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The Effect of Progesterone on Fetal and Placental Development in Normal and Protein-Energy-Restricted Rats

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Abstract. Maternal protein-energy restriction (25% of the *ad libitum* intake) during the first 10 days of pregnancy resulted in severely altered fetal growth rates. Fetal development was assessed by body weight, brain weight, brain DNA, and brain protein content on fetal days 16, 18, 20 and at term. The individual placentas were also examined (weight, DNA and protein content) on each of these fetal days. Progesterone was administered commencing with day 3 of pregnancy until the day Caesarian section was done, in an attempt to rehabilitate placental development. This treatment did not improve placental development on fetal days 16 or 18. However, fetal development was significantly improved on day 16 and day 20, as compared to the dietary-restricted group without progesterone.

The development of the body and brain of the fetus can be altered *in utero* by various types of experimental manipulations. Experimental alterations of the intrauterine environment can result in enhanced fetal development, as is the case when litter size is surgically reduced (8–10). Fetal development is dependent on blood supply, and experimental ischemia in individual fetuses resulted in restricted development of fetal body and brain development as reported by us (11).

Alterations in maternal dietary intake during pregnancy, such as a restriction in protein, energy, or protein-energy intake, and vitamin or mineral deficiency, result in altered fetal development (see review in 18). Previous reports from this laboratory have dealt with the effects of protein deprivation during specific time periods of gestation on neonatal brain development (19) and on fetal body and brain development (12).

We have reported a high incidence of failure to maintain pregnancy during early (0–10 days) protein deprivation (55% of the positively mated females failed to litter) (12). It has been reported that this failure is caused by faulty implantation due to a lack of progesterone and estrogen (3–6, 14). However, we

found that injections of 1.0 μg estrogen and 4.0 mg progesterone simultaneously from day 3 of gestation during maternal protein deprivation did not facilitate maintenance of pregnancy in our rats (12). We also investigated the effects of an early maternal protein-energy restriction (day 0–10) on fetal development and on the length of gestation (13). Since implantation occurs in our rats on day 6 post coitum, the actual time during which the developing embryo was exposed to maternal dietary restriction was only 4 days. During this time the amount of nutrients needed to build the small amount of tissue of the fetuses and their supporting tissue is negligible. Thus, the reduction in the fetal body weight is likely to be caused by lack of adequate placental development and not due to a nutritional insult. The importance of progesterone for placental development has been reported (6).

In the present study we have investigated fetal and placental development on days 16, 18, and 20 of gestation and on the day of spontaneous delivery, following early maternal protein-energy restriction, with or without progesterone administration. We have used fetal body weight, cerebral weight, cerebral DNA and protein content as assessments of fetal development. Normal neuron and glia cells at birth are essentially diploid and the amount of DNA per diploid cell of a given species is constant (16); the actual number of cerebral cells could be calculated by dividing by the constant DNA content per cell.

It has been reported that in the rat at birth the cerebrum predominantly contains neurons (neuroblasts) and that proliferation ceases at this time, with the possible exception of the short axon neurons (1). Thus, the determination of cerebral DNA content is a convenient and reliable quantitative method for cell, and possibly neuron, enumeration in the rat. Protein/DNA ratio has been repeatedly used as an index of cell size.

The assessment of placental development and its function is difficult one; factors such as permeability, exchange surface, blood flow, etc., would have to be taken into account. However, we have reported significant correlations between placental weight, its DNA (cell number), protein content, and cell number in the neonatal brain (20). The relationship between placental weight and newborn body weight following surgical litter restriction in the rat has been investigated by us (9).

Materials and Methods

All animals used in this study are from our colony of Sprague-Dawley-derived rats which have been randomly bred as a closed colony for 38 generations in our laboratory. 3-month-old virgin females with an average body weight of 231 ± 18 g were mated during the morning hours and this day was considered day 0 of pregnancy. All animals were housed individually in a temperature- and humidity-controlled room. The light cycle was adjusted to 12 h of light and 12 h of darkness. The mated animals were randomly assigned to either

the control (28 females) or to the following experimental groups: protein-energy restriction (P/E), 20 females; protein-energy restriction plus administration of progesterone (P/E + PROG), 67 females; progesterone injections only (PROG), 21 females.

The control animals and the females in group PROG were maintained on a standard pelleted laboratory chow and tap water *ad libitum*. The food intake was measured by supplying known amounts and weighing the remainder, including any spillage, every other day. The standard laboratory chow (Wayne Mousebreeder Blox, Allied Mills, Chicago, Ill.) contained 20% protein and an energy value of 2,882 kcal/1,000 g.

For the experimental groups of animals, groups P/E and P/E + PROG, the following dietary regime was imposed: the maternal protein and energy intake was reduced to 25% of the normal intake during the first 10 days of pregnancy by feeding $^1/_4$ of the normal *ad libitum* food intake of the control group. Starting with the evening of the 10th day of pregnancy, the animals were allowed to eat *ad libitum*, and again their food intake was measured as in the control group.

The females in groups P/E + PROG and PROG were daily injected (subcutaneously) with 4 mg of progesterone (Δ^4-Pregnene-3,20-dione) in 0.1 ml sesame oil, commencing with the 3rd day of pregnancy until 1 day prior to sacrifice.

All females were weighed on days 0, 10 and 15, and on the day of sacrifice. Caesarian sections were performed on day 16, 18, and 20 post coitum, or the mothers were allowed to deliver their litters spontaneously. All fetuses and newborns were weighed and decapitated and the cerebrum was dissected, weighed and stored at -10 °C for subsequent analyses. The respective placentas were dissected free of all membranes, blotted, weighed and stored. The cerebrum and the placentas were analyzed for total DNA (21) and protein content (7).

Results and Discussion

Maternal body weight changes and food intakes are represented in table I. Protein-energy restriction during the first 10 days resulted in maternal body weight loss regardless of whether progesterone was administered or not. Progesterone administration to females on *ad libitum* food intake did not affect maternal body weight gain. On the other hand, food intakes during days 11–21 in the P/E + PROG group were increased, compared to all other groups. Total food intake in the P/E + PROG group was reduced by 26%, whereas in the P/E group the total food intake was reduced by 35% in comparison to the *ad libitum* control group.

The pregnancy maintenance rates for all four groups of animals are represented in table II. Protein-energy restriction during days 0–10 did not decrease pregnancy maintenance rates as compared to the *ad libitum* control group in which 93% of females remained pregnant. Progesterone administration resulted in a decrease of pregnancy rates, but 86% of the females were pregnant, which is within the range of the control group. That the total amount of progesterone administered is not related to resorptions can be demonstrated in the females allowed to litter spontaneously: of those females which had the highest total dose administered (72 mg) 100% still littered. In contrast, the combination of protein-energy restriction and progesterone administration

Table I. Maternal body weights and food intakes

Experiment	Body weight[1], g			Food intake, g/24 h			
	day 0	day 10	day 15	days 0–10	days 11–15	days 16–21	total days 0–21
P/E + PROG	235 ± 22	206 ± 19	262 ± 25	3.8 ± 0 (−75%)[2]	19.7 ± 2.0 (+ 25%)	19.3 ± 1.7 (+ 21%)	252.3 ± 1.2 (−26%)[2]
P/E	220 ± 20	196 ± 8	259 ± 20	3.8 ± 0 (−75%)[2]	17.4 ± 1.0 (+ 10%)	16.2 ± 0.9 (+ 1%)	222.2 ± 0.6 (−35%)[2]
PROG	232 ± 15	248 ± 27	277 ± 25	12.8 ± 1.5 (−15%)	16.7 ± 1.0 (+ 6%)	17.1 ± 1.4 (+ 7%)	314 ± 0.8 (−8%)[2]
Control	227 ± 20	246 ± 21	276 ± 33	15.1 ± 0.9	15.8 ± 0.9	16.0 ± 1.0	341.8 ± 0.9

P/E = Protein-energy restriction; PROG = progesterone.
[1] Mean ± SD; differences with respect to control are given in parentheses.
[2] Probability less than 0.001.

Table II. Pregnancy maintenance rates

	P/E + PROG	P/E	PROG	Control
16th day	5/5 100%	4/5 80%	4/4 100%	7/8 88%
18th day	9/22 41%	5/5 100%	4/6 67%	7/7 100%
20th day	10/17 59%	4/4 100%	6/7 86%	7/7 100%
Litter	9/23 39%	6/6 100%	4/4 100%	5/6 83%
Total	33/67 49%	19/20 95%	18/21 86%	26/28 93%

severely decreased pregnancy maintenance: only 49% of the positively mated females were pregnant at the time of examination.

The majority of females from this group showed resorption sites in the uterus when examined on the day of Caesarian section. Again it is doubtful if the actual amount of progesterone was the cause: on day 18, 41% were pregnant following 56 mg of progesterone, whereas on day 20, 59% were found to be pregnant, at which time 64 mg of progesterone had been injected. The most

Table III. Fetal measurements on day 16

Experiment	Number of mothers	Number of fetuses	Body weight g[1]	Cerebrum			Placenta		
				weight, mg	DNA, μg	protein, mg	weight, mg	DNA, μg	protein, mg
P/E + PROG	5	50	0.4340 ± 0.0431 (−8%)	37.5 ± 7.4 (+ 10%)	199 ± 44 (−13%)[2]	1.44 ± 0.38 (−9%)	253.2 ± 46.7 (−29%)[2]	583 ± 101 (−29%)[2]	20.23 ± 4.30 (−35%)[2]
P/E	4	44	0.3040 ± 0.0324 (−35%)[2]	18.5 ± 4.0 (−46%)[2]	134 ± 30 (−41%)[2]	0.89 ± 0.23 (−44%)[2]	277.0 ± 41.8 (−22%)[2]	584 ± 71 (−28%)[2]	19.12 ± 3.09 (−39%)[2]
PROG	4	39	0.4409 ± 0.0582 (−6%)	32.2 ± 3.9 (−5%)	241 ± 62 (+ 6%)	1.70 ± 0.41 (+ 7%)	307.2 ± 45.9 (−14%)[2]	674 ± 99 (−17%)[2]	25.19 ± 4.39 (−19%)[2]
Control	7	60	0.4700 ± 0.1308	34.0 ± 5.5	228 ± 38	1.59 ± 0.23	356.0 ± 69.8	816 ± 87	31.11 ± 5.28

P/E = Protein-energy restriction; PROG = progesterone.
[1] Mean ± SD; differences with respect to control are given in parentheses.
[2] Probability less than 0.001.

severe underdevelopment of the placenta was found on day 16 in the P/E + PROG group; on day 18, placenta development in this group was still the most restricted one among all three groups of animals. Thus it is possible that underdevelopment of the placenta, which was found in group P/E + PROG, was due to the progesterone administration, and since there was a concomitant dietary insufficiency, no placental rehabilitation occurred, resulting in resorptions of the dead fetuses.

The effects of the three experimental treatments on litter size were also investigated. A total of 327 offspring were examined in the P/E + PROG group with an average litter size calculated as 9.9, 167 offspring in the P/E group resulting in 8.8 offspring per female, 172 offspring in the PROG group with an average litter size of 9.6, and a total of 237 offspring in the control group with an average litter size of 8.8.

From these data it is evident that none of the three treatments resulted in an increased fetal death since all litter sizes were within the range of that of the *ad libitum* control group.

The effects of maternal protein-energy restriction with or without concomitant progesterone injections, on fetal development are presented in tables III–V. On fetal day 16, all fetal and placental measurements were significantly lower than in the controls as a consequence of a 10-day protein-energy restriction (group P/E) as reported previously by us (13).

In comparison, the offspring of the females receiving daily progesterone injections (P/E + PROG group) were much less affected by the maternal protein-energy restriction. Wet weight of the cerebrum was well within the range of that of the normal control fetuses and was increased by 103% compared to that in the P/E group. The total DNA (index of cell number) was the only fetal parameter which was highly significantly lower in the P/E + PROG group as compared to the control group, but the reduction in DNA content was not of the same magnitude as in the P/E group. The cerebrum DNA content in the P/E + PROG group was increased by 49% as compared to that in the P/E group. Placental weight, its cell number (DNA) and total protein content were equally reduced (regardless of the progesterone injections) in both experimental groups (P/E and P/E + PROG).

Progesterone administration to normal *ad libitum*-fed females did not affect fetal development when examined on the 16th day post coitum, but did significantly inhibit normal placental development. Yet by the 18th fetal day all measured placental parameters in the PROG group were not significantly different from those of the control group (table IV). The fetal weight and the protein content of the cerebrum in group PROG at this time were significantly increased compared to the control.

On the 18th day all measured fetal parameters in the P/E group were significantly increased (except cerebrum protein content). The placenta showed

Table IV. Fetal measurements on day 18

Experiment	Number of mothers	Number of fetuses	Body weight g[1]	Cerebrum			Placenta		
				weight, mg	DNA, μg	protein, mg	weight, mg	DNA, μg	protein, mg
P/E + PROG	9	84	1.3275 ± 0.2026 (Ø)	57.3 ± 11.2 (−3%)	331 ± 53 (−13%)[2]	2.83 ± 0.81 (−14%)[2]	401.9 ± 77.8 (−6%)[2]	708 ± 119 (−13%)[2]	33.93 ± 7.14 (−6%)
P/E	5	37	1.5400 ± 0.3800 (+17)[2]	66.0 ± 10.8 (+11%)[2]	431 ± 56 (+13%)[2]	3.73 ± 0.94 (+13%)	452.7 ± 89.7 (+6%)	739 ± 93 (−9%)	39.15 ± 9.55 (+9%)
PROG	4	33	1.5540 ± 0.1478 (+18%)[2]	64.3 ± 6.2 (+8%)	358 ± 21 (−6%)	4.20 ± 0.67 (+27%)[2]	453.2 ± 44.1 (+6%)	784 ± 70 (−4%)	40.53 ± 5.97 (+12%)
Control	7	63	1.3147 ± 0.2382	59.3 ± 8.1	381 ± 61	3.30 ± 0.62	426.9 ± 70.9	814 ± 108	36.08 ± 6.41

Table V. Fetal measurements on day 20

Experiment	Number of mothers	Number of fetuses	Body weight g[1]	Cerebrum			Placenta		
				weight, mg	DNA, μg	protein, mg	weight, mg	DNA, μg	protein, mg
P/E + PROG	10	102	3.19 ± 0.66 (−19%)[2]	99.4 ± 13.9 (−9%)[2]	445 ± 64 (−14%)[2]	5.59 ± 1.10 (−5%)	487.8 ± 82.1 (−7%)	708 ± 109 (Ø)	40.57 ± 8.30 (Ø)
P/E	4	31	2.81 ± 0.38 (−28%)[2]	88.8 ± 7.9 (−19%)[2]	430 ± 41 (−17%)[2]	4.99 ± 0.47 (−15%)[2]	473.3 ± 80.0 (−10%)	670 ± 98 (−5)	37.27 ± 6.48 (−8%)
PROG	6	65	3.47 ± 0.36 (−12%)[2]	105.0 ± 7.9 (−4%)	518 ± 36 (Ø)	5.87 ± 0.64 (Ø)	489.4 ± 41.3 (−7%)	683 ± 51 (−4%)	46.92 ± 8.99 (+16%)[2]
Control	7	62	3.93 ± 0.72	109.7 ± 12.2	515 ± 45	5.90 ± 0.77	525.3 ± 123.2	708 ± 82	40.40 ± 4.16

P/E = Protein-energy restriction; PROG = progesterone.

[1] Mean ± SD; differences with respect to control are given in parentheses.

[2] Probability less than 0.001.

a concomitant catch-up growth when examined on the 18th fetal day. The measured fetal parameters in the P/E group were significantly above those of the control diet group on day 18; this clearly demonstrates the enhanced *in utero* growth rates during days 16–18 in that group. This type of growth spurt has also occurred in the P/E + PROG group, but in that group the rapid growth rate was *prior* to the 16th fetal day and can only be attributed to the hormone administration. In the P/E + PROG group the fetal cerebrum weight, its cell number (DNA content) and protein content did not demonstrate any accelerated growth rate during the last 2 days. In this group placental development was less reduced on day 18 than it was when examined on the 16th day. It should be pointed out that only 41% of the positively mated females were maintaining pregnancy past day 16. We must assume that only those females maintained their pregnancy which were able to rehabilitate placental development, possibly due to a better feed efficiency.

That all three experimental treatments resulted in alterations of the *in utero* growth rates was further demonstrated on the 20th day of pregnancy (table V). In all three groups fetal body weights were significantly less than in the normal control. The weight and DNA content of the cerebrum were significantly lower in both the P/E + PROG and the P/E groups. In the P/E + PROG group the differences were greater on the 20th than the 18th day. On the other hand, placental development was found to be within the range of that of the control group. It is known (17) that placental development in the rat reaches its plateau around day 19 and is afterwards not closely correlated with fetal development.

We have reported previously that early protein-energy restriction causes a prolongation of gestation (13); this prolongation was also observed in the P/E + PROG and PROG groups (table VI). During this additional time *in utero,* the fetuses were actively growing and, when examined at the time of natural birth, all measured neonatal parameters (except DNA content in the P/E group) had reached or even surpassed control values.

In conclusion, we have found that early maternal short-time protein-energy restriction caused altered fetal growth rates and that the gestational period was prolonged. The administration of exogenous progesterone with or without concomitant protein-energy restriction also changed fetal growth rates, resulting in three different patterns of development for P/E, P/E + PROG, and PROG. When examined on the 16th fetal day the fetuses in the P/E + PROG group demonstrated highly significant ($p < 0.001$) improvement above the P/E group in all the measured parameters. Body weight was + 42%, brain weight + 102%, brain DNA content + 49% and brain protein content + 62% as compared to the P/E group. That this improved fetal development was only transient can be demonstrated with the data on the 18th day of gestation. This advantage was not found on the 18th day of gestation in the P/E + PROG (table IV). The alterations in fetal growth rates reported here during late pregnancy were

Table VI. Newborn measurements

Experiment	Number of mothers	Number of newborns	Length of gestation days	Body weight g[1]	Cerebrum		
					weight, mg	DNA, μg	protein, mg
P/E + PROG	4	45	23	5.85 ± 0.50 (−4%)	173.7 ± 21.0 (+ 6%)	535 ± 71 (Ø)	8.43 ± 0.96 (−5%)
P/E	6	55	23	6.50 ± 0.50 (+ 7%)	176.4 ± 4.7 (+ 8%)[2]	513 ± 25 (−4%)[2]	9.22 ± 0.61 (+ 3%)
PROG	4	35	23	6.29 ± 0.54 (+ 3%)	186.0 ± 24.4 (+ 14%)[2]	569 ± 30 (+ 7%)[2]	10.09 ± 1.14 (+ 13%)[2]
Control	5	52	21.5	6.10 ± 0.78	163.4 ± 16.9	532 ± 21	8.91 ± 1.17

P/E = Protein-energy restriction; PROG = progesterone.
[1] Mean ± SD; differences with respect to control are given in parentheses.
[2] Probability less than 0.001.

probably not due to the direct action of progesterone, for it has been reported that the maternal contribution to fetal progesterone levels accounts for less that 10% (15). However, since progesterone is a precursor for corticosteroids, an effect of the latter cannot be excluded at the present. In view of the reported rigid time schedule during which brain cell proliferation and migration takes place, these alterations can be of utmost importance and significance even if it does appear that the total number of brain cells reaches that of normal untreated controls.

Administration of progesterone also prolongs the gestational time period; this is in accordance with previous reports that in many mammalian species parturition is preceded by a decrease in maternal progesterone secretion (2). Our data suggest that during this additional time period of prolonged gestation, the fetuses are actively growing and are compensating for a previous growth deficit.

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