

Statistics for the Sciences

Analysis of Covariance

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January 18, 2025

Outline

- Analysis of Covariance
 - ▶ Introduction
 - ▶ ANCOVA
 - ▶ Example
- Lab

Introduction

- ANCOVA (Analysis of Covariance) is really “ANOVA with covariates” or, more simply, a combination of ANOVA and regression used when you have some categorical factors and some quantitative predictors.
- The predictors (X variables on which to perform regression) are called “covariates” in this context.
- The idea is that often these covariates are not necessarily of primary interest, but still their inclusion in the model will help explain more of the response, and hence reduce the error variance.

Introduction

- Example (`partridge.csv`): Partridge and Farquhar (1981) studied the effect of number of mating partners on **longevity** of fruitflies. There were five **treatments**: one virgin female per day, one newly inseminated (pregnant) female per day, eight virgin females per day, eight newly inseminated (pregnant) females per day, and a control group with no females. There were 25 males, individually kept in separate vials, in each group. The **thorax length** of each individual fly was also recorded as a **covariate**. If thorax length explains some of the variation in longevity, then the evaluation of the effect of treatment on longevity adjusted for thorax length will be more precise.
- The purpose of the study is to check effect of partner treatment on longevity of male fruitflies, adjusting for thorax length.
 - ▶ Response: `longev`
 - ▶ Factor: `treatment` with 5 levels
 - ▶ Covariate: `thorax`

Introduction

##	partners	type	treatment	longev	thorax
## 1	8	0	preg8	35	0.64
## 2	8	0	preg8	37	0.68
## 3	8	0	preg8	49	0.68
## 4	8	0	preg8	46	0.72
## 5	8	0	preg8	63	0.72
## 6	8	0	preg8	39	0.76
## 7	8	0	preg8	46	0.76
## 8	8	0	preg8	56	0.76
## 9	8	0	preg8	63	0.76
## 10	8	0	preg8	65	0.76
## 11	8	0	preg8	56	0.80
## 12	8	0	preg8	65	0.80
## 13	8	0	preg8	70	0.80
## 14	8	0	preg8	63	0.84
## 15	8	0	preg8	65	0.84
## 16	8	0	preg8	70	0.84
## 17	8	0	preg8	77	0.84
## 18	8	0	preg8	81	0.84
## 19	8	0	preg8	86	0.84
## 20	8	0	preg8	70	0.88
## 21	8	0	preg8	70	0.88
## 22	8	0	preg8	77	0.92
## 23	8	0	preg8	77	0.92
## 24	8	0	preg8	81	0.92
## 25	8	0	preg8	77	0.94
## 26	0	9	none	40	0.64
## 27	0	9	none	37	0.70
## 28	0	9	none	44	0.72
## 29	0	9	none	47	0.72
## 30	0	9	none	47	0.72
## 31	0	9	none	47	0.76
## 32	0	9	none	68	0.78
## 33	0	9	none	47	0.80
## 34	0	9	none	54	0.84
## 35	0	9	none	61	0.84
## 36	0	9	none	71	0.84
## 37	0	9	none	75	0.84
## 38	0	9	none	89	0.84
## 39	0	9	none	58	0.88

Introduction

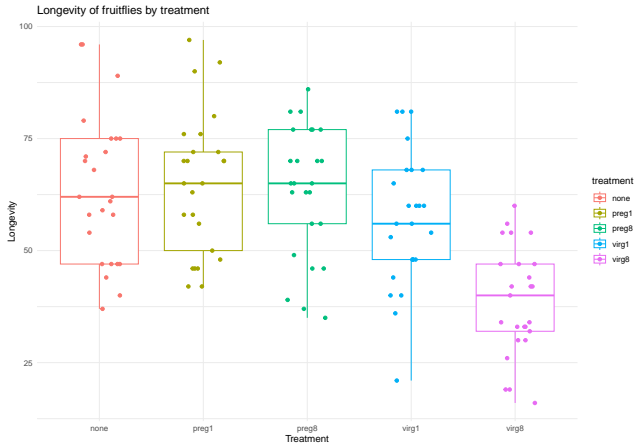
- Levels of treatment

```
## [1] "none" "preg1" "preg8" "virg1" "virg8"
```

Introduction

- The primary concern in their study was ensuring that groups were comparable at baseline. This allows any observed differences in longevity among treatment groups to be attributed to the treatment itself rather than potential confounding factor thorax.
 - ▶ Thorax length was considered as a covariate because it was hypothesized to explain some of the variation in longevity.
- By including thorax length as a covariate in the analysis, the study aimed to improve the precision of evaluating the effect of treatment on longevity while adjusting for potential baseline differences in thorax length across groups.

Introduction



Introduction

- Summarize the data by treatment

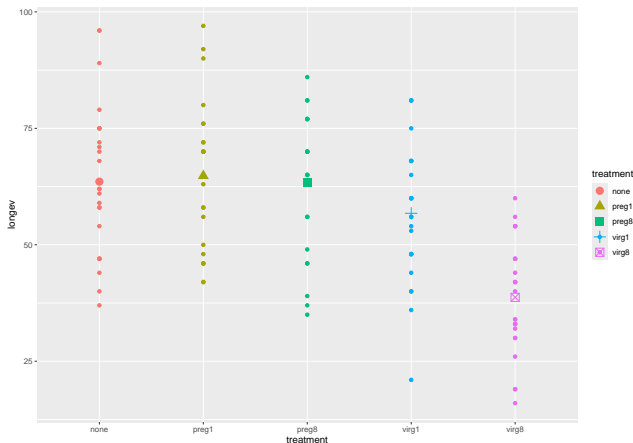
##	treatment	mean_longev	sd_longev	mean_thorax	sd_thorax
## 1	none	63.56	16.45215	0.8360	0.08426150
## 2	preg1	64.80	15.65248	0.8256	0.06988562
## 3	preg8	63.36	14.53983	0.8056	0.08155162
## 4	virg1	56.76	14.92838	0.8376	0.07055022
## 5	virg8	38.72	12.10207	0.8000	0.07831560

Introduction

Several statistical models can be proposed and fitted:

- Model 1: 1-way ANOVA, no covariates are involved

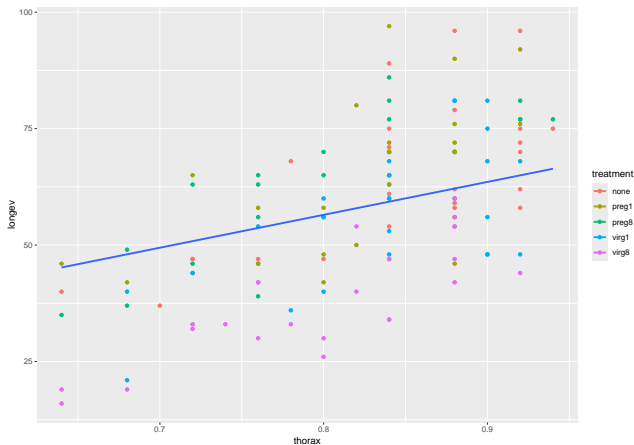
$$H_1 : Y_{ij} = \mu_i + \varepsilon_{ij}.$$



Introduction

- Model 2: SLR model

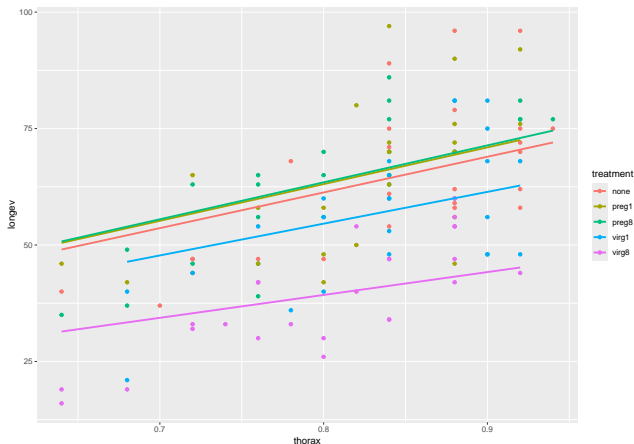
$$H_2 : Y_{ij} = \mu + \beta x_{ij} + \varepsilon_{ij}.$$



Introduction

- Model 3: Three SLR models with the same slope

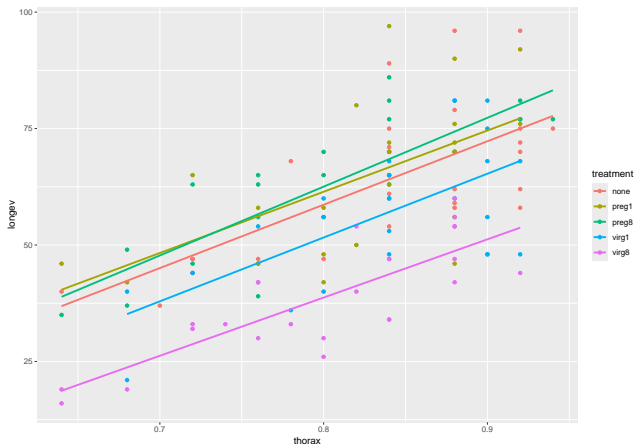
$$H_3 : Y_{ij} = \mu_i + \beta x_{ij} + \varepsilon_{ij}.$$



Introduction

- Model 4 (Five **independent** SLR models):

$$H_4 : Y_{ij} = \mu_i + \beta_i x_{ij} + \varepsilon_{ij}.$$



Analysis of Covariance

Table 1: Data from 1-way design with covariates

Trt 1		...		Trt a	
y	x			y	x
y_{11}	x_{11}	\cdots	\cdots	y_{a1}	x_{a1}
y_{12}	x_{12}	\cdots	\cdots	y_{a2}	x_{a2}
\vdots	\vdots	\vdots	\vdots	\vdots	\vdots
y_{1n}	x_{1n}	\cdots	\cdots	y_{an}	x_{an}

Analysis of Covariance

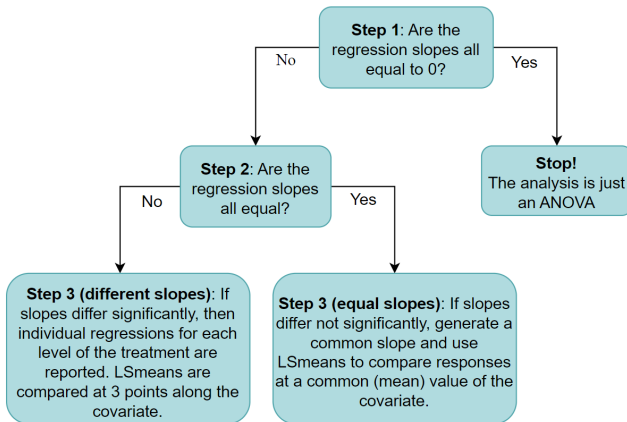
- **Homogeneity of regression slopes** assumption: ANCOVA assumes a constant effect of the covariate across levels of the categorical factor.
 - ▶ ANCOVA is assuming model H_3 and test for H_2
 - ▶ If the assumption is violated, use model H_4 .
- ANCOVA model for a balanced design

$$Y_{ij} = \mu + \tau_i + \beta(x_{ij} - \bar{x}_{..}) + \varepsilon_{ij}, i = 1, \dots, a; j = 1, \dots, n.$$

Table 2: Analysis of Covariance Table

Source	df	SS	MS	F
Regression	1	SS_{cov}	SS_{cov}	MS_{cov}/MSE
Treatments	$a - 1$	SS_T	$SS_T/(a - 1)$	MS_T/MSE
Error	$a(n - 1) - 1$	SSE	$\frac{SSE}{a(n-1)-1}$	
Total	$an - 1$	SS_{total}		

Analysis of Covariance



Example

- Fit a full linear model with interaction: formula `longev ~ thorax+ treatment +thorax*treatment`
 - ▶ The large p-value for the interaction shows that the relationship between the covariate and the dependent variable does NOT vary across levels of the categorical factor.

```
## Analysis of Variance Table
##
## Response: longev
##              Df Sum Sq Mean Sq  F value    Pr(>F)
## thorax         1 15496.6  15496.6  136.0170 < 2.2e-16 ***
## treatment      4   9611.5   2402.9   21.0905 4.617e-13 ***
## thorax:treatment 4    42.5    10.6    0.0933  0.9844
## Residuals     115 13102.1    113.9
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Example

- Fit a model without interaction `longev ~ thorax+ treatment`
- ANOVA table

```
## Analysis of Variance Table
##
## Response: longev
##           Df  Sum Sq Mean Sq F value    Pr(>F)
## thorax      1 15496.6 15496.6 140.293 < 2.2e-16 ***
## treatment   4  9611.5  2402.9  21.753 1.719e-13 ***
## Residuals 119 13144.7   110.5
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Example

- Model fit of `longev ~ thorax+ treatment`

```
##
## Call:
## lm(formula = longev ~ thorax + treatment, data = partridge)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -26.189  -6.599  -0.989   6.408  30.244
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -49.984    10.609  -4.711 6.73e-06 ***
## thorax        135.819    12.439  10.919 < 2e-16 ***
## treatmentpreg1  2.653     2.975   0.891  0.3745
## treatmentpreg8  3.929     2.997   1.311  0.1923
## treatmentvirg1 -7.017     2.973  -2.361  0.0199 *
## treatmentvirg8 -19.951     3.006  -6.636 1.00e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 10.51 on 119 degrees of freedom
## Multiple R-squared:  0.6564, Adjusted R-squared:  0.6419
## F-statistic: 45.46 on 5 and 119 DF, p-value: < 2.2e-16
```

Example

- Adjusted means: group means adjusted for the effect of the covariate.

```
##
## treatment effect
## treatment
##      none      preg1      preg8      virg1      virg8
## 61.51729 64.16980 65.44617 54.49998 41.56676
##
## Lower 95 Percent Confidence Limits
## treatment
##      none      preg1      preg8      virg1      virg8
## 57.33868 60.00608 61.26686 50.31769 37.37270
##
## Upper 95 Percent Confidence Limits
## treatment
##      none      preg1      preg8      virg1      virg8
## 65.69590 68.33353 69.62549 58.68227 45.76081
```

Example

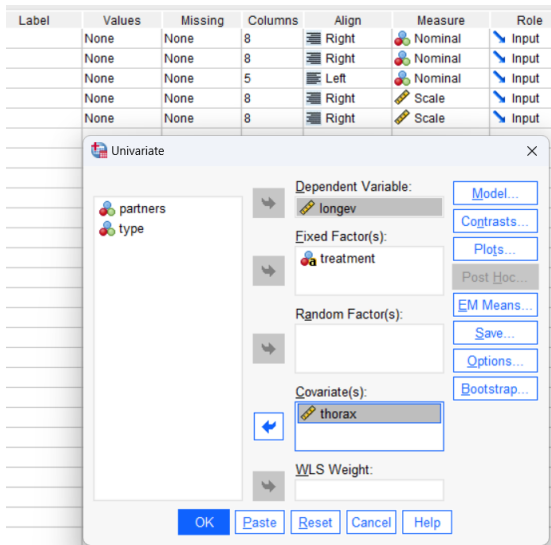
- Post hoc tests

- ▶ We conduct the test if " H_0 : All group means (adjusted) are equal" can be rejected and which group means are different

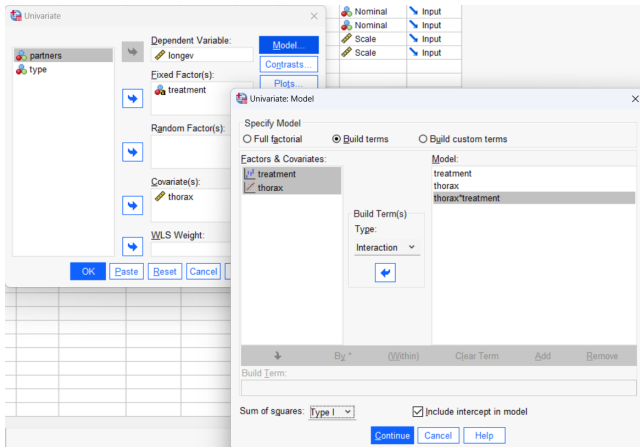
```
##
## Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
## Fit: lm(formula = longev ~ thorax + treatment, data = partridge)
##
## Linear Hypotheses:
##              Estimate Std. Error t value Pr(>|t|)
## preg1 - none == 0      2.653      2.975   0.891 0.899548
## preg8 - none == 0      3.929      2.997   1.311 0.684850
## virg1 - none == 0     -7.017      2.973  -2.361 0.133584
## virg8 - none == 0    -19.951      3.006  -6.636 < 1e-04 ***
## preg8 - preg1 == 0      1.276      2.983   0.428 0.992926
## virg1 - preg1 == 0     -9.670      2.976  -3.249 0.012868 *
## virg8 - preg1 == 0    -22.603      2.990  -7.560 < 1e-04 ***
## virg1 - preg8 == 0    -10.946      2.999  -3.650 0.003484 **
## virg8 - preg8 == 0    -23.879      2.973  -8.031 < 1e-04 ***
## virg8 - virg1 == 0    -12.933      3.009  -4.298 0.000361 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

Lab

- After importing the data, click on Analyze → General Linear Model → Univariate...



- Consider the full factorial model to check the assumption of Homogeneity of regression slopes



- Consider the full factorial model to check the assumption of Homogeneity of regression slopes

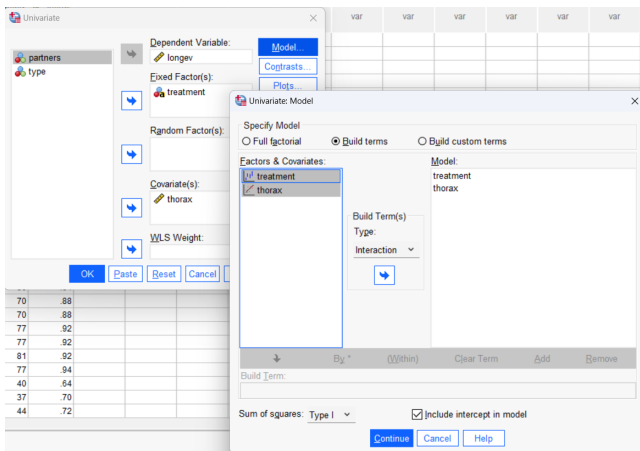
Tests of Between-Subjects Effects

Dependent Variable: *longev*

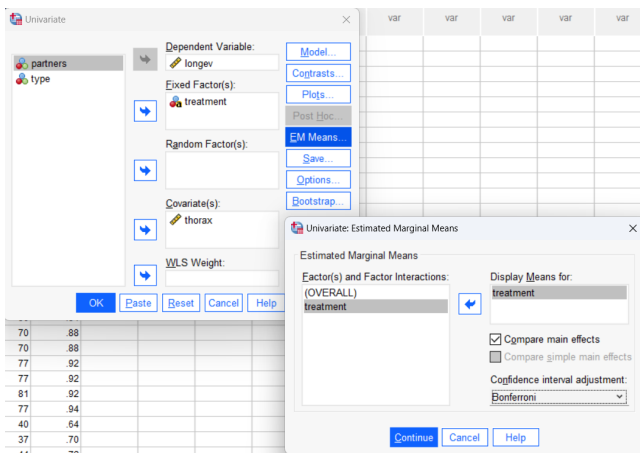
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25150.657 ^a	9	2794.517	24.528	<.001
Intercept	412419.200	1	412419.200	3619.882	<.001
treatment	11939.280	4	2984.820	26.198	<.001
thorax	13168.853	1	13168.853	115.586	<.001
treatment * thorax	42.523	4	10.631	.093	.984
Error	13102.143	115	113.932		
Total	450672.000	125			
Corrected Total	38252.800	124			

a. R Squared = .657 (Adjusted R Squared = .631)

- Now go back fit the model without interaction



- To compare adjusted means



The image shows two SPSS dialog boxes. The background box is the 'Univariate' dialog, and the foreground box is the 'Univariate: Estimated Marginal Means' sub-dialog.

Univariate Dialog:

- Dependent Variable:** longev
- Fixed Factor(s):** treatment
- Random Factor(s):** (empty)
- Covariate(s):** thorax
- WLS Weight:** (empty)
- Buttons:** Model..., Contrasts..., Plots..., Post Hoc..., EM Means... (highlighted), Save..., Options..., Bootstrap...

Univariate: Estimated Marginal Means Dialog:

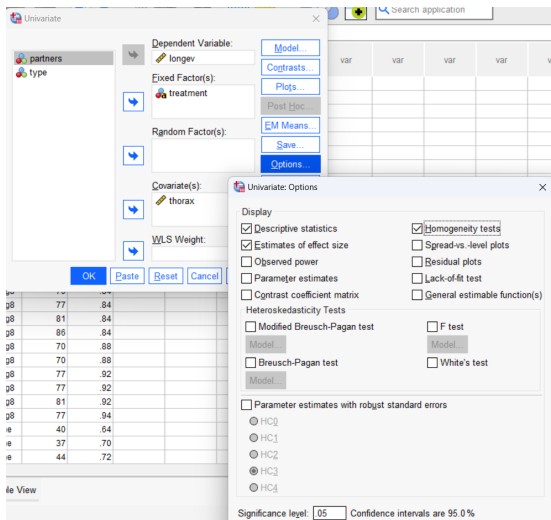
- Estimated Marginal Means**
- Factor(s) and Factor Interactions:** (OVERALL), treatment
- Display Means for:** treatment
- ☒ Compare main effects
- ☐ Compare simple main effects
- Confidence interval adjustment:** Bonferroni
- Buttons:** Continue, Cancel, Help

Data Table (bottom left):

70	.88			
70	.88			
77	.92			
77	.92			
81	.92			
77	.94			
40	.64			
37	.70			
44	.73			

Lab

- Set up some options



Descriptive Statistics

Dependent Variable: longev

treatment	Mean	Std. Deviation	N
none	63.56	16.452	25
preg1	64.80	15.652	25
preg8	63.36	14.540	25
virg1	56.76	14.928	25
virg8	38.72	12.102	25
Total	57.44	17.564	125

Levene's Test of Equality of Error Variances^a

Dependent Variable: longev

F	df1	df2	Sig.
1.861	4	120	.122

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + treatment + thorax

Tests of Between-Subjects Effects

Dependent Variable: longev

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25108.133 ^a	5	5021.627	45.461	<.001
Intercept	412419.200	1	412419.200	3733.673	<.001
treatment	11939.280	4	2984.820	27.022	<.001
thorax	13168.853	1	13168.853	119.219	<.001
Error	13144.667	119	110.459		
Total	450672.000	125			
Corrected Total	38252.800	124			

a. R Squared = .656 (Adjusted R Squared = .642)

Estimated Marginal Means

treatment

Estimates

Dependent Variable: longe

treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
none	61.517 ^a	2.110	57.339	65.696
pregl	64.170 ^a	2.103	60.006	68.334
pregs	65.446 ^a	2.111	61.267	69.625
vugl	54.500 ^a	2.112	50.318	58.682
vugs	41.567 ^a	2.118	37.373	45.761

a. Covariates appearing in the model are evaluated at the following values: thorax = .8210.

Pairwise Comparisons

Dependent Variable: longe

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig. ^b	95% Confidence Interval for Difference ^b	
					Lower Bound	Upper Bound
none	pregl	-2.653	2.975	1.000	-11.163	5.858
	pregs	-3.929	2.997	1.000	-12.500	4.642
	vugl	7.017	2.973	.199	-1.486	15.520
	vugs	19.951 [*]	3.006	<.001	11.352	28.549
pregl	none	2.653	2.975	1.000	-5.858	11.163
	pregs	-1.276	2.983	1.000	-9.809	7.256
	vugl	9.670 [*]	2.976	.015	1.156	18.183

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