PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 435–441 (1988)

Testicular Involvement in Peripubertal Gonadotropin Levels and Accessory Sex Organ Weight of Male Hamsters (42685)

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Abstract. This study evaluates the influence of testicular secretions during development in male hamsters on peripubertal gonadotropin levels. Castration or sham operations were performed on the day of birth (Day 1), Day 5, 10, or 20 of life. Repeated plasma samples on Days 20–60 at 10-day intervals were taken via orbital sinus puncture. Castrated animals received a subcutaneous testosterone capsule on Day 60 and were killed on Day 70. In addition, seminal vesicles and ventral prostate weights were taken in all animals at Day 70. Castrated animals, regardless of day of castration, had higher gonadotropin levels and suppressed sexual accessory organ weights. Animals castrated on the day of birth had lower luteinizing hormone (LH) levels than animals castrated on other days. Castration on Day 10 resulted in lower follicle stimulating hormone (FSH) levels. Males castrated on Day 20 were most sensitive to the negative feedback effect of testosterone on LH secretion, while Day 10 castrates had elevated FSH levels after testosterone exposure. Sexual accessory weights also differed depending upon the day of castration. Results point out the importance of testicular secretions on the developmental processes as well as the differing ages at which various systems may be influenced.

The peripubertal pattern of gonadotropin secretion in male and female hamsters is distinctly different (1) as has been shown for a number of species (2, 3). The role of testicular secretions in the development of a variety of sexually dimorphic characteristics, including morphology (4–6), behavior (7, 8), and reproductive hormone regulation (9–11), has been well documented. While it is clear that masculine behavioral potential of male hamsters is influenced by the hormonal condition around the time of birth (12), less is known about the influence of early hormones on other reproductive functions. In particular, is the developmental pattern of gonadotropin secretion "programmed" by testicular secretions in this species? Our earlier work showed that elevating the neonatal levels of steroid hormones in intact male hamsters altered adult behavior and gonadotropin levels (13). The present study examines the effects of eliminating neonatal testicular secretions on the development of gonadotropins around the time of puberty in male hamsters.

Materials and Methods. *Animals.* The animals used in this study were male hamsters born in the laboratory of Charles River Lakeview (Wilmington, MA) stock (LVG-LAK). Colony conditions included a 14:10 hr LD cycle with lights on at 0500 hr, constant temperature, and food and water available *ad libitum.* On the day of birth (Day 1) or Day 5, 10, or 20 of life males were castrated or sham operated through a mid ventral incision. On Days 1, 5, or 10, surgery was performed under ice anesthesia. The skin and body wall were closed with silk suture and flexible colloidin. On Day 20, ether anesthesia replaced ice while wound clips were used instead of colloidin. Sham surgery was identical to castration except that the testes were visualized but not removed. When animals were rewarmed, and had regained consciousness, they were returned to their mothers. Animals were weaned on Day 20 of life and housed in groups of two to four animals per cage.

Samples. Beginning on Day 20 and at 10-day intervals through Day 60, blood samples were taken via orbital sinus puncture under ether anesthesia. All samples were collected between 0800 and 1400 hr with the vast majority collected between 1000 and 1200 hr. The Day 20 samples for males who were castrated or sham operated on Day 20 were taken just prior to surgery. Samples were collected using heparinized, 75-mm microhematocrit tubes (i.d. = 1.10 mm; o.d. = 1.50 mm). Capillary tubes were plugged

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with Critoseal and were spun in a microhematocrit centrifuge for 8 min. Tubes were broken at the cell/plasma interface and 30-μl aliquots of plasma were pipetted into 12 × 75-mm polystyrene culture tubes. Plasma was diluted with 470 μl of 0.01 M phosphate-buffered saline with 1% egg white and frozen at −20°C until assay. Each animal had one sample assayed for LH and one for FSH at each of the sample days.

Following collection of the Day 60 sample, castrated animals were implanted with a Silastic capsule containing crystalline testosterone. Capsules were 1.18 mm i.d. × 3.18 o.d. packed with 8 mm of testosterone. The ends were plugged with wooden doweling and were sealed with silicone adhesive. On Day 70 of age, all animals were killed by decapitation. Trunk blood was collected and allowed to clot overnight at 4°C. After removal of the clots, serum was centrifuged at 2500*g* for 20 min. Serum was removed from the cells, aliquoted (30 μl), diluted, and frozen as above.

At autopsy seminal vesicles and ventral prostate were dissected from all animals and wet weight was recorded. At the same time, completeness of castration was verified.

RIA. Radioimmunoassays were run using a double antibody system. For LH the ovine:ovine system of Niswender *et al.* (14) was used. Antiserum was a gift from Dr. G. D. Niswender, Colorado State University. Hormone for radiolabeling was generously provided by Dr. L. E. Reichert of the Albany Medical College. FSH was measured using kits provided by NIADDK and the National Pituitary Agency. LH potencies are expressed as nanogram equivalents of NIADDK RP-1; FSH is expressed as NIADDK RP-2. All samples for each hormone were run in the same assay.

Statistical analysis. LH or FSH levels in plasma for Days 20–60 of life in shams and castrates were analyzed using multivariate analysis of variance with repeated measures (BMDP). Further comparisons among means were achieved using Tukey's test (15). Ventral prostate weight and seminal vesicle weight were each analyzed using two-way ANOVA followed by Tukey's test. Serum LH or FSH levels in castrates on Day 70 following implantation of testosterone capsules vs Day 60 values were compared using a paired Student's *t* test. In all cases, differences were considered to be significant if $P \leq 0.05$.

Results. *LH.* Figure 1 shows the levels of LH in the plasma of male hamsters castrated or sham operated on Days 1, 5, 10, or 20 of life. The number of animals per point is given in Tables I and II. Samples were taken every 10 days from Days 20 through 60 of life. Multivariate analysis of variance with repeated measures revealed a significant effect of treatment, i.e., castration ($F(1,78) = 63.97, P < 0.0001$), an interaction between treatment and age of treatment ($F(3,78) = 2.72, P = 0.05$) and age at which samples were taken ($F(4,75) = 24.83, P < 0.0001$). Further analysis with Tukey's test showed that castrated animals had elevated levels of LH as compared to sham-operated animals

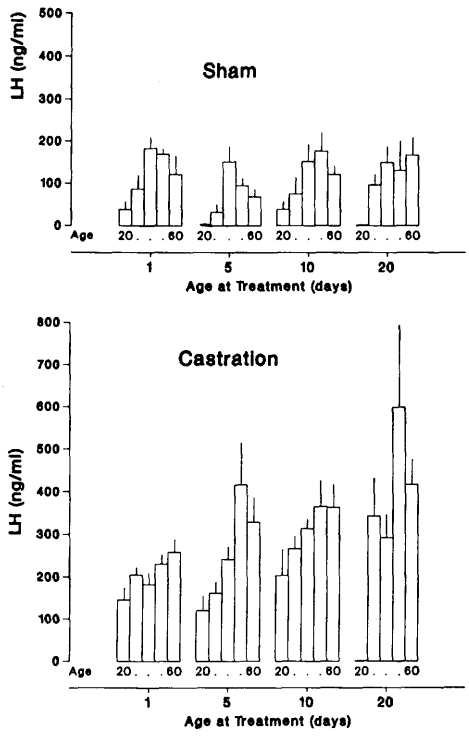

FIG. 1. Mean (±SEM) plasma luteinizing hormone (LH) levels in male hamsters castrated or sham operated on Day 1 (day of birth), 5, 10, or 20 of life. Repeated blood samples were taken on Days 20, 30, 40, 50, or 60 of life (age 20 and 60 days are indicated on the abscissa). Numbers of samples per point are given in Table II.

($P < 0.01$). Animals castrated on Day 1 had lower LH levels than those of other days ($P < 0.05$) while animals castrated on Days 5, 10, or 20 did not differ from each other. Samples collected on Days 50 or 60 contained more LH than other samples ($P < 0.01$). On all other sample days, LH was higher than on Day 20 of age ($P < 0.01$).

FSH. Plasma FSH levels after castration or sham operation at different ages through the peripubertal period are shown in Fig. 2. Statistically significant effects were revealed using multivariate analysis of variance. There was a significant effect of treatment ($F(1,78) = 149.50$, $P < 0.0001$), age of castration ($F(3,78) = 22.15$, $P < 0.0001$), an interaction of treatment with age of treatment ($F(3,78) = 3.13$, $P = 0.03$) and a significant effect in the repeated measure, i.e., age of sampling ($F(4,75) = 11.74$, $P < 0.0001$). Multiple comparisons among the means were accomplished using Tukey's test. FSH levels of castrates were higher than those of sham-operated animals ($P < 0.01$). Animals castrated on Day 10 of life had lower levels of FSH than the other 3 days ($P < 0.05$). Samples taken on Day 20 were not different from those taken on Day 60.

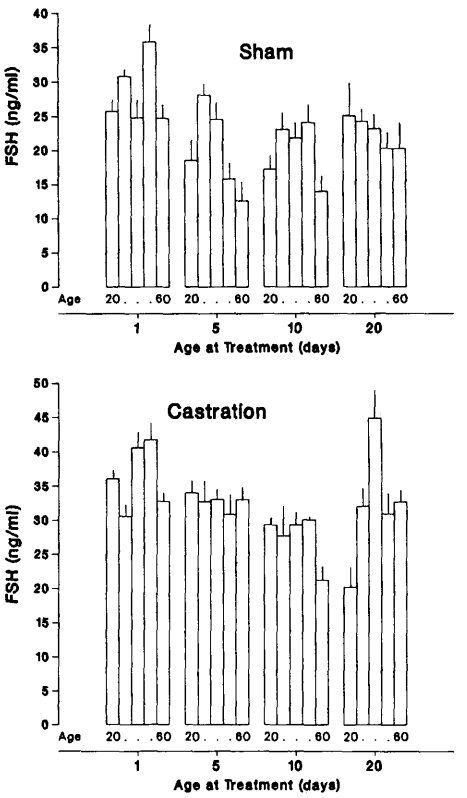

FIG. 2. Mean (±SEM) plasma follicle stimulating hormone (FSH) levels in male hamsters castrated or sham operated on Day 1 (day of birth), 5, 10, or 20 of life. Blood samples were taken on Days 20–60 at 10-day intervals. Numbers of samples per point are given in Table II.

Feedback of testosterone. Table I gives the levels of LH and FSH in castrated animals which had received an 8-mm Silastic capsule filled with testosterone 10 days prior to being killed on Day 70. A paired Student's t test (two-tailed) was used to compare the Day 70 values with those on Day 60 (just before implant). Males castrated on Day 20 had significantly lower LH levels after testosterone exposure than before ($t = 2.64$, $df = 8$, $P < 0.05$). No other differences in LH were found for other days of castration. FSH levels were significantly increased in animals which had been castrated on Day 10 ($t = 5.39$, $df = 12$, $P < 0.001$) and Day 20 ($t = 3.25$, $df = 8$, $P < 0.02$). Animals castrated at other ages did not change in FSH levels as a result of testosterone exposure.

Organ weights. Wet weights of ventral prostate and seminal vesicles (bilateral) from sham-operated and castrated animals are given in Table II. Two-way analysis of variance of ventral prostate weights showed that there was a significant effect of castration ($F(1,88) = 693.64$, $P < 0.0001$) of age at treatment ($F(3,88) = 6.90$, $P < 0.0003$) without a significant interaction term. Analysis of seminal vesicle weight also revealed a statistically significant effect of castration ($F1,88 = 451.40$, $P < 0.0001$), of age of treatment ($F3,88 = 10.96$, $P < 0.0001$) with a significant interaction of age at treatment ($F3,88 = 6.58$, $P < 0.0005$). Tukey's test of ventral prostate or seminal vesicle weights showed that all castrated animals had smaller accessory organs than sham-operated animals ($P < 0.01$). Animals castrated on Day 10 or 20 had higher seminal vesicle weights than those castrated on the day of birth ($P < 0.01$) but were not different from those castrated on Day 5. Ventral prostates of animals castrated on Day 20 of life were significantly smaller than those from animals castrated on Day 10

TABLE I. MEAN (±SEM) SERUM LEVELS OF LH AND FSH ON DAY 70 OF LIFE IN ANIMALS CASTRATED AT VARIOUS AGES WHICH HAD RECEIVED A SILASTIC CAPSULE OF TESTOSTERONE ON DAY 60[a]

	Castration day			
	1	5	10	20
LH (ng/ml)	205.3 ±25.0	290.3 ±49.5	574.3 ±67.0	212.3[b] ±47.5
n	24	12	13	9
FSH (ng/ml)	32.67 ±1.72	34.22 ±3.53	37.41[d] ±2.66	43.22[c] ±3.93
n	24	12	13	9

[a] Day 60 values in castrated males, with which these values are compared, are shown in Figs. 1 and 2.
[b] $P < 0.05$, [c] $P < 0.002$, [d] $P < 0.001$. Values significantly different from day 60 plasma levels using a paired, two-tailed Student's *t* test.

($P < 0.05$). Sham-operated animals did not differ.

Discussion. In the present study, sham-operated male hamsters exhibited patterns of LH and FSH secretion around puberty similar to those previously reported (1, 16). Intact male hamsters on an L:D, 14:10 hr cycle show increasing levels of LH between Day 20 and Days 40–50 with a decline thereafter. FSH levels peak at around Days 35–40 and then decline. Although the gonadotropin developmental patterns in the control animals are similar, absolute values of LH and FSH in the present study differ from the two previous studies. In each case, either a different assay system (rat:rat vs ovine:ovine) or different reference preparations (FSH RP1 vs RP2) can account for any discrepancy. As has been shown (16), prepubertal castration led to increased circulating gonadotropin levels compared to sham-operated males. In addition, we showed that the presence of the testis for varying lengths of time prior to the initial prepubertal blood sample had effects on actual gonadotropin levels, the subsequent developmental pattern of gonadotropins, sensitivity to testosterone-negative feedback, and sexual accessory organ weights.

The overall increase in gonadotropin levels differs depending on the day of castration and on which gonadotropin is considered. Hamsters which were castrated on the day of birth had lower LH levels than those castrated at increasing ages. In contrast, FSH levels in animals castrated on Day 10 were lower compared to animals castrated on other days. Furthermore, the pattern of subsequent development of gonadotropin around the time when puberty would occur also showed interesting differences. While LH levels increased between Days 20 and 50 in castrates, regardless of the day of castration, a decline later did not occur. FSH levels in castrates did not appear to change peripubertally. The observed increases in LH peripubertally, in the absence of gonads, may be support for a so-called "steroid-independent or intrinsic" regulation of pubertal gonadotropin levels (reviewed, 16). In rats, the ability of the hypothalamic–pituitary system to respond to either negative or positive feedback of steroids is influenced by hormones early in life. Depriving a male rat of testicular androgen from birth results in an attenuated gonadotropin release to withdrawal of exogenous steroid later in life (17). Neonatal gonadectomy alters both the negative and the positive response to estradiol in male and female rats (18, 19). Perinatal androgen exposure masculinizes the response of female rats

TABLE II. BILATERAL WET WEIGHTS IN GRAMS ($\bar{X}$ ± SEM) OF SEMINAL VESICLES (SV) AND VENTRAL PROSTATES (VP) FROM MALE HAMSTERS CASTRATED (CAST) OR SHAM OPERATED AT VARIOUS AGES AND KILLED ON DAY 70 OF LIFE

			Castration or sham age			
Organ	Treatment		1	5	10	20
VP	Cast[a]	$\bar{X}$	106	130	158	84
		SEM±	6	11	11	15
		n	19	12	13	10
	Sham	$\bar{X}$	429	388	435	407
		SEM±	21	26	21	25
		n	10	11	12	10
SV	Cast[a]	$\bar{X}$	60	90	139	152
		SEM±	4	7	7	11
		n	19	12	13	10
	Sham	$\bar{X}$	308	324	381	283
		SEM±	26	18	23	15
		n	10	11	12	10

[a] Castrated animals had a subcutaneous testosterone capsule implanted on Day 60 (10 days prior to being killed).

to steroid withdrawal (17, 20). Perinatal androgen also masculinizes the response of males or females to an estradiol challenge (19, 21). It appears that in hamsters as well, the presence or absence of testicular secretions during neonatal life is involved in the development of the male's gonadotropin levels. Although both steroid-dependent and "intrinsic, steroid-independent" mechanisms are supported in the present study, other studies favor the former (16).

Studies in the neonatal male rat have shown that testosterone, which surges around the time of birth (22, 23), is probably involved in the sexual differentiation of hypothalamic gonadotropin regulation. Elimination of this testosterone surge affects a variety of physiological and behavioral functions (19, 23, 24). In our hamsters, castration occurred within a few hours of birth, but not as early as in the above rat studies. While a study to detail the testosterone levels at birth has not been done in hamsters, we have shown that fetal and early neonatal hamster testes are capable of secreting testosterone (25). Perinatal studies are needed to determine if hamsters are "programmed" at birth as rats seem to be (19).

Silastic capsules containing testosterone were implanted into castrated animals and left in place from Day 60 to 70 of life to test the ability of the system to respond to testosterone feedback effects on gonadotropin secretion. The capsule size which we used (8 mm) was based on reported intermediate suppression of LH and FSH in castrated hamsters on the same photoperiod (26). In the present study, male hamsters castrated on Day 20 of life were more sensitive to feedback suppression of testosterone on LH than were animals castrated earlier in life. However, FSH levels increased in animals which had been castrated on Days 10 or 20 of life. In castrated, male rats it is not unusual to find that testosterone has differential effects on FSH and LH secretion (27, 28). In fact, testosterone can facilitate FSH release in castrated males (29) as we found here with hamsters. Lack of effect of testosterone in animals castrated on other days could possibly reflect the dose of testosterone used (26), the fact that testosterone may not be the most effective feedback agent (29), or the length of time since testes were present (30–32). In these hamsters, the correlation of androgen levels to Silastic capsule length is not known. Without measuring the androgens, it is difficult to be certain that 8-mm capsules released enough testosterone to adequately challenge the pubertal hamster's feedback system. Unfortunately, the orbital sinus sampling technique did not produce enough plasma for this determination. In castrated male hamsters, a 4-mm capsule produced androgen levels that were roughly equal to intact controls between 3 and 5 weeks of age (16). At later ages these levels remained constant while intact androgen levels increased. In the present study larger capsules may have had a greater effect on circulating gonadotropins.

The effect of castration on ventral prostate or seminal vesicle weights was to reduce their size compared to sham-operated animals. This size reduction occurred regardless of the day of castration. At the dose of testosterone used, tissues did not recover their size in 10 days of treatment. Depending on the day of castration and the tissue weighed, a differential response to the exogenous testosterone was evident. The seminal vesicles of animals castrated on the day of birth were less responsive to testosterone than those in animals castrated on Day 10 or 20. Ventral prostate response to testosterone was not different in animals castrated on Days 1–10 of life. However, ventral prostates of males castrated on Day 10 grew larger than those of animals castrated on Day 20.

The differential responsiveness of the two tissue types in hamster may not be surprising because of evidence in other species. In rats, elimination of testicular secretions by castration early in life (Day 4) is reported to reduce the responsiveness of the prostate to testosterone (33) without affecting the seminal vesicles. In a different study, castration on Day 3 affected the sensitivity of both tissues (34). Castration on the day of birth, followed by androgen replacement neonatally, protected the ability of seminal vesicles to respond to adult androgen but did not affect the ventral prostate (35). Elimination of endogenous androgen in mice by treatment with the antiandrogen, cyproterone acetate, reduces seminal vesicle weights (36, 37). If treatment

with cyproterone acetate occurred later in life seminal vesicles were better able to grow in response to endogenous androgen than if treatment were begun neonatally (37). Androgen exposure during similar early periods in rats suppresses the size of these sexual accessories (38, 39). Estradiol given neonatally to rats caused a differential growth in seminal vesicle (increases) and ventral prostate (decreases) (40). Taken together, these studies point to the importance of a male rodent's testicular function during the neonatal period in the ability of sexual accessories to respond to androgens later in life.

Perinatal testicular secretions have been variously described as "programming, imprinting or organizing" the neural gonadotropin regulatory system in males. Among the possible ways (reviewed, 41) that steroids might mediate such permanent developmental changes in reproductive regulation are the following: by inducing frank structural changes; by changing receptor profiles; by altering enzymatic pathways; by changing neurotransmitter systems; or by altering other aspects of the membranes. Presumably similar results could account for differences in peripheral structures as well. While the present study was not designed to choose among these possibilities, nor does it determine which testicular secretions are important, it is clear that different systems (e.g., LH regulation vs sexual accessory sensitivity) are influenced by testicular secretions at somewhat different times during development. Whether different (or similar) developmental mechanisms are involved remains to be investigated.

This study was funded by the Marquette University Committee on Research and NIH Biomedical Research Support Grant (RR07196). The authors wish to thank Jodie J. Rady for assistance with statistical analysis and figure preparation; Barbara DeNoyer for typing the manuscript; Dr. G. D. Niswender for the gift of LH antiserum; and Dr. L. Reichert for generously providing the LH for iodination.

1. Vomachka AJ, Greenwald GS. The development of gonadotropin and steroid hormone patterns in male and female hamsters from birth to puberty. Endocrinology **105**:960–966, 1979.
2. Ramaley JA. Development of gonadotropin regulation in the prepubertal mammal. Biol Reprod **20**:1–31, 1979.
3. Ojeda SR, Andrews WW, Advis JP, Smith White S. Recent advances in the endocrinology of puberty. Endocr Rev **1**:228–257, 1980.
4. Arnold AP, Gorski RA. Gonadal steroid induction of structural sex differences in the central nervous system. Annu Rev Neurosci **7**:413–442, 1984.
5. Gorski RA. Critical role for the medial preoptic area in the sexual differentiation of the brain. In: DeVries GJ, DeBruin JPC, Uylings HBM, Corner MA, Eds. Sex Differences in the Brain. Progress in Brain Research. New York: Elsevier, Vol **61**:pp129–146, 1984.
6. Jost A. Sexual organogenesis. In: Alder N, Pfaff D, Goy RW, Eds. Reproduction. Handbook of Behavioral Neurobiology. New York: Plenum, Vol **7**:pp3–19, 1985.
7. Goy RW, McEwen BS. Sexual Differentiation of the Brain. Cambridge, MA: MIT Press, 1980.
8. Feder HH. Hormones and sexual behavior. Annu Rev Psychol **35**:165–200, 1984.
9. Harlan RE, Gordon JH, Gorski RA. Sexual differentiation of the brain: Implications for neuroscience. Rev Neurosci **4**:31–71, 1979.
10. Barraclough CA. Sex differentiation of cyclic gonadotropin secretion. In: Kaye AM, Kaye M, Eds. Development of Responsiveness to Steroid Hormones. New York: Pergamon, pp433–450, 1980.
11. Handa RJ, Corbier P, Shryne JE, Schoonmaker JN, Gorski RA. Differential effects of the perinatal steroid environment on three sexually dimorphic parameters of the rat brain. Biol Reprod **32**:855–864, 1985.
12. Clemens LG, Witcher JA. Sexual differentiation and development. In: Siegel HI, Ed. The Hamster: Reproduction and Behavior. New York: Plenum, pp155–171, 1985.
13. Vomachka AJ, Ruppert PH, Clemens LG, Greenwald GS. Adult sexual behavior deficits and altered hormone levels in male hamsters given steroids during development. Physiol Behav **26**:461–466, 1981.
14. Niswender GD, Midgley AR, Monroe SE, Reichert LE. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine-LH ^{131}I. Proc Soc Exp Biol Med **128**:807–811, 1968.
15. Keppel G. Design and Analysis: A Researcher's Handbook. Englewood Cliffs, NJ: Prentice-Hall, p 658, 1973.
16. Sisk CL, Turek FW. Developmental time course of pubertal and photoperiodic changes in testosterone negative feedback on gonadotropin secretion in the golden hamster. Endocrinology **112**:1208–1216, 1983.
17. Johnson LM, Gay VL. Influence of perinatal androgenization on the castration response of adult rats. Biol Reprod **29**:819–823, 1983.
18. Weiland NG, Barraclough CA. Neonatal castration of male and female rats affects luteinizing hormone

responses to estrogen during adulthood. Biol Reprod **31**:942–949, 1984.
19. Corbier P. Sexual differentiation of positive feedback: Effect of hour of castration at birth on estradiol-induced luteinizing hormone secretion in immature male rats. Endocrinology **116**:142–147, 1985.
20. Damassa DA, Rabii J, Sawyer CH. Responses of serum LH and FSH to the removal of steroid feedback inhibition. Neuroendocrinology **37**:122–130, 1983.
21. Gogan F, Beattie IA, Hery M, Laplante E, Kordon C. Effect of neonatal administration of steroids or gonadectomy upon oestradiol-induced luteinizing hormone release in rats of both sexes. J Endocr **85**:69–74, 1980.
22. Corbier P, Kerdelhue B, Picon R, Roffi J. Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. Endocrinology **103**:1985–1991, 1978.
23. Gogan F, Slama A, Bizzini-Koutznetzova B, Dray F, Kordon C. Importance of perinatal testosterone in sexual differentiation in the male rat. J Endocr **91**:75–79, 1981.
24. Corbier P, Roffi J, Rhoda J. Female sexual behavior in male rats: Effects of hour of castration at birth. Physiol Behav **30**:613–616, 1983.
25. Vomachka AJ, DiMario KB. Perinatal hamster gonadal androgen and estradiol production *in vitro* and response to hCG. Biol Neonate **52**:40–47, 1987.
26. Ellis GB, Turek FW. Time course of the photoperiod-induced change in sensitivity of the hypothalamic-pituitary axis to testosterone feedback in castrated male hamsters. Endocrinology **104**:625–630, 1979.
27. Mahesh VB, Muldoon TG, Eldridge JC, Korach KS. The role of steroid hormones in the regulation of gonadotropin secretion. J Steroid Biochem **6**:1025–1036, 1975.
28. Campbell CS, Schwartz NB. Steroid feedback regulation of luteinizing hormone and follicle-stimulating hormone secretion rates in male and female rats. J Tox Environ Health **3**:61–95, 1977.
29. Juneja HS, Motta M, Vasconi F, Martini L. Negative and positive effects of androgens on gonadotropin release. In: Martini L, Motta M, Eds. Androgens and Antiandrogens. New York: Raven Press, pp127–135, 1977.
30. Odell WD, Swerdloff RS. Etiologies of sexual maturation: A model system based on the sexually maturing rat. In: Greep RO, Ed. Recent Progress in Hormone Research. New York: Academic Press, Vol **32**:pp245–288, 1976.
31. Conne BS, Scaglioni S, Lang U, Sizonenko PC, Aubert ML. Pituitary receptor sites for gonadotropin-releasing hormone: Effect of castration and substitutive therapy with sex steroids in the male rat. Endocrinology **110**:70–79, 1982.
32. Weiland NG, Barraclough CA, Catt KJ. Effects of long- and short-term gonadectomy on the hypothalamo-hypophysial (LH-releasing hormone-LH) system in oestrogen-treated male and female rats. J Endocr **110**:367–373, 1986.
33. Larsson K. Effects of neonatal castration upon the development of the mating behavior of the male rat. Z Tierpsychol **23**:867–873, 1966.
34. Larsson K. Effects of neonatal castration and androgen replacement therapy upon the development of the mating behavior of the male rat. Z Tierpsychol **24**:471–475, 1967.
35. Chung LWK, Ferland-Raymond G. Differences among rat sex accessory glands in their neonatal androgen dependency. Endocrinology **97**:145–153, 1975.
36. Jean-Faucher CH, Berger M, DeTurckheim M, Veyssiere G, Jean Cl. Sexual maturation in male mice treated with cyproterone acetate from birth to puberty. J Endocr **102**:103–107, 1984.
37. Jean-Faucher CH, Berger M, DeTurckheim M, Veyssiere G, Jean Cl. Permanent changes in the functional development of accessory sex organs and in fertility in male mice after neonatal exposure to cyproterone acetate. J Endocr **104**:113–120, 1985.
38. Baranao JLS, Chemes HE, Tesone M, Chiauzzi VA, Scacchi P, Calvo JC, Faigon MR, Moguilevsky JA, Charreau EH, Calendra RS. Effects of androgen treatment of the neonate on rat testis and sex accessory organs. Biol Reprod **25**:851–858, 1981.
39. Piacsek BE, Hostetter MW. Neonatal androgenization in the male rat: Evidence for central and peripheral defects. Biol Reprod **30**:344–351, 1984.
40. Gayton F, Bellido C, Aguilar R, Lucenda MC. Morphometric analysis of the rat ventral prostate and seminal vesicles during prepubertal development: Effects of neonatal treatment with estrogen. Biol Reprod **35**:219–225, 1985.
41. Toran-Allerand CD. On the genesis of sexual differentiation of the central nervous system: Morphogenetic consequences of steroidal exposure and possible role of α-fetoprotein. In: DeVries GJ, DeBruin JPC, Uylings HBM, Corner MA, Eds. Sex Differences in the Brain. Progress in Brain Research. New York: Elsevier, Vol **61**:pp63–98, 1984

Received September 1, 1987. P.S.E.B.M. 1988, Vol. 187.
Accepted December 7, 1987.