PHYSIOLOGICAL ANATOMICAL RODENT EXPERIMENT (PARE.04) FEASIBILITY TEST 2

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Abstract. The objective of this feasibility study was to subject pregnant rats of the same age, strain, and size that will be utilized in a shuttle flight experiment to all flight conditions except the unique microgravity of space flight and determine the feasibility of the proposed experimental design to meet the experimental objectives. The study utilized facilities at NASA, Ames Research Center, Moffett Field, CA to subject the rats to the gravitational stresses of a simulated shuttle launch and simulated shuttle landing. One hundred pregnant rats were received on gestation day (G) 2 (day 1 = day of vaginal sperm) and on G7, eighty rats were laparotomized to determine the condition of pregnancy and allow assignment to test groups. The five test groups (N=10 each group) were as follows: Group 1, Nominal Flight; Group 2, Laparotomy Control; Group 3, Hysterectomy Control; Group 4, Vivarium Control; Group 5, Delayed Recovery. On G9, animals in groups 1,2,3,and 5 were subjected to a shuttle launch simulation. On G18, groups 1,2, and 3 were subjected to a shuttle landing simulation and on this same day groups 1 and 2 were subjected to unilateral hysterectomy to obtain fetuses and placentas for evaluation. Fetal crown-rump length and fetal weight of the Nominal Flight group was significantly less than the Laparotomy Control group, but placentas were similar. On G20, group 5 was subjected to a shuttle landing simulation and on this day this group received a unilateral hysterectomy and fetuses and placentas were weighed. Animals in all groups were allowed to go to term and all animals delivered between 06:00 hours G22 and 18:00 hours G23. After delivery, a blood sample was taken from each experimental dam, and they were euthanized and the thymus and adrenal glands weighed. The thymus weight from all experimental group dams was decreased relative to the Vivarium Control group but adrenal glands and hormone values in dam plasma was similar in all groups. Pups from experimental groups were tattooed for identification, the anogenital distance of male pups was measured, and all pups placed with foster dams and litter sizes were standardized to 10. There was no difference in anogenital distances between male pups from different test groups. Pups delivered from Delayed Recovery animals were smaller than pups delivered from Nominal Flight animals. On neonatal day 7, all pups were euthanized and pup adrenal glands and thymus weighed. There was no difference in weights of thymus and adrenal glands in pups euthanized at neonatal day 7. Collectively, these data confirm the feasibility of the experimental design to meet objectives of the studies proposed for shuttle flight.

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INTRODUCTION

The Physiological Anatomical Rodent Experiment (PARE) .04, also referred to as NIH.R1, is a shuttle payload currently scheduled for launch October 27, 1994. The payload will be devoted to developmental biology experiments and will be the first US payload to fly pregnant rats. The experimental design for the PARE .04 studies was thoughtfully formulated to acquire the maximum statistically valid scientific yield; however, the resulting design requires that rats be subjected to a series of environmental/treatment stresses.

The general objective of the present study (PARE .04 Feasibility Test 2) was to subject pregnant rats of the same age, strain, and size that will be utilized in the planned shuttle flight experiment to all flight conditions except the unique microgravity of space flight and determine the feasibility of the proposed experimental design to meet the experimental objectives. The present test utilized facilities at Ames Research Center to subject the rats to the gravitational stresses of a simulated shuttle launch at gestational day (G) 9 and a simulated shuttle landing at G18 or G20. Acoustic stresses mimicking a shuttle launch were also incorporated. In addition, shuttle mission environmental and operational parameters were simulated, including pre- and post-flight surgical procedures and animal enclosure module (AEM) housing.

Other specific objectives of the present study included: (1) To corroborate the pregnancy success rate for the vendor (Taconic) supplied timed-pregnant rats and re-evaluate the effects of shipment at G2 on pregnancy viability; (2) To determine whether unilateral hysterectomy at G18 or G20 would interfere with the maintenance of pregnancy and the occurrence of vaginal delivery; (3) To determine whether the

addition of increased gravitational forces and simulated acoustics, typical of shuttle launch, and increased gravitational forces, typical of shuttle recovery, would evoke stress responses evidenced in neonatal pups and in hormonal levels of maternal plasma or maternal organ weights.

All studies contained in this report were reviewed and approved by the NASA Ames Research Center Animal Care and Use Committee during meetings in January, 1994.

METHODS AND MATERIALS

Animals: General. One hundred nulliparous pregnant Sprague-Dawley rats weighing between 165 and 205 grams (Taconic Farms, Germantown, NY) were received at Ames Research Center on G2 of pregnancy. The G2 rats were received in two groups on two successive days: Shipment group 1 (N=60) and Shipment group 2 (N=40). The five experimental groups were selected from these shipments. Seventy-three rats at G16 (N=42), G17 (N=25) or G18 (N=6) were also received in a single shipment and served as foster dams. In all cases, gestation day 1 = day of vaginal plug or sperm. The animals were transported from the vendor in Germantown, NY to New York, NY (LaGuardia airport) and shipped air freight (Delta Airlines) non-stop to San Francisco International Airport ("Delta Dash"). Rats were picked up from the San Francisco airport between 20:00 and 22:00 hours and transported to Ames Research Center and individually housed. Ambient temperatures in New York at the times of shipment were approximately 20° F.

Animals were housed at Ames Research Center in a room with controlled

lighting (lights on 06:00 - 18:00 hours) and temperature (approximately 22° C) and provided food and water ad libitum. Food for animals received at G2 consisted of both laboratory chow and NASA food bars through G8. From G9 through delivery, animals assigned to test groups received only the food bars. Food for foster dams consisted of rat chow throughout the study. Body weights and food and water consumption were recorded daily from G2 - G7. These parameters were also recorded at G19 and G20 (Groups 1,2,3, and 4) or G20 (Group 5). Daily food, water, and body weight measurements were terminated at G20 in all rats, in order to not interrupt preparturient nesting behavior.

Animals: Cages. Animals received at G2 were housed individually in clear polycarbonate cages, approximately 19 inches long, 10 inches wide and 8 inches high, and the bedding was ground corncob. At G9 through G18, animals assigned to Nominal Flight, Laparotomy Control, and Hysterectomy Control groups were housed in an animal enclosure module (AEM) with the same dimensions and configuration as utilized in shuttle flights. At G9 through G20, animals assigned to the Delayed Recovery group were also housed in AEMs. Each of the four aforementioned experimental groups of 10 animals were housed in two AEMs (5 rats per AEM). After G18 (Nominal Flight, Laparotomy Control, and Hysterectomy Control) or G20 (Delayed Recovery) rats were housed individually in clear polycarbonate cages. Animals in the Vivarium Control group were housed individually in clear polycarbonate cages for the duration of the study.

Animals: Experimental Groups. One hundred rats received on G2 were housed singly upon receipt. On days G2 - G8, between 06:00 and 09:00 hours, body weights, and consumption of food (food bar and chow) and water were recorded. On

G7, 80 rats were subjected to laparotomy to confirm pregnancy and determine the number of decidual swellings per uterine horn. The intent was to ensure that rats assigned to Nominal Flight, Hysterectomy Control and Delayed Recovery groups had at least five decidual swellings per uterine horn. Thirty-eight of 80 rats (48%) laparotomized at G7 met these criteria. Rats were randomly assigned to the Laparotomy Control and Vivarium Control groups without laparotomy per the experimental design. The five test groups (N=10 each group) were as follows:

Group 1: Nominal Flight (received laparotomy at G7 and unilateral hysterectomy at G18, AEM habitat between G9 and G18)

Group 2: Laparotomy Control (received unilateral hysterectomy at G18, AEM habitat between G9 and G18)

Group 3: Hysterectomy Control (received laparotomy at G7, AEM habitat between G9 and G18)

Group 4: Vivarium Control (no laparotomy or unilateral hysterectomy, vivarium cages throughout gestation)

Group 5: Delayed Recovery Control (received laparotomy at G7 and unilateral hysterectomy at G20, AEM habitat between G9 and G20)

Animals: Anesthesia for Surgery. All animals were anesthetized with isoflurane (IsoFlo, Abbott Labs, North Chicago, IL) vapor utilizing a non-rebreathing rodent anesthesia unit (Viking Products, Medford Lakes, NJ). The major components of the anesthesia unit include an oxygen source and regulator, oxygen flow meter, a vaporizer calibrated for isoflurane, a co-axial breathing apparatus for maintaining rat surgical anesthesia, an induction chamber, and a scavenger system to eliminate the anesthetic from the operating area. The induction chamber was charged initially for 15

minutes with 5% isoflurane and then subsequently the percentage of anesthetic was lowered to 2-3%. This concentration of isoflurane is sufficient for subsequent induction and maintenance of surgical anesthesia in rats.

Animals: Laparotomy at G7. For laparotomy at G7, aseptic technique was employed. Rats were anesthetized with isoflurane, hair was clipped, and the ventral abdomen was scrubbed with betadine and 70% ethyl alcohol. Veterinary ophthalmic ointment (Bacitracin-neomycin-polymyxin veterinary ophthalmic ointment, Pharmaderm, Melville, NY) was applied to the cornea and the rats were injected subcutaneously with an antibiotic (Microcillin-AG, Anthony Products Co., Arcadia CA, 10,000 I.U. per rat) and an analgesic (Butorphanol Tartrate, Fort Dodge Labs, Inc., Fort Dodge, IA, 10 mg/kg). A sterile drape was utilized with each rat. Instrument packs for each surgical procedure were steam sterilized initially, and subsequently, between each procedure, each instrument pack was cleaned and soaked at least 15 minutes in a germicide solution (Cetylcide, Pennsauken, NJ) followed by a 5 minute soak in 70% ethyl alcohol, and finally a saline rinse. The ventral skin, muscle wall, and parietal peritoneum were cut approximately 2 cm cranial to the pubis and the incision extended cranially 2-3 cm. To optimize observation and recording of individual decidual swellings, each uterine horn was gently manipulated by grasping the uterine horn between decidual swellings with thumb forceps. The number of decidual swellings was recorded and subsequently, the peritoneum and muscle layer were sutured (interrupted sutures) with monofilament nylon (3-0 Ethilon, Ethicon, Somerville, NJ), the skin closed with 9 mm wound clips (Autoclip, Clay Adams, Parsippany, NJ), and the animals allowed to recover. Each laparotomy procedure took approximately 10 minutes. On G8, body weights of all animals were recorded and rats subjected to laparotomy the previous day were closely examined. Three to five wound clips on each of 12 of the 80 rats that had experienced

surgery the previous day were found to be loose or missing at this time and the clips were removed or replaced as necessary. Subsequently, on G9, all 80 rats were closely examined again and in all cases all wound clips were secure. Also, on G8, nine of 80 rats had a small (5-10 mm diameter) granuloma on the nape of the neck at the site of the antibiotic injection. Application of zinc oxide ointment topically for 2-4 days alleviated these granulomas in the rats so affected. On G8, thirty of the rats with at least 5 decidual swellings per uterine horn, and devoid of wound clip and granuloma problems, were selected for assignment to groups 1, 3, and 5 (N = 10 each group).

Animals: Shuttle Launch Simulation. On G9, at 09:00 - 10:00 hours, rats in groups 1, 2, 3, and 5 were carefully inspected by the Ames Research Center Veterinarian, placed in AEMs with food bars, and subsequently the AEMs were fitted into transfer cages and transported via a van to the 20 G centrifuge facilities. The AEMs containing the rats were secured in a special carrier on the centrifuge arm and exposed to a launch simulation. The acceleration and acoustic ranges experienced by the rats during this simulation was formulated from previous launch data (Figs. 26 and 27). The launch simulation lasted approximately 8 minutes, varied from 1 to 3 Gs, and acoustics reached a maximum of 108 dB, and ranged from 31.5 - 2500 Hz. At the conclusion of the simulated launch, the AEMs containing the rats were transported back to a special animal holding room (Bldg 240, room 136D) and the AEMs were assembled to mimic shuttle mission housing (12:12 light cycle, lights on 06:00 - 18:00 hours; temperature raised to 26° C). During the period of simulated shuttle mission housing, the rats were observed briefly daily at 08:00 hours and notes on animal behavior were recorded. Water consumption during the simulated shuttle mission housing period was also recorded.

Animals: Shuttle Recovery Simulation. At G18 (Groups 1 and 2) or G20 (Group 5), at 09:00 - 10:00 hours, the AEMs containing the rats were transported back to the Ames Research Center 20 G Centrifuge Facility and exposed to a shuttle landing simulation. This simulation lasted approximately 16 minutes, and varied from 1 to 2 Gs (Fig. 29). There was not an acoustic component of the landing simulation. After completion of the landing simulation, the rats were transported back to Building 240, inspected by the Ames Research Center veterinarian, and a nasopharyngeal swab and fecal sample were taken. Subsequently, the rats were transported to the surgery area for unilateral hysterectomy.

Animals: Unilateral Hysterectomy at G18 or G20. On G18, in Groups 1 and 2, and on G20, in Group 5, rats were subjected to unilateral hysterectomy. For this procedure, animals were anesthetized with isoflurane and prepared for surgery, including injection of analgesic and antibiotic and application of ophthalmic ointment to the cornea as previously described for laparotomy at G7. The uterine cornu were exteriorized by extending the original mid ventral abdominal incision utilized at G7 for laparotomy. The number of live/resorbing fetuses in each horn was recorded. The uterine horn to be removed was alternated between rats in each of the three experimental groups. The horn was ligated cranially and caudally with black braided silk (2-0, Ethicon, Sommerville, NJ), and excised. Procedures for closing the incision were identical to those utilized at the G7 surgery. Fetuses and placentas were quickly dissected from the uterine horn, weighed, and weights recorded. The crown-rump length of each fetus was also recorded. The unilaterally hysterectomized rats were singly caged and returned to the animal room. After unilateral hysterectomy, body weights, food bar consumed, and water consumed, were recorded daily for each rat in each of the five test groups. One rat assigned to the Laparotomy Control group (Group

2) was not pregnant at the G18 surgery and was euthanized with an overdose of isoflurane at this time and eliminated from the study.

Animals: Natural Delivery. All rats were allowed to go to term and beginning on G21, all rats were observed for the initiation of delivery at hourly intervals from 06:00 hours to 18:00 hours; however no rat initiated delivery before 10:00 hours on G22. Rats were not disturbed during the dark cycle between 18:00 hours, G22 and 06:00 hours, G23. Thus the exact time of initiation/duration of delivery of foster and test animals with young in the cage at 06:00 hours on G23 was not known. During the light phase of G23, animals were again observed at hourly intervals beginning at 06:00 hours. During the light phase of the cycle, the time when pups were first found in the cage was designated as the time of the initiation of delivery. When the number of pups in the cage did not change at two successive hourly checks, parturition was designated complete and the dams and pups were utilized according to the experimental design. Also, animals that were in the process of delivery at 18:00 hours on G22 were designated to have completed delivery at 06:00 hours on G23 and were utilized according to the experimental design.

Animals: Cross Fostering. Once test animals had completed delivery, the number and sex of pups was recorded and each pup was numbered with a green tattoo on the dorsal side for identification. All tattoos were applied with a Spaulding Special Electric Tattoo Marker, Model SSEMK110 (Spaulding & Rogers, Voorheesville, NY). Subsequently, the green numbered pups were placed with a foster dam. Foster dams which received experimental pups had delivered their own pups within the past 12 hours. Pups delivered by the foster dams were numbered with a black tattoo on the dorsal side for identification. All pups were weighed after tattooing. The total litter

number (natural pups plus foster pups) of all foster dams was adjusted to 10. Experimental groups 1 and 2 had unilateral hysterectomy on G18, and group 5 had unilateral hysterectomy on G20. Since these three groups delivered young from only one horn, all live pups delivered were cross fostered. Rats in experimental groups 3 and 4 retained both uterine horns and often delivered more than 10 pups. In these groups, all male pups and female pups necessary to total 9, were placed with foster dams. The foster dam was always allowed to retain at least one of her natural pups. In one test animal from group 3, which delivered 13 live pups, all 13 pups were retained and crossed to two foster mothers.

Animals: Male Pup Anogenital Distance Measurements. On the day pups were delivered, prior to cross fostering, the anogenital distance in all male pups in all experimental groups, and in all male pups from foster dams, was photographed. The anogenital distance is defined as the length of tissue separating the anus and genital papilla. This distance is an index of sexual differentiation and in male pups experiencing prenatal stress, this distance is decreased transiently in the postnatal period. The anogenital distance was photographed with an Olympus OM-1 camera attached to an Olympus Operation Microscope (Model MTX, Olympus Optical Co., Tokyo, Japan). The film used was Kodak T-Max, ASA 100, and the magnification on the film was 2X. Black and white prints were enlarged to a total magnification of 16X, and the anogenital distance was measured from the prints with a six inch dial caliper and subsequently calculated and recorded.

Animals: Experimental Dam Tissues and Blood. While pups from experimental dams were being tattooed and having photomicrographs made of the anogenital distance, experimental dams were anesthetized with isoflurane, the

abdominal cavity opened, and the aorta exposed. At the aortic bifurcation, blood (7-9 mls) was drawn into a heparinized 10 ml syringe fitted with a 21 gauge 1 1/2 inch needle. If animals did not die from exsanguination, they were euthanized with an overdose of isoflurane. The plasma was separated immediately in a refrigerated centrifuge, decanted, and stored in a cryovial at -70° C. The thymus and adrenal glands were removed, connective tissue was carefully trimmed, and the organ weights recorded.

Frozen plasma was shipped in dry ice via Federal Express to Nichols Institute (Los Angeles, CA) for determination of plasma concentration of progesterone, estradiol, corticosterone, and catecholamines (dopamine, norepinephrine, and epinephrine).

Animals: Pups. The body weight of pups from test dams and foster dams was recorded when the pups were tattooed (day 0). Subsequently, pups were placed with dams and weighed on neonatal days 2 through 7. On day 7, the pups were anesthetized with isoflurane, decapitated, and the thymus and adrenal glands removed, connective tissue was trimmed, and organ weights recorded.

Statistics. Numerical data were expressed as mean +/- standard error of mean. Data in different groups were examined with one-way analysis of variance (ANOVA) and if significance was found with ANOVA, group comparisons of means were made with the Newman-Keuls test. The Student's t test was used to analyze data between two samples.

RESULTS

Animals: General. The docile, yet sturdy nature of the pregnant Taconic Sprague Dawley rats used in this feasibility study was corroborated. Animals appeared relaxed and content when housed singly. When housed in groups of five, they appeared biocompatible.

There was a high pregnancy rate in rats shipped on G2: Only 5 of 100 rats shipped at this stage of pregnancy were subsequently shown to not be pregnant. All 73 rats serving as foster dams and shipped at G16-G18 were pregnant.

Animals: Weight Gain G2-G7. One hundred rats received at G2 weighed approximately 186 grams. They were provided food and water *ad libitum* and by G7 they weighed approximately 222 grams (Fig 1). Laparotomy at G7 caused the animals to loose approximately 5 grams (Figs. 2,4,6) at G8. In contrast, animals without the laparotomy (Laparotomy Control, Fig. 3 and Vivarium Control, Fig. 5) gained approximately 5 grams between G7 and G8.

Animals: Food and Water Consumed G2-G7. During G2-G7, rats were allowed free choice of laboratory chow or food bars and water *ad libitum.* When both foods were made available, rats consumed 3-4 times more laboratory chow daily than food bars (compare Figs. 7- 12 with Figs 13-18). The total food consumed daily was approximately 22-23 grams. Laparotomy reduced total food consumption approximately 5-6 grams on the day of surgery (Figs. 8, 10, 12, 14, 16, 18).

Animals drank approximately 25-30 grams of water daily on days G2-G7 (Figs. 19-24). Laparotomy did not appear to alter water consumption on the day of surgery

(Figs. 20, 22, 24).

Animals: G7 Laparotomy. The number of decidual swellings per uterine horn in rats laparotomized at G7 and subsequently assigned to test groups is illustrated in Fig. 25. In all cases, rats assigned to the Nominal Flight, Hysterectomy Control and Delayed Recovery groups had at least 5 (usually 6) well defined decidual swellings in each horn.

Animals: G9 Launch Simulation. On G9, animals were taken by van from the animal holding facility to the NASA 20 G centrifuge facility. Rats assigned to the four test groups, *viz.* Nominal Flight, Laparotomy Control, Hysterectomy Control, and Delayed Recovery, were subjected to a shuttle launch simulation. This simulation included both acceleration and acoustic parameters and lasted approximately 8 minutes. The profile for the launch simulation was derived from previous shuttle launch data. Acceleration ranged from 1 to 3 Gs (Fig. 26) and acoustics had a maximum of 108 dB and a range of 31.5-2500 Hz (Fig. 27). During the simulation, animals were observed via a video monitor. During periods of increased G forces and increased noise levels, animals tended to crouch and not move. Otherwise, there were no discernable effects of the launch simulation on the rats.

Animals: Water Consumption by Four Experimental Groups Housed in AEMs. Water consumed by the four experimental groups housed in AEMs subsequent to shuttle launch simulation is shown in Fig. 28. Water consumption increased as pregnancy progressed. During mid pregnancy (G10), rats consumed approximately 30 mls of water daily. By G17, rats consumed 40-50 mls of water daily.

Animals: G18 or G20 Recovery Simulation. On G18, animals in Nominal Flight, Laparotomy Control and Hysterectomy Control groups were subjected to a recovery simulation. On G20, animals in the Delayed Recovery group were subjected to a recovery simulation. All animals were taken by van to the NASA 20 G centrifuge facility and exposed to a shuttle landing simulation which lasted approximately 16 minutes. This landing simulation utilized 1 to 2 Gs (Fig. 29) and the profile for the G forces was derived from previous shuttle landing data. The naturally occurring acoustics of the centrifuge environment was believed to be similar to shuttle landing acoustics. Therefore there was not a separate acoustics parameter for the landing simulation. During the simulation, animals were observed via a video monitor. Animals exhibited exploratory behavior during this simulation, except for periods when the G forces were increased (Fig. 29). During these times, the animals were less active.

Animals: G18 Unilateral Hysterectomy (Nominal Flight and Laparotomy Control Groups) or G20 Unilateral Hysterectomy (Delayed Recovery Group). The number of live fetuses in the left and right uterine horns at G18 and G20 unilateral hysterectomy is shown in Fig. 30. Only 9 of the 10 animals assigned to the Laparotomy Control group were pregnant. Also, there were fewer fetuses in the left horn of the Laparotomy Control group.

Nominal Flight and Delayed Recovery groups were both subjected to laparotomy at G7 and unilateral hysterectomy at G18 (Nominal Flight) or G20 (Delayed Recovery). Since the number of decidual swellings was recorded at G7, and the number of live fetuses recorded at unilateral hysterectomy, conceptus wastage during this interval of pregnancy could be ascertained. There was no significant difference in the total number (both horns) of decidual swellings at G7 and the total number of live fetuses in

the same animal at G18 or G20 (Fig. 31). Similarly, there was no difference when the data were expressed per horn (Fig. 32). These data indicate that laparotomy at G7 does not affect subsequent fetal development/viability at G18 or G20.

The data also show that there were less fetuses present at G18 in the Laparotomy Control group (Fig. 31).

Following unilateral hysterectomy, the removed uterine horn was dissected, the fetuses and placentas weighed, and the crown-rump length of the fetuses recorded. The crown-rump length and weight of fetuses in the Nominal Flight group were significantly ($p < 0.05$) less than the Laparotomy Control group (Figs. 33, 34). The placentas in the Nominal Flight Group also weighed less than placentas in the Laparotomy Control but this difference was not statistically significant ($p > 0.05$).

Between G18 and G20, fetuses grew rapidly and crown-rump length was approximately 50% greater at G20 (Fig. 33). Also, by G20, fetal weight was more than doubled over that recorded at G18 (Fig. 34). Lastly, weight of placentas was increased at G20 as compared to G18 (Fig. 35).

Animals: Body Weight G18-G21. Rats subjected to unilateral hysterectomy at G18 (Nominal Flight, Laparotomy Control) were approximately 10 grams lighter in body weight at G19. By G20, these rats were gaining weight again (Fig. 36). Unilateral Hysterectomy at G20 reduced body weight more than 30 grams at G21 (Fig. 36). In contrast, test groups without unilateral hysterectomy (Hysterectomy Control, Vivarium Control), gained weight at the rate of approximately 10 grams per day during the G18-G21 time period (Fig. 36).

Animals: Food and Water Consumed at G19 and G20. Food and water consumption of the four test groups was recorded at G19 and G20. Unilateral hysterectomy (Nominal Flight, Laparotomy Control groups) decreased food bar consumption during this period (Fig. 37). Unilateral hysterectomy at G18 also decreased water consumption at G19, but by G20, water consumption had increased (Fig. 38).

Animals: Initiation of Delivery. Parturition was initiated and completed in animals in all test groups between 06:00 hours G22 and 18:00 hours G23 (Fig. 39). In animals observed hourly during the light phase of the cycle, the duration of delivery ranged from 1-3 hours, with most animals completing delivery in less than 2 hours.

Foster dams (N = 48) initiated delivery on the morning of G22 and all animals had completed delivery by the afternoon of G23 (Fig. 39).

Most animals delivered during the light phase of the cycle on G22 or G23; however, 70% of the Delayed Recovery group (unilateral hysterectomy on G20) delivered during the dark period between 18:00 hours G22 and 06:00 hours G23 (Fig. 39).

The number of live fetuses remaining *in utero* at G18 or G20, after unilateral hysterectomy, was recorded in Nominal Flight, Laparotomy Control and Delayed Recovery groups. Thus the number of young to be born could be anticipated in animals in these groups. Figure 40 shows the number of live fetuses recorded at G18 (Nominal Flight, Laparotomy Control) or G20 (Delayed Recovery) and the number of live pups delivered in these same animals. There is a decrease in the number of live pups delivered in each of the three test groups (Fig. 40); however, this decrease is not

significant ($p > 0.05$).

Organ Weights and Plasma Hormone Levels of Dams Assigned to Test Groups. There was no significant difference in combined adrenal weights of dams in any of the test groups (Fig. 41). Thymus weights of dams in Nominal Flight, Laparotomy Control, Hysterectomy Control and Delayed Recovery groups were similar and in all four groups, significantly reduced ($p < 0.05$) relative to the Vivarium Control group (Fig. 42). There was no significant difference in plasma concentrations of progesterone, estradiol, corticosterone, and catecholamines (dopamine, norepinephrine, epinephrine) between dams in any of the test groups (Figs. 43-49).

Anogenital Distance of Male Pups from Test Dams on Day of Delivery. There was no significant difference in the anogenital distance in pups from dams in any of the test groups or from pups derived from foster dams (Fig. 50).

Body Weight of Pups Neonatal Days 0-7. Pups born to dams in test groups were placed with foster dams after the test dam was euthanized. Some of the foster dam's natural pups were retained in each litter of test pups assigned to a foster mother. All litter sizes were adjusted to 10. At the time of cross fostering (day 0), pups from experimental groups ranged in weight from 5.88 to 6.84 grams. Pups from the Delayed Recovery test dams weighed significantly less ($p < 0.05$) at neonatal day 0 than pups from the Nominal Flight test dams (5.88 grams vs 6.44 grams). Pups derived from foster dams and subsequently assigned to litters ranged in weight from 6.46 to 7.1 grams at neonatal day 0. Pups from test dams raised with natural pups from foster dams, in the same litter, grew at similar rates up to day 7 (Figs. 51-54). Pups derived from the Delayed Recovery group, who weighed less at neonatal day 0, had a different

weight gain curve from foster dam-derived pups raised in the same litters (Fig. 55).

Pup Organ Weights on Neonatal Day 7. There was no difference in the weight of the thymus (Fig. 56) or adrenal gland (Fig. 57) in pups derived from test or foster dams and euthanized at neonatal day 7.

Discussion and Conclusions

Animals: General. Taconic Farms outbred Sprague Dawley rats were shown to be an excellent strain of rat for these studies. There was a 95% pregnancy rate in animals shipped at G2 and 100 % pregnancy rate in animals shipped at G16, G17, or G18. In all cases, animals were received in excellent health and were generally docile and easy to handle during the various experimental procedures.

When the rats had free access to both laboratory chow and food bars, they consumed 3-4 times more laboratory chow than food bars. However, if the food bar was the only food source, it was readily consumed. Thus, there is probably no advantage to giving animals both the food bar and laboratory chow.

Animals: Surgery. The use of isoflurane (with a vaporizer and scavenger system) as an anesthetic for the surgical procedures was validated. Surgical anesthesia was quickly induced, easily maintained, and animals recovered rapidly, with no apparent side effects with this system.

The use of wound clips for closing ventral skin incisions in rats was corroborated. Rats readily accept the clips when applied properly and do not chew them. The technique for wound clip application is critical in order to prevent subsequent loosening

of clips. Two people are suggested: One person should carefully approximate the cut edges of the skin and the other person should apply the wound clips.

There was a minor incidence of granulomas at subcutaneous injection sites in the current study. It was believed that these granulomas resulted from partial injection of antibiotic intradermally, rather than subcutaneously. These granulomas can thus be prevented with careful technique.

Laparotomy at G7 transiently affects food and water consumption and thus weight gain, however there is no evidence that this procedure stresses the animals or alters subsequent fetal development. Eighty animals were laparotomized at G7 and 38 (48%) of these animals were found to have at least 5 decidual swellings in each uterine horn. Thus, the laparotomy procedure is necessary in order to qualitatively and quantitatively assess pregnancy and choose rats which meet the specified criteria for the proposed tests.

Unilateral hysterectomy at G18 or G20, like laparotomy at G7, transiently depresses food and water consumption and thus weight gain. This altered weight gain curve subsequent to this surgery can be attributed in part to the weight of the removed uterine horn and its contents. The mean number of live pups subsequently delivered by rats subjected to unilateral hysterectomy was not significantly less than the number of fetuses retained in the remaining horn at unilateral hysterectomy. Thus the unilateral hysterectomy procedure at G18 or G20 appears compatible with the proposed experimental design for the shuttle flight.

Animals: Initiation of Delivery. This ground based study confirmed that laparotomy at G7 and unilateral hysterectomy at G18 or G20 did not alter

characteristics of parturition. All animals delivered vaginally between 06:00 hours G22 and 18:00 hours G23. The study also showed that the shuttle launch and recovery simulations did not alter vaginal delivery.

Fetuses dissected from uterine horns following unilateral hysterectomy in the Nominal Flight group at G18 weighed less and had a shorter crown rump length than those fetuses removed from Laparotomy Control rats at G18. This finding differs from results observed in feasibility test 1 and the reasons for this discrepancy are not apparent. Perhaps the combination of laparotomy followed by shuttle launch and landing stresses may depress fetal growth, transiently. However, by the time fetuses are delivered from the remaining horn of the Nominal Flight animals at birth, there is no difference in fetal size in this group.

The thymus weight of dams in four test groups was decreased relative to the Vivarium Control group. This observation is an enigma. Adrenal gland weights of the five groups of dams were not significantly different and neither were any of the hormone concentrations believed to be correlated with stress. In the classical stress syndrome (Selye, 1936, Brit. J. Exptl. Pathol. 17: 234), the adrenal glands hypertrophy and the thymus involutes. Additional evidence for the absence of a generalized stress syndrome in the current study is provided by the data on male pup anogenital distances. This distance is decreased in male pups stressed *in utero* (Ward, 1972, Science 175: 82) but in the current study anogenital distances from male pups from all experimental groups were similar.

The weight of pups born to animals in the Delayed Recovery Group was slightly, but significantly, less than the weight of pups born to the Nominal Flight group. Fetal growth is occurring rapidly at this stage of pregnancy (G20) and the data suggest that

unilateral hysterectomy at this time may slightly reduce birth weight. Pups delivered by the Delayed Recovery group grew at a steady rate, but weighed less than littermates through neonatal day 7.

General Conclusion. Collectively, the data reported in these studies confirmed the feasibility, under conditions of simulated launch and recovery, and gravity, of the proposed experimental design for PARE .04.

Acknowledgements

Funded by NASA Cooperative Agreement NCC 2-810 to Hubert W. Burden. The cooperative assistance of Nichola K. Hawes in all phases of the design and performance of the study is gratefully acknowledged. The surgical expertise of Gary T. Price, the organizational skills of Amba Jonnalagadda, and the computer knowledge of Valerie Lynn ensured success of the study. This study was conducted at NASA, Ames Research Center, Moffett Field, CA and the contributions of the following personnel at this facility are acknowledged: Dana Leonard, Vera Vizir, Sunil Bhavsar, Sam Black, Mel Mack, Tom Fast, Samantha Edmonds, Laurie Dubrovin, Annette Ludtke, Celia Angell, Kevin O'Mara, Jack Vandendriesche, Joellen Jarvi, and Dr. Fred Rock.

FIG. 1. BODY WEIGHT (Mean +/- SEM) OF ALL ANIMALS (n=100) AT G2-G7

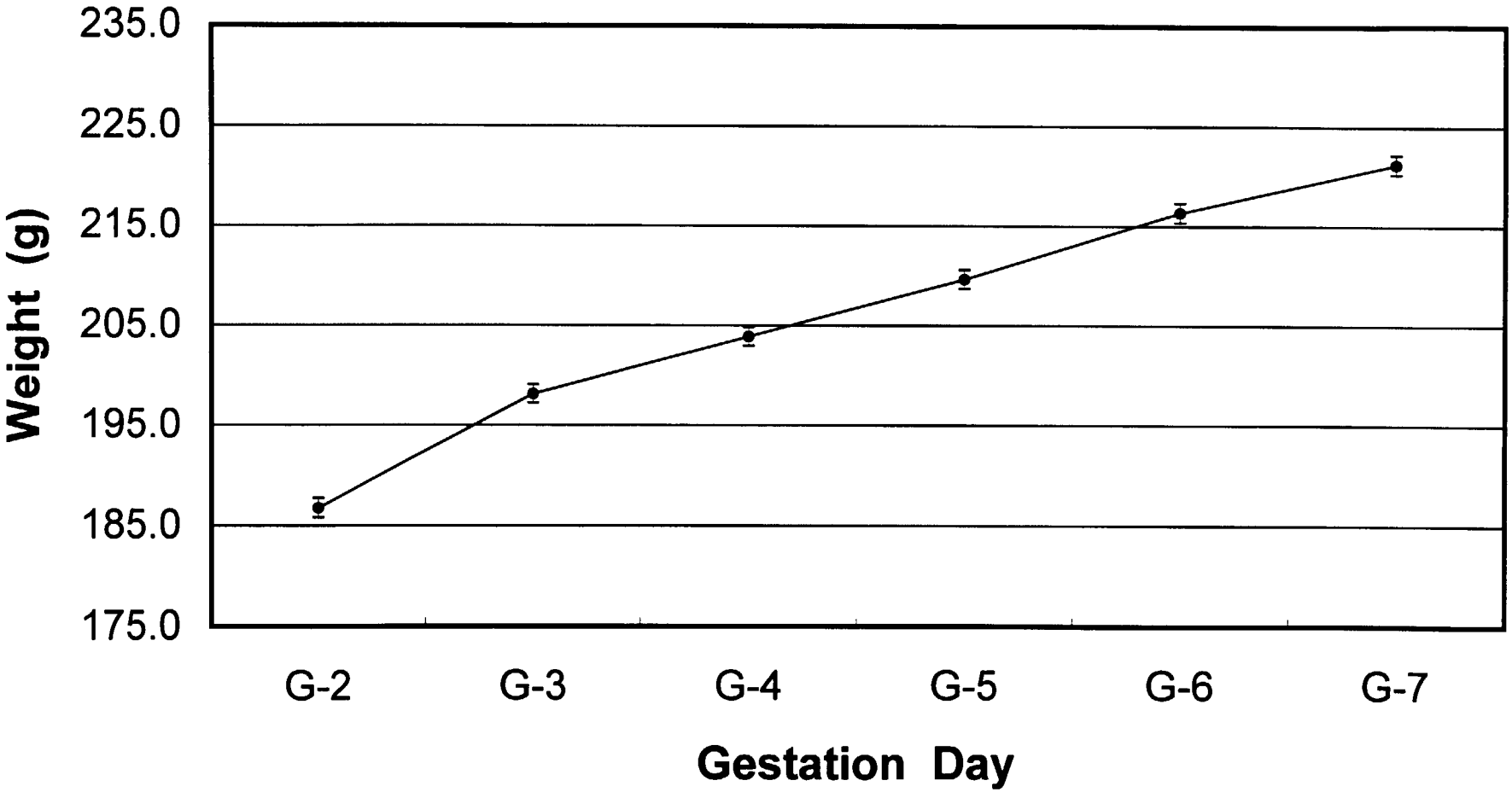

FIG. 2. BODY WEIGHT (Mean +/- SEM) OF NOMINAL FLIGHT GROUP (n=10) AT G2-G8

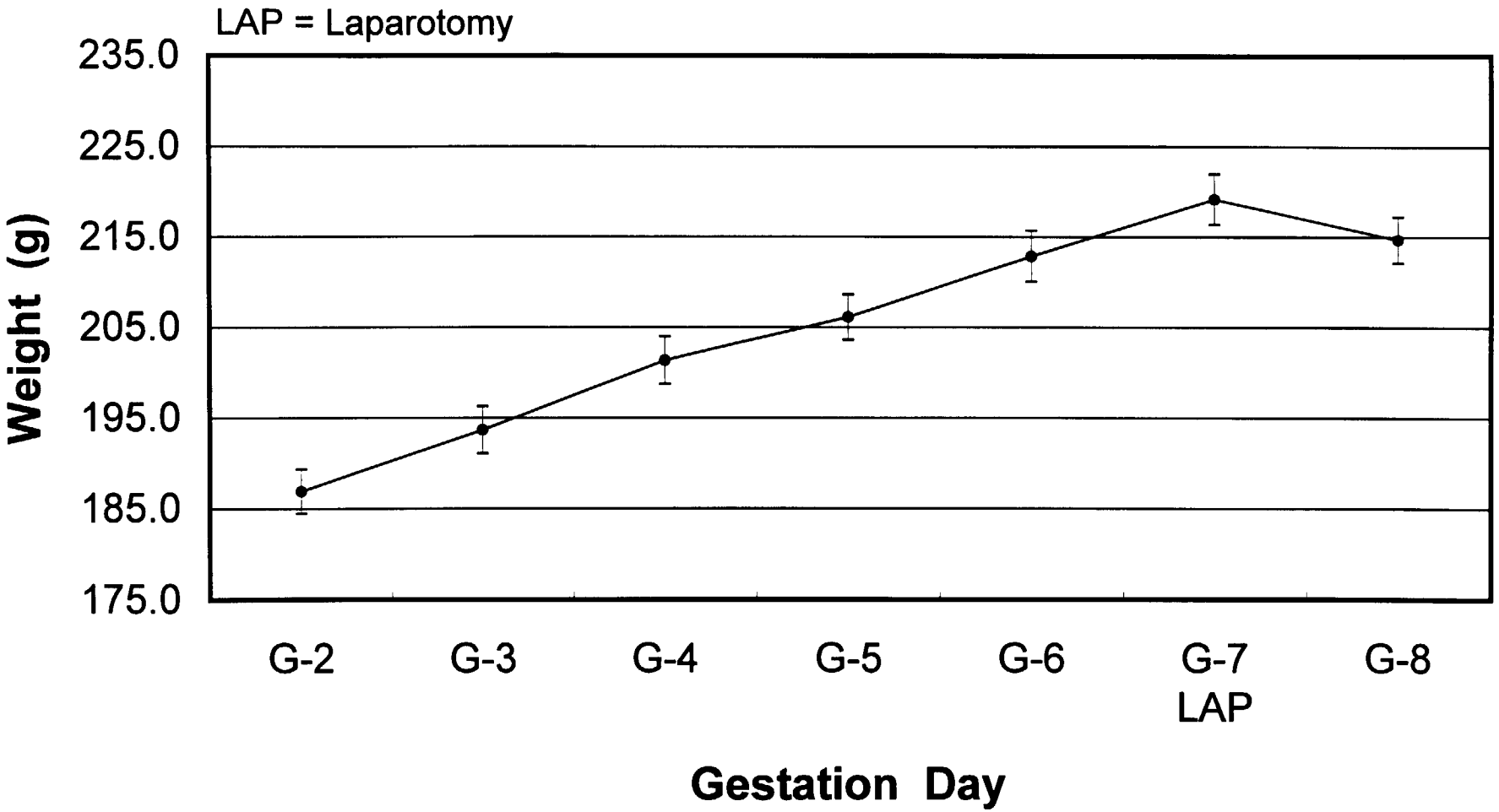

FIG. 3. BODY WEIGHT (Mean +/- SEM) OF LAPAROTOMY CONTROL GROUP (n=10) AT G2-G8

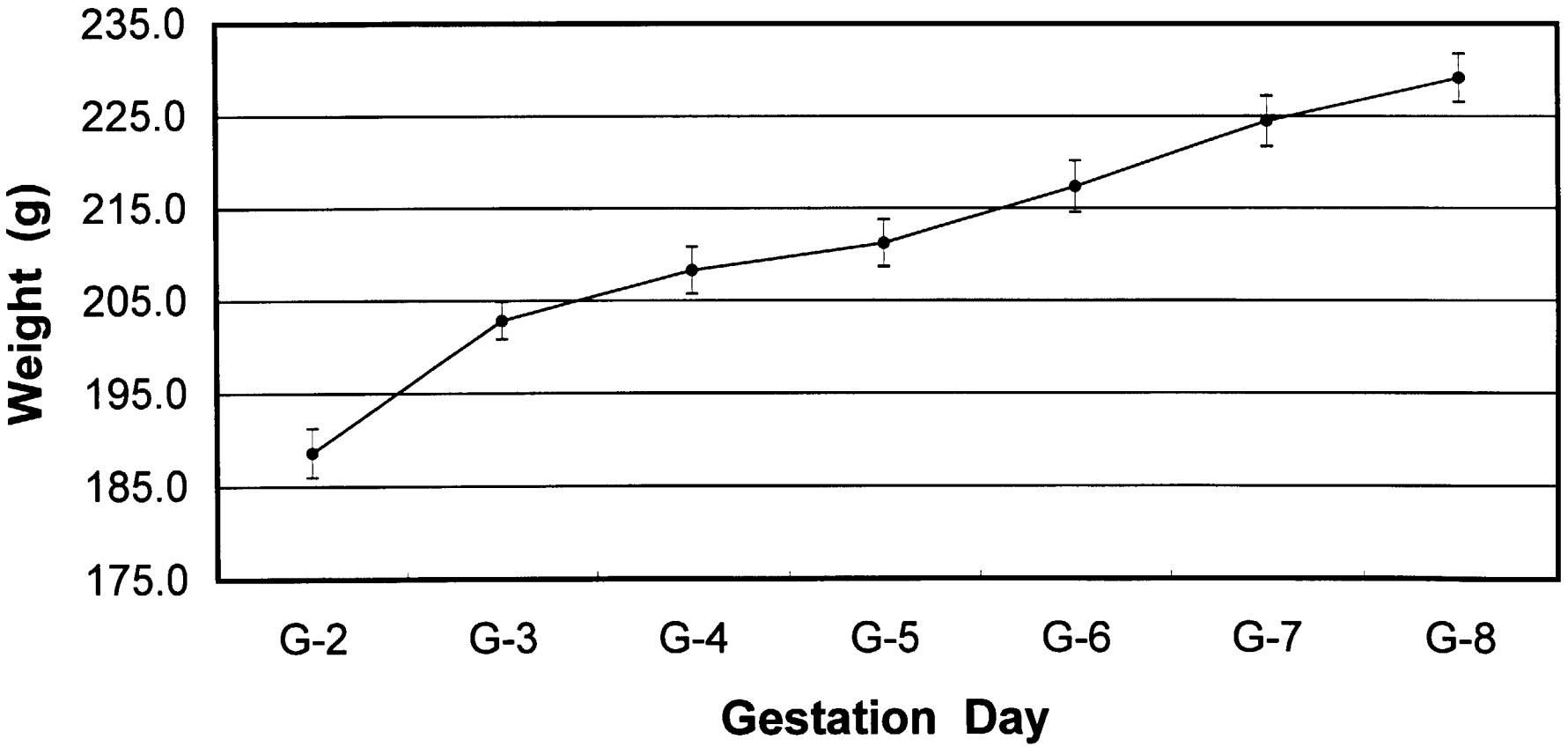

FIG. 4. BODY WEIGHT (Mean +/- SEM) OF HYSTERECTOMY CONTROL GROUP (n=10) AT G2-G8

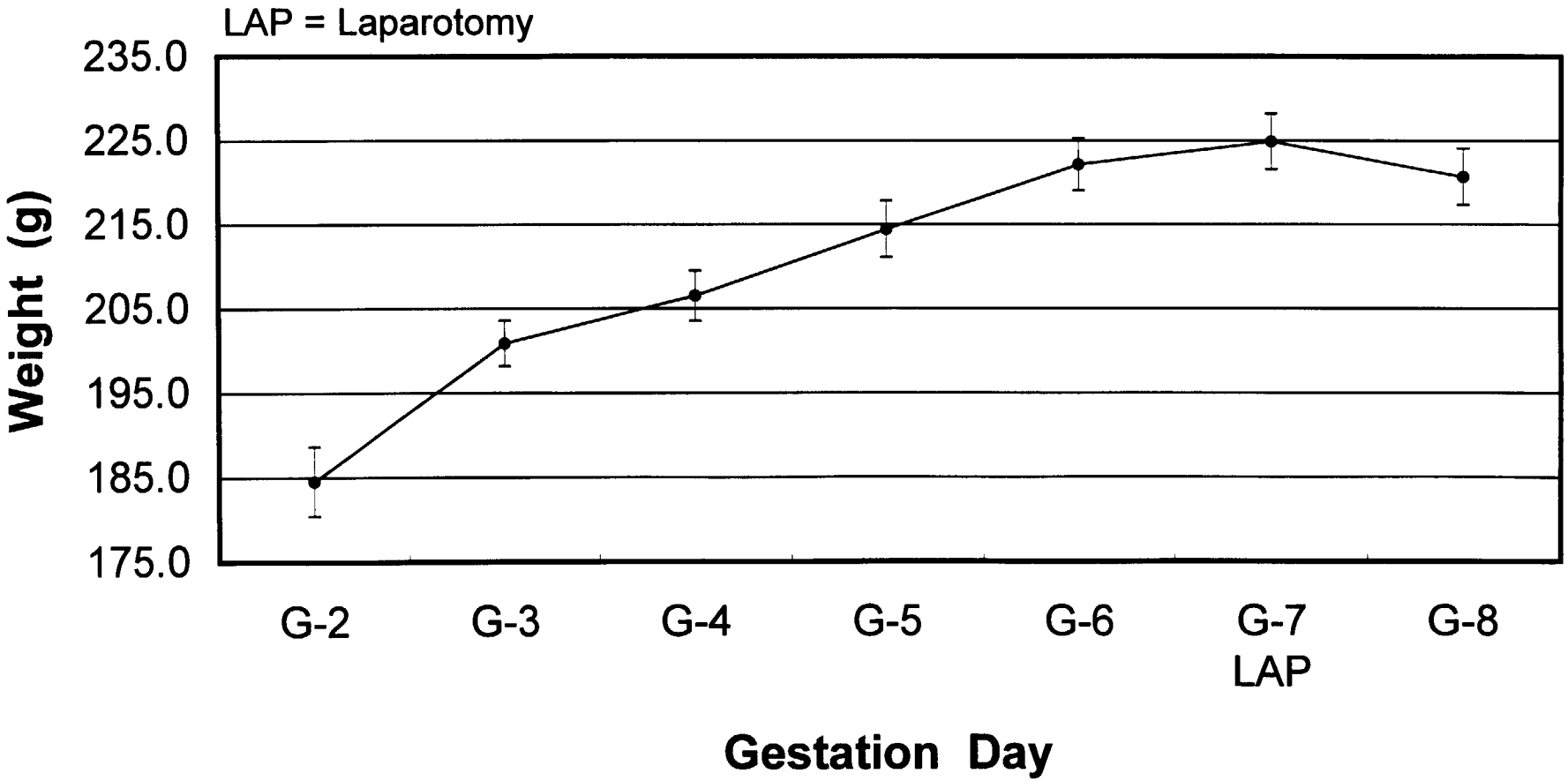

FIG. 5. BODY WEIGHT (Mean +/- SEM) OF VIVARIUM CONTROL GROUP (n=10) AT G2-G8

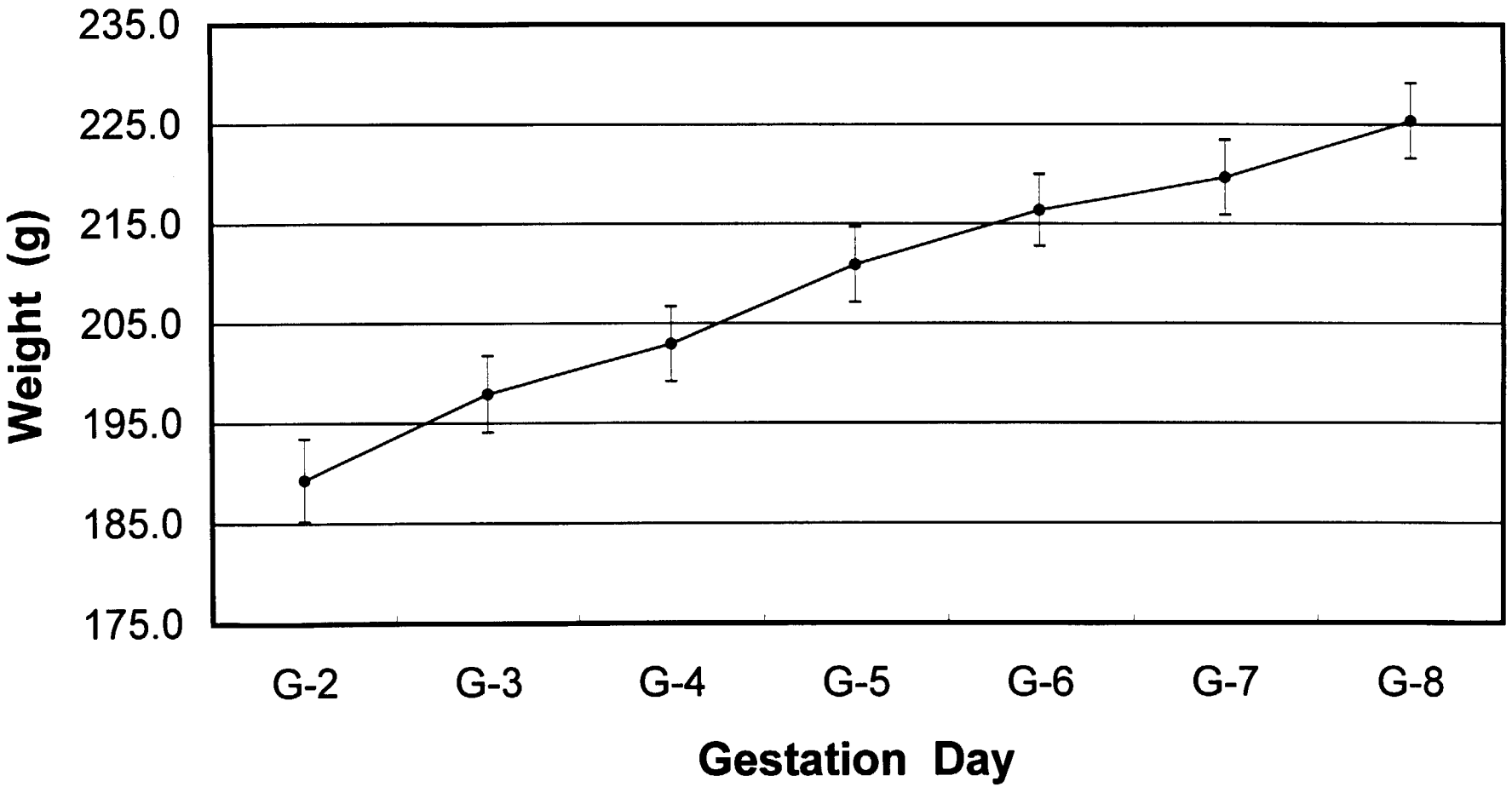

FIG. 6. BODY WEIGHT (Mean +/- SEM) OF DELAYED RECOVERY GROUP (n=10) AT G2-G8

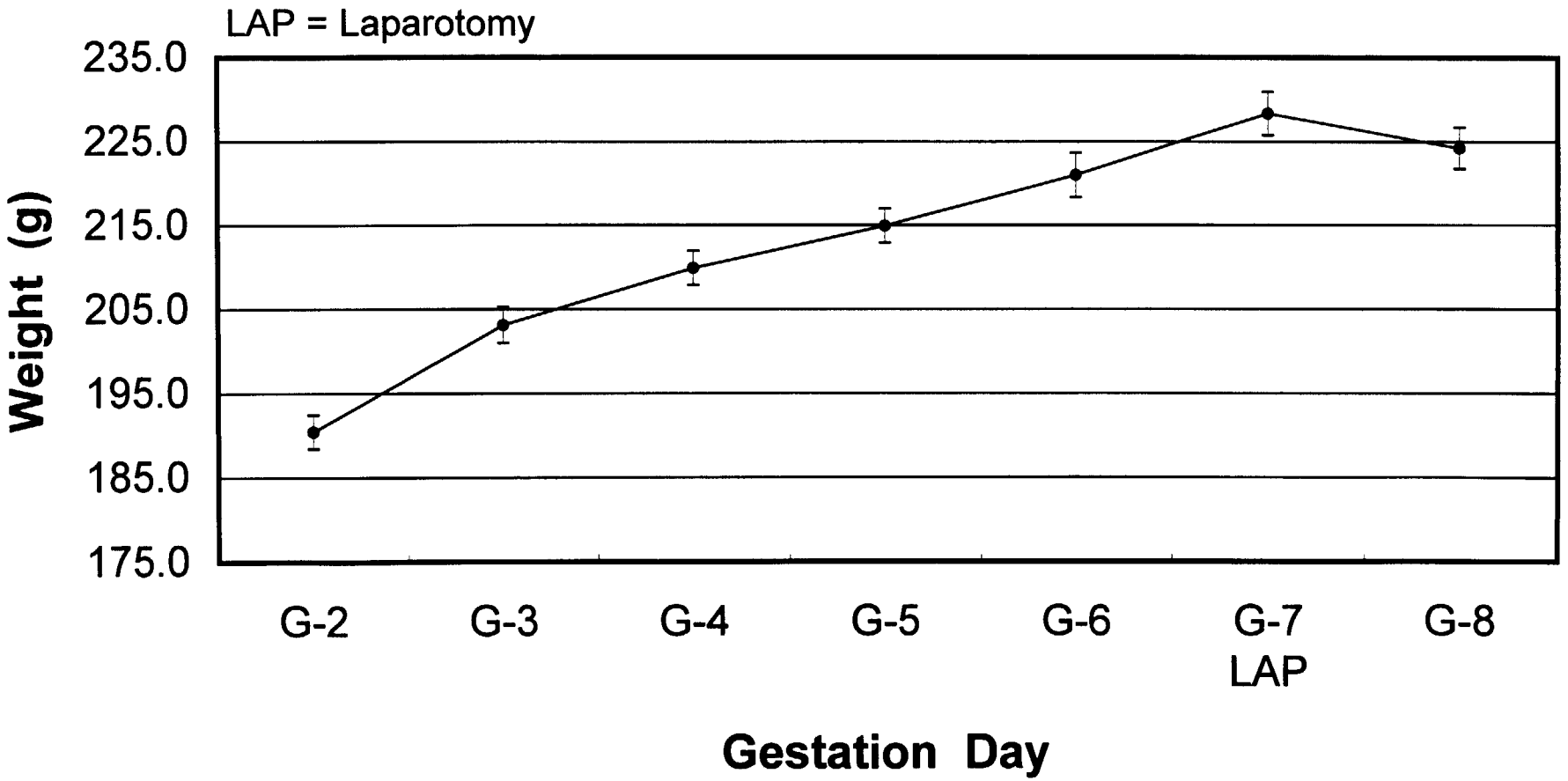

FIG. 7. LAB CHOW CONSUMED (Mean +/- SEM) BY ALL ANIMALS (n=100) AT G2-G7

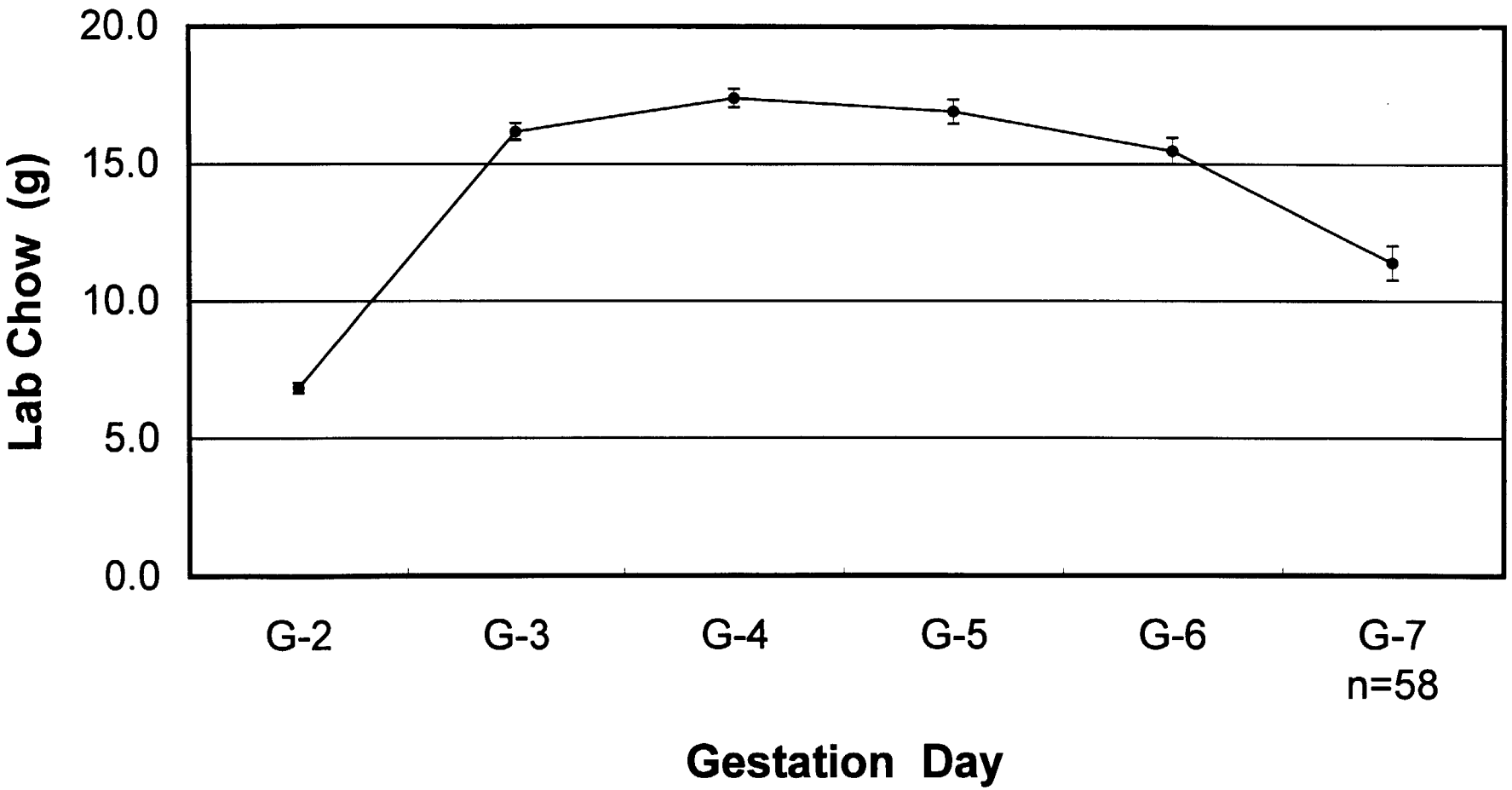

FIG. 8. LAB CHOW CONSUMED (Mean +/- SEM) BY NOMINAL FLIGHT GROUP (n=10) AT G2-G7

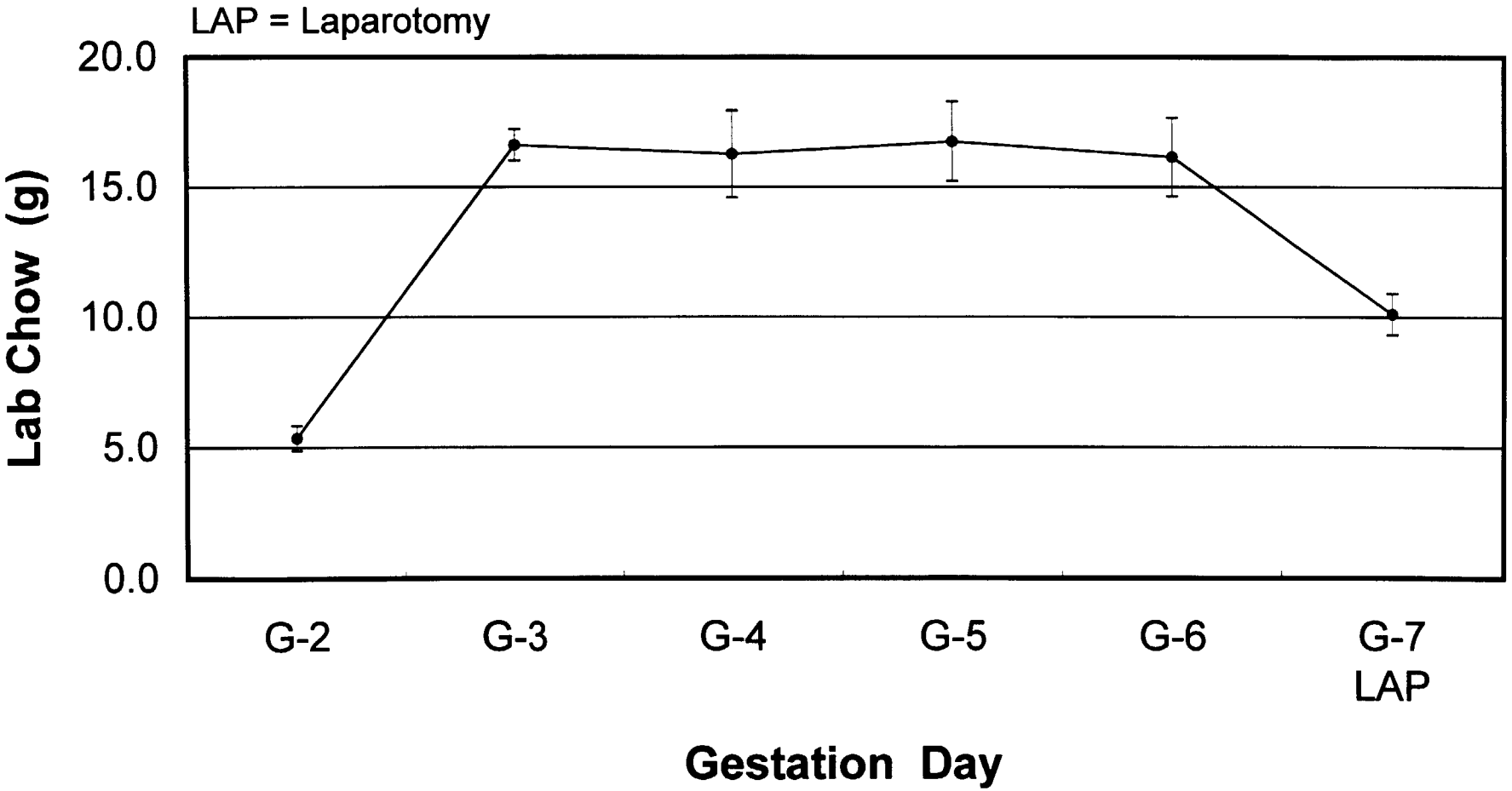

FIG. 9. LAB CHOW CONSUMED (Mean +/- SEM) BY LAPAROTOMY CONTROL GROUP (n=10) AT G2-G7

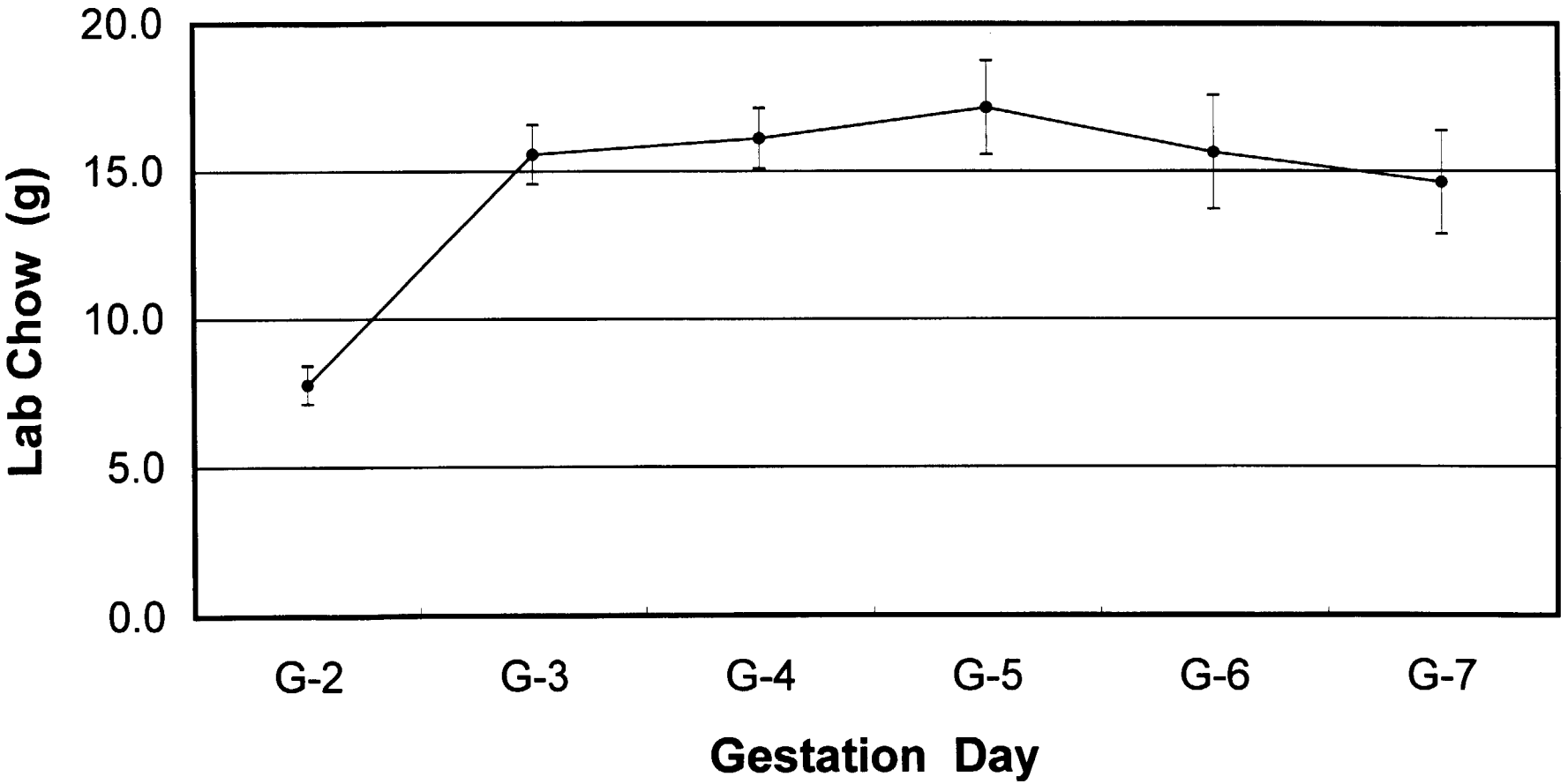

FIG. 10. LAB CHOW CONSUMED (Mean +/- SEM) BY HYSTERECTOMY CONTROL GROUP (n=10) AT G2-G7

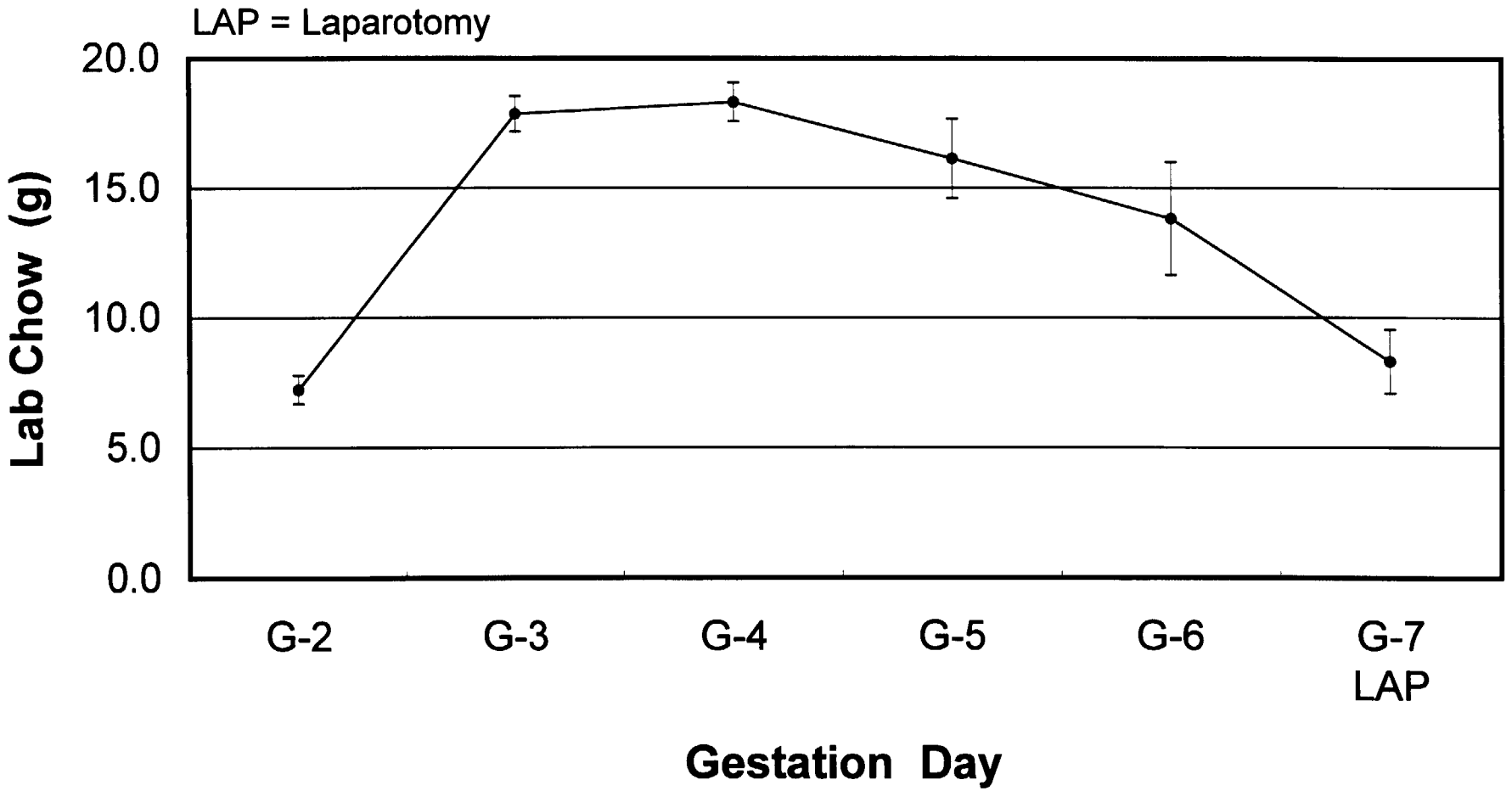

FIG. 11. LAB CHOW CONSUMED (Mean +/- SEM) BY VIVARIUM CONTROL GROUP (n=10) AT G2-G7

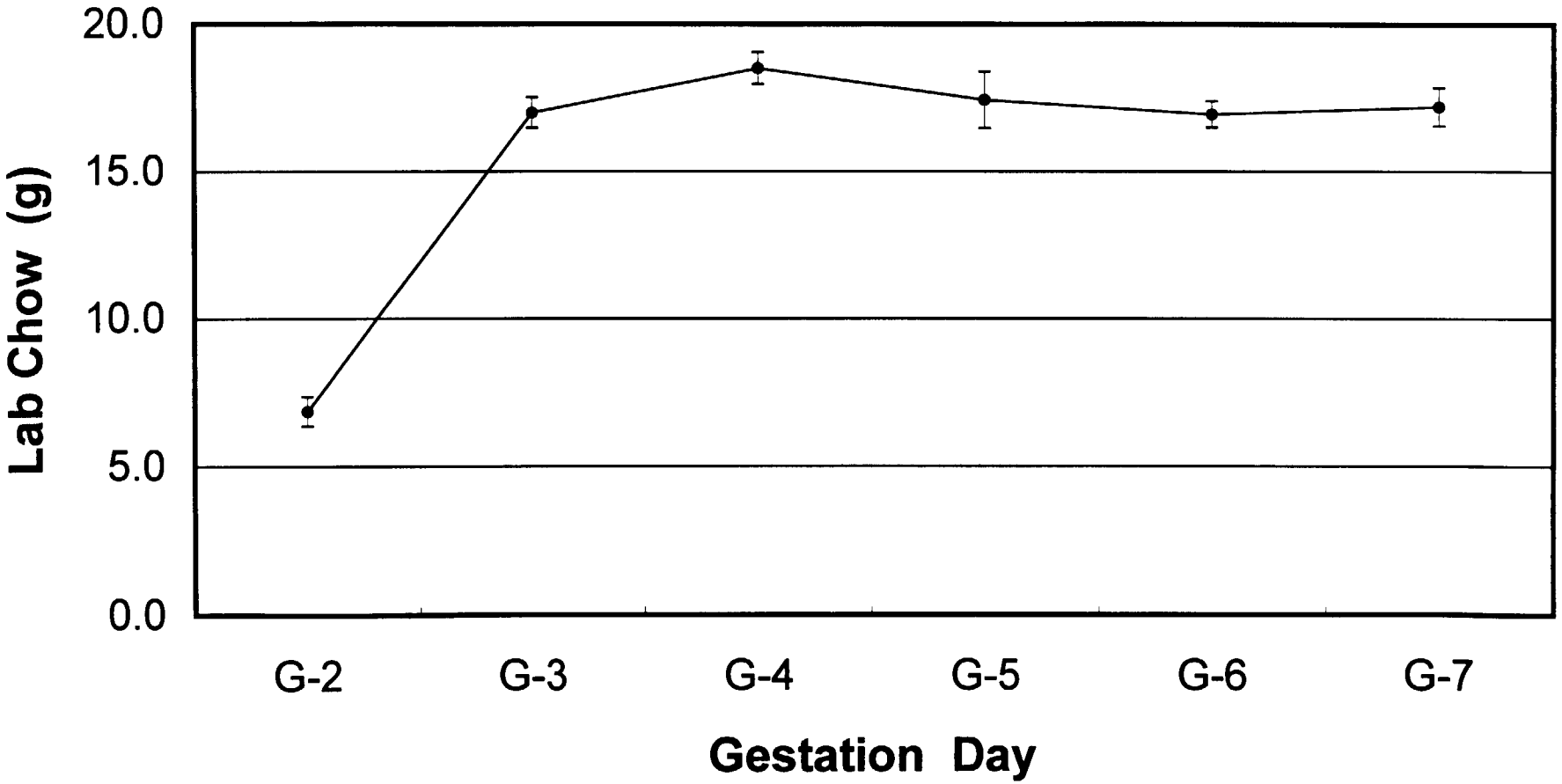

FIG. 12. LAB CHOW CONSUMED (Mean +/- SEM) BY DELAYED RECOVERY GROUP (n=10) AT G2-G7

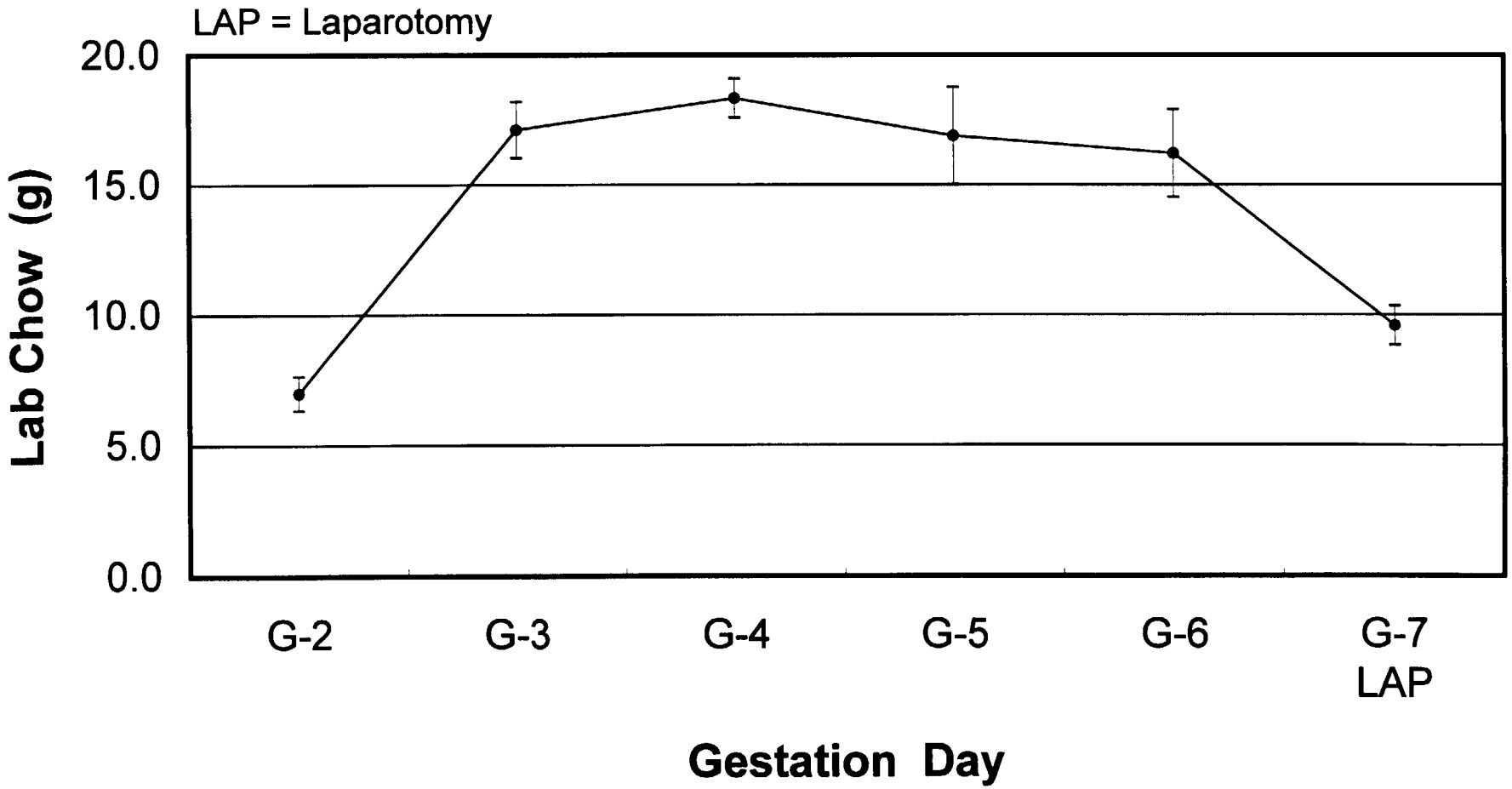

FIG. 13. FOOD BAR CONSUMPTION (Mean +/- SEM) OF ALL ANIMALS (n=100) AT G2-G7

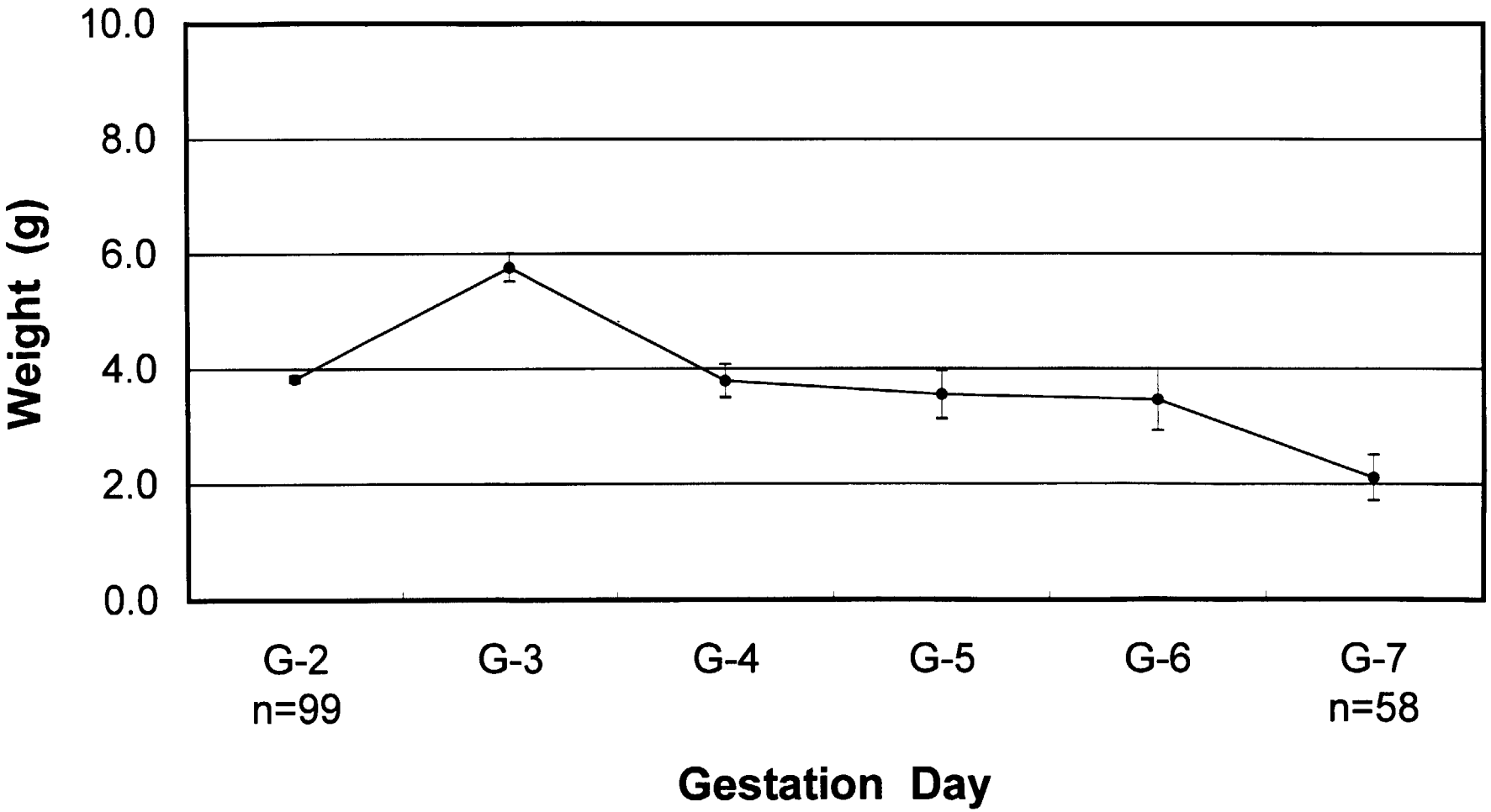

FIG. 14. FOOD BAR CONSUMPTION (Mean +/- SEM) OF NOMINAL FLIGHT GROUP (n=10) AT G2-G7

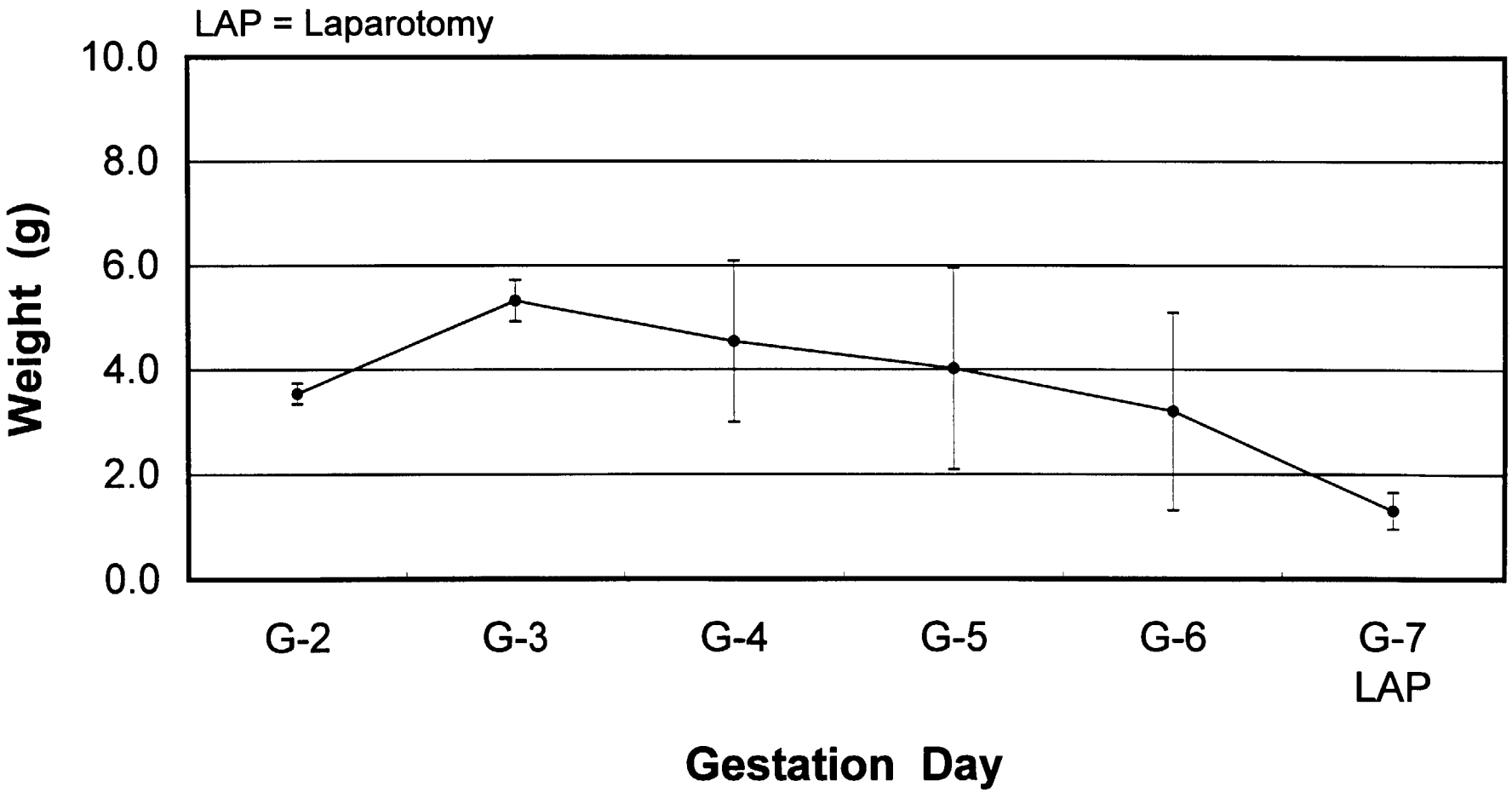

FIG. 15. FOOD BAR CONSUMPTION (Mean +/- SEM) OF LAPAROTOMY CONTROL GROUP (n=10) AT G2-G7

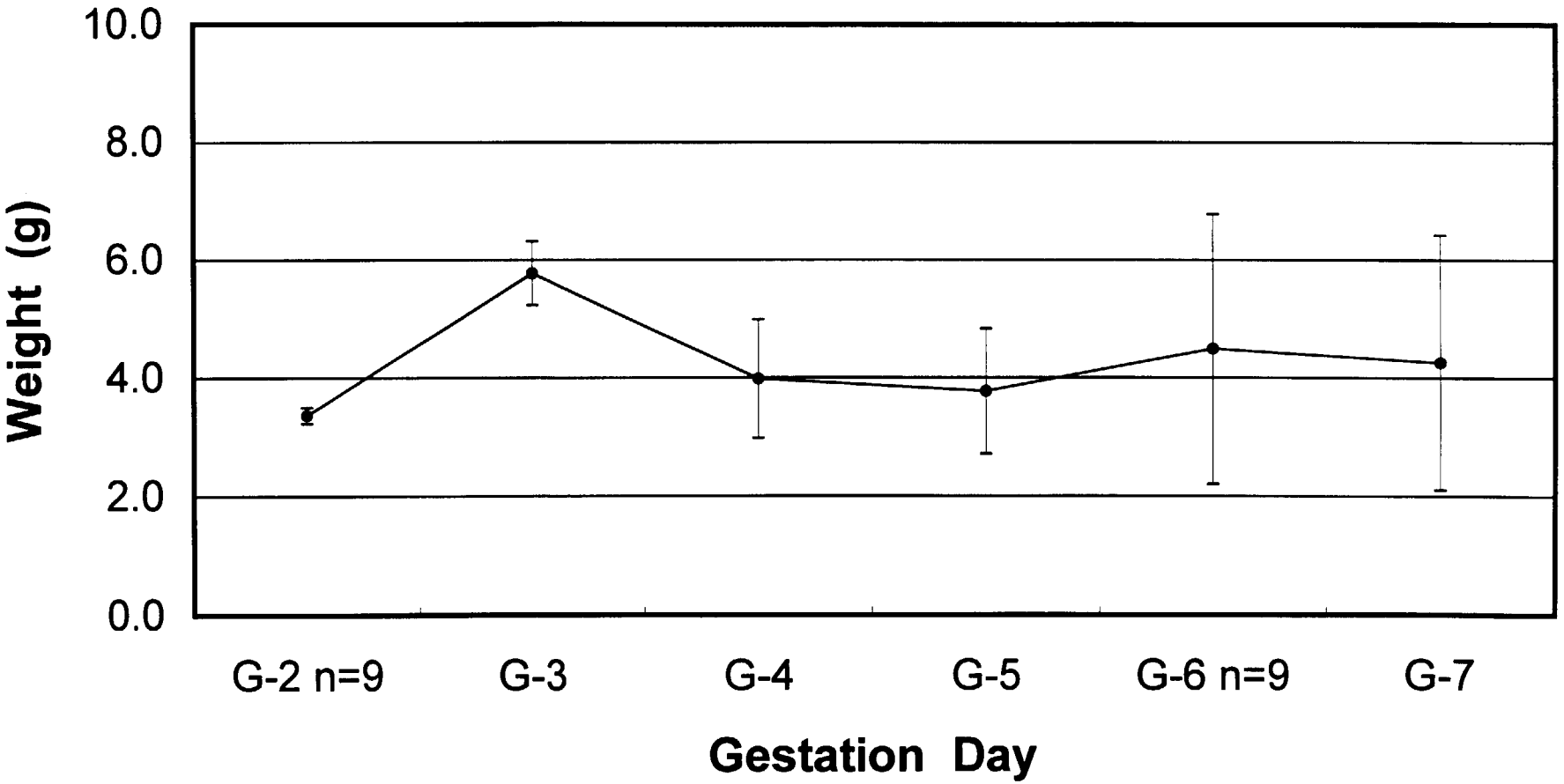

FIG. 16. FOOD BAR CONSUMPTION (Mean +/- SEM) OF HYSTERECTOMY CONTROL GROUP (n=10) AT G2-G7

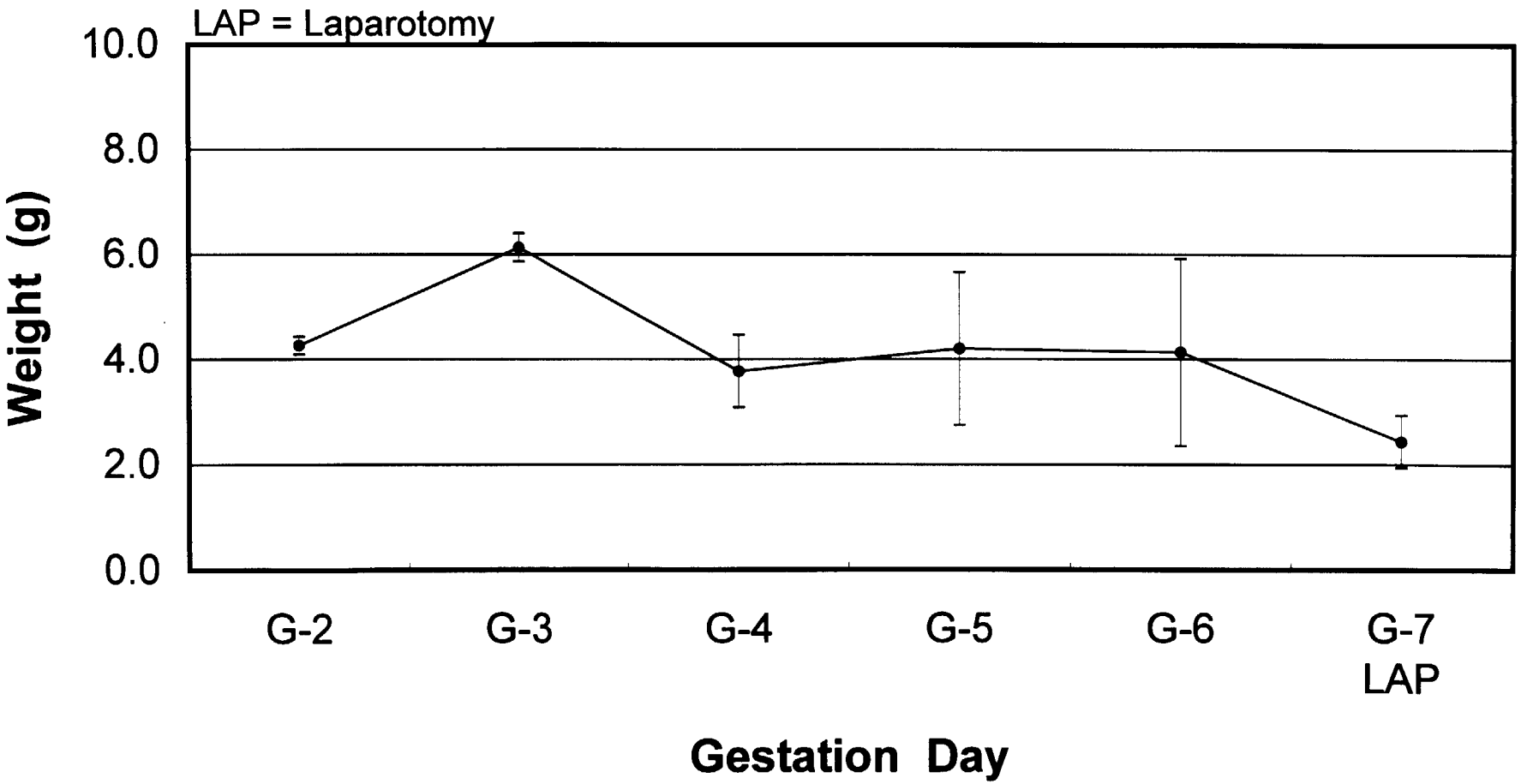

FIG. 17. FOOD BAR CONSUMPTION (Mean +/- SEM) OF VIVARIUM CONTROL GROUP (n=10) AT G2-G7

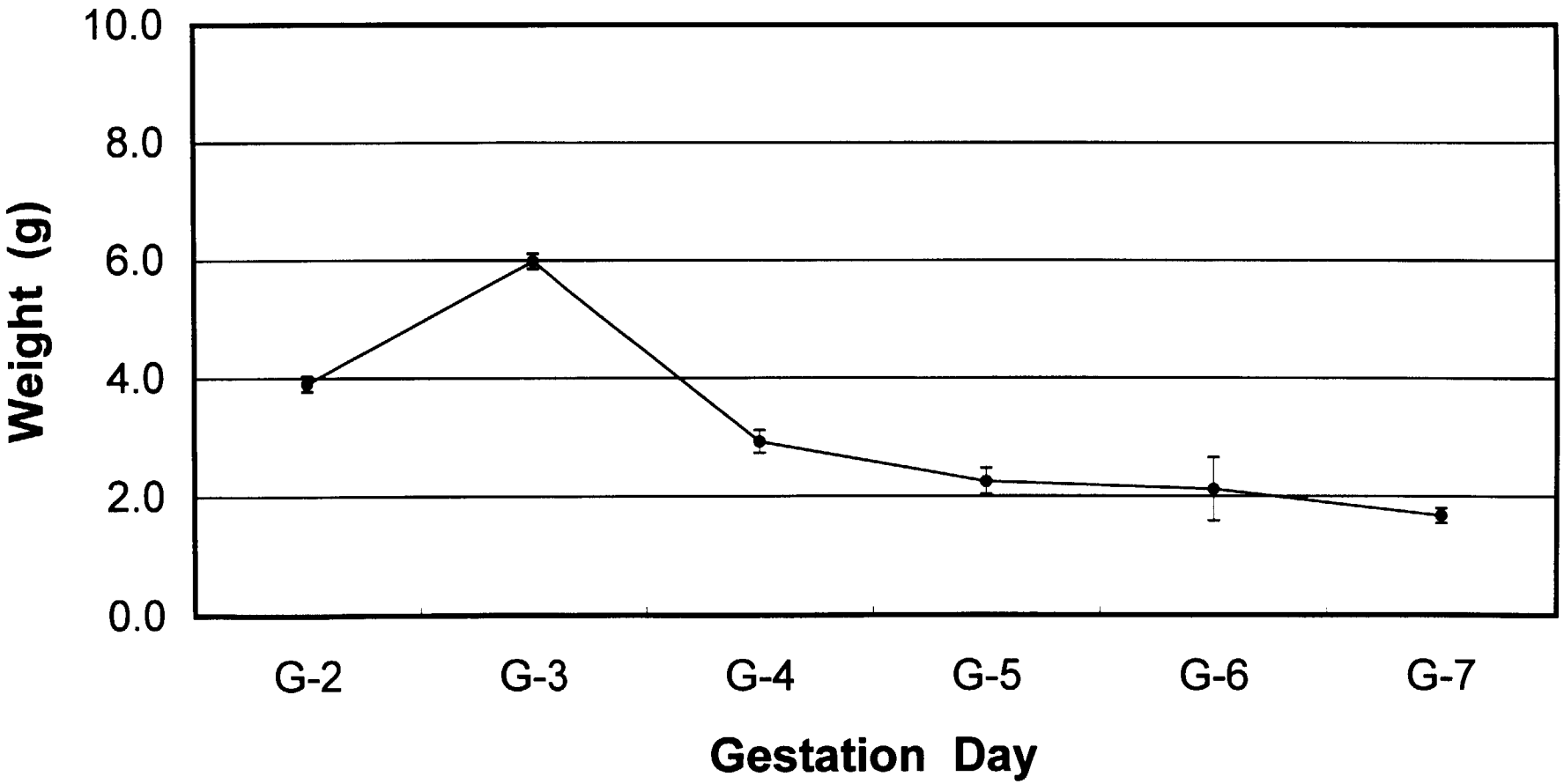

FIG. 18. FOOD BAR CONSUMPTION (Mean +/- SEM) OF DELAYED RECOVERY GROUP (n=10) AT G2-G7

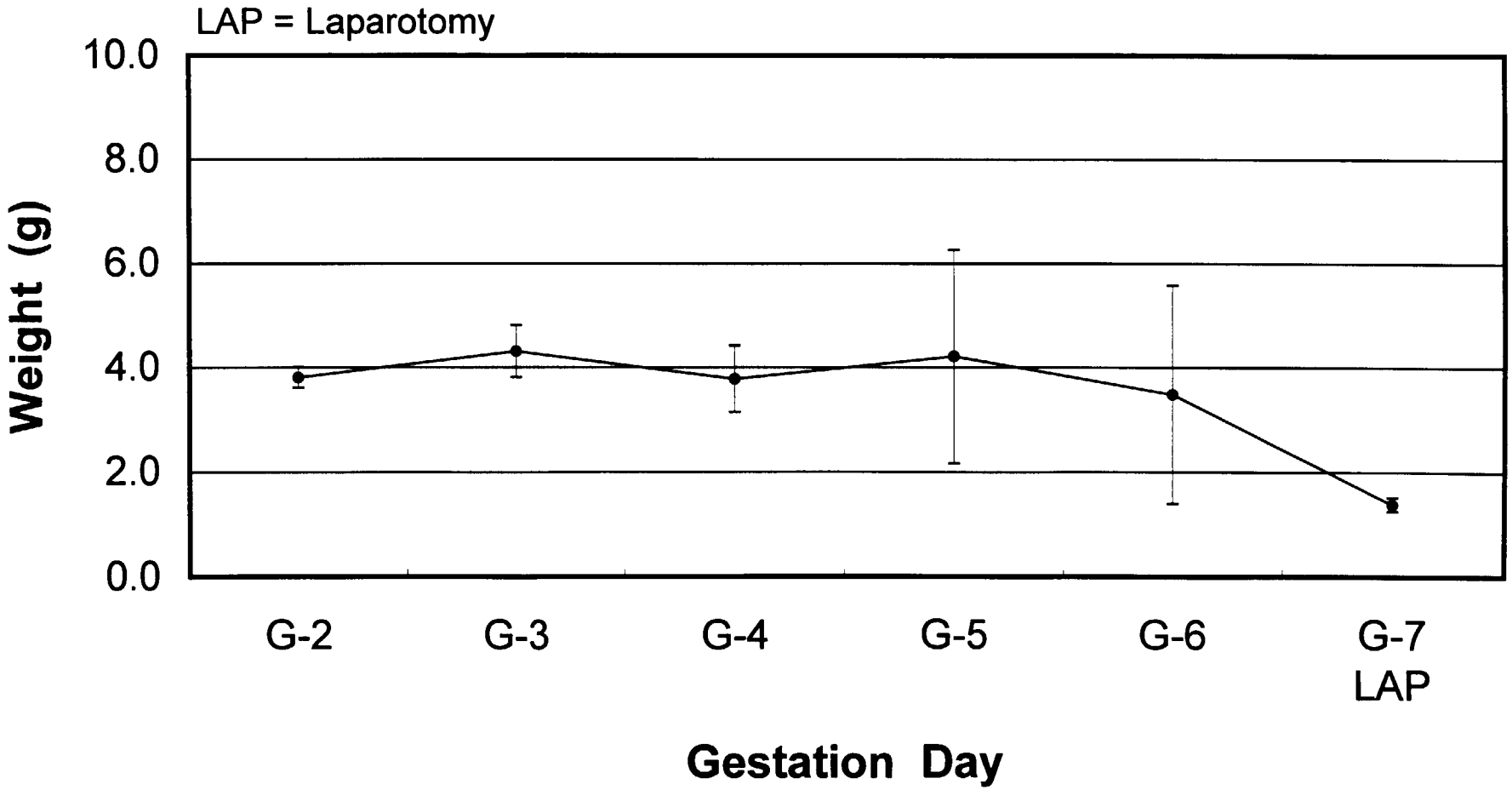

FIG. 19. WATER CONSUMED (Mean +/- SEM) BY ALL ANIMALS (n=100) AT G2-G7

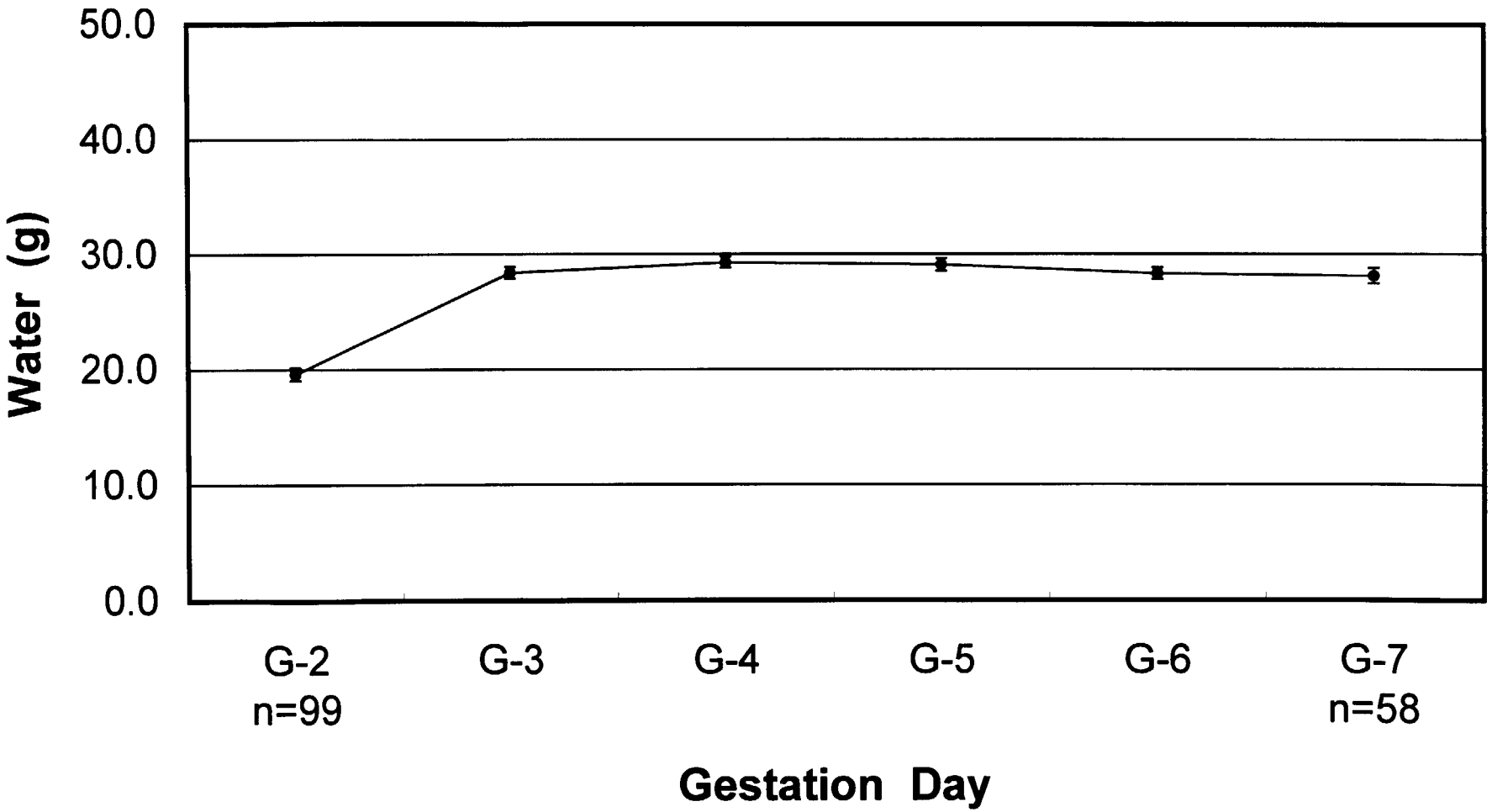

FIG. 20. WATER CONSUMED (Mean +/- SEM) BY NOMINAL FLIGHT GROUP (n=10) AT G2-G7

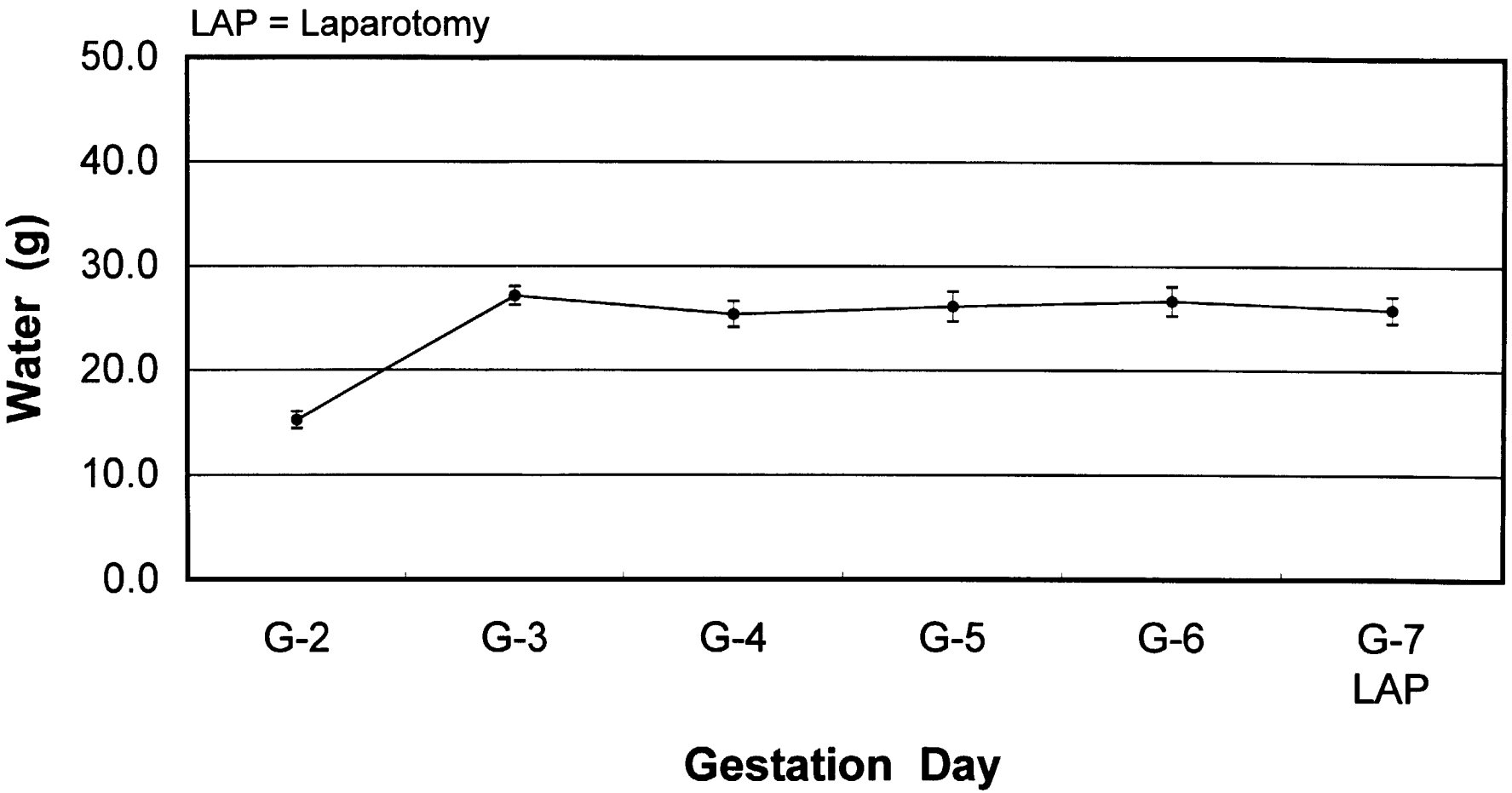

FIG. 21. WATER CONSUMED (Mean +/- SEM) BY LAPAROTOMY CONTROL GROUP (n=10) AT G2-G7

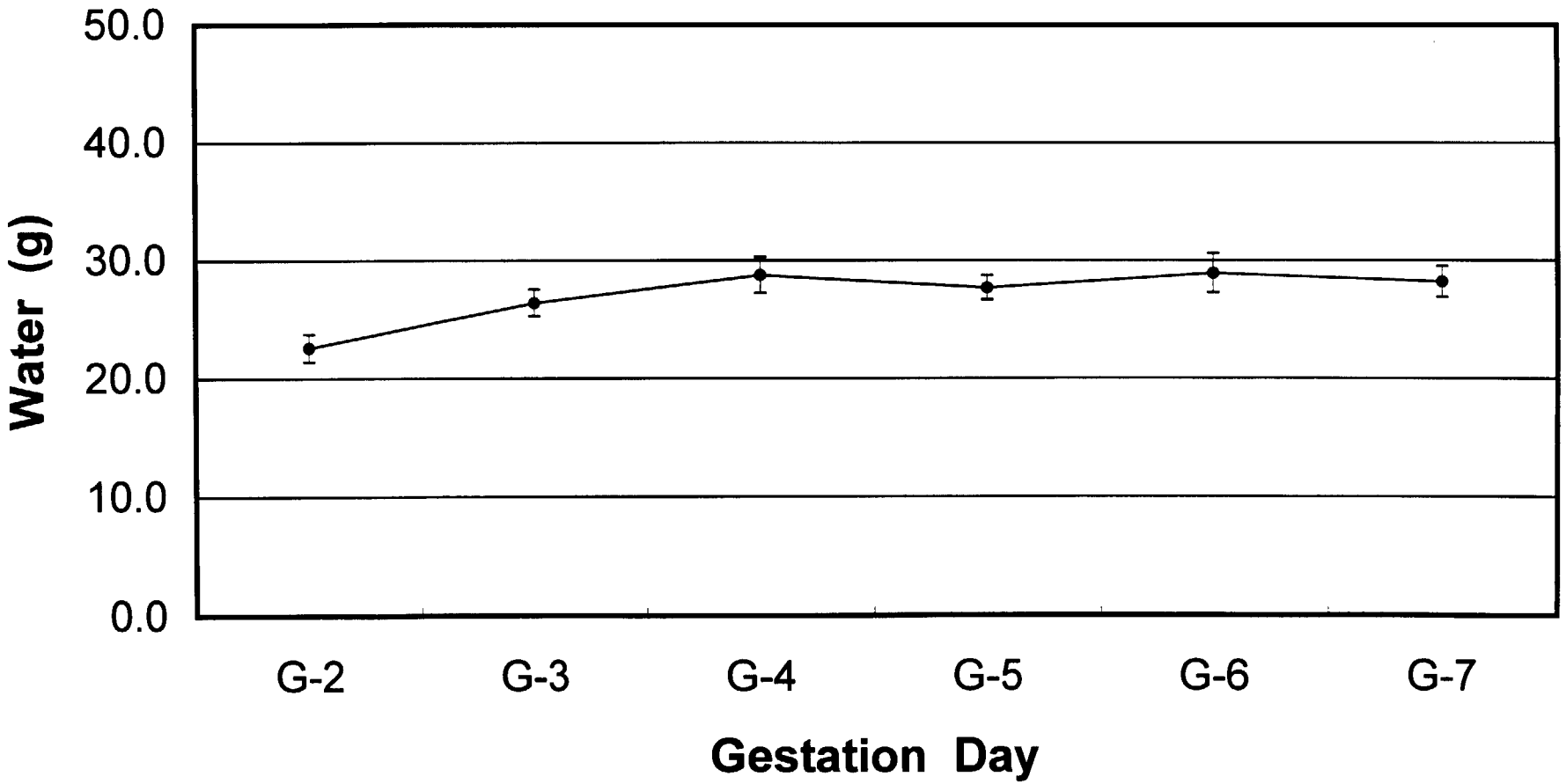

FIG. 22. WATER CONSUMED (Mean +/- SEM) BY HYSTERECTOMY CONTROL GROUP (n=10) AT G2-G7

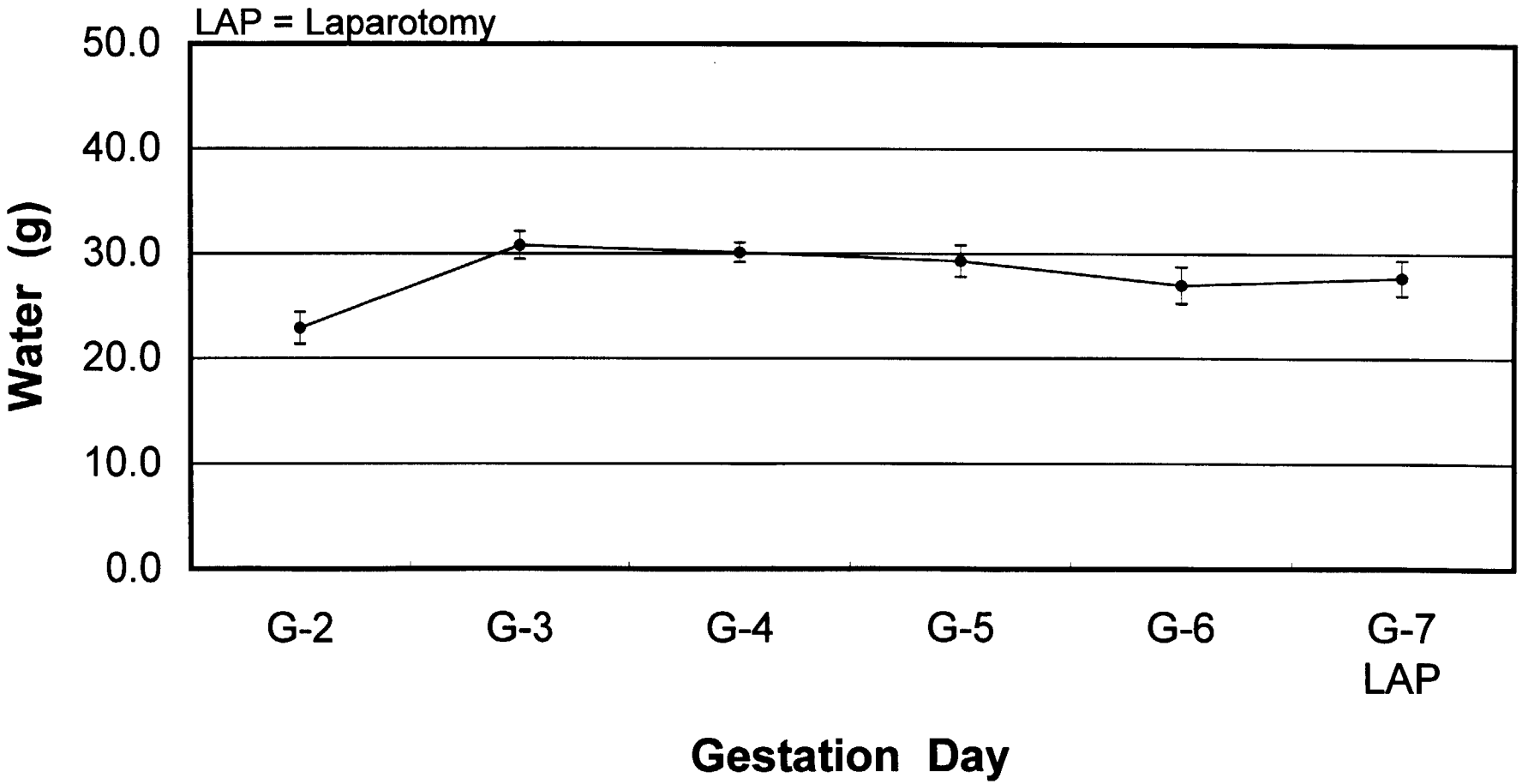

FIG. 23. WATER CONSUMED (Mean +/- SEM) BY VIVARIUM CONTROL GROUP (n=10) AT G2-G7

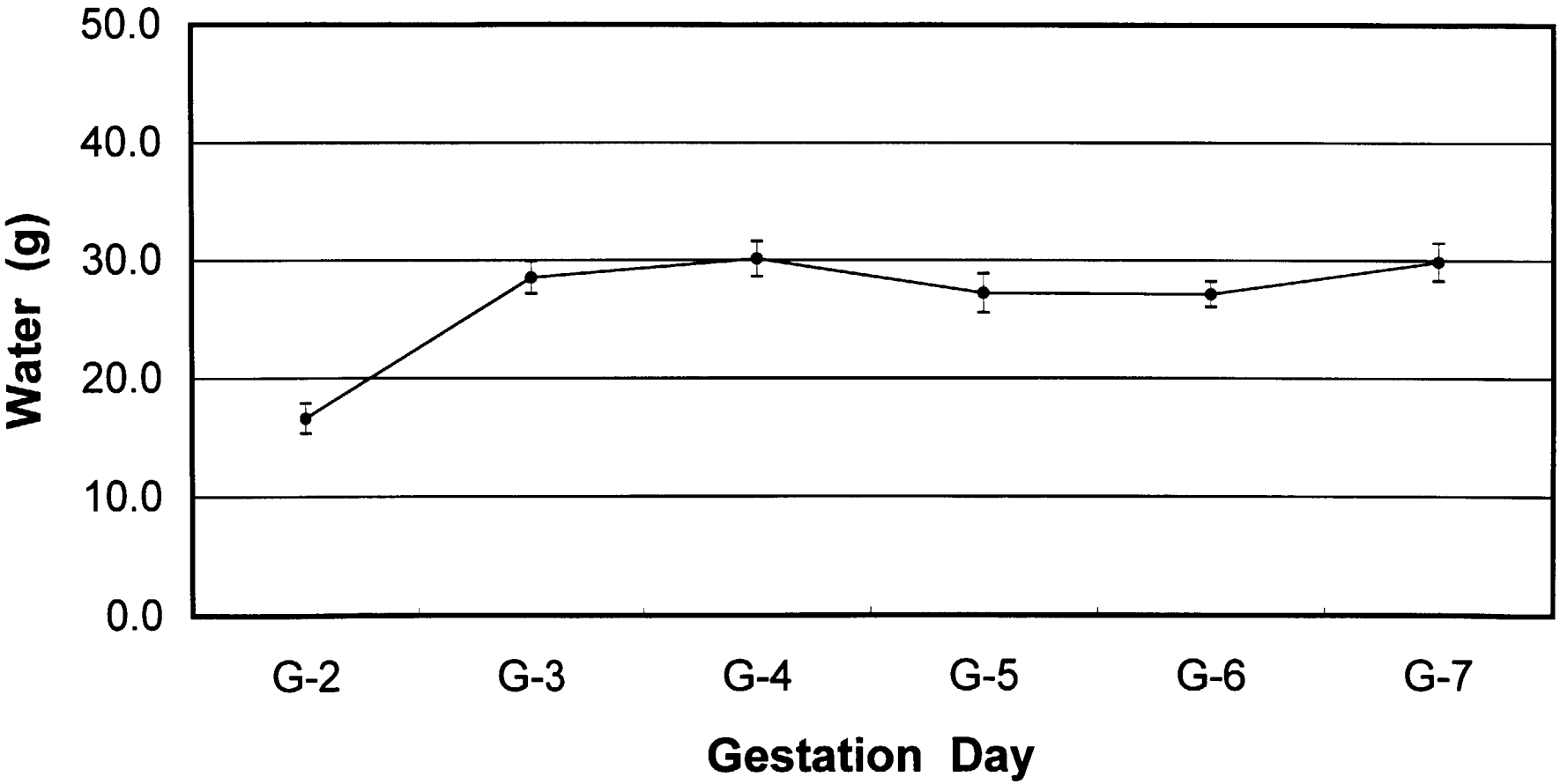

FIG. 24. WATER CONSUMED (Mean +/- SEM) BY DELAYED RECOVERY GROUP (n=10) AT G2-G7

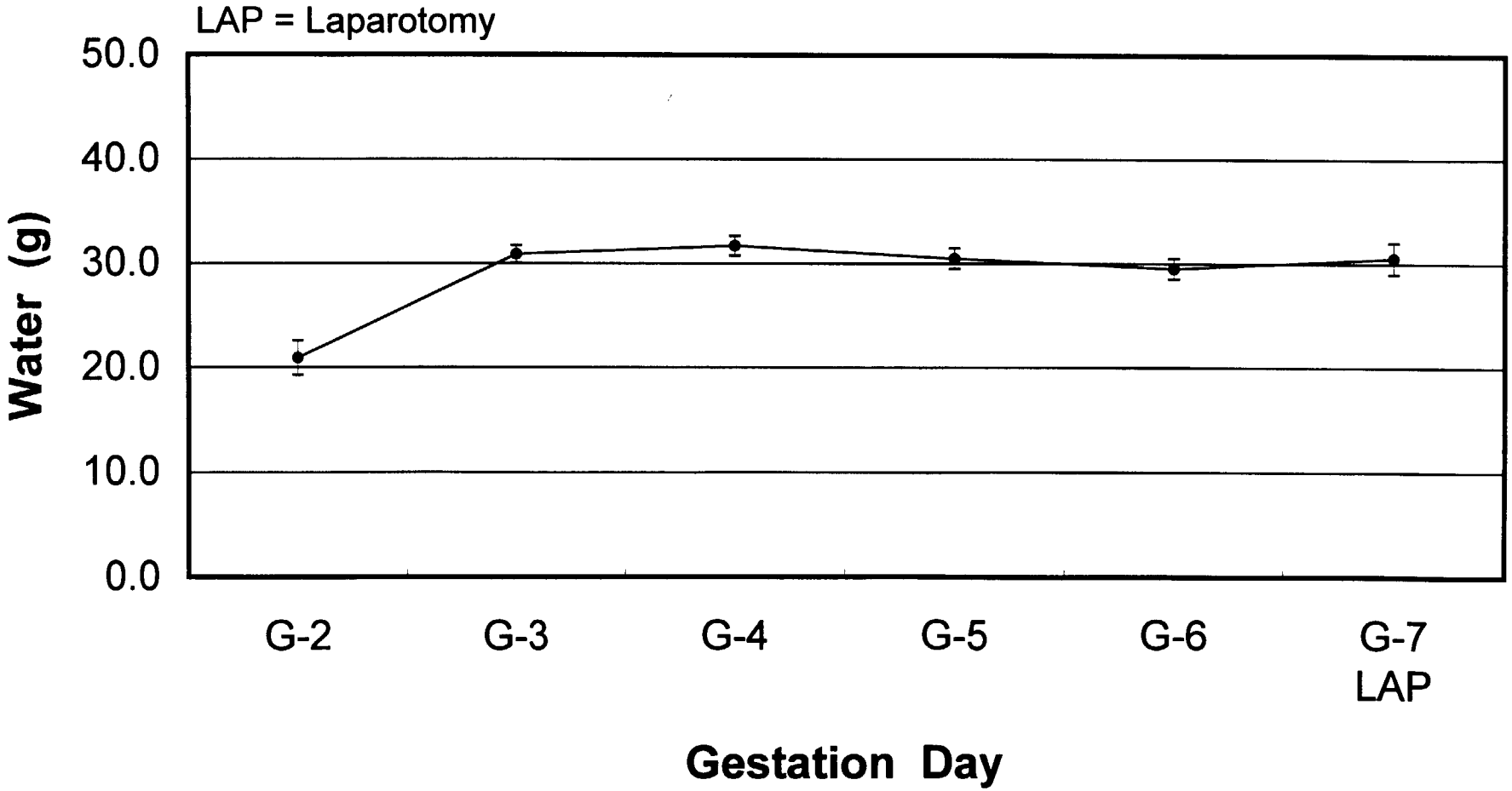

FIG. 25. DECIDUAL SWELLINGS (Mean +/- SEM) PER HORN IN RATS LAPAROTOMIZED AT G7 AND SUBSEQUENTLY ASSIGNED TO TEST GROUPS (n=10/group)

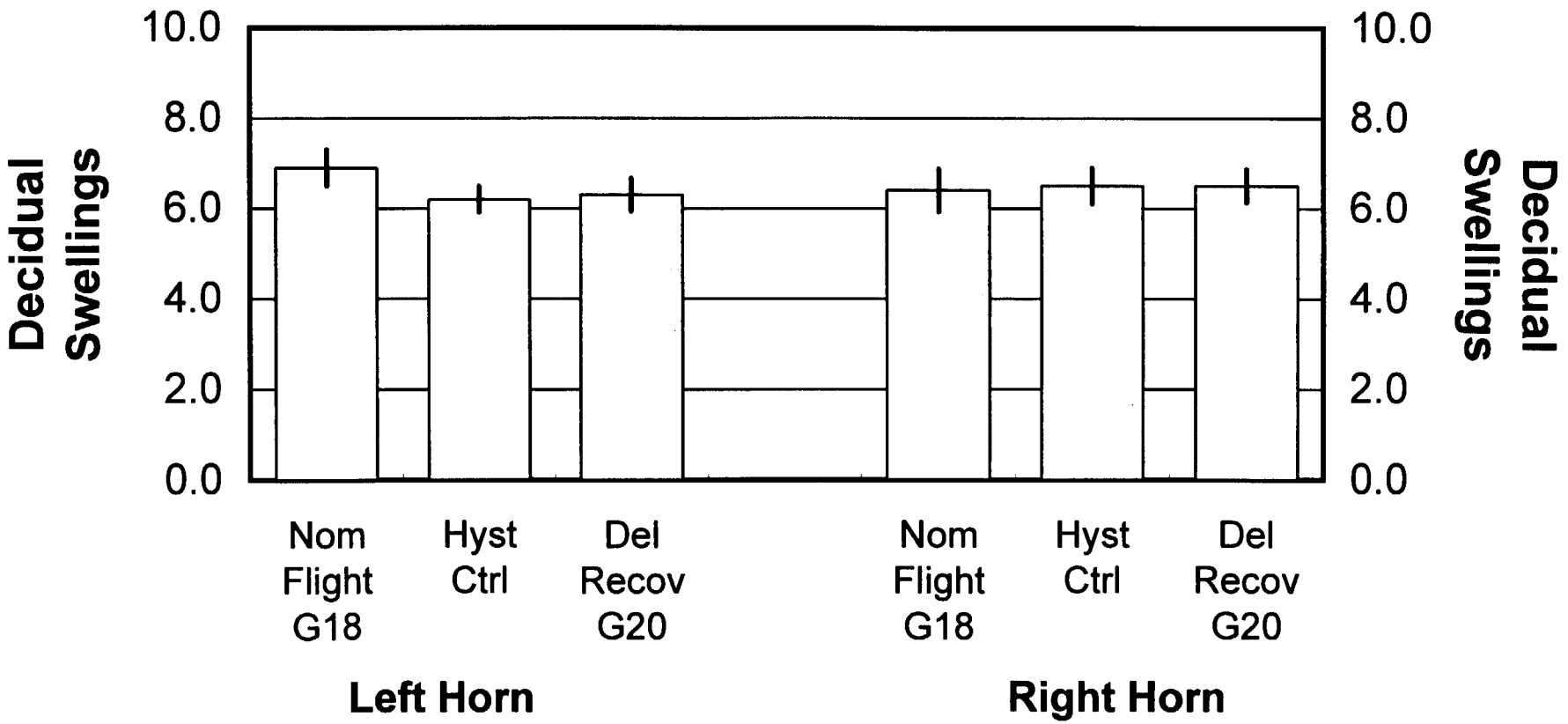

FIG. 26. NASA SUPPLIED PROFILE OF G-FORCES MIMICKING SHUTTLE LAUNCH

—— = PROFILE AS RUN •••••••••• = PROFILE AS PLANNED

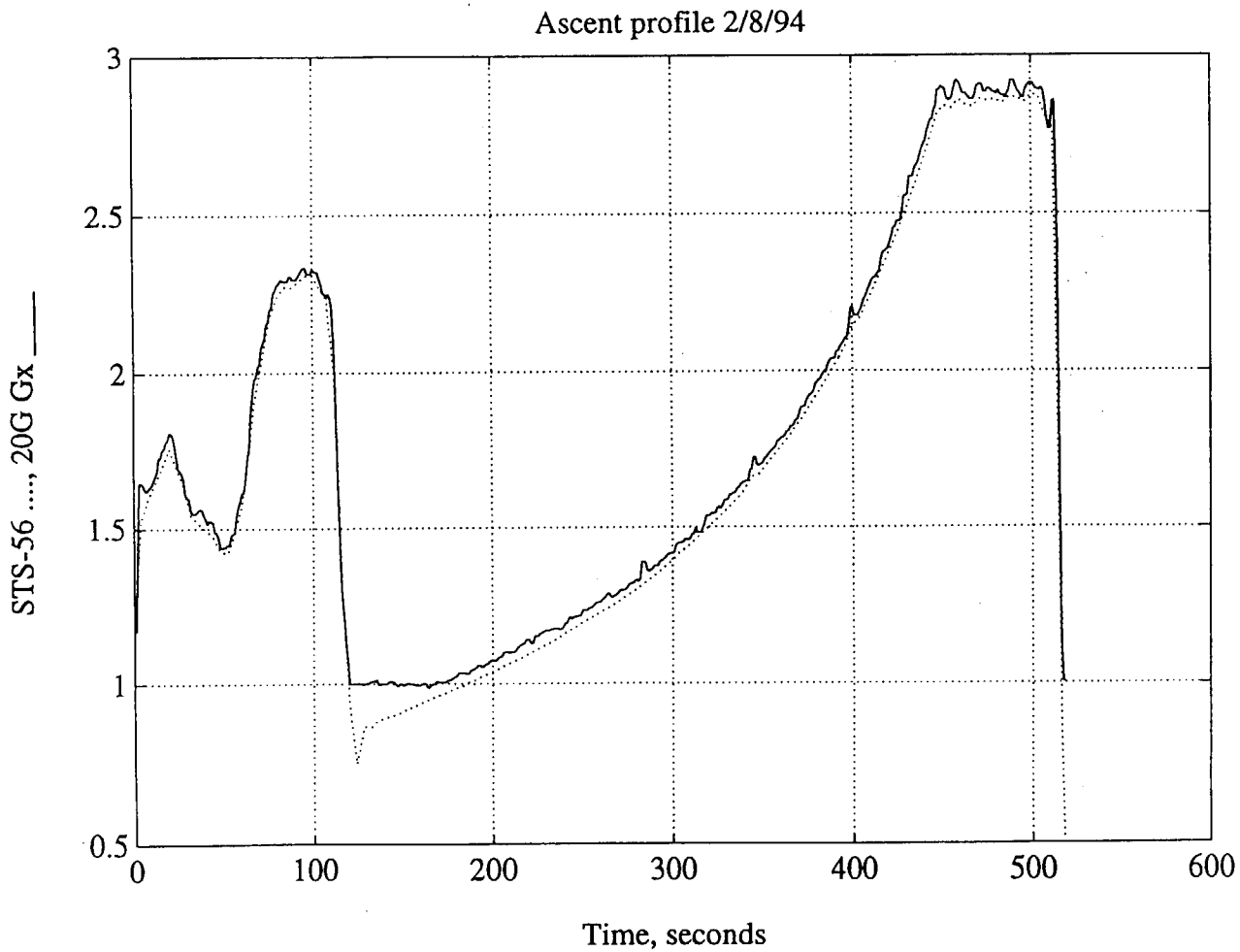

FIG. 27. NASA SUPPLIED PROFILE OF DECIBEL LEVELS MIMICKING SHUTTLE LAUNCH

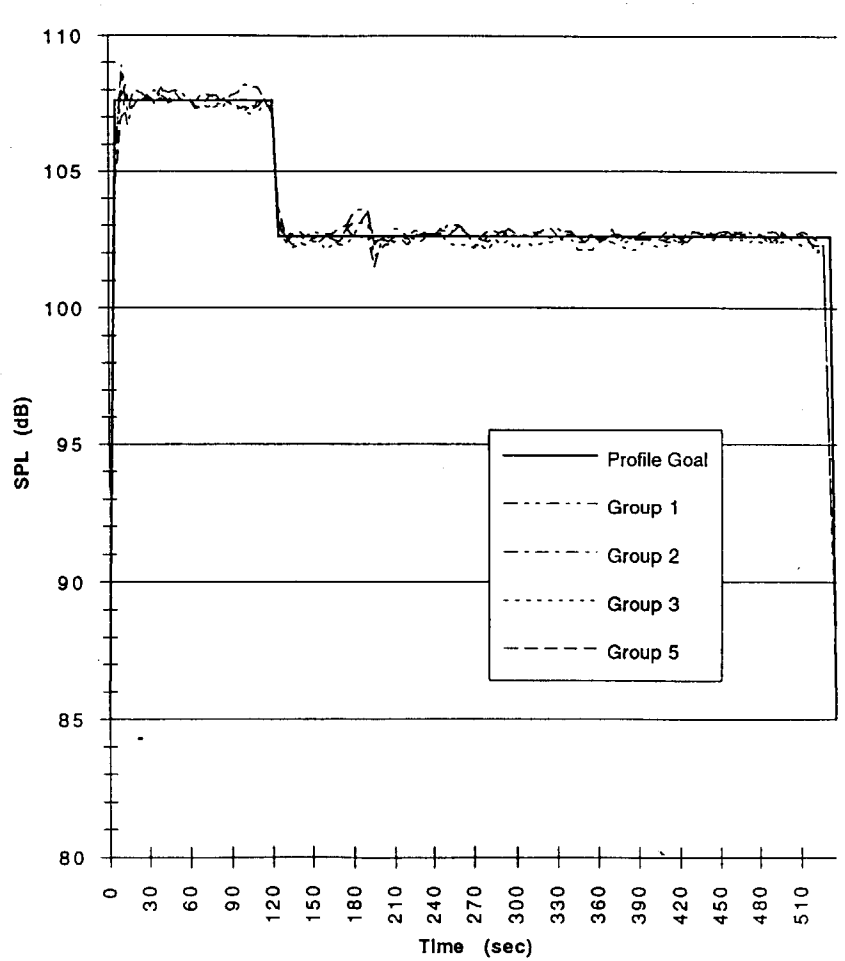

FIG. 28. WATER CONSUMED (Daily Mean of 10 rats/group) BY RATS HOUSED IN AEMS DURING PERIOD OF SIMULATED SHUTTLE FLIGHT

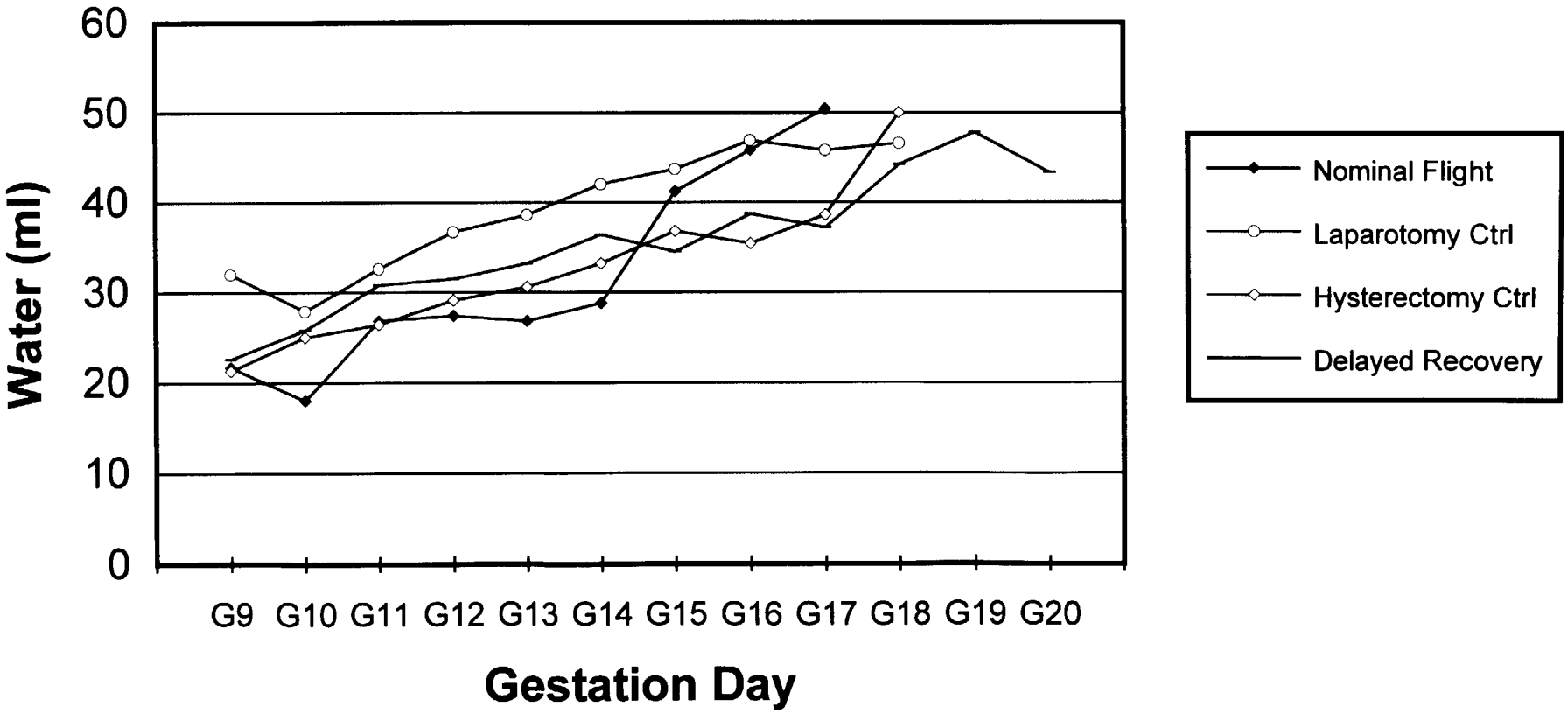

FIG. 29. NASA SUPPLIED PROFILE OF G-FORCES MIMICKING SHUTTLE LANDING

——= PROFILE AS RUN ••••••••= PROFILE AS PLANNED

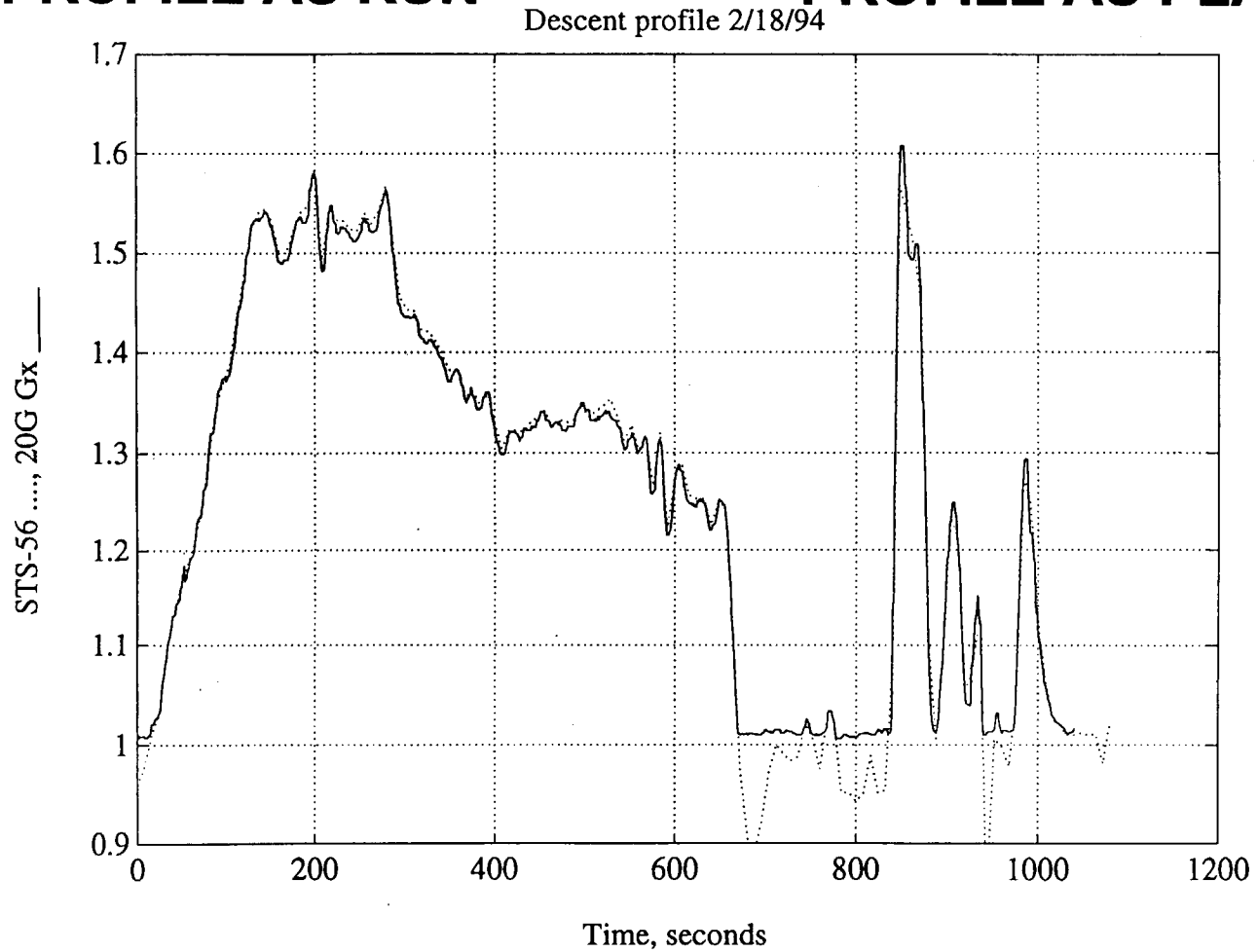

FIG. 30. LIVE FETUSES (Mean +/- SEM) PER HORN IN THE THREE TEST GROUPS UNILATERALLY HYSTERECTOMIZED AT G18 OR G20 (n=10/group)

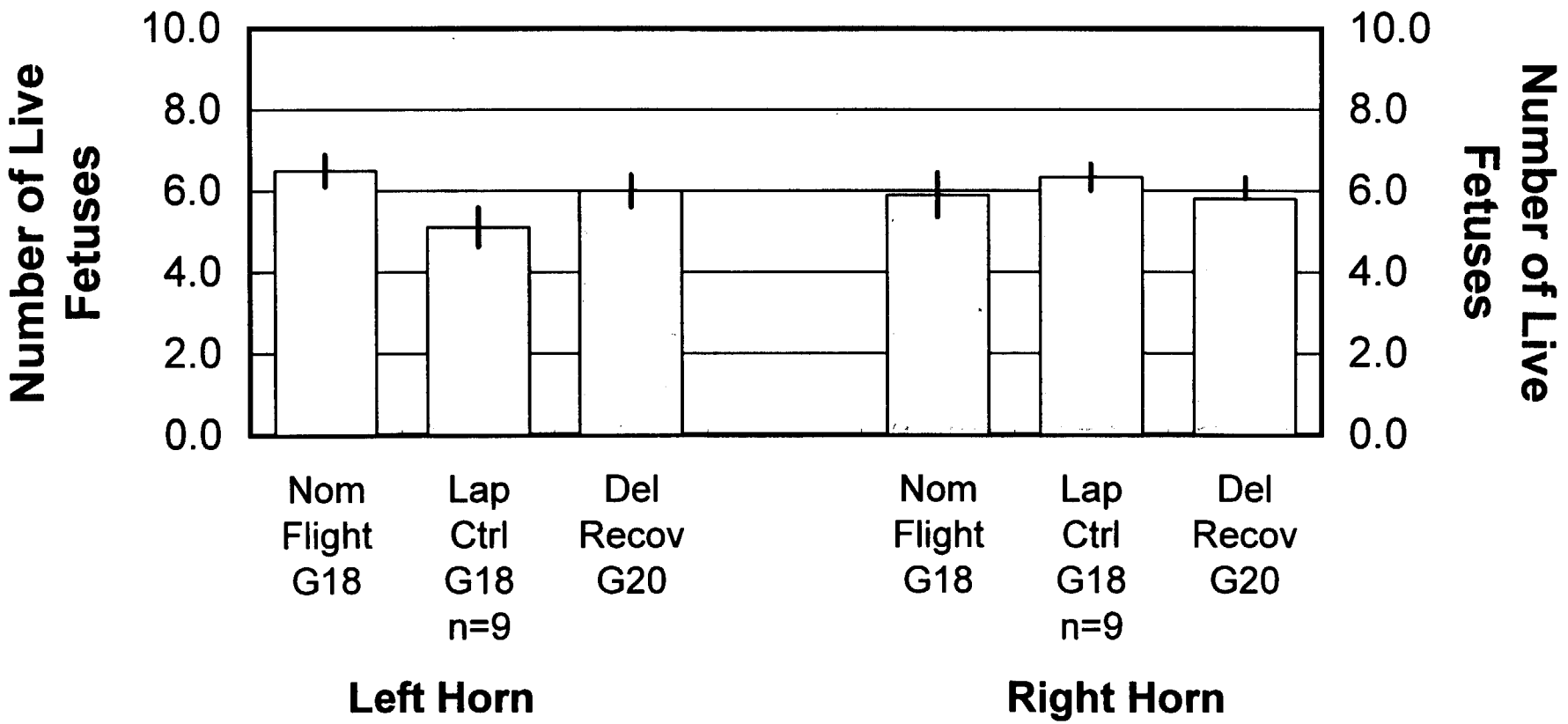

FIG. 31. TOTAL NUMBER (Mean +/- SEM) OF DECIDUAL SWELLINGS AT G7 COMPARED TO TOTAL NUMBER OF LIVE FETUSES AT G18 OR G20 (n=10/group)

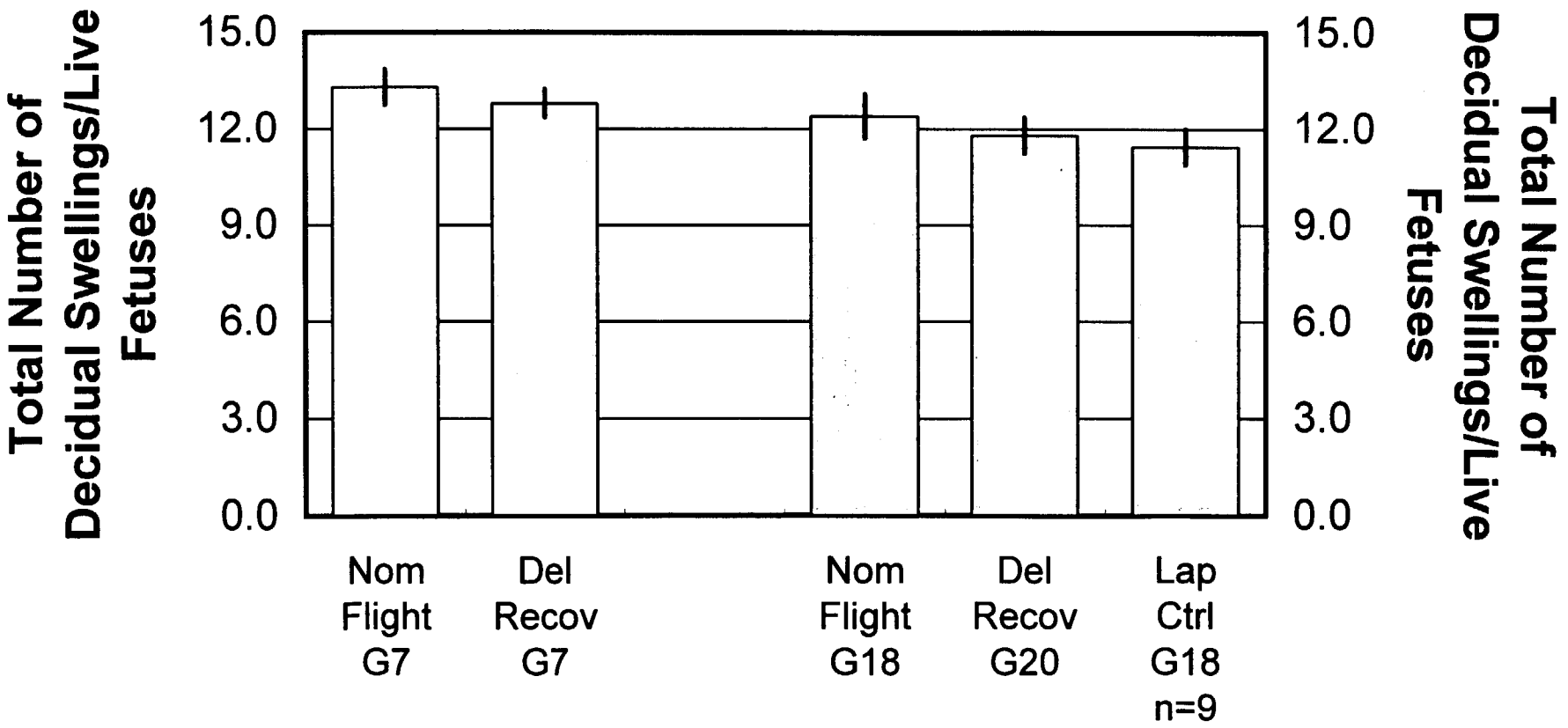

FIG. 32. DECIDUAL SWELLINGS (Mean +/- SEM) PER HORN AT G7 COMPARED TO LIVE FETUSES IN THE SAME HORN AT G18 OR G20 (n=10/group)

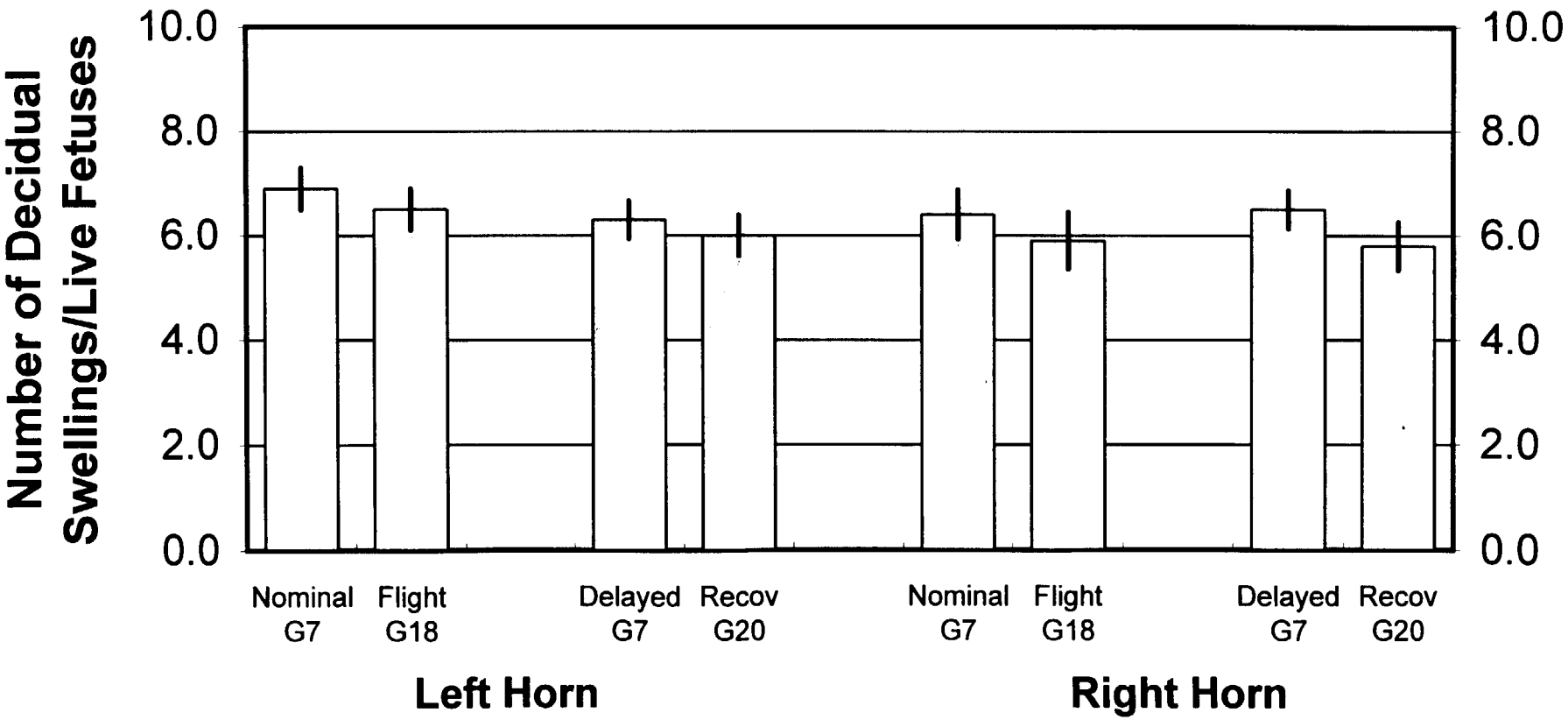

FIG. 33. CROWN-RUMP LENGTH OF FETUSES (Mean +/- SEM) AT UNILATERAL HYSTERECTOMY

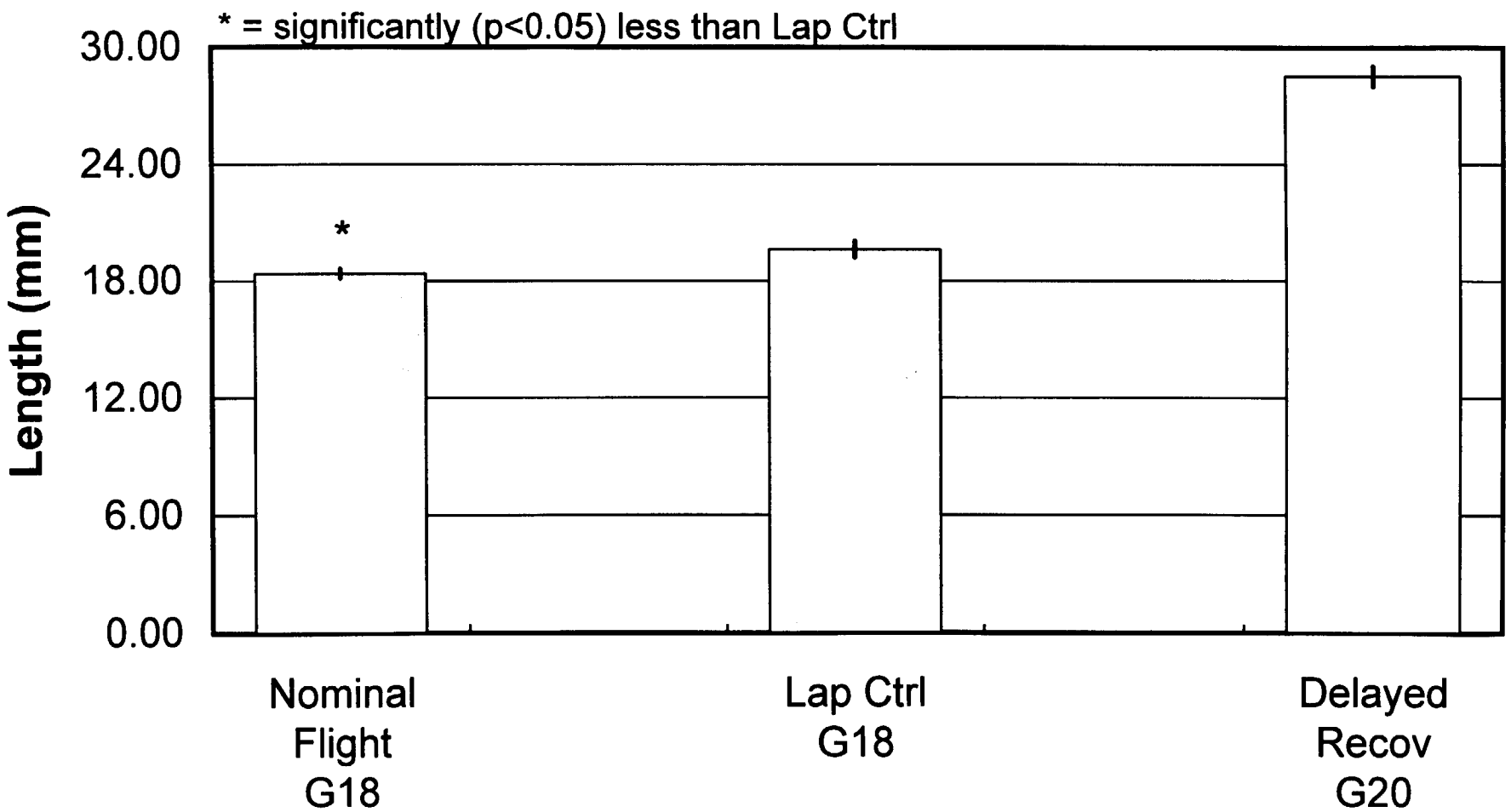

FIG. 34. WEIGHT OF FETUSES (Mean +/- SEM) AT UNILATERAL HYSTERECTOMY

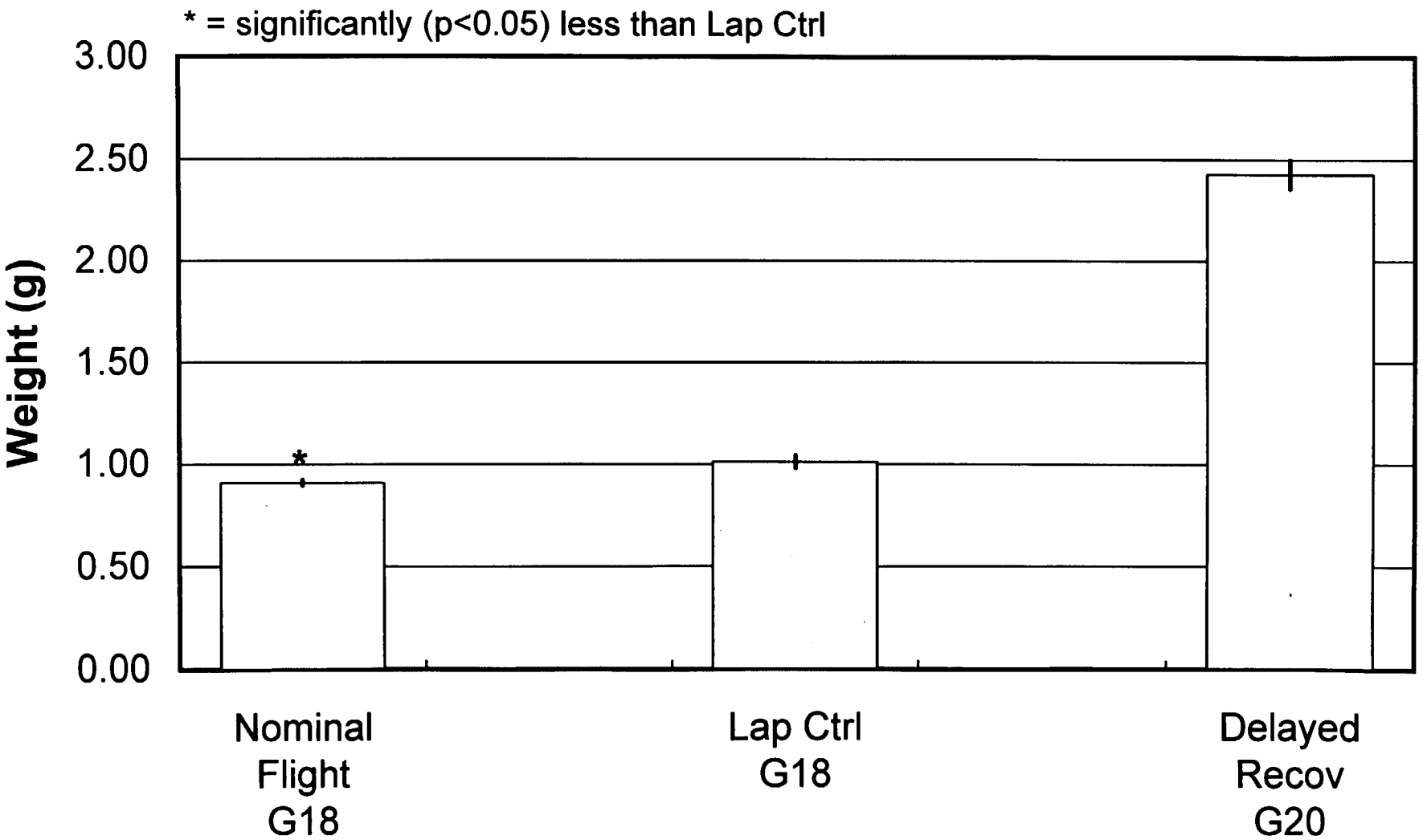

FIG. 35. WEIGHT OF PLACENTAS (Mean +/- SEM) AT UNILATERAL HYSTERECTOMY

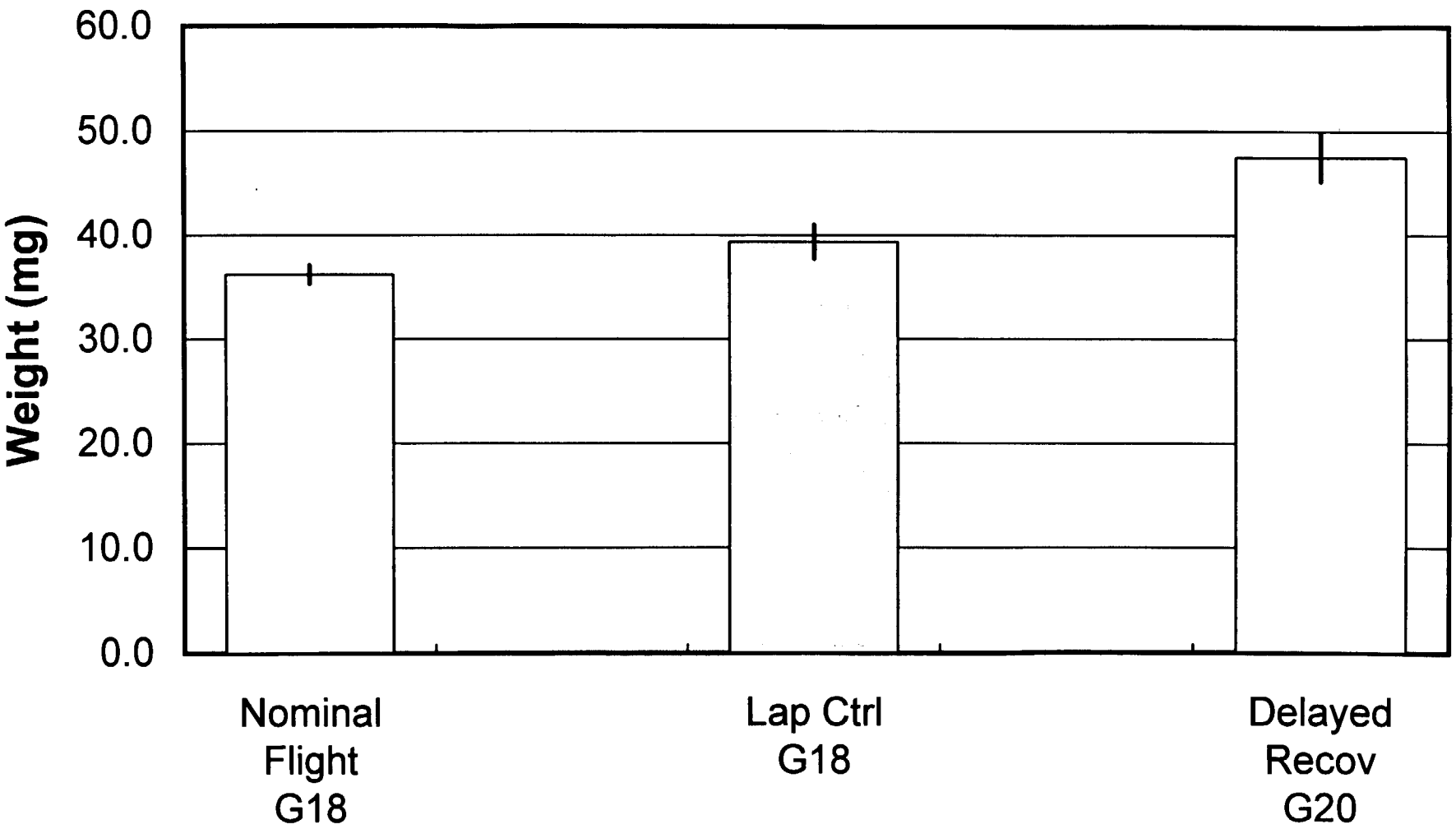

FIG. 36. BODY WEIGHT (MEAN) OF PREGNANT RATS G18 - G21

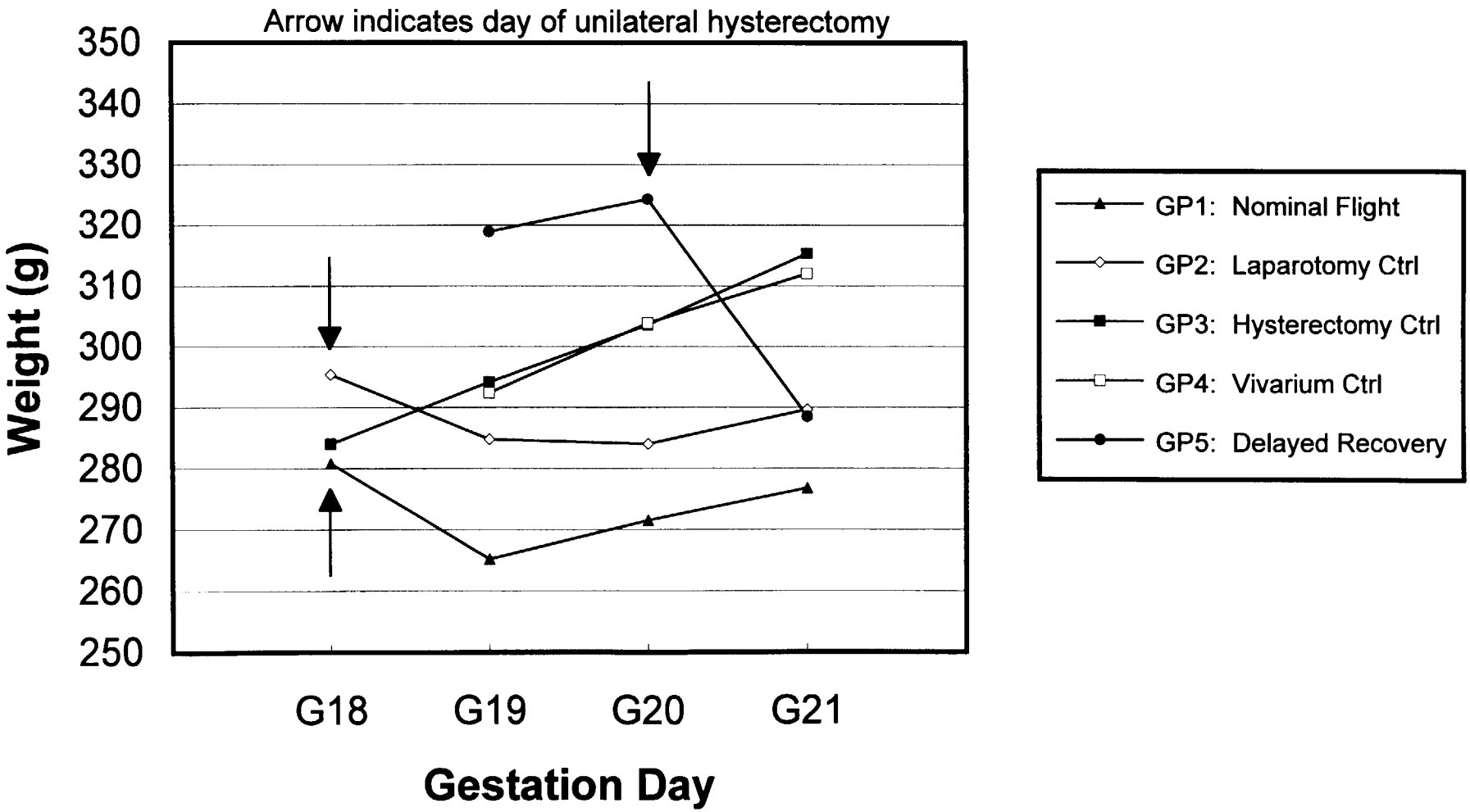

FIG. 37. FOOD BAR (MEAN) CONSUMED BY PREGNANT RATS G19 - G20

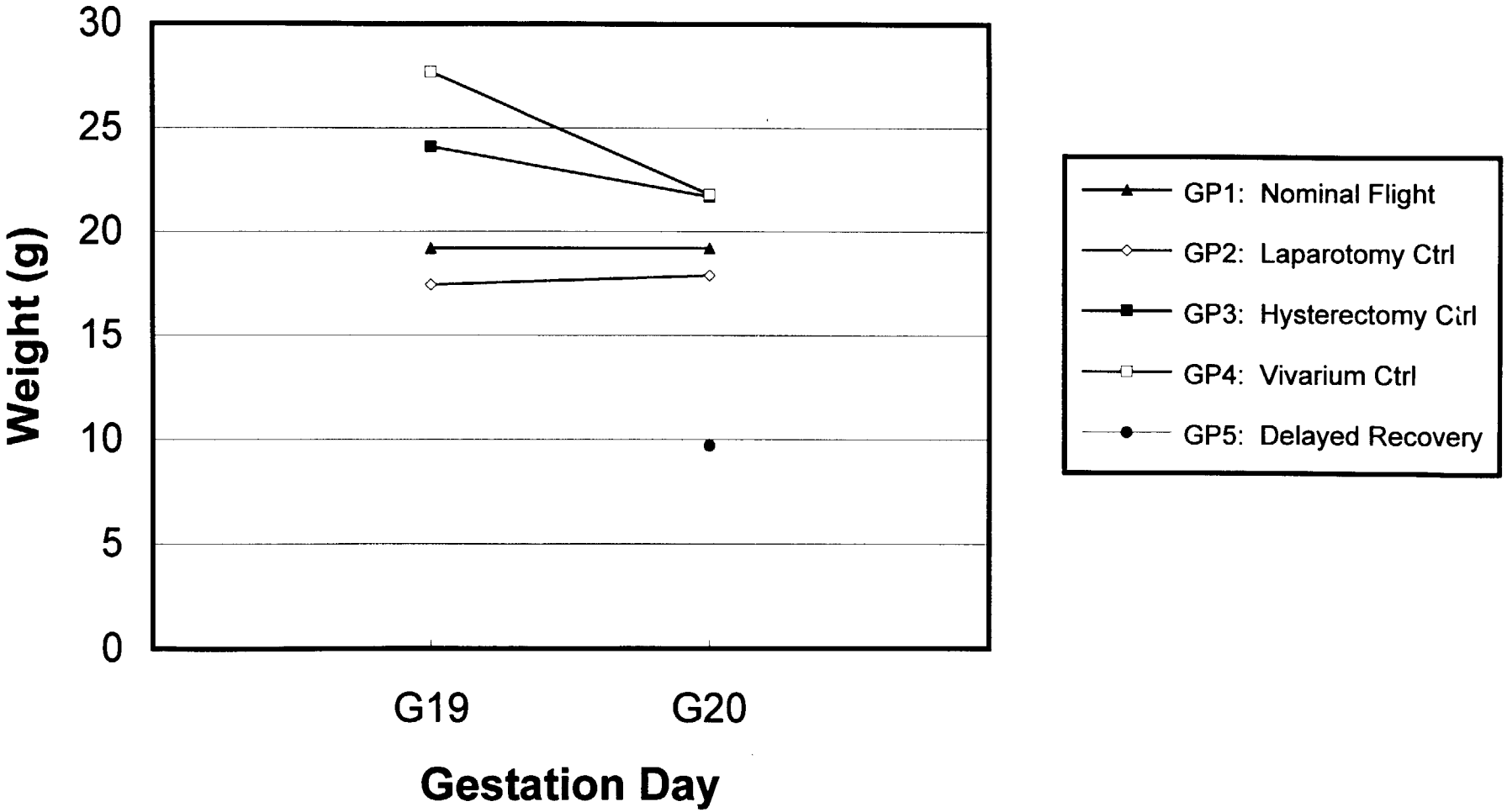

FIG. 38. WATER (MEAN) CONSUMED BY PREGNANT RATS G19 - G20

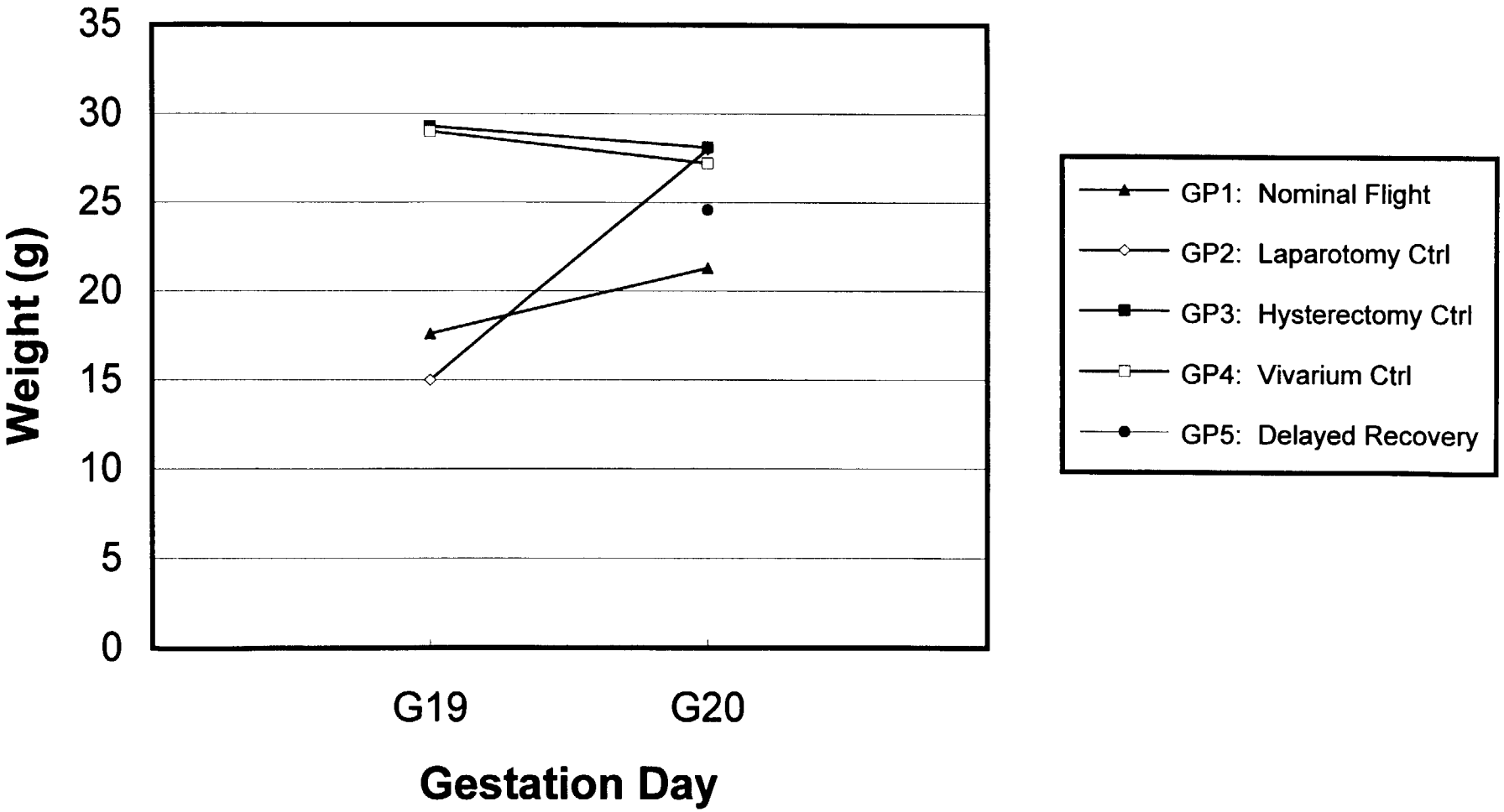

FIG. 39. INITIATION OF DELIVERY: EXPERIMENTAL GROUPS AND FOSTER DAMS

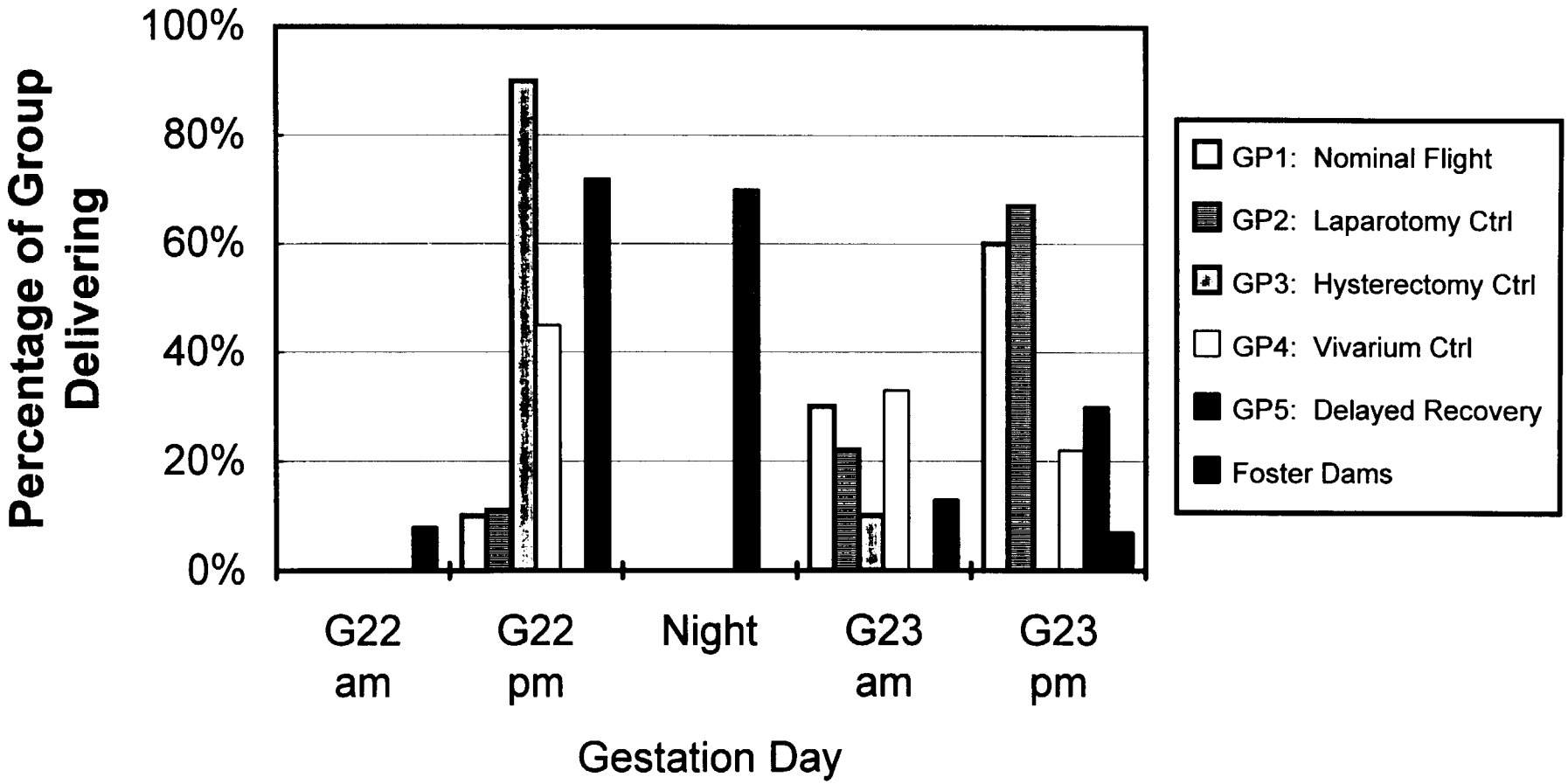

FIG. 40. NUMBER OF LIVE FETUSES (Mean +/- SEM) IN THE REMAINING HORN FOLLOWING UNILATERAL HYSTERECTOMY (G18 OR G20) COMPARED TO NUMBER OF LIVE PUPS DELIVERED (n=10/group)

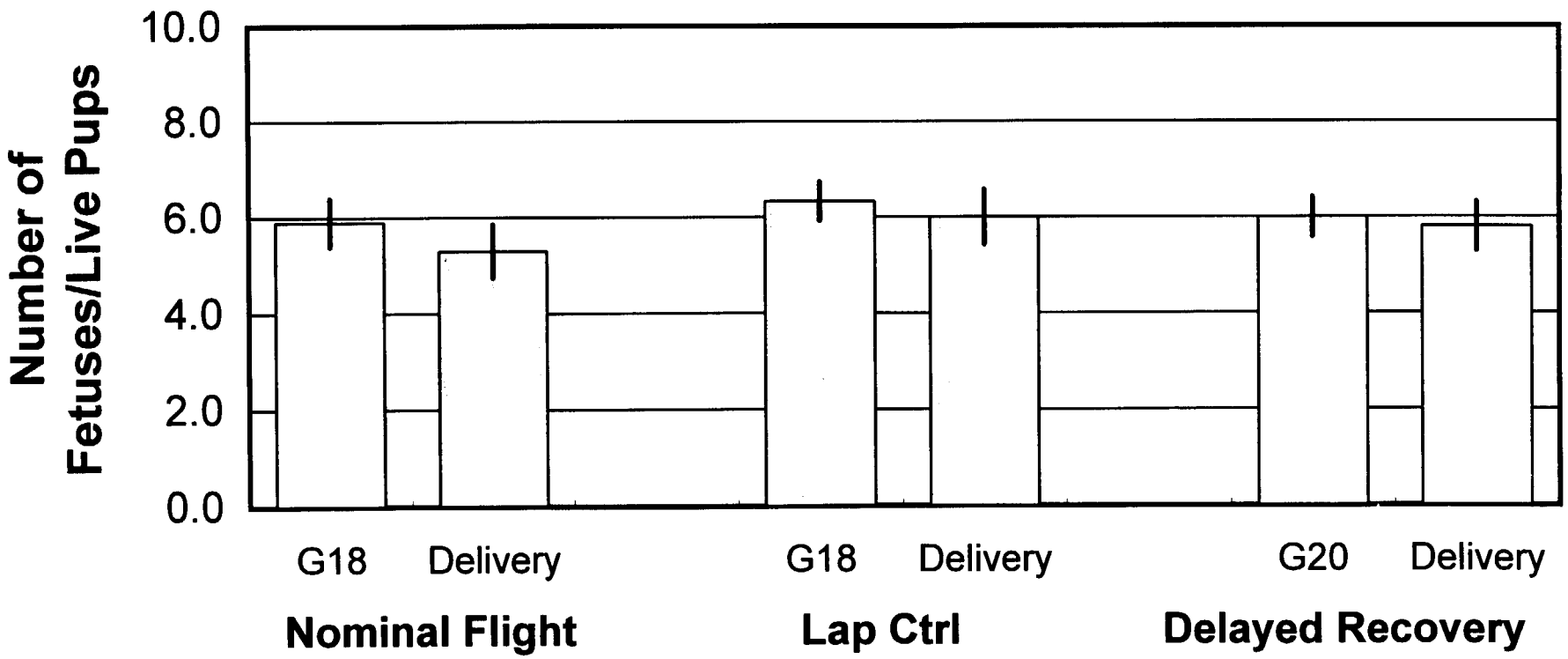

FIG. 41. COMBINED ADRENAL WEIGHT (Mean +/- SEM) OF DAMS AT DAY OF PARTURITION

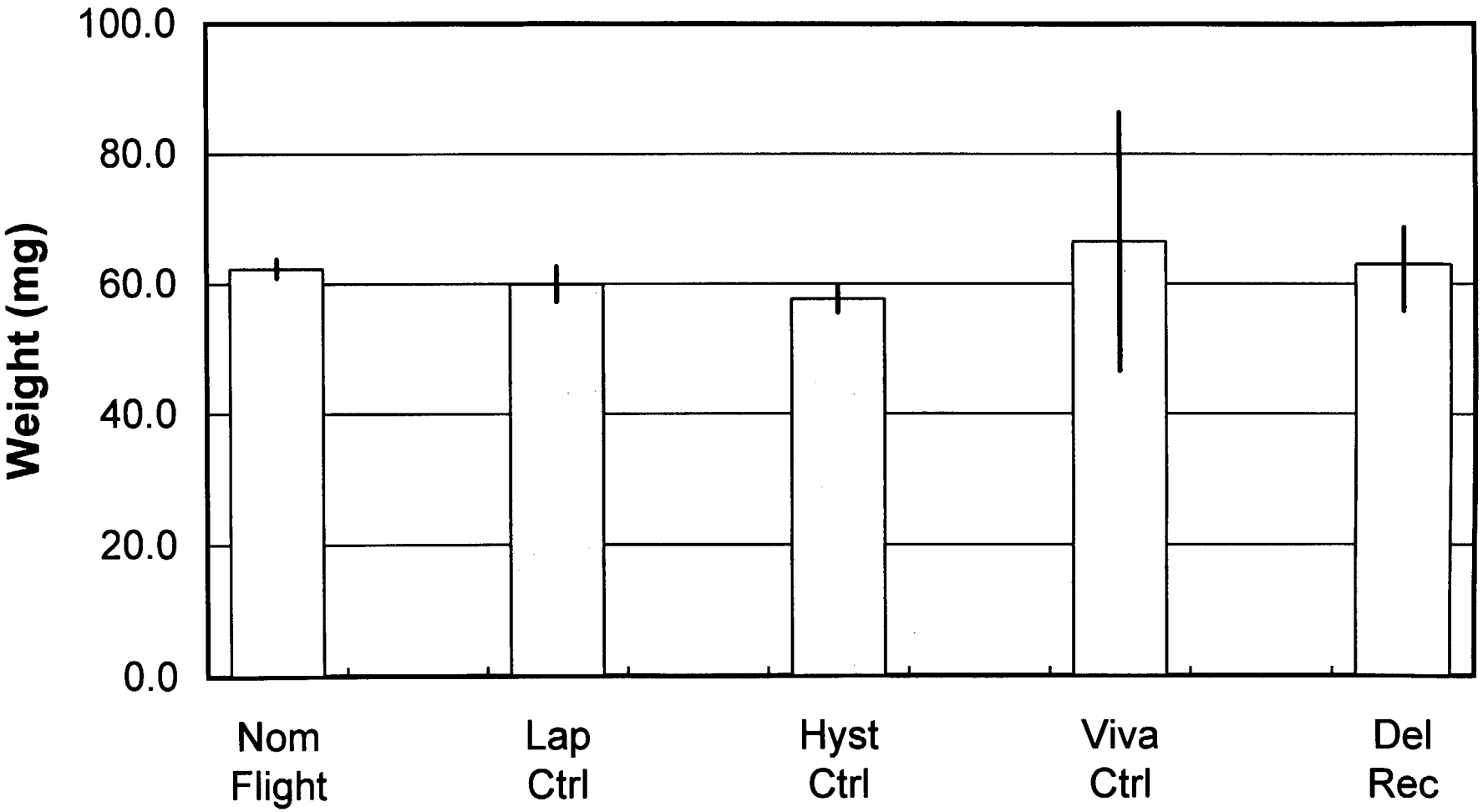

FIG. 42. THYMUS WEIGHT (Mean +/- SEM) OF DAMS AT DAY OF PARTURITION

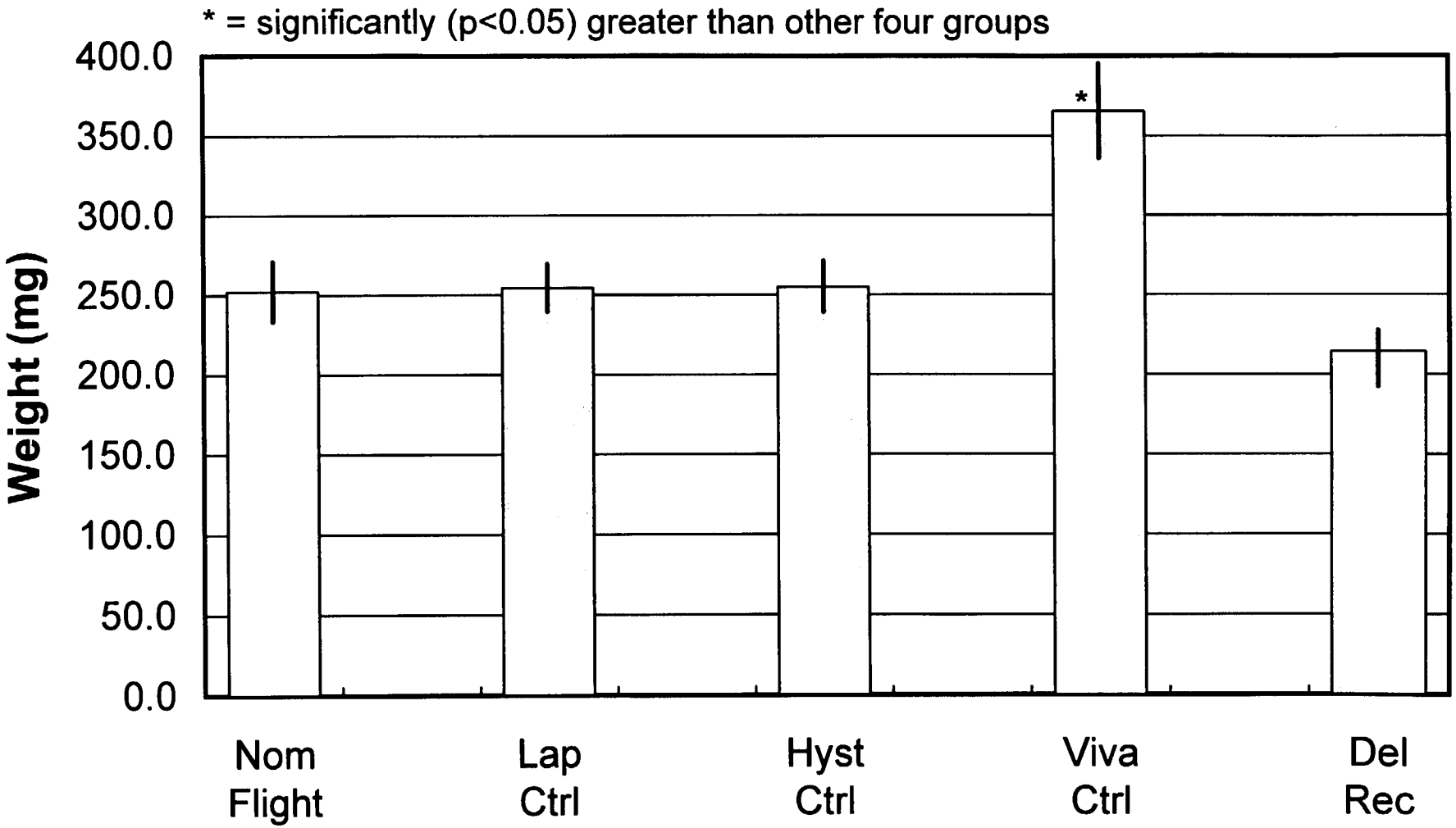

FIG. 43. PLASMA PROGESTERONE LEVELS (Mean +/- SEM) IN POST-PARTUM DAMS

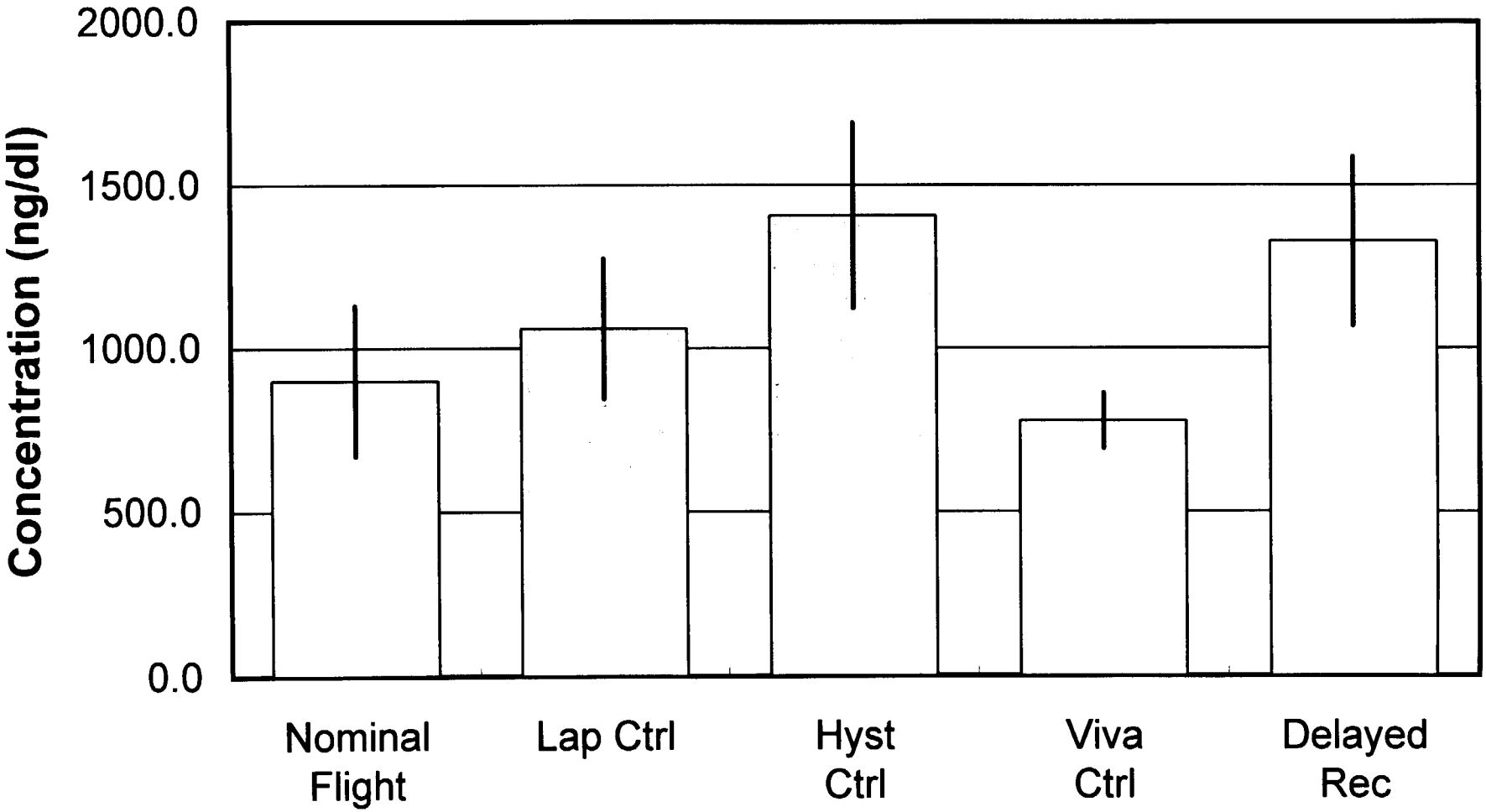

FIG. 44. PLASMA ESTRADIOL LEVELS (Mean +/- SEM) IN POST-PARTUM DAMS

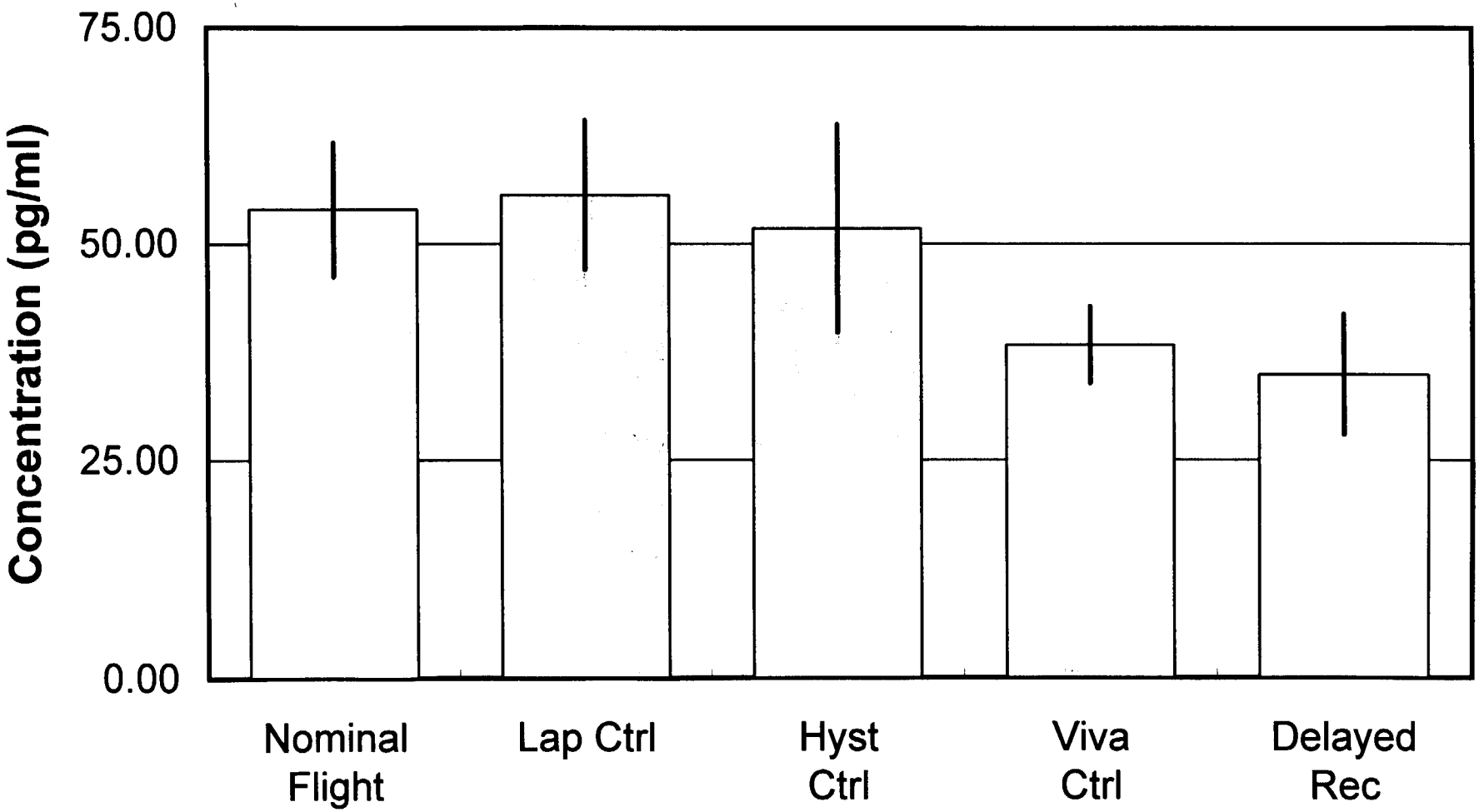

FIG. 45. PLASMA CORTICOSTERONE LEVELS (Mean +/- SEM) IN POST-PARTUM DAMS

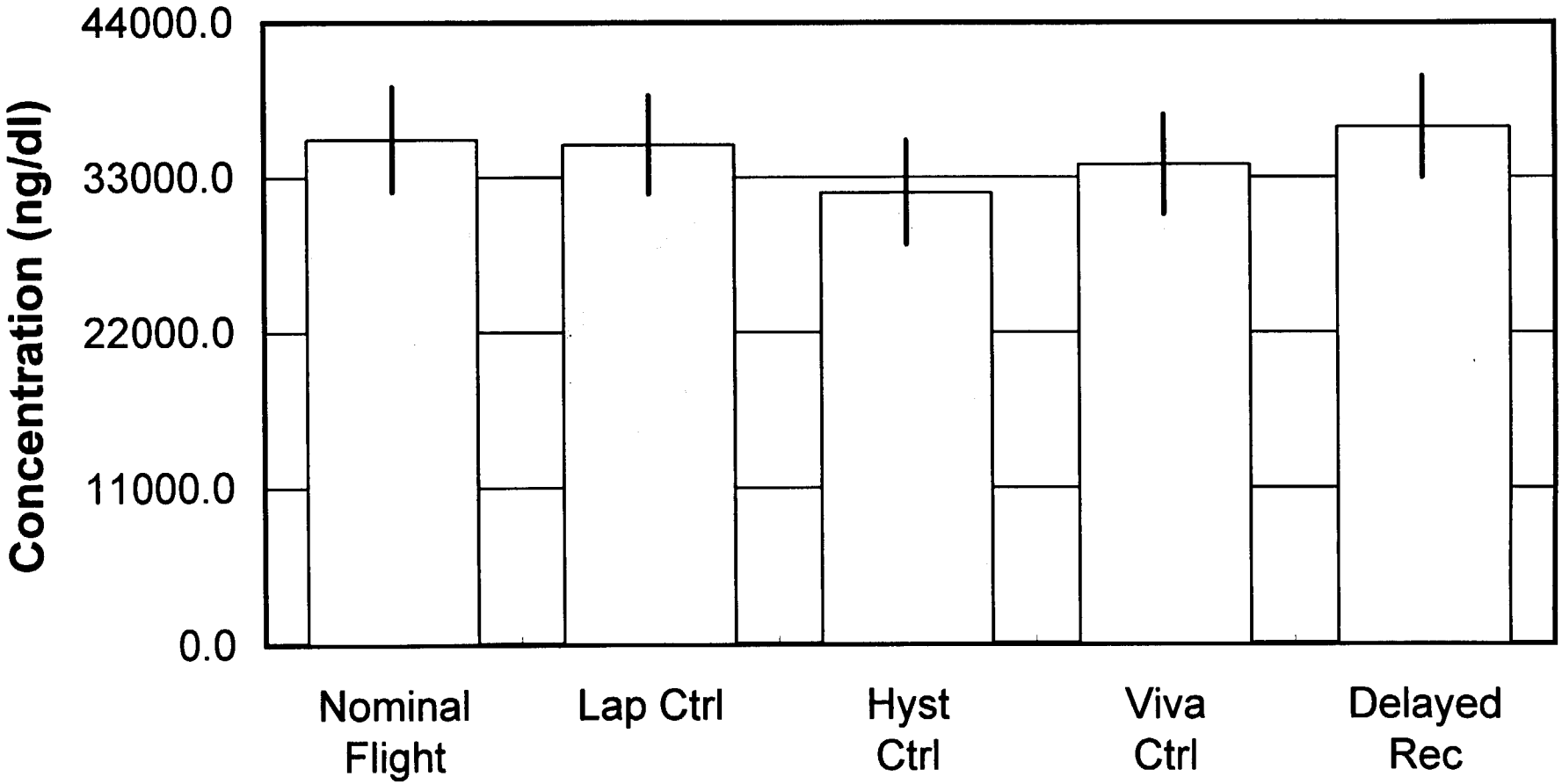

FIG. 46. TOTAL PLASMA CATECHOLAMINE LEVELS (Mean +/- SEM) IN POST-PARTUM DAMS

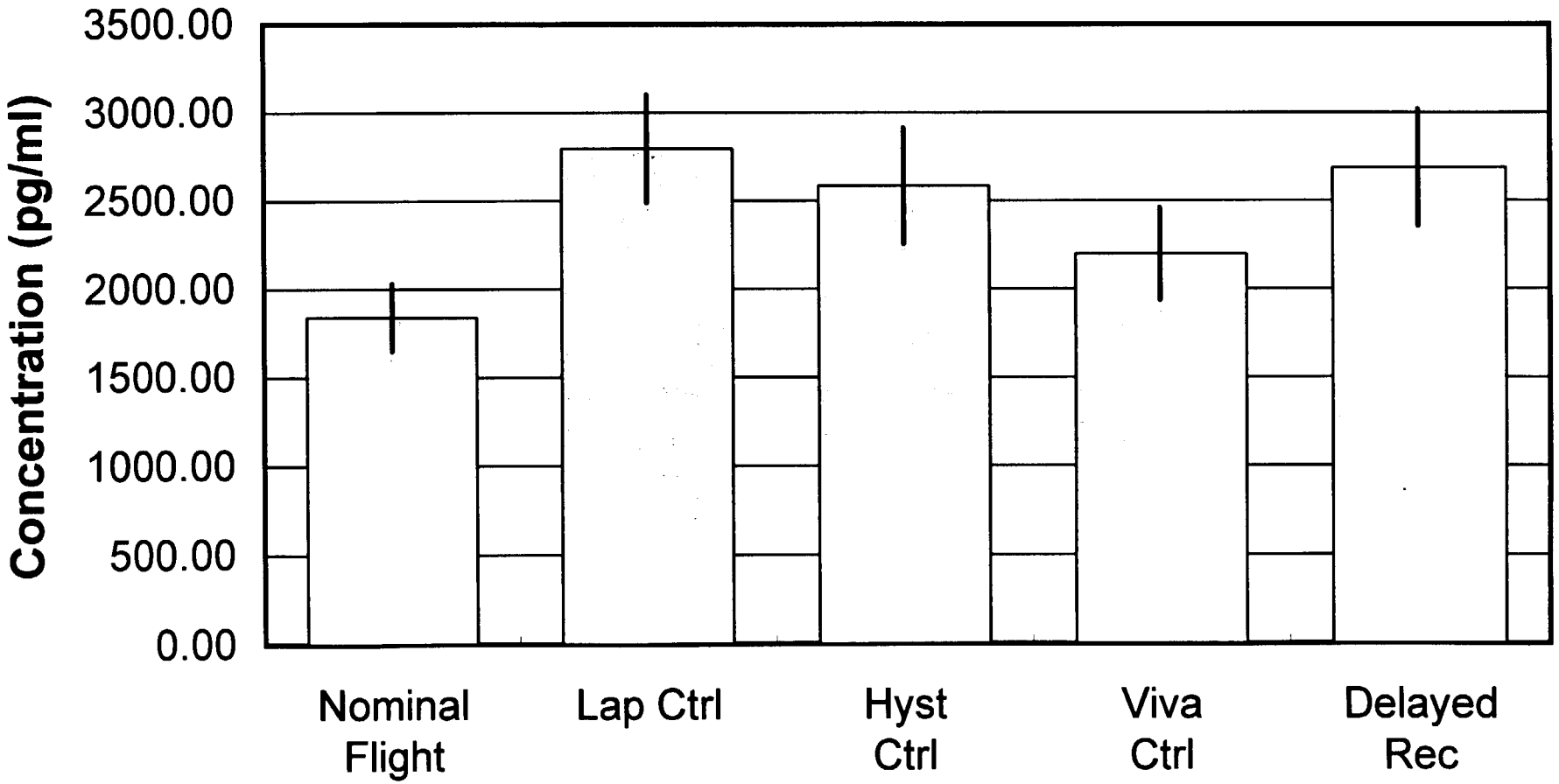

FIG. 47. PLASMA DOPAMINE LEVELS (Mean +/- SEM) IN POST-PARTUM DAMS

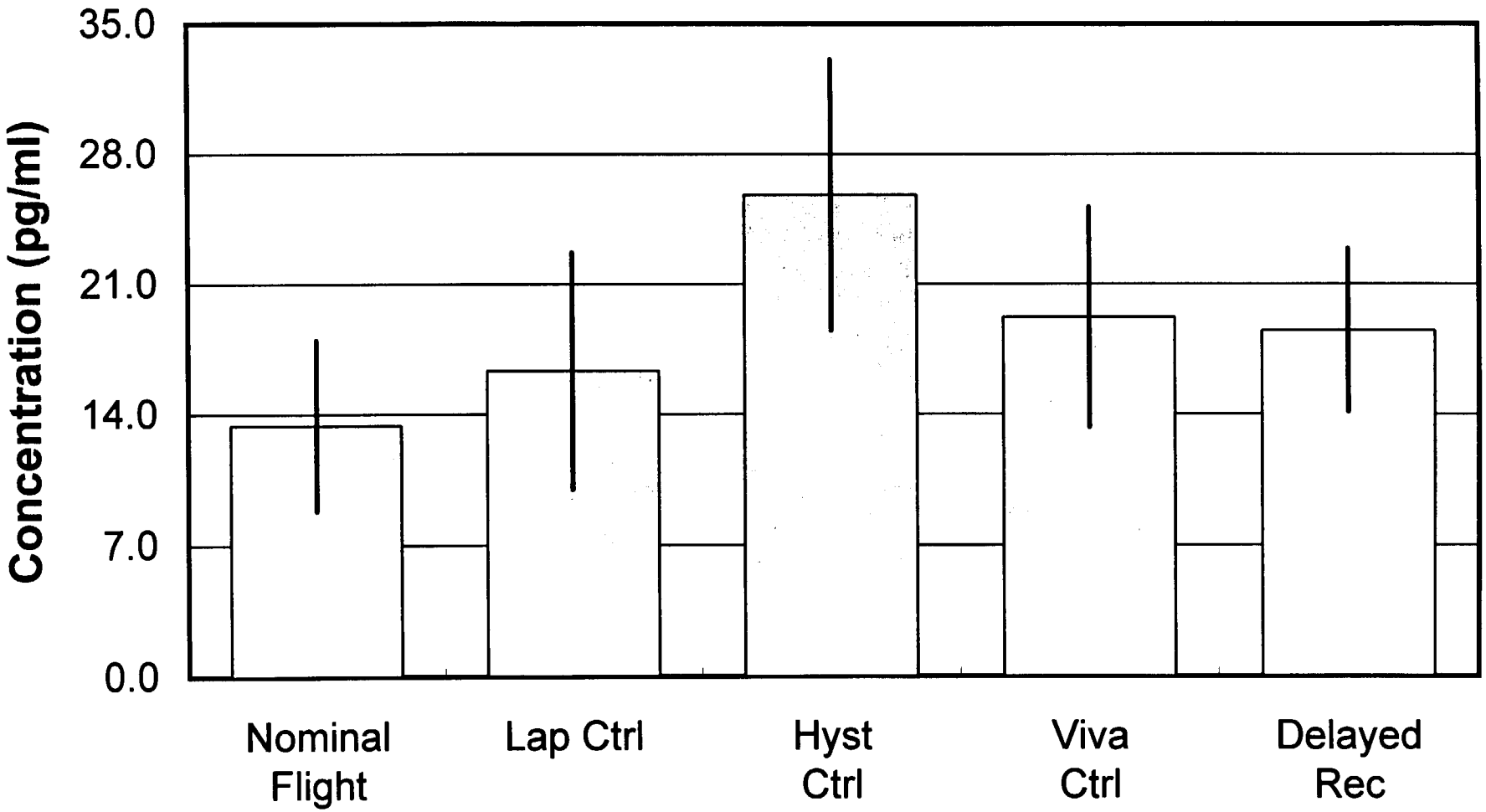

FIG. 48. PLASMA NOREPINEPHRINE LEVELS (Mean +/- SEM) IN POST-PARTUM DAMS

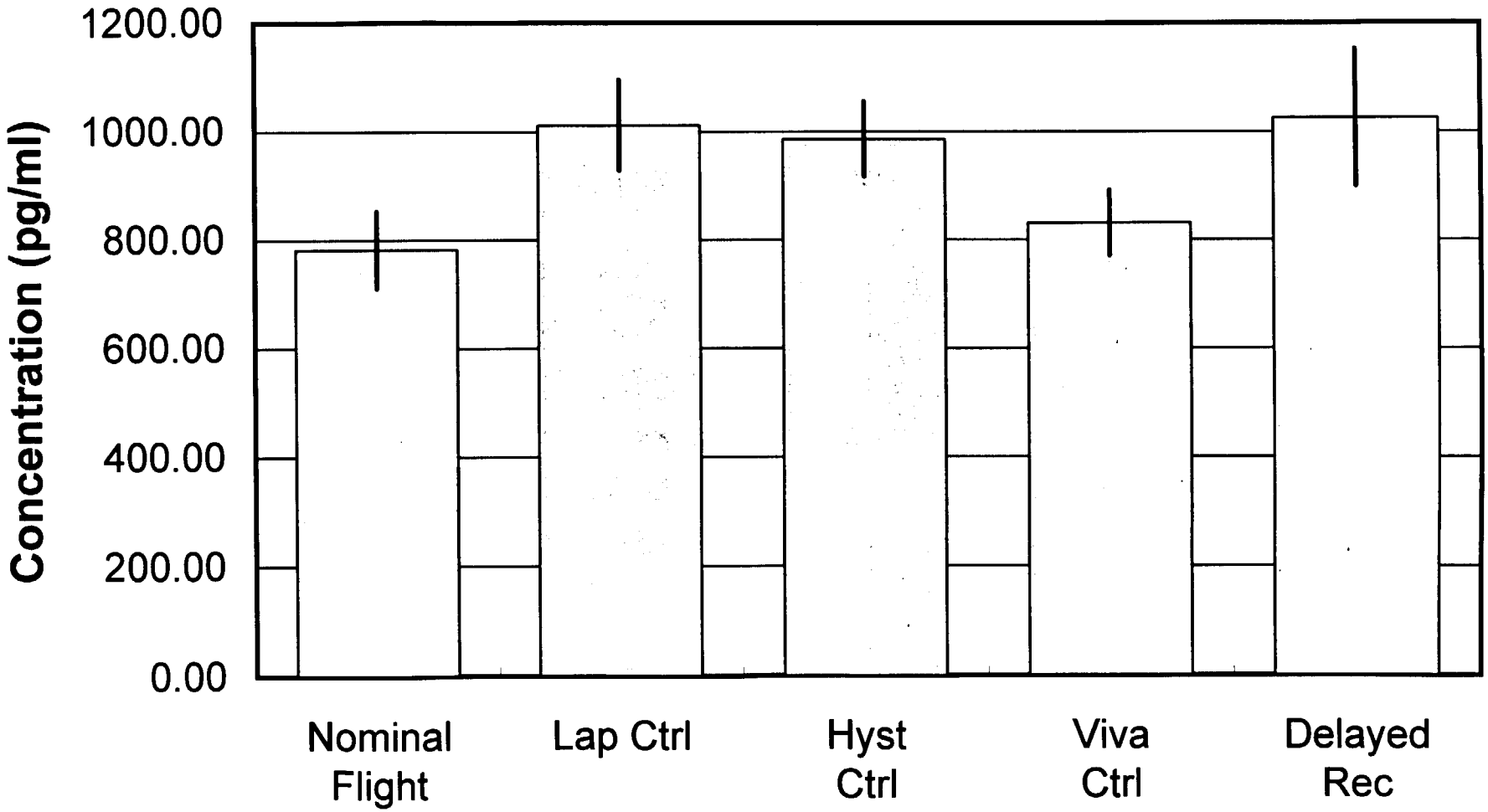

FIG. 49. PLASMA EPINEPHRINE LEVELS (Mean +/- SEM) IN POST-PARTUM DAMS

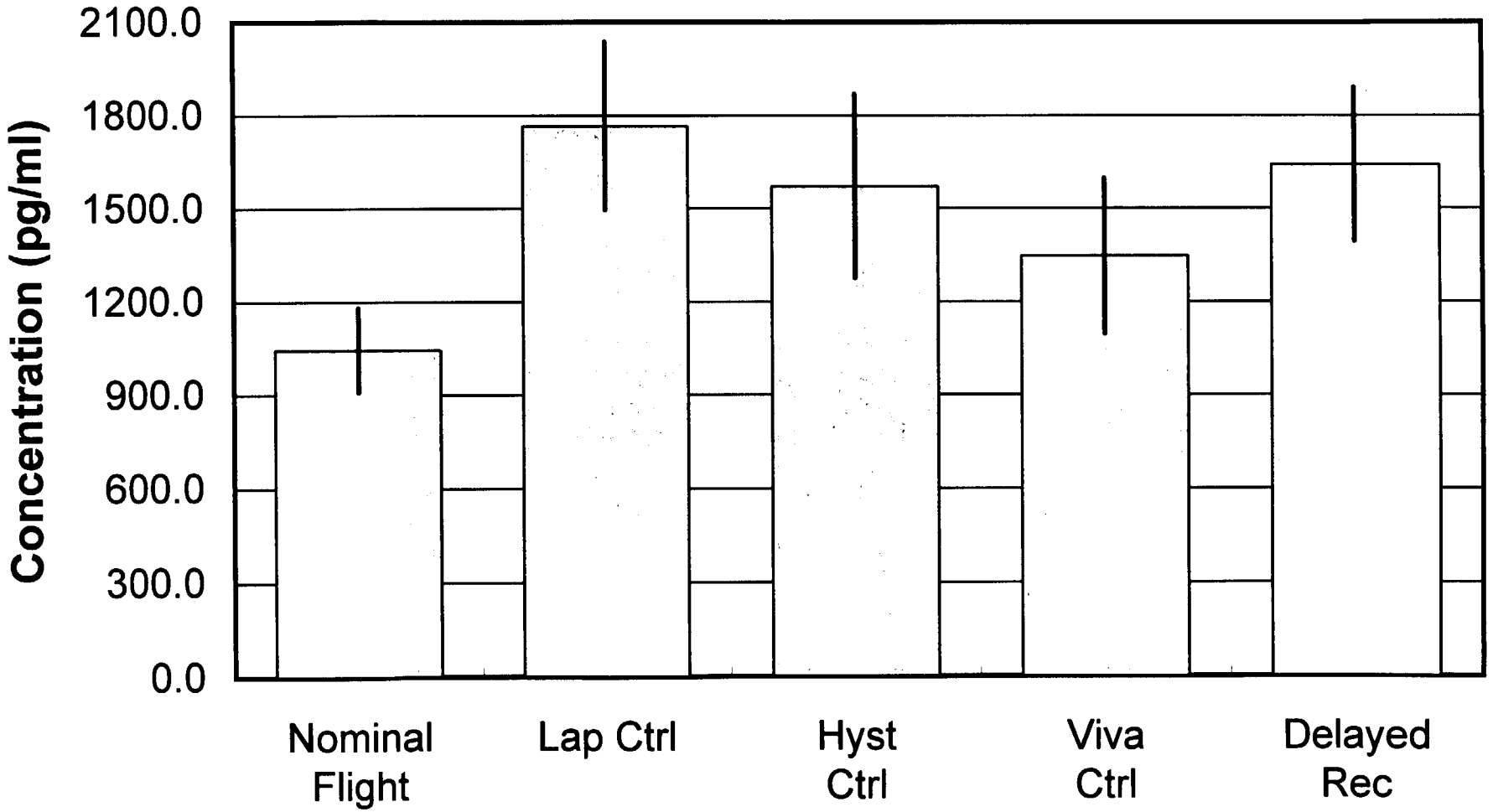

FIG. 50. ANOGENITAL DISTANCE (Mean +/- SEM) OF MALE PUPS ON DAY OF DELIVERY

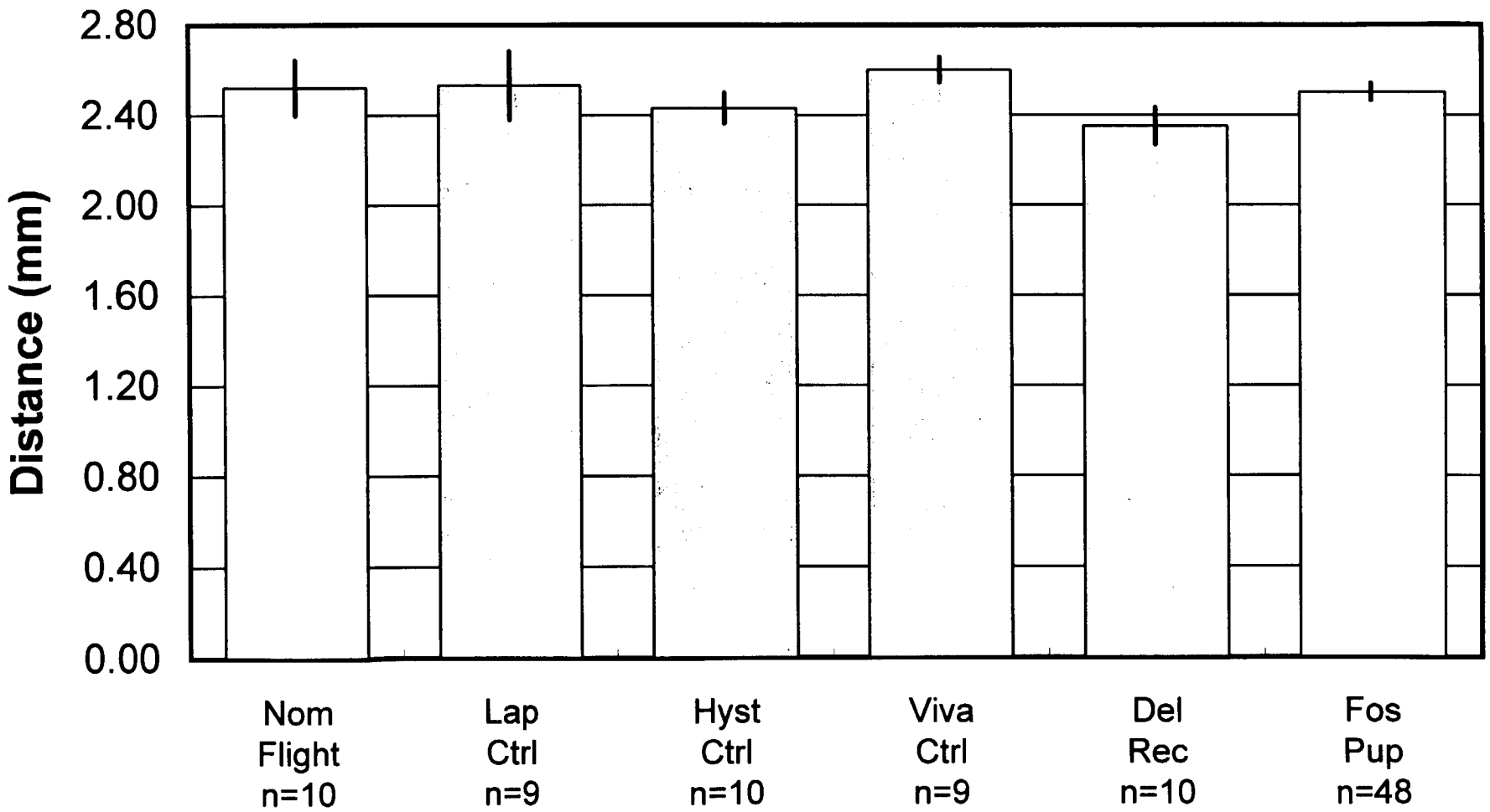

FIG. 51. BODY WEIGHT (Mean +/- SEM) OF NOMINAL FLIGHT PUPS AND FOSTER PUPS (indicated by arrow) FOR NEONATAL DAYS 0 - 7

Pups delivered by test dams from the Nominal Flight Group were transferred to a foster dam. An appropriate number of foster dam pups were retained such that the litter size = 10.

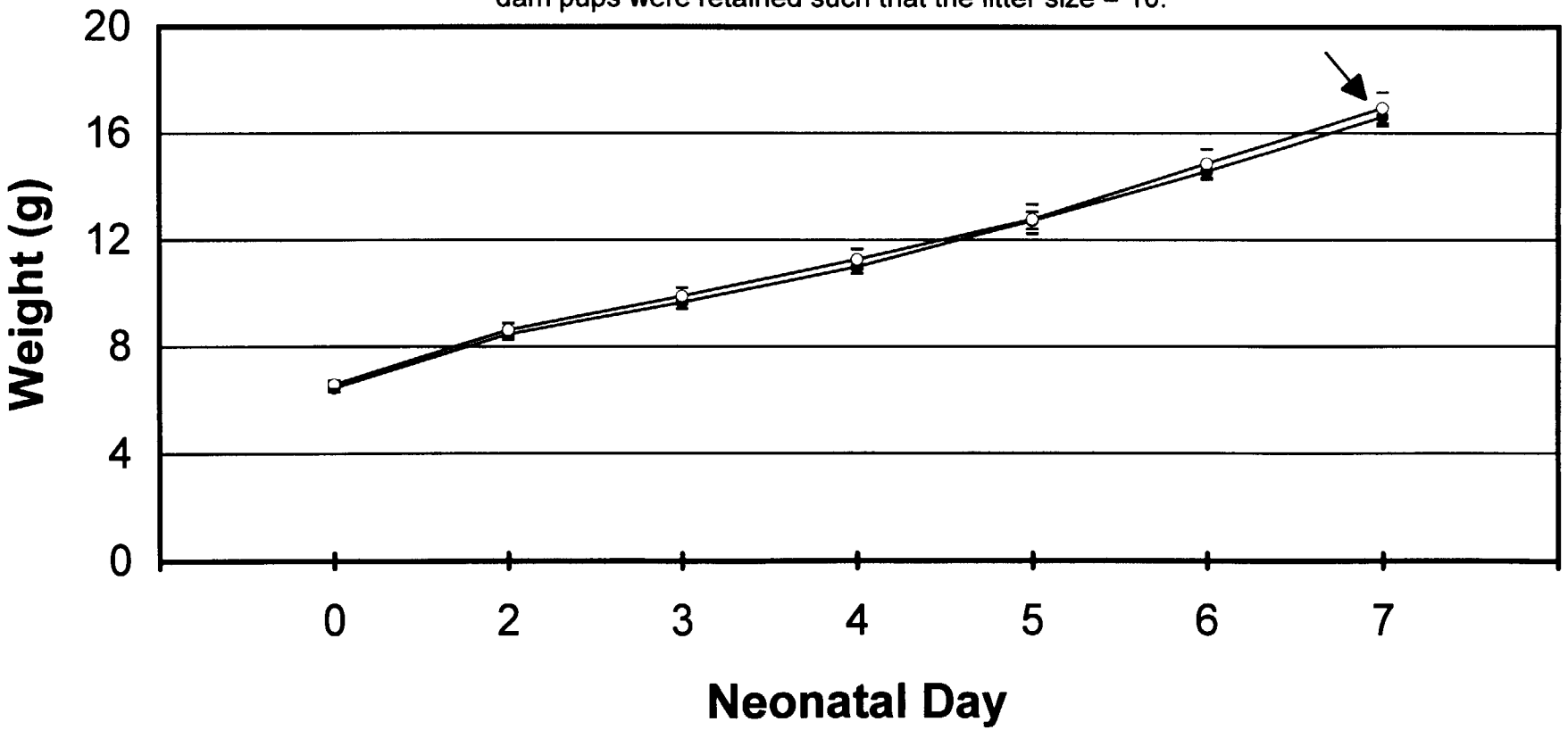

FIG. 52. BODY WEIGHT (Mean +/- SEM) OF LAPAROTOMY CONTROL PUPS AND FOSTER PUPS (indicated by arrow) FOR NEONATAL DAYS 0 - 7

Pups delivered by test dams from the Laparotomy Control Group were transferred to a foster dam. An appropriate number of foster dam pups were retained such that the litter size = 10.

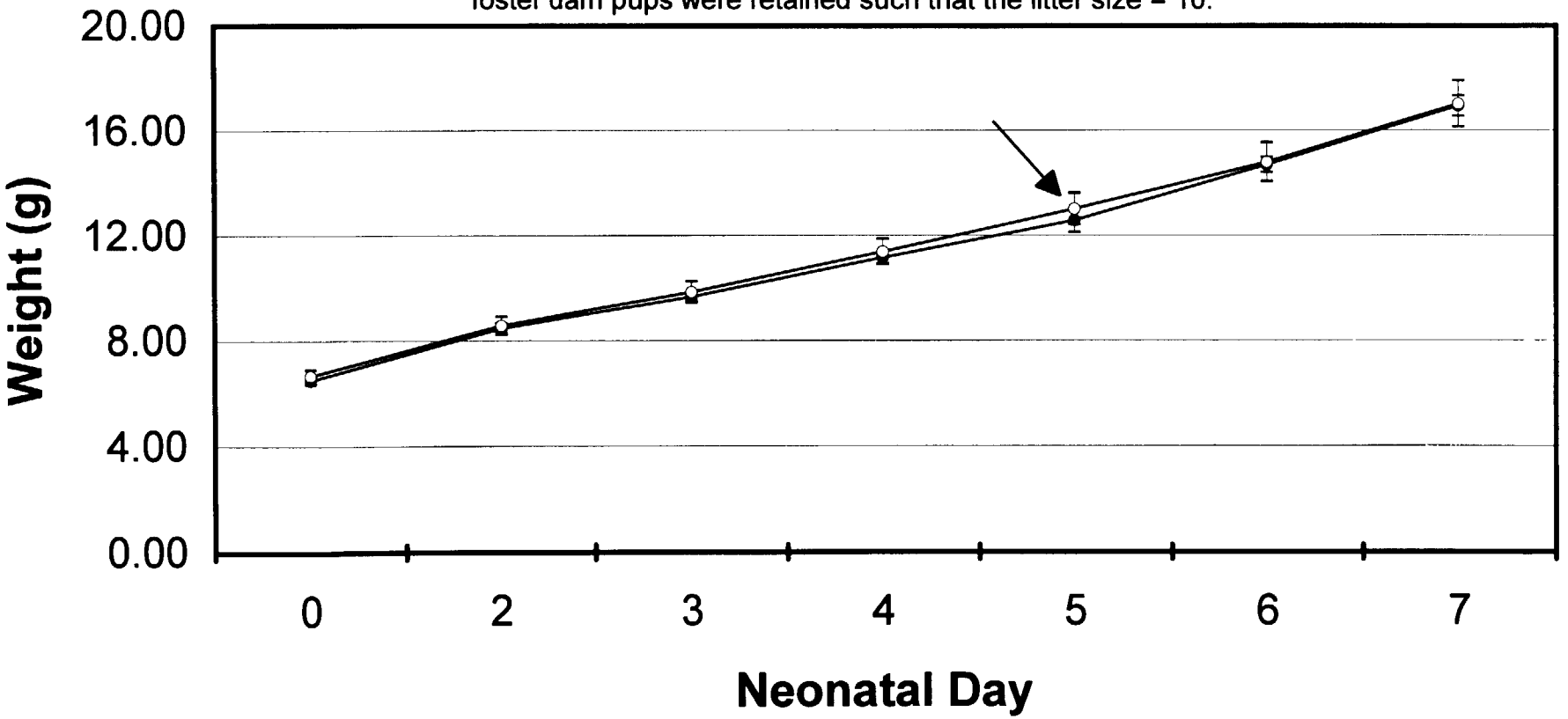

FIG. 53. BODY WEIGHT (Mean +/- SEM) OF HYSTERECTOMY CONTROL PUPS AND FOSTER PUPS (indicated by arrow) FOR NEONATAL DAYS 0 - 7

Pups delivered by test dams from the Hysterectomy Control Group were transferred to a foster dam. An appropriate number of foster dam pups were retained such that the litter size = 10.

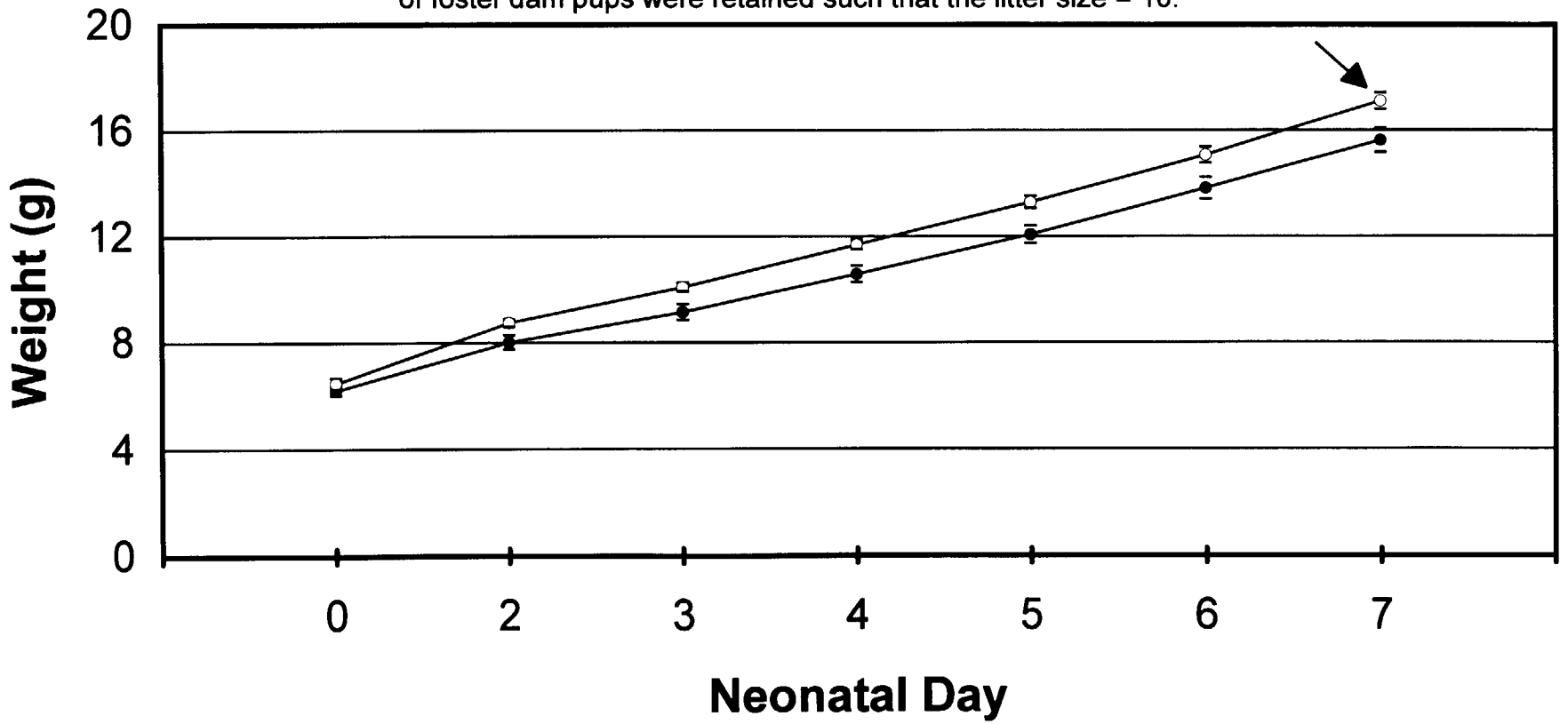

FIG. 54. BODY WEIGHT (Mean +/- SEM) OF VIVARIUM CONTROL PUPS AND FOSTER PUPS (indicated by arrow) FOR NEONATAL DAYS 0 - 7

Pups delivered by test dams from the Vivarium Control Group were transferred to a foster dam. An appropriate number of foster dam pups were retained such that the litter size 10.

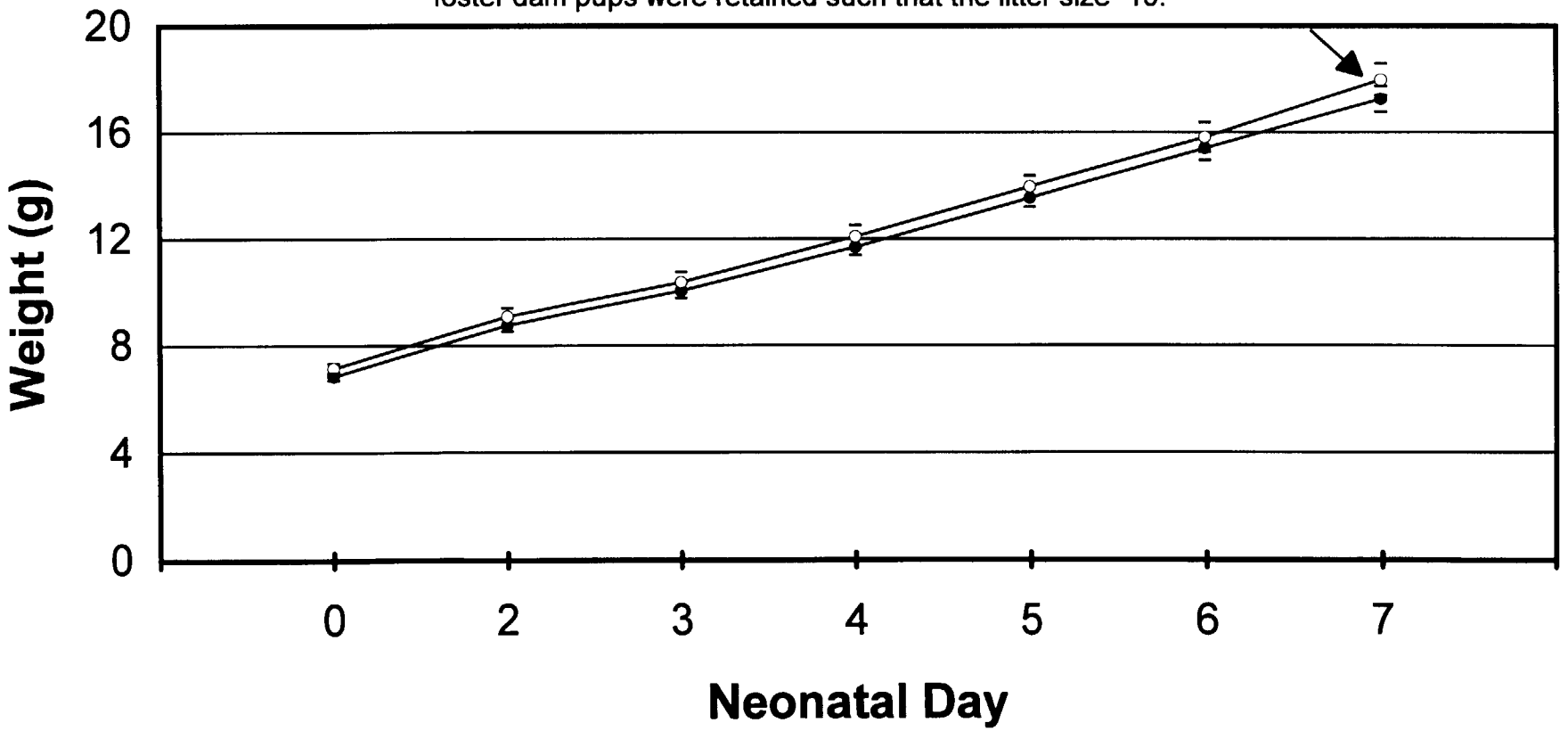

FIG. 55. BODY WEIGHT (Mean +/- SEM) OF DELAYED RECOVERY PUPS AND FOSTER PUPS (indicated by arrow) FOR NEONATAL DAYS 0 - 7

Pups delivered by test dams from the Delayed Recovery Group were transferred to a foster dam. An appropriate number of pups were retained such that the litter size = 10.

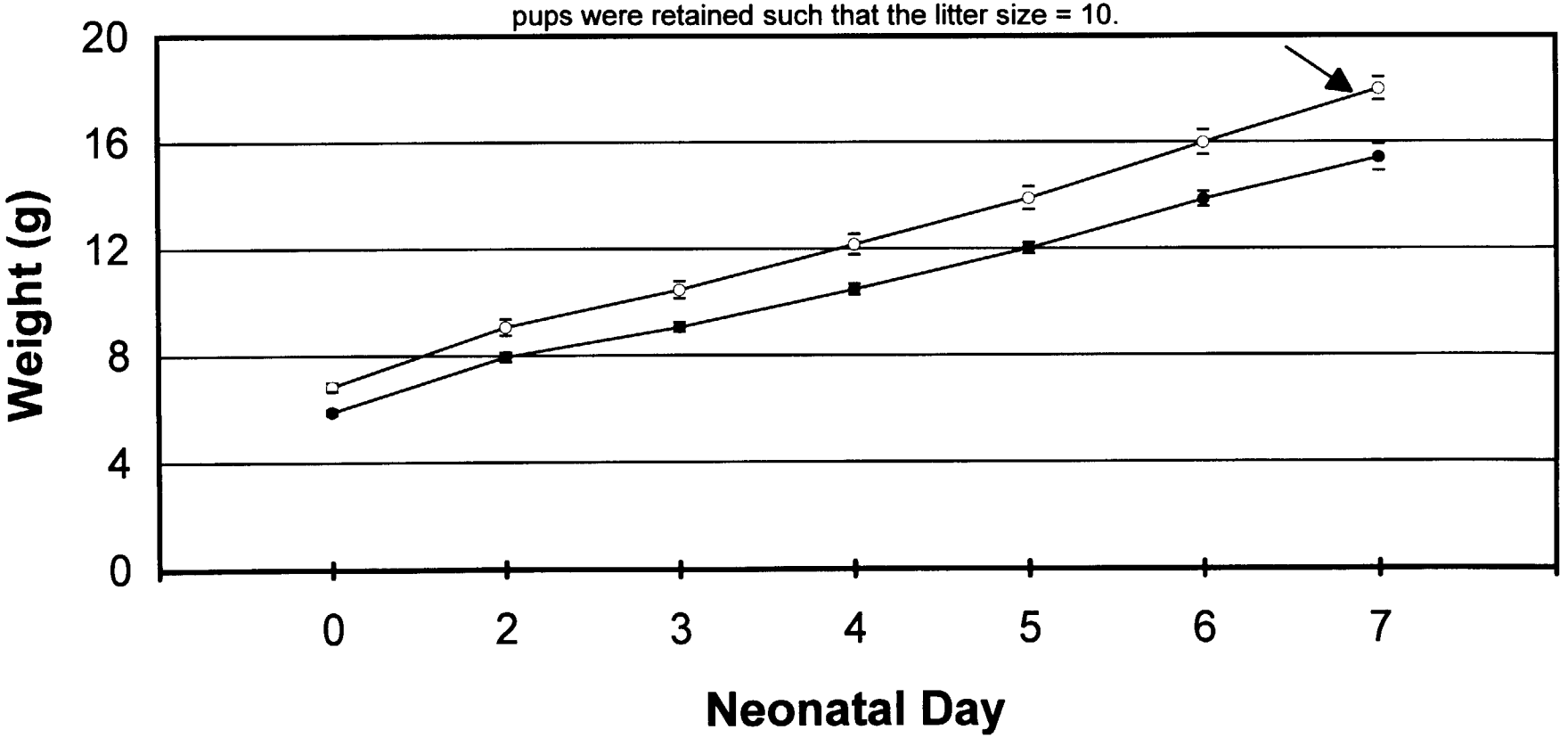

FIG. 56. THYMUS WEIGHT (Mean +/- SEM) OF PUPS AT NEONATAL DAY 7

Pups delivered by test dams were transferred to a foster dam. An appropriate number of foster dam pups were retained such that the litter size = 10.

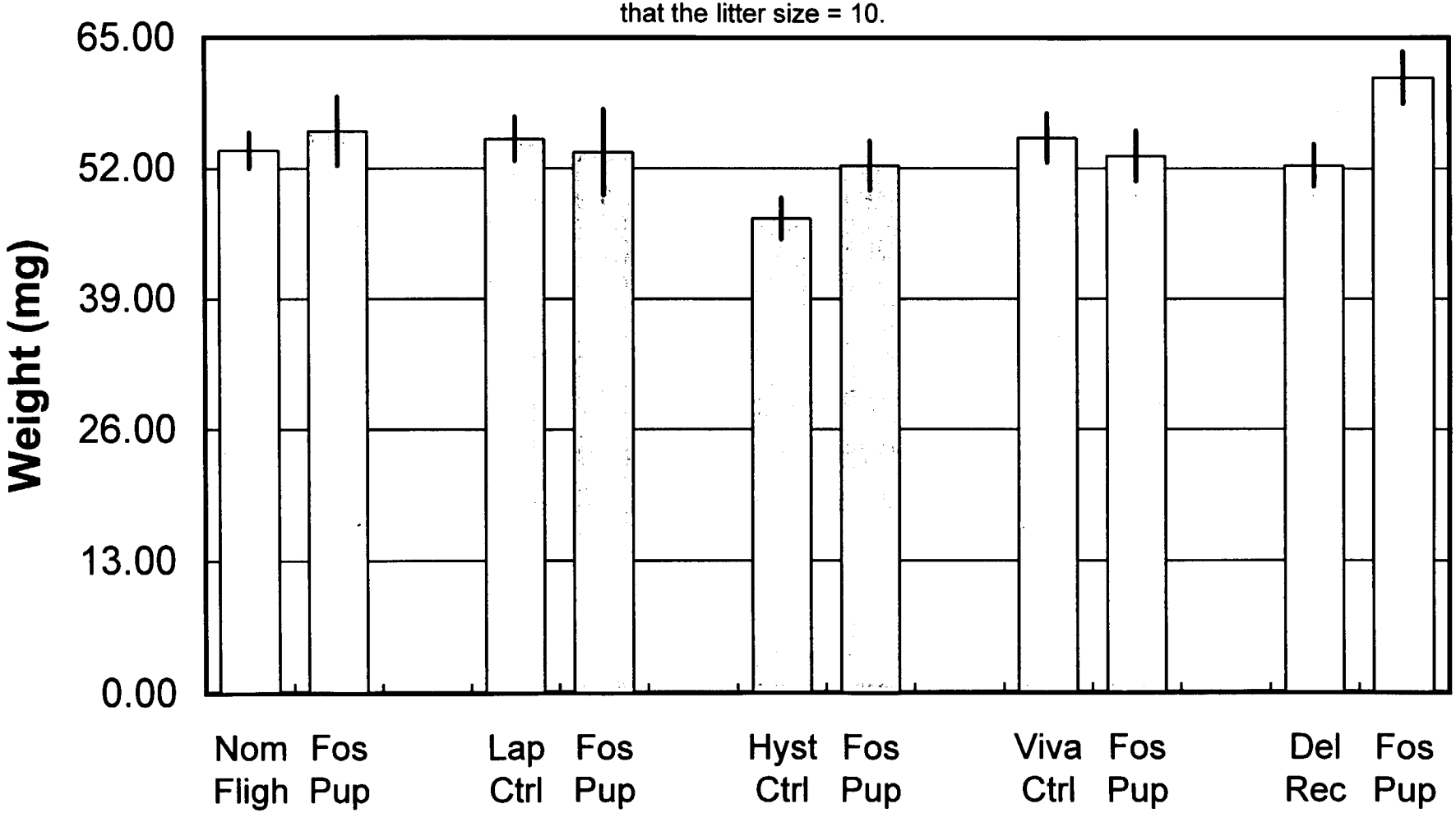

FIG. 57. COMBINED ADRENAL WEIGHT (Mean +/- SEM) OF PUPS AT NEONATAL DAY 7

Pups delivered by test dams were transferred to a foster dam. An appropriate number of foster dam pups were retained such that the litter size = 10