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RE: Protocol 418-020

Attached is the information on covariant analysis for study 418-020. Four different analyses were done for females and two for males.

Females:
1. Sexual maturation w/ gestational age at sexual maturation as covariate
2. Sexual maturation w/ body weight at maturation as covariate
3. Estrous stages w/ day 64 postweaning body weight as covariate
4. Estrous stages w/ gestational age at start of estrous testing as covariate

Males:
1. Sexual maturation w/ gestational age at sexual maturation as covariate
2. Sexual maturation w/ body weight at maturation as covariate

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We are defining "gestational age" in this context as the time in days from evidence of mating in the P generation until evidence of sexual maturation in the F1 generation. The reasoning is rats naturally delivery after 21 to 23 days of gestation. Pups that are delivered after 23 days of gestation tend to weigh more and appear to develop faster than pups of the same sex after 21 days of gestation. Both pups would be 1 day old but the first pup would be 23 gestational days old and the second pup only 21 gestation days old. If a test substance shortened pregnancy (reduced length of gestation or if more pups were delivered early (reduced parturition length) in one group, the pups may reach sexual maturation a day or two before pups in a group which had a longer gestation or parturition length. It would appear that the test substance was causing a delay in sexual maturation, when it actually affected the maternal animal by changing the time spent *in utero*. By using each pup's age from time of conception to sexual maturation as the covariant, the length of the stay *in utero* is factored out.

MR 59263

The Analysis of Covariance was used to evaluate preputial separation and vaginal patency of F1 generation male and female rats, respectively, using gestational age at sexual maturity and the body weight recorded on the day of preputial separation (male rats) or day of vaginal patency (female rats) as the covariates.

The Analysis of Covariance was also used to evaluate estrous cycling of F1 generation female rats, using gestational age at the start of estrous testing and day 64 postweaning body weight as the covariates.

Please review the attached information and if you have any questions, please do not hesitate to contact me.

ANCOVA (DUNNETT'S TEST) WITH BODY WEIGHT AS COVARIATE

SEX	GROUP	N	MEAN	SD
F	1	60	34.8833	2.07562
	2	58	35.5345	2.26503
	3	58	34.0862	2.56347
	4	58	34.8103	2.11475
	5	54	36.7222	2.43726 *
M	1	52	48.4615	2.13708
	2	58	49.4655	2.68991
	3	57	49.3333	3.42956
	4	58	49.7241	3.10513
	5	54	52.3333	3.02817 *

* Significantly different from Control group 1 ($p \leq 0.05$)

ANCOVA (DUNNETT'S TEST) WITH GESTATION AGE AS COVARIATE (#)

SEX	GROUP	N	MEAN	SD
F	1	60	34.8833	2.07562
	2	58	35.5345	2.26503
	3	58	34.0862	2.56347
	4	54	34.9630	2.08284
	5	52	36.7115	2.47605
M	1	52	48.4615	2.13708
	2	58	49.4655	2.68991
	3	57	49.3333	3.42956
	4	54	49.8333	3.19049
	5	53	52.2642	3.01376

(#) No statistically significant differences when compared with Control group 1 ($p>0.05$)

ANCOVA (DUNNETT'S TEST) WITH DAY 64 BODY WEIGHT AS COVARIATE

SEX	GROUP	N	MEAN	SD	
F	1	30	4.66667	0.88409	
	2	29	5.03448	0.90565	
	3	30	5.03333	0.80872	
	4	30	5.03333	1.06620	
	5	30	5.40000	0.67466	*(1)

ANCOVA (DUNNETT'S TEST) WITH ESTROUS AGE AS COVARIATE

SEX	GROUP	N	MEAN	SD	
F	1	30	4.66667	0.88409	
	2	29	5.03448	0.90565	
	3	30	5.03333	0.80872	
	4	28	5.03571	1.10494	
	5	28	5.39286	0.68526	*(1)

* Significantly different from Control group 1 ($p \leq 0.05$)
(1) Note: Results from Dunnett's test are reported without regard to the significance of the global treatment group effect in the overall ANCOVA (Haseman, Toxicological Sciences, June, 2001). In both analyses (covariate = day 64 body weight and covariate = estrous age) the global treatment group effect was not statistically significant in the overall ANCOVA.

SEX=F

The Mixed Procedure

Model Information

Data Set	WORK.AAA
Dependent Variable	DAY_MAT
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
GROUP	5	1 2 3 4 5

Dimensions

Covariance Parameters	1
Columns in X	7
Columns in Z	0
Subjects	1
Max Obs Per Subject	300
Observations Used	288
Observations Not Used	12
Total Observations	300

SEX=F

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	4.3642

Fit Statistics

-2 Res Log Likelihood	1246.4
AIC (smaller is better)	1248.4
AICC (smaller is better)	1248.4
BIC (smaller is better)	1252.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
BWT	1	282	59.63	<.0001
GROUP	4	282	9.50	<.0001

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	1	34.8248	0.2698	282	129.07	<.0001
GROUP	2	35.7142	0.2753	282	129.73	<.0001
GROUP	3	34.1967	0.2747	282	124.50	<.0001

SEX=F

The Mixed Procedure

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	4	34.8710	0.2744	282	127.07	<.0001
GROUP	5	36.4103	0.2871	282	126.80	<.0001

Differences of Least Squares Means

Effect	GROUP	_GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
GROUP	2	1	0.8893	0.3859	282	2.30	0.0219	Dunnett-Hsu	0.0736
GROUP	3	1	-0.6281	0.3853	282	-1.63	0.1042	Dunnett-Hsu	0.3006
GROUP	4	1	0.04619	0.3850	282	0.12	0.9046	Dunnett-Hsu	0.9999
GROUP	5	1	1.5855	0.3932	282	4.03	<.0001	Dunnett-Hsu	0.0003

SEX=M

The Mixed Procedure

Model Information

Data Set	WORK.AAA
Dependent Variable	DAY_MAT
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
GROUP	5	1 2 3 4 5

Dimensions

Covariance Parameters	1
Columns in X	7
Columns in Z	0
Subjects	1
Max Obs Per Subject	300
Observations Used	279
Observations Not Used	21
Total Observations	300

SEX=M

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	7.1683

Fit Statistics

-2 Res Log Likelihood	1344.4
AIC (smaller is better)	1346.4
AICC (smaller is better)	1346.4
BIC (smaller is better)	1350.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
BWT	1	273	53.38	<.0001
GROUP	4	273	17.77	<.0001

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	1	48.5554	0.3715	273	130.70	<.0001
GROUP	2	49.6708	0.3527	273	140.84	<.0001
GROUP	3	49.0207	0.3572	273	137.24	<.0001

---------- SEX=M ----------

The Mixed Procedure

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	4	49.5845	0.3521	273	140.84	<.0001
GROUP	5	52.5024	0.3651	273	143.81	<.0001

Differences of Least Squares Means

Effect	GROUP	_GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
GROUP	2	1	1.1154	0.5115	273	2.18	0.0301	Dunnett-Hsu	0.0964
GROUP	3	1	0.4653	0.5164	273	0.90	0.3684	Dunnett-Hsu	0.7726
GROUP	4	1	1.0291	0.5123	273	2.01	0.0456	Dunnett-Hsu	0.1409
GROUP	5	1	3.9469	0.5203	273	7.59	<.0001	Dunnett-Hsu	<.0001

SEX=F

The Mixed Procedure

Model Information

Data Set	WORK.AAA
Dependent Variable	DAY_MAT
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
GROUP	5	1 2 3 4 5

Dimensions

Covariance Parameters	1
Columns in X	7
Columns in Z	0
Subjects	1
Max Obs Per Subject	300
Observations Used	282
Observations Not Used	18
Total Observations	300

SEX=F

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	0.2779

Fit Statistics

-2 Res Log Likelihood	457.3
AIC (smaller is better)	459.3
AICC (smaller is better)	459.3
BIC (smaller is better)	462.9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
GEST_AGE	1	276	4990.64	<.0001
GROUP	4	276	3.21	0.0135

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	1	35.1763	0.06819	276	515.88	<.0001
GROUP	2	35.3426	0.06928	276	510.16	<.0001
GROUP	3	35.0133	0.07046	276	496.94	<.0001

SEX=F

The Mixed Procedure

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	4	35.2004	0.07182	276	490.11	<.0001
GROUP	5	35.3069	0.07576	276	466.01	<.0001

Differences of Least Squares Means

Effect	GROUP	_GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
GROUP	2	1	0.1663	0.09732	276	1.71	0.0887	Dunnett-Hsu	0.2639
GROUP	3	1	-0.1630	0.09749	276	-1.67	0.0956	Dunnett-Hsu	0.2816
GROUP	4	1	0.02402	0.09889	276	0.24	0.8083	Dunnett-Hsu	0.9978
GROUP	5	1	0.1306	0.1027	276	1.27	0.2048	Dunnett-Hsu	0.5273

SEX=M

The Mixed Procedure

Model Information

Data Set	WORK.AAA
Dependent Variable	DAY_MAT
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
GROUP	5	1 2 3 4 5

Dimensions

Covariance Parameters	1
Columns in X	7
Columns in Z	0
Subjects	1
Max Obs Per Subject	300
Observations Used	274
Observations Not Used	26
Total Observations	300

SEX=M

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	0.3806

Fit Statistics

-2 Res Log Likelihood	529.4
AIC (smaller is better)	531.4
AICC (smaller is better)	531.4
BIC (smaller is better)	535.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
GEST_AGE	1	268	5816.42	<.0001
GROUP	4	268	1.80	0.1290

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	1	49.7916	0.08731	268	570.26	<.0001
GROUP	2	49.9788	0.08129	268	614.84	<.0001
GROUP	3	49.7086	0.08186	268	607.21	<.0001

SEX=M

The Mixed Procedure

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	4	49.8734	0.08396	268	594.04	<.0001
GROUP	5	49.9531	0.09000	268	555.05	<.0001

Differences of Least Squares Means

Effect	GROUP	_GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
GROUP	2	1	0.1872	0.1183	268	1.58	0.1147	Dunnett-Hsu	0.3183
GROUP	3	1	-0.08298	0.1190	268	-0.70	0.4861	Dunnett-Hsu	0.8903
GROUP	4	1	0.08185	0.1211	268	0.68	0.4995	Dunnett-Hsu	0.9003
GROUP	5	1	0.1615	0.1295	268	1.25	0.2135	Dunnett-Hsu	0.5288

SEX=F

The Mixed Procedure

Model Information

Data Set	WORK.AAA
Dependent Variable	EST
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
GROUP	5	1 2 3 4 5

Dimensions

Covariance Parameters	1
Columns in X	7
Columns in Z	0
Subjects	1
Max Obs Per Subject	300
Observations Used	145
Observations Not Used	155
Total Observations	300

SEX=F

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	0.7707

Fit Statistics

-2 Res Log Likelihood	381.0
AIC (smaller is better)	383.0
AICC (smaller is better)	383.0
BIC (smaller is better)	385.9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
EST_AGE	1	139	4.04	0.0462
GROUP	4	139	1.90	0.1131

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	1	4.7408	0.1645	139	28.83	<.0001
GROUP	2	4.9751	0.1657	139	30.03	<.0001
GROUP	3	5.0098	0.1607	139	31.17	<.0001

SEX=F

The Mixed Procedure

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	4	5.0590	0.1663	139	30.42	<.0001
GROUP	5	5.3769	0.1661	139	32.37	<.0001

Differences of Least Squares Means

Effect	GROUP	_GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
GROUP	2	1	0.2343	0.2381	139	0.98	0.3266	Dunnett-Hsu	0.7202
GROUP	3	1	0.2690	0.2318	139	1.16	0.2479	Dunnett-Hsu	0.5956
GROUP	4	1	0.3182	0.2321	139	1.37	0.1725	Dunnett-Hsu	0.4501
GROUP	5	1	0.6361	0.2350	139	2.71	0.0076	Dunnett-Hsu	0.0267

SEX=F

The Mixed Procedure

Model Information

Data Set	WORK.AAA
Dependent Variable	EST
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information

Class	Levels	Values
GROUP	5	1 2 3 4 5

Dimensions

Covariance Parameters	1
Columns in X	7
Columns in Z	0
Subjects	1
Max Obs Per Subject	300
Observations Used	149
Observations Not Used	151
Total Observations	300

SEX=F

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	0.7394

Fit Statistics

-2 Res Log Likelihood	390.6
AIC (smaller is better)	392.6
AICC (smaller is better)	392.6
BIC (smaller is better)	395.6

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
BWT_64	1	143	6.80	0.0101
GROUP	4	143	2.11	0.0829

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	1	4.6807	0.1571	143	29.80	<.0001
GROUP	2	5.0123	0.1599	143	31.35	<.0001
GROUP	3	5.1143	0.1600	143	31.96	<.0001

SEX=F

The Mixed Procedure

Least Squares Means

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
GROUP	4	5.0455	0.1571	143	32.12	<.0001
GROUP	5	5.3143	0.1604	143	33.13	<.0001

Differences of Least Squares Means

Effect	GROUP	_GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
GROUP	2	1	0.3316	0.2244	143	1.48	0.1416	Dunnett-Hsu	0.3873
GROUP	3	1	0.4337	0.2235	143	1.94	0.0543	Dunnett-Hsu	0.1682
GROUP	4	1	0.3648	0.2220	143	1.64	0.1025	Dunnett-Hsu	0.2948
GROUP	5	1	0.6336	0.2253	143	2.81	0.0056	Dunnett-Hsu	0.0201