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Chronological Changes in Sex Steroid, Gonadotropin and Prolactin Secretion in Aging Female Rats Displaying Different Reproductive States[1]

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ABSTRACT

Longitudinal studies were performed in a colony of aging female rats, from 4–33 months of age, to determine the chronological change in reproductive patterns and the changes in sex steroid, prolactin and gonadotropin secretion associated with different reproductive states. The present study demonstrates that the incidence (65%) of irregular estrous cycles in aging rats increased abruptly from 10–12 months of age. Subsequently, female rats became chronically anovulatory with persistent vaginal cornifications and their ovaries contained developed follicles but no corpora lutea. The highest incidence (65%) of constant estrous (CE) rats occurred at the age of about 19 months. During the anovulatory state, CE rats displayed low to medium levels of serum estradiol, estrone, testosterone and androstenedione, low levels of progesterone and minimal levels of 20α-hydroxyprogesterone. Preovulatory increases in gonadotropin and prolactin release, similar to those seen in young cycling rats on proestrus, were not observed in CE rats. Whereas serum basal LH levels remained unaltered, morning FSH levels were increased in CE rats. The latter may account for the persistent follicular development in aging rats during chronic anovulatory state. Serum basal prolactin levels were normal in CE rats during the early phase (11–16 months of age) of the anovulatory state, but were subsequently increased 3 to 4-fold beyond 24 months of age. Moreover, ovariectomy at a young age prevented the increased pituitary prolactin release in old female rats. These results suggest that continuous exposure to medium levels of estrogens, particularly in the presence of sustained low progesterone secretion, may alter pituitary secretion of prolactin in aging rats.

With further advance of age and following many months of anovulatory function, aging female rats exhibited ovulatory activity at irregular intervals. After each ovulation, formed corpora lutea were maintained for a prolonged period, presumably due in part to the existing high prolactin levels in the circulation of older female rats. These corpora lutea in old "pseudopregnant (PSP)" rats were functional as indicated by active secretion of progestins, with 20α-hydroxyprogesterone levels greater than those of progesterone in the circulation. These results indicate that the ovaries of aging rats retain their functional capacity to develop follicles and corpora lutea and to secrete steroid hormones.

Although the cause(s) responsible for cessation of normal ovulatory cycles in aging female rats is unknown, the present study demonstrates that the chronic anovulatory state in aging female rats is characterized by significantly reduced ovarian secretion and the lack of cyclic increases in pituitary gonadotropin and prolactin release. The causal relationships between the decreased ovarian steroid production and the absence of preovulatory surges of gonadotropin release in aging CE rats remain to be determined.

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INTRODUCTION

The role of the pituitary-ovarian system in the regulation of normal reproductive function in adult females has been extensively studied. However, the changes in pituitary and ovarian function associated with decline in reproduction in aging mammals are understood poorly. Even less is known about the involvements of the hypothalamus, pituitary and

ovaries in the initiation of abnormal ovulatory cycles in aging animals. The female laboratory rat is a good model for studying reproductive endocrine changes associated with aging, since adult female rats exhibit well defined estrous cycles and aging rats display changes in reproductive patterns. Previous studies showed that irregular estrous cycles emerged in female rats beginning at about 1 year of age and these were followed in sequence by constant estrus (CE), repetitive pseudopregnancies (PSP) and anestrus (Ingram, 1959; Mandl, 1961; Aschheim, 1961; Meites and Huang, 1976). Longitudinal studies have not been performed in aging female rats to determine the quantitative patterns of change in reproductive states.

Changes in ovarian morphology in aging rats have been described elsewhere (Mandl, 1959; Mandl and Shelton, 1959; Meites and Huang, 1976; Crumeyrolle-Arias et al., 1976). To date, the functional capacity of aging rat ovaries to secrete steroid hormones has received only modest consideration (Chan and Leathem, 1977). It was reported most recently (Huang et al., 1978) that serum progesterone levels were higher in PSP than in CE rats at 24 months of age, whereas both PSP and CE rats had similar serum estradiol levels. These investigators also reported that estradiol concentrations as high as 30 pg/ml were found in the oldest anestrous rats with their atrophic ovaries containing little or no follicular or luteal elements (Huang et al., 1978; Meites and Huang, 1976). Changes in other sex steroids in aging female rats have not been studied. The work by Clemens and Meites (1972) indicated that old CE rats had lower pituitary LH but higher FSH and prolactin concentrations than did young cycling female rats. The study by Shaar et al. (1975) demonstrated that serum basal LH levels were about the same while prolactin levels were greater in old than in young female rats. A subsequent report by Huang et al. (1976a), however, showed that serum LH levels were higher in old CE than in PSP or young rats on estrus, whereas FSH levels were about the same. Despite previous efforts, systematic studies have not been performed in aging female rats to determine the chronological changes in pituitary and ovarian hormone secretion associated with different reproductive states. Since a chronic anovulatory state in aging female rats is displayed for a prolonged period of time, it is of interest to study the hormonal milieu shortly after aging rats become anovulatory and during subsequent long intervals of CE state. It was the purpose of the present study to determine the quantitative patterns of change in reproductive states in aging female rats and to measure the circulating levels of estrogens, androgens, progestins, gonadotropins and prolactin in aging rats displaying different reproductive states.

MATERIALS AND METHODS

Establishment of a Colony of Aging Female Rats

A total of 180 Long-Evans female rats, retired breeders, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. When originally received at 8–9 months of age, some of these aging female rats were pregnant and later delivered healthy pups. These aging rats and a group of 3-month-old female rats of the same strain were maintained in animal rooms under controlled temperature (25 ± 1°C) and artificial lighting regimens (lights on from 0600–2000 h daily). The rats were housed in groups of 4–8/cage and were given tap water and Purina rat chow *ad libitum*. Among the aging female rats, 72 were maintained beyond the age of 27 months, whereas the others were used in experiments prior to reaching the age of 17 months.

In both the old and young female rats, estrous cycle patterns were determined by daily examination of vaginal smears and the following patterns were observed: a) regular and successive estrous cycles of 4 or 5-day duration; b) regular estrous cycles and intermittent irregular cycles (the latter condition was shown by prolongation of smears of leucocytes or cornified epithelial cells, indicative of a prolonged phase of follicular maturation); c) persistent vaginal cornifications; d) prolonged diestrus with intermittent estrous cycles at irregular intervals; and e) persistent diestrus.

Experimental Procedures

Groups of 4 to 5-month-old female rats (controls) that had displayed 3 or 4 consecutive regular estrous cycles of 4-day duration were selected for experiments on the day of diestrus, proestrus, or estrus. Experiments also were performed on groups of 11 to 33-month-old female rats displaying different reproductive states. Aging rats that had exhibited persistent vaginal cornifications for a period of at least 15 days were considered to be in constant estrus (CE), whereas rats that had displayed diestrous smears for 15 consecutive days and had corpora lutea in the ovaries at autopsy were considered to be in PSP (Meites and Huang, 1976). Some aging female rats displayed persistent diestrous smears and had no corpora lutea in the ovaries. The hormone levels of persistent diestrous (PD) rats were reported separately from those of the PSP rats. The rats were killed by decapitation with a guillotine at 1030–1130 h. Blood samples were collected from the trunk and the serum was separated by centrifugation and stored at −20°C until assayed. Gross postmortem examination of old rats was made

to determine the presence of hemorrhagic pituitaries and the presence of corpora lutea and/or follicles in the ovaries. Some specimens of the ovaries were fixed in Bouin's fluid and sectioned for histological examination.

Steroid and Pituitary Hormone Radioimmunoassays

Serum concentrations of estradiol (E_2), estrone (E_1), testosterone (T), androstenedione (A), progesterone (P) and 20α-hydroxy-pregn-4-ene-3-one (20α-OH-P) were measured by radioimmunoassays. One ml of serum was extracted with 7 ml of diethyl ether, which was then evaporated and subjected to celite chromatography for separation of A, T, E_1 and E_2. The column system used was the celite:ethylene glycol system of Brenner et al. (1973) with some modifications. Elution was carried out stepwise using 5 ml of isooctane (A fraction), followed by another 5 ml of isooctane (discarded) and 5 ml of cyclohexane: benzene (90:10) (T fraction), 4 ml of 15% ethyl acetate in isooctane (E_1 fraction) and finally 4 ml of 30% ethyl acetate in isooctane (E_2 fraction). Androstenedione and T were measured by assays using antisera provided by Dr. G. E. Abraham. The assay procedures and characteristics of these antisera are described elsewhere (Abraham et al., 1975a). Estrone was assayed using a sheep antiserum against estradiol-17β-BSA (DeVane et al., 1975); E_2 was measured employing an antiserum generated in a rabbit against 6-ketoestradiol-17β-6-(0-carboxy-methyl)-oxine-BSA (provided by Dr. A. S. Bhatnagar).

Another 0.2 ml of serum was extracted similarly and applied to a celite column for separation of P and 20α-OH-P (Abraham et al., 1975b). Progesterone was collected in the first 3.5 ml of isooctane and 20α-OH-P was eluted from the column with 3.5 ml of 5% ethyl acetate in isooctane. Progesterone and 20α-OH-P were measured by assays employing antisera and procedures of Abraham et al. (1975b). To establish specificity of each steroid assay for rat serum extract, we measured profiles of immunoreactive and radioactive steroids and plotted them as illustrated by Anderson et al. (1976). Two to 3 antisera from different sources were tested in each steroid assay system for specific immunoreactivity. For all the steroids measured in the present study, peaks of radioactive and immunoreactive steroids coincided in the column fraction.

Serum levels of FSH, LH and prolactin were measured by double antibody radioimmunoassays (Daane and Parlow, 1971; Monroe et al., 1968; Niswender et al., 1969). The assay reagents were provided by Dr. A. F. Parlow through the NIH Rat Pituitary Hormone Program. The results were expressed in terms of the reference standards of NIAMDD rat FSH-RP-1, rat LH-RP-1 and rat prolactin-RP-1, respectively. Assay procedures for these hormone measurements have been validated in our laboratory as reported previously (Lu et al., 1976; Kobayashi et al., 1978).

Steroid and pituitary hormone data were treated by analysis of variance. Statistical significance between group means was determined by Student-Newman-Kuel's multiple range test and levels of $P<0.05$ were considered as significant.

RESULTS

Chronological Changes in Reproductive Patterns in Aging Female Rats (Fig. 1)

When received originally at about 9 months of age, 93% of the female retired breeders exhibited regular and successive estrous cycles of 4 or 5-day duration; only 7% displayed both regular and intermittent irregular estrous cycles. The incidence of irregularly cycling rats in the aging rat colony increased to 65% at 12 months of age, while the incidence of regularly cycling rats was concomitantly decreased to 30%. Thereafter, the percent of irregularly cycling rats in the colony was progressively but slowly decreased, because by 19 months of age, 65% of the aging female rats became CE and chronically anovulatory. By 25 months of age, 55% of the rats displayed prolonged diestrus and some showed intermittent estrous cycles at extremely irregular intervals. Those rats in persistent diestrus with their ovaries containing corpora lutea at autopsy were considered to be in PSP state. Thus, the highest incidence of irregularly cycling rats and CE rats occurred at about 12 and 19 months of age, respectively. A high incidence (75%) of persistent diestrus (PD) was found in the aging rats by 27 months of age, but the peak incidence of PD rats in this colony could not be determined, since many of the aging rats were used in experiments when 25–27 months of age.

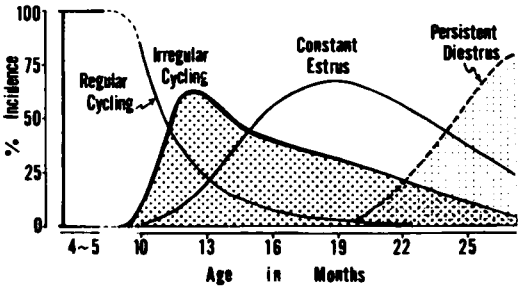

FIG. 1. Chronological change in reproductive patterns in a colony of aging female rats between 9–26 months of age. The incidence of regularly cycling female rats was precipitously decreased beginning at 9 months of age. Note that the highest incidence of irregularly cycling rats and constant estrous rats occurred at about 12 and 19 months of age, respectively. Many of the aging female rats displayed persistent diestrous smears prior to the age of 27 months.

Among a group of 65 old female rats examined postmortem, hemorrhagic pituitaries were found in 4 rats prior to the age of 25 months. Most of these hemorrhagic pituitaries were enlarged, but no histological examinations were performed on these pituitary tissues. Later, hemorrhagic pituitaries also were found in another 26 rats 27–33 months of age. Presumably, many of these hemorrhagic pituitaries observed in aging female rats were prolactin-secreting pituitary tumors as reported previously (Meites and Huang, 1976).

Changes in Circulating Levels of Estradiol (E_2) and Estrone (E_1) in Aging Female Rats (Fig. 4)

In young cycling female rats, serum levels of E_2 were 18 ± 7 and 11 ± 1 pg/ml on estrus and diestrus Day 1 (D-1), respectively; they were increased to 29 ± 3 pg/ml on diestrus Day 2 (D-2). In the morning of proestrus (PE), E_2 levels were increased further to 41 ± 5 pg/ml. The ovaries of CE rats contained several developed follicles but no corpora lutea (Fig. 2), indicative of persistent follicular development during chronic anovulatory state. During early phase of CE in aging female rats (11–13 months of age), serum levels of E_2 (17 ± 1 to 18 ± 2 pg/ml) were significantly lower than those in young cycling rats during D-2 and PE. Subsequently, E_2 levels in 16 to 30-month-old CE rats (29 ± 1 to 30 ± 4 pg/ml) were also significantly less than those seen in young cycling rats on PE. The ovaries of PSP rats contained many large corpora lutea and some maturing follicles (Fig. 3) indicative of ovulatory activity. Serum levels of E_2 in PSP rats (13 ± 3 pg/ml) were similar to those in young cycling rats during estrus and D-1, but were significantly lower than those in CE rats of comparable age. Estradiol levels in PD rats (17 ± 3 pg/ml) were similar to those in PSP rats.

Serum levels of E_1 were higher on D-2 (37 ± 5 pg/ml) than during the rest of the cycle (23 ± 2 to 26 ± 3 pg/ml) in young female rats. Estrone levels in 11 to 13-month-old CE rats (18 ± 2 to 24 ± 2 pg/ml) were similar to the low basal levels in young cycling rats, whereas the levels in 25 to 30-month-old CE rats (35 ± 4 pg/ml) were similar to those in young female rats on D-1. Estrone levels in PSP and PD rats were similar to those in CE rats of comparable age.

Changes in Circulating Levels of Testosterone (T) and Androstenedione (A) in Aging Female Rats (Fig. 5)

In young cycling female rats, serum basal T levels on D-2 (47 ± 8 pg/ml) were higher than those on estrus (20 ± 4 pg/ml) or D-1 (26 ± 7 pg/ml) and T levels were highest on PE (79 ± 9 pg/ml). This pattern of T secretion was similar

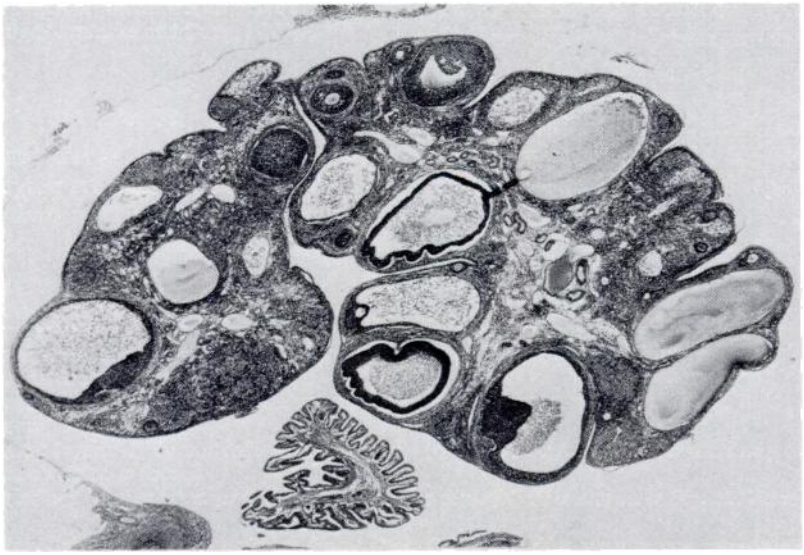

FIG. 2. Cross section of an ovary from 13-month-old constant estrous rat during chronic anovulatory state. The ovary contains several large, developed follicles but no corpora lutea. With further advance of age (17 months of age), many large, cystic follicles are found in the ovaries of constant estrous rats. ×20.

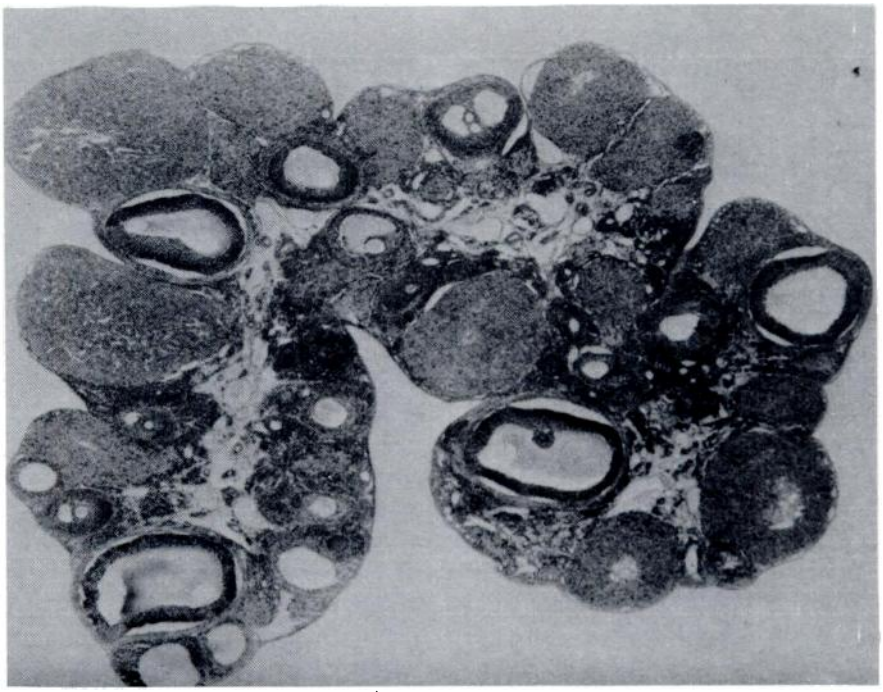

FIG. 3. Cross section of an ovary from 27-month-old female rat in repetitive pseudopregnant state. The ovary contains many large corpora lutea and maturing follicles indicative of follicular development and ovulatory activity. X 20.

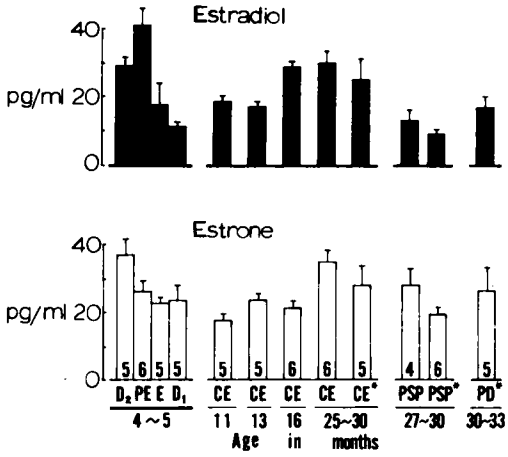

FIG. 4. Serum concentrations of estradiol and estrone in female rats displaying regular estrous cycles and during aging. Each column on the graph represents mean ± SEM of serum estrogen levels from a group of rats with the number of rats indicated at the bottom. D_2 = diestrus Day 2; PE = proestrus; E = estrus; D_1 = diestrus Day 1; CE = constant estrus; PSP = pseudopregnancy; PD = persistent diestrus; * = aging rats with hemorrhagic pituitaries. Note the cyclic pattern of serum estradiol levels displayed in young female rats and the higher serum estradiol levels displayed in aging constant estrous (CE) than in pseudopregnant (PSP) rats.

to that of E_2 during the regular estrous cycle (Fig. 4). Aging CE, PSP and PD rats all displayed low serum levels of T (26 ± 6 to 37 ± 5 pg/ml) as compared with young cycling female rats. Serum levels of T in CE rats with hemorrhagic pituitaries (64 ± 21 pg/ml) were significantly higher than those in CE rats with normal pituitaries (34 ± 4 pg/ml).

As in T secretion during the regular estrous cycle, serum levels of A in young female rats were highest on PE (66 ± 11 pg/ml). Androstenedione levels in aging CE and PD rats (31 ± 3 to 51 ± 13 pg/ml) were similar to those in young cycling rats on D-2, whereas the A levels in PSP rats (59 ± 5 pg/ml) were about the same as in young female rats on PE. Levels of A in CE rats with hemorrhagic pituitaries (90 ± 33 pg/ml) were higher than those in CE rats with normal pituitaries (48 ± 7 pg/ml).

Changes in Circulating Levels of Progesterone (P) and 20α-OH-P in Aging Female Rats (Fig. 6)

In young cycling rats, morning serum levels of P were lower on estrus (2.7 ± 1.1 ng/ml) than during the rest of the cycle (7.7 ± 4.1 to

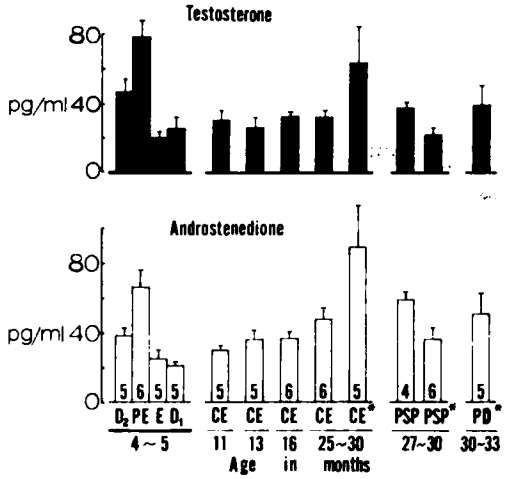

FIG. 5. Serum concentrations of testosterone and androstenedione in female rats displaying regular estrous cycles and during aging. The legends are the same as in Fig. 4. Note the cyclic pattern of serum androgen levels displayed in young female rats and the relatively low testosterone levels in aging constant estrous (CE) rats.

12.6 ± 3.0 ng/ml). Levels of P in aging CE rats (2.0 ± 0.3 to 5.2 ± 1.0 ng/ml) were similar to those low basal levels found in young cycling rats, whereas P levels in aging PSP rats (27.8 ± 5.6 ng/ml) were ∿5-fold greater than in CE rats of comparable age. Levels of P in PSP rats with hemorrhagic pituitaries (14.4 ± 3.2 ng/ml) were significantly lower than those in PSP rats with normal pituitaries. Levels of P in PD rats (3.9 ± 1.6 ng/ml) were similar to those in CE rats and neither CE nor PD rats had ovaries containing corpora lutea.

In young cycling rats, serum levels of 20α-OH-P (29.0 ± 12.5 to 47.8 ± 5.0 ng/ml) were about 4 to 8-fold greater than P levels and the pattern of 20α-OH-P release during the regular estrous cycle was less clear than that of P. Serum levels of 20α-OH-P were only minimal in aging CE and PD rats (1.0 ± 0.3 to 1.8 ± 0.4 ng/ml) in the absence of corpora lutea in their ovaries (Fig. 2). In aging PSP rats, ovulatory activity resumed at irregular intervals and resulted in the formation of many corpora lutea. The PSP rats displayed serum 20α-OH-P levels as high as those in regularly cycling young female rats.

Changes in Circulating Levels of Prolactin in Aging Female Rats (Fig. 7)

Basal serum prolactin levels in young cycling rats were higher on PE (80.2 ± 14.9 ng/ml) than during the rest of the estrous cycle (28.9 ± 3.5 to 48.7 ± 9.2 ng/ml). During the early phase of anovulatory state (11–16 months of age), aging CE rats displayed low serum prolactin levels (37.3 ± 1.8 to 48.6 ± 5.0 ng/ml) similar to those of young cycling rats. With further

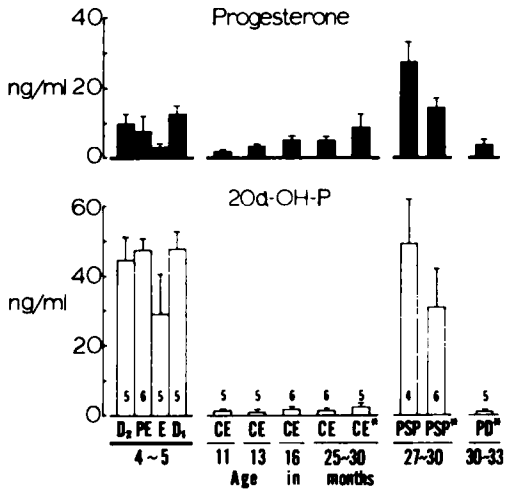

FIG. 6. Serum concentrations of progesterone and 20α-hydroxy-pregn-4-ene-3-one (20α-OH-P) in female rats displaying regular estrous cycles and during aging. The legends are the same as in Fig. 4. Note the minimal levels of 20α-OH-P in aging CE and PD rats and the higher serum levels of progesterone and 20α-OH-P in PSP than in CE rats.

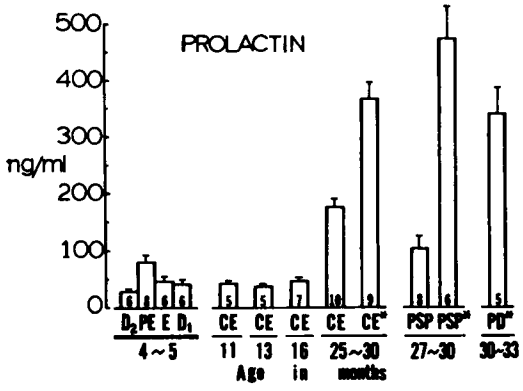

FIG. 7. Serum concentrations of prolactin in female rats displaying regular estrous cycles and during aging. The legends are the same as in Fig. 4. Note the marked increase in serum prolactin levels in older CE rats and the higher prolactin levels in aging rats with hemorrhagic pituitaries than those with normal pituitaries.

advance of age and following many months of anovulatory state, CE rats demonstrated 3 to 4-fold increases in prolactin levels (176.5 ± 17.4 ng/ml) from 25–30 months of age. Serum prolactin levels were even greater in CE rats with hemorrhagic pituitaries (368.4 ± 29.4 ng/ml) than in CE rats with normal pituitaries. Prolactin levels in PSP rats (104.7 ± 29.4 ng/ml) were significantly lower than those in CE rats of comparable age, but were higher than in young cycling rats. The PSP rats with hemorrhagic pituitaries had greater prolactin levels (475.5 ± 56.4 ng/ml) than did PSP rats with normal pituitaries. All the PD rats studied were found to have hemorrhagic pituitaries and had prolactin levels (340.5 ± 49.6 ng/ml) similar to those of CE rats.

Changes in Circulating Levels of FSH and LH in Aging Female Rats (Fig. 8)

In young cycling female rats, morning serum levels of FSH were significantly higher on D-1 (177 ± 20 ng/ml) than they were on PE (104 ± 14 ng/ml). During chronic anovulatory state, FSH levels in aging CE rats (143 ± 8 to 246 ± 20 ng/ml) were significantly higher than those in young cycling rats during the morning of PE, whereas aging PSP rats displayed serum FSH levels (137 ± 19 ng/ml) similar to those in young cycling rats on PE. Levels of FSH in PD rats (198 ± 28 ng/ml) and PSP rats with hemorrhagic pituitaries (219 ± 34 ng/ml) were similar to those in CE rats of comparable age.

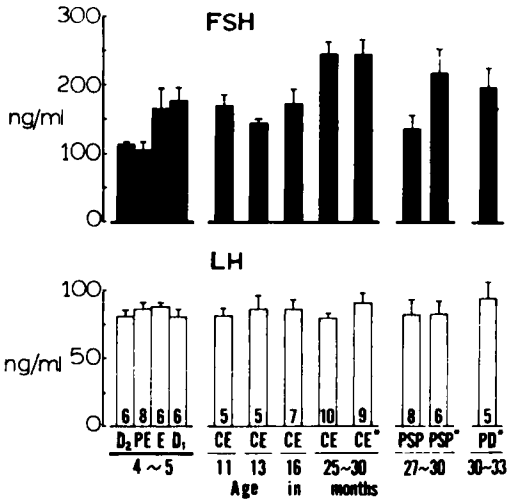

FIG. 8. Serum concentrations of FSH and LH in female rats displaying regular estrous cycles and during aging. The legends are the same as in Fig. 4. Note that morning serum basal FSH levels were increased in aging CE rats during chronic anovulatory state, whereas no significant change in morning serum basal LH levels was observed in aging female rats displaying different reproductive states. However, aging CE rats exhibited no cyclic increase in FSH or LH release.

No significant change in morning basal serum LH levels in young cycling rats was observed under the present conditions. Moreover, there was no significant difference in basal LH levels in aging female rats displaying different reproductive states.

Patterns of Gonadotropin and Prolactin Release in Aging CE rats (Table 1)

Groups of 12-month-old CE rats and 4-month-old cycling proestrous rats were killed at 1200, 1600 or 2000 h and changes in serum levels of FSH, LH and prolactin were determined. The CE rats were selected within 4 weeks after they had become chronically anovulatory. The data in Table 1 show that young cycling rats displayed marked increases in pituitary prolactin, LH and FSH release during late afternoon of PE, whereas CE rats exhibited no statistically significant increase in prolactin or gonadotropin release during the afternoon. However, aging CE rats displayed higher FSH levels in the morning than in the afternoon and showed small and insignificant rises in LH and prolactin levels at about 1600 h.

DISCUSSION

The present study demonstrates that aging female rats display chronological change in reproductive patterns and exhibit marked changes in sex steroid, gonadotropin and prolactin secretion. These results indicate that the incidence of irregularly cycling rats in the aging rat colony increased abruptly during 10–12 months of age. Subsequently, many aging rats became chronically anovulatory with their ovaries containing developed follicles but no corpora lutea. During anovulatory state, aging CE rats displayed low to medium levels of serum estradiol, estrone, testosterone and androstenedione, low levels of progesterone and minimal levels of 20α-OH-P, indicative of reduced ovarian steroid production. The noncyclic patterns of estrogen and progesterone secretion in aging CE rats resulted in persistent vaginal cornifications, although serum estrogen levels were not elevated during anovulatory

TABLE 1. Patterns of serum prolactin, LH and FSH levels (mean ± SEM) in aging constant estrous rats as compared to young cycling rats on proestrus.[a]

Time of day	Young cycling rats on proestrus (n = 4)	Aging constant estrous rats (n = 4)
	Prolactin, ng/ml	
1200 h	77.8 ± 18.0	48.6 ± 8.1
1600 h	721.0 ± 62.0	123.3 ± 62.5***
2000 h	234.0 ± 62.7	46.7 ± 5.0*
	LH, ng/ml	
1200 h	98.8 ± 8.6	77.8 ± 10.7
1600 h	364.5 ± 88.0	131.5 ± 24.5*
2000 h	805.8 ± 293.0	66.8 ± 14.7*
	FSH, ng/ml	
1200 h	73.2 ± 2.2	198.0 ± 31.7**
1600 h	152.1 ± 23.2	128.7 ± 24.8
2000 h	255.2 ± 29.3	119.2 ± 10.0*

[a]Hormone concentrations in constant estrous rats, which are significantly different from the corresponding values in young cycling rats on proestrus, as determined by Student's t test.

*P<0.05; **P<0.01; ***P<0.001.

state. The decreased steroid secretion by the ovaries may be causally related to the lack of preovulatory increases in gonadotropin and prolactin release in aging CE rats. Results similar to these were recently reported by Huang et al. (1978) and these investigators concluded from their study that the major cause for cessation of regular estrous cycles in aging rats lies in altered hypothalamic-pituitary function. While little is known about the involvements by hypothalamus, pituitary and ovaries in the initiation of abnormal ovulatory cycles in aging female rats (Meites et al., 1978), it has been shown that daily administration of progesterone reinitiates normal ovulatory cycles in some aging CE rats (Huang et al., 1976b). The mechanism by which progesterone restores ovulatory function in aging CE rats is unknown. In a similar fashion, previous studies also demonstrated that administration of LH or synthetic LHRH induced ovulation in aging CE rats (Aschheim, 1965; Meites et al., 1978), suggesting that lack of sufficient pituitary LH release in CE rats may be the immediate cause for anovulation (Lu et al., 1978).

With further advance of age and following many months of anovulatory state, female rats exhibited ovulatory activity at irregular and long intervals. As the result, many large corpora lutea and maturing follicles were present in the ovaries of old "pseudopregnant (PSP)" rats (Meites and Huang, 1976). Although the cause(s) for resumption of ovulatory activity in aging rats is unknown, the existing high serum prolactin levels may be responsible for the prolonged maintenance of corpora lutea after each ovulation. These corpora lutea were functional as indicated by active secretion of both progesterone and 20α-OH-P in PSP rats. A recent report by Huang et al. (1978) also revealed that serum progesterone levels were higher in old PSP than in CE rats. However, these investigators also reported that both old PSP and CE rats had about the same levels of serum E_2 (about 40 pg/ml) and that E_2 concentrations as high as 30 pg/ml were found in the oldest anestrous rats with their atrophic ovaries containing little or no follicular or luteal elements (Meites and Huang, 1976). Our present results demonstrate that serum E_2 levels were significantly greater in aging CE than in PSP or PD rats, substantiating the observed difference in ovarian morphology in aging rats. Although the cause(s) for cessation of normal ovulatory cycles in aging female rats is unknown, the present study demonstrates that the ovaries of aging rats retain functional capacity to develop follicles and corpora lutea and to secrete steroid hormones under appropriate conditions.

The present study demonstrates that serum levels of progesterone in aging CE rats were similar to the low basal levels in young cycling rats, whereas 20α-OH-P levels were minimal

during chronic anovulatory state. These observations substantiate a previous finding that 20α-OH-P production in female rats derives from enzymatic conversion of progesterone in previously formed corpora lutea (Hashimoto and Wiest, 1969) and suggest that some progesterone in the circulation of CE rats may come from an extraovarian source. It has been shown that the adrenals of female rats secrete substantial amounts of progesterone (Feder et al., 1968; Shaikh and Shaikh, 1975) and that after ovariectomy in rats 20α-OH-P is rapidly cleared from the circulation while progesterone of adrenal origin continues to circulate in substantial amounts (Feder et al., 1968). It is concluded from the present study that the adrenals of aging CE rats produce small amounts of progesterone while ovarian secretion of progesterone and 20α-OH-P is markedly reduced during chronic anovulatory state. These results of steroid hormone levels in aging female rats indicate, therefore, that greater amounts of progesterone and 20α-OH-P are present in the circulation of PSP than that of CE rats, whereas higher estradiol concentrations are found in CE than in PSP rats. Thus, the changing serum levels of estrogens and progestins in aging female rats represent major changes in follicular and luteal function of the ovaries associated with different reproductive states.

In aging female rats, changing serum levels of steroid hormones provide functional modulations for pituitary prolactin and gonadotropin release under different reproductive states. Our results are in agreement with previous reports that serum prolactin levels were higher in old CE than in young cycling female rats (Clemens and Meites, 1971; Shaar et al., 1975; Huang et al., 1976a). Our studies reveal further that increased pituitary prolactin release was first seen in aging CE rats between 16–25 months of age, whereas serum basal prolactin levels in 11 to 16-month-old CE rats were similar to those in young cycling rats. These observations are believed to indicate that increased pituitary prolactin release in older CE rats was a consequence of changes in hormonal milieu occurring during chronic anovulatory state. It is well established that estrogens stimulate prolactin secretion by acting at both hypothalamic and pituitary levels and that high doses of progesterone can prevent estrogen stimulation of prolactin secretion (Chen and Meites, 1970; Meites et al., 1972). Results from the present study demonstrate that serum levels of estradiol and estrone in aging CE rats were moderately increased from 16–30 months of age but not earlier. This may explain the increased pituitary prolactin release in older CE rats which was probably due to continuous exposure to medium levels of estrogens, particularly in the presence of sustained low progesterone secretion during chronic anovulatory state. Consonant with this, we also have found that ovariectomy at a young age prevented the increased prolactin release in aging female rats (Lu, unpublished). Prolactin levels in PSP rats were significantly lower than those in CE rats of comparable age, presumably due to modulations of pituitary prolactin release by lower serum estradiol and higher progesterone levels in PSP than in CE rats. As mentioned above, high doses of progesterone were shown to prevent estrogen stimulation of pituitary prolactin secretion (Chen and Meites, 1970). Earlier reports also showed that prolactin levels were lower in PSP than in CE rats (Wuttke and Meites, 1973; Huang et al., 1976a), but no explanation for this difference in prolactin release was offered.

Several investigators (Wuttke and Meites, 1973; Shaar et al., 1975; Huang et al., 1976a) reported high serum prolactin levels in old female rats by including rats both with hemorrhagic and normal pituitaries in their data analyses. It is demonstrated clearly in the present study that, in both aging CE and PSP rats, serum prolactin levels were 2 to 3-fold greater in rats with hemorrhagic pituitaries than in those with normal pituitaries. Presumably, many of these hemorrhagic pituitaries found in aging rats were prolactin-secreting pituitary tumors as previously reported by Ito et al. (1972) and by Meites and Huang (1976). Our present data indicate, however, that the higher serum prolactin levels in CE rats with hemorrhagic pituitaries were not directly related to estradiol or progesterone concentrations in the circulation. This suggests that the regulatory mechanism for prolactin release by hemorrhagic or tumorous pituitaries may be different from that by normal pituitaries.

In contrast to the marked change in pituitary prolactin release in aging female rats, changes in basal levels of serum gonadotropins were relatively small in aging rats displaying different reproductive states. However, the important change in the endocrine pattern of aging CE rats was the lack of cyclic increases in gonadotropin and prolactin release during

chronic anovulatory state. Basal levels of serum FSH were higher in the morning than in the afternoon and the morning FSH levels in CE rats were higher than the low basal FSH levels in young cycling rats. This pattern of FSH release in aging CE rats may account for the persistent follicular development during chronic anovulatory state. In perimenopausal women, increased FSH but not LH release was found to be associated with decreased estradiol secretion during the menstrual cycle (Sherman et al., 1976). It is not known whether the increase in FSH but not of LH levels found in aging CE rats is due similarly to a reduced estradiol secretion during anovulatory state.

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