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Serum Progesterone Levels in Pregnant Rats Fed a Protein-Free Diet (38227)

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An adequate amount of dietary protein is required to successfully maintain pregnancy in the rat (1). With diets containing less than 6% protein the immediate cause of pregnancy failure appears to be a decrease in ovarian steroid secretion since pregnancy can successfully be maintained with daily injections of progesterone and estrone (2). The ovarian failure has indirectly been attributed to reduced secretion of hypophysial luteotrophin because injection of prolactin (2) or a combination of FSH, LH and prolactin (3) can protect the pregnancy of protein-deprived rats. Absence of dietary protein decreases the pituitary and blood content of prolactin (4), however, the capacity of the pituitary to respond to stimuli with increased synthesis and release of prolactin is retained (5–8). The purpose of this study was to further explore the postulated pituitary mediated ovarian effect of a protein-free diet by directly measuring endogenous serum progesterone levels.

Material and Methods. Adult female rats weighing between 180–200 g were purchased from Charles River Breeding Laboratories and housed in humidity controlled air conditioned quarters with the lights adjusted to give 12 hr of darkness between 7 am and 7 pm. To obtain pregnant animals a fertile male was placed with 2 females between 10 am and 2 pm and checked immediately thereafter for spermatozoa (day 0 of pregnancy). The rats were then fed either a 20% casein or a 0% casein diet (9). 0.7 ml of blood was withdrawn from the jugular vein from each rat on days 5, 7, 9, 11, 13 and 15 of pregnancy. All animals were sacrificed on day 15 and pregnancy maintenance was noted. The serum was assayed for progesterone using the radioimmunoassay procedure of Abraham (10).

Serum from intact and hypophysectomized rats fed a protein-free diet and administered estrone, prolactin or a combination of both from days 5–12 of pregnancy were also assayed. Estrone was injected sc at a daily dose of 1.0 and 2.0 μg dissolved in sesame oil. Ovine prolactin (NIH-P-S-10) was dissolved in saline and injected sc twice daily at a total dose of 2.5 mg. Hypophysectomies were performed via the parapharyngeal approach, under ether anesthesia, on day 5 of pregnancy.

Progesterone concentration (pg/ml of serum) was calculated from a standard curve using the logit transformation method of Rodbard and Lewald (11). A typical standard curve plotted as the logit versus the log mass of progesterone is shown in Fig. 1. Each point represents the mean of 3 determinations ± S.E. Interassay and intraassay coefficients of variation for 5000 pg of progesterone added to the serum from adrenalectomized and ovariectomized animals were 8.1 and 7.7, respectively, with a mean recovery for 10 samples of 98%.

Results. Serum progesterone levels in pregnant rats fed a protein-free diet revealed a significant drop in progesterone concentration on day 11 of pregnancy and steroid levels remained low thereafter (Fig. 2). This was associated with a 100% pregnancy loss when the animals were examined on day 15. All rats fed the 20% casein diet satisfactorily maintained their pregnancies with typical serum progesterone levels (Fig. 2) confirming previously reported values (12).

When prolactin and estrone were injected

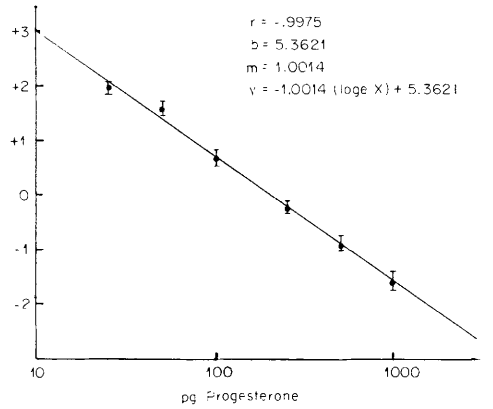

FIG. 1. Standard curve for progesterone plotted as logit vs log mass.

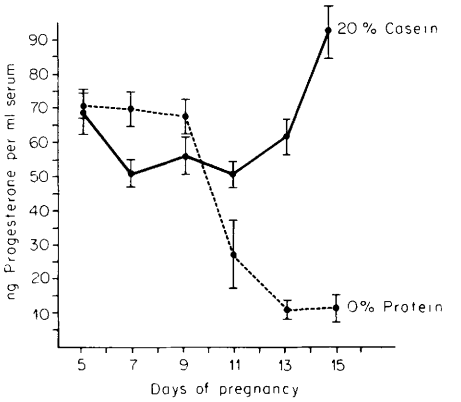

FIG. 2. Serum progesterone levels in pregnant rats fed at 20% casein or protein-free diet.

concomitantly into intact or hypophysectomized rats being fed the protein-deficient diet (Table I), pregnancies were maintained in 100% and 80% of these animals, respectively, and serum progesterone was comparable to the 20% casein fed rats. The injection of prolactin alone maintained pregnancy in 2 of 6 rats with serum progesterone decreasing on day 11 in those animals which aborted. Estrone alone which can cause prolactin release (13) maintained pregnancy in 50% of the animals, however serum progesterone levels on days 11 and 13 were not significantly reduced in those rats that had not maintained pregnancy. That estrone can indeed stimulate pro-

TABLE I. Effects of Prolactin and Estrone on Pregnancy and Serum Progesterone Levels in Intact and Hypophysectomized Rats Fed a Protein-Free Diet.[a]

Treatment* (daily dose)	No. rats bred	No. rats pregnant day 15	Avg. no. i.p. sites	Avg. no. resorp.	Day of preg.	ng Prog/ml Serum	
						Maintained	Aborted
None	8	0	0	—	9	—	65.3 ± 5.2
					11	—	27.0 ± 10.8
					13	—	11.1 ± 1.2
Estrone (2.0 μg)	6	3	10.0	7.7	9	75.7 ± 24.5	96.6 ± 23.0
					11	56.4 ± 11.7	42.5 ± 17.5
					13	56.6 ± 7.2	45.0 ± 16.9
Prolactin (1.25 mg × 2)	6	2	9.8	7.0	9	93.2 ± 19.5	63.8 ± 12.2
					11	60.7 ± 4.9	13.8 ± 4.1
					13	72.6 ± 6.4	13.4 ± 1.6
Prolactin (1.25 mg × 2) + Estrone (1.0 μg)	6	6	12.0	1.25	9	49.6 ± 6.1	—
					11	49.3 ± 6.4	—
					13	45.4 ± 13.1	—
Hypox + Prolactin (1.25 mg × 2) + Estrone (1.0 μg)	6	5	11.0	4.7	9	25.1 ± 4.8	69.8 ± 0
					11	52.2 ± 14.6	12.7 ± 0
					13	42.3 ± 13.0	15.3 ± 0
Hypox + Estrone (2.0 μg)	6	0	0	—	9	—	4.9 ± 0.8
					11	—	2.3 ± 0.3
					13	—	1.1 ± 0.4

[a] ± = Standard error of mean.
* = Days 5–12 of pregnancy.

lactin secretion in rats fed a protein-free diet is indirectly indicated by the negligible serum progesterone levels in the hypophysectomized estrone-treated rats.

Discussion. Pregnancy maintenance in the rat requires a minimum amount of dietary protein (1). Injections of prolactin (2), estrogen with and without progesterone (7), ACTH (14) or corticoids (6, 8) during early pregnancy have all been shown to afford some protection against protein deficiency. Our results have shown that in the absence of dietary protein serum progesterone levels become significantly diminished on day 11 of pregnancy resulting in complete loss of pregnancy. This can be prevented by administering prolactin and estrone concomitantly from days 5–12 of pregnancy into either intact or hypophysectomized animals. Injection of prolactin or estrone alone was only partially effective, thus confirming previously reported studies (5, 15). Rats that aborted despite treatment with prolactin all had depressed serum progesterone levels, whereas those treated with estrone did not. Therefore, pregnancy loss in estrone treatment would appear to be due to some other factor(s) than decreased ovarian production of progesterone.

The fact that progesterone does not drop until day 11 of pregnancy, the day on which the placenta assumes the luteotrophic function from the pituitary (16), seems to indicate that the primary cause of the pregnancy loss is inadequate placental development and function. Day 11 placentas from normally fed rats (17) or those fed a protein-free diet but maintained with progesterone and estrone (18) do retain their luteotrophic and mammotropic activities. Placental development must therefore be dependent upon early pituitary secretion of prolactin and ovarian secretion of estrogen and progesterone since substitution of prolactin and estrone into the hypophysectomized, pregnant rat on the protein-deficient diet does successfully maintain pregnancy and normal serum progesterone levels. It has been suggested that estradiol-17β is the regulator of placental growth in the rat (19), however whether or not estrogen deficiency alone is the initiating factor in the chain of events leading to abortion in the rats on a protein-free diet has yet to be determined. Since serum estrogen levels are very low during pregnancy (20), one must measure ovarian vein levels of E_2 in an attempt to answer this question.

Summary. Peripheral serum progesterone levels in rats fed a protein-free diet show a dramatic decrease on day 11 of pregnancy and remain low thereafter. This drop and the resultant abortion can be prevented by concomitant injections of prolactin and estrone in the intact or hypophysectomized animal. The decline in serum progesterone levels at day 11 of pregnancy suggests a defect in placental development and secretion of luteotrophin and that early secretion of adequate amounts of prolactin and ovarian steroids are required for placental formation and function.

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