BIOLOGY OF REPRODUCTION 27, 29–37 (1982)

Contribution of the Ovary Versus Hypothalamus-Pituitary to Termination of Estrous Cycles in Aging Rats Using Ovarian Transplants[1]

VICTORIA M. SOPELAK[2] and ROY L. BUTCHER[3]

Department of Obstetrics and Gynecology
West Virginia University Medical Center
Morgantown, West Virginia 26506

ABSTRACT

Vaginal smear patterns were monitored for 80 days after orthotopic ovarian transplants (OvTr) between young and old rats to examine the importance of the ovary and the hypothalamus-pituitary in the termination of estrous cyclic activity. At the end of this period, the rats were sacrificed at 2200 h, blood was collected for measurement of ovarian steroids and gonadotropins, and ovaries were prepared for histological study. A second experiment examined the effect of a reduction in ovarian tissue by unilateral ovariectomy of old rats on estrous cyclicity during the first 16 days following surgery.

During the 80 days of observation, 25% of the old and 0% of the young sham-operated rats showed irregular estrous cycles. This decrease in cyclicity followed a decrease in the total number of oocytes in the ovaries. Rats with OvTr did not cycle as regularly as young or old intact rats; and 75% of both young and old recipients with OvTr were acyclic by Day 80. Ovarian factors were implicated in the termination of cyclicity since: *1)* old ovaries in either old or young recipients were found to have a limited ability to maintain cyclicity compared to young or prepuberal ovaries; *2)* rats with OvTr which continued to cycle had more ovarian tissue and oocytes than rats which had become constantly estrous or anestrous, but fewer oocytes than intact rats; and *3)* in Experiment 2, a greater number of old unilaterally ovariectomized rats demonstrated irregular cycles by 16 days after surgery compared to old intact rats. Within the group of acyclic rats with OvTr, more ($P<0.05$) old than young recipients became constantly estrous, while more young than old recipients became anestrous. Since numbers of follicles were not different between rats with constant estrus and anestrus, this suggested that there also were alterations at the hypothalamic-pituitary axis with advancing age. As long as cyclicity was maintained, there were no significant differences in serum concentrations of the 6 measured steroids or prolactin at 2200 h on metestrus between intact rats and rats with OvTr. However, compared to intact rats, concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were increased in the cycling rats with OvTr. A further increase in LH and FSH occurred during constant estrus, with castration levels found in anestrous rats. The increase in gonadotropins and the decrease in estrous cyclic activity accompanied a decrease in the number of growing follicles. This increase in gonadotropins cannot be explained by changes in steroids alone, since a significant decline in ovarian steroids was not found at this particular sampling time, except in rats with OvTr which were anestrous.

It is concluded that a reduced amount of ovarian tissue and number of follicles plays a major role in the decline in reproductive function, probably through a reduction in inhibin. However, some difference at the hypothalamic-pituitary axis is responsible for the increased incidence of constant estrus in older rats.

Accepted February 12, 1982.

Received August 18, 1981.

[1] This investigation was supported by NIH Research Grant AG-02311 from the National Institute of Aging.

[2] Present address: Pregnancy Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205.

[3] Reprint requests: Dr. Roy L. Butcher, Dept. of Obstetrics and Gynecology, West Virginia University Medical Center, Morgantown, WV 26506.

INTRODUCTION

Aged rats show an increased incidence of irregular estrous cycles (Ingram, 1959; Mandl and Shelton, 1959) and a decline in fertility with advancing age (Talbert, 1968). The hypothalamus, pituitary, ovary and uterus have each been implicated as the primary site responsible for this age-related decline in reproduction (Adams, 1970; Finch, 1978; Talbert, 1978).

Studies of the initial changes in the hypo-

thalamus, pituitary and ovary which are associated with the decrease in fecundity, have often utilized animals in the terminal stages of reproductive senescence or ovariectomized acyclic rats which are steroid primed, and sometimes included rats with pituitary tumors. Thus, the end results rather than the initiating factors contributing to reproductive aging, have been studied.

Krohn (1962) suggested that the age of the ovary played a major role in maintenance of estrous cycles, since transplants of young ovaries into acyclic old CBA mice restored estrous cycles, while acyclic old ovaries transplanted into young mice resulted in irregular cycles or failure to cycle. Using young ovaries transplanted into acyclic old rats, Aschheim (1965) and Peng and Huang (1972) reported that the hypothalamus-pituitary was the primary site of aging which led to the termination of cyclic activity.

The present study was undertaken to investigate the effects of the age of the ovary and the age of the recipient on cyclic activity. Orthotopic transplants of prepuberal, young and old ovaries were utilized to study the effects of the age of the ovary on the decline in regularity of estrous cycles. Cycling young and old rats were used as ovarian transplant recipients and as controls to elucidate hypothalamic-pituitary changes which may have occurred prior to the onset of irregular estrous cycles. At the time of sacrifice, blood was collected for hormonal analysis and ovaries were saved for histological evaluation of the oocyte population in order to gain an understanding of the mechanism of reproductive aging. Control surgery or unilateral ovariectomies were performed on old rats to study the effect of a decrease in ovarian tissue on estrous cyclic activity.

MATERIALS AND METHODS

Animals

Female Holtzman rats were purchased at 21 days of age (prepuberal), 2 months of age (young adults) or >1 year of age (retired breeders). Rats were housed 6–10 per cage under controlled conditions of temperature and humidity with lights on 0600 to 1800 h. Estrous cycles of sexually mature rats were monitored by daily vaginal smears and only those rats exhibiting at least 3 consecutive 4- or 5-day cycles were utilized in this study. Old rats with tumors, irregular cycles or unthrifty appearance were discarded.

Ovarian Transplantation

In the first experiment, ovarian transplants (OvTr) were made orthotopically between young and old rats to study the role of ovarian age and recipient age (hypothalamo-hypophyseal age) on the termination of reproductive cyclicity. Sham-operated young and old rats served as controls for age effects, whereas young rats with young OvTr and old rats with old OvTr served as controls for the effects of transplantation.

The 8 treatments (N = 15–17 rats/group) included the following: *1)* young sham-operated controls; *2)* old sham-operated controls; *3)* young recipients with old ovaries; *4)* old recipients with young ovaries; *5)* young recipients with young ovaries; *6)* old recipients with old ovaries; *7)* young recipients with prepuberal ovaries; and *8)* old recipients with prepuberal ovaries. Donor and recipient rats were both at metestrus or diestrus at the time of OvTr, except prepuberal donors which were 22–32 days of age. Prepuberal ovaries were used because it was thought that a greater number of oocytes might survive transplantation, since the prepuberal ovaries contained more oocytes compared to young or old ovaries.

A mixture of 10 ml of 50 mg/ml of injectable Ketamine HCl (Parke, Davis & Co.) and 0.5 ml of 10 mg/ml of injectable acepromazine maleate (Ayerst Labs.) was given s.c. for anesthesia at a dose of 75 and 100 mg of Ketamine/kg body weight for old and young rats, respectively. Surgery was performed according to the method of Jones and Krohn (1960) with slight modifications. In all rats, the ovary and surrounding fat pad were exposed through a flank incision and examined. In the rats receiving transplants, a 5–6 mm semicircular incision was made through the fat pad and ovarian bursa, forming a flap and exposing the ovary. The ovary was ligated at the hilus with 6-0 nylon suture and dissected from the hilus. The appropriate ovary was then placed into the bursa without suturing and the bursal flap was sutured closed. The same procedure was repeated on the opposite side.

Vaginal smears were monitored daily after surgery. Cycling females were decapitated at 2200 h on the first metestrus after Day 80. Acyclic rats in anestrus or constant estrus were sacrificed at 2200 h on Days 81–86. Trunk blood was collected into 12 ml conical centrifuge tubes and stored overnight at 4°C. The sera were collected and stored frozen until assayed for estrone (E_1), estradiol (E_2), progesterone (P_4), testosterone (T), androstenedione (A), dehydroepiandrosterone (DHEA), LH, FSH, and prolactin. Ovaries were removed, weighed, fixed in Bouin's solution and prepared for histological evaluation. Pituitaries were weighed and examined for tumors.

Cyclic Activity

An estrous cycle was designated as a 3–9 day period extending from the first day of leukocytes (Lc) which was preceded by a cornified (C) vaginal smear until the end of the next sequence of C smears. To be considered a cycle, there could be not more than 4 consecutive days of epithelial (Ec) or C cell types. Pseudopregnancy (psp) was defined as a period of 10–16 days which consisted of Lc smears and ended with no more than 4 consecutive days of Ec or C cell types. Periods of >15 days of consecutive Lc smears were defined as anestrus and periods of >10 days of consecutive Ec or C smears were classified as constant estrus. Periods of ≥5 but ≤9 days of Ec or C vaginal

smears were designated as short constant estrus. Using these classifications, each rat's cyclic activity throughout the 80-day postoperative period was given a rating on a scale of 1 to 9 with long anestrus being 1 and regular cycles rated as 9. Rats which were anestrus were given a score of 1 (>25 days Lc) or 2 (>15 but ≤25 days Lc); constant estrus a score of 3 (>20 days C), 4 (>10 but ≤20 days C) or 5 (≥3 periods of C or Ec smears <10 days); irregularly cycling a score of 6 (≥3 cycles of 3 or 6–9 days, but including ≥2 psp), 7 (≥3 cycles of 3 or 6–9 days in length with ≤1 psp), or 8 (≤2 cycles of 3 or 6–9 days in length with ≤1 psp); and regular 4- or 5-day cycles a score of 9.

Histology

One ovary from the first 6–7 old and young intact rats and both ovaries from the first 6–7 rats in each transplant group were embedded in paraffin, serially sectioned at 10 μm and stained with hematoxylin and eosin. Oocytes and follicles were counted in the section containing the nucleolus and were classified as normal or atretic. Oocytes which were ≤30 μm and were surrounded by a single layer of flattened granulosal cells were classified as "resting" oocytes. Follicles >30 μm were classified as normal or atretic, antral or nonantral and by size. Follicles were classified as atretic if there were abnormalities in the oocytes (shrinkage, pseudocleavage or fragmentation), pyknotic cells present in the granulosal layer or free in the follicular fluid or hypertrophy of the theca. Measurements of follicular diameter were taken using a calibrated scale under the drawing tube of a microscope using 15X eyepieces and 10X objective.

Radioimmunoassays

Description and verification of the assays for LH, FSH, prolactin, E_2 and P_4 have been reported previously (Butcher et al., 1974; Butcher, 1977). Antisera were purchased for T (Miles-Yeda Ltd., catalog no. 61-315), A (Miles-Yeda Ltd., catalog no. 61-320) and DHEA (Radioassay Systems, catalog no. 1490). These assays were validated and cross-reactivity determined in this laboratory. Cross-reaction of anti-T-7α-BSA (bovine serum albumin) was 3% with A and 17% with dihydrotestosterone; anti-A-17α-BSA was 0.9% with T and 0.8% with 11-deoxycortisol; anti-DHEA-3β-HSA was 6% with A and 2% with E_1. Other steriods tested had cross-reactivities of <0.1%, and column chromatography was used to separate the steroids to be assayed. The steroids were extracted from 0.5 ml of serum with 3 ml diethyl ether and the P_4, E_1 and E_2 fractions eluted from Sephadex LH-20 columns with methylene chloride:methanol (95:5) as previously described (Butcher, 1977). The P_4 fraction from the LH-20 columns, which also contained A, T and DHEA, was further chromatographed on celite columns (Butcher, 1977) to separate each of the 4 steroids. The sample was added to the column in 0.4 ml of isooctane saturated with ethylene glycol. The P_4, A, DHEA and T were eluted in order with 2.5 ml isooctane, 2.5 ml isooctane, 4 ml isooctane:ethyl acetate (90:10) and 4.5 ml isooctane:ethyl acetate (85:15), respectively. The T, A and DHEA assays followed identical procedures as the estrogen assays and the ranges of standard curves were 2.5 to 1500, 15 to 1000 and 10 to 1000 pg/tube, respectively. The within assay coefficients of variation for E_1, E_2, P_4, A, T and DHEA were 19%, 11%, 9%, 21%, 9% and 12%, with between assay coefficients of variation of 17%, 14%, 10%, 29%, 9% and 14%, respectively, assaying duplicates of a pool of rat serum. Reported values have been corrected using recovery of H^3-steroids from duplicates of a pool of rat serum in each assay.

Plasma concentrations of prolactin, LH and FSH were determined by double-antibody radioimmunoassay as described previously (Butcher et al., 1974; Butcher, 1977). All samples were analyzed in a single assay for each gonadotropin. The concentrations of gonadotropins are expressed as ng of NIAMDD standard/ml serum: LH-RP-1 (0.03 X NIH-LH-S1), FSH-RP-1 (2.1 X NIH-FSH-S1) and prolactin-RP-2 (30 IU/mg). The within assay coefficients of variation for the gonadotropins were: prolactin 4.1%; FSH 4.1%; and LH 7.4%.

Unilateral Ovariectomy

In the second experiment, retired breeders which had exhibited at least 3 consecutive 4- or 5-day vaginal estrous cycles were used in this study. Sham surgery or unilateral ovariectomizes were performed on the morning of diestrus 1 on 103 and 123 rats, respectively. Vaginal smears were monitored for 16 days following surgery to determine the effect of a decrease in ovarian tissue on cyclic activity. Cycles of ≥6 days were considered abnormal.

Statistical Analysis

Data for estrous cyclic activity of the 125 rats throughout the 80 days after ovarian transplantation were evaluated using the above rating system. The scores were analyzed by age of recipient and type of ovary using orthogonal comparisons. If differences were significant, comparisons between group means were determined by the Student's t test, and a confidence level of 0.05 or less was considered to be statistically significant.

At the time of sacrifice, only 25% of the rats with OvTr were cycling and in metestrus. Inadequate numbers of rats in metestrus within some treatment groups and rats in constant estrus or anestrus in other groups limited the use of the preplanned 2 X 4 analysis of variance of recipient age versus ovarian type. Therefore, data on numbers of oocytes and follicles, hormonal concentration and ovarian weight for young and old recipients were pooled and analyzed by one-way analysis of variance according to whether rats were in metestrus, constant estrus, or anestrus when sacrificed. If the variance ratio (F) was significant, the Student's t test was used to test the significance of differences between means.

Data obtained from sham-operated rats sacrificed at metestrus were analyzed for effects due to age of animal. Since numbers of oocytes and follicles are similar in both ovaries in an intact rat (Arai, 1920), counts were done on only 1 ovary in the intact rats and were doubled in order to compare data on a rat basis. In rats with OvTr, numbers of oocytes and follicles were counted in both ovaries, since numbers of surviving oocytes were not uniform within both ovaries. Data on 25 rats were omitted from hormonal analysis because of morphological pituitary tumors with elevated prolactin concentrations, complete fail-

ure of ovarian transplants or sacrifice at a time other than metestrus, or established constant estrus or anestrus.

In the second experiment, chi-square analysis was used to compare the proportion of rats exhibiting abnormal estrous cycles of >6 days in length following unilateral ovariectomy or control surgery.

RESULTS

Vaginal smear patterns throughout the 80-day experiment indicated differences in cyclic activity due to age of recipient and age of the transplanted ovary (Fig. 1, Table 1). Groups of rats with OvTr received significantly lower cyclicity scores compared to intact rats, with recipients of old ovaries having the lowest rating. Among rats with old OvTr, the mean score for young recipients (1.7 ± 0.4) was significantly lower compared to old rats (3.2 ± 0.4) due to more young rats becoming anestrous. Table 1 shows that all of the young and 75% of the old intact rats continued to cycle. Although >98% of the OvTr became established, as evidenced by a return to estrus, only 26% of young and 25% of old rats with OvTr continued cyclic patterns. Of these cycling rats, only 9 of 12 young rats and 2 of 12 old rats with OvTr cycled regularly and received scores of 8 or 9. Prepuberal ovaries, with their higher complement of occytes at the time of transplantation, functioned no differently than young OvTr and maintained cyclic activity to the same extent as young OvTr. Among all rats with OvTr, more ($P<0.05$) recipients with young and prepuberal ovaries cycled (36%) than recipients with old ovaries (6%), indicating that old ovaries are not as capable of maintaining cyclicity as younger ovaries (Table 1). In the rats with OvTr which became acyclic, the distribution of constant estrus and anestrus was different ($P<0.05$). More old than young recipients became constantly estrous (56% vs. 15%), while more young than old recipients became anestrous (85% vs. 44%). These results provide evidence for age-related differences in

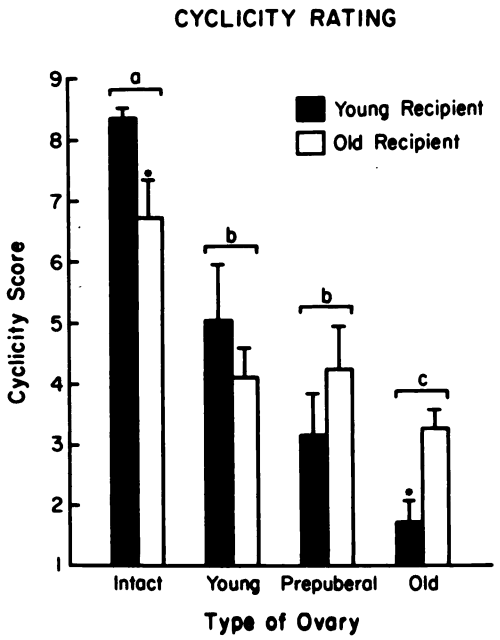

FIG. 1. Cyclicity rating ± SEM on a scale of 1 (anestrous) to 9 (regularly cycling) based on vaginal smear patterns throughout the 80-day experiment. See *Materials and Methods* for details of ratings. N = 15–17 rats/group. $^{a-c}$Mean of scores within type of ovary having a common superscript letter are not significantly different from each other ($P>0.05$). *Significant differences due to age of recipient within type of ovary ($P<0.05$).

TABLE 1. Number of rats by age of recipient, age of ovary and 80-day cyclicity pattern.

Recipient	Cyclicity pattern[a]	Type of transplant: Pre-puberal	Young	Old	Sham-operated
Young	Cycling	3	8	1	15
	Constant estrus	3	1	1	0
	Anestrus	9	6	14	0
Old	Cycling	5	6	1	12
	Constant estrus	4	5	11	2
	Anestrus	6	5	5	2

[a]Cyclicity patterns were based on rating scores as follows: Anestrus = 1–2, Constant estrus = 3–5, Cycling = 6–9. See *Materials and Methods* for details of scoring system.

both the ovary and the hypothalamic-pituitary axis.

When ovarian weights in intact rats were compared, old rats had significantly more ovarian tissue than young rats or rats with OvTr (Table 2). However, when ovarian weights were compared on a body weight basis between old and young intact rats (23 ± 1 vs. 24 ± 1 mg/100 g BW), there was no difference. Rats with OvTr showed a progressive decrease in amount of ovarian tissue when sacrificed at metestrus, at constant estrus or at anestrus (Table 2). Thus, the amount of ovarian tissue present tended to be reflected in the vaginal smear patterns.

Histological evaluation revealed that transplanted ovaries had fewer oocytes ($P<0.05$) than those from young or old intact rats (Table 3). Irrespective of age of the recipient, old OvTr contained fewer ($P<0.05$) oocytes (32 ± 11) compared to the number of oocytes in young (218 ± 66) or prepuberal (303 ± 84) transplanted ovaries. When data were pooled according to stage of cycle at the time of sacrifice, rats with OvTr sacrificed at metestrus had significantly fewer resting oocytes and normal proliferating follicles than either young or old intact rats at metestrus, but contained significantly more resting oocytes and normal follicles than rats with OvTr at either constant estrus or anestrus. Among the intact animals sacrificed at metestrus, old rats had fewer resting oocytes and normal proliferating follicles than young rats. However, the population of atretic follicles in the proliferating pool was strikingly similar in all metestrous rats, and was significantly decreased only when rats became constantly estrous or anestrous (Table 3). The percentage of proliferating follicles which became atretic increased dramatically from 12% in young intact rats and 19% in old intact rats to 39%, 68% and 77%, respectively, in cycling, constantly estrous and anestrous rats with OvTr. Therefore, it seems that the rate of atresia is not entirely dependent upon the total number of oocytes.

Since there were no age-related differences in the concentrations of the 6 steroids and 3 gonadotropins between young and old sham-operated rats sacrificed at metestrus, hormonal data for these rats were combined (Table 4). When concentrations of LH and FSH were compared at 2200 h during metestrus between intact rats and rats with OvTr, concentrations were significantly lower in intact rats. In rats with OvTr, the LH and FSH levels increased progressively from metestrus to constant estrus to anestrus (Table 4). Prolactin concentrations did not differ with cyclic condition and were 2.5 ± 0.4 ng/ml for intact metestrous rats and 3.3 ± 0.7, 2.4 ± 0.5 and 2.0 ± 0.4 ng/ml for metestrous, constantly estrous and anestrous rats with OvTr, respectively. There were no differences at metestrus in the concentrations of the 6 steroids tested between intact rats and rats with OvTr. A significant decline in steroids was found in rats with OvTr only during anestrus for E_2, A and DHEA and during constant estrus and anestrus for P_4. In rats with OvTr, T was elevated during constant estrus compared to metestrus or anestrus. The decline in steroids in anestrous rats is reflected in the increase in gonadotropins, as well as the decline

TABLE 2. Mean weight (± SEM) of ovarian tissue present in rats at 81 to 86 days after sham surgery or orthotopic ovarian transplantation.

	Number of rats	Total ovarian weight (mg)
Metestrus (sham)	25	81 ± 3[a]
Young	15	74 ± 4*
Old	10	90 ± 4
Metestrus (transplants)	18	54 ± 4[b]
Constant estrus (transplants)	21	33 ± 4[c]
Anestrus (transplants)	36	15 ± 3[d]

[a,b,c,d]Weights with different superscript letters are significantly different from each other ($P<0.05$).

*Ovarian weight compared to old sham was significantly different on an absolute basis, but not on a body weight basis: Young sham = 24 ± 1 mg/100 g BW; Old sham = 23 ± 1 mg/100 g BW.

TABLE 3. Total number of oocytes and follicles (mean ± SEM) present in sham-operated rats sacrificed at metestrus and in rats with orthotopic ovarian transplants sacrificed during metestrus, constant estrus and anestrus.

	Number of rats	Number/rat			
		Total oocytes	Total resting oocytes	Proliferative pool	
				Normal	Atretic
Metestrus (sham)*					
Young	6	5156 ± 700[a]	3224 ± 565[a]	1702 ± 268[a]	230 ± 40[a]
Old	5	3034 ± 430[b]	1806 ± 265[b]	990 ± 204[b]	238 ± 89[a]
Metestrus (transplants)	9	806 ± 218[c]	252 ± 118[c]	339 ± 107[c]	215 ± 34[a]
Constant estrus (transplants)	5	73 ± 51[d]	15 ± 13[d]	19 ± 15[d]	39 ± 23[b]
Anestrus (transplants)	14	155 ± 68[d]	14 ± 6[d]	33 ± 16[d]	108 ± 47[b]

[a,b,c,d]Numbers of oocytes and follicles within each column with different superscript letters are significantly different from each other ($P<0.05$).

*Numbers of oocytes and follicles were doubled since only 1 ovary/rat was counted in groups with sham surgery.

in ovarian tissue and population of oocytes seen in this group of rats.

In the second experiment, a decrease in ovarian tissue in aged rats brought about by unilateral ovariectomy, significantly increased the number of abnormal cycles (cycles >6 days in length) within 16 days after surgery compared to old intact rats (Table 5). When abnormal cycles occurred, they were characterized by >4 days of consecutive cornified vaginal smears in 15 of 20 old intact rats and 32 of 42 old unilaterally ovariectomized rats. In the other 5 old intact rats and 10 old unilaterally ovariectomized rats, the abnormal cycles consisted of periods of prolonged diestrus.

DISCUSSION

The age of the ovary and the age of the hypothalamus-pituitary both contributed to the termination of reproductive cyclicity in the present study. A decrease in number of oocytes, and therefore number of follicles, appears to be the ovarian aging factor, since a reduction in number of oocytes following OvTr is associated with an age-like reduction in estrous cyclicity. In the intact rats, the decline in cyclic estrous activity with advancing age was associated with a decrease in number of oocytes and follicles. Such a decrease in number of oocytes with age has been reported by Arai (1920) and Mandl and Shelton (1959).

Rats with transplanted ovaries had an even greater reduction in ovarian tissue and number of oocytes and did not cycle as regularly as the old intact rats. A similar decrease in oocyte population following orthotopic ovarian grafting has been noted by Jones and Krohn (1960) in mice. The fact that young rats with old transplanted ovaries failed to maintain regular cycles, agrees with work in CBA mice (Krohn, 1962), but appears to conflict with the reports of Aschheim (1965), Zeilmaker (1969) and Peng and Huang (1972), who often used acyclic rats as donors and recipients. The smaller oocyte population in old ovaries, combined with the additional reduction in number of oocytes brought about by necrosis during establishment of the graft, could account for the poor performance of old ovaries. Immunological reactions from the use of outbred rats in the present study also could have accelerated the loss of oocytes in the transplanted ovaries.

The rats with OvTr which continued to cycle had significantly fewer oocytes compared to

TABLE 4. Concentration of hormones (mean ± SEM) at 2200 h for sham-operated control rats sacrificed at metestrus and rats with ovarian transplants sacrificed at metestrus, constant estrus or anestrus.

	pg/ml						ng/ml		
	E_1*	E_2	A	DHEA	T	P_4	LH	FSH
Metestrus								
Controls (N = 25)	18.1 ± 1.3	13.9 ± 1.9	74.4 ± 5.3	77.5 ± 4.3	40.5 ± 5.1	16.9 ± 1.4	34.9 ± 3.0††	131 ± 16††
Transplants (N = 17)**	21.4 ± 3.0	11.4 ± 1.6	61.7 ± 6.6	64.2 ± 5.6	31.1 ± 5.6	15.5 ± 2.8[a]	72.3 ± 13.0[a]	451 ± 219[a]
Constant								
Estrus transplants (N = 21)	21.3 ± 2.5	14.3 ± 1.5	71.0 ± 11.9	55.0 ± 5.3	59.9 ± 12.5[a]	2.3 ± 0.3[b]	536.1 ± 92.0[b]	840 ± 94[b]
Anestrus transplants (N = 36)†	19.4 ± 1.0	5.0 ± 0.4[a]	36.8 ± 3.3[a]	41.5 ± 1.5[a]	23.8 ± 3.2	1.9 ± 0.1[b]	>1000.0	1580 ± 149[c]

[a,b,c]Values within each column with different superscripts for groups with transplants are significantly different from each other ($P<0.05$).

*Abbreviations are: E_2 – Estradiol, E_1 – Estrone, P_4 – Progesterone, A – Androstenedione, T – Testosterone, DHEA – Dehydroepiandrosterone.

**N = 16 for FSH.

†N = 31 for LH and FSH.

††Metestrous controls compared only to metestrous transplants ($P<0.05$).

intact rats, but significantly more oocytes than the constantly estrous or anestrous rats. From this we concluded that the amount of functional ovarian tissue plays a major role in the maintenance of cyclicity. The increased incidence of abnormal cycles in old rats following unilateral ovariectomy further emphasized the importance of amount of ovarian tissue in the maintenance of regular estrous cycles. Thus, the reduction in number of oocytes, and therefore, follicles, is likely the causative factor in the decline in cyclicity in aged rats, in rats with ovarian transplants and in old rats after unilateral ovariectomy.

From the present study, it was concluded that old rats with regular estrous cycles prior to receiving an OvTr, were capable of continuing cyclicity, provided that transplants of young or prepuberal ovaries were used. However, once the number of oocytes and follicles fell below the minimum number required for maintenance of estrous cycles, age-related hypothalamic-pituitary differences became evident. With the termination of estrous cycles, young rats more frequently became anestrous, whereas old rats more often became constantly estrous. Since numbers of oocytes and follicles were not different between anestrous and constantly estrous rats with OvTr, this suggests age-related differences at the hypothalamic-pituitary axis. Old rats which had a decreased amount of ovarian tissue due to unilateral ovariectomy, also tended to have abnormal cycles, 75% of which included short periods of constant estrus. Therefore, the tendency for aged rats to become constantly estrous was evident not only in old rats with OvTr but also in rats after unilateral ovariectomy. This tendency for aged rats to become constantly estrous and young rats to become anestrous when both have a similar number of oocytes, is likely due to a change in the sensitivity of the hypothalamic-pituitary axis to ovarian steroid feedback and could be caused by the duration of exposure to steroids such as estradiol (Brawer et al., 1980).

In this study, as long as cyclicity was maintained, there were no significant age-related differences in serum concentrations of E_1, E_2, P_4, DHEA, A, T, LH, FSH or prolactin between young and aged control rats sacrificed at 2200 h at metestrus. The hormonal values were similar to those reported for young rats by Butcher et al. (1974) for E_2, P_4, LH, FSH and prolactin, by Lu et al. (1979) for T and by

TABLE 5. Number of old intact and unilaterally ovariectomized rats which had abnormal estrous cycles of ≥6 days in length by 16 days after surgery.

Group	Number of rats	Rats with abnormal cycles (no.)
Old intact	103	20
Old unilaterally ovariectomized	123	42*

*Significantly different from intact rats ($P<0.01$).

Dupon and Kim (1973) for A. The fact that the serum concentrations of the 6 steroids were not different at metestrus in rats with OvTr and intact rats, indicated that even though the population of follicles was decreased in the rats with OvTr, the remaining follicles compensated and maintained similar serum concentrations of steroids. However, the greater concentrations of FSH and LH at metestrus in rats with OvTr compared to controls, did indicate the involvement of an ovarian factor in the control of LH and FSH secretion.

The failure to find a difference in steroid concentrations between intact rats and rats with OvTr at 2200 h of metestrus does not rule out the occurrence of differences at other times during the estrous cycle. Such differences in steroid levels at other times of the cycle in rats with OvTr could have affected LH and FSH secretion in metestrus. The further elevation in LH and FSH during constant estrus in rats with OvTr and values of LH and FSH in the castration range (Wise and Ratner, 1980) in anestrous rats with OvTr can be attributed to a decreased follicular population. A decrease in the number of follicles producing inhibin could account for the elevated concentration of FSH. The concentration of LH and FSH in rats with constant estrus and anestrus exceeded those reported by Lu et al. (1979) for old intact rats in constant estrus and anestrus. This discrepancy could be due to differences in age or strain of rats or to lack of secretion of some unidentified substance by the ovarian transplants.

Plasma E_1 appears to be mainly of adrenal origin since it did not vary according to reproductive state or decline with the decrease in ovarian tissue and number of follicles. As would be expected, rats which were in constant estrus or anestrus had low P_4 concentrations due to the absence of luteal tissue. Also, the larger and sometimes "cystic" follicles are likely responsible for the elevated serum T levels found in rats during constant estrus. The decreased concentrations of E_2, A and DHEA seen only in anestrous rats reflects significantly less ovarian tissue, but in the presence of a marked elevation in LH and FSH, and with a number of oocytes equal to that of rats in constant estrus. These elevated levels of gonadotropins associated with anestrus may not be appropriate for follicular growth and may produce early follicular atresia (Harman et al., 1975). Somewhat lower levels of LH and FSH in the presence of similar numbers of follicles could allow greater follicular development without proper stimulus for an ovulatory surge of LH, and result in the constant estrous condition. Wise and Ratner (1980) reported that old rats show an attenuated rise in plasma LH and FSH in response to ovariectomy, compared to young rats. In the present study, more old rats were classified as "constant estrus" while more young rats were anestrous, thus, the difference in magnitude of the elevation in LH and FSH between constantly estrous and anestrous rats, when both have a similar number of oocytes, could be attributed to age-related hypothalamic-pituitary differences. This difference in production of steroids could be due to differences between constantly estrous and anestrous rats in the responsiveness of the follicles and/or ovarian interstitial tissue to the elevated levels of LH and FSH. Therefore, the transition from constant estrus to anestrus could involve either development of an imbalance between number of follicles and gonadotropins resulting in early follicular atresia or the development of decreased responsiveness of the ovary with increasing LH and FSH secretion.

From this study, it appears that as the population of oocytes decreases, there is a concomitant decrease in cyclic activity and an increase in concentration of gonadotropins. This increase in gonadotropins cannot be explained by changes in steroids alone, since a significant decline in ovarian steroids was not found at this particular sampling time, except in rats with OvTr which were anestrous. Likewise, it appears that a reduced number of follicles and responsive ovarian tissue plays a major role in the decline in reproductive function. However, since young rats respond to decreases in

amount of ovarian tissue by becoming anestrous whereas old rats become constantly estrous, some nonovarian factor, probably due to differences at the hypothalamic-pituitary axis, is responsible for the increased incidence of constant estrus as numbers of follicles decline in older rats.

ACKNOWLEDGMENTS

The authors are indebted to the NIAMDD and to Dr. A. F. Parlow for supplying the RIA materials for assay of rat FSH and prolactin, Dr. Gordon Niswender for #15 antiserum to ovine LH, and to Dr. L. E. Reichert, Jr. for purified ovine LH (LER 1056-02). We are grateful to Mrs. Diana Kirkpatrick-Keller and Mrs. Pat Lutsie for their excellent technical assistance. Salary support for Dr. Sopelak was from Hatch Project 224 (NE 72) of the West Virginia University Agricultural and Forestry Experiment Station.

REFERENCES

Adams, C. E. (1970). Ageing and reproduction in the female mammal with particular reference to the rabbit. J. Reprod. Fertil. (Suppl.)12:1–16.

Arai, H. (1920). On the postnatal development of the ovary (albino rat), with especial reference to the number of ova. Am. J. Anat. 27:405–462.

Aschheim, P. P. (1965). Résultats fournis par la greffe hétérochrone des ovaires dans l'étude de la régulation hypothalamo-hypophyso-ovarienne de la ratte sénile. Gerontologia 10:65–75.

Brawer, J. R., Schipper, H. and Naftolin, F. (1980). Ovary-dependent degeneration in the hypothalamic arcuate nucleus. Endocrinology 107:274–279.

Butcher, R. L., Collins, W. E. and Fugo, N. W. (1974). Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94: 1704–1708.

Butcher, R. L. (1977). Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. Endocrinology 101:830–840.

Dupon, C. and Kim, M. H. (1973). Peripheral plasma levels of testosterone, androstenedione, and oestradiol during the rat oestrous cycle. J. Endocrinol. 59:653–654.

Finch, C. E. (1978). Reproductive senescence in rodents: Factors in the decline of fertility and loss of regular estrous cycles. In: The Aging Reproductive System, vol. 4 (E. L. Schneider, ed.). Raven Press, New York, pp. 193–212.

Harman, S. M., Louvet, J. P. and Ross, G. T. (1975). Interaction of estrogen and gonadotropins on follicular atresia. Endocrinology 96:1145–1152.

Ingram, D. L. (1959). The vaginal smear of senile laboratory rats. J. Endocrinol. 19:182–188.

Jones, E. C. and Krohn, P. L. (1960). Orthotopic ovarian transplantation in mice. J. Endocrinol. 20: 135–146.

Krohn, P. L. (1962). Review lectures on senescence. II. Heterochronic transplantation in the study of ageing. Proc. R. Soc. Lond. (Biol.) 157:128–147.

Lu, K. H., Hopper, B. R., Vargo, T. M. and Yen, S.S.C. (1979). Chronological changes in sex steroid, gonadotropin and prolactin secretion in aging female rats displaying different reproductive states. Biol. Reprod. 21:193–203.

Mandl, A. M. and Shelton, M. (1959). A quantitative study of oocytes in young and old nulliparous laboratory rats. J. Endocrinol. 18:444–450.

Peng, M. and Huang, H. (1972). Aging of hypothalamic-pituitary-ovarian function in the rat. Fertil. Steril. 23:535–542.

Talbert, G. B. (1968). Effect of maternal age on reproductive capacity. Am. J. Obstet. Gynecol. 102: 451–477.

Talbert, G. B. (1978). Effect of aging of the ovaries and female gametes on reproductive capacity. In: The Aging Reproductive System, vol. 4 (E. L. Schneider, ed.). Raven Press, New York, pp. 59–83.

Wise, P. N. and Ratner, A. (1980). Effect of ovariectomy on plasma LH, FSH, estradiol, and progesterone and medial basal hypothalamic LHRH concentrations in old and young rats. Neuroendocrinology 30:15–19.

Zeilmaker, G. H. (1969). Effects of prolonged feeding of an ovulation inhibitor (Lyndiol) on aging of the hypothalamic-ovarian axis and pituitary gland tumorigenesis in rats. J. Endocrinol. 43:xxi.