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Wernsing, Danyel R., "Fetal and Placental Development in GHR-KO Mice" (1999). *Honors Theses.* Paper 78.

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Fetal and Placental Development in GHR-KO mice

Danyel Wernsing

In this research, I worked with growth hormone receptor knockout mice to examine embryo survival, fetal and placental development in homozygous GHR/BP(KO) and normal females. First, I mated the KO and normal females with normal males and checked for plugs every day. When the females were at various stages of pregnancy, I would kill them with CO_2 and dissected them. Upon killing these mice at various stages, I found the number and conditions of the fetuses. Also, placental weight, fetus weight, and crown-rump length of the fetus were compared between the KO and normal females. Therefore, I could detect, if there was a significant difference between the fetuses of the KO and normal females.

GHR-KO mice came from Ohio University in Athens, Ohio. These mice were made by a disrupted GHR/binding protein (GHR/BP) gene through a homologous gene targeting approach. Essentially, these animals are mammalian models for Laron syndrome in humans. Furthermore, the KO mice have characteristics that resemble the phenotype of people with Laron syndrome. These characteristics consist of dwarfism, absence of the GHR and GH binding protein, postnatal growth retardation, decreased serum insulin-like growth factor I and elevated serum GH concentrations. The KO mouse obtained by disrupting the GHR/BP gene through homologous recombination. After two chimeric males transferred the disrupted allele to their offspring which the genotype GHR/BP$^{+/-}$, heterozygotes. Then, the FI generation of GHR/BP$^{+/-}$ mated to produce homozygous GHR/BP$^{-/-}$ mice(KO). Thus, the generation of GHR-KO mice produced with GHR/BP gene knockout, the Laron mouse

(Zhou, 1997).

The only fertility information given before the start of my experiment was the report of litter size, perinatal mortality, and maturation of the KO(homozygous) and normal(heterozygous) females. The litters of normal females reported was 6.90 to 6.57. Yet, the litters of the KO on the average are 2.71. The perinatal mortality was higher in the KO than the normal. Furthermore, the maturation of the normal females was six weeks, but the maturation in the KO females was 10 weeks. The reason given for the smaller litter and higher mortality maybe related to placentation, "obstetrical problems" due to maternal-fetal size mismatch, and insufficient lactation to nourish pups (Zhou, 1997).

In my study, I assumed that the fetuses of the KO females would be smaller than the normal females. The data was collected over a period of two months. After checking plugs and determining which stage of pregnancy, the mice were killed. I started from day 14 of pregnancy to day 18. Also, the KO females usually had to obtain a plug twice before getting pregnant. Table 1 shows the number of mice killed per day. The litter sizes of the normal females were 7.125 pups, and the KO females were 4.833(Table 2). All the fetuses showed to be in good condition, but some KO females gave birth to dead litters. For instance, the KO females that did not have a plug date carried to term, but one out of seven had live pups. From the normal females, only one litter out of three died. The corpus luteum was looked at in every female. It has cells that produce

progesterone to maintain pregnancy until the placenta can produce enough progesterone to take over for the corpus luteum. When dissecting the mice, I observed the corpus luteum for ruptured follicles which accounted for the fetuses, or absorbed fetuses and non-implanted embryos. In the normal females, the non-implanted eggs were found in only two of the females, but in the KO females, it was found in four of the mice. Table 2 shows the data of the absorbed fetuses of the normal and KO females. There was not a significant difference between the females.

The data of the placental weight, fetal weight, and fetal length was analyzed on ANOVA. The graphs showed the mean and standard of each day compared with normal and KO females. Also, the p value had to show a value less than 0.05 to be statistically significant. From the placental weight, the data showed day 16 and day 17 to have a significant difference. Furthermore, the placental weight of the KO females was larger than the normal females. The suspected reason for the larger placental weight is that the KO mice are secrete a lower level of glucose, so the larger placenta maybe compensating for the lack of glucose. The fetus weight displayed a significant difference at day 14 and day 17. With the fetus length the significant difference was found on day 17 and day 18. The graphs and statistics are attached at the end of the paper.

To better the results, more animals mated to get a larger number of fetuses. Also, the normal females and normal males need to be tested for their genotype, to know if they are +/+ or +/-. Knowing the genotype of the parents, the study would show

if fetuses are both KO and normal mice. If the fetuses are both KO and normal, the genotype of the fetuses can be found by taking samples of the fetus tissue.

TABLE 1

Day	KO	Normal	Total Number of Fetuset:	Normal	KO
14	4	3		21	18
15	3	3		27	13
16	4	3		21	23
17	3	3		19	17
18	4	4		28	15

TABLE 2

Type	Litter size mean(range)	Absorbed Fetus mean(range)
KO	4.833(2 to 8)	1.875(1 to 4)only in 8 KOs
Normal	7.125(4 to 11)	2(1 to 3)only in 8 normals

Works Cited

Zhou, Yihua., et al. "A mammalian model for Laron syndrome

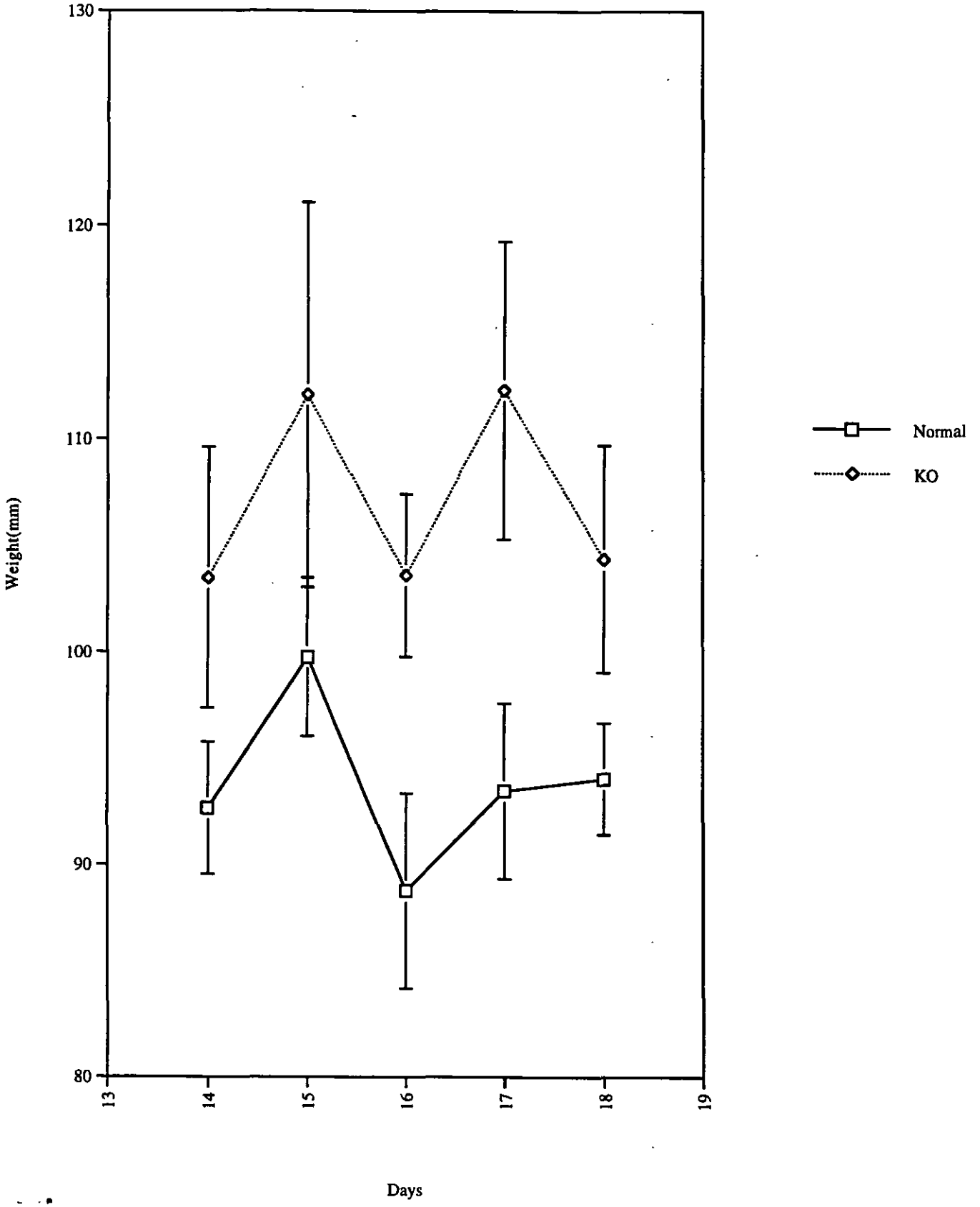
Another project I was working on included working with the ames dwarf mice. In this experiment, I was to find out the effects of replacement therapy with progesterone on pregnancy. For several weeks, I injected the df/df females with T_4, three times a week. The concentration of the T_4 was 20ug/ml, and the amount injected was 0.05ml. I mated the mice with normal males and checked them daily for plugs. After a second plug was obtained in a df/df female, the mouse was treated with progesterone daily. When the df/df reached 14 days, the mouse was killed and dissected. Nine mice killed and showed no sign of pregnancy. Therefore, the mice did not carry pregnancy with injections of progesterone in sesame oil once a day.

Works Cited

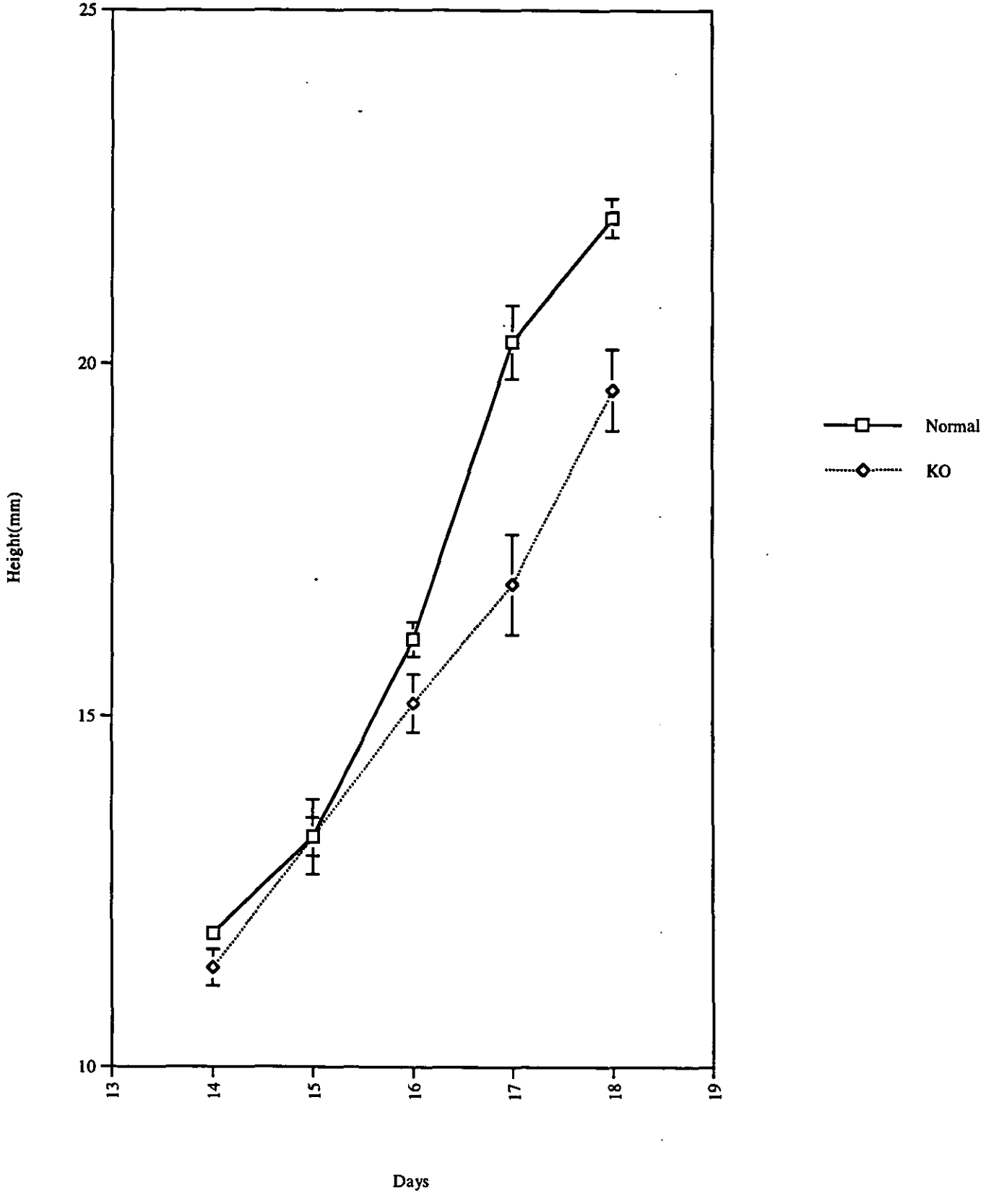
Zhou, Yihua., et al. "A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse)." The National Academy of Sciences 1997: 13215-13220.

Appandix 1

Placenta Weight

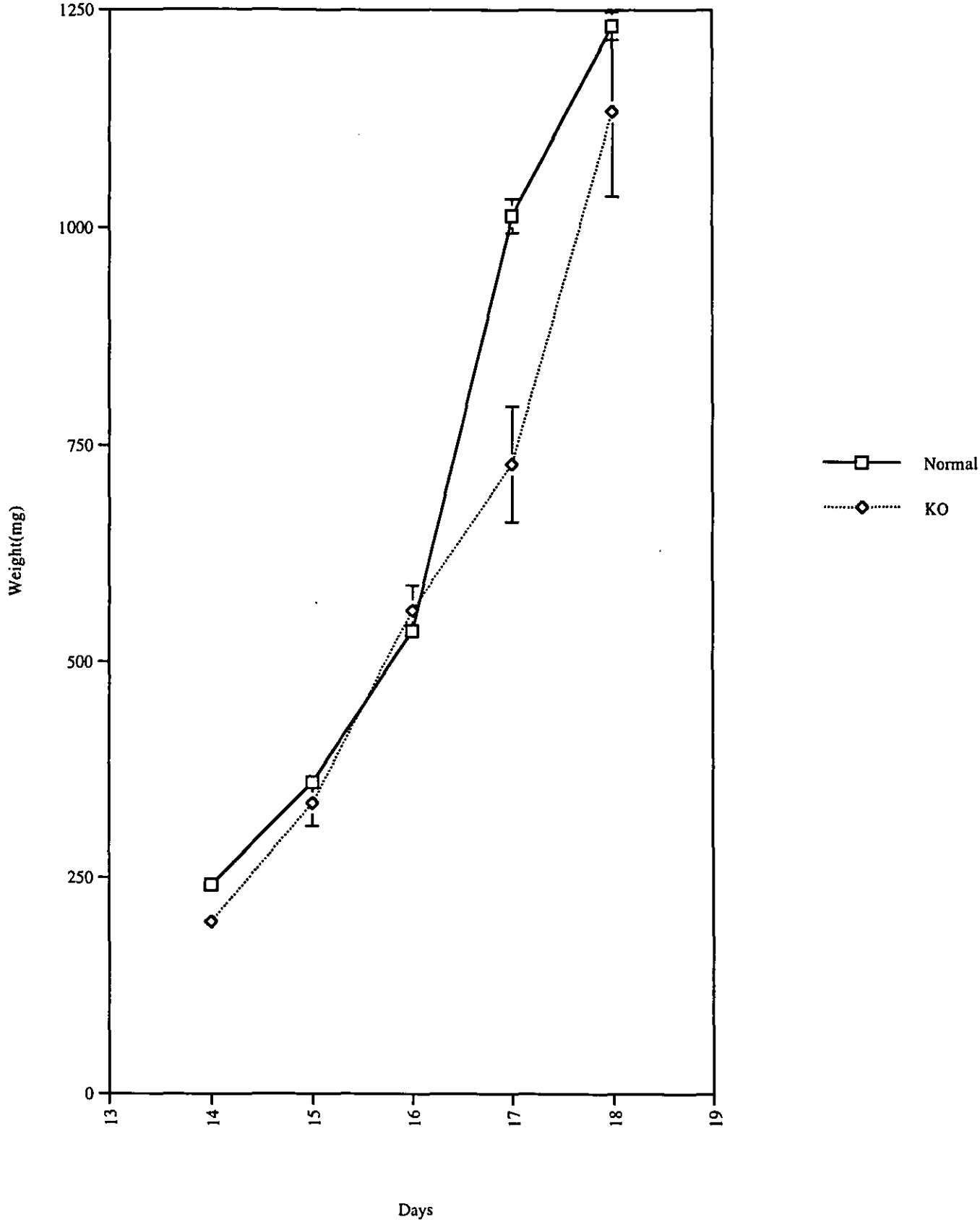


Fetus Height



Appendix 3

Fetal Weight



Day 14

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	1139.167	1139.167	2.715
Within groups	37	15523.181	419.545	p = .1079
Total	38	16662.348		

Model II estimate of between component variance = 37.123

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	21	92.664	14.259	3.112
KO	18	103.506	25.96	6.119

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	-10.841	13.331	2.715	1.648

Day 15

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	1323.446	1323.446	2.246
Within groups	38	22394.971	589.341	p = .1423
Total	39	23718.417		

Model II estimate of between component variance = 41.829

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	27	99.783	19.383	3.73
KO	13	112.064	32.438	8.997

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	-12.281	16.59	2.246	1.499

Day 16

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	2419.467	2419.467	6.274
Within groups	42	16197.534	385.656	p = .0162
Total	43	18617.001		

Model II estimate of between component variance = 92.637

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	21	88.785	21.008	4.584
KO	23	103.631	18.304	3.817

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	-14.846	11.962*	6.274*	2.505

* Significant at 95%

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	3176.043	3176.043	5.656
Within groups	34	19091.338	561.51	p = .0232
Total	35	22267.381		

Model II estimate of between component variance = 145.702

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	19	93.488	18.04	4.139
KO	17	112.303	28.759	6.975

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	-18.815	16.077*	5.656*	2.378

* Significant at 95%

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	1042.662	1042.662	3.82
Within groups	41	11191.83	272.971	p = .0575
Total	42	12234.492		

Model II estimate of between component variance = 39.401

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	28	94.066	13.894	2.626
KO	15	104.398	20.667	5.336

One Factor ANOVA X_1: Group Y_1: Placenta Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	-10.332	10.676	3.82	1.954

Day 14

One Factor ANOVA X_1: Group Y_3: Fetus Height

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	2.354	2.354	2.83
Within groups	37	30.783	.832	p = .101
Total	38	33.137		

Model II estimate of between component variance = .079

One Factor ANOVA X_1: Group Y_3: Fetus Height

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	21	11.91	.713	.156
KO	18	11.417	1.101	.26

One Factor ANOVA X_1: Group Y_3: Fetus Height

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	.493	.594	2.83	1.682

One Factor ANOVA X_1: Group Y_3: Fetus Height

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	6.410E-6	6.410E-6	2.523E-6
Within groups	38	96.53	2.54	p = .9987
Total	39	96.53		

Model II estimate of between component variance = -.145

One Factor ANOVA X_1: Group Y_3: Fetus Height

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	27	13.278	1.41	.271
KO	13	13.277	1.934	.536

One Factor ANOVA X_1: Group Y_3: Fetus Height

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	.001	1.089	2.523E-6	.002

One Factor ANOVA X_1: Group Y_3: Fetus Height

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	9.056	9.056	3.456
Within groups	42	110.056	2.62	p = .07
Total	43	119.112		

Model II estimate of between component variance = .293

One Factor ANOVA X_1: Group Y_3: Fetus Height

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	21	16.095	1.114	.243
KO	23	15.187	1.968	.41

One Factor ANOVA X_1: Group Y_3: Fetus Height

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	.908	.986	3.456	1.859

Day 17

One Factor ANOVA X_1: Group Y_3: Fetus Height

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	105.769	105.769	15.551
Within groups	34	231.25	6.801	p = .0004
Total	35	337.019		

Model II estimate of between component variance = 5.515

One Factor ANOVA X_1: Group Y_3: Fetus Height

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	19	20.316	2.268	.52
KO	17	16.882	2.944	.714

One Factor ANOVA X_1: Group Y_3: Fetus Height

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	3.433	1.769*	15.551*	3.943

* Significant at 95%

Day 18

One Factor ANOVA X_1: Group Y_3: Fetus Height

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	58.061	58.061	18.805
Within groups	41	126.59	3.088	p = .0001
Total	42	184.651		

Model II estimate of between component variance = 2.814

One Factor ANOVA X_1: Group Y_3: Fetus Height

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	28	22.071	1.458	.275
KO	15	19.633	2.224	.574

One Factor ANOVA X_1: Group Y_3: Fetus Height

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	2.438	1.135*	18.805*	4.336

* Significant at 95%

Day 14

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	17754.494	17754.494	27.71
Within groups	37	23707.008	640.73	p = .0001
Total	38	41461.501		

Model II estimate of between component variance = 882.853

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	21	242.342	25.857	5.642
KO	18	199.542	24.657	5.812

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	42.8	16.474*	27.71*	5.264

* Significant at 95%

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	5241.182	5241.182	1.391
Within groups	38	143213.156	3768.767	p = .2456
Total	39	148454.339		

Model II estimate of between component variance = 83.898

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	27	361.065	36.243	6.975
KO	13	336.625	95.333	26.441

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	24.439	41.954	1.391	1.179

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	5957.545	5957.545	.5
Within groups	42	500182.87	11909.116	p = .4833
Total	43	506140.415		

Model II estimate of between component variance = -271.086

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	21	535.993	52.097	11.368
KO	23	559.289	142.367	29.685

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	-23.296	66.471	.5	.707

Day 17

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	731903.155	731903.155	18.707
Within groups	34	1330245.955	39124.881	p = .0001
Total	35	2062149.11		

Model II estimate of between component variance = 38606.839

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	19	1014.361	84.799	19.454
KO	17	728.749	273.954	66.444

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	285.612	134.2*	18.707*	4.325

* Significant at 95%

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	1	94588.305	94588.305	1.779
Within groups	41	2180481.388	53182.473	p = .1897
Total	42	2275069.693		

Model II estimate of between component variance = 2119.584

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Normal	28	1231.953	84.886	16.042
KO	15	1133.545	376.632	97.246

One Factor ANOVA X_1: Group Y_2: Fetus Weight

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Normal vs. KO	98.408	149.021	1.779	1.334