

Genetic and Litter Size Effects on Serum Placental Lactogen in the Mouse

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ABSTRACT

Placental lactogen (PL) and progesterone are important hormones of pregnancy in the mouse. The purpose of this study was to investigate the influence of genetic differences and litter size on serum PL levels in the mouse. Serum progesterone levels were also measured in some experiments. Two features of serum PL levels under genetic control were identified: 1) the gestational profile of serum PL and 2) absolute serum PL levels. Breeding combinations that resulted in profound effects on serum PL levels were without significant effect on serum progesterone levels. Serum concentrations of PL and progesterone were directly proportional to litter size. Conceptus number was found to significantly affect ovarian progesterone release. The presence of uterine tissue antagonized the luteotropic actions of the conceptus but did not affect serum PL levels.

INTRODUCTION

Placentas from a number of rodent species have been shown to produce prolactin-like hormones. These hormones are presumed to be involved in the gestational control of luteal function and mammary gland development (see Talamantes et al., 1980 for a review). Although the factors that regulate placental hormone secretion are poorly understood, substantial evidence exists in rodents that interbreeding of genetically different animals affects placental size (Billington, 1964; McLaren, 1965; Rogers and Dawson, 1970). Whether there also are effects on serum placental lactogen (PL) levels under these circumstances is unknown. Litter size is known to affect serum PL levels in the rat (Robertson and Friesen, 1981; Voogt et al., 1982) and is believed to also affect PL levels in the mouse (Markoff and Talamantes, 1981). PL has recently been purified and a homologous radioimmunoassay developed for its measurement in the mouse (Colosi et al., 1982; Soares et al., 1981). It is essential to use a radioimmunoassay specific for PL because there are other lactogens of conceptus and pituitary

origin in the mouse and rat which interfere with bioassays and radioreceptor assays for lactogenic hormones (Robertson et al., 1982; Soares et al., 1983). The purpose of this investigation was to evaluate the effects of litter size and interbreeding genetically different mouse strains on serum PL levels. Since PL is hypothesized to be a luteotropic agent, serum progesterone levels were also measured in some experiments.

MATERIALS AND METHODS

General

Mice were maintained on a schedule of 14L:10D (lights on at 0600 h). Food and water were available ad libitum. Timed pregnancies were determined by daily examinations of the vaginas of females housed with adult male mice. The presence of a vaginal plug was designated Day 0 of pregnancy. Mice of the C3H/HeN, Balb/c, and C57BL strains, 2-3 months of age, were used in the experiments.

Blood samples were obtained by decapitation under conditions designed to minimize stress-related hormone responses (Barkley et al., 1978; Markoff and Talamantes, 1981). The blood was allowed to clot at room temperature, centrifuged and the serum stored at -20°C until assayed for PL and progesterone.

Radioimmunoassays

Serum progesterone levels were estimated by employing a sheep antiserum to progesterone-11-bovine serum albumin (BSA) obtained from Dr. G. D. Niswender, Colorado State University, Fort Collins, CO (#337). The specificity of this antiserum has been previously reported (Gibori et al., 1977). Ten-microliter aliquots of serum were extracted in 15 volumes of

Accepted March 7, 1983.

Received December 16, 1982.

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hexane and assayed without further purification. The lower limits of detectability of the progesterone assay (90% of buffer control) ranged from 5 to 10 pg/tube. [1,2,6,7-³H]progesterone (96 Ci/mMol) was obtained from New England Nuclear (Boston, MA). Nonradioactive progesterone was purchased from Sigma Chemical Co. (St. Louis, MO). Extraction recoveries averaged over 90%. Final serum hormone concentrations were not adjusted for losses incurred during the extraction procedure.

Serum mouse PL (mPL) was measured with a specific radioimmunoassay as previously described (Soares et al., 1982). Sera were assayed in duplicate at two dilutions (1 and 10 μ l for serum from Days 8 and 10 of pregnancy and 0.1 and 1 μ l for serum from Days 12 to 18 of pregnancy). The lower limits of detectability for the mPL radioimmunoassay ranged from 5 to 10 pg/tube.

Genetic Effects on Serum PL and Progesterone

Virgin female mice of the Balb/c strain were mated with male mice of either the Balb/c or C3H strain and virgin females of the C3H strain were mated with males of the Balb/c, C3H, or C57BL strains. On Day 18 of pregnancy the females were sacrificed and blood collected for later measurement of PL. Placentas were also removed at the time of autopsy, lyophilized and the dry weight measured to the nearest 0.1 mg. In another experiment, virgin female mice of the C3H strain were mated with either Balb/c or C3H males. The females were sacrificed on Day 8, 10, 12, 14, 16 or 18 of pregnancy and blood was collected for later measurement of PL and progesterone. Mice from both experiments were killed between 1400 and 1600 h. Litter size in both experiments ranged from 6 to 9 fetuses and was not significantly affected by the breeding combination.

Litter Size Effects on Serum PL and Progesterone

For these experiments, female mice of the C3H strain were mated to C3H males. On Day 7 of pregnancy, females with at least 8 conceptuses were subjected to litter size manipulation. The females were anesthetized with Nembutal (60 mg/kg BW) and the litters were adjusted to 1-2, 3-4, or 8-10 conceptuses. Access to the uterus was achieved via a midventral laparotomy. A small incision was then made on the antimesometrial surface of a section of uterus corresponding to a conceptus and the contents removed with forceps. On Day 15 of pregnancy the females were sacrificed, the number of viable fetoplacental units recorded, and blood collected for later measurement of PL and progesterone.

In a second experiment, females from Day 7 of pregnancy were subjected to litter size adjustment. Two experimental groups of animals were used: a) 1-2 conceptuses and b) 8-10 conceptuses. On Day 15 of pregnancy the females were sacrificed and their ovaries were removed for short-term incubation. Each ovary was cut into four equally sized explants. Ovarian explants from each animal were incubated together. The ovaries were preincubated for 1 h in vials containing 2 ml of α -MEM (Grand Island Biological Co., Grand Island, NY) culture medium supplemented with Garamycin (50 μ g/ml; Schering Corporation, Kenilworth, NJ) at 37°C in a shaking water bath (50

revolutions/min) under an atmosphere of 95% O₂/5% CO₂. After the preincubation, the incubation media were removed and replaced with 2 ml of fresh media. Following a 6-h incubation, media were collected and processed for progesterone measurement. Preliminary experiments indicated that it was not necessary to extract incubation media before assaying for progesterone.

A third experiment was conducted to evaluate the effects of the presence of the uterus on the litter size-dependent progesterone and PL changes. Females from Day 7 of pregnancy had their litters adjusted to: a) 1-2 conceptuses or b) 8-10 conceptuses. The females that were subjected to the major adjustment of the number of their conceptuses (females with 1-2 conceptuses remaining) were divided into two groups. In one group the uterine tissue associated with the conceptuses was also removed and in the other group only the conceptuses were removed as in the first two experiments. On Day 15 of pregnancy the females were sacrificed, the number of viable fetoplacental units recorded, and blood collected for later measurement of PL and progesterone.

Animals from these experiments were sacrificed between 1400 and 1600 h.

Statistical Analyses

Data were analyzed by analysis of variance and post hoc comparisons were conducted with the Dunn multiple comparison test (Keppel, 1973).

RESULTS

Genetic Effects on Serum PL and Progesterone

Table 1 contains Day 18 serum PL levels and placental weights from females bred to males of the same strain and to different strains. PL levels were significantly higher in females bred to males of a heterotypic strain than in those mated with males of the same strain ($P < 0.01$ for all). Females of the C3H strain bred to either Balb/c or C57BL males had significantly larger placentas than did C3H females bred to C3H males ($P < 0.01$ for both). Placental weight in Balb/c females was not influenced by the genetic makeup of the sire.

A two-way classification analysis of variance indicated that serum PL levels were significantly influenced by the genetic constitution of the sire and the day of gestation ($P < 0.01$ for both, Fig. 1). There was also a significant interaction effect between these variables ($P < 0.01$). PL levels were significantly higher in C3H females bred to Balb/c males than in C3H females bred to C3H males on Days 10 to 18 of pregnancy ($P < 0.05$ for Days 10 and 12; $P < 0.01$ for Days 14, 16 and 18). Serum PL levels in the heterotypic cross (Balb/c male \times C3H female)

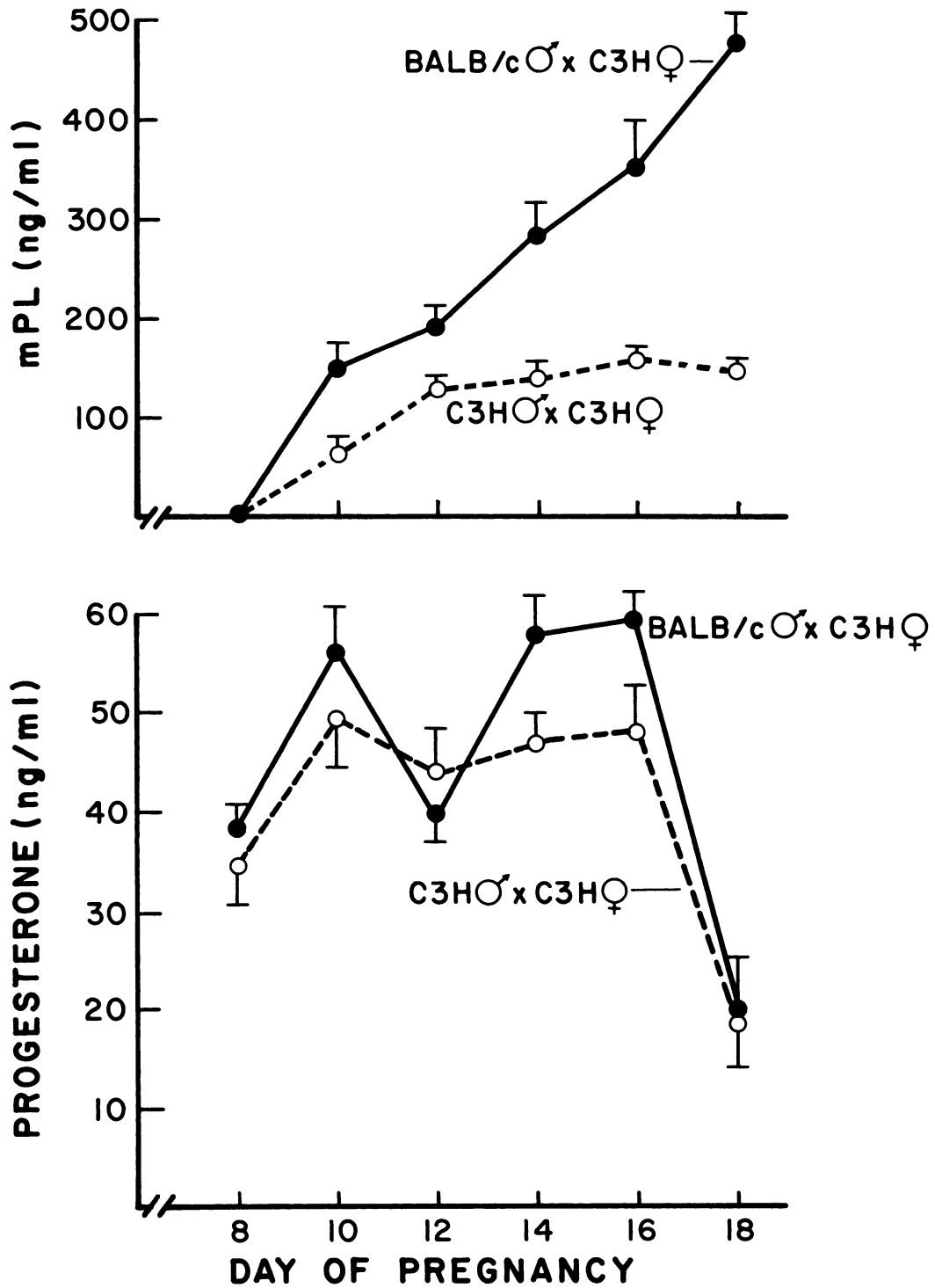


FIG. 1. Gestational pattern of serum placental lactogen and progesterone levels in females of the C3H strain mated to either males of the Balb/c or C3H strain. Each point represents the mean from measurements of 7-9 mice and the vertical lines refer to the standard error of the mean.

TABLE 1. Genetic effects on Day 18 serum placental lactogen levels and placental weights (mean \pm SEM).

Breeding combination (male \times female)	N	Litter size	Placental wt (mg)	Serum mPL (ng/ml)
Balb/c \times Balb/c	9	6.6 \pm 0.3	11.2 \pm 0.3	231.3 \pm 36.5
C3H \times Balb/c	11	7.5 \pm 0.5	10.7 \pm 0.3	531.2 \pm 54.3 ^a
C3H \times C3H	12	6.9 \pm 0.5	9.1 \pm 0.3 ^b	135.3 \pm 10.9 ^b
Balb/c \times C3H	9	6.8 \pm 0.4	11.3 \pm 0.3 ^c	510.5 \pm 80.5 ^c
C57BL \times C3H	8	7.1 \pm 0.4	10.8 \pm 0.4 ^c	338.7 \pm 31.9 ^c

^aValues are significantly different from values for the Balb/c \times Balb/c breeding combination, $P < 0.01$.

^bValues are significantly different from values for the Balb/c \times Balb/c breeding combination, $P < 0.05$.

^cValues are significantly different from values for the C3H \times C3H breeding combination, $P < 0.01$.

increased throughout gestation (Day 12 vs. Day 18, $P < 0.01$), whereas PL levels for the homozygous cross (C3H male \times C3H female) showed minimal changes from Day 12 to Day 18 of pregnancy. Serum progesterone levels were not significantly affected by the genetic constitution of the sire but did change significantly as a function of the day of gestation ($P < 0.01$). A significant decrease in serum progesterone was noted on Day 18 of pregnancy (Day 16 vs. Day 18, $P < 0.01$).

Litter Size Effects on Serum PL and Progesterone

Inspection of the number of viable fetoplacental units on Day 15 of pregnancy indicated that 8 of 13 females in the 1–2 conceptus group, 8 of 11 females in the 3–4 conceptus group, and 9 of 11 females in the 8–10 conceptus group had retained enough

conceptuses to maintain their classification in one of the experimental groups. Only the animals that successfully retained their conceptuses were used in the analysis. One-way classification analyses of variance indicated a significant effect of litter size on serum PL and progesterone ($P < 0.01$ for both, Table 2). Females with 1–2 conceptuses had significantly lower serum PL and progesterone levels than females with 3–4 conceptuses ($P < 0.01$ for both) and females with 8–10 conceptuses had significantly greater serum levels of PL and progesterone than did the aforementioned groups (PL: $P < 0.01$ for both comparisons; progesterone: 1–2 conceptuses vs. 8–10 conceptuses, $P < 0.01$ and 3–4 conceptuses vs. 8–10 conceptuses, $P < 0.05$).

In vitro ovarian progesterone release was significantly greater by ovaries obtained from females bearing 8–10 conceptuses than from females bearing 1–2 conceptuses (1–2 con-

TABLE 2. Effects of litter size on serum placental lactogen and progesterone levels in the C3H mouse (mean \pm SEM).

Conceptus number	N	Day 15 serum	
		mPL ^a (ng/ml)	Progesterone (ng/ml)
1–2	8	25.9 \pm 2.4	22.0 \pm 3.4
3–4	8	70.7 \pm 7.3	35.8 \pm 2.5 ^b
8–10	9	138.6 \pm 7.3	45.4 \pm 2.0 ^c

^aValues for all treatments are significantly different from each other, $P < 0.01$ for all.

^bValues for animals bearing 3–4 conceptuses are significantly different from values for animals bearing 1–2 conceptuses ($P < 0.01$) and animals bearing 8–10 conceptuses ($P < 0.05$).

^cValues for animals bearing 8–10 conceptuses are significantly different from values for animals bearing 1–2 conceptuses ($P < 0.01$).

ceptuses: 26.2 ± 3.4 ng/ovary per 6 h, $n=8$ vs. 8–10 conceptuses: 44.2 ± 4.4 ng/ovary per 6 h, $n=9$, $P<0.01$). In this experiment, 8 of 13 females in the 1–2 conceptus group and 9 of 11 females in the 8–10 conceptus group had retained enough fetoplacental units to maintain their classification in the appropriate experimental group.

In Experiment 3, one-way classification analyses of variance indicated significant treatment effects for both serum PL and progesterone levels ($P<0.01$ for both, Table 3). Post hoc comparisons indicated that the presence of the uterus did not have a significant effect on serum PL levels in females bearing 1–2 conceptuses but serum PL levels were significantly lower in these females than in females bearing 8–10 conceptuses ($P<0.01$ for both comparisons). Females from both treatment groups with 1–2 conceptuses had significantly lower progesterone levels than did females with 8–10 conceptuses ($P<0.01$ for both comparisons). The presence of the uterus in females bearing 1–2 conceptuses was associated with a significant reduction in serum progesterone levels when compared to females lacking the bulk of their uterus ($P<0.05$). In this experiment, 9 of 16 females in the 1–2 conceptus with uterus group, 9 of 14 females in the 1–2 conceptus without uterus group, and 9 of 12 females in the 8–10 conceptus group had retained enough fetoplacental units to maintain their classification in the appropriate experimental group.

DISCUSSION

The results presented in this report and in our previous report (Soares et al., 1982) indicate that there is a pronounced genetic influence on serum PL levels in the mouse. We have identified two characteristics of serum PL levels under genetic control: 1) the gestational profile of serum PL and 2) absolute serum PL levels. Interestingly, the gestational profile of serum PL levels in female C3H mice bred to Balb/c males resembled the profile of Balb/c females bred to Balb/c males rather than the pattern of C3H females bred to C3H males (Soares et al., 1982). The data suggest that the gestational pattern of serum PL is independent of the maternal environment but dependent upon the genotype of the fetoplacental unit. The increase in absolute levels of serum PL in females bred to males of a heterotypic strain may be related to morphological changes in the placenta previously found under similar conditions (Billington, 1964; McLaren, 1965) but do not seem to be dependent on changes in placental size (present study). McLaren (1975) has interpreted the changes in placental morphology accompanying genetic interbreeding to be a consequence of hybrid vigor (heterosis) caused by increased heterozygosity. On the other hand, some of these genetic differences in circulating PL levels may be related to genetic factors involved in the metabolic clearance of the hormone as previously reported for pituitary prolactin and growth hormone in the mouse (Sinha et al., 1979a,b). Genetic differences in

TABLE 3. Uterine influences on the serum placental lactogen and progesterone responses to litter size adjustment in the C3H mouse (mean \pm SEM).

Treatment	N	Day 15 serum	
		mPL (ng/ml)	Progesterone (ng/ml)
1–2 conceptuses with uterus	9	22.4 ± 3.2	20.8 ± 1.8
1–2 conceptuses with uterine tissue removed	9	25.3 ± 2.2	28.0 ± 1.9^a
8–10 conceptuses	9	115.2 ± 9.8^b	42.8 ± 2.1^b

^aValues for animals bearing 8–10 conceptuses are significantly different from values for animals bearing 1–2 conceptuses ($P<0.01$).

^bValues for animals bearing 1–2 conceptuses with uterine tissue removed are significantly different from animals bearing 1–2 conceptuses with an intact uterus ($P<0.05$).

serum PL have been reported in cattle (Bolander et al., 1976). Bolander and co-workers found that dairy cattle had significantly higher PL levels than did beef cattle. Butler et al. (1981) have also reported that genetic factors influence serum PL levels in sheep. Interpretation of the results from this latter study is somewhat troublesome, in that an effect of crossbreeding could not be separated from a possible breed difference in serum PL levels. Strain differences for other reproductive hormone levels during pregnancy have been demonstrated in the mouse (Michael et al., 1975; Barkley et al., 1979).

The elevated serum PL levels found in females bred to males of a heterotypic strain did not have a significant effect on serum progesterone levels. The impact of varied serum PL concentrations on the physiology of gestation in genetically disparate mice is unknown. Strain differences in lactational performance (Yanai and Nagasawa, 1971), maternal behavior (Broida and Svare, 1982; Schneider et al., 1982), and serum progesterone levels (Michael et al., 1975; Barkley et al., 1979) have been reported and may be a direct or indirect reflection of altered serum PL levels. Of course, the genetic differences in serum PL levels may be a compensatory adjustment for a strain difference in target tissue responsiveness to PL.

In contrast to a previous report in the mouse (Simon et al., 1978), litter size did significantly influence serum progesterone levels as well as *in vitro* ovarian progesterone output. In the earlier report, conceptus number was not experimentally adjusted and observations were limited to serum progesterone measurements from mice bearing 7–15 conceptuses. Kato and co-workers (1979) have shown that in the rat serum progesterone levels are affected minimally by the presence of more than six conceptuses. This latter finding provides a possible explanation of the discrepancy between our results and those of Simon et al. (1978). Litter size was also positively correlated with serum PL levels which is consistent with a number of previous studies (mouse: Markoff and Talamantes, 1981; rat: Robertson and Friesen, 1981; Voogt et al., 1982; sheep: Butler et al., 1981). PL may be the factor responsible for maintaining ovarian progesterone production; however, placental androgens are potential luteotropic candidates as well (Soares and Talamantes, 1982). We are currently investigating this issue in our laboratory. Results from our

studies also provide evidence that the pregnant, mouse uterus produces a factor that antagonizes the luteotropic actions of the conceptus. This finding is consistent with the observations of Crister and co-workers (1980 and 1982). Further experimentation is necessary to identify a possible interplay between placental and uterine regulators of luteal function.

In conclusion, the effects of genotype and litter size on serum PL and progesterone levels in the mouse have been described. These results have important implications on the interpretation of experiments pertaining to the physiology of pregnancy in the mouse. Our findings indicate that the hormonal milieu of pregnancy can be modified significantly by litter size and by the genotype of the sire and dam. Although females bearing different size litters, of different genetic strains, or bred to males of different strains may all be considered "pregnant," their pregnancies are not the same. Target tissues from "pregnant" animals where genotype and litter size are not controlled may be exposed to tremendous differences in circulating hormone levels.

ACKNOWLEDGMENTS

We thank Paula Folger for her excellent care of the animals used in these experiments and Dr. Linda Ogren for her review of the manuscript. The authors gratefully acknowledge Dr. G. D. Niswender for antiserum used in the progesterone RIA. Our special thanks to Dr. Josef Skarda, a visiting scientist from the Institute of Animal Physiology and Genetics, Czechoslovak Academy of Sciences, for his valuable advice and helpful discussions during the course of this study. This work was supported by NIH grants RR08132 and HD14966 to F. T. M.J.S. was supported by an NRSA postdoctoral fellowship, HD06363.

REFERENCES

- Barkley, M. S., Bradford, G. E. and Geschwind, I. I. (1978). The pattern of plasma prolactin concentration during the first half of mouse gestation. *Biol. Reprod.* 19:291–296.
- Barkley, M. S., Geschwind, I. I. and Bradford, G. E. (1979). The gestational pattern of estradiol, testosterone, and progesterone secretion in selected strains of mice. *Biol. Reprod.* 20: 733–738.
- Billington, W. D. (1964). Influence of immunological dissimilarity of mother and foetus on size of placenta in mice. *Nature* 202:317–318.
- Bolander, F. F., Ulberg, L. C. and Fellows, R. E. (1976). Circulating placental lactogen levels in dairy and beef cattle. *Endocrinology* 99: 1273–1278.

- Broida, J. and Svare, B. (1982). Strain-typical patterns of pregnancy-induced nestbuilding in mice: maternal and experiential influences. *Physiol. Behav.* 25:153-157.
- Butler, W. R., Fullenkamp, S. M., Cappiello, L. A. and Handwerger, S. (1981). The relationship between breed and litter size in sheep and maternal serum concentrations of placental lactogen, estradiol and progesterone. *J. Anim. Sci.* 53:1077-1081.
- Colosi, P., Marr, G., Lopez, J., Haro, L., Ogren, L. and Talamantes, F. (1982). Isolation, purification, and characterization of mouse placental lactogen. *Proc. Natl. Acad. Sci. USA* 79:771-775.
- Crister, E. S., Rutledge, J. J. and French, L. R. (1980). Role of the uterus and the conceptus in regulating luteal lifespan in the mouse. *Biol. Reprod.* 23:558-563.
- Crister, E. S., Savage, P. J., Rutledge, J. J. and French, L. R. (1982). Plasma concentrations of progesterone and 13,14-dihydro-15-keto prostaglandin F-2 α in pregnant, pseudopregnant and hysterectomized pseudopregnant mice. *J. Reprod. Fertil.* 64:79-83.
- Gibori, G., Antczak, E. and Rothchild, I. (1977). The control of estrogen in the regulation of luteal progesterone secretion in the rat after Day 12 of pregnancy. *Endocrinology* 100:1483-1495.
- Kato, H., Morishige, W. K. and Rothchild, I. (1979). A quantitative relation between the experimentally determined number of conceptuses and corpus luteum activity in the pregnant rat. *Endocrinology* 105:846-850.
- Keppel, G. (1973). *Design and Analysis*. Prentice-Hall, Englewood Cliffs, NJ.
- Markoff, E. and Talamantes, F. (1981). Serum placental lactogen in mice in relation to day of gestation and number of conceptuses. *Biol. Reprod.* 24:846-851.
- McLaren, A. (1965). Genetic and environmental effects on foetal and placental growth in mice. *J. Reprod. Fertil.* 9:79-98.
- McLaren, A. (1975). Antigenic disparity: does it affect placental size, implantation or population genetics? In: *Immunobiology of the Trophoblast* (R. G. Edwards, C.W.S. Howe and M. H. Johnson, eds.). Cambridge University Press, London, pp. 255-273.
- Michael, S. D., Geschwind, I. I., Bradford, G. E. and Stabenfeldt, G. H. (1975). Pregnancy in mice selected for small litter size: reproductive hormone levels and effect of exogenous hormones. *Biol. Reprod.* 12:400-407.
- Robertson, M. C. and Friesen, H. G. (1981). Two forms of rat placental lactogen revealed by radioimmunoassay. *Endocrinology* 108:2388-2390.
- Robertson, M. C., Gillespie, B. and Friesen, H. G. (1982). Characterization of the two forms of rat placental lactogen (rPL): rPL-I and rPL-II. *Endocrinology* 111:1862-1866.
- Rogers, J. F. and Dawson, W. D. (1970). Foetal and placental size in a *Peromyscus* species cross; *J. Reprod. Fertil.* 21:255-262.
- Schnedider, J. E., Lynch, C. B., Possidente, B. and Hegmann, J. P. (1982). Genetic association between progesterone-induced and maternal nesting in mice. *Physiol. Behav.* 29:97-105.
- Simon, N. G., Bridges, R. S. and Gandelman, R. (1978). Correlation among foetal number, corpora lutea and plasma progesterone in Rockland-Swiss mice. *Endokrinologie* 72:247-249.
- Sinha, Y. N., Baxter, S. R. and Vanderlaan, W. P. (1979a). Metabolic clearance rate of prolactin during various physiological states in mice with high and low incidences of mammary tumors. *Endocrinology* 105:680-684.
- Sinha, Y. N., Baxter, S. R. and Vanderlaan, W. P. (1979b). Metabolic clearance rate of growth hormone in mice during various physiological states. *Endocrinology* 105:685-689.
- Soares, M. J. and Talamantes, F. (1982). Gestational effects on placental and serum androgen, progesterone and prolactin-like activity in the mouse. *J. Endocrinol.* 95:29-36.
- Soares, M. J., Colosi, P. and Talamantes, F. (1982). The development and characterization of a homologous radioimmunoassay for mouse placental lactogen. *Endocrinology* 110:668-670.
- Soares, M. J., Colosi, P., Ogren, L. and Talamantes, F. (1983). Identification and partial characterization of a lactogen from the midpregnant mouse conceptus. *Endocrinology* 112:1313-1317.
- Talamantes, F., Ogren, L., Markoff, E., Woodward, S. and Madrid, J. (1980). Phylogenetic distribution, regulation of secretion, and prolactin-like effects of placental lactogens. *Fed. Proc.* 39:2582-2587.
- Voogt, J., Robertson, M. and Friesen, H. (1982). Inverse relationship of prolactin and rat placental lactogen during pregnancy. *Biol. Reprod.* 26:800-805.
- Yanai, R. and Nagasawa, H. (1971). Mammary growth and placental mammatropin during pregnancy in mice with high or low lactational performance. *J. Dairy Sci.* 54:906-910.