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Effects of Pre-Conception Parental Stress on Sex Ratio in Rat Litters

Pamela L. Parent
College of William & Mary - Arts & Sciences

EFFECTS OF PRE-CONCEPTION PARENTAL STRESS ON SEX RATIO IN RAT LITTERS

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Pamela L. Parent

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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Ellen F. Rosen, Ph.D., Chairperson

E. Rae Harcum, Ph.D.

Deborah G. Hartley, Ph.D.

Linda C. Petty, Ph.D.
Hampton Institute

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For my mother, Grace Cloutier Parent,
her mother, Eva Lambert Cloutier, and
for all my foremothers, whose names
have been ignored and lost by history.

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ABSTRACT

Relationships between pre-conception parental stress and litter sex ratio, weights, implantation rates, and litter size were explored. Stress consisted of confinement in wire cocoons prohibiting movement for 4 hours daily for 5 consecutive days. Pilorection, distress vocalization, excessive defecation and urination resulted from the stress procedure. Following the stress sequence, each male (stressed or non-stressed) was bred to 2 females (1 stressed, 1 non-stressed). Females were killed on gestational day 20, the pups counted, weighed, sexed, and the number of corpora lutea and implantation sites recorded.

Analyses of variance were conducted upon the various measures with no significance found although some definite trends appeared. Control groups averaged more pups per litter than any of the experimental groups and, as expected, groups with both parents stressed produced the fewest number of pups. The question of differential effects of the stress due to disproportionality between the size of the stress cages and the individual rats was raised. Sex ratio was not affected by the stress condition. It appeared that, for many female rats, the consistent stress precluded conception altogether. A possible interaction was observed in the percentage of male and female pups per litter and in the number of pups to the number of implantation sites.

EFFECTS OF PRE-CONCEPTION PARENTAL STRESS ON SEX RATIO IN RAT LITTERS

INTRODUCTION

The importance of the influence of stress upon psychological measures in the individual has long been interesting to psychologists (Selye, 1936). Selye considered his theories on stress as an extension of the Bernard-Cannon theories dealing with adaptive and homeostatic reactions of organisms (Cofer & Appley, 1964). This blending of theories suggests that organisms have an innate tendency to psychologically resist serious changes in their environment but that the adaptation of the organism "- acquiring stability in the face of disturbance - is achieved automatically" (Cofer & Appley, 1964).

An exact definition of the word "stress" is elusive and various definitions have been dependent upon the individual interests and biases of the various investigators. Pascal (1951), as reported by Cofer & Appley (1964, p. 437), first advanced a definition that included perceptions by the affected organism when he explained stress "in terms of a perceived environmental situation which threatens the gratification of needs". Miller (1953) sees stress as "any vigorous, extreme or unusual stimulation which, being a threat, causes some significant change in behavior...", while others see stress as closely akin to frustration (Lazarus, Deese, & Osler, 1952).

Cofer & Appley (1964) in an attempt to integrate the various definitions of stress proposed the following:

> "Psychological stress is defined
> as the state of an organism in

> any situation where he perceives that his well-being is endangered, and that he must devote all of his energies to its protection. It is proposed that stress is one stage of an arousal continuum, defined by successive instigation, frustration and stress thresholds, and followed by an exhaustion threshold marking a decline in arousal.."

This definition was important since it synthesized all previous definitions in such a manner that there was a clearer distinction between the concepts of stress, frustration, and conflict. Stress was now placed into the position of being a reaction to an untenable situation rather than the definition of the situation or of its components.

Physiological Effects of Stress

Selye, since the 1930's has been developing a theory of stress which views stress "as the state of the organism following failure of the normal homeostatic regulatory mechanisms of adaptation" (Cofer & Appley, 1964). Selye (1936) used the name General Adaptation Syndrome (GAS) in order to describe the systemic reactions of the organism to stress. Selye's operational definition of stress was "a state manifested by a syndrome which consists of all the nonspecifically induced changes in a biologic system" (Selye, 1936). The first stage of the GAS was the alarm reaction during which time mobilization of the body's emergency reactions (increased heart rate, increased number of red blood cells in order to carry the extra oxygen needed, releases of liver-stored sugar to the muscles, increased blood supply to the muscles and brain, etc) are activated by a large rise in the amount of adrenalin secreted by the adrenal medulla. During the second stage, the stage of resistance, the importance of the adrenal medulla decreases as the production of the adrenal cortex hormone drastically increases (Selye, 1956). The adrenal cortex manufactures a large number of steroid hormones such as the glucocorticoids, the mineralocorticoids, and sex hormones (androgens, estrogens, and progesterone-like compounds) (Hafez, 1965). The release of these steroids from the adrenal cortex is under the influence of adrenocorticotrophic hormone (ACTH), which is emitted in larger-than-normal quantities by the anterior pituitary during "emotional arousal" (Grossman, 1971). At the same time, there is a decreasing amount of thyroid-stimulating hormone (TSH) released by the anterior pituitary which causes a decrease in thyroid activity and a slow-down of body growth related to decreased somatotrophic hormone (STH) secretion. Additionally, prolonged severe stress can cause a reduction in the secretion of gonadotrophic hormones by the anterior pituitary (Gray, 1971).

Effects of Stress on Reproduction

As a post-conception phenomenon, the importance of maternal stress has long been recognized (Wigglesworth, 1964; Alexander and Williams, 1971; Williamson, 1971; Widdowson and McCance, 1975). In animals, it is often caused by overcrowded conditions. In humans, maternal hypertension (often related to maternal stress) is well accepted as a causative factor in the birth of "small-for-date" infants (Dawes, 1976).

Stressing pregnant rats has also been shown to produce feminized behavior patterns in male pups born from these mothers (Ward, 1972). Pregnant rats were stressed with a combination of immobilization and light-stress (200 foot candles of light directed at them while they were immobilized) and the male pups tested with a number of behavioral measures. Their male pups at maturity were found to exhibit frequent male counting behavior and generally to present a female response to other male rats. This response is due to the fact that ACTH, along with the corticosteroids, can permeate the placenta (Beck, 1967), and increase the amount of androgen secreted by the adrenal cortex while decreasing the plasma and urine testosterone levels as well as reducing testicular size (Ward, 1972).

Gray (1971) reports that stress in the female results in a reduced secretion of gonadotrophic hormones which in turn causes estrous cycle disruption, impairment of ovulation, implantation difficulties, and decreased uterine weight. In the male, Gray states that there may be reduced testosterone secretion and decreased sperm production due to the reaction of the animal to the stress. Although these effects caused by stress may seem intuitively correct, Gray (1971) offers no details on the type of stress producing these reactions in the animals.

Effects of Stress on Sex Ratio

Schuster and Schuster (1969; 1972) noted abnormal sex ratios in litters born to female rats stressed by immobilization for one hour per day for four consecutive days. To account for these results they proposed a theory in which they evoked what they called a "Darwinian survival-of-the-fittest concept". They hypothesized that the parent under the least amount of stress at the time of conception will reproduce its own sex. For example, an unstressed male and a stressed female would produce a significantly higher proportion of male pups than would be expected normally since the male would be the least stressed of the pair. In the 1969 study they used 36 male and 36 female rats divided into four groups: both parents stressed, the female stressed, the male stressed, and neither parent stressed. The stress procedure consisted of immobilization for one hour per day for four consecutive days. The rat was made to crawl into the point of a wire-screen funnel and the funnel was stapled shut behind the rat. Vaginal smears were performed and females in the stress group were stressed for the four consecutive days immediately following estrous. On the fifth day they were bred with either a stressed or an unstressed male. Gestation was uninterrupted and pups were sexed as soon as possible following birth and then again at weaning. Schuster and Schuster report results supporting their hypothesis on pre-conception parental stress to be significant at the .01 level for both groups with only one parent stressed, and significant at the .05 level for the interaction between the parental stress variables. Schuster and Schuster performed the original study in 1969, with a subsequent report in 1972 in an attempt to clarify some of the severe methodological problems present in the first study. Specifically these were problems relating to the ambient temperature in the areas where the experimental animals were housed, stressed and bred. Due to mechanical failures and oversights, the ambient room temperature fluctuated from 72°F to 68°F.

The results of this study are, however, inconclusive because of several severe methodological problems above and beyond that of varying temperature, the problem they attempted to resolve with the 1972 replication of the study. Study of the results indicates that data were collected from a total of only 11 litters, with no consistency of size among groups (one "group" contained a grand total of one litter). Additionally, pregnancies were allowed to run full-term and since parturition was unobserved, it was impossible to assume that the number of pups surviving birth were actually representative of the number born and the sex ratio at birth.

An alternative theory was proposed by Lane (1969). She hypothesized that as the stress of the mother increases so will the proportion of female offspring conceived increase. Directly in contrast to the Schuster and Schuster (1969) theory, this theory predicts that an unstressed male and a stressed female will produce significantly higher proportion of female offspring. Lane (1969) found that schizophrenic women, psychotic at the time of conception, ultimately gave birth to a disproportionately large number of female children (an important assumption in her theory is that psychotic schizophrenic women are under a tremendous amount of stress).

In their 1972 study, Lane and Hyde found that female rats stressed by immobilization prior to conception gave birth to significantly fewer male pups and had significantly smaller litters than did the females of the unstressed control groups. No males were stressed in this study. These results led to Lane's suggestion that the male zygote or fetus was, therefore, "less likely" to survive when the prenatal environment is "less than ideal". Money (1972) has suggested that male zygotes and fetuses are more likely to abort spontaneously as it may be that this is not an abnormal occurence that Lane has postulated. However methodological problems also flaw the Lane and Hyde study. Once again, only post-parturition sex ratios were obtained, allowing no information on resorption rates or even the number of pups born dead or killed at birth. Lane and Hyde (1972) mention that, to the best of their knowledge, there were no pups born dead or consumed by the mothers, but this is an unsupportable statement given the design of their experiment. Additionally, no males were stressed and all were allowed multiple breedings which could possibly have affected sperm production. Males who breed infrequently are more likely to produce a larger volume of Y chromosomes than males who breed frequently, thereby affecting the number of male pups possibly conceived (McCary, 1973). Goldstein (1967) discusses the theory that there are two distinct types of sperm, the X-bearing gynosperm and the Y-bearing androsperm, with the gynosperm producing an XX zygote and the androsperm producing an XY zygote.

Shettles (1961) has advanced an even more controversial theory that states that androsperm, as compared to gynosperm, have less specific gravity, longer tails, smaller and rounder heads, greater "swimming" speed, and are more susceptible to environmental changes. From these observations, Shettles concludes that conception of males must take place as close as possible to the time of ovulation since during this period the vaginal fluids are at their least acid stage, and therefore least hostile to the faster, but more frail androsperm. Numerous criticisms have arisen regarding this theory (Goldstein, 1967) including the rather simple observation that Shettles postulates are erroneous because he is basing his conclusions upon microscopic observations which may well have no validity in the living organism.

Hypotheses and Predictions

Cramer and Gill (1975) have suggested that reductions in the number of pups per litter may be due to environmental effects not only on the development of the fetus but also due to effects on the ovulation rate, the rate of preimplantation mortality of the zygote, and the rate of postimplantation mortality of the embryo. If Gray's (1971) theory expecting general degradation and impairment of reproductive functions proves viable then it would be expected that a number of areas would be affected. It may be expected that the stressed females will exhibit a lower number of corpora lutea per ovary due to reduced secretion of gonadotrophic hormones by the anterior pituitary (Gray, 1971). Intuitively, it would seem that stressed females would exhibit a higher number of resorption sites than would the unstressed females. An unfavorable uterine environment would be expected to cause the premature death of a number of embryos, or prevent their implantation altogether. If Money (1972) is correct and indeed the male zygotes are the most vulnerable then it would be expected that in the stressed females there would be a disproportionately high number of female pups present, in addition to a large number of resorption sites.

This study will employ four groups -- a control group (NxN) which is not subject to any experimental manipulation, and three groups followed under varied experimental conditions; male stressed, female unstressed control (SxN), male unstressed control, female stressed (NxS), and both male and female stressed (SxS). The smallest number of pups per litter is expected to occur in the group pairing stressed male and unstressed female. Again following Gray's (1971) hypotheses, the stress would be expected to affect not only the female but also the reduction of number and viability of the sperm would be expected to cause a reduced litter size. Given the physiological changes induced by consistent, low level preconception stress, it is also expected that the weights of the pups in the stressed groups will differ significantly from that of the pups in the control group. With a decrease in maternal TSH and STH it would seem probable that the pups would experience a slower-than-normal intrauterine growth pattern.

METHOD

Subjects

Subjects consisted of 20 male and 40 nulliparous female Holtzman albino rats (Holtzman Laboratories, Madison, Wisconsin). All subjects were from 47-57 days of age upon arrival and approximately 110-120 days of age at the time of use. Both males and females were housed in same-sex pairs until the onset of experimental manipulations, at which time the males were placed in individual cages (8x7x7). Purina Laboratory Rat Chow and water were available ad libitum, with a 12:12 artificial light-dark schedule in effect (light from 7A.M. to 7P.M.). Oxtetracycline HCl (Sussex Drug Products Co., Edison, New Jersey) was given to the rats in a 1 tbs. to 2 quarts water concentration for approximately 50% of the experimental period. This occurred in response to an outbreak of hemolytic streptococcus within another rat colony housed in the same room. No data was collected from any ill animals.

Stress

Twelve male and twenty-four female rats were subjected to experimental stress which consisted of confinement of the rats within individual immobilizing hardware-cloth cocoon-like cages (10x3x3), for four hours daily (Normally from 11A.M. until 3P.M.) for a period of five consecutive days. All animals were weighed daily for a seven day period beginning the day before the onset of the stress sequence and ending with the day after the stress sequence. Loss of weight is an accepted indicator of stress reaction in rats (Sines & McDonald, 1968).

Breeding Program

Prior to the stress procedure, each male was allowed to mate with one multiparous female rat in order to counteract the low conception rate found in matings where both rats are sexually inexperienced (Schuster and Schuster, 1969). Throughout the stress sequence, a vaginal lavage was examined daily from each female and a record kept of the course of her estrous cycle. The lavage was obtained by flushing cells from the vaginal tract of the female by inserting an eyedropper containing approximately one drop of water and withdrawing it repeatedly until a proper smear was obtained.

Immediately following the last stress period each male was placed into a breeding cage (24x9x7) with two female cagemates, one stressed and one an unstressed control. Vaginal lavages were performed daily to determine the presence of sperm. A sperm-positive lavage was considered indicative of pregnancy (day 0) and the female was placed into an individual cage (8x9x7). Four breeding combinations were utilized: stressed male x stressed female, unstressed male x stressed female, unstressed male x unstressed female, and stressed male x stressed female. Each male bred both females placed into his cage, i.e., each stressed male bred both a stressed female and an unstressed female, and each unstressed male bred both a stressed female and an unstressed female. Any groupings that did not result in impregnation of both females were dropped from the study and their data not utilized.

Autopsy Procedure

On gestational day 20, the pregnant rat was killed with an overdose of ether. An incision was made through the abdominal wall extending ventrally to the thoracic cavity, the horns of the uterus were exposed and the number of implantation and resorption sites were recorded. For the purposes of this study, an implantation site was defined as the presence of a fully developed fetus or any indication of the former presence of an implantation, i.e., a resorption site. Resorption sites are recognized by the presence of a metrial gland. Jordan (1972) explains metrial glands as "highly vascularized yellowish nodules which are found along the mesometrial margin of the uterine horns where they mark any original implantation site, whether the embryo or fetus with that site survives or not. Accordingly there is a metrial gland at the base of each placental attachment, but there may be metrial glands which are no longer associated with placentae and which represent sites at which embryos or fetuses have undergone prior death or resorption".

After this procedure, the ovaries were removed from each uterine horn and placed in saline solution for further examination, which consisted of counting and recording the number of corpora lutea on each ovary. A corpus luteum is normally formed on the ovary where an ovum is shed, and is readily apparent to the naked eye. The corpus luteum functions early in gestation by secreting progesterone which prepares the uterus for the embedding of the now traveling ova (Fox & Laird, 1970). A single incision was then made through the length of the left horn of the uterus and the uterine contents exposed, the fetuses were removed in serial order beginning at the left ovary and continuing in order down the left horn, across the cervix and up the right horn to the right ovary. Each implantation was assigned an identifying number and a record kept of the position of each fetus or resorption site and its position within the uterus. The individual fetuses were then removed from the uterus and examined for any gross abnormalities such as limb deformities, cleft palate, hydrocephaly, and micrognathia. Finally, the sex of the fetus was determined by observing the distance between the genital tubercle and the anus; in male pups at birth it is normally about 2.8 mm while in females the distance is about 1.2 mm (Farris & Griffith, 1942), while Jordan (1972) reports distances of approximately 2.0 mm in male pups and approximately 1.0 mm in female pups removed from the uterus at gestational day 20.

When the entire left horn of the uterus had been completed, the right horn was exposed and the same procedure repeated. All pups were killed with ether immediately following the autopsy procedures.

RESULTS

Six two-by-four analyses of variance with repeated measures on one factor (pre-parturitive litter size) were employed to analyze the data. Contrary to expectations, no significant difference between groups were found.

Table 1 details the results of the analysis with reference to the number of pups per litter in each of the four groups. It would appear that stress on either parent has some tendency to reduce litter size with the control group exhibiting an average number of 15.000 ($\pm$1.414) pups per litter. All experimental groups contained at least 3.667 less pups with a standard deviation of at least 1.841 larger than the control group. The interaction F ratio of 3.2040 does approach the .20 level of significance (F=3.078) suggesting the possibility that a larger number of subjects may have yielded clearer results. Further supporting this view is an r_m value ($.5 < r_m > .45$) showing a moderate magnitude of effect on litter size.

Analysis of Table 2, which explores the relationship between the number of male pups present in the litter and the stress administered, or not administered, to their parents reveals an F value for stress effect of only 3.0496 ($p > .20$) while exhibiting a moderate ($.35 < r_m > .30$) magnitude of effect. The mean number of male pups per litter ranged from 7.750 ($\pm$1.714) for the control group to 5.417 ($\pm$2.812) for the SxS group. Although the average number of male pups per litter was lower than the control group, the SxN combination yielded a standard deviation (1.863) quite close to the NxN standard deviation (1.774).

The number of female pups per litter (Table 3), while not significant ($p > .20$) did show a moderate difference ($.45 < r_m > .40$) from the stress procedures. The large difference between the mean number of female pups in the control litters (7.250 ± 2.271) and the mean number in the NxS grouping (4.500 ± 3.041) suggests the possibility of a differential effect of the stress upon the various females. This is suggested due to perusal of the raw data which indicates that 6 of the 8 NxS litters were conceived during the first half of the study. Since data was not collected from incomplete groups (groups which did not contain 2 impregnated females) it may be that the study did not include those females who were so severely affected by the stress that their normal estrous cycle was interrupted.

The relationship of the number of <u>corpora lutea</u> to the presence of absence of stress (see Table 4) showed no relationship or interaction whatsoever, with no F values even approaching 1.000 ($p > .20$) and a small magnitude of effect ($r_m > .30$). Although the 18.000 average number of <u>corpora lutea</u> for the SxS group is rather close to the 19.250 for the NxN group, the standard deviation of 8.485 for SxS and 4.815 for NxN are highly variant. The large standard deviation for the SxS group suggests once again possible differential effects of the stress upon the females.

The number of implantation sites (Table 5) shows no effect from the stress ($F=1.800$, $p > .20$) although examination of the averages from the various groups suggests otherwise and, indeed, exhibits a moderate effect from the stress ($r_m = .35$). The least affected was the relationship of stress to the number of resorption sites (Table 6) which showed no significant ($p > .20$) and little, if any, magnitude of effect ($r_m > .20$). Especially for this aspect, with such low numbers concerned and an N of no more than 12, the number of animals employed should be increased.

Table 7 reveals that while there is little variation between the number of days required for impregnation, there is a notably larger standard deviation for both the SxS and SxN groups.

Table 1

Number of Pups Per Litter

Male		Female	Mean		Standard Deviation
Stressed	x	Stressed	11.333	±	3.255
Stressed	x	Non-stressed	11.250	±	3.490
Non-stressed	x	Stressed	10.375	±	4.846
Control			15.000	±	1.414

Table 2

Number of Male Pups Per Litter

Male		Female	Mean		Standard Deviation
Stressed	x	Stressed	5.417	±	2.812
Stressed	x	Non-stressed	5.833	±	1.863
Non-stressed	x	Stressed	5.875	±	2.891
Control			7.750	±	1.714

Table 3

Number of Female Pups Per Litter

Male	Female	Mean		Standard Deviation
Stressed	x Stressed	5.917	±	2.691
Stressed	x Non-stressed	5.417	±	2.253
Non-stressed	x Stressed	4.500	±	3.041
Control		7.250	±	2.271

Table 4

Number of Corpora Lutea

Male		Female	Mean		Standard Deviation
Stressed	x	Stressed	18.000	±	8.485
Stressed	x	Non-stressed	17.333	±	5.071
Non-stressed	x	Stressed	16.375	±	5.977
Control			19.250	±	4.815

Table 5

Number of Implantation Sites

Male		Female	Mean		Standard Deviation
Stressed	x	Stressed	11.833	±	4.741
Stressed	x	Non-stressed	11.917	±	3.121
Non-stressed	x	Stressed	11.500	±	5.099
Control			15.250	±	1.199

Table 6

Number of Resorption Sites

Male		Female	Mean		Standard Deviation
Stressed	x	Stressed	0.500	±	0.675
Stressed	x	Non-stressed	0.333	±	0.623
Non-stressed	x	Stressed	0.625	±	0.695
Control			0.250	±	0.434

Table 7

Number of Days with Male Until Impregnation

Male		Female	Mean		Standard Deviation
Stressed	x	Stressed	4.45	±	3.09
Stressed	x	Non-stressed	4.72	±	3.53
Non-stressed	x	Stressed	4.50	±	2.06
Control			4.83	±	2.03

DISCUSSION

This exploration of the efforts of pre-conception parental stress on litter sex ratios indicates that while litter size may be affected, the actual sex ratio in the litter appears not to be altered appreciably. There were no statistically significant differences in the litters from the four groups of animals (SxS, SxN, NxS, NxN) although several of the factors studied (most notably the effects of the stress on size of the litter and upon the number of female pups it contained) exhibited r_m values indicating a moderate effect, suggesting further study with a larger number of subjects. From the results of this study it would also appear though that the stress procedures had little or no effect upon the number of corpora lutea found on the ovaries or on the number of resorption sites discovered in each uterus. It would appear that the stress effects occurred in the pre-implantation stages of gestation bringing to mind Shettles (1972) theory on the greater susceptibility of androsperm to environmental changes.

Previous investigators have furnished an extremely contradictory view of the results that might be expected from this type of inquiry, with one investigator reporting that more female offspring would be produced by a female under stress (Lane and Hyde, 1973) and another reporting that a similar mating would produce predominately male offspring (Schuster and Schuster, 1969, 1971). It appeared that as yet the primary question posed by the studies (e.g., the effects of pre-conception stress on litter sex ratios) had not been adequately explored. Methodological problems within both studies further complicated interpretation of their data resultant conclusions to be drawn about their findings. In the Schuster and Schuster (1969) report, conclusions are drawn from one all-male litter which by itself constitutes an entire group. Lane and Hyde (1973) offered an improvement in numbers of animals per group but they reduced the number of groups and did not deal with the effects of stress on the male. Additionally, both studies reported post- rather than pre-parturitive sex counts which could not account for any pups born dead or consumed by the mother. The use, in this study, of sex determination of the pups prior to birth gives an accurate view of the true litter sex ratio. It appears that although the litter sizes produced by two stressed animals is slightly smaller, the sex ratio remains relatively consistent with that found in the control litters. The pre-parturitive sex counts employed in this study also strongly suggest that stress applied prior to conception does not increase the number of dead pups or resorption sites encountered.

The obviously smaller size of the experimentally manipulated litters points to an effect of the stress on the fertilization process itself since the number of <u>corpora</u> <u>lutea</u> and resorption sites are not affected. This would support Shettles (1971) postulate that there are different types of sperm and that they are affected by the vaginal climate of the female. Additionally, this concept blends well with Gray's (1971) development of a theory of reduced gonadotrophic hormone secretion in the female and reduced testosterone and sperm production in the male under stress.

The results obtained in this study would indicate that the stressed or non-stressed condition of the parents at conception does not affect the sex ratio of the resultant litter. It may be that after a certain amount of consistent stress, the effects of the stress on the male or female precludes conception altogether. The results of the stress are quite apparent in analysis of the variability of the number of days needed for the impregnation of both females by the male. The average for the control group was 4.83 ± 2.03, while the SxS mean was 4.45 ± 3.09, SxN equalled 4.72 ± 3.53, and NxS averaged 4.50 ± 2.06 (Table 7). This would point to some effect influencing impregnation solely related to the male, since the SxS and SxN group numbers exhibited the greatest variation. Vaginal lavages of animals during the stress sequence did not reveal estrous cycle interruption suggesting the possibility of hormone levels reduced enough to prevent pregnancy but high enough to maintain normal cycling in the rat.

It seems reasonable to replicate this study employing a much larger number of animals and utilizing immobilization cages whose size is determined by the size and weight of the individual animal so that for each animal the stress is proportionately the same. One question raised by this study is whether, indeed, all animals were subject to the same amount of stress.

An interesting point is that of all the measures, those least significant were those involving the number of <u>corpora lutea</u> detected and the number of resorption sites discovered. This would indicate that the females were not strongly affected by the stress, in which case the condition of the male would be the determining factor affecting the sex ratio. Retrospectively, it appears that the male rats may have reacted more seriously to the stress confinement than did the females.

The uniform size of the stress cages may have allowed a sense of mobility to the female rats that was not afforded to the males due to their greater size. The stress procedure could have affected the sperm production and sperm motility of the males. As Gray (1971) has reported, it is possible for sperm viability and production to be reduced by continuous stress such as immobilization.

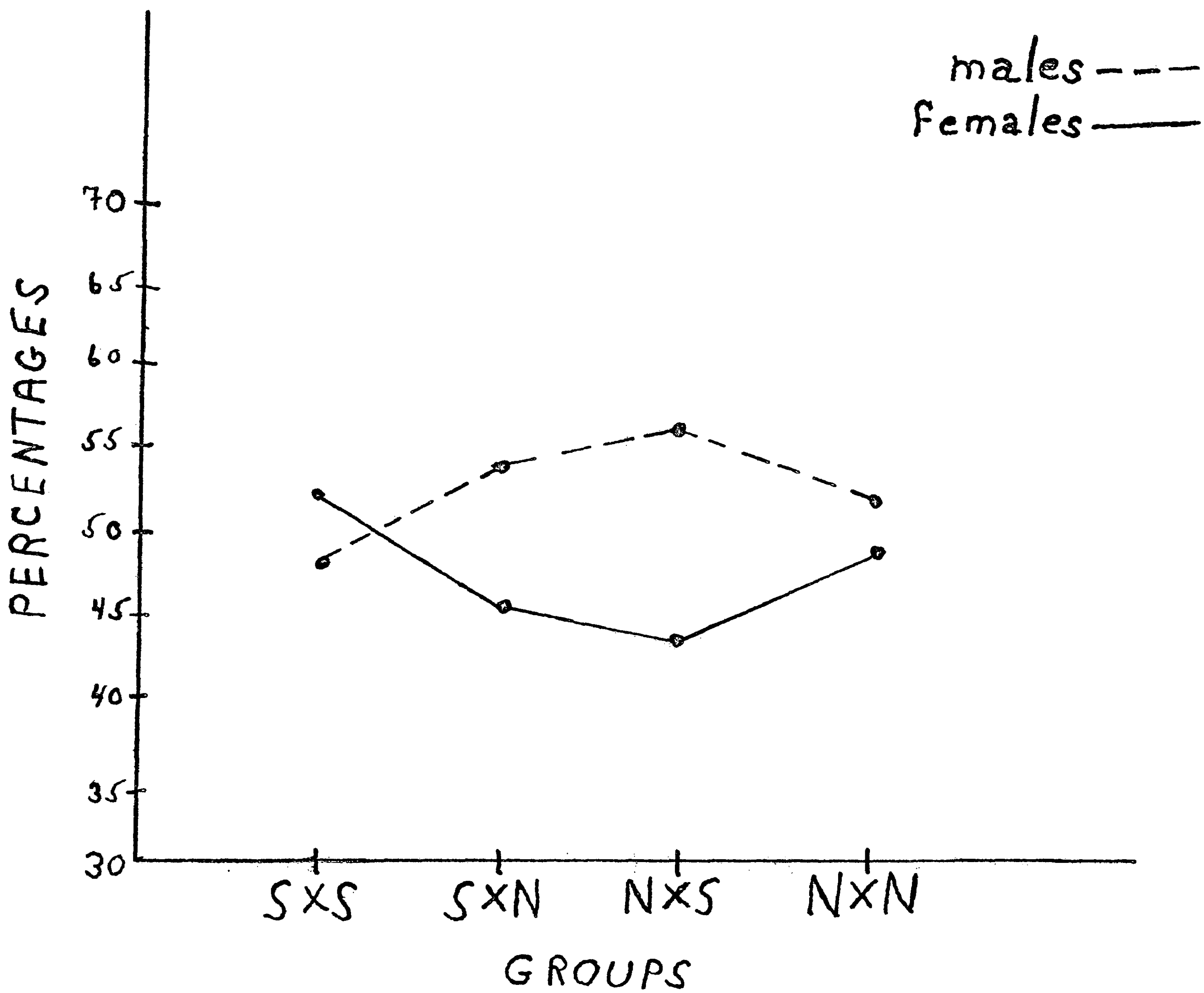
Some interaction was observed in the percentage of male and female pups per litter and in the ratio of male and female pups to the number of implantation sites (Figs. 1 and 2). As suggested previously, it would be advantageous to investigate these results using a larger subject pool. The value of the results from the cells containing 8 animals appears to lie in the questions raised by the trends they exhibit.

An accurate assessment of the sex ratios induced by varying parental stress aid in understanding the various physiological mechanisms involved in the effects stress on reproductive functions.

FIGURE 1

PERCENTAGE OF MALE AND FEMALE PUPS PER LITTER

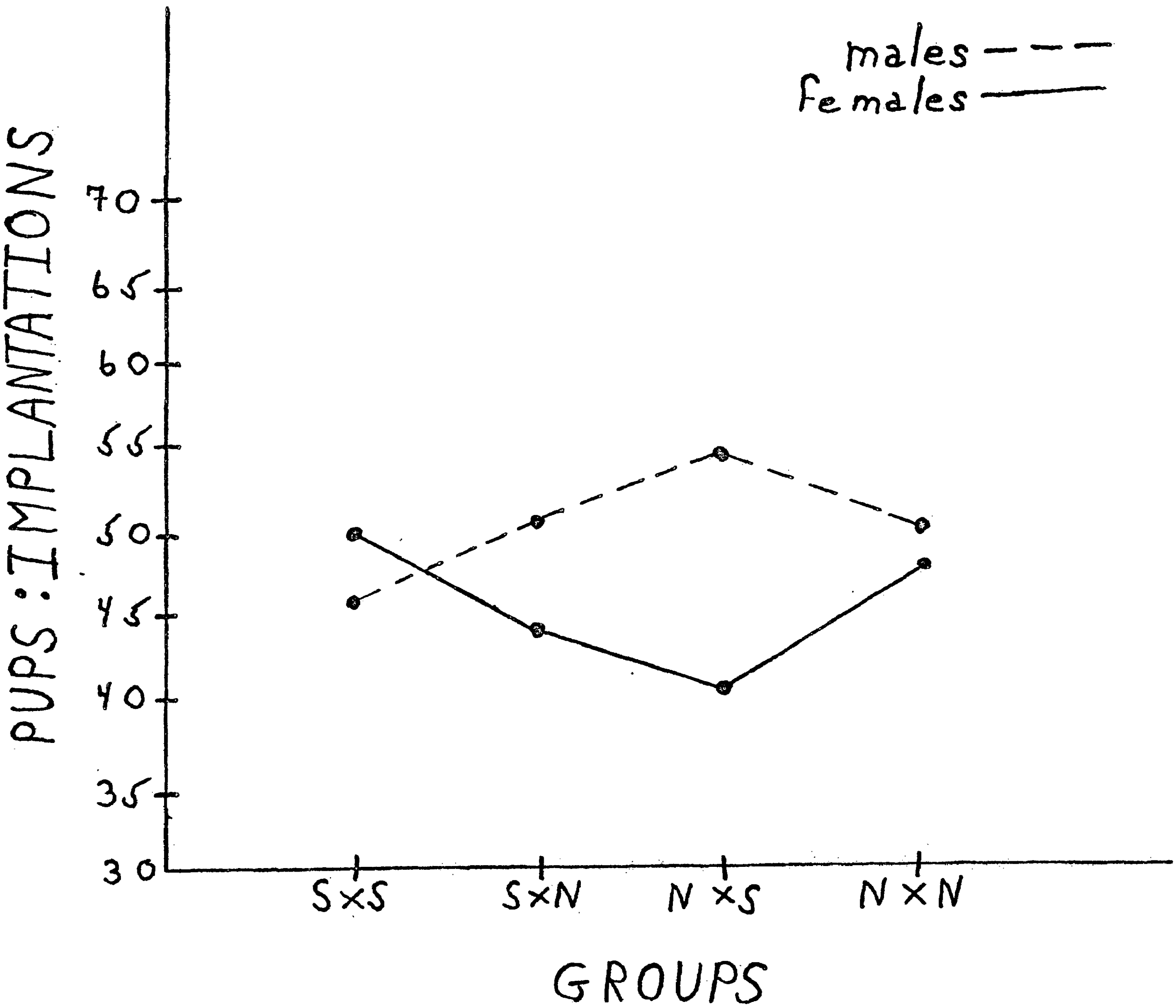
WITHIN EACH GROUP



males - - -
Females ——
PERCENTAGES
70
65
60
55
50
45
40
35
30
SXS
SXN
NXS
NXN
GROUPS

FIGURE 2

RATIO OF MALE AND FEMALE PUPS TO NUMBER OF IMPLANTATION SITES WITHIN EACH GROUP



males
females
PUPS:IMPLANTATIONS
70
65
60
55
50
45
40
35
30
S×S
S×N
N×S
N×N
GROUPS

APPENDIX

ANOVA – Number of Pups Per Litter

Source	SS	df	MS	F Ratio
Between	332.600	19.0		
A	19.704	1.0	18.704	1.073
Error A	313.895	18.0	17.438	
Within	384.500	20.0		
B	49.504	1.0	49.504	2.981
A*B	53.204	1.0	53.204	3.204
Error B	298.895	18.0	16.605	
Total	717.100	39.0		

ANOVA - Number of Male Pups Per Litter

Source	SS	df	MS	F Ratio
Between	166.100	19.0		
A	13.537	1.0	13.537	1.597
Error A	152.562	18.0	8.475	
Within	89.500	20.0		
B	12.604	1.0	12.604	3.050
A*B	5.104	1.0	5.104	1.235
Error B	74.395	18.0	4.133	
Total	255.600	39.0		

ANOVA - Number of Female Pups Per Litter

Source	SS	df	MS	F Ratio
Between	69.500	19.0		
A	0.416	1.0	0.416	0.109
Error A	69.083	18.0	3.837	
Within	210.000	20.0		
B	12.150	1.0	12.150	1.227
A*B	25.350	1.0	25.350	2.560
Error B	178.250	18.0	9.903	
Total	279.500	39.0		

Number of Corpora Lutea

Source	SS	df	MS	F Ratio
Between	1042.475	19.0		
A	0.204	1.0	0.204	0.004
Error A	1042.270	18.0	57.903	
Within	637.500	20.0		
B	11.704	1.0	11.704	0.350
A*B	30.104	1.0	30.104	0.901
Error B	601.770	18.0	33.431	
Total	1679.975	39.0		

Number of Implantation Sites

Source	SS	df	MS	F Ratio
Between	280.975	19.0		
A	21.600	1.0	21.600	1.499
Error A	259.375	18.0	14.409	
Within	403.000	20.0		
B	35.266	1.0	35.266	1.831
A*B	32.266	1.0	32.266	1.675
Error B	346.708	18.0	19.261	
Total	683.975	39.0		

Number of Resorption Sites

Source	SS	df	MS	F Ratio
Between	3.275	19.0		
A	0.004	1.0	0.004	0.023
Error A	3.270	18.0	0.181	
Within	12.500	20.0		
B	0.704	1.0	0.704	1.077
A*B	0.104	1.0	0.104	0.159
Error B	11.770	18.0	0.653	
Total	15.775	39.0		

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VITA

Pamela L. Parent

The author was born January 25, 1950, in Lewiston, Maine, and graduated from Lewiston High School. She received the degree of Bachelor of Science in Animal and Veterinary Sciences from the University of Maine, Orono, in 1971. The author entered the College of William and Mary in Virginia, where she is a candidate for the degree of Master of Arts in Psychology.

The author worked in teratology research at the Medical College of Virginia in Richmond, Virginia from May, 1972, until June, 1974. She served from September, 1974, through August, 1975, as a psychologist intern at Eastern State Hospital and from September, 1975, through May, 1976, as a graduate teaching assistant in the Department of Psychology at the College of William and Mary.