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Orthotopic Ovarian Transplantations in Young and Aged C57BL/6J Mice

T. A. PARKENING,[1] T. J. COLLINS and F.F.B. ELDER[2]

Departments of Anatomy, Obstetrics and Gynecology, and Pediatrics[2]
The University of Texas Medical Branch at Galveston
Galveston, Texas 77550

ABSTRACT

Orthotopic ovarian transplantations were done between young (6-wk-old) and aged (17-mo-old) C57BL/6J mice. The percentages of mice mating following surgery from the four possible ovarian transfer combinations were as follows: young into young, 83%; young into aged, 46%; aged into young, 83%; and aged into aged, 36%. The percentages of these mice that were pregnant 10 days following the presence of a vaginal sperm plug were as follows: young into young, 58%; young into aged, 9%; aged into young, 50%; and aged into aged, 0%. Some of the fetuses derived from matings of the above mice were dissociated and their cells prepared for chromosomal smears. No evidence of aneuploidy or mosaicism was found in fetuses derived from ovaries of young or aged mice. Aged ovaries, transferred to either young or aged recipients, were found to have fewer developing follicles and lower weight, which was most apparent in recipients that failed to mate or to get pregnant. Concentrations of luteinizing hormone, follicle-stimulating hormone (FSH), and prolactin in plasma from each of the pregnant recipients were analyzed by radioimmunoassay. The only statistical differences found between the transfer groups occurred in FSH concentrations. Plasma FSH was markedly elevated ($P<0.005$) in young recipients with ovaries transplanted from aged donors, in comparison to young recipients with ovaries from young donors. These data indicate that the aging ovary and uterus play a secondary role in reproductive failure and that the aging hypothalamic-hypophyseal complex is primarily responsible for the loss of fecundity in older female C57BL/6J mice.

INTRODUCTION

Aging begins at birth or even before birth. Recognizing that all cells age, it is still apparent that some cells or organs age at a faster rate than others. The thymus gland and ovary, for example, age at a faster rate than other organs. For the last several years, we have been engaged in studies on the aging reproductive system of laboratory rodents to determine if one particular organ or cell (such as the oocyte) is primarily responsible for reproductive senescence. The problem in deciphering age-related changes in such organs lies in determining whether the changes are the primary cause of the dysfunction or merely a secondary reflection of the aging process in another organ. One approach to determining this is organ transplantations between young and aged animals.

Orthotopic ovarian transplantations were conducted between young and aged C57BL/6J mice to determine estrous cyclicity, ability to mate, viable and resorbing implantation sites on Day 10 of pregnancy, fetal chromosomal anomalies, and circulating concentrations of plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (Prl).

MATERIALS AND METHODS

Animals and Accommodations

Orthotopic ovarian transplantations were done between 24 young (6-wk-old) and 24 aged (17-mo-old) C57BL/6J mice. The mice were first-generation offspring of parents purchased from the Jackson Laboratory (Bar Harbor, ME) and were born within 3 days of one another. They were provided with food and water ad libitum and kept in a room under controlled temperature (21–23°C) and illumination (14L:10D). Before surgery all of the young mice had 4–5-day (4.8 ± 0.8) estrous cycles and all of the aged mice had irregular vaginal smears (cycle length 7.9 ± 1.2 days).

Ovarian Transplantations

The procedure for transplanting ovaries was patterned after that of Stevens (1957). Two female mice were anesthetized with Diabutal at the same time, and dorsolateral incisions were made to expose each ovary. A small cut with an iridectomy scissors

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[1] Address for correspondence: Dr. T. A. Parkening, Dept. of Anatomy, The University of Texas Medical Branch, Galveston, Texas 77550.

was made in the ovarian bursa (side away from the infundibulum), and the freshly exposed ovary was excised at the hilus. Each ovary was either left intact upon removal, or cut in half with a sterile razor blade and then placed in a culture dish containing Medium 199 that had been warmed on a slide warmer (35°C). The ovaries were then exchanged between the two animals by inserting a new ovary into each empty bursa.

A dozen mice were used in each of the following four types of transplantations: ovaries from young donors into young recipients; from young donors into aged recipients; from aged donors into young recipients; and from aged donors into aged recipients. One-third of each group received whole-ovary transplants and two-thirds received half-ovary transplants. Surgery was done only when the donor and recipient mouse exhibited a diestrous vaginal smear. The transplantations were done on 6 consecutive days with 8 mice in a sequential manner so that 2 mice/day were done in each transfer group.

Three weeks after surgery, 2–3 recipients were placed with a 5–6 mo-old male C57BL/6J mouse shown previously to be fertile. Each morning, the females were examined for the presence of a vaginal sperm plug, and a vaginal smear was taken. Those that had mated were judged to be pregnant according to three criteria: weight gain according to measurements taken on Days 1, 5, and 10 after the sperm plug was found; evidence of implantation sites from palpation; and an atypical estrous cycle smear taken on Day 10.

In these experiments, there was no way of determining for certain if a small amount of ovarian tissue left in the bursae of transplanted mice produced the embryos. However, a recent study in this laboratory (Parkening et al., 1984), in which identical operating procedures were used but a marker gene was added, revealed that every one of the 47 embryos examined by chromosomal analysis came from transplanted ovaries. Therefore, it is highly unlikely that the embryos derived by the same techniques in the present study developed from residual ovarian tissue of the recipients.

Bleeding and Preparation of Ovarian Tissue

The transplanted recipients that were considered to be pregnant were bled, unanesthetized, by cardiac puncture, between 1000 and 1200 h on Day 10 of their pregnancy. Those that failed to become pregnant after being with male mice for 2.5 mo were killed in the same manner. The entire reproductive tract was excised immediately after killing in both groups, and the numbers of implantation sites and resorptions were recorded. The ovaries were placed in Bouin's fixative and later embedded in paraffin wax. They were then serially sectioned at 5 μm and stained with hematoxylin and eosin. The number and type of ovarian follicles were classified according to Pedersen and Peters (1968) with two modifications: 3b follicles were considered to be small follicles since they still contained a single layer of granulosa cells and 5b follicles were considered to be medium since no antrum had started to form.

Pituitary glands were macroscopically examined for the presence of tumors. None were found in mice with ovarian transplantations; however, 3 aged control mice had tumors and were excluded from the study.

Chromosomal Preparations

The viable fetuses were removed from implantation sites and were prepared individually for chromosomal analysis. This was done by dissociating fetal cells by rapidly pipetting the fetus in 3 ml of Minimal Essential Medium (Grand Island Biological Company, Grand Island, NY) containing 300 μl of colcemid. The cell suspension was then incubated at 37°C for 1.5 h, centrifuged, and resuspended in fresh fixative, and then drops of the cell suspension were placed on glass slides. After allowing the slides to air dry, the metaphase preparations were stained with a 2% Giemsa solution.

Radioimmunoassays

Blood plasma from each mouse was analyzed for LH, FSH, and Prl by radioimmunoassays described in detail elsewhere (Parkening et al., 1980, 1982). Luteinizing hormone and FSH were measured with rat NIAMDD kits, and Prl with the NIAMDD mouse kit. All samples for a particular hormone were measured in a single assay, and the intraassay coefficients of variation were less than 10%.

Superovulation

Some of the mice with transplanted ovaries that failed to become pregnant were superovulated to determine whether the ova were capable of reaching the oviducts. These mice, on the day they exhibited a prominent leukocytic vaginal smear, were injected intraperitoneally with 5 IU of pregnant mare's serum gonadotropin followed 48 h later with an intraperitoneal injection of 5 IU human chorionic gonadotropin. These dosages were chosen to prevent hyperstimulating the ovary and to induce ovulation in a manner similar to that occurring during natural ovulation.

RESULTS

Numbers of Mice Mating and Pregnant after Ovarian Transplantations

The numbers of recipients that mated, those that became pregnant, and the number of fetuses are shown in Table 1. Initially, 12 mice were operated on in each of the four categories of transplantation. All of the recipients survived the surgery, although two of the aged recipients, one that received young ovaries and one that received aged ovaries, died 1 and 2 mo, respectively, into the experiment (Table 1). Neither of these mice had mated.

The young recipients with orthotopically transplanted ovaries from young donors typically had a 4–5-day (5.2 ± 0.3) estrous cycle. The cycles in aged recipients with ovaries from aged donors were highly irregular. Three of these mice exhibited only leukocytic vaginal smears for a month before they were killed and

TABLE 1. Numbers of pregnancies and fetuses and plasma concentrations of LH, FSH, and Prl in C57BL/6J mice with orthotopic ovarian transplantations.

Type of ovarian transplantation	No. of mice	No. that mated (%)	No. pregnant (%)	Total implants	Total resorptions (%)	Plasma ng/ml (mean ± SEM)		
						LH	FSH	Prl
Young into young	12	10 (83)	7 (58)	37 (4.6)[a]	5 (14)	7.5 ± 1.7	452.9 ± 90.9	15.5 ± 5.6
Young into aged	11	5 (46)	1 (9)	5 (5.0)	5 (100)	9.7	455.0	71.2
Aged into young	12	10 (83)	6 (50)	27 (5.3)	0 —	21.0 ± 7.8	1125.7 ± 168.5[b]	34.8 ± 7.6
Aged into aged	11	4 (36)	0 (0)	0	— —	—	—	—

[a]Mean number of normal fetuses in parentheses.

[b]Significantly different from young into young: $P<0.005$.

they were considered acyclic. The remaining 8 mice had a cycle length of 11.6 ± 3.2 days. The estrous cycle in young recipients with ovaries from aged donors was 6.3 ± 0.4 days; the cycle in aged recipients with young ovaries was 9.8 ± 2.4 days.

The number of young recipients that became pregnant did not differ greatly, whether the donors were young (58% pregnant) or aged (50%); 5 of the 7 with ovaries from young donors and 5 of the 6 with ovaries from aged donors became pregnant at the first mating. The remaining recipients in both groups became pregnant after the second mating. The two groups produced approximately the same number of viable fetuses (Table 1). Only one of the aged recipients with ovaries from young donors became pregnant after its first mating, and all of the fetuses had died and were being resorbed 10 days after impregnation.

The group with the smallest percentage of matings (36%) was aged recipients with ovaries from aged donors (Table 1). None were pregnant after 10 days. Two of the mice mated once and 2 mated twice. The latter 2 were killed 10 days after the second copulatory plug was found to look for uterine resorptions, but none were found.

Ovarian Weight in Pregnant and Nonpregnant Recipients

In young recipients with ovaries from young donors, the ovarian weights of pregnant and nonpregnant mice were comparable to those in control young mice (Table 2). However, all of the nonpregnant mice were induced to ovulate; therefore, the ovarian weight in these mice was probably greater than would be expected, because of the presence of corpora lutea. The ovarian weight (1.3 ± 0.1 mg) for 6 superovulated mice (4 half-ovary, 2 whole-ovary) in the aged-to-aged transplantation group was higher ($P<0.05$) than that of nonstimulated mice (0.7 ± 0.3 mg). The ovaries from both of these groups still weighed considerably less ($P<0.001$) than those of aged control ovaries. The ovarian weight of nonpregnant young-to-aged transplantations was less ($P<0.05$) than that of young control ovaries (Table 2). Use of whole or half ovaries did not produce any statistical differences in ovarian weight in any transfer group of pregnant or nonpregnant (including superovulated) mice. Whole-ovary transfers among all 4 groups resulted in 25% pregnancies (4 of 16),

TABLE 2. Ovarian weights of C57BL/6J mice with orthotopic ovarian transplantations.

Type of transplantation	Pregnant Mice				Nonpregnant Mice			
	Whole-ovary transplant		Half-ovary transplant		Whole-ovary transplant		Half-ovary transplant	
	No. of mice	Weight/ Ovary (mg)[a]	No. of mice	Weight/ ovary (mg)[a]	No. of mice	Weight/ ovary (mg)[a]	No. of mice	Weight/ ovary (mg)[a]
Young into young	2	4.6 ± 1.0	5	4.3 ± 0.7	2	4.3 ± 0.3[b]	3	3.7 ± 0.6[b]
Young into aged	1	3.7 ± 0.7		—	3	2.5 ± 0.6[e]	7	2.1 ± 0.7[e]
Aged into young	1	1.9 ± 1.2	5	3.0 ± 0.7	3	2.1 ± 0.7	3	1.7 ± 0.4
Aged into aged		—		—	4	1.1 ± 0.2[c,f]	7	0.9 ± 0.2[d,f]
Nontransferred young					12	4.1 ± 0.3		
Nontransferred aged					12	2.4 ± 0.1		

[a] Mean ± SEM.
[b] Superovulated.
[c] Two were superovulated.
[d] Four were superovulated.
[e] Significantly different from nontransferred young ($P<0.05$).
[f] Significantly different from nontransferred aged ($P<0.001$).

and half-ovary transfers resulted in 33% pregnancies (10 of 30). The mean number of embryos derived from whole-ovary transfers was 3.8 ± 1.0, compared to 5.4 ± 1.1 from half-ovary transfers.

Numbers of Ovarian Follicles in Transplanted Ovaries

The numbers of follicles in ovaries were counted for all of the recipient–ovarian donor combinations (Fig. 1). The one pregnant, aged recipient with ovaries from a young donor had almost the same number of follicles as the young recipients from young donors. Pregnant young recipients with ovaries from young donors had more follicles ($P<0.001$) than those that were not pregnant and had been superovulated. Pregnant young recipients with ovaries from aged donors also had more follicles than those that were not pregnant ($P<0.005$). A control group of young and aged diestrous mice, identical in age to the nonpregnant mice when they were killed, had more follicles than the transplant recipients (Fig. 1). This would be expected since some necrosis occurs in a newly transplanted ovary before its blood supply can be reestablished (Mussett and Parrott, 1961).

Analysis of Chromosomes

Chromosomal smears were prepared from normal 10-day-old fetuses from each young mouse that became pregnant. Twenty-four fetuses derived from aged donors and 12 fetuses derived from young donors were studied. Between 5 and 22 metaphases were examined from all but 6 of the fetuses (in 2 fetuses from young donors and 4 fetuses from aged donors, less than 5 metaphases were read because of improper spreading of chromosomes). There was no evidence of aneuploidy or mosaicism in any of the fetuses.

Plasma Concentrations of LH, FSH, and Prl

Concentrations of plasma LH, FSH, and Prl for the mice that became pregnant after transplantation are shown in Table 1. Plasma FSH was significantly higher ($P<0.005$) in young recipients with ovaries from aged donors than in young recipients with ovaries from young donors. Plasma LH in the young recipients with aged ovaries was also more than twice as high as in young recipients with young ovaries or aged recipients with young ovaries, but this difference was not statistically significant.

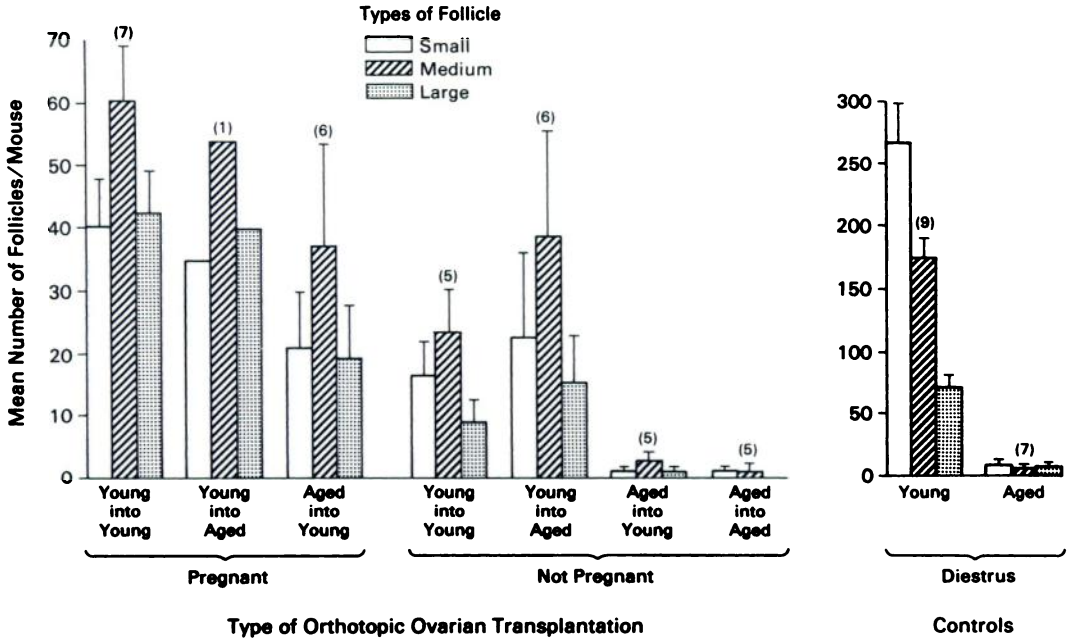

FIG. 1. Represented are the mean (± SEM) numbers of small, medium, and large follicles present in orthotopically transplanted ovaries of C57BL/6J mice. The number in parentheses over each set of bars represents the number of mice in that group. Recipients that became pregnant, those that did not become pregnant, and diestrous controls (without ovarian transplants) are represented. The ages of the diestrous controls were the same as mice receiving ovarian transplants. At the termination of the study the young recipients were 5 mo old and the aged recipients were 20 mo old. The follicles were classified, with minor modifications, according to a scheme devised by Pedersen and Peters (1968): small (3a, 3b), medium (4–5b), and large (6–8). All follicles were counted irrespective of atresia.

Five of the aged recipients with ovaries from aged donors (two that had mated) were killed during diestrus. Their LH concentration was 30.5 ± 19.4; FSH was 745.8 ± 148.3; and Prl, 28.4 ± 8.7 ng/ml. The remaining 6 aged recipients with aged ovaries and the 5 young recipients with young ovaries that had not become pregnant were superovulated, in order to determine whether ovulated eggs were capable of reaching the Fallopian tubes. None of the aged recipients and only one of the young ones had any ova in the oviducts. An examination of the excised ovaries before fixation, and of histologic sections of the ovaries, indicated that all of the younger recipients had ovulated, and we believe that lesions from the transplant surgery blocked access of the eggs into the oviduct. Three of the 6 aged recipients ovulated and, at examination, their ovaries still contained different stages of follicles. The other 3 aged recipients had no evidence of ovulation and no small, medium, or large follicles were detected in histologic sections of their ovaries. The concentration of LH in the young superovulated mice was 115.8 ± 32.2; of FSH, 680.5 ± 122.5; and of Prl, 29.1 ± 6.9. The concentration of LH in the aged superovulated mice was 109.1 ± 31.8; of FSH, 1310.0 ± 258.7; and of Prl, 23.4 ± 6.7.

DISCUSSION

Techniques for the orthotopic transplantation of mouse ovaries have been known for many years (Robertson, 1940; Stevens, 1957). Krohn (1962, 1966) first used the method to study the effects of aging on the mouse (CBA × A strain) reproductive system. After transplantation of young ovaries into old mice, he concluded (from the histologic appearance of the ovaries and the presence of degenerating embryos) that the uterus of the aged mouse was the primary limitation of reproduction. After reciprocal transplantations (Krohn, 1966), he concluded that ova from older mice were not the limiting factor in pregnancy failure, since litter sizes increased when ovaries from aged mice were transplanted into young mice. Data from the present study would support the latter

hypothesis since many of the limited number of oocytes remaining in the C57BL/6J mouse are still capable of producing normal fetuses. Normal-appearing morulae and blastocysts derived from aged C57BL mice have also been shown to survive, as well as those from younger mice when transferred into the uteri of younger pseudopregnant recipients (Talbert and Krohn, 1966; Gosden, 1974).

Gosden et al. (1983), from studies on aging in mice, and Sopelak and Butcher (1982), from studies in rats, have suggested that a reduction in the amount of ovarian tissue and a decrease in the number of follicles adversely affect reproductive function. Data from the present study agree with those suppositions. Ovaries of aged mice that had been transplanted into aged mice weighed less than the ovaries in any other type of transplantation, and this was the only transfer group in which there were no pregnancies. All but one of the mice that became pregnant in the other transfer groups had a mean ovarian weight greater than that found in the nonpregnant groups (Table 2). As a part of the study design, half an ovary rather than a whole ovary was transferred in two-thirds of the mice in each transplant group, because Stevens (1957) had achieved the largest number of offspring from half-ovary grafts. He speculated that half-ovary grafts vascularized sooner than whole-ovary grafts, so there was less necrosis. That is probably true, since the weights of the half- and whole-ovary transfers in our study were similar among all pregnant and nonpregnant transfer groups. A higher percentage of pregnancies and a larger mean number of young/litter was achieved with half-ovary transfers. For technical reasons, as well, the half-ovary transplants are better, because it is easier to insert half an ovary than a whole ovary into an empty bursa.

The extent of revascularization of the transplanted ovary seems to be an important factor in this study. The weights of ovaries from young mice transplanted into young recipients were similar to weights of ovaries from young control mice that did not have transplants. In contrast, when ovaries from young mice were transplanted into aged recipients, the ovarian weight was significantly lower than in young controls. Also, when ovaries of aged mice were transferred into aged recipients, the ovarian weight was significantly lower than in aged controls. Therefore, in older mice there is probably a greater degeneration of ovarian tissue, before a new blood supply is established. In young mice, however, many more oocytes still remain when their ovaries are transplanted, and a large number of developing follicles are still capable of developing upon transplantation into an older host (Fig. 1).

The elevated levels of FSH found in young recipients with ovaries from aged donors were similar to the levels found in aged, nontransplanted mice (Parkening et al., 1980, 1982). Although the rise in FSH may result from aging defects in the ovary itself, it probably occurs because fewer ovarian follicles are producing sufficient quantities of inhibin to feed back and control the release of FSH.

As a result of the study of the number of developing follicles, it became apparent that few follicles were developing in aged ovaries that had been transplanted into aged recipients, and in fact, no large follicles were found in nonstimulated mice (Fig. 1). Three aged recipients of ovaries from aged donors that were superovulated also had no large follicles present, although the number of small follicles was five times greater than that in the nonstimulated transplanted ovaries. Yet some aged ovaries transferred into younger recipients were capable of producing viable ova, and the mice that became pregnant had all types of developing follicles present in their ovaries. Fewer developing follicles were found, but the number was not statistically different from those found in young ovaries transplanted into young recipients.

The number of developing follicles found in the ovaries of intact individual aging mice varies considerably (Parkening et al., 1980; Gosden et al., 1983). However, it still seems unlikely that this variation alone accounts for the differences observed between ovaries of aged donors when transferred into young or into aged recipients. The aged mice were all born within 3 days of one another and consisted of several sets of litter-mates that were divided equally between the two types of transfers, yet 50% of the ovaries from aged donors transferred to young recipients yielded fetuses, whereas no pregnancies resulted from aged-to-aged transfers. No attempt was made in this study to count primordial follicles, although we did have the impression that the ovaries in pregnant young recipients of ovaries from aged donors had fewer primordial follicles. Statistically, there were certainly more developing follicles in ovaries of pregnant young recipients with ovaries from aged donors than in the ovaries of aged recipients with ovaries

from aged donors. Perhaps the environment of the young mouse and its more "finely tuned" hypothalamic-hypophyseal complex serve to stimulate the ovary from the aged donor into responding in a manner reminiscent of its performance during youth.

The primary question is, if the ovary of the young donor is capable, upon transplantation into an aged recipient, of developing sufficient ovarian follicles, why was there only one pregnancy? Why did these recipients not then have a 4–5-day estrous cycle and why did only 46% of them mate? The defect must reside within the hypothalamic-hypophyseal complex of the older mouse. Aschheim (1964/5) performed heterotopic ovarian transplants between young and aged rats. Ovaries from prepubertal donors that were grafted into aged recipients in constant estrus remained in constant estrus, whereas ovaries from aged donors in constant estrus that were grafted into young recipients assumed normal estrous cycles. Aschheim concluded that the chronologic age of the ovary was not the primary cause for its loss of cyclic activity.

Felicio et al. (1983) did heterotopic ovarian transplantation (renal capsule) in which ovaries from 3–5-mo-old C57BL/6J mice were transferred to ovariectomized 5-, 17-, 25-, and 30-mo-old mice. Some differences existed between the 17–30-mo-old mice depending upon whether they were acutely ovariectomized or had been ovariectomized for many months prior to the ovarian graft; however, all of the older mice either failed to resume an estrous cycle or exhibited an estrous cycle significantly longer than that of 5-mo-old mice receiving ovarian grafts. The failure of the older mice to regain the degree of cyclicity found in younger mice suggests that an age-related impairment exists within the hypothalamic-pituitary axis.

Sopelak and Butcher (1982) did orthotopic ovarian transplantations between young and aged rats. They found that old recipients with transplanted ovaries from young or prepubertal donors maintained a regular estrous cycle longer than aged recipients with transplants from aged donors. Both groups of transplanted rats gradually stopped cycling, over an 80-day observation period, as the number of oocytes and follicles declined. The younger rats entered anestrus and the aged rats entered constant estrus. Since the numbers of oocytes and follicles remaining in the ovaries of the two age groups were not considered to be significantly different, their cyclicity was attributed to differences in the hypothalamic-hypophyseal axis.

Peng and Huang (1972) confirmed Aschheim's early studies on heterotopic ovarian transplantation between young and aged rats. They also attempted orthotopic pituitary transplantations between young and aged rats and found that pituitaries from some irregularly cycling and anestrous aged rats were capable of maintaining an estrous cycle in young rats, which suggested age-related changes in the hypothalamus. The transplantation of hypothalamic tissue from newborn rats into the third ventricle or periventricular parenchyma of hypothalami of 21–30-mo-old rats restored ovarian function in 4 of 7 rats (Matsumoto et al., 1984). In the present studies, it is not known if the hypothalamus is malfunctioning in older mice, although previous research (Collins et al., 1981) in our laboratory on intact aged C57BL/6 mice has shown that the anterior pituitary gland is less sensitive to gonadotropin-releasing hormone stimulation than the pituitaries of young mice.

Another organ of the reproductive system that must be considered in this type of study is the aging uterus. Krohn (1962) killed 13 aged mice (during midpregnancy) that had been shown to be incapable of producing litters before they received orthotopic ovarian grafts from young mice. Two normal embryos, 2 questionably normal embryos and 43 dead resorbing embryos were found in their uteri and 104 corpora lutea were detected on their ovaries. A deteriorating uterine environment was considered to play a critical role in the ability of the aging mouse to produce offspring. In the present study, the single aged mouse that became pregnant after receiving ovaries from a young mouse contained five dead resorbing fetuses. Although the aging uterus is obviously playing a role in the waning reproduction of the mouse, it appears to be a secondary factor considering the small number of aged mice in this transplant group that mated.

The quality of the ova from the ovaries of old donors should also be considered, since previous studies on mice (Parkening et al., 1980) and Syrian hamsters (Parkening and Soderwall, 1975) have shown a greater preimplantation loss with increased age. A reduction in chiasma frequency and increased univalent formation has been reported for aging mice (Henderson and Edwards, 1968; Luthardt et al., 1973). After examining 765 newborn mice, however, Goodlin (1965) could find no correlation be-

tween aneuploidy and maternal age. The differences in these studies may simply be a reflection of the developmental stage chosen for chromosomal smears, since Goodlin (1965) examined newborns, whereas the other investigators examined ovarian oocytes. Since litter size is reduced with increasing age, preimplantation and early postimplantation loss may have already eliminated the aneuploid embryos. This may be an explanation for the absence of aneuploidy in the 24 fetuses derived from ovaries of aged mice in the present study, or it may simply be that the sample size was too small.

Orthotopic ovarian transplantation studies on CBA × A mice (Krohn, 1962) and heterotopic ovarian transplantation studies on Wistar rats (Aschheim, 1964/5) are in direct conflict with one another (Krohn, 1966; Aschheim, 1976). Krohn (1962) considered the aging uterus to be the limiting factor in reproductive function, whereas Aschheim (1964/5) considered the aging hypothalamic-hypophyseal complex to be the major cause of reproductive failure. Our laboratory has conducted an orthotopic ovarian transplantation study on CBA/J mice (Parkening et al., 1984) that was similar to the one just described for C57BL/6J mice. The CBA/J mouse is a special strain that prematurely loses its ovarian oocytes, which renders the animal virtually sterile shortly after 1 yr of age. The results of that study showed that the aging ovary and its loss of oocytes is probably the limiting factor in reproduction, although the hypothalamic-hypophyseal complex was several months younger in the CBA/J mice used in that study in comparison to the oldest C57BL/6J mice used in the present study. We suggest that the CBA/J mouse is a unique genetic strain, because of its early loss of oocytes, and that this accounts for the differences in the Krohn (1962) and Aschheim (1964/65) transplantation studies that were conducted on aging mice and rats.

Orthotopic ovarian transplantations between young and aged C57BL/6J mice indicate that there are age-related changes occurring throughout the reproductive system. However, the primary defect limiting reproductive function seems to reside within the hypothalamic-hypophyseal complex or higher central nervous system, whereas secondary defects arise with age from the ovary and uterus.

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