TWO STUDIES OF BIOLOGICAL EFFECTS DUE TO ULTRASONIC IRRADIATION

BY

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THESIS

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CHAPTER I - INTRODUCTION

A. INTRODUCTION

The applications of ultrasound have been expanding since the discovery of the piezoelectric effect in 1880.[1] Sonar applications for military purposes were one of the first acoustic developments of the piezoelectric element.[2,3] Soon thereafter the effects on biological systems were noted when it was noticed that small fish were killed if they swam into the sound beam.[3] Early real time ultrasonic imaging in 1929 and low intensity ultrasonic therapeutics in 1939 marked the beginning of medical applications of ultrasound. Today, the largest diagnostic medical application has been in obstetrics and gynecology. However, therapeutic and other diagnostic applications include ophthomology, cardiology, and surgery to name a few.

Within obstetrics and gynecology its uses include fetal imagery scanning to diagnose fetal abnormalities, such as malformation, fetal position, and age of the fetus. The most common ultrasonic diagnostic tool is a continuous wave Doppler instrument.[1] The Doppler shifted backscattered signal produces audible sounds which are used to monitor the fetal heart during delivery. The fetal heart rate is a good indication of stress, thus giving the obstetrician valuable information about the status of the fetus.

A survey conducted with the help of the American Institute of Ultrasound in Medicine, the Biomedical Engineering Society, the Bureau of Radiological Health, the United States Public Health Service, and the United Kingdom Medical Research Council, showed that there was an average annual increase of about 10 percent per year in the use of clinical ultrasound for the period between 1963 to 1971.[4] At this annual increase it has been estimated that virtually the entire population will be exposed to ultrasonic energy *in utero* by the year 1984.[5] This is an alarming situation should some problem later arise as was the case of X-rays.

To help the clinician, bioeffect studies such as the ones reported herein should be used to examine the risk *vs* benefit. It is not possible to prove ultrasound safe, that is an absence of effect or not involving risk. Instead these studies aid in the overall assessment of risk associated with exposure to ultrasound.[5] While much useful diagnostic information is obtained with ultrasound, it is necessary to justify its use in low-risk situations. This justification must come from the physician that administers the ultrasound only after the evaluation of the benefit-risk assessment.

This thesis concentrates on two studies, *viz.*, the effects of ultrasonic irradiation on fetal and post partum weight changes after *in utero* exposure and the effects of functional impairment after testicular irradiation (herein referred to as Weight Study and Fecundity Study respectively).

Other investigators have done similar studies as those reported in this thesis. O'Brien previously reported fetal weight changes as the result of *in utero* ultrasonic irradiation at spatial averaged intensities of 0.5 W/cm^2 to 5.5 W/cm^2 for time durations of 10 to 300 seconds.[6] On the same strain of mice, Stratmeyer[7] found a post partem weight gain but no significant weight change of the fetus at the same day of necropsy O'Brien used. The spatial averaged intensities of 0.25 W/cm^2 and 0.8 W/cm^2 for 120 seconds were used by Stratmeyer.[7,8]

The study reported here examines both fetal and post partem weight changes but on a different strain of mouse than those used by O'Brien and Stratmeyer. The spatial peak intensity used was 2.5 W/cm^2 for 20 seconds as described in the Irradiation Parameters Section of the Weight Study. (Chapter II, Section A, Part 1c.)

Ultrasound is a technique which is considered non-surgical for imaging scrotal swellings.[9] This clinical assessment enables abnormalities as small as a few millimeters to be displayed.[9] Scrotal gray scale ultrasonography is also being used clinically in cases where differential diagnosis of the enlarged scrotum is difficult.[10]

It has previously been reported[11] that gross morphological damage occurs as a result of ultrasonic irradiation at the same intensity reported in this thesis. The disruption of testicular tissues from ultrasound raises questions as to whether a functional impairment is present. Others have reported that after testicular irradiation on rats at a reported intensity of 4 W/cm^2 for durations up to four minutes, the animals showed no desire to reproduce during the first three to four days.[12] The paper was unclear as to the intensity parameter specified. It was also reported in the study that the potency of those rats who had been irradiated for three and four minutes did not return.

Fry reported a statistically significant reduction in litter size on the same strain of mice as used in this thesis after testicular irradiation.[13] The spatial peak and temporal peak intensity was 1525 W/cm^2 for a duration of 20 seconds, however the source was pulsed and burst time was varied to yield 30.5 W/cm^2 and 68.6 W/cm^2 average intensities. A reduction in litter size suggests that spermatogenesis is disrupted. Others investigated the effects of ultrasonic testicular irradiation on spermatogenesis by examining sperm counts in the rat.[14] It was reported that atspatial averaged intensities of up to 4 W/cm^2 for durations of 5 to 10 minutes, no effects on reproductive function were observed as determined from sperm counts, reproductive organs weights, and testicular histology.

This thesis describes separately the two studies. Chapter II comprises the Weight Study which includes sections on Methods of Procedure, Data and

Results, and Discussion of Results. Chapter III comprises the Fecundity Study, including sections on Methods of Procedure, Analysis, and Discussion of Results. Since there is some duplication in the instrumentation of the two studies, the balance is found in Chapter II for purposes of conciseness.

CHAPTER II - WEIGHT STUDY

A. METHODS OF PROCEDURE

To investigate fetal and post partum weight change ~~due~~ to *in utero* ultrasonic irradiation in the mouse the following procedures were established. Six month old proven LAF_1/J mice (Jackson Lab, Bar Harbor, Maine) were used. Proving was done after the mice reached the age of 13 weeks by placing one male with three females in a cage for a period of approximately two weeks. The females were examined to determine pregnancy. If any females were pregnant, that female and the male in that cage were considered proven and eligible for use in the study.

The mating procedure used in the study was as follows. Approximately 10 proven females and five proven males were placed in a cage. After two hours the males were removed and the females inspected for the presence of a vaginal plug which is suggestive of coitus. These animals were ultrasonically irradiated on the eighth day of gestation, day zero being the day of mating.

1. Irradiation Procedure

The irradiation procedure can be divided into three aspects, induction of anesthesia, irradiation preparation, and irradiation parameters.

a. Induction of Anesthesia

On the eighth day of gestation the females were anesthetized with Metofane (methoxyfluorane, Pitman-Moore, Inc., Washington Crossing, NY 09560), an ether based liquid which is sufficiently volatile to easily obtain a gaseous mixture suitable to administer to the animals. The anesthetic equipment is shown in Figure 1. The chamber shown in the left foreground of Figure 1 was used for initial induction of anesthetic for both studies reported in this

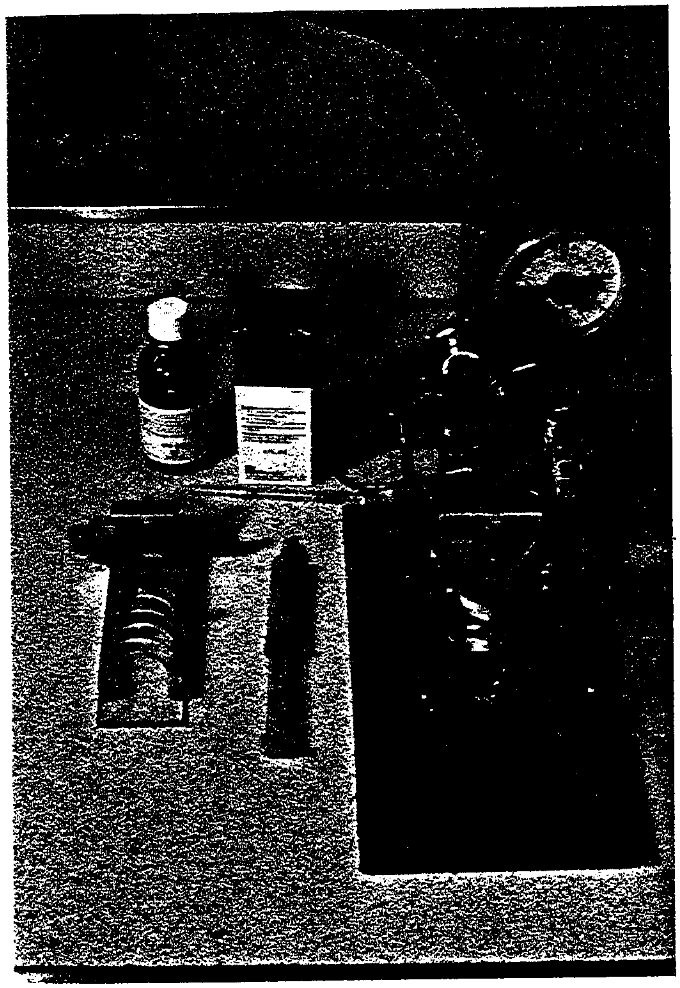


FIGURE 1 Anesthetic equipment for induction and maintenance of anesthesia throughout irradiation procedures

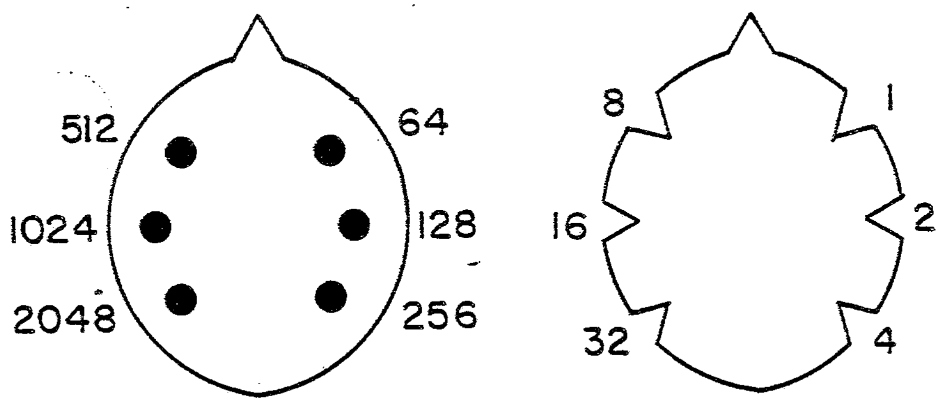
thesis. The animal, handled by its tail, naturally runs into the holding chamber once its head was inserted into the open end. With the animal in position, a gate was inserted nearly sealing the chamber from the external environment. Tension must be applied to the mouse's tail until it was sufficiently anesthetized to prevent the mouse from turning around in the chamber, thus maintaining the animal's nose in close proximity to the anesthetic chamber. The partition was removed allowing the gas to enter the holding chamber while a perforated stainless steel wall and copper wire screen prevents the mouse from coming into direct contact with the Metofane soaked cotton. The rate and depth of respiration, which were closely monitored visually, were used as subjective indicators of anesthetic level. At the desired anesthetic level the animal was removed from the holding chamber.

b. Irradiation Preparations

The following is the chronological procedure used to prepare the mice for irradiation after induction of anesthetic. This irradiation preparation consisted of sequentially numbering the animals by using a binary coded ear punch system shown in Figure 2 which allows 4096 (2^{12}) individual animals to be identified without repetition. Anesthetic level was maintained during this time by using a syringe containing a Metofane soaked cotton wad (center of Figure 1) which was periodically slipped over the head of the animal.

To insure good coupling from the transmitting medium (37°C degassed mammalian Ringers) into the abdominal cavity, the females were shaved from the sternum, posteriorly on both ventral and dorsal surfaces, excluding the legs, and a commercial depilatory (Neet) was applied for a period of three minutes to remove stubble. To assure thorough wetting of the skin surface the animals were immersed in a detergent solution (Prell) and rinsed in warm tap water.

The animals were mounted on the holder in spread eagle fashion as shown



BINARY CODED EAR PUNCH SYSTEM

FIGURE 2

in Figure 3 with the anesthetic hood placed over its head and positioned in the irradiation tank as shown in Figure 4. An alignment pointer was used to define the origin in the two dimensional array of exposures to be administered by the computer. The 1 cm disc on the pointer's end was positioned so that the upper edge of the disc was at the posterior end of the xiphoid process as shown in Figure 4.

c. Irradiation Parameters

In utero irradiation was performed with a 0.95 cm diameter aperture transducer operating at an ultrasonic frequency of 1 MHz. The ultrasonic intensity reported herein represents the free field value determined in the far field at 12.5 cm from the transducer, where the animal is positioned but without it in place.

The calibration facility utilizes two techniques to obtain the ultrasonic field parameters. The radiation force technique, the primary calibration system, uses a stainless steel ball suspended by bifilar nylon monofilaments.

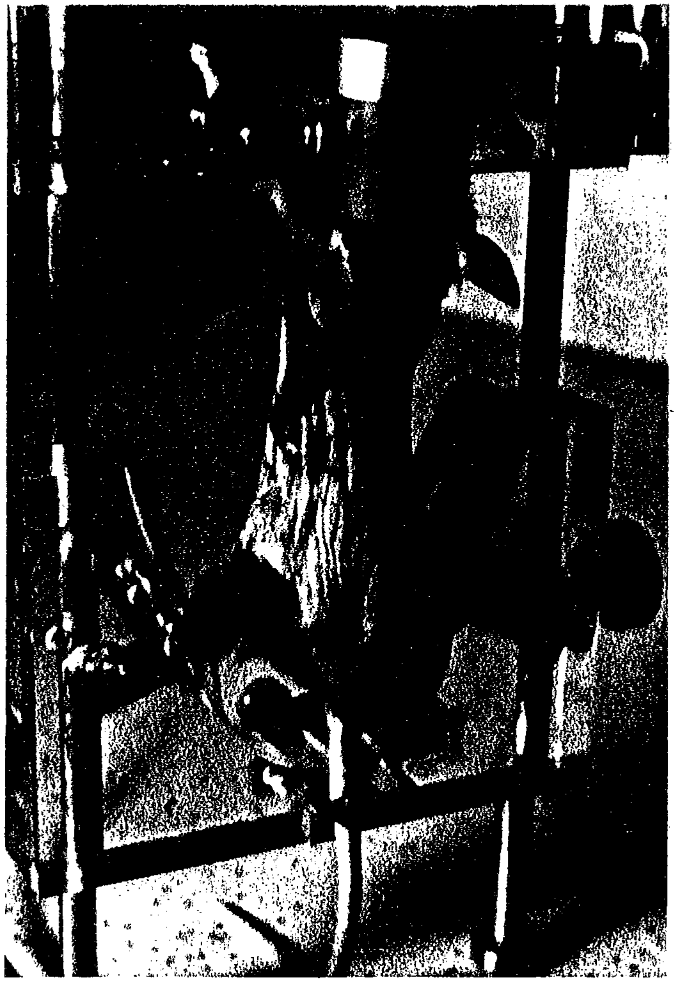


FIGURE 3 Female mounted in constraint system

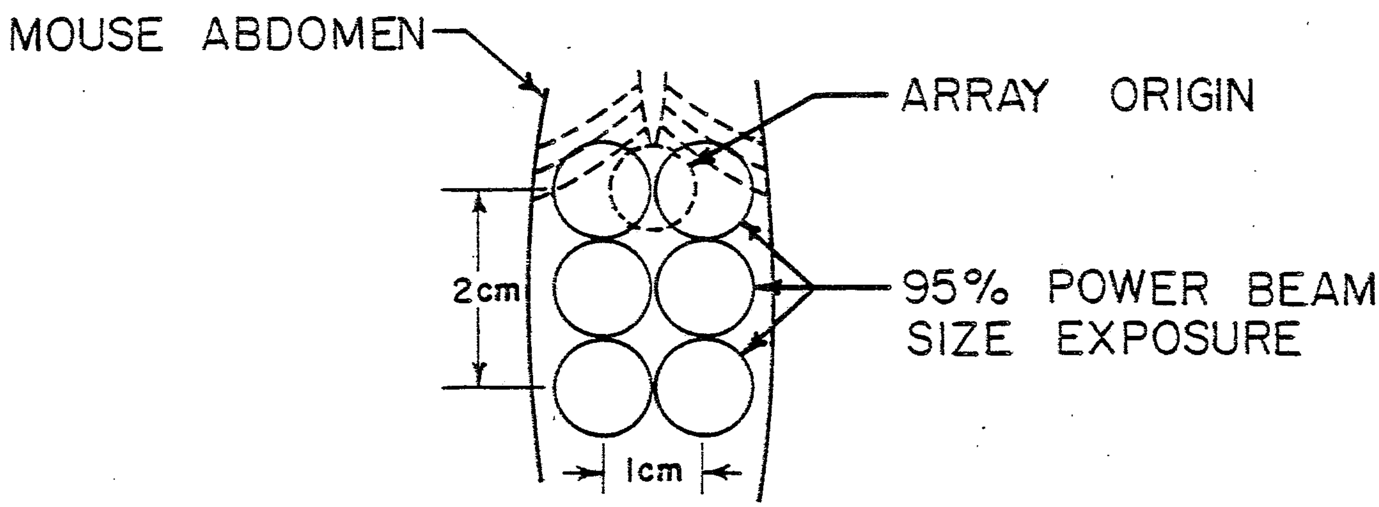
FIGURE 4 Irradiation tank with pointer at exposure array origin

The acoustic intensity is related to the radiation force and can be determined by measuring the displacement of the ball.[15,16]

The intensity determined with the radiation force technique is referred to as the spatial peak intensity. The second technique is used to obtain the spatial distribution of the field. The transient thermoelectric technique utilizes a thermocouple embedded in Dow Corning 710 acoustic absorbing fluid. The output of the thermocouple is related to the acoustic intensity[16,17,18,19] and by moving the thermocouple across the beam axis an intensity distribution profile is obtained. The precision of the reported ultrasonic intensities is ± 2% by utilizing these techniques.[16] The estimated overall uncertainty in determining the acoustic intensity for this field is 5 percent. The unfocused wave source has a half-power beam width of 20 mm at the distance the mouse was placed.

The irradiation parameters are programmed into a PDP-8 minicomputer which contains a library of arrays the user selects to be implemented for irradiation. The specific array utilized in this experiment was a six exposure array (Figure 5) permitting exposure over the entire abdominal region. The computer permits a blind study by selecting in a pseudo-random fashion the irradiated and sham animals. The exposure system also makes transducer voltage and positional checks prior to irradiation, minimizing erroneous exposures or operator error. These periodic checks also allow the system to respond to equipment failure and also operator initiated interrupt signals.

Appendix A is an example of a typical exposure parameter specification available to the user *via* the teletype. Note that ultrasonic intensity and exposure time of each shot in the designated array can be chosen. Also specified is the number of mice to be incorporated in the irradiation procedures and the number to be pseudo-randomly selected for irradiation.



MOUSE EXPOSURE ARRAY

FIGURE 5

The spatial peak intensity for each exposure was 2.5 W/cm^2 and 0 W/cm^2 (sham) for a duration of 20 seconds.

Sham irradiated mice received identical preparations and exposure durations but did not receive ultrasonic energy. After the irradiation the mice were removed from the holding assembly, wrapped in a tissue to help keep the mice from becoming chilled during their recovery, and placed in individual cages.

A control group was also included in the study and these animals were randomly selected after mating and placed into individual cages identical to the irradiated and sham groups but did not leave the animal room except to be weighed.

2. Scoring of Data

The fetuses of each group were weighed at a gestational age of 18 days. To obtain the weight data prior to delivery (18th day of gestation) the mothers were sacrificed and fetuses extracted by laparotomy. The position of the fetuses, early and late resorptions, anomalies, and empty sites in the uterus were recorded in addition to the weight of each fetus. The post partum data was obtained by weighing the pups at 21, 29, and 42 days post conception. These mothers were allowed to deliver and individual pup weights were recorded on each of the development days.

The data sheets shown in Appendix B facilitated data transfer for computer analysis. The time of day of each weighing was recorded and this parameter was maintained nearly constant so that each group was weighed at approximately the same point of development, that is between 10 AM to 2 PM. Other data scored included mouse identifier number, days post conception, and date.

B. DATA AND RESULTS

Table 1 summarizes the average fetal and pup weight data and the results of the statistical analysis.

The histograms shown in Figures 6a, 6b, 6c, and 6d represent the distribution of individual pup weights that were obtained for each weight group. The number at the very top of each histogram is the average weight of that group. The underlined number to each side represents plus and minus 30% of the average which establishes an indication of the norm, that is the circled number is the number of animals which are either considered stunted or giants. The histograms of the individual pup weights was done to investigate overall trends and follow weight developments of the pups.

The data was analyzed by administering various statistical tests to determine the significance of any effect from *in utero* ultrasonic exposure on the weight of the developing mice. The first statistical test performed on the data was a two-factor mixed design: repeated measures on one factor[20] which tests the significance of differences of overall weight gain, and rate of weight gain between groups.

Apprendix C lists the FORTRAN program written for the IBM 360 which performed the statistical test and determined various other parameters. Examples of the program printouts are shown in Appendix C. This program computed standard deviations, average pup weights, and the number of pups included in each litter at the time of the weighing. The number of pups weighed is an important aspect to follow throughout the successive weighings, in that it shows cannibalism due to handling of the mice or developmental problems which could have possibly been caused from the irradiation.[13] A linear regression analysis was done and the average pup weight *vs* time of weighing was plotted. This examined weight development trends and any possible difference in trends.

TABLE 1

DATA AND RESULTS

IRRADIATED

Day of Weighing (DPC)	Average Pup Wt./Litter	Stand. Dev.	# of Litters	P Value
18	1.020	0.176	13	NS
21	1.797	0.148	21	< .5
29	5.983	0.806	21	NS
42	12.818	1.778	21	NS

SHAM

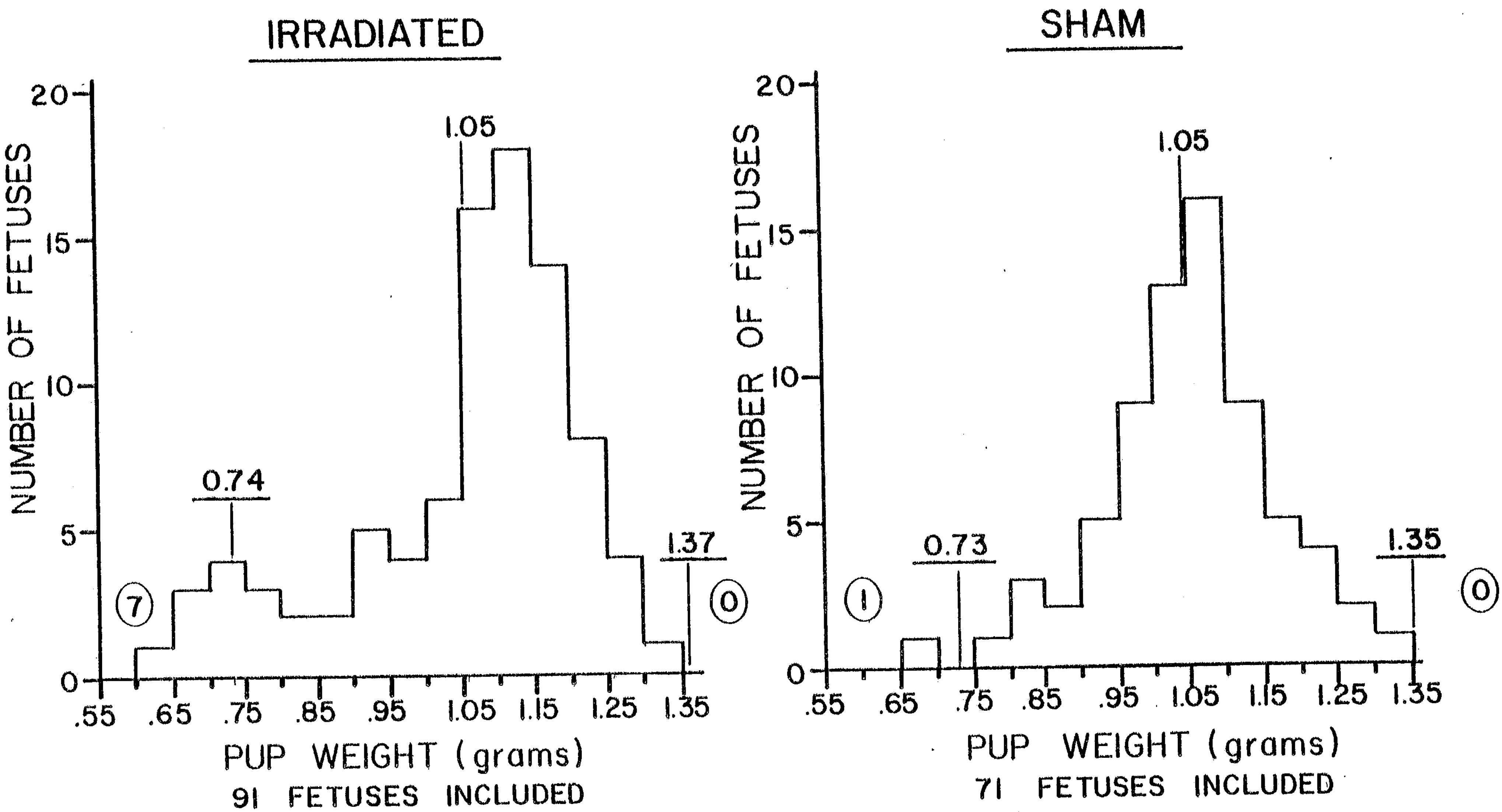
Day of Weighing (DPC)	Average Pup Wt./Litter	Stand. Dev.	# of Litters
18	1.036	0.075	10
21	1.869	0.167	17
29	5.947	0.551	17
42	12.819	1.126	17

IRRADIATED

Day of Weighing (DPC)	Average Individ. Wt.	St. Dev.	# of Pups	P Value
18	1.053	0.163	91	NS
21	1.778	0.245	184	< .01
29	5.780	0.904	179	< .5
42	12.409	1.909	179	< .1

SHAM

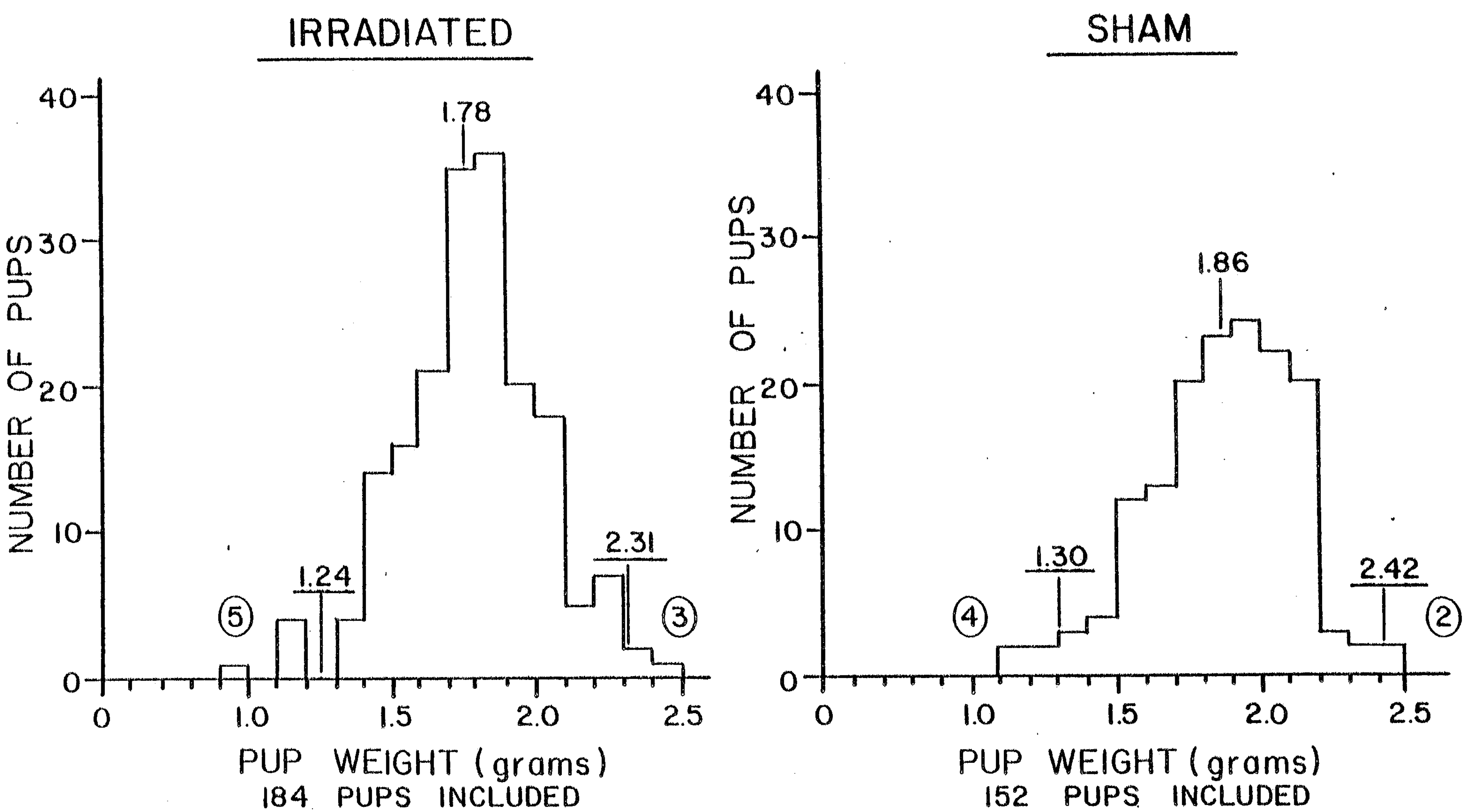
Day of Weighing (DPC)	Average Individ. Wt.	St. Dev.	# of Pups
18	1.045	0.118	71
21	1.861	0.249	152
29	5.884	0.768	152
42	12.739	1.695	152



18 DAY POST CONCEPTION INDIVIDUAL WEIGHT HISTOGRAM

FIGURE 6a

1) Number at the top is the average weight
2) Underlined numbers are plus and minus 30% of average weight
3) Circled numbers are stunted and giant animals



21 DAY POST CONCEPTION INDIVIDUAL WEIGHT HISTOGRAM

FIGURE 6b

1) Number at the top is the average weight
2) Underlined numbers are plus and minus 30% of average weight
3) Circled numbers are stunted and giant animals

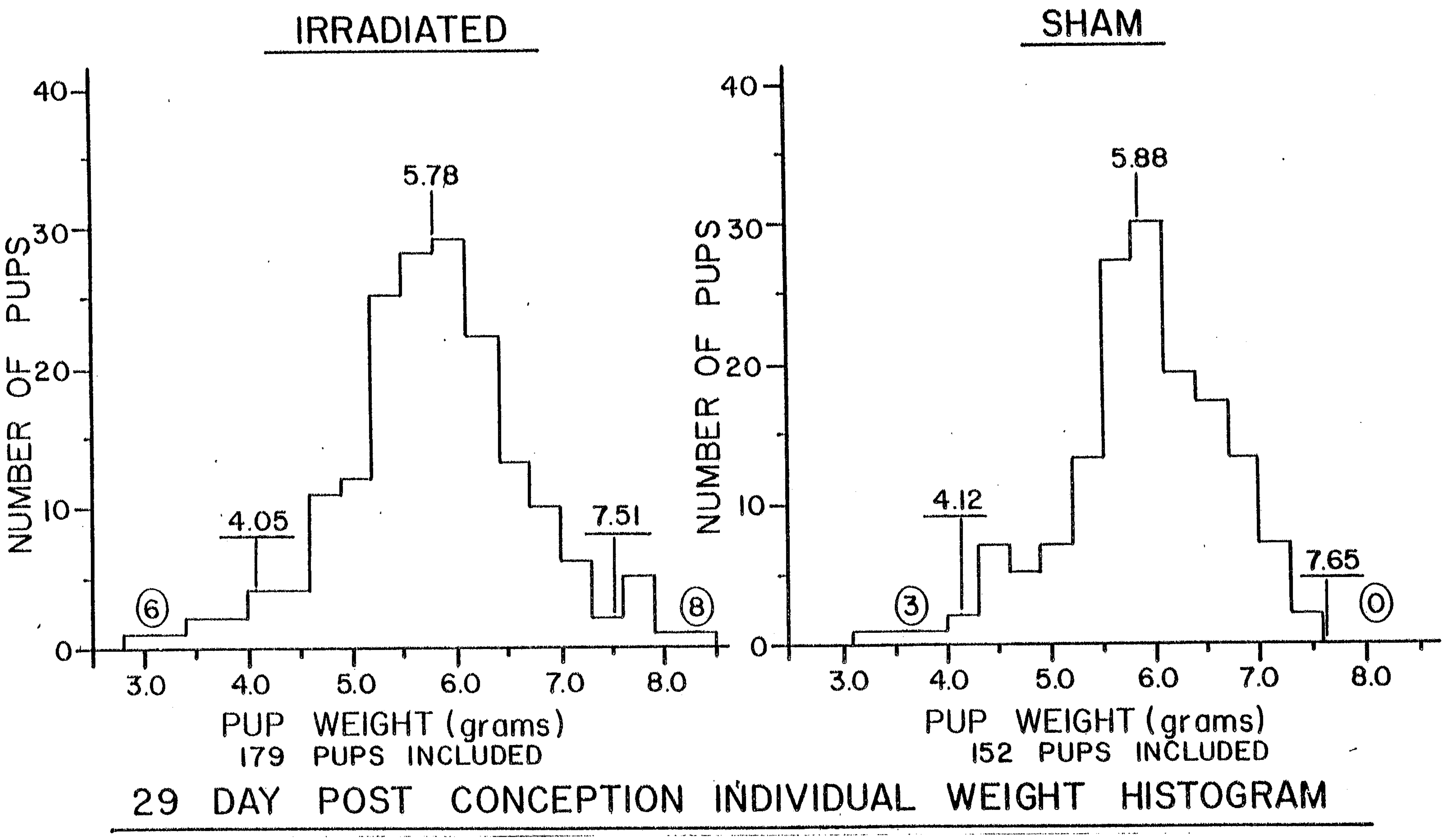
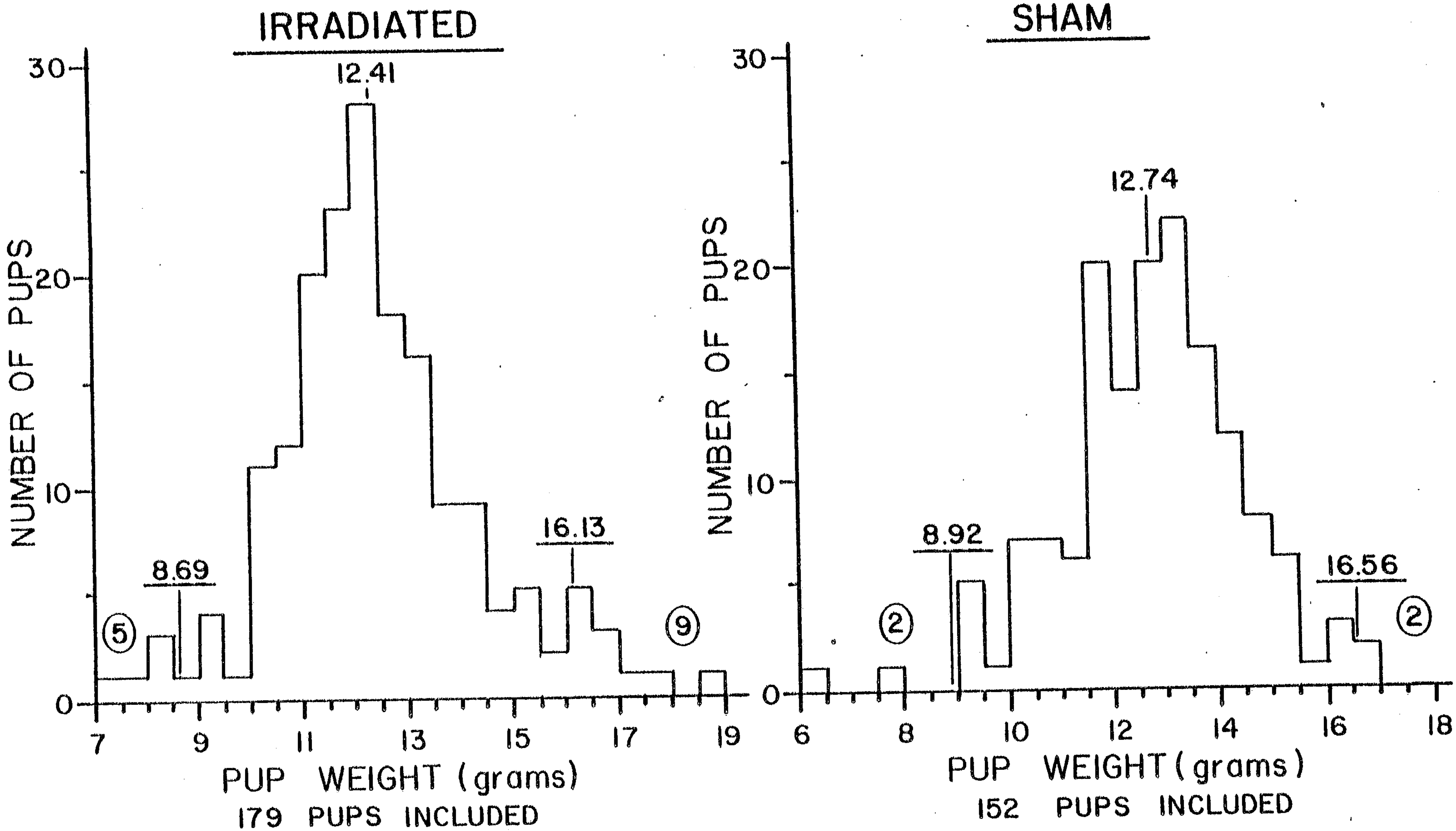


FIGURE 6c

1) Number at the top is the average weight
2) Underlined numbers are plus and minus 30% of average weight
3) Circled numbers are stunted and giant animals



42 DAY POST CONCEPTION INDIVIDUAL WEIGHT HISTOGRAM

FIGURE 6d

1) Number at the top is the average weight
2) Underlined numbers are plus and minus 30% of average weight
3) Circled numbers are stunted and giant animals

The other statistical test used on the data was a t-test for a difference between two independent means.[21] Here the objective was to determine if a weight difference was present between *in utero* ultrasonic irradiation at 2.5 W/cm^2 and 0 W/cm^2 (sham exposure group) at each weighing. A FORTRAN program (Appendix D) performed the t-test and compared the 18th day weight data of the irradiated group to that of the sham group. The program also compared the 21st, 29th, and 42nd day post conception weighings and printed out the t-test values and degrees of freedom from which the level of significance was determined.

C. DISCUSSION OF RESULTS

No significant difference between groups was found for the overall weight gain or for the rate of weight gain as determined from the two-factor mixed design statistical test. The question arises as to whether or not there is any significant weight difference at any of the intermediate weighings. To test this, the average pup weight per litter was tested using the t-test. Examining the individual pup weight using the t-test showed greater significance than the average pup weight per litter. A statistically significant level is indicated by a P-value of 5% or less. Of all the weighing days, 18, 21, 29 and 42 days post conception, the only significant weight difference between irradiated and sham occurs for the 21st day weighing. The mean of the individual weights for the irradiated and sham are 1.78 and 1.86, respectively. This weight reduction in the irradiated group is significant ($p_\alpha < 0.01$).

The histograms in Figures 6a, 6c, and 6d show that in these weight day groups the irradiated groups have more animals considered outside the norm. The number of animals included in each histogram is indicated on its respective figure.

While the number of animals in the irradiated groups is larger for each pair of histograms, this difference does not account for the increase in stunted and giants seen in the 18, 29, and 42nd day post conception weighings. Even though there is not a significant weight difference found for the 18, 29, and 42nd day weighings, the histograms and standard deviations at these days suggest that there is an expansion of the weight distribution due to ultrasonic irradiation.

CHAPTER III - FECUNDITY STUDY

A. INTRODUCTION

It has been shown that gross morphological damage occurs as a result of *in vivo* ultrasonic irradiation to mammalian testis.[11] A normal histological section of mouse testis appears in Figure 7. At least two types of damage occur after ultrasonic irradiation. One type is shown in Figure 8 wherein an increase in the interstitial space between the tubules is observed. A second type is shown in Figure 9 wherein a detachment of the germinal epithelium from the basement membrane and free floating cells into the lumen of the tubule is observed. These findings pose the question as to whether the damage observed morphologically has an effect on testicular function. To investigate this, the following study was proposed using nearly identical experimental procedures and ultrasonic exposure variables as those used in the morphological studies.[11,22]

B. METHODS OF PROCEDURE

In vivo testicular irradiation of six month old LAF_1/J mice (Jackson Lab, Bar Harbor, Maine) is accomplished with a 2.54 cm aperture transducer shown in Figure 10. At 0.995 MHz the unfocused continuous wave source has a half-power beam width of 10 mm at 12.5 cm from the source. The spatial peak intensity used was 10 W/cm^2 and the exposure duration applied to each testis was 30 seconds. Each testis was sequentially irradiated without any attempt to shield acoustically the opposing testis. The alignment of the transducer was performed manually with the center of the alignment disc positioned over each testis (as shown in Figure 11). The alignment pointer was then swung out of position during the irradiation.

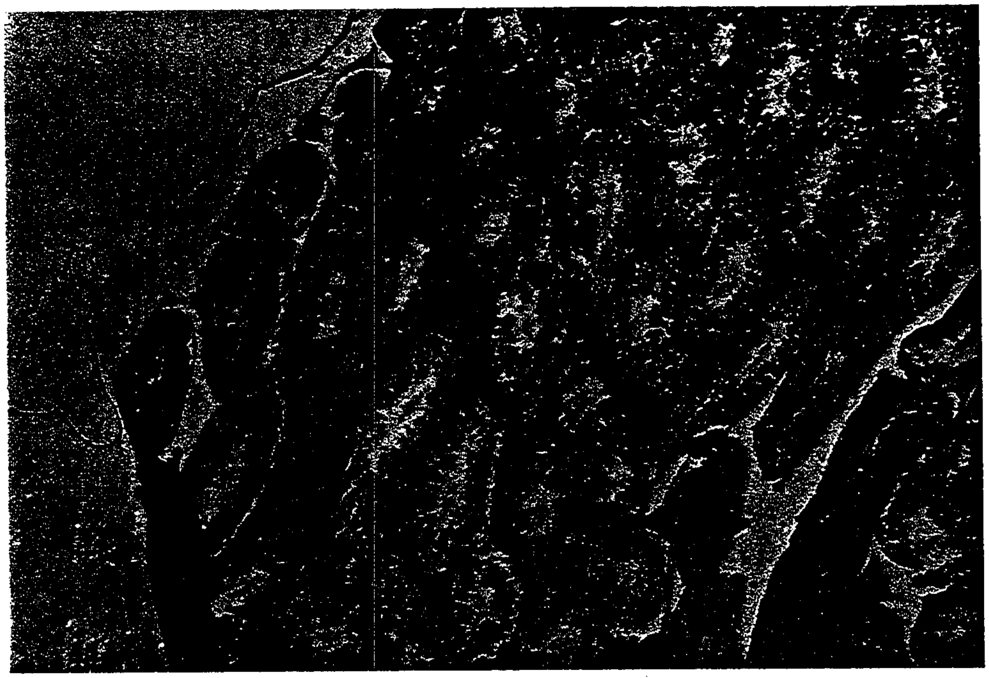


FIGURE 7 Normal histological section of mouse testis

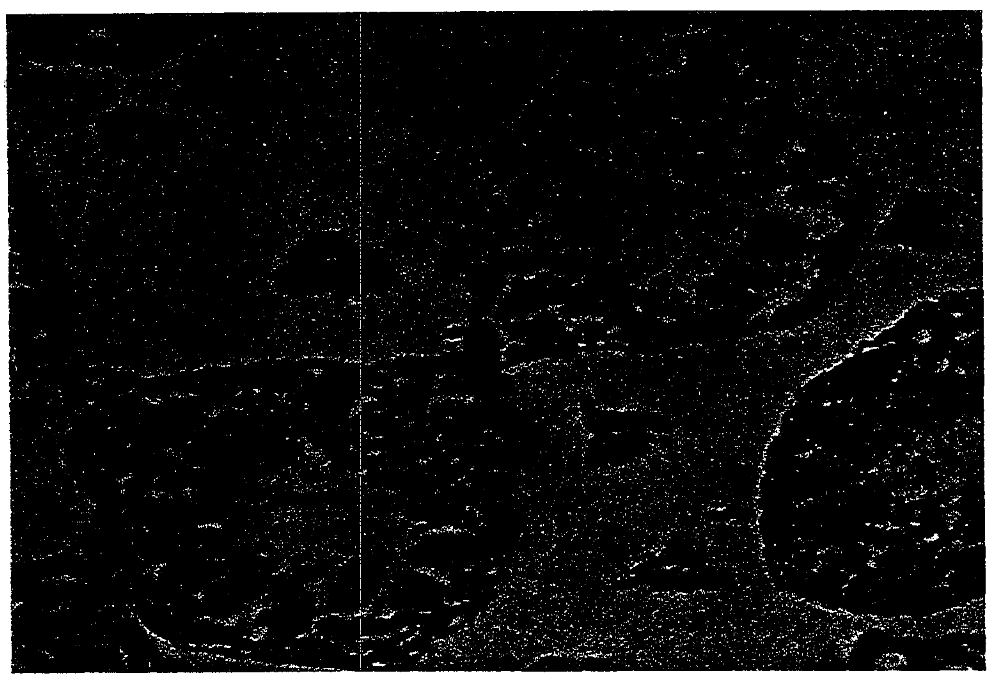


FIGURE 8 Histological section of irradiated mouse testis showing increase in interstitial space

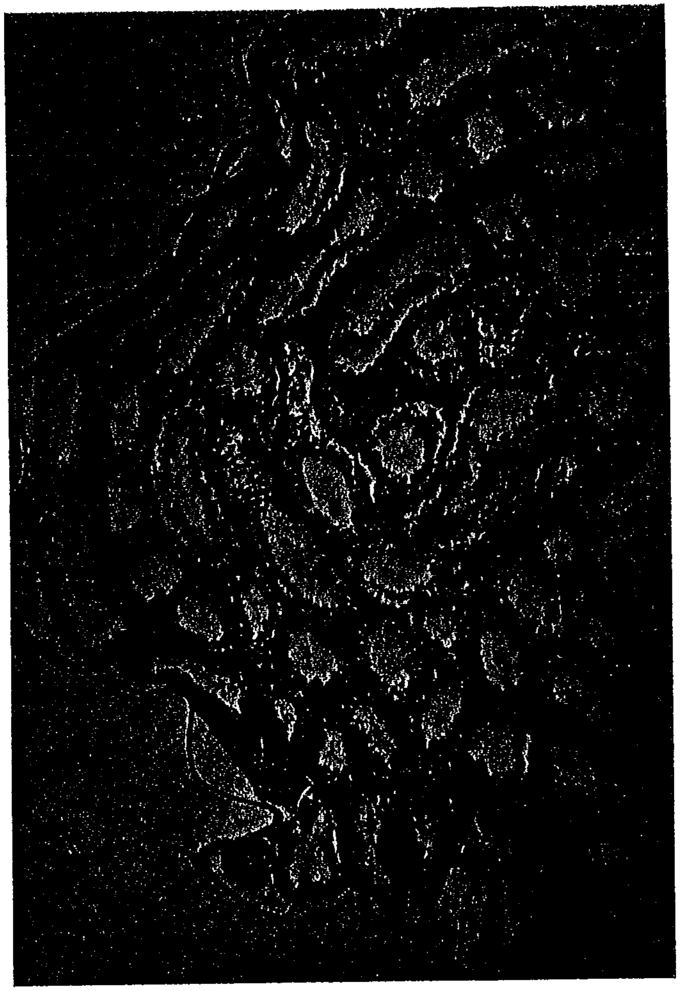


FIGURE 9 Histological section of irradiated mouse testis showing detachment of germinal epithelium from basement membrane

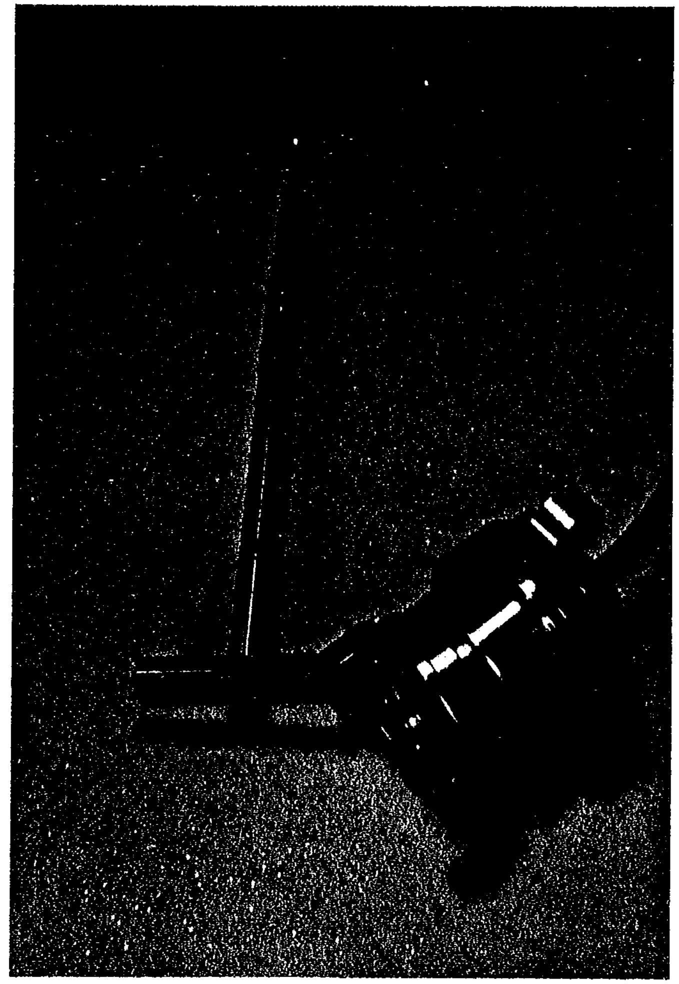


FIGURE 10 2.54 cm aperture, plane wave acoustic transducer

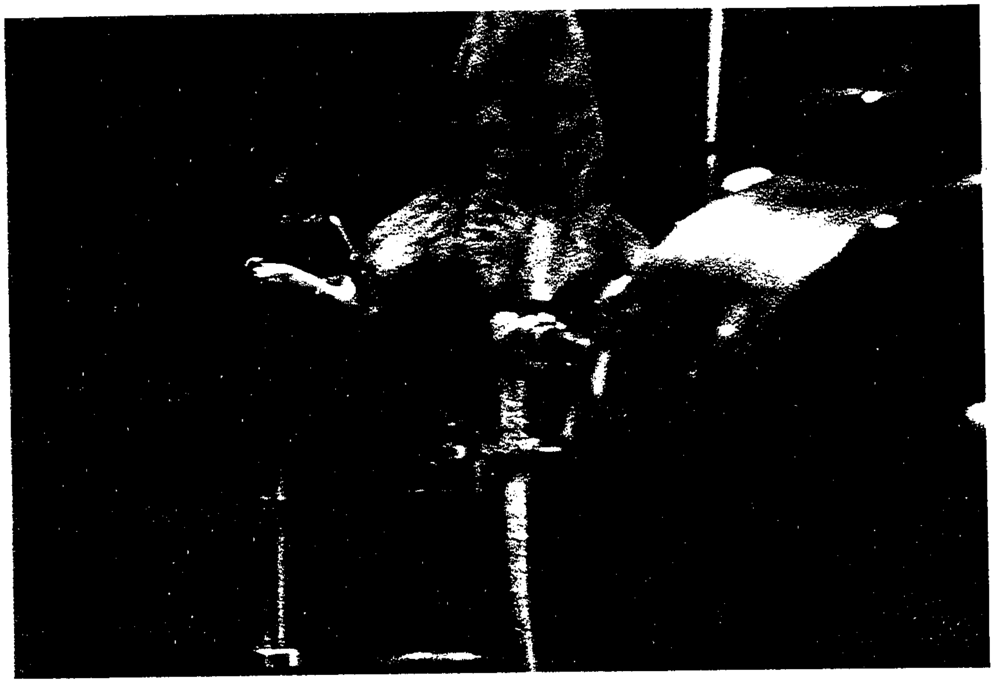


FIGURE 11 Male in irradiation tank with pointer aligned for testicular exposure

1. Irradiation Preparations

Prior to irradiation the males were anesthetized with methoxyfluorane (metofane, Pittman Moore, Inc., Washington Crossing, NJ 09560). The induction system and procedures were identical to those used in the Weight Study (Figure 1). The scrotum and surrounding inguinal regions were shaved and a ligature was tied to secure the testis and prevent retraction of the testis into the abdomen. The shaved region was bathed in a mild detergent solution to assure wetting of the surface for medium coupling and reflection minimization. The surfaces were rinsed lightly to remove excess suds and the animal was mounted in a specially designed support structure in spread eagle fashion to restrict movement (see Figure 12). The holder was positioned in a lucite irradiation tank and 37°C degassed mammalian Ringers solution served as the coupling medium with acoustic absorbing material (SOAB, B. F. Goodrich) lining portions of the tank to minimize reflections and prevent standing waves from developing (see Figure 11)

2. Groupings

The animals were randomly placed into one of three groups. Animals in the irradiated group received ultrasonic energy at an intensity of 10 W/cm^2 and exposure time of 30 seconds (each testis) as described in the Methods of Procedure (Chapter III, Section B). Animals in the sham group were prepared identically to the irradiated group, however no ultrasound was administered (0 W/cm^2). Animals placed in the control group did not leave the animal room. After recovering from the anesthesia each male was placed in a cage with three normal six month old proven females.

3. Scoring of Data

The cages were examined daily for pups. If a litter was found in the

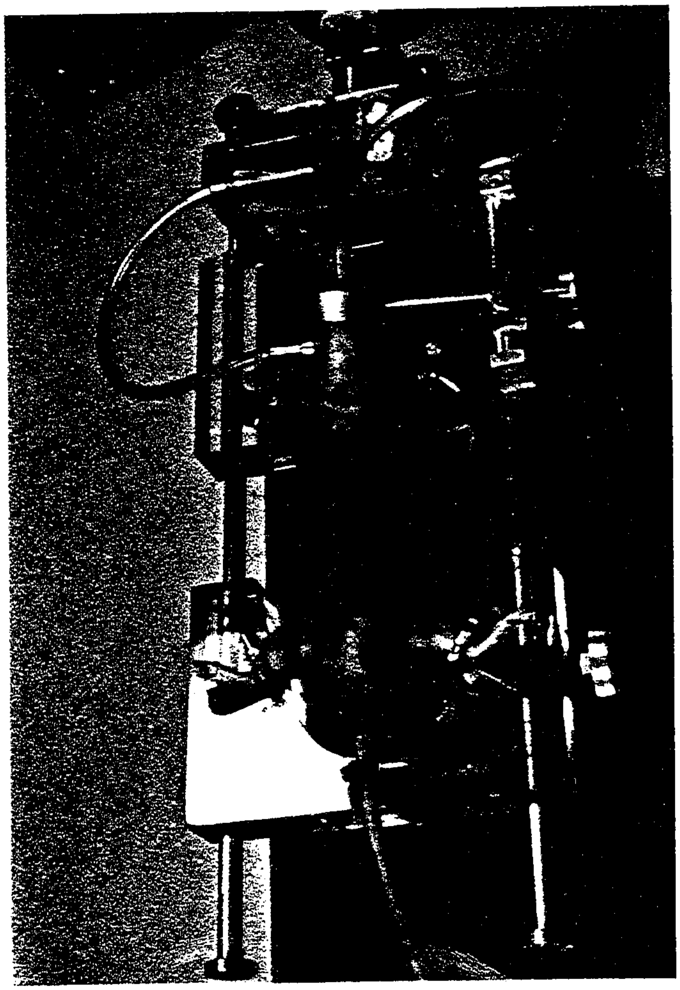


FIGURE 12 Male mounted in constraint system and testes ligated

cage the date and size of the litter were scored on a card. The pups were removed to prevent accumulation and to discontinue lactation in the mother. The mother's estrous cycle resumed and the females were again receptive to mating. This daily scoring continued for approximately six months at which time the males were sacrificed and the testis fixed in formalin for future histological examination.

C. ANALYSIS AND RESULTS

Three of the seven irradiated animals died within the first 12 days post irradiation, however one male impregnated a female prior to death. One control animal was not included in the analysis due to an obvious problem in mating as indicated by the small number of litters and high mortality rate of the pups. Of the initial seven animals in each group, all seven are included in the sham group (Figure 14), six in the control group (Figure 15), and four in the irradiated group (Figure 13).

The period of time between litters was compared to the known gestational period of about 19-20 days for the mouse in order to assign each litter with a specific female, thus assessing whether or not each female had been mating and was fertile. For example, if three litters were found in the cage within the period of 19 days, that is, one gestational period, all females were considered fertile. With this determination, a cumulative record of the number of pups per female was made with each litter receiving equal weight in the analysis. The cumulative pups per female as a function of days post irradiation was plotted and a linear regression and least squares analysis was performed to obtain the best fit linear and quadratic curves. The analysis package used was a SOUPAC program for least squares on the IBM 360 computer (Appendix E). Table 2 is data exerpted from Appendix F and shows how the data

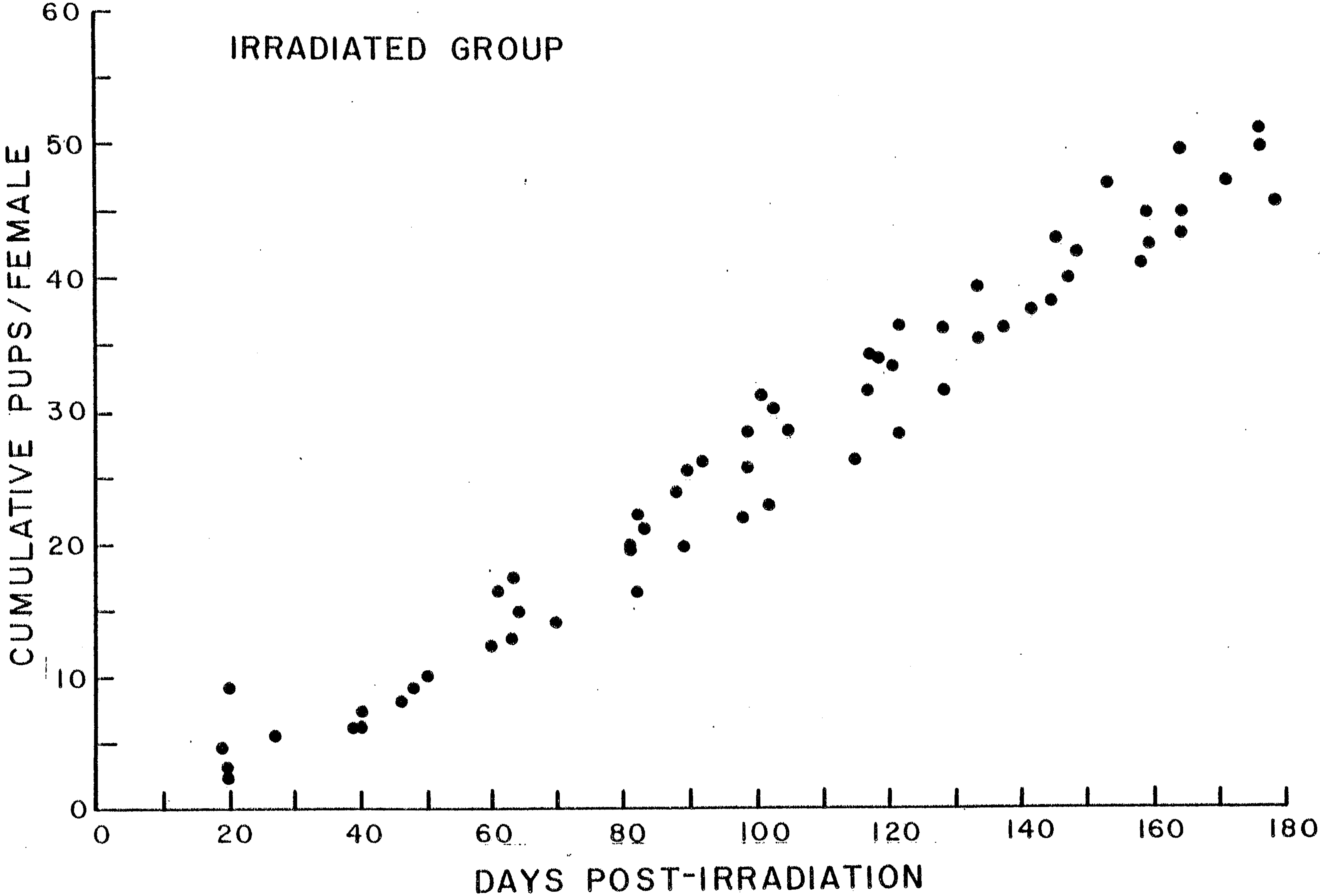


FIGURE 13 Cumulative pups/female vs. days post irradiation

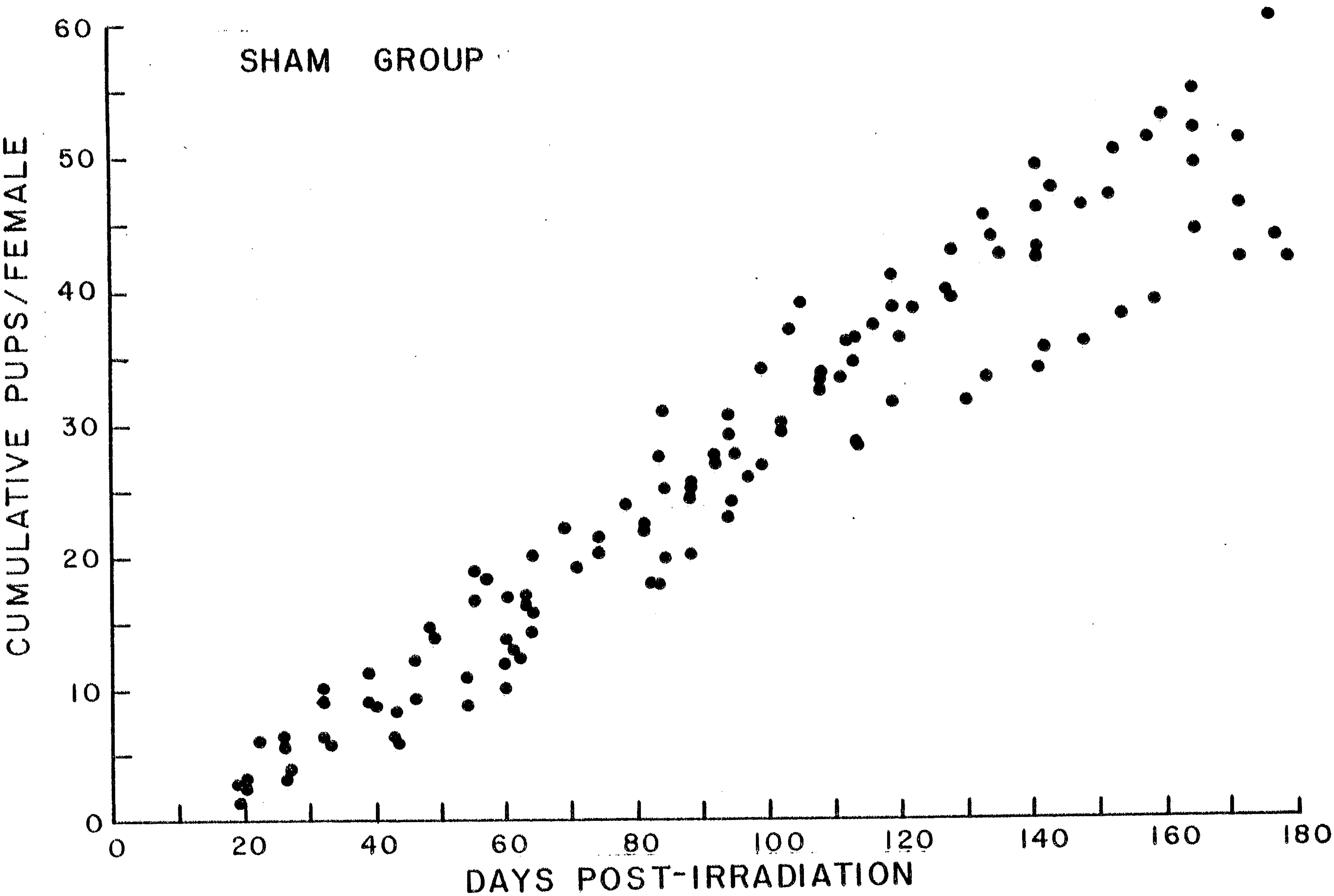


FIGURE 14 Cumulative pups/female vs. days post irradiation

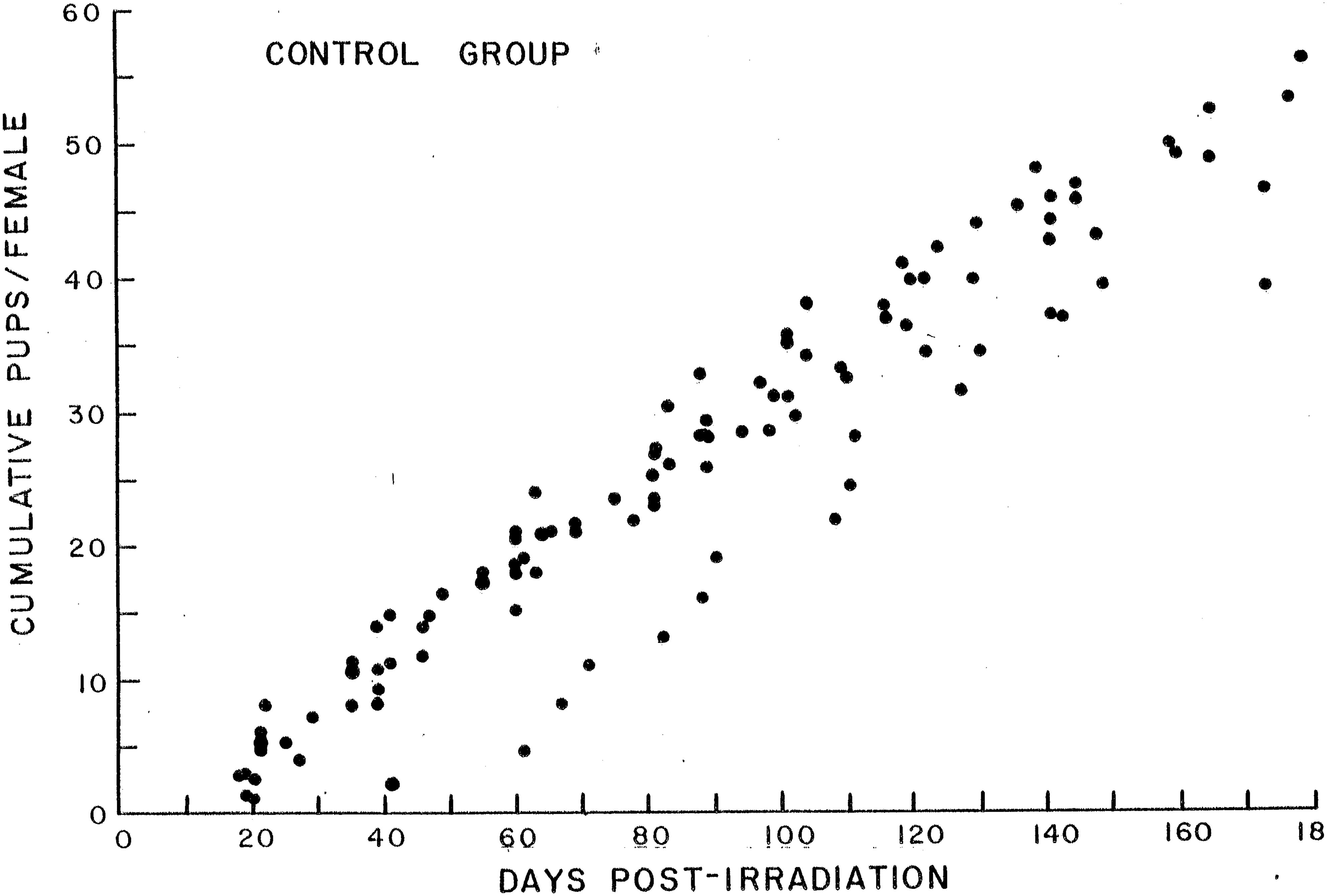


FIGURE 15 Cumulative pups/female vs. days post irradiation

was scored and computed.

TABLE 2

DATA EXAMPLE

DAY OF IRRADIATION 6/8

SCORED DATA			
LITTER DATE	LITTER SIZE (PUPS/LITTER)	DAYS POST IRRADIATION	CUM PUPS/ FEMALE
6/28	4	20	1.33
7/20	3	42	2.33
8/9	7	62	4.67
8/15	11	68	8.33
8/19	8	72	11.00

From the scored data the day of the litter was converted to days post irradiation. As an example, the first litter which occurred on 6/28 is 20 days post irradiation relative to 6/8, the day of irradiation. To calculate the cumulative pups per female each litter size was normalized by the number of fertile females and summed. For the data in Table 2 there were three fertile females since litters occurred at 8/9, 8/15, and 8/19. The first litter of four was normalized to 1.33 and the second litter of three to 1.00. Their sum of 2.33 represents the second data point. The rest of the litters were normalized and summed to yield the cumulative pups per female data found in the last column of Table 2. The data, plotted for each of the three groups, irradiated (Figure 13), sham (Figure 14), and control (Figure 15), were encoded on data cards and submitted for SOUPAC least squares analysis. Figure 16 shows the first order linear regression plots for control, sham, and irradiated. The time intercept of the first order cumulative pups/female curve (Figure 16) gives an indication of the time coitus was initiated and the slope may be considered a measure of the males' mating capabilities referred to as fecundity. The first order equations are:

$$\text{control cumulative pups} = 0.317t - 1.98 \tag{1}$$

$$\text{time intercept} = 6.27$$

$$\text{sham cumulative pups} = 0.322t - 3.679 \tag{2}$$

$$\text{time intercept} = 11.44$$

$$\text{irradiated cumulative pups} = 0.297t - 4.046 \tag{3}$$

$$\text{time intercept} = 13.63$$

A comparison of the time intercepts of the linear regression equations of cumulative pups/female above, shows that the irradiation procedures administered to the sham group introduced a time delay of 5.2 days as compared with the control group. The irradiated group showed an additional delay of 2.2 days, indicating a longer recovery period prior to coitus initiation. A comparison of the slopes show the control and sham groups have virtually the same slope. The irradiated group has a lesser slope suggesting a decrease in mating capability post irradiation. To test the significance of the data the two-factor mixed design: repeated measures on one factor test used in the Weight Study was applied.[20] To use this test it was necessary to block the data into 30 day groups. The cumulative pups data was grouped into six groups and analyzed similar to the weighings of the previous study. None for the data was significant at at least the 5% level.

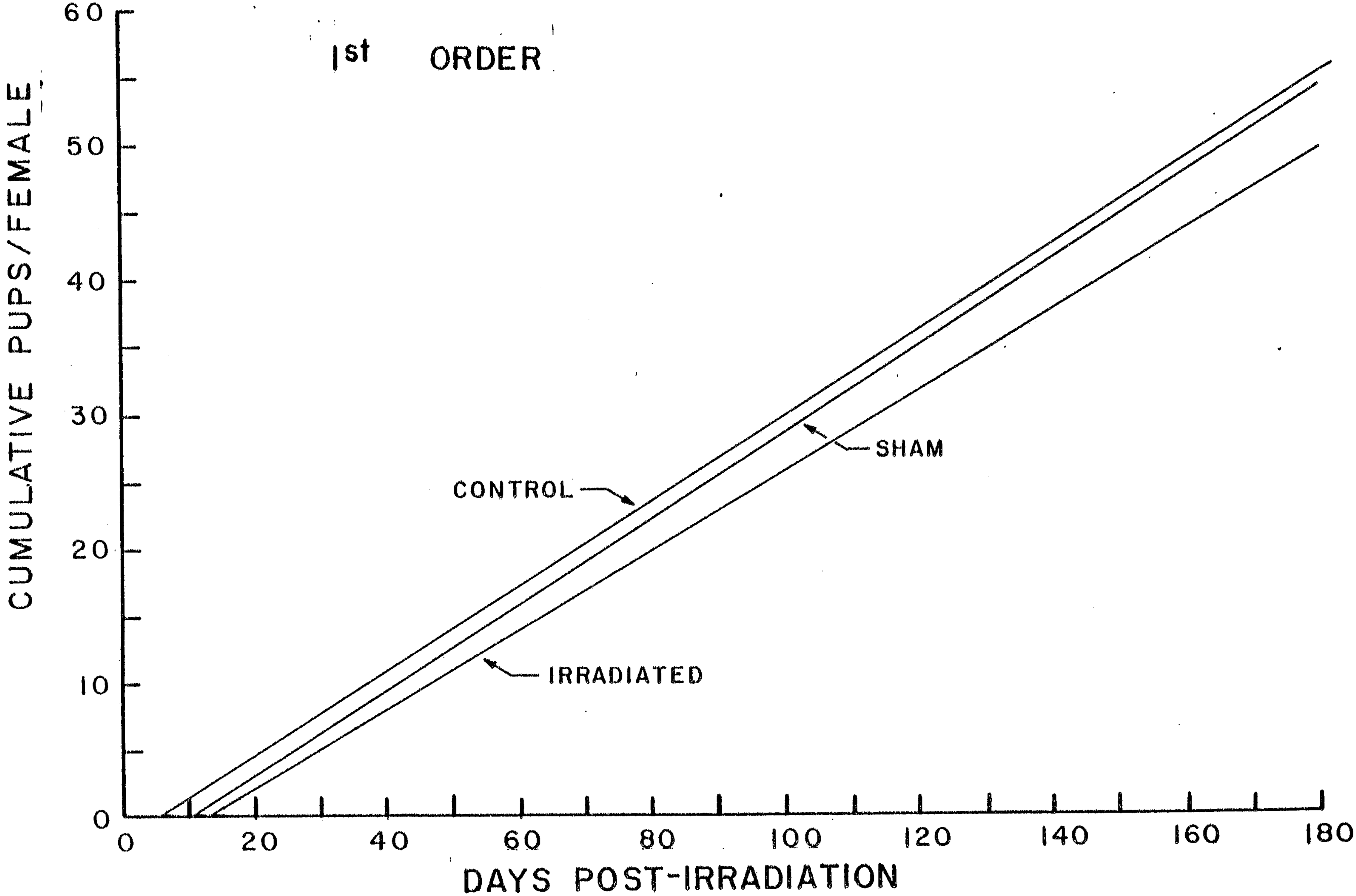


FIGURE 16 First order analysis of cumulative pups/female vs. days post irradiation

Examination of the data suggested that a first order analysis did not completely describe it. A second order analysis revealed additional information about a time varying slope indicating the changing delivery rate of pups.

The trends of the sham and control groups showed the rate of cumulative pups decreasing. The derivative of the second order equations for cumulative pups/female yielded quantitative information reflecting the rates of accumulation. Below are the second order equations which represent the curves found in Figure 17 and their time derivative equations (Figure 18).

<u>Cumulative Pups/Female (t in days)</u>

<u>Controls</u>

$$\text{cumulative pups} = -\,0.00039t^2 + 0.38824t - 4.53729$$ (4) Figure 17

$$\frac{d(\text{cumulative pups})}{dt} = -\,0.00078t + 0.38824$$ (5) Figure 18

<u>Sham</u>

$$\text{cumulative pups} = -\,0.00035t^2 + 0.3901t - 6.3228$$ (6) Figure 17

$$\frac{d(\text{cumulative pups})}{dt} = -\,0.0007t + 0.3901$$ (7) Figure 18

<u>Irradiated</u>

$$\text{cumulative pups} = 0.00013t^2 + 0.27136t - 3.05949$$ (8) Figure 17

$$\frac{d(\text{cumulative pups})}{dt} = 0.00026t + 0.27136$$ (9) Figure 19

As shown in Figure 18, the slope of the irradiated group increases with increasing time. The rate in the first 110 days was less than the sham and control group but increased in the latter 70 days. This suggests that the effect seen initially recovered and the rate returned to that of the control. This effect could be due either to a decrease in litter size suggesting a

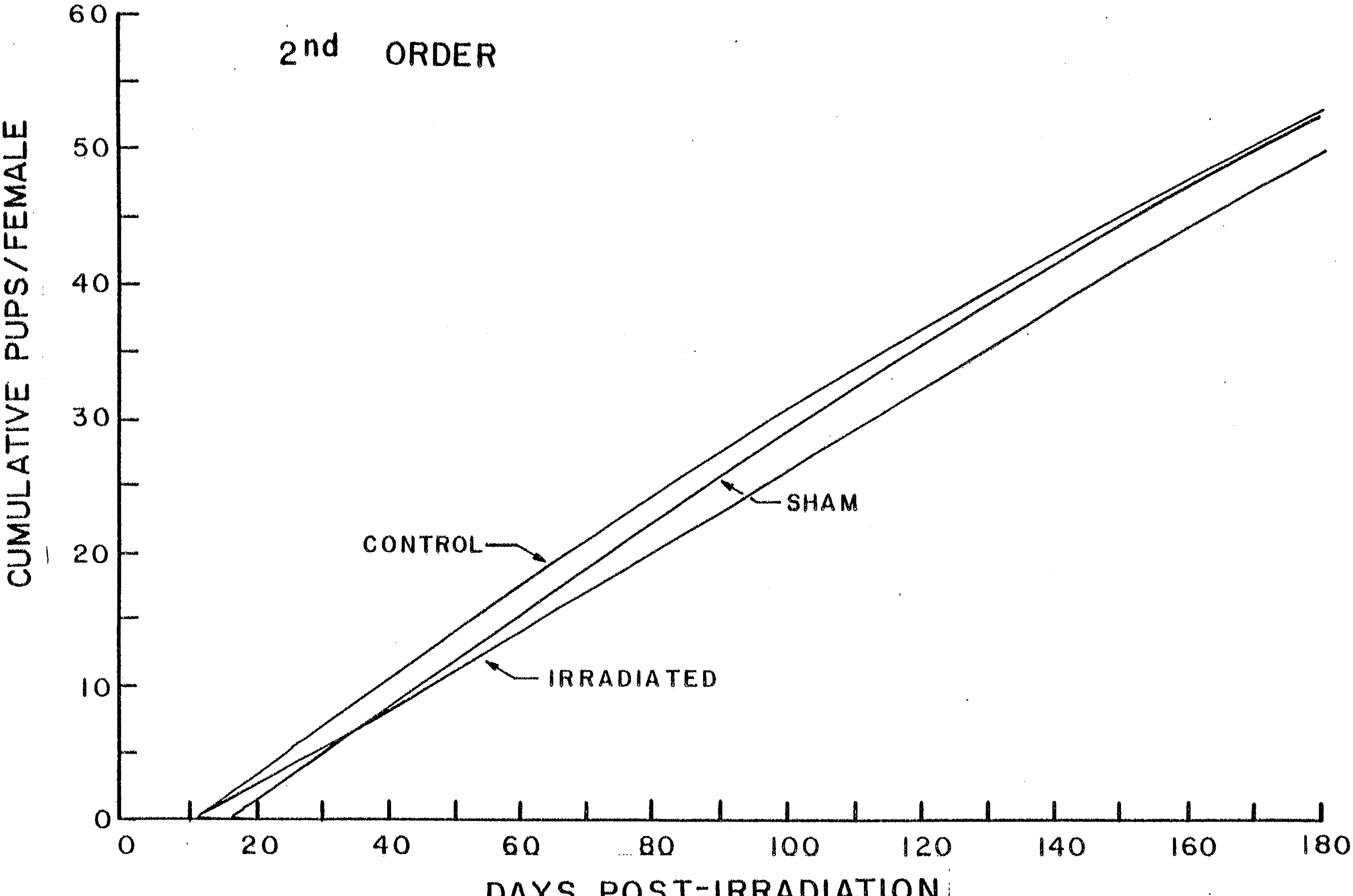


FIGURE 17 Second order anaylsis of cumulative pups/female vs. days post irradiation

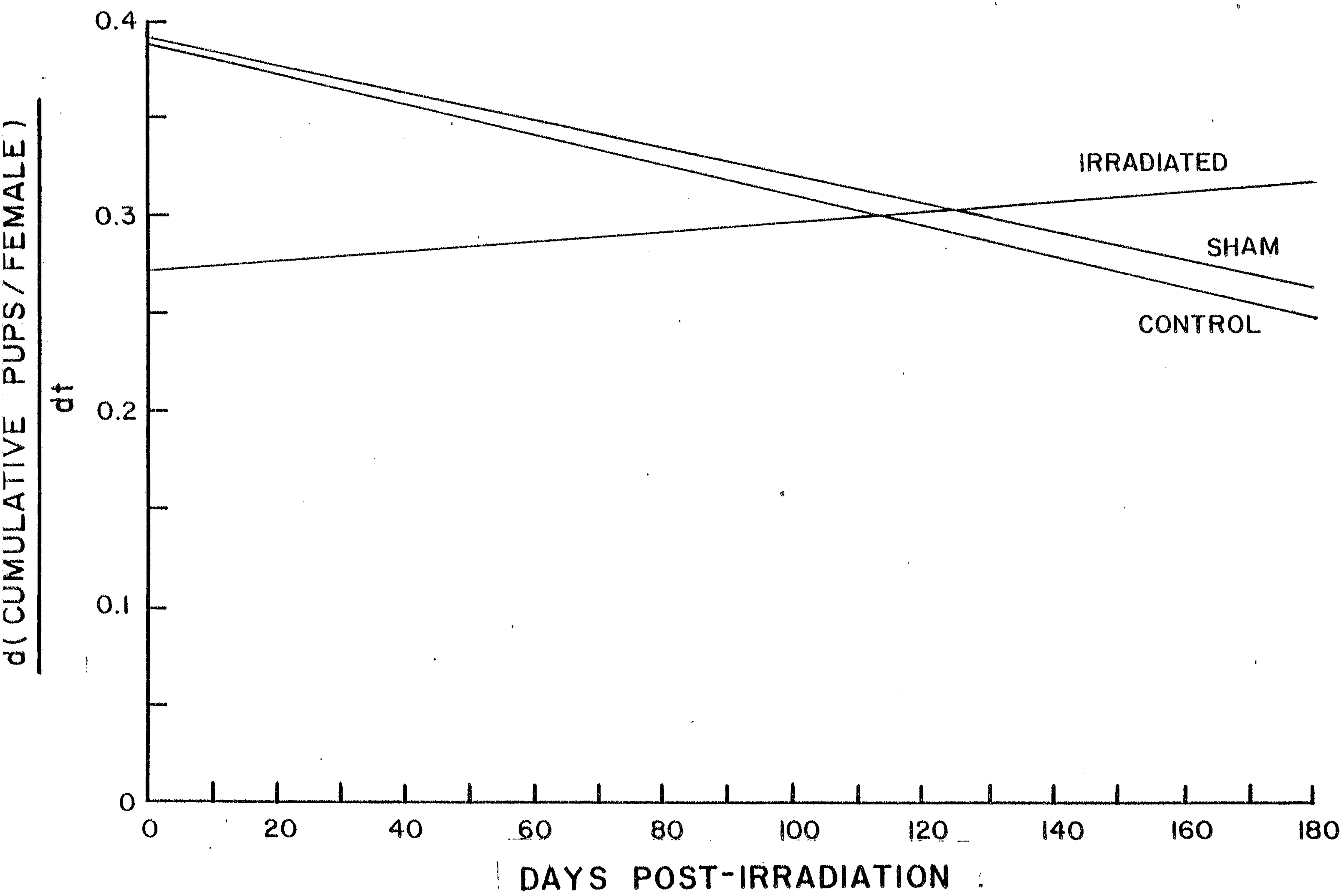


FIGURE 18 Derivatives of second order cumulative pups/ female vs. days post irradiation

change in spermatogenesis or to a decrease in litter frequency suggesting a decrease in sex drive of the male. To examine this, pups per litter *vs* days and cumulative litters per female *vs* days were tabulated as shown in Table 2 and 3, respectively. First and second order equations and their derivatives for pups per litter are:

Pups/Litter *vs* Days

Control

$$\text{pups/litter} = -\ 0.00244t + 8.23302 \qquad (10)\ \text{Figure 19}$$

$$\text{pups/litter} = 0.00001t^2 - 0.00417t + 8.29483 \qquad (11)\ \text{Figure 20}$$

$$\frac{d(\text{pups/litter})}{dt} = 0.00002t - 0.00417 \qquad (12)\ \text{Figure 21}$$

Sham

$$\text{pups/litter} = -\ 0.0083t + 9.25877 \qquad (13)\ \text{Figure 19}$$

$$\text{pups/litter} = -\ 0.00016t^2 + 0.02391t + 8.01615 \qquad (14)\ \text{Figure 20}$$

$$\frac{d(\text{pups/litter})}{dt} = -\ 0.00032t + 0.02391 \qquad (15)\ \text{Figure 21}$$

Irradiated

$$\text{pups/litter} = -\ 0.01047t + 9.41921 \qquad (16)\ \text{Figure 19}$$

$$\text{pups/litter} = -\ 0.00015t^2 + 0.01945t + 8.25642 \qquad (17)\ \text{Figure 20}$$

$$\frac{d(\text{pups/litter})}{dt} = -0.00030t + 0.01945 \qquad (18)\ \text{Figure 21}$$

The linear regression plot (Figure 19) of pups/litter as a function of days post irradiation shows a slight decrease in litter size with time of the sham, control, and irradiated groups. For the pups per litter *vs* days post irradiation the litter size is independent of the number of fertile females, so no normalization was necessary. Both the first and second order plots (Figures 19 and 20) show that the irradiated and sham pups/litter are nearly equivalent

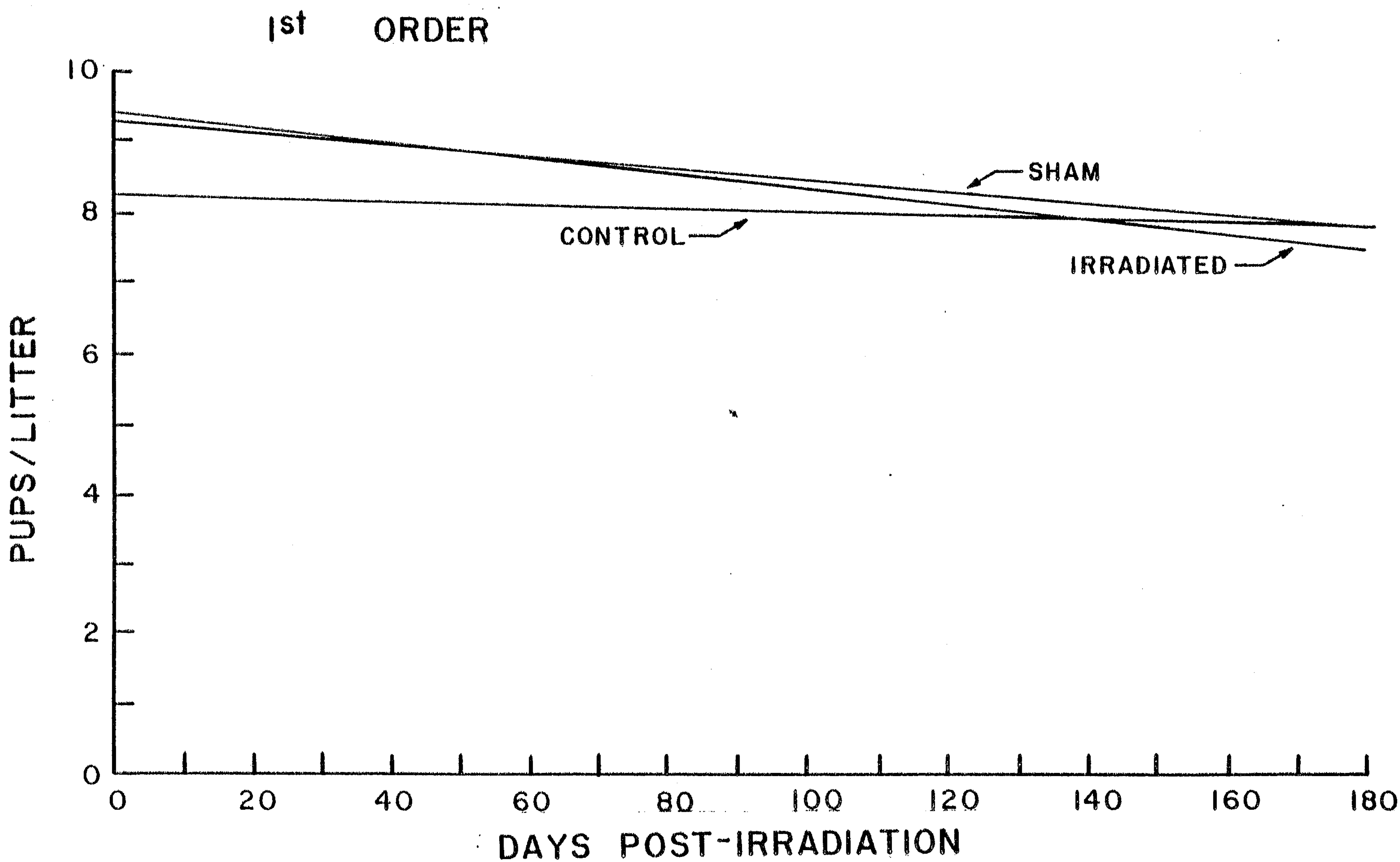


FIGURE 19 First order analysis of pups/litter vs. days post irradiation

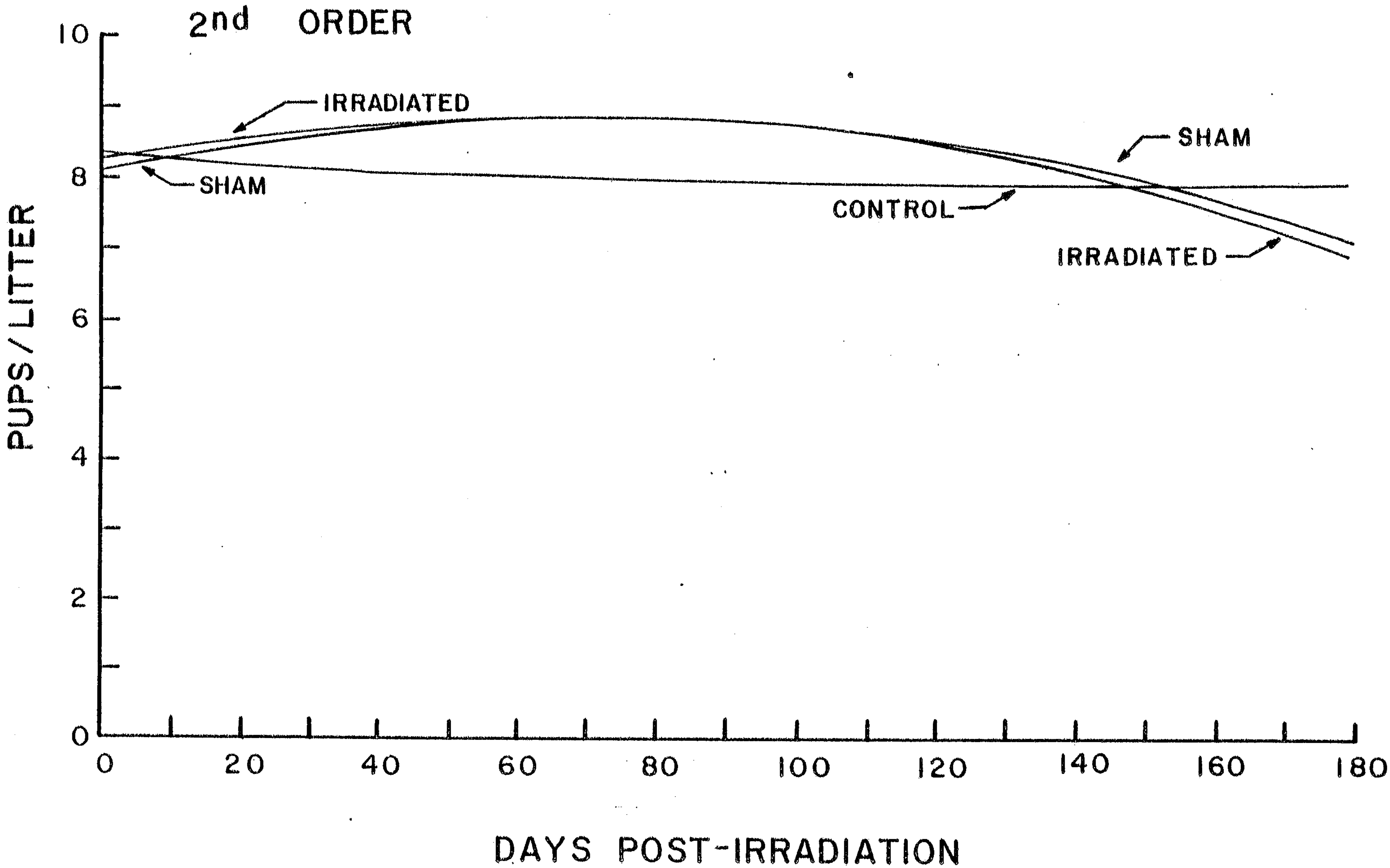


FIGURE 20 Second order analysis of pups/litter vs. days post irradiation

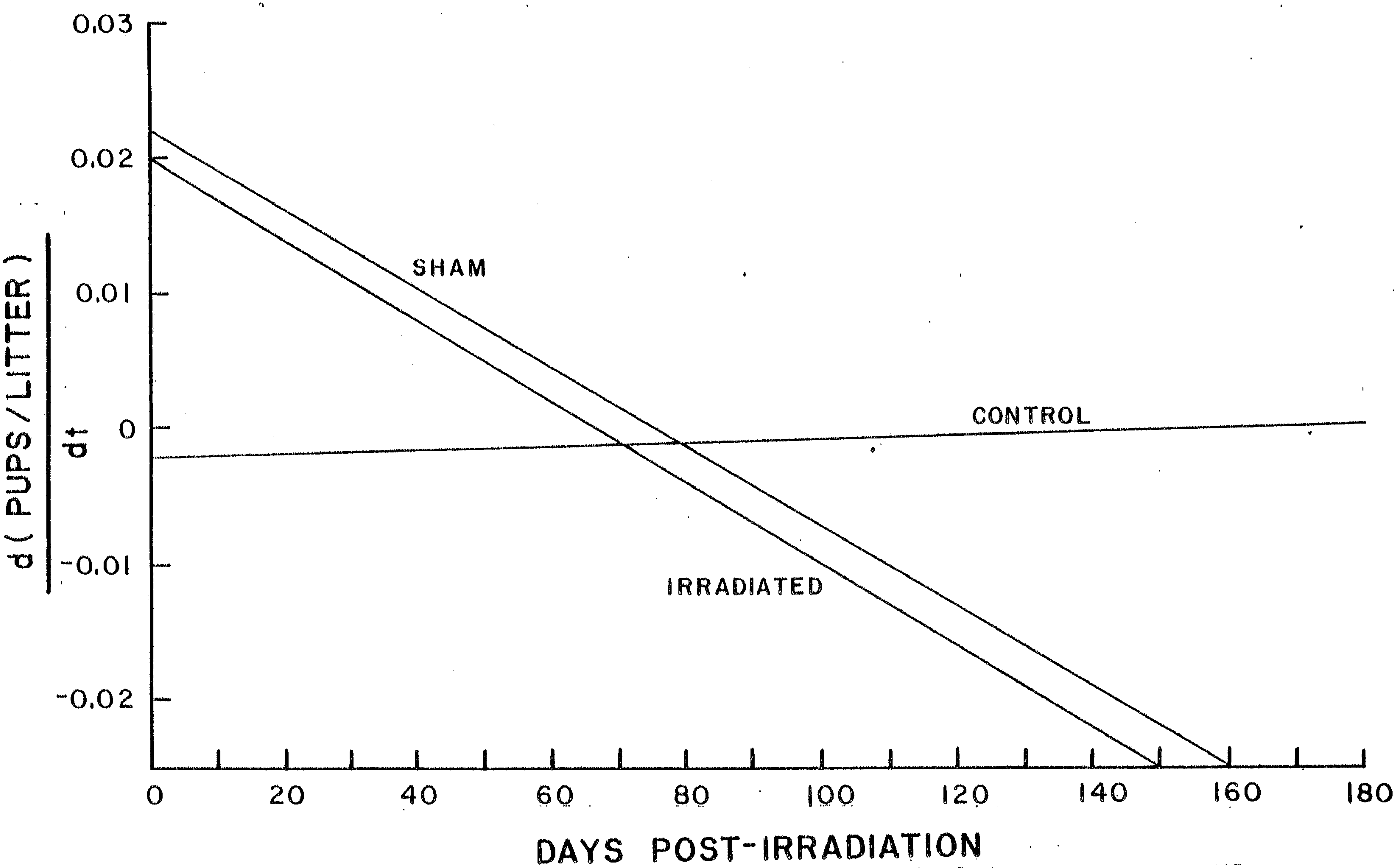


FIGURE 21. Derivatives of second order pups/litter vs. days post irradiation

or greater than the control group at any time during the study. Examination of the second order approximation (Figure 20) reveals that the control group pups/litter is nearly linear and decreasing slightly with time. Also shown on Figure 20 the sham and irradiated curves are nearly identical and differ from the control group in their non-linear concave down orientation. To examine the frequency of litters the data was prepared as shown in Table 3.

TABLE 3

DATA EXAMPLE

SCORED DATA			
LITTER DATE	LITTER SIZE (PUPS/LITTER)	DAYS POST IRRADIATION	CUM PUPS/ FEMALES
6/28	4	20	0.33
7/20	3	42	0.67
8/9	7	62	1.00
8/15	11	68	1.33
8/19	8	72.	1.67

The cumulative litters per female is the sum of the number of litters divided by the number of fertile females. This data was subjected to SOUPAC analysis resulting in the following equations. The time derivatives were calculated from the second order equations.

Cumulative Litters Per Female *vs* Days

Control

cumulative litters = 0.039t - 0.266 (19) Figure 22

time intercept = 6.77

$$\text{cumulative litters} = -0.00004t^2 + 0.0472t - 0.54925 \quad (20)$$ Figure 23

$$\frac{d(\text{cumulative litters})}{dt} = -0.00008t + 0.0472 \quad (21)$$ Figure 24

Sham

cumulative litters = 0.038t - 0.480 (22) Figure 22

time intercept = 12.7

$$\text{cumulative litters} = -0.00002t^2 + 0.04098 - 0.60434 \quad (23)$$ Figure 23

$$\frac{d(\text{cumulative litters})}{dt} = -0.00004t + 0.04098 \quad (24)$$ Figure 24

Irradiated

cumulative litters = 0.03612t - 0.6156 (25) Figure 22

time intercept = 17.04

$$\text{cumulative litters} = 0.00005t^2 + 0.02542t - 0.19963 \quad (26)$$ Figure 23

$$\frac{d(\text{cumulative litters})}{dt} = 0.0001t + 0.02542 \quad (27)$$ Figure 24

The curves of cumulative litters per female (Figures 22 and 23) resemble closely the curves of cumulative pups per female (Figure 16 and 17 respectively). The relative positions of control, sham, and irradiated linear regression curves are similar for both cases. Also, the time delay of the sham cumulative litters (Figure 22) is shown to be six days in relations to the control group. This is compared to 5.2 days for cumulative pups (Figure 16). Similarly, the irradiated group lags the sham group's time intercept for both the cumulative litters and cumulative pups.

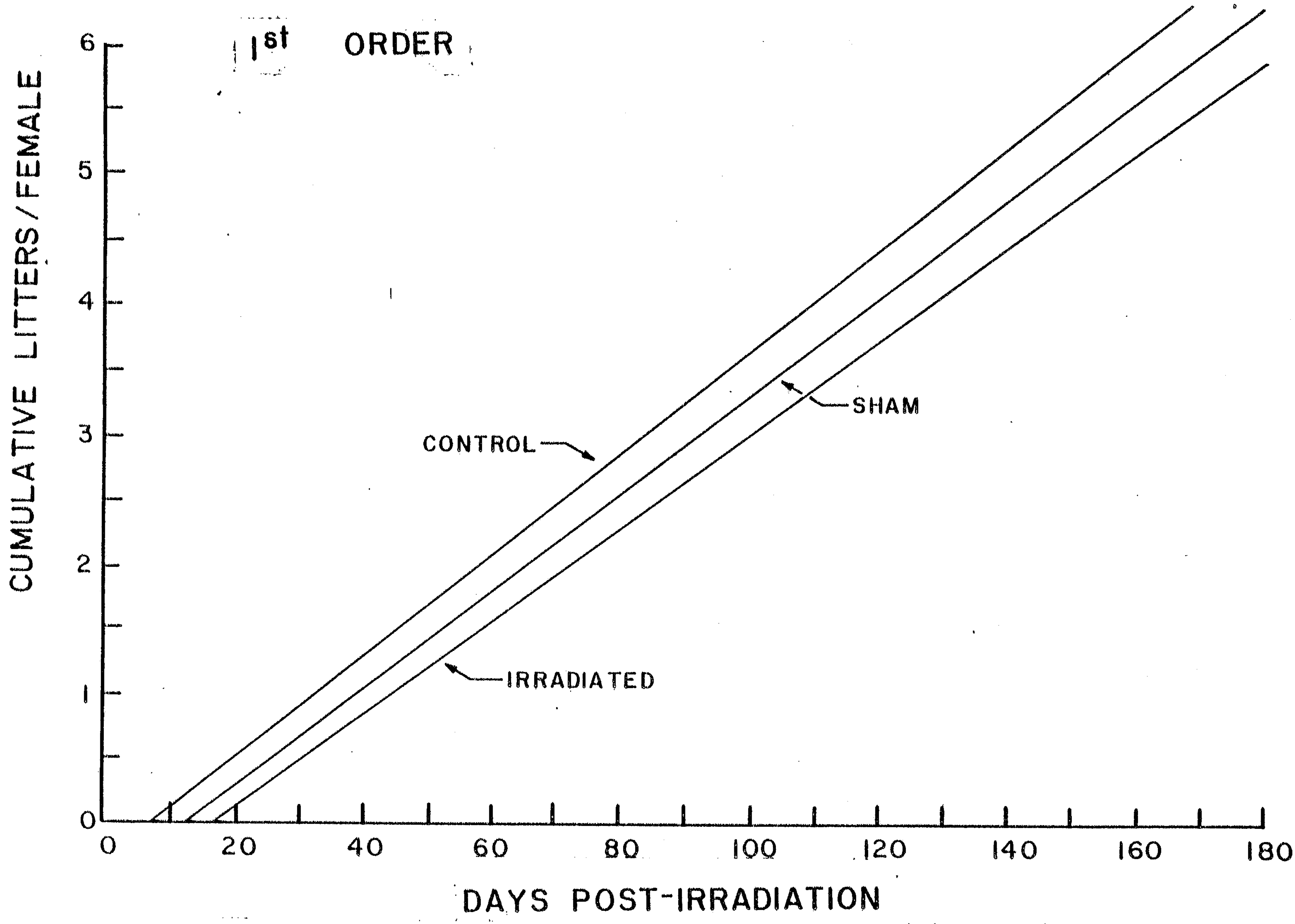


FIGURE 22 First order analysis of cumulative litters/female vs. days post irradiation

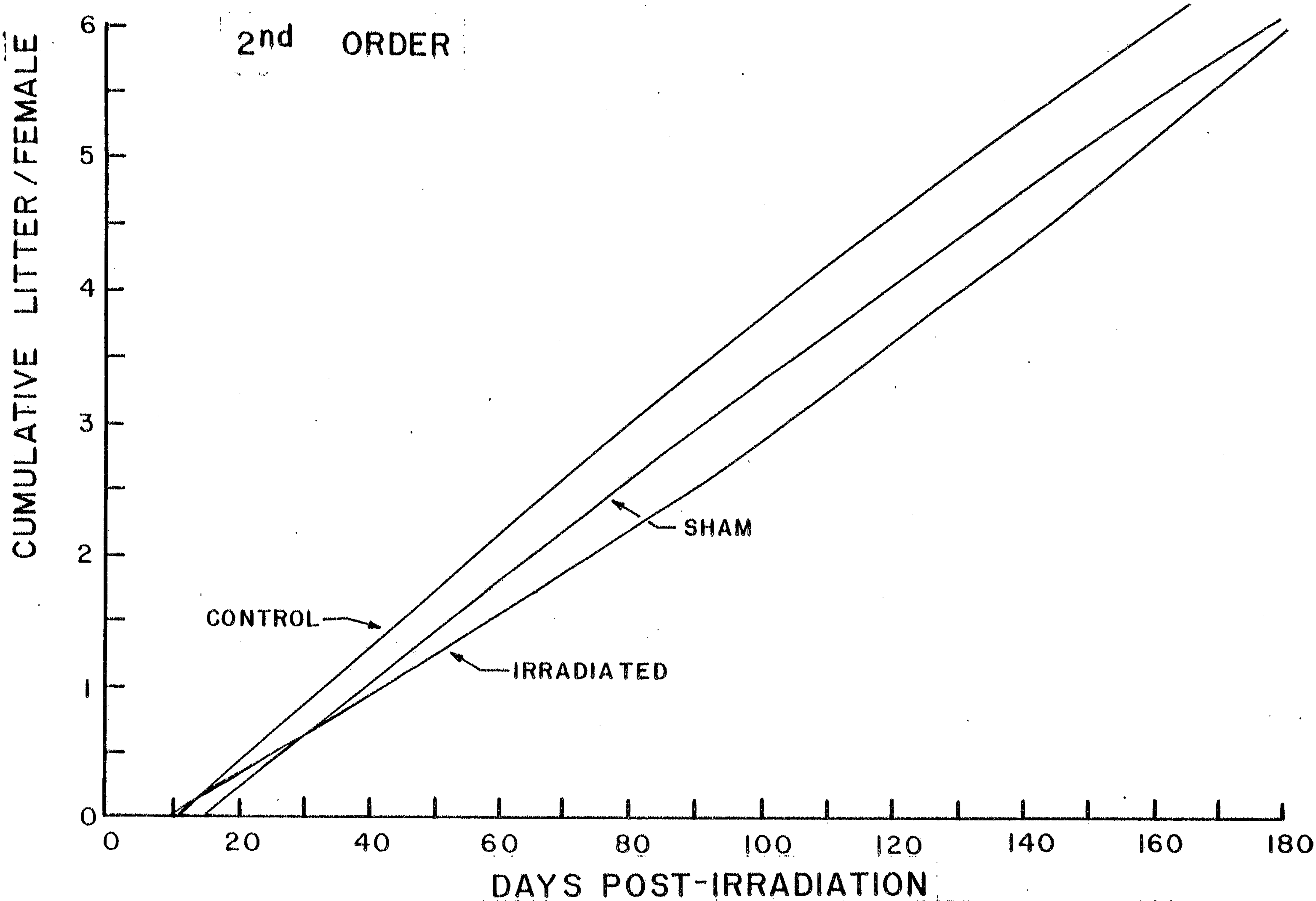


FIGURE 23 Second order analysis of cumulative litters/female vs. days post irradiation

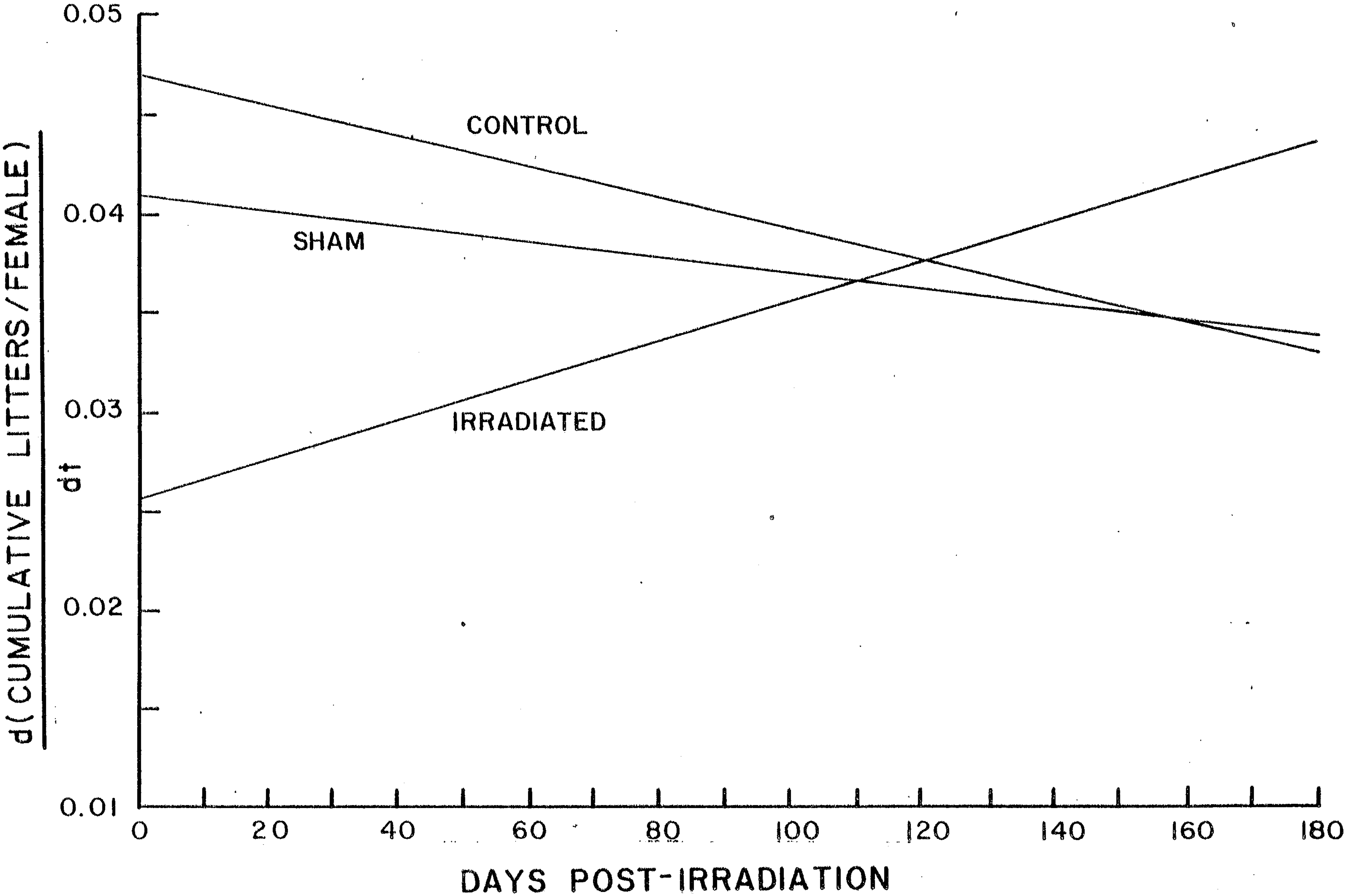


FIGURE 24 Derivatives of second order cumulative litters/female vs. days post irradiation

The second order curves (Figure 23) of cumulative litters also confirm the similarity with the cumulative pups (Figure 17). The control and sham group rates of littering were constantly decreasing while the rate of the irradiated littering increased in time. These rates were verified by examining the derivatives of the cumulative litter curves (Figure 24).

D. DISCUSSION OF RESULTS

The first order plots of litter size (Figure 19) all show a slight decrease in litter size as a function of time. This trend is expected as the females age throughout the study.[23]

A summary of the trends seen in Figures 17, 20, and 23 are shown in Figure 25. Note that the trends of cumulative pups/female and cumulative litters/female are identical. The curves of sham and irradiated are nearly identical for the pups/litter curves as previously stated. This indicates that there is some effect on the litter size due to irradiation procedures (anesthesia, etc.) but not due to the ultrasonic irradiation.

The trends of male fecundity post irradiation appear to be caused by the number of litters produced. This may be a result of various effects, possibly a decrease in sex drive due to change in hormonal levels or painful coitus. Visual examination of the testis from the males which died showed a darkening of the tunica albuginea. This discoloration seems to be present in all the animals which died within the two weeks post irradiation. These findings contradict those inferred by the damage to the tubules seen in Figures 8 and 9 and also those found in other ultrasonic toxicity studies.[13] These references suggest that a change in spermatogenesis affecting litter size is present and do not infer a change in frequency of littering. Based upon the suggested trends and the small number of animals this study is being repeated with

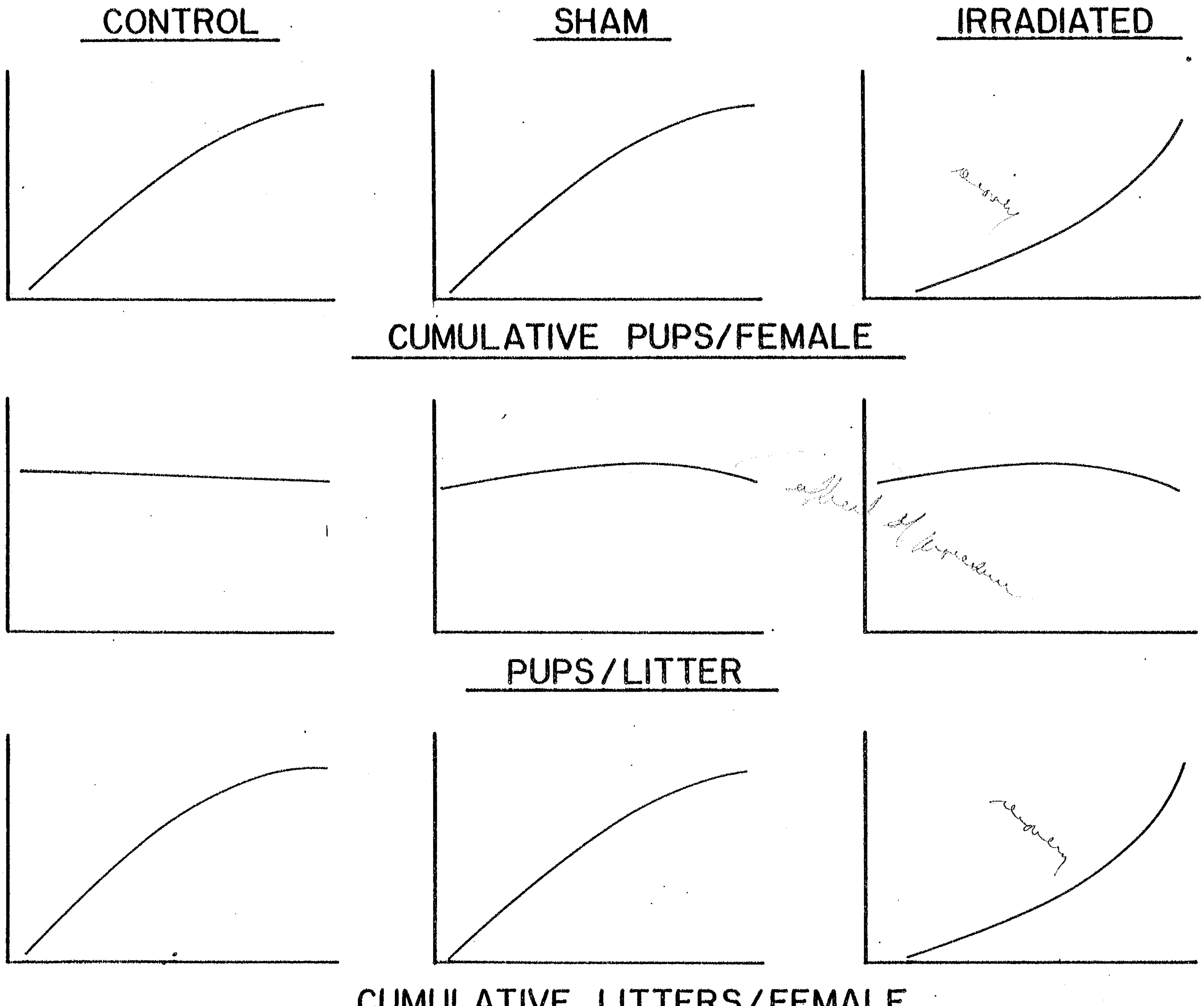


FIGURE 25. Summary of trends observed in second order analy

larger numbers of animals to verify the reported findings.

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APPENDICES

APPENDIX A

EXPOSURE PARAMETER SPECIFICATIONS

```
                    MOUSE IRRADIATION PROGRAM

MODIFY DOSE FOR DESIRED ARRAY?
TYPE Y OR N FOR YES OR NO      YES

                  KEYBOARD DOSE MODIFICATION
 MOVE NO.     4

 INTENSITY(WATTS/CM**2)    2.5
 TIME(SEC.)    20

 DATE:  7 24 78
 TRANSDUCER NO.  2
 CALIBRATION VOLTS  5540
 CALIBRATION W ATTS/CM**2  5.5
 NO. OF FIRST MOUSE  281
 REMARKS:  7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 MOVE # 4
       INTENSITY    2.50 WATTS PER CM**2
       TIME    20  SECONDS

           X        Y         Z
  1)     0.00    -0.50     0.00
  2)     0.00    -0.50    -1.00
  3)     0.00    -0.50    -2.00
  4)     0.00     0.50    -2.00
  5)     0.00     0.50    -1.00
  6)     0.00     0.50     0.00


 NUMBER OF MICE  7
 NUMBER TO BE IRRADIATED 4
 PRINT TABLE? NO.  TURN ON HIGH SPEED PUNCH.  PRESS CONTINUE.


NEXT MOUSE IS     288
 REMARKS:
 MOVE # -2


$IRRADIATION TABLE
   281*   282    283*   284    285*   286    287*
```

(Optional)

APPENDIX A

IRRADIATION ANIMAL SEQUENCE

```
                MOUSE IRRADIATION PROGRAM

 DATE:    7 24 78
 TRANSDUCER NØ.      2
 SET CS TO 1ØØ

 MOUSE NO.     281
   7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 READY?G
**** CS ERROR OF.-13ØØ.4  CALC.=  692.6  MEAS.= -6Ø7.8G
***R-F VOLTAGE TOO HIGH!G
***R-F VOLTAGE TOO HIGH!G
***R-F VOLTAGE TOO HIGH!G
***R-F VOLTAGE TOO HIGH!G
***R-F VOLTAGE TOO HIGH!G
***R-F VOLTAGE TOO HIGH!G

 MOUSE NO.     282
   7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 READY?G

 MOUSE NO.     283
   7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 READY?G

 MOUSE NO.     284
   7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 READY?G

 MOUSE NO.     285
   7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 READY?G

 MOUSE NO.     286
   7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 READY?G

 MOUSE NO.     287
   7 MICE 4 IRRADIATED AT 2.5 W/CM**2
 READY?G

 MOUSE NO.     288

***IRRADIATION COMPLETE!
```

APPENDIX B COMPUTER DATA TRANSFER SHEETS

PRE PARTUM ABNORMALITY STUDY

(ANALYSIS DATA SHEET-1)

MOTHER'S NUMBER ____ ____ ____ ____
1 2 3 4

DATE OF ANALYSIS ____ MO ____ | ____ DA ____ | ____ YR ____
5 6 7 8 9 10

HOUR OF ANALYSIS ____ ____
11 12

GESTATIONAL AGE ____ ____
13 14

MOTHER'S WEIGHT ____ ____ ____ ____ ____
15 16 17 18 19

MATERNAL DAMAGE
(1-YES, 2-NO) ____
20

PREGNANT
(1-YES, 2-NO) ____
21

POS MAXIMUM ____ ____
22 23

CORPUS LUTEUM - LEFT ____ ____
24 25

CORPUS LUTEUM - RIGHT ____ ____
26 27

Description of Maternal Damage:

Description of Abnormal Fetuses:

Cond Legend:

N - Normal
C - Cervix
I - Empty Site

E - Early Resorption
L - Late Resorption

H - Head Abnormality
B - Body Abnormality
D - Digit and/or Limb Abnormality
S - Size Abnormality
O - Other Abnormality

APPENDIX B

PRE PARTUM ABNORMALITY STUDY
(ANALYSIS DATA SHEET-2)

MOTHER'S NUMBER ___ ___ ___ ___
(1) (2) (3) (4)

DO YOU EXCEED MAXIMUM DATA LINE (Y-YES; N-NO) ___
(5)

LEFT OVARY TO RIGHT OVARY

POS	COND	SEX (M/F)	WEIGHT (GMS)		
1	___ 6	___ 7	___ 8	. ___ 9	___ 10
2	___ 11	___ 12	___ 13	. ___ 14	___ 15
3	___ 16	___ 17	___ 18	. ___ 19	___ 20
4	___ 21	___ 22	___ 23	. ___ 24	___ 25
5	___ 26	___ 27	___ 28	. ___ 29	___ 30
6	___ 31	___ 32	___ 33	. ___ 34	___ 35
7	___ 36	___ 37	___ 38	. ___ 39	___ 40
8	___ 41	___ 42	___ 43	. ___ 44	___ 45
9	___ 46	___ 47	___ 48	. ___ 49	___ 50
10	___ 51	___ 52	___ 53	. ___ 54	___ 55
11	___ 56	___ 57	___ 58	. ___ 59	___ 60
12	___ 61	___ 62	___ 63	. ___ 64	___ 65
13	___ 66	___ 67	___ 68	. ___ 69	___ 70
14	___ 71	___ 72	___ 73	. ___ 74	___ 75
15	___ 76	___ 77	___ 78	. ___ 79	___ 80
MAXIMUM - - - - DATA LINE					
16	___ 1	___ 2	___ 3	. ___ 4	___ 5
17	___ 6	___ 7	___ 8	. ___ 9	___ 10

APPENDIX B

POST PARTUM WEIGHT STUDY

(WEIGHT DATA SHEET)

Time ____________

Wt. of Box = ____________

MOTHER'S NUMBER ___ ___ ___ (1 2 3)

MOTHER'S WEIGHT ___ ___ .___ ___ ___ (4 5 6 7 8)

DAYS POST CONCEPTION ___ ___ ___ (9 10 11)

DATE ___ ___ ___ ___ ___ ___ (12 13 14 15 16 17)
Month | Day | Year

PUP WEIGHT ___ ___ .___ ___ ___ (18 19 20 21 22)

PUP WEIGHT ___ ___ .___ ___ ___ (23 24 25 26 27)

PUP WEIGHT ___ ___ .___ ___ ___ (28 29 30 31 32)

PUP WEIGHT ___ ___ .___ ___ ___ (33 34 35 36 37)

PUP WEIGHT ___ ___ .___ ___ ___ (38 39 40 41 42)

PUP WEIGHT ___ ___ .___ ___ ___ (43 44 45 46 47)

PUP WEIGHT ___ ___ .___ ___ ___ (48 49 50 51 52)

PUP WEIGHT ___ ___ .___ ___ ___ (53 54 55 56 57)

PUP WEIGHT ___ ___ .___ ___ ___ (58 59 60 61 62)

PUP WEIGHT ___ ___ .___ ___ ___ (63 64 65 66 67)

PUP WEIGHT ___ ___ .___ ___ ___ (68 69 70 71 72)

PUP WEIGHT ___ ___ .___ ___ ___ (73 74 75 76 77)

Time ____________

Wt. of Box = ____________

MOTHER'S NUMBER ___ ___ ___ (1 2 3)

MOTHER'S WEIGHT ___ ___ .___ ___ ___ (4 5 6 7 8)

DAYS POST CONCEPTION ___ ___ ___ (9 10 11)

DATE ___ ___ ___ ___ ___ ___ (12 13 14 15 16 17)
Month | Day | Year

PUP WEIGHT ___ ___ .___ ___ ___ (18 19 20 21 22)

PUP WEIGHT ___ ___ .___ ___ ___ (23 24 25 26 27)

PUP WEIGHT ___ ___ .___ ___ ___ (28 29 30 31 32)

PUP WEIGHT ___ ___ .___ ___ ___ (33 34 35 36 37)

PUP WEIGHT ___ ___ .___ ___ ___ (38 39 40 41 42)

PUP WEIGHT ___ ___ .___ ___ ___ (43 44 45 46 47)

PUP WEIGHT ___ ___ .___ ___ ___ (48 49 50 51 52)

PUP WEIGHT ___ ___ .___ ___ ___ (53 54 55 56 57)

PUP WEIGHT ___ ___ .___ ___ ___ (58 59 60 61 62)

PUP WEIGHT ___ ___ .___ ___ ___ (63 64 65 66 67)

PUP WEIGHT ___ ___ .___ ___ ___ (68 69 70 71 72)

PUP WEIGHT ___ ___ .___ ___ ___ (73 74 75 76 77)

MOTHER'S NUMBER ___ ___ ___ (1 2 3)

MOTHER'S WEIGHT ___ ___ .___ ___ ___ (4 5 6 7 8)

DAYS POST CONCEPTION ___ ___ ___ (9 10 11)

DATE ___ ___ ___ ___ ___ ___ (12 13 14 15 16 17)
Month | Day | Year

PUP WEIGHT ___ ___ .___ ___ ___ (18 19 20 21 22)

PUP WEIGHT ___ ___ .___ ___ ___ (23 24 25 26 27)

PUP WEIGHT ___ ___ .___ ___ ___ (28 29 30 31 32)

PUP WEIGHT ___ ___ .___ ___ ___ (33 34 35 36 37)

PUP WEIGHT ___ ___ .___ ___ ___ (38 39 40 41 42)

PUP WEIGHT ___ ___ .___ ___ ___ (43 44 45 46 47)

PUP WEIGHT ___ ___ .___ ___ ___ (48 49 50 51 52)

PUP WEIGHT ___ ___ .___ ___ ___ (53 54 55 56 57)

PUP WEIGHT ___ ___ .___ ___ ___ (58 59 60 61 62)

PUP WEIGHT ___ ___ .___ ___ ___ (63 64 65 66 67)

PUP WEIGHT ___ ___ .___ ___ ___ (68 69 70 71 72)

PUP WEIGHT ___ ___ .___ ___ ___ (73 74 75 76 77)

MOTHER'S NUMBER ___ ___ ___ (1 2 3)

MOTHER'S WEIGHT ___ ___ .___ ___ ___ (4 5 6 7 8)

DAYS POST CONCEPTION ___ ___ ___ (9 10 11)

DATE ___ ___ ___ ___ ___ ___ (12 13 14 15 16 17)
Month | Day | Year

PUP WEIGHT ___ ___ .___ ___ ___ (18 19 20 21 22)

PUP WEIGHT ___ ___ .___ ___ ___ (23 24 25 26 27)

PUP WEIGHT ___ ___ .___ ___ ___ (28 29 30 31 32)

PUP WEIGHT ___ ___ .___ ___ ___ (33 34 35 36 37)

PUP WEIGHT ___ ___ .___ ___ ___ (38 39 40 41 42)

PUP WEIGHT ___ ___ .___ ___ ___ (43 44 45 46 47)

PUP WEIGHT ___ ___ .___ ___ ___ (48 49 50 51 52)

PUP WEIGHT ___ ___ .___ ___ ___ (53 54 55 56 57)

PUP WEIGHT ___ ___ .___ ___ ___ (58 59 60 61 62)

PUP WEIGHT ___ ___ .___ ___ ___ (63 64 65 66 67)

PUP WEIGHT ___ ___ .___ ___ ___ (68 69 70 71 72)

PUP WEIGHT ___ ___ .___ ___ ___ (73 74 75 76 77)

APPENDIX C TWO FACTOR MIXED DESIGN FORTRAN PROGRAM

```
$JOB
      INTEGER DPC,DATE,SIZE,PUPNUM,GEST
      INTEGER ST7GR,ST7A,DFMEAS,DFSUB,DFGR,DFSSB,DFSSW,DFERR,DFTRC,DFS
      REAL MOTWT
      DIMENSION PUP(13),GEST(3),AVE(3),SIZE(3)
      DIMENSION SD(3)
      DIMENSION GR1TR1(30),GR1TR2(30),GR1TR3(30)
      DIMENSION ST4(30),S14T1G(3),S14T2G(3)
      DIMENSION S14T3G(3),ST5A(30)
      DIMENSION ST3GR(3),ST5GR(3)
      DIMENSION S2T1G(3),S2T2G(3),S2T3G(3)
      DIMENSION ST7GR(3),ST4GR1(30),ST4GR2(30),ST4GR3(30)
      MNUMOL=0
      IOMS=0
      ST9C=0.
      ST14A=0
      ST5GR(1)=0
      ST5GR(2)=0
      ST5GR(3)=0
      N=0
      ST5=0.
      ST6=0.
      ST7A=0.
      ST9A=0.
      ST9B=0.
      ST13T1=0.
      ST13T2=0.
      ST13T3=0.
    1 READ(5,100,END=99)MOTNUM,MOTWT,DPC,DATE,PUP
  100 FORMAT(I3,F5.2,I3,I6,12F5.2,F1.0)
      IF(PUP(13).EQ.1)GO TO 49
      GO TO 52
   49 READ 51,PUP(13)
   51 FORMAT(F5.2)
   52 IF(MNUMOL.EQ.0)GO TO 15
      IF(MOTNUM.NE.MNUMOL)GO TO 14
   15 IF(MOTNUM.NE.MNUMOL)J=1
      IF(MOTNUM.EQ.MNUMOL)J=J+1
      IF(PUP(13).NE.0) I=14
      IF(PUP(13).NE.0)GO TO 11
      DO 10 I=1,13
      IF(PUP(I).EQ.0)GO TO 11
   10 CONTINUE
   11 PUPNUM=I-1
      SUM=0
      SUMSQ=0.
      DO 12 L=1,PUPNUM
      SUM=SUM+PUP(L)
      SUMSQ=SUMSQ+PUP(L)**2
   12 CONTINUE
      AVE(J)=SUM/PUPNUM
      SD(J)=SQRT(SUMSQ/PUPNUM-(SUM/PUPNUM)**2)
      SIZE(J)=PUPNUM
      GEST(J)=DPC
      MNUMOL=MOTNUM
      GO TO 1
```

APPENDIX C

```
   14 IF(J.EQ.3)GO TO 20
      M1=J+1
      DO 21 M=M1,6
      AVE(M)=0.0
      SIZE(M)=0
      GEST(M)=0
      SD(M)=0.
   21 CONTINUE
   20 MOMS=MOMS+1
      GR1TR1(MOMS)=AVE(1)
      GR1TR2(MOMS)=AVE(2)
      GR1TR3(MOMS)=AVE(3)
      ST4(MOMS)=AVE(1)+AVE(2)+AVE(3)
      ST5A(MOMS)=AVE(1)**2+AVE(2)**2+AVE(3)**2
      PRINT 19
   19 FORMAT('-','MOTHER S# DPC=')
      PRINT 16,GEST
   16 FORMAT('+',17X,I3,10X,I3,10X,I3,10X,I3,10X,I3,10X,I3)
      PRINT 17,MNUMOL,AVE
   17 FORMAT(' ',I3,3X,'AVE.WT.=  ',F5.2,8X,F5.2,8X,F5.2,8X,F5.2,8X,F5.
     1,8X,F5.2)
      PRINT 70,SD
   70 FORMAT(' ',5X,'ST. DEV.=  ',F5.2,8X,F5.2,8X,F5.2,8X,F5.2,8X,F5.2,
     1X,F5.2)
      PRINT 18,SIZE
   18 FORMAT(' ',9X,'SIZE=',5X,I2,11X,I2,11X,I2,11X,I2,11X,I2,11X,I2)
      IF(MOTNUM.EQ.0)GO TO 22
      GO TO 15
   22 ST2TR1=0.
      ST2TR2=0.
      ST2TR3=0.
      DO 23 I=1,MOMS
      ST2TR1=GR1TR1(I)+ST2TR1
      ST2TR2=GR1TR2(I)+ST2TR2
      ST2TR3=GR1TR3(I)+ST2TR3
      PRINT 61,ST2TR1,ST2TR2,ST2TR3
   23 CONTINUE
      N=N+1
      ST3GR(N)=ST2TR1+ST2TR2+ST2TR3
      PRINT 61,ST3GR(N)
      IF(N.EQ.1)GO TO 28
      IF(N.EQ.2)GO TO 29
      IF(N.EQ.3)GO TO 30
   28 DO 31 M6=1,MOMS
      ST4GR1(M6)=ST4(M6)
   31 CONTINUE
      GO TO 50
   29 DO 32 M7=1,MOMS
      ST4GR2(M7)=ST4(M7)
   32 CONTINUE
      GO TO 50
   30 DO 33 M8=1,MOMS
      ST4GR3(M8)=ST4(M8)
   33 CONTINUE
```

APPENDIX C

```
   50 CONTINUE
      S2T1G(N)=ST2TR1
      S2T2G(N)=ST2TR2
      S2T3G(N)=ST2TR3
      DO 25 M3=1,MOMS
      ST5GR(N)=ST5A(M3)+ST5GR(N)
   25 CONTINUE
      ST7GR(N)=MOMS
      MOMS=0
      MNUMOL=MOFNUM
      GO TO 1
   99 CONTINUE
      DO 24 M2=1,3
      ST6=ST3GR(M2)+ST6
   24 CONTINUE
      DO 26 M4=1,3
      ST7A=ST7GR(M4)+ST7A
   26 CONTINUE
      ST7=ST6**2/ST7A/3
      DO 27 M5=1,3
      ST5=ST5GR(M5)+ST5
   27 CONTINUE
      ST8=ST5-ST7
      N1=ST7GR(1)
      DO 34 M9=1,N1
      ST9A=ST4GR1(M9)**2+ST9A
   34 CONTINUE
      N2=ST7GR(2)
      DO 35 M10=1,N2
      ST9B=ST4GR2(M10)**2+ST9B
   35 CONTINUE
      N3=ST7GR(3)
      DO 36 M11=1,N3
      ST9C=ST4GR3(M11)**2+ST9C
   36 CONTINUE
      ST9D=ST9A+ST9B+ST9C
      ST9E=ST9D/3
      ST9=ST9E-ST7
      ST10G1=ST3GR(1)**2/ST7GR(1)/3
      ST10G2=ST3GR(2)**2/ST7GR(2)/3
      ST10G3=ST3GR(3)**2/ST7GR(3)/3
      ST10A=ST10G1+ST10G2+ST10G3
      ST10=ST10A-ST7
      ST11=ST9-ST10
      ST12=ST8-ST9
      DO 37 M12=1,3
      ST13T1=S2T1G(M12)+ST13T1
      ST13T2=S2T2G(M12)+ST13T2
      ST13T3=S2T3G(M12)+ST13T3
   37 CONTINUE
      ST13A1=ST13T1**2/ST7A
      ST13A2=ST13T2**2/ST7A
      ST13A3=ST13T3**2/ST7A
      ST13A=ST13A1+ST13A2+ST13A3
      ST13=ST13A-ST7
```

APPENDIX C

```
      DO 38 M13=1,3
      S14T1G(M13)=S2T1G(M13)**2/ST7GR(M13)
      S14T2G(M13)=S2T2G(M13)**2/ST7GR(M13)
      S14T3G(M13)=S2T3G(M13)**2/ST7GR(M13)
      PRINT 62,S14T1G(M13),ST7GR(M13),S2T1G(M13)
   62 FORMAT('0',F8.2,I4,F8.2)
   38 CONTINUE
      DO 39 M14=1,3
      ST14A=S14T1G(M14)+S14T2G(M14)+S14T3G(M14)+ST14A
   39 CONTINUE
      ST14=ST14A-ST7-ST10-ST13
      ST15=ST12-ST13-ST14
      DFMEAS=ST7A*3-1
      DFSUB=ST7A-1
      DFGR=2
      DFSSB=DFSUB-DFGR
      DFSSW=DFMEAS-DFSUB
      DFSTR=2
      DFTRC=DFSTR*DFGR
      DFERR=DFSSW-DFSTR-DFTRC
      ST1710=ST10/DFGR
      ST1711=ST11/DFSSB
      ST1713=ST13/DFSTR
      ST1714=ST14/DFTRC
      ST1715=ST15/DFERR
      F1=ST1710/ST1711
      F2=ST1713/ST1715
      F3=ST1714/ST1715
   61 FORMAT('0',9F8.1)
      PRINT 61,ST5,ST6,ST7,ST8,ST9,ST9D,ST9E,ST10
      PRINT 40
   40 FORMAT('1',6X,'SOURCE',12X,'SS',4X,'DF',5X,'MS',3X,'F')
      PRINT 41,ST8,DFMEAS
   41 FORMAT('0','TOTAL',15X,F6.1,3X,I3)
      PRINT 42,ST9,DFSUB
   42 FORMAT(' ',2X,'BETWEEN SUBJECTS',3X,F5.1,3X,I3)
      PRINT 43,ST10,DFGR,ST1710,F1
   43 FORMAT(' ',4X,'CONDITIONS',7X,F5.1,3X,I3,4X,F5.2,4X,F5.2)
      PRINT 44,ST11,DFSSB,ST1711
   44 FORMAT(' ',4X,'ERRORB',11X,F5.1,3X,I3,4X,F5.2)
      PRINT 45,ST12,DFSSW
   45 FORMAT(' ',2X,'WITHIN SUBJECTS',3X,F6.1,3X,I3)
      PRINT 46,ST13,DFSTR,ST1713,F2
   46 FORMAT(' ',4X,'TRIALS',10X,F6.1,3X,I3,2X,F7.2,2X,F7.2)
      PRINT 47,ST14,DFTRC,ST1714,F3
   47 FORMAT(' ',4X,'TRIALS*CONDITIONS',F5.1,3X,I3,4X,F5.2,4X,F5.2)
      PRINT 48,ST15,DFERR,ST1715
   48 FORMAT(' ',6X,'ERRORW',9X,F5.1,3X,I3,4X,F5.2)
      PRINT 61,ST14A,ST14,ST15,ST1714,F3
      STOP
      END
```

APPENDIX C

TWO FACTOR MIXED DESIGN PROGRAM PRINTOUTS

$ENTRY

MOTHER S#				
	DPC=	21	29	42
73	AVE.WT.=	2.00	6.15	11.77
	ST. DEV.=	0.27	0.81	1.85
	SIZE=	3	8	8

MOTHER S#				
	DPC=	21	29	42
95	AVE.WT.=	1.96	6.62	13.19
	ST. DEV.=	0.24	0.45	1.36
	SIZE=	9	9	9

MOTHER S#				
	DPC=	21	29	42
103	AVE.WT.=	1.72	4.62	11.56
	ST. DEV.=	0.26	0.84	1.79
	SIZE=	13	13	13

SOURCE	SS	DF	MS	F
TOTAL	3010.3	143		
BETWEEN SUBJECTS	81.0	47		
CONDITIONS	1.4	2	0.68	0.38
ERRORB	79.6	45	1.77	
WITHIN SUBJECTS	2929.3	96		
TRIALS	2378.9	2	1439.47	2685.45
TRIALS*CONDITIONS	2.1	4	0.53	1.00
ERRORW	48.2	90	0.54	

APPENDIX D   t-TEST BETWEEN TWO INDEPENDENT MEANS FORTRAN PROGRAM

```
 $JOB
          INTEGER ST11A
          REAL NG(2)
          REAL MN21G(2),MN29G(2),MN42G(2)
          DIMENSION S521G(2),S529G(2),S542G(2)
          J=0
        2 N=0
          SMSQ21=0
          SMSQ29=0
          SMSQ42=0
          SUM21=0
          SUM29=0
          SUM42=0
        1 READ 100,DAY21,DAY29,DAY42
      100 FORMAT(3F5.2)
          PRINT 3,DAY21,DAY29,DAY42
        3 FORMAT(' ',3F6.2)
          IF(DAY21.EQ.0)GO TO 5
          N=N+1
          SUM21=DAY21+SUM21
          SUM29=DAY29+SUM29
          SUM42=DAY42+SUM42
          SMSQ21=DAY21**2+SMSQ21
          SMSQ29=DAY29**2+SMSQ29
          SMSQ42=DAY42**2+SMSQ42
          GO TO 1
        5 J=J+1
          S4A21=SUM21**2
          S4A29=SUM29**2
          S4A42=SUM42**2
          STP421=S4A21/N
          STP429=S4A29/N
          STP442=S4A42/N
          STP521=SMSQ21-STP421
          STP529=SMSQ29-STP429
          STP542=SMSQ42-STP442
          S521G(J)=STP521
          S529G(J)=STP529
          S542G(J)=STP542
          MN21G(J)=SUM21/N
          MN29G(J)=SUM29/N
          MN42G(J)=SUM42/N
          NG(J)=N
          PRINT 20,SUM21,SMSQ21,STP421,STP521,N
       20 FORMAT(' ',4F8.1,I3)
          IF(J.EQ.2)GO TO 6
          GO TO 2
        6 ST1021=S521G(1)+S521G(2)
          ST1029=S529G(1)+S529G(2)
          ST1042=S542G(1)+S542G(2)
          PRINT 7,ST1021,ST1029,ST1042
        7 FORMAT(' ',3F10.2)
          ST11A=NG(1)+NG(2)-2
```

APPENDIX D

```
      ST1121=ST1021/ST11A
      ST1129=ST1029/ST11A
      ST1142=ST1042/ST11A
      PRINT 10,ST11A,ST1121,ST1129,ST1142
   10 FORMAT(' ',I4,3F10.2)
      ST12A=(1/NG(1))+(1/NG(2))
      ST1221=ST1121*ST12A
      ST1229=ST1129*ST12A
      ST1242=ST1142*ST12A
      PRINT 11,NG(1),NG(2)
   11 FORMAT(' ',2F6.1)
      PRINT 9,ST12A,ST1221,ST1229,ST1242
    9 FORMAT(' ',4F10.2)
      ST1321=SQRT(ST1221)
      ST1329=SQRT(ST1229)
      ST1342=SQRT(ST1242)
      ST1521=MN21G(2)-MN21G(1)
      ST1529=MN29G(2)-MN29G(1)
      ST1542=MN42G(2)-MN42G(1)
      ST1621=ST1521/ST1321
      IF(ST1329.EQ.0)ST1629=999.99
      IF(ST1329.EQ.0)GO TO 8
      ST1629=ST1529/ST1329
      IF(ST1342.EQ.0)ST1642=999.99
      IF(ST1342.EQ.0)GO TO 8
      ST1642=ST1542/ST1342
    8 PRINT 21,ST1021,ST11A,ST1121,ST12A,ST1221,ST1321,MN21G(1)
   21 FORMAT(' ',F8.2,I3,5F8.2)
      PRINT 22,MN21G(2),ST1521,ST1621
   22 FORMAT(' ',3F8.2)
      PRINT 23,ST1621,ST11A
   23 FORMAT(' ','T TEST VALUE 21 DAYS=',F6.2,'DEGREES OF FREEDOM=',I3
      PRINT 24,ST1629,ST11A
   24 FORMAT(' ','T TEST VALUE 29 DAYS=',F6.2,'DEGREES OF FREEDOM=',I3
      PRINT 25,ST1642,ST11A
   25 FORMAT(' ','T TEST VALUE 42 DAYS=',F6.2,'DEGREES OF FREEDOM=',I3
      STOP
      END
```

t-TEST PROGRAM PRINTOUT

```
T TEST VALUE 21 DAYS=  1.41DEGREES OF FREEDOM= 36
T TEST VALUE 29 DAYS= -0.16DEGREES OF FREEDOM= 36
T TEST VALUE 42 DAYS=  0.00DEGREES OF FREEDOM= 36
```

APPENDIX E

1. SOUPAC POLYNOMIAL FITTING PROGRAM

```
ID CARD #1
ID CARD #2
/*ID REGION=(170K,20K)
//EXEC SOUPAC
//SYSIN DD *
POLY(C)(2)(P).         ((2) SPECIFIES DEGREE OF POLYNOMIAL)
ENDS
DATA(2)(F3.0,1X,F5.2)          (DATA FORMAT)

DATA CARDS (x,y)

END#
/*
```

2. DATA SEQUENTIAL SORTING PROGRAM FOR WEIGHT STUDY HISTOGRAMS

```
ID CARD #1
ID CARD #2
// EXEC SORT
//SORTOUT DD SYSOUT=A
//SYSIN DD *
      SORT FIELDS=(1,5,CH,A)
      RECORD TYPE=F,LENGTH=(80,80)
      END
/*

DATA CARDS
//SORTIN DD *
/*
```

APPENDIX F

DATA FROM FECUNDITY STUDY - IRRADIATED 6/8/77
DATE GIVEN IN FORM (DAY OF LITTER--SIZE OF LITTER)

IRRADIATED

Animal #1	Animal #3	Animal #5	Animal #7
6/29-7	6/29-8	6/29-9	6/28-9
7/18-11	7/6-8	7/19-9	6/30-9
7/19-4	7/25-8	7/27-9	8/18-10
8/8-8	8/11-14	8/8-10	8/29-11
8/9-7	8/11-14	8/9-12	9/16-11
8/12-7	8/31-11	8/29-10	9/20-9
8/30-5	9/5-8	8/30-8	10/8-6
9/6-10	9/9-7	9/7-9	10/16-6
9/15-6	9/22-7	9/16-9	11/5-11
9/19-3	10/4-9	9/18-8	11/16-6
10/2-10	10/5-7	10/6-8	
10/9-6	10/25-7	10/9-8	
10/16-6	11/1-6	10/21-8	
10/21-11	11/15-8	11/2-11	
10/29-7	11/21-7	11/10-12	
11/4-7	12/5-7	11/21-8	
11/16-8		12/3-4	
11/21-7			
11/28-7			
12/3-8			

APPENDIX F

SHAM

Animal						
#2	#4	#6	#8	#10	#12	#14
7/5-10	6/29-8	6/29-9	6/29/8	6/29-9	6/28-4	6/28-8
7/5-9	7/6-3	7/1-9	7/11-11	7/22-10	7/12-13	7/5-9
7/11-11	7/22-7	7/11-9	7/19-7	7/25-9	7/18-10	7/22-8
7/28-11	8/2-8	7/18-7	8/2-6	8/10-9	7/25-9	8/8-5
8/3-8	8/8-9	7/27-10	8/8-9	8/11-11	8/8-14	8/9-8
8/3-7	8/12-8	8/5-11	8/11-10	8/19-9	8/12-10	8/12-9
8/17-10	8/22-8	8/22-9	8/22-9	8/29-9	8/26-11	8/31-6
9/1-9	8/30-11	9/5-11	8/29-5	9/5-8	8/31-11	9/5-7
9/11-11	9/1-5	9/9-10	9/5-8	9/9-8	9/1-10	9/11-12
9/28-13	9/11-9	9/11-11	9/12-9	9/19-7	9/16-9	9/26-8
9/30-9	9/14-9	9/25-8	9/19-5	9/25-10	9/20-9	9/30-5
10/6-7	9/30-8	10/3-12	9/25-9	9/29-8	9/22-6	10/17-9
10/28-11	10/6-8	10/14-8	9/30-7	10/9-8	10/6-6	10/20-5
11/21-7	10/28/8	10/21-12	10/7-5	10/15-13	10/22-5	11/4-8
11/28-5	10/29-5	11/8-9	10/15-9	10/20-8	10/28-10	11/10-6
	11/15-10	11/21-8	10/28-11	10/30-6	10/28-10	11/28-13
	12/5-9		11/4-10	11/9-8	11/14-6	12/3-5
			11/21-9	11/16-8	11/21-2	
			11/28-5	11/21-6	12/3-7	
				12/3-8		
				12/3-8		

APPENDIX F

CONTROLS

Animal					
#15	#16	#18	#19	#20	#21
6/28-4	6/28-8	6/27-8	6/29-3	6/29-8	6/29-8
7/30-3	6/30-9	6/30-8	7/6-9	6/30-7	7/4-8
8/9-7	7/18-11	7/1-8	7/18-13	7/8-7	7/14-8
8/15-11	7/25-7	7/14-10	7/20-9	7/14-10	7/18-9
8/19-8	8/8-10	7/20-10	7/26-10	7/18-10	7/25-9
8/30-6	8/11-9	8/3-9	8/8-9	7/28-7	8/8-12
9/5-9	8/12-8	8/8-8	8/8-9	8/3-12	8/12-8
9/7-9	8/29-7	8/11-10	8/17-1	8/9-6	8/29-8
9/25-8	8/31-9	8/29-11	8/26-2	8/17-8	9/6-7
9/27-8	9/5-8	8/31-9	8/29-15	8/23-5	9/11-8
9/28-11	9/18-9	9/5-7	9/6-7	8/29-6	9/16-8
10/14-10	9/21-9	9/18-8	9/14-9	9/6-8	9/27-4
10/17-9	10/3-11	9/21-7	9/18-10	9/15-1	10/9-6
10/30-7	10/6-9	10/9-6	10/3-4	9/19-4	10/28-8
11/4-8	10/17-9	10/11-7	10/7-9	9/26-10	11/29-6
	10/23-4	10/28-7	10/28-9	10/6-10	
	10/26-8	11/1-4	10/28-10	10/16-10	
	11/16-6	11/21-9	11/1-4	11/4-10	
	12/3-10		11/16-7	11/29-10	
	12/5-9		11/21-9		