)
mpt$donor <- as.factor(mpt$donor)
mpt$treat <- as.factor(mpt$treat)
names(mpt) <- c("donor", "time", "prog", "total", "treat")
mpt$prog_frac <- mpt$prog / mpt$total
mpt$prog_perc <- mpt$prog_frac * 100
# Selected model
mpt_m2 <- glmer(cbind(prog, total - prog) ~ treat * time + (1 | donor),
  data = mpt, family = binomial(link = "logit"))
```

```r
mpt_conc_effect <- as.data.frame(effects::predictorEffect(mpt_m2,
  predictor = "time")[c("x", "fit")])
time_b <- ggplot(data = mpt) +
  geom_jitter(aes(x = time, y = prog_perc, col = treat),
    width = 0.035, alpha = 0.5, size = 1.2) +
  #geom_line(aes(x = time, y = fitted(mpt_m2) * 100, col = treat),
  #  alpha = 0.5) +
  geom_line(data = mpt_conc_effect, aes(x = x.time,
    y = boot::inv.logit(fit) * 100, col = x.treat), size = 0.8) +
  stat_summary(aes(x = time, y = prog_perc, col = treat), geom = "point",
    fun = "mean", shape = 19, size = 2.5) +
  #stat_summary(aes(x = time, y = prog_perc, col = treat),
  #  geom = "errorbar", fun.data = "mean_sdl", fun.args = list(mult = 1),
  #  alpha = 0.7, width = 0.15, position = "dodge") +
  ylim(25, 100) +
  scale_color_manual(values = c("darkgray", "red", "navyblue", "royalblue1")) +
  labs(x = "Time (h)", y = "Progressive motile sperm (%)") #+
  #guides(colour = guide_legend(title = "Treatment", nrow = 1))
```

```
time <- ggpubr::ggarrange(time_a, time_b, labels = "AUTO",
  common.legend = TRUE, legend = "bottom", hjust = -0.5, vjust = 1.5,
  font.label = list(size = 14, color = "black", face = "plain", family = NULL))
time
```
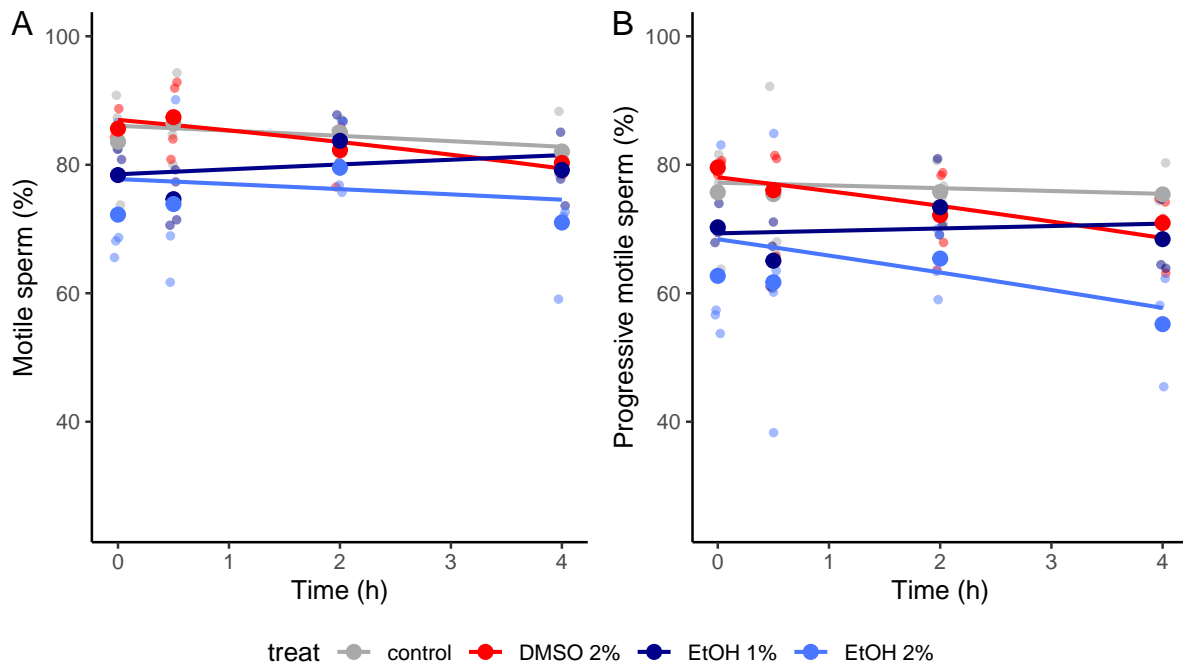


```
ggsave("../figures/time.pdf", plot = time)
```

```
Saving 7 x 4 in image
```