BIOLOGY OF REPRODUCTION 27, 523–529 (1982)

# Placental and Serum Hormone Changes During the Second Half of Pregnancy in the Hamster

MICHAEL J. SOARES and FRANK TALAMANTES[1]

*Thimann Laboratories*
*Division of Natural Sciences*
*University of California,*
*Santa Cruz, California 95064*

## ABSTRACT

The concentrations of androgen, estrogen, progesterone, and prolactin-like hormones in serum, placentae, and media from placental incubations during the second half of pregnancy in the hamster were determined. Concentrations of prolactin-like activity in serum, placentae, and media from placental incubations increased from Day 8 to Day 14 of gestation. On Day 16 of pregnancy the content and in vitro release of placental prolactin-like activity declined; however, serum prolactin-like activity increased. The in vitro release of $^{3}$H-protein by hamster placentae showed gestational changes resembling the pattern of in vitro placental lactogen release. Serum and placental content of progesterone increased from Day 8 to Day 14 of gestation and declined from Day 14 to Day 16. In vitro placental progesterone release increased from Day 8 to Day 12 and declined from Day 12 to Day 16. Serum estrogen levels were elevated during the second half of pregnancy, whereas serum androgen levels were minimal. Neither estrogen nor androgen production by hamster placentae could be detected.

## INTRODUCTION

The hormonal control of pregnancy involves interactions between the anterior pituitary, ovaries, and placentae (Greenwald and Rothchild, 1968). In the hamster, our understanding of the role of the anterior pituitary and the ovaries in the control of pregnancy has progressed much further (Greenwald, 1967, 1973) than our understanding of the role of placentae in this process.

The endocrinology of the hamster placenta has received little attention. Hamster placentae contain enzymes involved in the synthesis and metabolism of steroid hormones (Legrand, 1977; Marchut, 1980, 1981); however, their endocrine activities are not sufficient to maintain pregnancy in the absence of the ovaries (Klein, 1938). The presence of placentae are essential for the maintenance of corpora lutea function during the second half of pregnancy (Klein, 1938; Greenwald, 1974). A prolactin-like hormone identified in the hamster placenta (Talamantes, 1975; Kelly et al., 1976) may be one of the luteotropic factors responsible for sustaining corpora lutea activity during the last half of pregnancy (Greenwald and Rothchild, 1968). The relationships between serum steroid and prolactin-like hormone patterns and the in vitro placental secretion profiles of these hormones have not been described.

The purpose of this investigation was to determine the concentrations of androgen, estrogen, progesterone, and prolactin-like hormones in serum, placentae, and media from placental incubations during the second half of pregnancy in the hamster.

## MATERIALS AND METHODS

### *Animals*

Pregnant hamsters were obtained from Eagle Laboratory Animals, Inc. (Farmersburg, IN) on Day 5 of gestation (Day 1 of pregnancy is defined as the day in which spermatozoa were present in the vaginal lavage). Animals were maintained on a 14L:10D lighting schedule (lights on at 1600 h). Food and water were available ad libitum.

### *Radioimmunoassays for Androgen, Estrogen, and Progesterone, and Radioreceptor Assay for Prolactin-Like Activity*

Androgen, estrogen, and progesterone were measured with techniques previously described (Soares and Hoffmann, 1981; Campbell et al., 1977; Gibori et al., 1977). The antisera used for the androgen (GDN #250), estrogen (GDN #244), and progesterone (GDN #337) assays were obtained from Dr. Gordon Niswender. The androgen radioimmunoassay measures

Accepted April 9, 1982.
Received January 11, 1982.
[1] Reprint requests.

primarily testosterone (100% cross-reactivity) and 17β-hydroxy-5α-androstane-3-one (78.4% cross-reactivity). [1,2,6,7,16,17-$^3$H] testosterone (135 Ci/mMol), [2,4,6,7,16,17-$^3$H] estradiol (137.1 Ci/mMol), and [1,2,6,7-$^3$H] progesterone (96.5 Ci/mMol) were purchased from New England Nuclear (Boston, MA). Nonradioactive steroids were obtained from Sigma Chemical Co. (St. Louis, MO). Sera and supernatants from placental homogenizations were extracted with 15 vol of benzene:hexane (1:2 v/v), 10 vol of diethylether, or 15 vol of hexane for the androgen, estrogen, and progesterone assays, respectively. Extraction recoveries averaged over 90% for all three procedures. Final serum and placental concentrations were not adjusted for losses incurred during the extraction procedure. The sensitivities of the steroid assays were: Androgen, 2.5 to 5.0 pg/tube; progesterone, 5 to 10 pg/tube; estrogen, 2.5 to 5.0 pg/tube. Preliminary experiments indicated that it was not necessary to extract the incubation media before assaying for progesterone. Androgen and estrogen could not be detected in either placental homogenates or media from placental incubations.

Sera, supernatants from placental homogenizations, and media from the placental incubations were assayed for prolactin-like activity by the lactating rabbit mammary gland radioreceptor assay as previously described (Shoer et al., 1978; Markoff and Talamantes, 1981). Ovine prolactin (NIAMDD-P-S13, 30 IU/mg) was obtained from the National Institute of Arthritis and Metabolic Digestive Diseases (Bethesda, MD) and used both for iodination and as a reference standard.

### *Blood Collection*

Blood samples were obtained from female hamsters on Days 8, 10, 12, 14, and 16 of pregnancy. The 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 androgen, estrogen, progesterone, and prolactin-like activity. All hamsters were sacrificed between 0800 and 0830 h.

### *Placental Hormone Content*

The fetal portions of freshly dissected placentae from Days 8, 10, 12, 14, and 16 of pregnancy were lyophilized individually, weighed, and homogenized with a Teflon-coated pestle in a 5-ml glass grinding vessel immersed in ice. Tissues were homogenized in 0.1 M ammonium bicarbonate buffer, pH 9.4, containing 10 mM EGTA, 10 mM EDTA, 20 mM benzamidine HCl, and 1% Triton X-100. The homogenate was centrifuged at 4000 × *g* for 20 min and the supernatants were stored at −20°C until assayed. Ten placentae were used for each day of gestation and were randomly obtained from 6 to 8 female hamsters.

### *Placental Incubations*

Hamsters were sacrificed on Days 8, 10, 12, 14, and 16 of pregnancy and their placentae were dissected and the fetal portions utilized for explant culture. Day 8 placentae were cut in half and cultured in pairs. Placentae from the remainder of gestation were incubated individually and either quartered (Days 10 and 12) or cut into six equal explants (Days 14 and 16) to facilitate diffusion. The placentae were incubated in vials containing 2 ml of alpha-MEM (Grand Island Biological Co., Grand Island, NY) supplemented with 50 μg/ml of Garamycin (gentamycin sulfate; Schering Corp., Kenilworth, NJ). Explants were preincubated for 1 h at 37°C in a shaking water bath (50 revolutions per min) under an atmosphere of 95% $O_2$/5% $CO_2$. After the preincubation, the incubation media were removed and replaced with 2 ml of fresh media. The explants were incubated for 12 h. The media recovered from the incubations were centrifuged at 4000 × *g* for 10 min and stored at −20°C until assayed. At the end of the incubation, the explants were lyophilized and their dry weights were determined. Ten placentae were used for each day of gestation and were randomly obtained from 6 to 8 female hamsters.

Gestational changes in placental protein synthesis were assessed by culturing placentae from different days of gestation with [$^3$H] leucine and measuring the incorporation of [$^3$H] leucine into trichloroacetic acid (TCA) precipitable protein. Explants of placentae from Days 8, 10, 12, 14, and 16 of gestation were prepared as described above, preincubated for 1 h in 2 ml of leucine-free MEM culture medium (Grand Island Biological Co.,) supplemented with Garamycin (50 μg/ml) and then incubated for 12 h in 2 ml of fresh leucine-free MEM supplemented with 10 μCi of [$^3$H] leucine (60 Ci/mM, Schwarz-Mann, Orangeburg, NY). A 0.1 ml aliquot of medium was incubated with 2 ml of 10% (w/v) TCA, 250 μg bovine serum albumin, and 1 mg of L-leucine per ml. The precipitated protein was collected by centrifugation at 2000 × *g* for 10 min at 4°C. The precipitate was solubilized in 0.1 ml of 1.0 N sodium hydroxide and counted in 10 ml of scintillation fluid (Scintisol, Isolabs Inc., Akron, OH) using a Beckman liquid scintillation spectrometer. The nonspecific adsorption of [$^3$H] leucine to proteins was determined by precipitating 0.1 ml of culture medium containing an appropriate amount of [$^3$H] leucine. The values obtained for nonspecific adsorption were subtracted from the values obtained for the placental incubations. Ten placentae obtained from 6 to 8 female hamsters were used for each of the days of gestation evaluated.

### *Statistical Analysis*

Data were analyzed by a randomized block, one-way classification analysis of variance. Post hoc analyses were conducted with Duncan's test (Keppel, 1973).

## RESULTS

### *Prolactin-Like Activity in Serum, Placentae, and Media from Placental Incubations*

Parallel displacement curves were found with pregnant hamster serum, placental extracts, media from placental incubations, and ovine prolactin reference standards in the radioreceptor assay for prolactin-like activity (Fig. 1). Analyses of variance indicated that prolactin-like activity varied significantly during

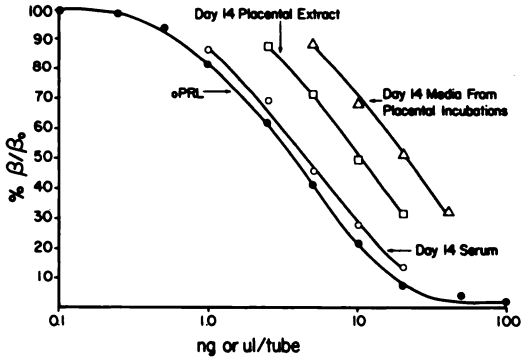


FIG. 1. Displacement curves for ovine prolactin (oPrl), serum from Day 14 of pregnancy, Day 14 placental extracts, and medium from Day 14 placental incubations in the receptor assay for prolactin-like activity. $B/B_0$ refers to the ratio of bound to total bound. Each point represents the mean of 5 determinations.

gestation when measured in serum, placental extracts, or media from placental incubations ($P<0.01$ for all, Fig. 2). Serum levels increased significantly from Day 8 to Day 12 of gestation ($P<0.01$), did not change significantly from Day 12 to Day 14, and then increased significantly from Day 14 to Day 16 ($P<0.05$). The patterns of prolactin-like activity in placentae and released by placentae during gestation were similar. Placental content and release of prolactin-like activity increased significantly from Day 8 to Day 14 ($P<0.01$ for both). Placental content of prolactin-like activity decreased significantly from Day 14 to Day 16 of gestation ($P<0.01$), whereas placental release of prolactin-like activity did not change significantly during this interval.

### *Placental $^3$H-Protein Release and Placental Weights*

The patterns of $^3$H-protein released by placentae and placental weights varied significantly during gestation ($P<0.01$ for both, Fig. 3). Placental $^3$H-protein release increased from Day 8 to Day 14 ($P<0.01$) and decreased significantly from Day 14 to Day 16 ($P<0.01$). Placental weights increased significantly from Day 8 to Day 16 of gestation ($P<0.01$).

### *Progesterone Levels in Serum, Placentae, and Media from Placental Incubations*

Analyses of variance for serum, placental, and placental incubation media concentrations of progesterone displayed significant variations during the second half of pregnancy ($P<0.01$ for all, Fig. 4). The profiles of serum and placental progesterone were similar. Progesterone levels showed minimal changes from Day

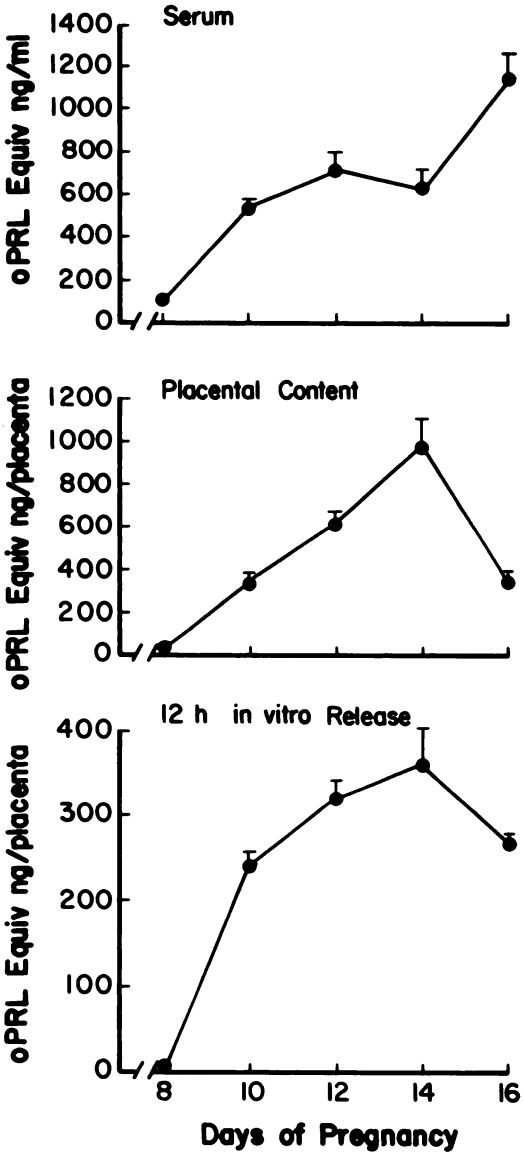


FIG. 2. Serum, placental content and in vitro placental release of prolactin-like activity during the second half of gestation. Each point for the serum measurements represents the mean of 6 to 8 animals. Each point for the placental content and in vitro placental release figures represents the mean measurements from 10 placentae obtained from 6 to 8 hamsters. The *vertical lines* indicate the standard error of the mean.

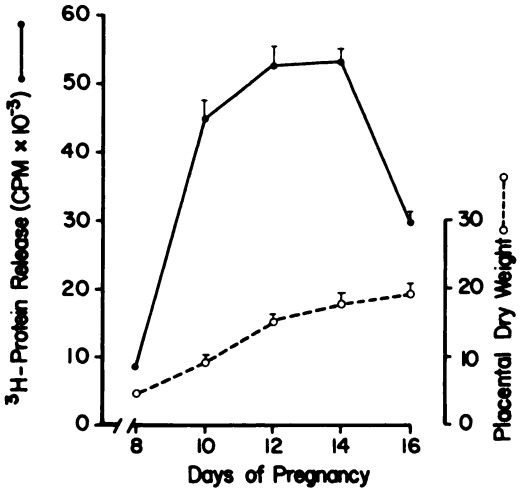


FIG. 3. In vitro placental $^3$H-protein release and placental dry weights during the second half of gestation. Each point represents the mean measurements from 10 placentae obtained from 6 to 8 hamsters. The *vertical lines* indicate the standard error of the mean.

8 to Day 12, increased significantly from Day 12 to Day 14 ($P<0.01$ for both), and decreased significantly from Day 14 to Day 16 ($P<0.01$ for both). Release of progesterone by placental explants increased significantly from Day 8 to Day 12 ($P<0.01$) and decreased from Day 12 to Day 16 ($P<0.01$).

### *Androgen and Estrogen Activity During the Second Half of Gestation*

Androgen and estrogen could not be detected in placental extracts or in media from placental incubations. Serum androgen levels did not vary significantly during the second half of gestation (Fig. 5). Analysis of variance indicated that serum estrogen levels varied significantly during the second half of gestation ($P<0.01$, Fig. 5). Serum estrogen levels increased from Day 8 to Day 14 of gestation ($P<0.05$) and decreased significantly from Day 14 to Day 16 of gestation ($P<0.01$).

## DISCUSSION

We have characterized the in vitro placental secretory profile for progesterone and prolactin-like activity during the second half of pregnancy in the hamster and have been unable to detect androgen or estrogen production by hamster placentae in vitro.

The serum profile for prolactin-like activity during the second half of pregnancy was similar to a previous report by Kelly et al. (1976). The increase in serum prolactin-like activity from Day 8 to Day 14 of gestation corresponded to similar patterns of placental prolactin-like hormone content and in vitro release. On Day 16 of pregnancy the content and in vitro release of placental prolactin-like activity declined; however, serum prolactin-like activity increased. Whether this discrepency in placental and serum prolactin-like activities is indicative of the absence of an important regulator of placental lactogen secretion in vitro or is suggestive of another source of serum prolactin-like activity on Day 16 of pregnancy remains to be evaluated. Based on the serum levels of pituitary prolactin during the latter days of

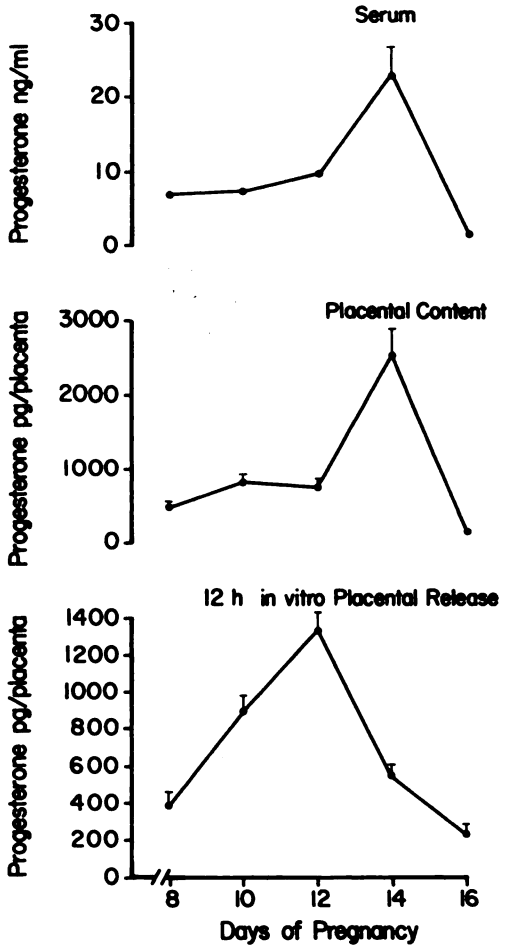


FIG. 4. Serum, placental content and in vitro placental release of progesterone during the second half of gestation. See Fig. 2 for further details.

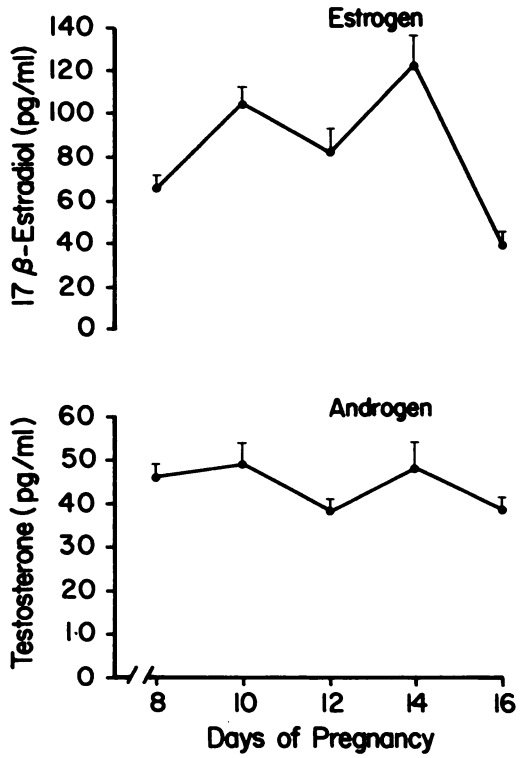


FIG. 5. Serum estrogen and androgen levels during the second half of gestation. See Fig. 2 for further details.

gestation in the hamster (Bast and Greenwald, 1974) and the potency of hamster pituitary prolactin in the radioreceptor assay (Colosi et al., 1981), it is unlikely that the elevation in serum prolactin-like activity measured with the radioreceptor assay on Day 16 could be attributed to pituitary prolactin.

The levels of serum prolactin-like activity during gestation measured in our laboratory were lower than those measured by Kelly and co-workers. This discrepency may be explained by differences in the hamsters used in the two studies, in the time of blood sampling, or in the method of blood sampling. The latter point is particularly relevant since Kelly et al. (1976) conducted their blood collections under ether anesthesia, a known stimulator of pituitary prolactin release in rodents (Neill, 1970; Shin, 1979; Goldman et al., 1981).

The in vitro release of $^{3}$H-protein by hamster placentae showed gestational changes resembling the pattern of in vitro placental lactogen release. A similar relationship between in vitro placental protein synthesis and placental lactogen release is also evident in the mouse (Soares and Talamantes, unpublished findings).

Serum progesterone levels during the second half of gestation were in agreement with progesterone profiles previously reported for the hamster (Leavitt and Blaha, 1970; Lukaszewska and Greenwald, 1970; Baranczuk and Greenwald, 1974). The gestational changes in placental progesterone content coincided with gestational changes in serum progesterone levels; however, the profile of placental progesterone release was not consistent with these profiles. The significance of the differences in these patterns neeeds to be evaluated. The relative contribution of placental progesterone secretion to circulating progesterone levels in the hamster is unknown, as is the biological relevance of placental progesterone production.

Serum estrogen levels during the latter half of pregnancy were similar to levels previously reported for the hamster (Baranczuk and Greenwald, 1974). The source of circulating estrogen does not appear to be the placenta, which is consistent with its lack of aromatase activity (Marchut, 1981). Serum estrogen levels on Days 8 to 14 of gestation were considerably higher than serum androgen levels during this same period. This finding is in contrast to gestational patterns of serum estrogen and androgen levels for the rat (Gibori and Sridaran, 1981) and mouse (Barkley et al., 1979). The placenta appears to be the source of serum androgen during the second half of pregnancy in the rat (Gibori and Sridaran, 1981; Sridaran et al., 1981) and mouse (Soares and Talamantes, unpublished findings); however, in the hamster, serum androgen levels are low during the second half of gestation and their placentae do not secrete detectable quantities of androgen nor are they capable of synthesizing androgens from C-21 steroid precursors (Marchut, 1980). Estrogen has been demonstrated to be an important luteotropic hormone during pregnancy in the rat and the rabbit (see Keyes et al., 1979, for review) and aromatizable androgens are luteotropic, as well, via their capacity to be converted to estrogens by the corpus luteum (Gibori and Keyes, 1978; Gibori et al., 1978; Gibori et al., 1979; Keyes et al., 1980). A role for estrogen as a luteotropic hormone during the second half of pregnancy in the hamster has not been demonstrated. If estrogen is indeed luteotropic in the hamster, the lack of placental estrogen or androgen production during the second half of pregnancy would

necessitate an ovarian source for these hormones. The dependency of ovarian androgen and estrogen biosynthesis on the anterior pituitary (see Leung and Armstrong, 1980, for a review) may explain the need for the anterior pituitary throughout pregnancy in the hamster (Greenwald and Rothchild, 1968). Further experimentation will be necessary in order to clarify the regulation of luteal activity during the second half of pregnancy in the hamster.

## ACKNOWLEDGMENTS

We thank Greg Marr and Brad Hunter for excellent technical assistance and Dr. Linda Ogren for critical review of the manuscript. The authors gratefully acknowledge Dr. Gordon D. Niswender for his generous supply of antisera for the androgen (No. 250), estrogen (No. 244), and progesterone (No. 337) radioimmunoassays, the NIAMDD and Dr. A. Parlow for providing ovine prolactin (NIAMDD-P-S13). For assistance in the preparation of the manuscript the authors express their thanks to Marlene M. Soares. This work was supported by NIH grants RR08132 and HD14966 to Dr. Frank Talamantes.

## REFERENCES

Baranczuk, R. and Greenwald, G. S. (1974). Plasma levels of oestrogen and progesterone in pregnant and lactating hamsters. J. Endocrinol. 63:125–135.

Barkley, M. S., Geschwind, I. I. and Bradford, G. E. (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.

Bast, J. D. and Greenwald, G. S. (1974). Daily concentrations of gonadotrophins and prolactin in the serum of pregnant or lactating hamsters. J. Endocrinol. 63:527–532.

Campbell, C. S., Schwartz, N. B. and Firlit, M. G. (1977). The role of adrenal and ovarian steroids in the control of serum LH and FSH. Endocrinology 101:162–172.

Colosi, P., Markoff, E., Levy, A., Ogren, L., Shine, N. and Talamantes, F. (1981). Isolation and partial characterization of secreted hamster pituitary prolactin. Endocrinology 108: 850–854.

Gibori, G. and Keyes, P. L. (1978). Role of intraluteal estrogen in the regulation of the rat corpus luteum during pregnancy. Endocrinology 102: 1176–1182.

Gibori, G., Antczak, E. and Rothchild, I. (1977). The role of estrogen in the regulation of luteal progesterone secretion in the rat after Day 12 of pregnancy. Endocrinology 100:1483–1495.

Gibori, G., Chatterton, R. T. and Chien, J. L. (1979). Ovarian and serum concentrations of androgen throughout pregnancy in the rat. Biol. Reprod. 21:53–56.

Gibori, G., Keyes, P. L. and Richards, J. S. (1978). A role for intraluteal estrogen in the mediation of luteinizing hormone action on the rat corpus luteum during pregnancy. Endocrinology 103: 162–169.

Gibori, G. and Sridaran, R. (1981). Sites of androgen and estradiol production in the second half of pregnancy in the rat. Biol. Reprod. 24:249–256.

Goldman, B. D., Matt, K. S., Roychoudhury, P. and Stetson, M. H. (1981). Prolactin release in golden hamsters: Photoperiod and gonadal influences. Biol. Reprod. 24:287–292.

Greenwald, G. S. (1967). Luteotropic complex of the hamster. Endocrinology 80:118–130.

Greenwald, G. S. (1973). Further evidence for a luteotropic complex in the hamster: Progesterone determinations of plasma and corpora lutea. Endocrinology 92:235–242.

Greenwald, G. S. (1974). Modifications in ovarian and pituitary function in the hysterectomized pregnant hamster. J. Endocrinol. 61:45–51.

Greenwald, G. S. and Rothchild, I. (1968). Formation and maintenance of corpora lutea in laboratory animals. J. Anim. Sci. 29: suppl. 1, pp. 139–162.

Kelly, P. A., Tsushima, T., Shiu, R.P.C. and Friesen, H. G. (1976). Lactogenic and growth hormone-like activities in pregnancy determined by radioreceptor assays. Endocrinology 99:765–774.

Keppel, G. (1973). Design and Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Keyes, P. L., Yuh, K.C.M. and Miller, J. B. (1979). Estrogen action in the corpus luteum. In: Ovarian Follicular and Corpus Luteum Function (C. P. Channing, J. M. Marsh and W. A. Sadler, eds.). Plenum Press, New York, pp. 447–463.

Keyes, P. L., Gibori, G., Possley, R. M and Brown, J. M. (1980). Early changes in luteal function associated with luteotrophic effect of testosterone in the pregnant rat. Biol. Reprod. 22:1142–1148.

Klein, M. (1938). Relation between the uterus and the ovaries in the pregnant hamster. Proc. Roy. Soc. (Lond.) Ser. B 125:348–364.

Leavitt, W. W. and Blaha, G. C. (1970). Circulating progesterone levels in the golden hamster during the estrous cycle, pregnancy, and lactation. Biol. Reprod. 3:353–361.

Legrand, C. (1977). Histochemical distribution of 5-3$\beta$ and 17$\beta$-hydroxysteroid dehydrogenases in hamster trophblast. J. reprod. Fertil. 51: 405–408.

Leung, P.C.K. and Armstrong, D. T. (1980). Interactions of steroids and gonadotropins in the control of steroidogenesis in the ovarian follicle. Ann. Rev. Physiol. 42:71–82.

Lukaszewska, J. H. and Greenwald, G. S. (1970). Progesterone levels in the cyclic and pregnant hamster. Endocrinology 86:1–9.

Marchut, M. (1980). Steroid metabolism in pregnant hamster. I. Metabolism of 4-$^{14}$C pregnenolone and 4-$^{14}$C progesterone by hamster placental tissue *in vitro*. Endocrinol. Exp. 14:121–135.

Marchut, M. (1981). Steroid metabolism in pregnant hamster. II. The metabolism of androgens by the hamster placenta *in vitro*. Endocrinol. Exp. 15:111–128.

Markoff, E. and Talamantes, F. (1981). Serum placen-

tal lactogen in mice in relation to day of gestation and number of conceptuses. Biol. Reprod. 24:846–851.

Neill, J. D. (1970). Effect of "stress" on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat. Endocrinology 87: 1192–1197.

Shin, S. H. (1979). Prolactin secretion in acute stress is controlled by prolactin releasing factors. Life Sci. 25:1829–1836.

Shoer, L. F., Shine, N. R. and Talamantes, F. (1978). Isolation and partial characterization of secreted mouse pituitary prolactin. Biochim. Biophys. Acta 537:336–347.

Soares, M. J. and Hoffmann, J. C. (1981). Seasonal reproduction in the mongoose, *Herpestes auropunctatus*. I. Androgen, luteinizing hormone, and follicle stimulating hormone in the male. Gen. Comp. Endocrinol. 44:350–358.

Sridaran, R., Basuray, R. and Gibori, G. (1981). Source and regulation of testosterone secretion in pregnant and pseudopregnant rats. Endocrinology 108:855–861.

Talamantes, F. (1975). Comparative study of the occurrence of placental prolactin among mammals. Gen. Comp. Endocrinol. 27:115–121.