THE INFLUENCE OF INTRAUTERINE FACTORS ON THE FETAL WEIGHT OF RABBITS

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A lethal dwarf mutation in the rabbit, which appeared during the course of experiments designed for other purposes, has been the subject of a previous report (1) from this laboratory. These dwarfs, though born alive, all die within a few days. They are delicately formed and appear to be fully developed, but the bones of the calvarium are usually incompletely calcified. The observations on these animals appeared to indicate an incomplete recessive mutation which is characterized morphologically by a dwarfing effect and functionally by disorders in both heterozygous and homozygous individuals.

The occurrence of this dwarf mutation emphasized the desirability of a general consideration of the factors influencing the birth weight of the rabbit. Studies pursued with this end in view had, however, the particular purpose of ascertaining to what extent environmental influences could account for the abnormally low weights of the animals denominated dwarfs. In approaching this problem, it was believed desirable first to determine whether intrauterine factors were operative in producing variations in fetal weight at or near term. An analysis of data accumulated with this end in view, together with certain collateral findings, forms the basis of the present report. Future communications will deal with the influence of gestation period, litter size, age and weight of the doe, season, and breed, on the birth weight of the rabbit.

Material and Methods

The observations on 475 fetuses carried by 71 pregnant rabbits form the basis of the present report. The majority of the does, all of which were bred in the colony, were the progeny resulting from miscellaneous hybrid matings, but a few were descended in pure line from original standard bred stock. Similarly, the stud bucks were for the most part derived from hybrid ancestry, although an occasional standard bred animal was represented. The rabbits in the colony are all housed in individual cages, and the diet comprises a standard uniform ration. Matings are personally supervised by members of the staff. To procure the desired mating, the doe is placed with the buck in the latter's cage, and after copulation, returned to its own cage. It is possible after the lapse of a 10 day interval to determine the pregnant or non-pregnant state of the doe by abdominal palpation. If pregnant, it is permitted to go to term; if non-pregnant, the desired mating is repeated. In this way the date of the copulation which resulted in fertilization is obtained.

An effort was made in the present investigation to examine the fetuses as near term as possible. At intervals ranging from 23 to 31 days after the fertile copulation, the pregnant does were sacrificed by air injection into the marginal ear vein. The pelvic organs were immediately exposed and the uterine horns opened. Each fetus was removed from its membranes, and the placentae carefully detached from the uterine wall. The weight of each fetus and of the corresponding placenta was then determined by means of a Toledo automatic balance calibrated in 1 gm. intervals. In addition, careful records were made of the uterine horn in which the fetus was located, that is, whether right or left, and of the fetal presentation and position or order. By presentation is meant that part, head or breech, which is directed toward the uterine outlet. The position or order indicates the relative locus of the fetus in the horn, the first position being that nearest the Fallopian tube. In summary, the following information was available with reference to each fetus: age, weight, weight of placenta, horn, presentation, and position or order.

Statistical Analysis.—In deriving the significance of the difference between the right and left uterine horns with regard to such variables as number of fetuses, total weight of fetuses, mean weight of fetuses, etc., the mean difference and its standard error were calculated. The mean difference (Md) was obtained by the formula $\frac{\Sigma d}{n}$, and its standard error from the formula

$$\sqrt{\frac{\Sigma(d)^2 - \Sigma d \cdot Md}{n(n-1)}},$$

d representing the difference between the numerical observations on the right and left horns of any doe. This method of comparison eliminates the variation due to fetal age, breed, maternal weight, and other uncontrolled variables. In a few instances comparisons were made by the use of the standard error of the mean and the standard error of the difference between means.

The χ^2 test of homogeneity is described by Fisher (2) who also gives tables for translating various levels of χ^2 into terms of probability. The method of analysis of variance in which the ratio F is used is that described by Snedecor (3), F being a symbol for the ratio of the larger to the smaller variance. The term "variance" as employed by Fisher, indicates the square of the standard deviation, *i.e.*, $V = \sigma^2$. The simple zero order correlations were calculated by the usual statistical methods, the text by Wallace and Snedecor (4) being employed as a guide. For determining the significance of these correlation coefficients, the formula

$$t = \frac{r}{\sqrt{1 - r^2}} \cdot \sqrt{n' - 2}$$

was employed. Published tables (2) are available for transferring t into probability terms. In all statistical procedures, significance has been attached to values of $t \geq 2.5$ or $P \leq 0.01$; that is, when the probability of an event occurring by chance alone was 1 or less than 1 in 100, the result was considered significant.

In a few instances the use of absolute values was abandoned in favor of relative values. This procedure was adopted in order to place the observations on an equal basis by reducing them all to percentage terms. In this way the observations on the weights of fetuses 28 days old, for instance, could be summated with those of 30 day fetuses since age as a variable was eliminated.

A description of the method employed for the graphic presentation of the observations has been presented in detail elsewhere (5). The horizontal broken lines in the figures block off intervals on the ordinate which are equal to the estimated value for the standard error of the depicted means. When the heights of any two vertical bars are separated by at least two and a half such intervals, then the difference between the represented means is significant.

RESULTS

The material on which the analysis is based is presented in Table I. There were 475 fetuses carried by the 71 does, the number per doe ranging from 1 to 12, with a modal value of 7. The number of fetuses per uterine horn varied from 0 to 7, with 4 as the modal class.

Fetal Weight and Age.—The average gestation period of the rabbit is 31 and a fraction days (6). As shown in Table II, 63 or 88.7 per cent of the 71 does were sacrificed from 28 to 31 days after the last fertile mating, and these does carried 401 or 88.2 per cent of the total of 455 fully developed fetuses. The weights of 20 degenerated fetuses were not included. The relative daily increase in mean fetal weight was as follows: from 27 to 28 days, 16.3 per cent; from 28 to 29 days, 26.4 per cent; from 29 to 30 days, 8.1 per cent; from 30 to 31 days, 6.5 per cent. These values represent an average relative daily increase of 14.3 per cent for gestation periods ranging from 27 to 31 days. It is of interest to note that as the gestation period approaches its normal limit of 31 days, the relative daily increase is progressively retarded. Whether this retardation is the result of an inherent growth potentiality of the developing fetus, or is due to an exhaustion of the nutritive and tensile capacity of the maternal uterus, is a question which cannot be answered on

TABLE I

The Weights of Rabbit Fetuses and Corresponding Placentae

Doe's No. in series	Gestation period	Presentation	Horn and position	Weight	
				Fetus	Placenta
	days			*gm.*	*gm.*
1	27	H	L1	27.5	5
		H	L2	29	4.5
		B	R1	26	5
			R2	D	D
		B	R3	31	6
		B	R4	25	4.5
2	28	B	L1	30	6.5
		H	L2	29	4.5
		H	L3	28	4.5
		H	R1	32.5	7
3	28	H	L1	37	6
		H	L2	36	5
		H	L3	37	5
		H	L4	34	6
		H	L5	40	5.5
		B	R1	36	5.5
		B	R2	38	6
4	27	B	L1	25	4
		H	L2	32.5	6
		H	L3	27	4
		B	L4	26	4
		B	R1	28	4.5
		B	R2	29	6
		B	R3	30	5
		H	R4	28.5	4.5
5	29	H	L1	46	3.5
		H	L2	49	6
		H	R1	48	5
		B	R2	46	4
		B	R3	44	4
			R4	D	D
		H	R5	46	4.5
		B	R6	50	5.5
6	28	H	L1	35	6
		H	L2	38	6.5
		H	R1	36	5.5
7	28	H	L1	29	4.5
		B	L2	29	4.5
		H	L3	23	4
		B	L4	25	3.5
		H	L5	21	3.5
		H	L6	25	4
		B	R1	24	4
8	28	H	L1	28	4
		H	L2	27	5
		H	L3	22	5
		B	L4	25	3
		H	L5	21	3
		B	L6	23	3
		B	R1	30	5
		B	R2	29	4
			R3	D	D
			R4	D	D
			R5	D	D
9	28	H	L1	31	4.5
		B	L2	27	4
		B	L3	30	4
		B	L4	25	4
		H	R1	31	5
		H	R2	32	6
		B	R3	26.5	4
		H	R4	29	4.5
10	28	H	L1	40	7.5
		H	L2	37	4.5
		B	L3	36	7.5
		H	L4	33	5.5
		H	R1	31	5
		H	R2	35	7
		B	R3	30	6
		H	R4	33.5	5.5
		H	R5	28	5.5
		H	R6	33	6

H = Head; B = Breech. L1 = left horn, first position; R3 = right horn, third position, etc. D = Degenerated.

TABLE I—*Continued*

Doe's No. in series	Gestation period	Presentation	Horn and position	Weight: Fetus	Weight: Placenta
	days			*gm.*	*gm.*
11	28		L1	34	5.5
			L2	34	4
			L3	34	4
			L4	34.5	4.5
			R1	38.5	6
			R2	34	6.5
12	28	H	L1	38.5	5
		B	L2	32	4
		H	L3	28	4
			R1	D	D
		B	R2	36	5
13	27	H	L1	25	4.5
		H	L2	23.5	3.5
		B	L3	25	4
		H	L4	24	4
		B	L5	27	4
		H	R1	25.5	4.5
		H	R2	26	4.5
		B	R3	28	5
		B	R4	28	4.5
14	27	H	L1	28	4.5
		H	R1	25	4
		H	R2	21.5	4
		B	R3	21.5	3
		H	R4	26	4
		H	R5	28	4.5
15	27	H	L1	34	6
		H	L2	30	5
		H	L3	33	6
		H	L4	31	5
16	28		L1	30	5
			L2	27	4
			L3	31	5
			L4	28	5
			R1	33	5
			R2	29	5
17	28	B	L1	32	5
		H	L2	37	6.5
		B	L3	38	7
		B	L4	40	8
		B	R1	36	7
		H	R2	38	8
		H	R3	27	5
		H	R4	29	5
18	28	B	R1	35.5	10
19	28	H	L1	33	4.5
		H	L2	27	3
		H	L3	32	4
		H	L4	23	3
			L5	D	D
		B	R1	27.5	3
		H	R2	30	4
		H	R3	25	3.5
		H	R4	27	4
20	29	H	L1	38.5	4.5
		B	L2	34.5	4
		B	L3	34	4
		H	R1	42	5
		H	R2	40	4
		B	R3	38	4
		H	R4	37	4
21	27	H	L1	32	4
		H	L2	27	4
		H	L3	24.5	3.5
			L4	D	D
		H	L5	30	4
		B	L6	28	4
		B	L7	24	3
		H	R1	31	4
		H	R2	30	4
		B	R3	26.5	4

TABLE I—*Continued*

Doe's No. in series	Gestation period	Presentation	Horn and position	Weight		Doe's No. in series	Gestation period	Presentation	Horn and position	Weight	
				Fetus	Placenta					Fetus	Placenta
	days			*gm.*	*gm.*		*days*			*gm.*	*gm.*
22	28	B	L1	32	5	26	29	B	R2	31.5	4
		B	L2	30	5			B	R3	35.5	4.5
		H	R1	32.5	5.5			B	R4	29	4
		B	R2	30	4.5						
		H	R3	30	5	27	29	H	L1	43	4
		B	R4	26	3.5			H	L2	50	6.5
								H	L3	36	3
23	28	B	L1	31	4			H	L4	43	4
		B	L2	26	3.5			B	R1	45.5	5.8
		B	L3	25	3			H	R2	47	4
		H	L4	25.5	3				R3	D	D
		H	R1	32	5			H	R4	41	3.5
		H	R2	35	5						
		B	R3	28	3	28	30	H	L1	49.5	4
		H	R4	29	3			H	L2	49	3.5
		B	R5	21	3			B	R1	43	3
		H	R6	24	2			B	R2	50	4
		B	R7	30	4			H	R3	50	4
24	29	B	L1	36	4	29	30	H	L1	40	4
		H	L2	34	4			H	L2	28.5	3.5
		B	L3	30	3.5			B	L3	35	4
		H	L4	30	3.5			H	L4	27	3
		B	L5	34	3.5			H	L5	35	4
		B	R1	47	5.8			H	R1	36	4
		H	R2	38	4			B	R2	31.5	4
		B	R3	38.5	4			B	R3	36	3
		H	R4	38.5	4						
						30	30		L1	D	D
25	29	H	L1	41.5	5			H	L2	54	5.5
		B	L2	36	4			B	R1	50	5.5
		B	L3	41	4.5			H	R2	47	5
		B	L4	37.5	4			H	R3	50	6
		H	L5	33.5	4						
		H	R1	45	5	31	31	H	L1	49	8
		B	R2	45.5	5.5			B	L2	51	7
		H	R3	42	5.5			B	R1	48	7
26	29	H	L1	33.5	5	32	31	H	L1	51	5.5
		H	L2	32	4			H	L2	53	6
		B	L3	35	5			H	L3	43	5
		H	R1	38	5.5			B	L4	45.5	5
								B	R1	50	6

TABLE I—*Continued*

Doe's No. in series	Gestation period	Presentation	Horn and position	Weight: Fetus	Weight: Placenta
	days			*gm.*	*gm.*
33	26	B	L1	24	5
		H	L2	23	5.5
		H	L3	22	5
		H	L4	20	4.5
		H	L5	21	5
			R1	D	D
			R2	D	D
		B	R3	22.5	5
		H	R4	22	5
34	23	H	L1	9	3
		H	L2	10	3
		H	R1	9	3
		H	R2	8.5	2.5
		H	R3	10	3
		B	R4	10	3
35	30	B	L1	44	4
		H	L2	40	4
		B	L3	45	3
		B	R1	46	4.5
		H	R2	41	4
		B	R3	45	4.5
		B	R4	45	4
36	30	B	L1	48	4.5
		H	L2	43	4.5
		H	R1	44	4.5
		H	R2	40	4
		H	R3	39	4
		B	R4	40	4.5
37	29	H	L1	41	5
		H	L2	40	5
		H	R1	38	5
		B	R2	39	4
		H	R3	36	4
		B	R4	36	4
		H	R5	35	4
38	29	H	L1	46	6
		H	L2	47	6
		H	L3	43	5
		H	L4	33	4
		H	R1	44	5
		H	R2	42	5
		B	R3	46	5
		B	R4	39	4
39	29	H	L1	37.5	5
		B	L2	37.5	5
		B	R1	41	5
		B	R2	38.5	5.5
		B	R3	36	4
		H	R4	34	3
40	28	H	L1	33	4
		H	L2	39	5
		B	R1	35	5
		B	R2	37	6
		H	R3	36	5
41	29	B	L1	40	5.5
		B	L2	48	6.5
		B	R1	40	6
		B	R2	40	4.5
		H	R3	42	4.5
		H	R4	41	5
		B	R5	42	5.5
42	28	B	L1	36	5
		B	R1	40	6
		H	R2	38	5
		H	R3	39	6
43	31	B	L1	52	7
		B	L2	50	6.5
		B	L3	45	6
		H	R1	52	7

TABLE I—*Continued*

Doe's No. in series	Gestation period	Presentation	Horn and position	Weight	
				Fetus	Placenta
	days			gm.	gm.
44	31	B	L1	34	5
		B	L2	31	4
		B	L3	38	5.5
		B	L4	32	4.5
		H	L5	29	4
		B	R1	38	5.5
45	29	H	L1	42	4
		H	L2	32	4
		H	L3	35	4
		B	R1	37	5
		B	R2	28	3
		B	R3	34	5
		H	R4	30	3
		H	R5	35	5
46	29	B	L1	44	7.5
		B	L2	38	6
		B	L3	38	5
		B	R1	40	6
		H	R2	40.5	6
		B	R3	39	5.5
		H	R4	40	5
47	29	B	L1	38	5
			L2	D	D
		B	L3	36	5
		B	L4	29	4
		B	L5	35	4
		H	R1	40	4.5
		H	R2	36	4.5
		H	R3	27	3.5
48	29	H	L1	48	6
		B	L2	39	4
		B	L3	40	5
		B	R1	43	5
		B	R2	38	4
49	29	B	L1	50	7
		H	L2	43	5
		H	L3	46	5.5
		B	L4	43	6
49	29	H	R1	46	6
		B	R2	44	6
		B	R3	44	5.5
		H	R4	47	5.5
		H	R5	44	6
50	29	H	L1	46	6
		H	L2	45	5
		H	L3	43	5
		B	R1	45	6
		H	R2	43	6
		H	R3	41	5
		H	R4	42	5
51	29	H	L1	52	10
			L2	D	D
			L3	D	D
		H	R1	43	7
		B	R2	48	9
		B	R3	47	8
52	29	H	L1	45	7
		B	L2	45	8
		B	L3	47	7
		H	L4	42	7
		H	L5	44	6
		H	R1	47	6
		H	R2	40	7
		H	R3	42	8
		H	R4	44	7
		H	R5	37	6
		H	R6	33	4
		B	R7	36	6
53	29	H	L1	43	6
		H	L2	25	2.5
		H	L3	33	4
		B	L4	38	4.5
		B	L5	35	4
		B	R1	42	5
		B	R2	36	4
		H	R3	36	4

TABLE I—*Continued*

Doe's No. in series	Gestation period	Presentation	Horn and position	Weight		Doe's No. in series	Gestation period	Presentation	Horn and position	Weight	
				Fetus	Placenta					Fetus	Placenta
	days			*gm.*	*gm.*		*days*			*gm.*	*gm.*
54	29	B	L1	45	8	60	29	H	L1	53	8
		B	L2	47	7			H	L2	43	5
		H	R1	48	7			B	L3	45	6
		H	R2	42	6			B	L4	32	4
		B	R3	40	6			B	R1	45	7
								H	R2	48	8
55	29	B	L1	40	5			B	R3	48	6
		B	L2	40	5						
		B	L3	43	5	61	29	H	L1	45	5
		B	L4	39	5			H	L2	50	6
		H	L5	39	5			H	R1	50	7.5
		B	R1	43	7			H	R2	46	6
		B	R2	38	5			H	R3	39	4
56	29	B	L1	33	5	62	31	H	R1	63	6
		B	L2	33	4				R2	D	D
		B	R1	36	5			B	R3	67	6
		H	R2	34	6			H	R4	53	5
		H	R3	32	5						
		B	R4	39	5	63	28	H	L1	29	4
								H	L2	30	4
57	29	H	L1	40	5			B	L3	31	6
		B	L2	32	5			H	L4	33	4
		H	R1	47	8			B	R1	34	4
		H	R2	34	6						
						64	30	H	L1	50	7
58	31	B	L1	44	6			H	L2	52	6
		H	L2	47.5	9			B	L3	47	5
		B	L3	43	5			H	L4	47	5
		H	R1	46	7			H	R1	51	6
		H	R2	37	4.5			B	R2	48	5
		H	R3	38	6						
		B	R4	42	6	65	30	H	L1	52	6
								H	L2	47	6
59	31	B	L1	54	6			B	L3	40	4
		B	R1	60	6.5			H	L4	33	4
			R2	D	D			B	L5	45	5
			R3	D	D			H	R1	50	6
		H	R4	44	4			B	R2	48	7
		H	R5	51	5			B	R3	47	6

TABLE I—*Concluded*

Doe's No. in series	Gestation period	Presentation	Horn and position	Weight: Fetus	Weight: Placenta	Doe's No. in series	Gestation period	Presentation	Horn and position	Weight: Fetus	Weight: Placenta
	days			*gm.*	*gm.*		*days*			*gm.*	*gm.*
66	29	H	L1	37	5	69	29	H	L1	38	6
		B	L2	35	4			H	L2	34	5
		B	L3	36	4			H	L3	28	5
		B	L4	35	4			H	L4	28	5
		H	L5	42	5			H	L5	31	4
		H	R1	40	7			B	R1	43	8
		H	R2	38	4				R2	D	D
						70	28	H	L1	35	7
67	28	B	L1	34	4			B	L2	35	7
			L2	D	D			H	L3	34	6
		B	L3	35	5			B	R1	46	6.5
		H	R1	31	4.5			B	R2	33	7
		B	R2	36	4.5			H	R3	35	6
		B	R3	37	5			B	R4	27	5
								B	R5	32	6.5
68	29	H	L1	40	5	71	28	B	L1	37	5
		H	L2	38	4.5			H	L2	35	5
		H	L3	36	4			H	R1	26	5.5
		B	L4	30	3			H	R2	30	5
		B	R1	40	6			B	R3	30	5
		H	R2	36	5			H	R4	30	5
		B	R3	34	3			B	R5	33	5.5

TABLE II

*The Relation between Fetal Weight and Age**

Age, *days*	23	26	27	28	29	30	31
No. of does	1	1	6	21	28	7	7
No. of fetuses	6	7	41	131	194	44	32
Mean weight of fetuses, *gm.*	9.42	21.6	27.0	31.4	39.7	42.9	45.8
Standard deviation of mean, *gm.*	—	—	0.45	0.42	0.40	0.79	1.62

* The weights of 20 degenerated fetuses are not included.

the basis of the present observations. Draper (7) has noted a similar retardation in the fetal growth of the guinea pig. In this species, fetal growth occurs rapidly from the 15th to the 25th day, and thereafter the relative growth rate decreases rapidly at first and then more slowly for the rest of the gestation period.

Comparison of the Number of Fetuses in Each Uterine Horn.—A total of 475 fetuses were observed. Of these, 236 were in the right horn and 239 in the left. The mean difference of 0.042 ± 0.140 was statistically insignificant. In Ibsen's (8) observations on the guinea pig there was likewise no difference between the number of fetuses in the right and left horns. 32 does of the present series had a greater number in the right than in the left horn, 31 had a greater number in the left horn, and 8 had an equal number in each horn. These values are not any different from those one would expect by the operation of chance alone. 20 or 4.2 per cent of the 475 fetuses were degenerated and non-viable. 7 of these were located in the left horn as compared with 13 in the right. ($\chi^2 = 3.6$, not significant.) The 20 degenerated fetuses were found in 15 different does; in 6 of these the degenerated individuals were located in the left horn and in 9 in the right. The difference between these values is not significant ($\chi^2 = 1.2$). In summary, the observations indicated that there was no difference between the number of fetuses in each uterine horn; that in any particular doe the chances were equal that the number of fetuses in either horn would be greater or less than the number in the other horn; that when degeneration of a fetus occurred, it had an equal chance of taking place in either horn; and that if a given doe had a degenerated fetus, there was an equal chance that it would be found in either horn.

The Frequency of Head and Breech Presentation, and the Influence of Right and Left Sidedness on Presentation.—Presentation is here taken to mean that part of the fetus which is directed toward the uterine outlet, *i.e.*, head or breech. Transverse presentations were not encountered. The presentation of 443 fetuses carried by 69 does was recorded. The head was the presenting part in 247 or 55.7 per cent of these, and the breech in 196 or 44.3 per cent. The difference between these values is statistically significant ($\chi^2 = 11.56$, $P < 0.01$), indicating that in our material, head presentation was significantly more frequent than breech. Ibsen (8) has noted a similar disproportion between head and breech presentation in the guinea pig.

There were 219 fetuses in the right uterine horn; 115 or 52.5 per cent had the head as the presenting part. Of the 224 in the left horn, 132 or 58.9 per cent were head presentation. The difference between these values is statistically not significant ($\chi^2 = 1.84$). Thus the uterine horn in which the fetus was located had no influence on its presentation.

The Relation between Presentation and Position.—The order of the fetus in the uterine horn, whether first, second, third, etc., has been denominated "position." The first position is that highest in the uterine horn, nearest the Fallopian tube. Table III presents the relation between the presentation and position of the fetuses. An analysis of these values indicates that the ratio between the frequency of head and breech presentations in the several positions is probably significantly different from the ratio represented in the totals ($\chi^2 = 7.95$, $P = 0.05$). It is evident that the principal discrepancy exists in the third position, in which there is an excess of fetuses with breech presentation. The reason for this discrepancy is not at present understood.

Comparison of the Total and Mean Fetal Weight in Each Horn.—Considering only the 56 does with fetuses which were all normally developed and showed no evidence of degeneration, the total fetal weight in one horn as compared with the other was not significantly different although the weight in the left horn was slightly greater than that in the right (Mean difference = 5.83 ± 4.73 gm.). The total fetal weight was greater in the left than in the right uterine horn in 26 does, and in 30 does the total fetal weight in the right horn was greater than in the left. The difference between these findings is not significant ($\chi^2 = 0.56$). Since it has already been seen that the total number of fetuses in each uterine horn was essentially the same, and since the horns showed no difference with regard to the total fetal weight in each, one would expect that the mean fetal weight in each horn should be similar. This actually was the case. Of 28 does bearing three or more fetuses in each horn,

TABLE III

*The Relation between Fetal Presentation and Position**

Presentation	Position								Total
	1		2		3		4 and over		
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	
Head	76	73.8	70	64.1	40	51.9	61	57.4	247
Breech	56	58.2	45	50.9	53	41.1	42	45.6	196
Total	132	132	115	115	93	93	103	103	443
x	2.2		5.9		11.9		3.6		
$S\frac{x^2}{m}$	0.15		1.12		6.18		0.50		7.95

x = difference between observed and expected value. m = expected value. S = sum.

* 12 fetuses of 2 does were omitted because their presentation was not recorded.

the mean difference between the average fetal weights in the right and left horns was 0.73 ± 0.41 gm., a statistically insignificant value. In 16 of these does the mean fetal weight in the right horn was greater than the mean fetal weight in the left; in 10 the reverse was found, and in 2 the mean weight in each horn was the same. These values are not any different from those one would expect by the operation of chance alone ($\chi^2 = 2.76$). To summarize, there was no difference between the horns with regard to total fetal weight; in any doe, the chances were equal that the total fetal weight in one horn would be greater or less than the total fetal weight in the other; in a group of does each bearing three or more fetuses in each horn, the average fetal weights in one horn were no different from the average fetal weights in the other; and the chances were equal that in any doe bearing three or more fetuses in each horn, the mean fetal weight in one horn would be greater or less than the mean fetal weight in the other.

The Influence of Presentation on Fetal Weights.—To determine whether there existed any relationship between fetal presentation and weight, two methods of analysis were employed. The first was based on the fetal weights in litters containing at least two fetuses with each variety of presentation. There were 51 such litters; for each of these, the average weight was calculated for those individuals with head and for those with breech presentation. The average weight of head presenting fetuses was greater than the average for breech presenting fetuses in 20 litters; the opposite was the case in 28 litters, and the average weights for head and for breech individuals were the same in 3 litters. There is no significant difference between these values ($\chi^2 = 2.68$). The mean difference between the averages for head and breech fetuses also was statistically insignificant

TABLE IV

The Relation between Fetal Weight and Order of Implantation in Uterine Horn Combined Right and Left Horns*

Position or order	1	2	3	4 and 4+	Total
No. of fetuses	89	87	88	95	359
Mean weight, *per cent*	105.99	99.89	98.12	95.81	99.88

Analysis of Variance

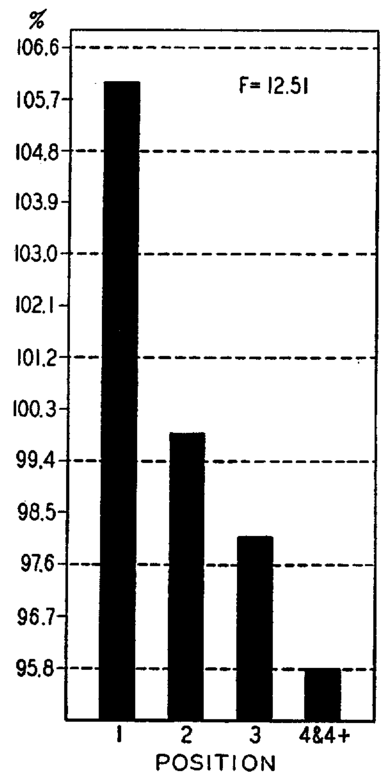
Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	358	57,320.9	160.1
Between means of position classes	3	5,483.8	1827.9
Within position classes	355	51,837.1	146.0

* Fetal weight is expressed as a ratio to the mean fetal weight in the corresponding horn. Only those horns containing 3 or more fetuses are included.

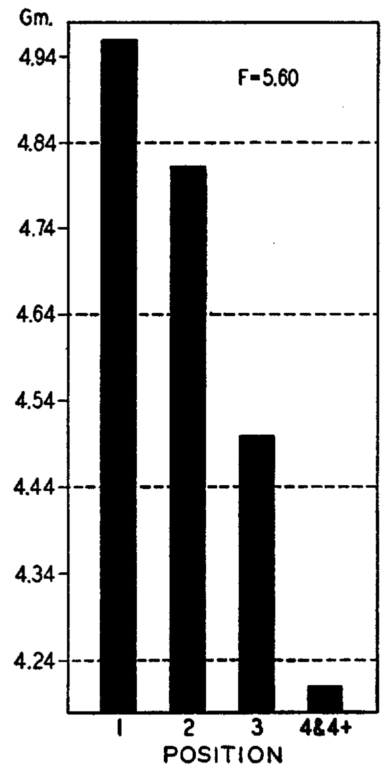
although the weight of breech was slightly less than that of head fetuses (Mean difference = 0.164 ± 0.24 gm.).

Since by the above method the average weight was in some cases determined by only two values, and since such an average was less accurate than others based on four or five values, an alternate method of analysis was employed. There were 29 does in which a head and a breech presenting fetus were found in the first position. In this group there were 18 fetuses with breech presentation in the left horn as compared with 11 in the right. There is no significant difference between these values, indicating that with respect to the distribution of head and breech fetuses in each horn the series is homogeneous. In 16 of these 29 pairs the weight of the head presenting fetus was greater than that of its breech presenting mate; in 11 the opposite was observed, and in 2 the weights of both were similar. These values do not differ from chance distribution ($\chi^2 = 0.64$). The mean difference, 0.40 ± 0.51 gm., between the weights of breech and head fetuses is not significant, but the mean weight of the breech fetuses was slightly greater than that of the head fetuses. The analysis thus indicates that fetal weight was not influenced by presentation.

The Influence of Position on Fetal Weight.—A preliminary survey indicated that



TEXT-FIG. 1



TEXT-FIG. 2

TEXT-FIG. 1. The relation between fetal weight and position.

TEXT-FIG. 2. The relation between placental weight and position.

Fetal weight is expressed as a ratio to the average weight in the corresponding horn. In each case the horizontal dotted lines intersect intervals on the ordinate equal to the estimated value for the standard error of the difference between any two means. When the heights of any two vertical bars are separated by at least two and a half such intervals, the populations represented are significantly different.

the weight of the fetus is significantly influenced by its position in the uterine horn. Considering only the first 33 does in the series, there were 54 uterine horns containing two or more fully developed fetuses. In three of these horns the first and last position fetuses had identical weights. However, 35 or 68.6 per cent of the remaining 51 first position fetuses were heavier than their mates occupying the last position in the corresponding horn, and this is significantly greater than would be expected by random sampling alone ($\chi^2 = 14.16, P < 0.01$, significant). In terms of absolute weight values, the mean difference between the weights of the first and last fetus in the left horn was $+2.2 \pm 0.51$ gm. ($t = 4.3, P < 0.01$, significant), and in the right horn $+1.92 \pm 0.52$ gm. ($t = 3.7, P < 0.01$, significant).

The above observations indicated the desirability of a more precise analysis of the relation between position and fetal weight. This is presented in Table IV and Text-fig. 1. Each uterine horn of the 71 does was considered individually, and those containing fewer than three fetuses were excluded. The fourth and succeeding fetuses were classified into one group, and the weights of each individual were expressed as a ratio to the mean fetal weight in the respective horn. The employment of relative instead of absolute fetal weight values eliminates any variability which might be due to fetal age, maternal weight, breed, etc., and places all observations on a comparable basis. It is seen that there is a progressive decrease in mean fetal weight from a high value of 106.0 per cent in the first position to a low of 95.8 per cent in the fourth and over position. By the method of analysis of variance it was found that the variance between the mean values for each position was significantly greater than the variance of the values within the position classes ($F = 12.51$, significant). This demonstration of heterogeneity between position classes indicates that the weight of the fetus is significantly influenced by position.

Since the above analysis considered only those uterine horns containing three or more fetuses, it does not indicate the influence of position on fetal weight when there are only two or only three or only four fetuses in a horn. A further analysis was undertaken with this consideration in mind.

1. Two Fetuses in a Horn.—25 uterine horns of 24 does contained only two fetuses each. 14 of these, occupying the first position had weights greater, and 9 had weights less than their mates in the same horn. In two horns both fetuses had identical weights. There is no significant difference between these values ($\chi^2 = 2.50$). The mean difference between the weights of the first and second position fetus was $+0.74 \pm 0.60$ gm., an insignificant difference. Thus, when there were only two fetuses in any uterine horn, position did not materially influence their weights.

2. Three Fetuses in a Horn.—There were 28 uterine horns of as many does in each of which three fetuses were found. Here again, by employing relative weights obtained from the ratio of the absolute weight of a fetus to the mean fetal weight in the horn, there was noted a progressive decrease in weight with increasing distance from the Fallopian tube. The mean relative weight in position 1 was 104.37 per cent, in position 2, 98.87 per cent, and in position 3, 96.88 per cent.

The variance between the means for each position was significantly greater than the variance of the values comprising each position class ($F = 11.17$, significant). From this it follows that when there were three fetuses in a horn, position significantly influenced fetal weight, fetuses in the first position in general weighing most, those in the third position weighing least, and those in the second position having an intermediate weight.

3. Four Fetuses in a Horn.—29 does had 34 uterine horns containing four fetuses each. The weight of each fetus was again expressed as a ratio to the mean fetal weight in the horn. The mean ratio for each position ranged from a high value of 105.55 per cent in the first position, to a low value of 94.83 per cent in the fourth position, with intermediate values of 101.04 per cent in the second and 98.38 per cent in the third positions. There was significant heterogeneity between each position as indicated by the significant value of $F = 13.31$. It is evident that when there were only four fetuses in a horn (and it will be recalled that four represented the modal class for number of fetuses per horn), their weights were significantly influenced by their position in the uterine horn, those located highest in the horn having in general the greatest weights, those lowest in the horn near the outlet having the lowest weights, and those occupying intermediate positions having intermediate weights. Ibsen (8) noted in the guinea pig that in the 65 day stage those fetuses nearest the ovaries weighed approximately the same as those nearest the vagina. During the later stages, however, the fetuses nearest the ovary and the vagina averaged more in weight than those between these extremes.

DISCUSSION

Of major interest in the foregoing analysis was the observation that fetal weight at or near term was significantly influenced by the position or order in the uterine horn. In general, the weights of fetuses implanted high up in the horn nearest the Fallopian tube were greater than those developing nearest the outlet, and fetuses occupying intermediate positions had intermediate weights. In attempting to arrive at an explanation for this phenomenon, our attention was directed to the placenta.

A preliminary analysis indicated that there was a high degree of relationship between fetal weight and placental weight. The weights of the heaviest and lightest fetus and of the corresponding placentae were recorded for each of the first 32 litters. The mean difference between the weights of the placentae corresponding to the heaviest fetus and the weights of the placentae corresponding to the lightest fetus was $+1.37 \pm 0.128$ gm., a highly significant value ($t = 10.7$, $P < 0.01$). Moreover, in 30 of the 32 litters the placenta of the heaviest fetus weighed more than the placenta of the lightest fetus, and the difference varied from 0.5 to 3.0 gm. There thus appeared to be a high positive correlation between fetal and placental weights.

The character of this correlation was investigated further by the method of linear correlation. The coefficient of correlation between fetal weight and placental weight was +0.3521 ($n = 161$, $t = 4.7$, $P < 0.01$), which is highly significant. In determining this coefficient, the 161 observations on the first 28 litters in the series were employed, and fetal weight was expressed as a percentage of the mean weight of the corresponding litter. Absolute values for placental weight were employed however. Had placental weight been placed on the same relative basis as fetal weight, a still higher correlation coefficient would have been noted. This statement is based on a comparative analysis of the 57 fetuses and placentae of the first 9 does. When both the fetus and the placenta were given a relative value based on the ratio to the mean weight in the doe, the coefficient of correlation was +0.4582 ($n = 57$, $t = 3.8$, $P < 0.01$), while when relative values were employed for the fetus only and absolute values for the placenta, the smaller coefficient of +0.3378 ($n = 57$, $t = 2.6$, $P < 0.01$) was obtained.

This finding of a high positive correlation between fetal and placental weight merely indicates that in general heavy fetuses and heavy placentae, and light fetuses and light placentae are associated. From these observations one would expect that placental weight should be influenced by the order of implantation in the uterine horn in exactly the same manner that fetal weight was so influenced. This in fact was found to be the case. Considering only the first 33 does, and grouping the observations without regard to uterine horn, the following results were obtained: 54 placentae in position 1 had a mean weight of 4.96 ± 0.133 gm.; 55 placentae in position 2 had a mean weight of 4.81 ± 0.149 gm.; 44 placentae in position 3 had a mean weight of 4.50 ± 0.155 gm.; and 55 placentae in position 4 and over had a mean weight of 4.21 ± 0.136 gm. (Text-fig. 2). The variance between the means of classes was significantly greater than the variance within classes ($F = 5.60$), indicating significant heterogeneity between each position.

In the final analysis the explanation for the decreasing weight of fetus and of placenta with increasing distance from the Fallopian tube cannot be found in the present data. Two hypotheses, however, are suggested. The first is that fetal weight is determined in a large measure by placental mass, and that placentae high up in the horn are larger than those lower down because of the differential in the vascularity and nutritive efficiency of the uterus in these locations. Whether this gradient of vascularity actually exists, however, is not known. The second hypothesis is that fetal and placental weights are correlated with each other, and that variability in both is caused by a third factor, namely age. On this basis, fetuses and their corresponding placentae which are located nearest to the Fallopian tube are older than those developing lower down in the uterine horn and, therefore, weigh more. This hypothesis suggests that there is a time differential in the maturation, discharge and fertilization of the ova, that the first ovum to be fertilized is in general implanted in that portion of the horn nearest the ovary, and that the last ovum to be fertilized is implanted nearest the uterine outlet. The test of this hypothesis must await further investigation. It should be stated, however, that Walton and Hammond (9), in experiments involving direct observation of the maturing ovarian follicle of the rabbit, noted that the follicles of the same batch do not rupture at exactly the same time, for in several cases where observations began about 10 hours after coitus, one or more follicles had already ruptured, while others ruptured subsequently under observation.

An earlier communication (10) has reported the criterion for the diagnosis of dwarfism on the basis of weight at birth. Birth weights have been obtained in this laboratory by the following procedure. Each morning rabbits born during the previous 24 hour period are identified by color markings or toe markings and weighed to the nearest gram on a Toledo automatic balance calibrated in 1 gm. intervals. The average elapsed time from birth to weighing is 12 hours, the midpoint of the 24 hour interval between determinations. It was apparent that this routine introduces an error which is dependent on the amount of nourishment which each individual in the litter has obtained between birth and the weight determination. This error varies with the length of the intervening period and also with the number in the litter and the relative strength or weakness of the newborn. The weaklings are frequently overpowered by their stronger sibs in the struggle for nourishment and, moreover, the doe sometimes disregards her weaker offspring and refuses to nurse them. As a result, the recorded weights may deviate from the actual birth weights in a negative or positive direction according to the relative strength or weakness of the animals comprising the litter. In order to determine to what extent these factors might account for the occurrence of dwarfs, an analysis was made of two large groups of rabbits. The first group consisted of the individuals in normal non-dwarf containing litters, and the second comprised the members of litters each containing at least one dwarf. It was concluded that when the birth weight of a rabbit as determined by the routine procedure described above is less than 50.1 per cent of the weight of its

TABLE V

Frequency Distribution of Weights of Three Groups of Rabbits
Weight of Each Individual Expressed as a Percentage of the Weight of the Heaviest Litter Mate

Group	Description	100.0–85.1%	85.0–70.1%	70.0–50.1%	50.0–20.1%	Total
I	Fetal weight—normal litters	312	123	19	0	454
II	Birth weight—normal litters	378	146	37	4	565
III	Birth weight—dwarf litters	252	77	19	140	488

heaviest sib, a diagnosis of dwarfism is warranted. Since the birth weights of the unusually small, non-viable individuals all fell below the 50.1 per cent class, they were regarded as true dwarfs in the sense that their abnormally low weights could not be ascribed to environmental and nutritive factors operative in the interval between birth and the weight determination.

It will be recalled that the present observations were initiated for the particular purpose of ascertaining to what extent intrauterine factors could account for these dwarf individuals. The quantity of nutriment obtained by the fetus is independent of its own efforts, so that in a consideration of fetal weight at or near term one element which might account for variability in birth weight, namely, nursing, is absent. Table V presents a comparison of the weights of three groups of individuals. Group I comprises the fetal weights recorded in the present study; group II consists of the birth weights of rabbits in normal non-dwarf containing litters; and group III includes the birth weights of individuals in litters each containing at least one dwarf. In each case the weight of each individual was expressed as a ratio to the weight of its heaviest sib. The material in groups II and III has been presented elsewhere (10) in slightly different form.

An analysis of these observations by the χ^2 test of homogeneity indicates that there is no significant difference between groups I and II ($\chi^2 = 5.67$), and that both of these differ significantly from group III (I and III, $\chi^2 = 155.87$; II and III, $\chi^2 = 176.16$). Of particular importance is the fact that in no case was the weight of the smallest fetus in a litter less than 50.1 per cent of that of its heaviest litter mate, while the birth weights of all of the dwarfs fell below the 50.1 per cent class. These findings show conclusively, first, that those factors which produce variability in fetal weight at or near term cannot account for the abnormally low weights of dwarf individuals and, second, that the error introduced by our method of obtaining birth weights does not appreciably affect the relative variability in the weights of rabbits at the precise moment of birth. It is to be expected, however, that the weight of dwarf individuals would be affected by the position occupied by such individuals in the uterus.

SUMMARY

Observations were made on 475 fetuses carried by 71 pregnant rabbits. 63 or 88.7 per cent of the 71 does were sacrificed from 28 to 31 days after the last fertile mating, and these does bore 401 or 88.2 per cent of the total of 455 fully developed fetuses. The following information was available with reference to each fetus: age, weight, weight of corresponding placenta, horn, *i.e.*, right or left, presentation, and position or order. The presentation indicated that part, head or breech, which was directed toward the vagina, and position or order, the relative locus of the fetus in the horn, the first position being that nearest the ovary.

As the gestation period approached its normal limit of 31 days, the relative daily increase in mean fetal weight was progressively retarded.

There was no significant difference between the number of fetuses in each uterine horn.

Head presentation was significantly more frequent than breech, but the uterine horn in which the fetus was located had no influence on its presentation.

A greater relative number of breech presenting fetuses was observed in the third position than in the other positions.

Presentation did not exert a significant influence on fetal weight.

Fetal weight at or near term was significantly influenced by the position or order in the uterine horn. In general, the weights of fetuses implanted high up nearest the ovary were greater than those developing nearest the outlet, and fetuses occupying intermediate positions had intermediate weights. When, however, only two fetuses were present in a horn, position had no effect on their weights.

A significant positive coefficient of correlation was observed between fetal and placental weights. Moreover, placental weight was influenced by position in the uterine horn in exactly the same manner that fetal weight was so influenced.

The factors which produced variability in fetal weight at or near term, did not account for the abnormally low birth weights of the dwarf rabbits observed in this laboratory.

BIBLIOGRAPHY

1. Greene, H. S. N., Hu, C. K., and Brown, W. H., *Science*, 1934, **79**, 487.
2. Fisher, R. A., Statistical methods for research workers, London, Oliver & Boyd, 1930.
3. Snedecor, G. W., Calculation and interpretation of analysis of variance and co-variance, Ames, Iowa, Collegiate Press, Inc., 1934.
4. Wallace, H. A., and Snedecor, G. W., Correlation and machine calculation, Ames, Iowa, Iowa State College Official Publication, revised edition, 1931, **30**, No. 4.
5. Rosahn, P. D., *Human Biol.*, 1935, **7**, 267.
6. Rosahn, P. D., Greene, H. S. N., and Hu, C. K., *J. Exp. Zoöl.*, 1935, **72**, 195.
7. Draper, R. L., *Anat. Record*, 1920, **18**, 369.
8. Ibsen, H. L., *J. Exp. Zoöl.*, 1928, **51**, 51.
9. Walton, A., and Hammond, J., *Brit. J. Exp. Biol.*, 1928, **6**, 190.
10. Rosahn, P. D., and Greene, H. S. N., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1580.