The Role of the Time Allowed for Mating on Variability of Fetal Weight in Rats

MASON BARR, JR.
Department of Pediatrics, University of Michigan, Ann Arbor, Michigan 48104

ABSTRACT In rats variation of fetal weights among litters is significantly greater than variation of fetal weights within litters. This phenomenon is called the litter effect. Twenty litters of Wistar rats were studied to determine whether or not variation in the duration of the period allowed for copulation (1.5 hours versus 15 hours) was a cause of the litter effect. Analyses of variance showed that the duration of the mating period did not significantly influence mean fetal weight, intralitter variability, or interlitter variability. It is concluded that the use of a restricted mating period as opposed to the commonly used overnight mating period offers no advantage from the standpoint of fetal weight variability. The need to consider the sample size of both litters and fetuses in teratologic and fetal growth studies is stressed.

A common practice in teratologic and fetal growth studies has been to consider a sample of fetuses from several litters as coming from one large litter. Jensh and Brent ('67) and Jensh et al. ('70) demonstrated that in rats the variation in mean fetal weight among litters is significantly greater than within litters, and therefore greater than that expected to occur within "one large litter." The interlitter variability has been called the *litter effect.*

There are several possible causes of the litter effect. Although mean fetal weight and litter size are correlated the litter effect persisted when differences in fetal number were controlled (Jensh et al., '70). The female rats used by Jensh et al. were mated with males during a 15-hour period. This suggests that spread in the time of conception among litters might cause variation in fetal weight when measured near term. This paper presents the results of an investigation of the role of the duration of the time allowed for copulation (mating period) in the causation of the litter effect.

MATERIALS AND METHODS

CFN Wistar rats, obtained from a commercial supplier, were used. Virgin females weighing 200–300 g were caged with males from either (a) 9:30 PM to 11 PM (short mating period) or (b) 5 PM to 8 AM (long mating period). Pregnancy was considered to have started (day 0) at 11 PM during the mating period if vaginal smears showed sperm the following morning. Each inseminated female was laparotomized at day 7.5, under pentobarbital anesthesia (35 mg/kg), and the number and intrauterine positions of implantations were recorded and the litter size was reduced to eight (four per horn) by crushing the excess implantations with blunt forceps.

At day 21.5 the pregnant females were again anesthetized with pentobarbital and the fetuses were delivered by cesarean section. The fetuses were kept under saline-soaked gauze until they could be separated from their placentas and membranes. The umbilical cords were severed at the fetal body wall by electrocautery to prevent fetal blood loss. The cleaned fetuses were then transferred to closed plastic boxes containing moist sponges to prevent air drying. The placentas were cleaned of all remnants of membranes and umbilical cord and placed in the boxes with the fetuses.

As soon as possible after delivery each fetus was quickly blotted dry on absorbent toweling and weighed to the nearest milligram. The placentas were similarly weighed. The fetuses were then labeled for future identification and subsequently

dissected and examined for malformations using Wilson's ('65) cross-section method.

The fetal and placental weight data were analyzed using *t* tests for comparing means and analyses of variance for comparing inter- and intralitter variations.

RESULTS

Forty-six pregnant females were used, 20 for the short mating period (SMP) and 26 for the long mating period (LMP). At laparotomy on day 7.5 and day 21.5 seven and 17 litters, respectively, had fewer than four implantations in one or both uterine horns (7.5-SMP, 3, LMP, 4; 21.5-SMP, 6, LMP, 11) and were excluded from the study. One litter in each group contained malformed fetuses and these litters were also excluded. Ten litters in each mating-period group satisfied the criterion of having four normal fetuses in each uterine horn. The mean fetal and placental weights of these litters are presented in table 1. There were no statistically significant differences between the mean fetal weights ($P > 0.10$) or the mean placental weights ($P > 0.90$) of the two groups. There was no significant difference ($P > 0.10$) between the maternal day-0 weights. The amount of weight gained during pregnancy was not significantly different between the two groups of animals $P > 0.40$).

The analyses of variance (table 2) showed that for both groups variations in fetal and placental weights were greater among than within litters thus demonstrating that variation in the duration of the mating period is not the cause of the litter effect. Aside from the litter effect there were no significant differences in placental weight between the two groups.

The fetal weight data were tested for intralitter variability between the SMP and LMP groups. The mean standard deviations of the two groups were similar ($P > 0.70$). The mean absolute deviation of fetal weights from the mean litter weight was essentially the same in both ($P > 0.90$). Thus it is apparent that variation in the duration of the mating period had no influence on variability of fetal weights within litters.

The data were tested to determine whether the variability of mean fetal weight among litters in the LMP group was greater than in the SMP group. The mean fetal weights for the two groups were not significantly different (table 1), but the standard deviation of the LMP group was larger than that of the SMP group indicating a wider dispersion of litter mean fetal weights about the common mean. Also suggesting this possibility was the *F* ratio obtained on the analysis of variance (table 2) for the LMP group, which was greater than that obtained for the SMP group. While there was greater dispersion of the litter means about the common mean in the LMP group a *t* test

TABLE 1

Fetal and placental weight data from litters of Wistar rats in which the litter size was controlled experimentally to eight (four fetuses per uterine horn). Comparison of short and long mating periods

Mean fetal weight ± SD [1] (g)		Mean placental weight ± SD (g)	
Short mating	Long mating	Short mating	Long mating
5.224 ± 0.212	4.756 ± 0.300	0.489 ± 0.067	0.491 ± 0.051
5.207 ± 0.245	4.979 ± 0.358	0.536 ± 0.052	0.497 ± 0.046
4.885 ± 0.313	5.160 ± 0.425	0.467 ± 0.034	0.426 ± 0.054
5.380 ± 0.171	5.588 ± 0.184	0.417 ± 0.044	0.527 ± 0.019
4.902 ± 0.265	5.216 ± 0.161	0.463 ± 0.029	0.481 ± 0.043
5.050 ± 0.215	5.095 ± 0.189	0.420 ± 0.026	0.449 ± 0.038
5.292 ± 0.256	5.004 ± 0.189	0.499 ± 0.045	0.475 ± 0.043
5.100 ± 0.347	4.520 ± 0.277	0.499 ± 0.037	0.419 ± 0.033
4.837 ± 0.205	5.118 ± 0.147	0.440 ± 0.054	0.475 ± 0.028
5.212 ± 0.268	4.930 ± 0.411	0.485 ± 0.064	0.474 ± 0.038
Mean	Mean	Mean	Mean
5.109 ± 0.298	5.036 ± 0.379	0.471 ± 0.057	0.471 ± 0.049
$t_{158} = 1.342$		$t_{158} = 0.012$	
$0.20 > P > 0.10$		$P > 0.90$	

[1] SD, standard deviation.

on the mean absolute deviation of mean litter weights from the mean weight of all fetuses showed that the difference between the LMP and SMP groups was not statistically significant ($P > 0.50$). This finding was confirmed by the more sensitive analysis of variance method ($P > 0.10$). Two litters in the LMP group were responsible for the wider dispersion of the litter means (fig. 1). The mean fetal weight

TABLE 2

Analyses of variance among litters versus within litters. Computed for fetal weight and placental weight for litters of rats with short or long mating periods

	Sum of squares	df	Mean square	F ratio
Fetal weights (g)				
Short mating period				
Among	2.488	9	0.276	4.257
Within	4.546	70	0.065	
Long mating period				
Among	5.782	9	0.642	8.058
Within	5.582	70	0.080	
Placental weights (g)				
Short mating period				
Among	0.103	9	0.011	5.134
Within	0.156	70	0.002	
Long mating period				
Among	0.077	9	0.009	5.176
Within	0.116	70	0.002	

$F_{0.9995}(9,70) = 3.90$

All tests show differences "among" are greater than "within," $P < 0.0005$

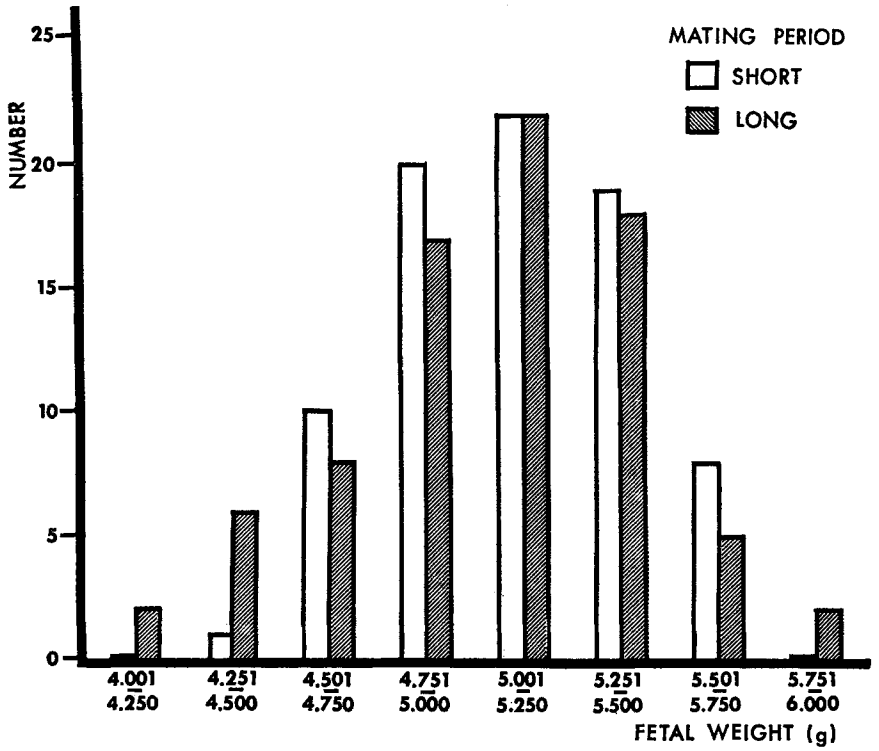

Fig. 1 Distribution of fetal weights for litters with short and long mating periods.

of one litter (5.588 ± 0.184 g) was considerably higher than the group mean (5.036 ± 0.379 g) while that of the other (4.520 ± 0.277 g) was considerably lower. It is possible that these two litters represent variations in the time of conception, early and late respectively, during the long mating period.

DISCUSSION

A common approach to teratologic and fetal growth studies is to consider each group of experimental fetuses as representing one large litter. The number of fetuses studied is usually considered of primary importance and little attention is directed to the number of litters involved. Jensh and Brent ('67) and Jensh et al. ('70) showed that even in inbred populations there are individual maternal factors that influence the development of the fetus which should not be ignored. There are considerable differences in mean fetal weight among litters, and these differences are of significantly greater magnitude than intralitter weight differences. This variability of fetal weights among litters in excess of the intralitter variability has been called the litter effect.

It is apparent that there may be several components to the litter effect. That the number of fetuses in the litter and the number in a single uterine horn influence fetal weight is beyond dispute (Eckstein et al., '55; McLaren, '65; Barr et al., '70). However, the data reported by Jensh et al. ('70) and in this study demonstrate that even when both the number of fetuses per litter and per horn are controlled the litter effect is still present. Data on maternal weight gain during pregnancy and their effect on fetal weight revealed no significant correlations (unpublished data).

A frequent practice in experiments involving the breeding of rats for timed pregnancies is to allow the animals to mate during an overnight period. Theoretically some animals might copulate and conceive early in the mating period (e.g., 6 PM) while others might copulate and conceive later (e.g., 6 AM). Although it would be erroneous to assume that copulation-fertilization or copulation-implantation intervals are inflexible a 12-hour difference in the time of copulation might be apparent in fetal weight at day 21 of pregnancy. However, the results of this study indicate that fetal weight and more particularly the dispersion of litter mean fetal weights about the common mean did not differ significantly according to the duration of the mating period. Lisk ('69) reported that most copulatory activity in rats occurred between 3.5 and 5.5 hours after the beginning of the dark phase of the light cycle. In the present study the males and females in the short-mating-period group were together from 4.5–6 hours after the start of the dark phase. Those in the long-mating-period group were together for the entire dark phase. If variation in the time of copulation is reflected in variation in fetal weight at day 21 the data suggest that in at least 18 of the 20 animals conception took place within a relatively short span of time. It is concluded that the use of a restricted mating period, as opposed to the commonly used overnight mating period, offers no advantage from the standpoint of reducing fetal weight variability.

The fact that there was a litter effect indicates that there were individual maternal variations among the rats which exerted important influences on fetal growth. The rats used in this study, while not strictly inbred by rigid genetic criteria, were at least from a relatively inbred strain and, therefore, the likelihood that the litter effect was due to genetic variability is not great. Even if the nature of the maternal variations could be determined it is not at all sure that it would be practical to control them and thus eliminate the litter effect.

The litter effect appears to be an important variable which has received little attention to teratologic and fetal growth studies. Unless statistical methods designed to eliminate the litter effect (Barr et al., '69, '70) are used in the analysis of fetal data the sample size of litters studied would appear to be of greater importance than the sample size of fetuses. The actual number of litters needed in a given study to override the litter effect statistically will depend on the characteristics of the population and of the measurements being made.

LITERATURE CITED

Barr, M., Jr., R. P. Jensh and R. L. Brent 1969 Intrauterine position and fetal weight in rats. Teratology, 2: 241–246.

——— 1970 Prenatal growth in the albino rat. Effects of number, intrauterine position and resorptions. Am. J. Anat., *128:* 413–428.

Eckstein, P., T. McKeown and R. G. Record 1955 Variation in placental weight according to litter size in the guinea-pig. J. Endocr., *12:* 108–114.

Jensh, R. P., and R. L. Brent 1967 An analysis of the growth retarding effects of trypan blue in the albino rat. Anat. Rec., *159:* 453–458.

Jensh, R. P., R. L. Brent and M. Barr, Jr. 1970 The litter effect as a variable in teratologic studies of the albino rat. Am. J. Anat., *128:* 185–191.

Lisk, R. D. 1969 Cyclic fluctuations in sexual responsiveness in the male rat. J. Exp. Zool., *171:* 313–320.

McLaren, A. 1965 Genetic and environmental effects on foetal and placental growth in mice. J. Repr. Fert., *9:* 79–98.

Wilson, J. G. 1965 Methods for administering agents and detecting malformations in experimental animals. In: Teratology: Principles and Techniques. J. G. Wilson and J. Warkany, eds. Univ. of Chicago Press, Chicago, pp. 262–277.