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Transplantation of Young Ovaries Restored Cardioprotective Influence in Postreproductive-Aged Mice

Jeffrey B. Mason^{1,a,*}, Shelley L. Cargill^{1,b}, Stephen M. Griffey³, J. Rachel Reader³, Gary B. Anderson¹, and James R. Carey²

¹ Department of Animal Science, University of California, Davis

² Department of Entomology, University of California, Davis

³ Comparative Pathology Laboratory, School of Veterinary Medicine, University of California, Davis

Summary

The female cardioprotective advantage, present in mammals of a reproductively-competent age, is lost during the transition to a postreproductive state. The role of reproductive hormones in this transition is most evident in women with premature ovarian failure, where reduced estrogen production has been associated with an increased incidence of early death from cardiovascular disease. Previously, we reported that postreproductive-aged mice that received young ovaries displayed an increased life span. Subsequent histopathological analysis suggested the presence of a cardioprotective effect associated with the restoration of ovarian influence. This restoration in postreproductive-aged mice produced a sharp decrease in evidence of significant cardiomyopathy at death, compared with sham-transplanted mice (36.0% vs. 73.3%, respectively). Within the intact transplant group, evidence of cardiomyopathy at death was decreased in mice that were reproductively cycling at the time of transplant, compared with acyclic mice (26.7% vs. 50.0%, respectively). This observation reflects the importance of timing in restoration of ovarian influence in this study. Transplantation of young ovaries to intact, postreproductive-aged female mice provided significant, long-term restoration of a cardioprotective benefit, similar to that previously present during a reproductively-competent age. In these mice, restoration of ovarian influence through ovarian transplantation may, in effect, have postponed the advance of age-associated cardiomyopathy to a point where the disease did not reach a clinically-relevant threshold during the lifetime of the recipients. These results offer support for previous clinical observations suggesting that hormone replacement therapy can produce divergent results if initiated during the perimenopausal period compared with the postmenopausal ages.

Keywords

Sex hormones; Mouse models; Endocrinology; Estradiol; Senescence

*Address correspondence to: Jeffrey B. Mason, PhD, Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348. Phone: 610-925-6541, Fax: 610-925-8121, masonjef@vet.upenn.edu.

^aCurrent contact information; Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania.

^bCurrent contact information; Department of Biological Sciences, San Jose State University.

Introduction

Cardiovascular disease (CVD) is relatively uncommon in premenopausal women, compared with similarly aged men (Murray and Lopez, 1997; Mathers and Loncar, 2006; Thom et al., 2006). Unfortunately, this advantage disappears after menopause, as the incidence of CVD increases rapidly at the transition to a postreproductive state (Hodis and Mack, 2009; Shuster et al., 2010). This phenomenon has led to the concept that reproductive hormones protect women from CVD and that CVD risks increase after the postmenopausal drop in hormone levels. Further support for the role of reproductive hormones in CVD is seen in women with premature ovarian failure (POF), where reduced estrogen production is associated with an increased incidence of early death from CVD (Jacobsen et al., 1999). Observations from the study of POF support the role of estrogen-loss, in addition to aging per se, as a contributing factor in the increased risk of CVD in older women (Joakimsen et al., 2000; Lobo 2007).

The dilemma of estrogen-loss in older women is often addressed with hormone replacement therapy (HRT). Results of observational studies suggest that HRT can provide cardioprotective effects in postmenopausal women. While these observations are well supported in the literature, they are not well supported by clinical trials. Observational studies provide support for the use of HRT for its potential long-term cardioprotective effects, management of osteoporosis, dementia and the acute symptoms associated with the menopausal transition (Colditz et al., 1987; Mendelsohn and Karas, 1999). In clinical trials, some researchers have suggested an increased risk of CVD in HRT users (Rossouw et al., 2002; Ballard and Edelberg, 2005) and advised that HRT be eliminated as a tool to fight increasing rates of CVD in postmenopausal women (Dull, 2006). Conclusions drawn from observational and clinical studies suggest that endogenous ovarian hormones provide cardioprotective effects when supplied by the ovaries, but exogenous hormones can produce suboptimal and potentially harmful results. If the endogenous ovarian influence present in a reproductively-competent female could be recapitulated in a postreproductive female, she may benefit from a similar level of cardioprotection that was present during her reproductively-competent state.

Transplantation of young ovaries to postreproductive-aged females is an obvious methodological choice to examine this question. Previously, we reported that prepubertally ovariectomized mice that received young, transplanted ovaries at a postreproductive age displayed a 40% increase in life expectancy (Cargill et al., 2003). In a subsequent experiment, postreproductive-aged mice that were ovary-intact at the time of transplant displayed an increase in life span (Mason et al., 2009), improved aging-associated body condition and altered rates of body-weight gain and loss (Mason et al., 2010), compared with control mice. Reported here are the results of the histopathological investigation of these mice at necropsy, focusing on the cardiovascular consequences of increasing and decreasing long-term ovarian influence through ovariectomy and ovarian transplantation.

Results

Cardiomyopathy at Death

Evidence of significant cardiomyopathy at death was used as a contributing factor for determining if restoration of a cardioprotective environment occurred under various modes of ovarian influence (Fig. 1.). Intact mice that received ovarian transplants at 11 months displayed decreased evidence of significant cardiomyopathy at the time of death, compared with sham-transplanted mice ($P=0.005$, Fig. 2.). Recipients that were cycling at the time of transplant displayed the greatest decrease in evidence of cardiomyopathy at death, compared with all other groups (Tab. 1). No change in cardiomyopathy was observed in

postreproductive-aged, transplant recipients that were ovariectomized prepubertally, compared with sham-transplanted, ovariectomized mice.

Age at Death with Evidence of Significant Cardiomyopathy

Decreased evidence of significant cardiomyopathy at death was accompanied by an increase in life span for intact transplant mice ($P=0.031$, Fig. 3A). Interestingly, the mean age at death for mice that died with significant cardiomyopathy varied by only 13 days between groups, compared with 65 days between groups when considering all causes of death. Neither ovarian transplantation nor ovariectomy notably affected the mean age at death in mice that died with evidence of significant cardiomyopathy (Tab. 1). An exception to this 13-day window was found when the intact transplant mice were divided into cycling and non-cycling groups, subsequently doubling the window to 24 days between these two groups (Fig. 3B).

Incidence of Atrial Thrombosis at Death

Evidence of atrial thrombosis at death in intact transplant recipients was decreased, compared with intact shams (Fig. 4). Atrial thrombosis at death was decreased even further for recipient mice that were acyclic at the time of transplant, compared with cycling mice (Tab. 1). An important qualitative difference between transplant and sham mice was the presence of chronic thrombus formation, found in transplant recipients, but not in the intact sham mice. As mice found dead were subject to autolysis prior to necropsy, some acute thrombotic events may have been lost, although evidence of chronic thrombosis is typically conserved. Ovariectomized sham mice were not different from intact sham mice in regard to evidence of atrial thrombosis at death. Ovarian transplantation produced no change in evidence of atrial thrombosis at death in ovariectomized mice.

Incidence of Myocardial Fibrosis at Death

Increased evidence of myocardial fibrosis at death was observed with intact mice that received ovarian transplants, compared with sham-transplanted mice ($P=0.004$, Fig. 5). This phenomenon was not observed in the ovariectomized mice, as ovariectomized mice in both transplant and sham groups displayed increased evidence of fibrosis, compared with intact sham mice (Tab. 1). The evidence of fibrosis in the ovariectomized mice appeared to be independent of chronological age.

Incidence of Mammary Lesions at Death

Similar to results from previous reports, none of our prepubertally ovariectomized mice developed mammary lesions, compared with a 6.3% lesion incidence in our intact mice (Cori, 1927; Lebowitz and Zujewski, 2003). Increasing ovarian influence by transplantation of young ovaries in either ovariectomized or intact experimental groups produced no change in the incidence of mammary lesions (Tab. 1). While this observation is not directly related to cardiovascular health, the lack of a tumorigenic response to the putative increase in ovarian hormones in postreproductive-aged mice may be significant for hormone replacement therapies.

Discussion

The female cardioprotective advantage, present in mammals of a reproductively-competent age, is lost during the transition to a postreproductive state. Transplantation of young ovaries to intact, postreproductive-aged female mice provided restoration of a cardioprotective benefit, similar to that previously present during the reproductively-competent ages. This phenomenon was most evident with intact, postreproductive-aged recipients that displayed a

significant decrease in evidence of cardiomyopathy at the time of death. This decrease was accompanied by a significantly increased life span in transplant recipients. A longer life span coupled with decreased evidence of age-associated cardiomyopathy advocates for a superior cardioprotective environment in transplant recipients. In contrast, the transplantation of young ovaries to ovariectomized mice produced no positive cardiovascular effects. The basis for the lack of a cardioprotective response in ovariectomized mice is unknown, but the negative effects associated with prepubertal ovariectomy may have influenced the positive potential of the transplanted ovaries.

The cardioprotective effects described in premenopausal women are often attributed to the effects of reproductive hormones, although some contradictory information has been reported and varies according to the form of hormone used and the path of delivery. In many clinical HRT trials, conjugated equine estrogen and medroxyprogesterone acetate were utilized, which behave differently from endogenous hormones (Kuhl, 2005). Many of these clinical trials were also burdened by the detrimental effects associated with an oral route of administration (Hulley et al., 1998; Rossouw et al., 2002). Ovarian transplantation provides delivery of natural hormones through a natural pathway with controlled delivery, and with the dosage regulated through endogenous mechanisms. These endogenous mechanisms may have provided a cardioprotective effect superior to many exogenous therapy regimes.

Heterochronic ovarian transplantation has previously been used to manipulate the hypothalamic-pituitary-gonadal axis to study ovarian influence in neuroendocrine aging (Finch et al., 1984). The ovary does not act independently; rather it acts upon and is acted upon by other regulatory entities including the hypothalamus, pituitary and adrenal glands, etc. Normally, when a mouse ceases cycling, she is acyclic until death. Interestingly, in our intact sham mice, where ovaries were simply removed and replaced to their original ovarian bursae, 30% of the mice that had ceased cycling before surgery began cycling again after the sham surgery. The ovary was no different, but it appears that a brief change in signaling within the neuroendocrine axis had 'kicked' the ovary back into gear. The axis was once again responsive to the presence of the same ovary it previously ignored.

Our ovarian transplant recipients benefited from a significantly increased mean life span. However, among mice that died with evidence of significant cardiomyopathy, the mean age at death for transplant recipients mirrored that found in the sham-transplanted mice, with only a 13-day variation between experimental groups. One might be tempted by this statistic to embrace this narrow range of ages as dedicated for 'death-by-CVD'. An observation providing a disconnect between age at death and CVD is seen when the intact transplant group is divided into mice that were cycling and mice that were acyclic at transplant. Cycling recipients displayed only a modest increase in mean life span, but displayed the greatest decrease in significant cardiomyopathy at death. In contrast, mice that were acyclic produced the greatest increase in mean life span, but displayed only a slight decrease in cardiovascular-related pathology. These observations correlate well with results of previous clinical studies that suggest HRT, started during the perimenopausal (cycling) period, produces superior cardioprotective effects, compared with HRT started during the postmenopausal (acyclic) period (Prentice et al., 2006). Among these two groups, transplant recipients with the largest decrease in evidence of cardiomyopathy also displayed the smallest amount of life span extension. The opposite is true for atrial thrombosis.

Decreased evidence of thrombosis at death closely correlated with the observed increase in life span. Atrial thrombosis is relatively common in aging rodents and has been shown to be influenced by ovarian hormones (Fry et al., 1965; Sichuk et al., 1965; Maita et al., 1988). Cycling status at the time of surgery had no effect on evidence of atrial thrombosis at death for intact sham mice. The story was different for intact transplant recipients, where cycling

status had a huge effect on atrial thrombosis. Acyclic mice had far less evidence of atrial thrombosis at death, compared with mice still cycling at the time of surgery. These observations suggest that 1) ovarian transplantation had a major influence on evidence of atrial thrombosis at death and 2) this effect was only realized by mice that had ceased cycling by the time they received the young, transplanted ovaries.

Interestingly, when the groups were divided by age-at-death, mice that died at a very old age or a very young age displayed less evidence of atrial thrombosis than mice that died at a moderate age. No mice over the age of 820 days or under the age of 685 days showed evidence of atrial thrombosis at death. It would be a stretch to suggest that this broad range of ages is similar to the previously-described narrow range of ages as dedicated for 'death-by-CVD'. The age range for atrial thrombosis is 135 days, much broader than the 13-day variation in mice with significant cardiomyopathy. The fact that chronic thrombi were found only in the transplant mice suggests these mice were able to contend with the presence of thrombi to a greater degree than intact sham mice. A key factor in thrombosis and atherosclerosis is damage to the endothelium. In women, the menopause-related decrease in estrogen has been associated with a decrease in the numbers of circulating endothelial precursor cells (Baker et al., 2003). Estrogen replacement has been shown to increase responsiveness of endothelial stem cell repair mechanisms, to increase levels of circulating endothelial precursor cells and to promote migration and proliferation of endothelial precursors (Wilson et al., 1970; Strehlow et al., 2003; Ballard and Edelberg, 2005; Imanishi et al., 2005), suggesting an increased capacity to repair damaged endothelium.

The myocardial repair process differs significantly from endothelial repair. Reparative fibrosis is often pronounced in rodents with clinical signs of heart failure (Conrad et al., 1995; Brilla et al., 1996). Our intact transplant recipients displayed a two-fold increase in evidence of myocardial fibrosis at death, compared with sham mice. Increased life span and increased fibrosis were closely correlated in the intact groups. In contrast, among ovariectomized mice, both transplant and sham groups displayed increased fibrosis, compared with intact shams. The extensive fibrosis observed in the ovariectomized groups may have been due, in part, to increased levels of adrenal influence in the absence of ovarian feedback (Pearce, 1906; Kahn et al., 1969; Brilla et al., 1994; Muders and Elsner, 2000), or to the ovariectomized mice being subjected to two separate surgical procedures (prepubertal and 11 months of age). No differences were seen in myocardial fibrosis or any other cardiovascular-related parameter between untouched-control mice and sham-operated mice, suggesting little influence of the surgery at 11 months on incidence of fibrosis.

By utilizing death as an endpoint in our experiments, it was possible to observe the results of increased ovarian influence (ovarian transplantation) and decreased ovarian influence (ovariectomy) over the life span of the animal. Prepubertal ovariectomy removed ovarian influence during the normally reproductively-competent ages in ovariectomized mice and was associated with an increase in myocardial fibrosis. Subsequent transplantation of young ovaries produced no significant cardiovascular benefits in ovariectomized mice. In postreproductive-aged, ovary-intact recipients, restoration of youthful levels of ovarian influence with new ovaries produced a sharp decrease in evidence of significant cardiomyopathy at death. Restoration of ovarian influence may, in effect, have postponed the advance of age-associated cardiomyopathy to a point where the disease was subclinical during the lifetime of the recipients. As many strains of mice are resistant to atherosclerosis, cardiovascular degeneration is more often attributed to chronological age than to loss of reproductive function in female mice. In the current study, ovarian transplantation and ovariectomy clearly demonstrated the influence of reproductive function on cardiovascular health. It would not be prudent at this point to credit estrogen alone for the numerous changes observed in mice that received ovarian transplants, but these and other results have

shown that the relationship between chronological aging and cardiovascular disease can be improved and that reproductive function is a likely candidate for future research in prevention of cardiovascular-related pathologies.

Experimental procedures

Mice

The CBA strain (used in the current study) and the DBA strain of mice are unique in that they prematurely lose their ovarian follicles, becoming reproductively senescent by 10–11 months of age (Thung et al., 1956; Jones and Krohn, 1961; Faddy et al., 1987). Since a reduction of ovarian follicles in the human may be associated with the onset of menopause, it has been suggested that CBA and DBA mice may serve as appropriate experimental models to study age-related changes in the human reproductive system (Thung et al., 1956; Gosden et al., 1978; Barnett et al., 2006).

Vicarious, non-cyclic hormone production by the adrenal glands has been described in CBA mice, but does not occur in C57BL/6 mice. In C57BL/6 mice, signs of estrus in old age in intact females would likely be due to ovarian activity, whereas in aged, intact CBA females signs of estrus (but not estrous) could be ascribable to adrenal hyperplasia in addition to ovarian activity. At a comparatively early age, vaginal smears in CBA mice tend toward an anestrus condition, whereas C57BL/6 mice on losing their normal cycles revert to a prolonged subestrous rather than an anestrus condition with a high frequency of metestrous smears. These strain differences should be kept in mind when reviewing the manuscript.

Adult (40g) CBA/J strain female mice (Jackson Laboratory, Bar Harbor, ME) were provided ad libitum access to feed (Purina Mouse Chow 5008: 23.5% protein, 6.5% fat, Purina Mills, St. Louis, MO; <http://labdiet.com/pdf/5008.pdf>) and water (deionized) and were housed under conditions of constant temperature ($21^{\circ}\text{C} \pm 2^{\circ}\text{C}$), humidity (min. 50%), and lighting (14L: 10D, lights-on at 0700 h). Individual pups were weaned and ear-notched at 21 days (day of birth = 0 days). All female weanlings were housed individually, with added enrichment, in a $26 \times 17 \times 13$ cm shoebox cage in a specific-pathogen-free (SPF) colony where pathology on sentinel mice was done quarterly and pathological results showed no breach in this status. Mice were maintained in an American Association for Accreditation of Laboratory Animal Care (AAALAC)-approved facility in accordance with NIH animal-use guidelines. Animal care and use protocols were developed under National Research Council guidelines found in the Guide for the Care and Use of Laboratory Animals. This project was approved by the University of California, Davis Institutional Animal Care and Use Committee.

Experimental Design

Animals were randomly assigned to Control, Sham-transplant or Ovarian-transplant groups as follows (Fig. 6.):

Controls consisted of

IT: Controls included intact animals that were not subjected to any surgical procedures.

Shams consisted of

IT-S: Intact sham animals remained intact to 11 months, at which time they underwent a sham surgery where we removed and replaced both endogenous ovaries.

OX-S: Ovariectomized sham animals were ovariectomized at 21 days and subsequently, at 11 months, underwent a sham surgery where we removed the glass beads previously placed into the ovarian bursae during the 21-day ovariectomy procedure.

Ovarian Transplants consisted of

IT-TX: Intact transplant animals remained intact to 11 months, at which time their endogenous ovaries were removed and replaced with a pair of donor ovaries from a 60-day-old mouse.

OX-TX: Ovariectomized transplant animals were ovariectomized at 21 days and subsequently, at 11 months, the glass beads previously placed into the ovarian bursae during the 21-day ovariectomy procedure were removed and replaced with a pair of donor ovaries from a 60-day-old mouse. Sham treatments served as surgery controls for transplant procedures. All animals in each experimental group remained virgin until death.

Age at Manipulation

Mice of the CBA/J strain become reproductively competent between 45 and 60 days of age. Ovariectomy at 21 days of age was chosen to avoid major up-regulation of the reproductive system at the onset of puberty and to eliminate other influences the female gonad might have in addition to direct effects of gonadal hormones. These influences may include positive or negative feedback mechanisms or system-wide “imprinting” influences the ovary may normally provide after 21 days of age. Rodents do not undergo menopause, but instead have an estropause-like decrease in reproductive function. Reproductive decline in CBA/J mice usually begins with irregular cycles at 8–10 months of age. At 11 months of age, many CBA/J mice have become reproductively incompetent (Cargill et al., 2003) with a complete loss of oocytes by 12 months of age. Ovarian transplantation and sham surgeries were conducted at 11 months of age, as many females in this line of mice have reached a point of reproductive failure by this time.

Surgical Procedures

Bilateral ovariectomies at 21 days and ovarian transplantation and sham surgeries at 11 months were performed as previously described (Cargill et al., 1999). Briefly, the ovaries were exposed by paralumbar incision under sodium pentobarbital anesthesia and removed by incising the ovarian bursa opposite the ovarian hilum. The ovary was gently removed from the ovarian bursa and excised by clamping the ovarian hilum to prevent bleeding. Excised ovaries were placed in cold saline prior to transfer/replacement. The ovarian bursa was closed with one to three sutures of 10-0 Ethilon monofilament (Ethicon, Inc.). The abdominal wall was sutured with 5-0 chromic gut (Ethicon, Inc.), and the skin was closed with 9 mm wound clips (MikRon Precision, Inc.). When we performed ovariectomies at 21 days of age, a sterile 1-mm diameter glass bead was inserted into each empty ovarian bursa to keep it open for future ovarian transplantation or sham surgery.

At 11 months of age, bilateral ovarian transplantation and sham surgeries were performed as previously described (Cargill et al., 1999). Briefly, OX-S animals were subjected to sham ovarian transplant surgery in which the glass bead placed at 21 days of age was removed, but no ovary was transplanted. Intact sham animals underwent sham ovarian transplant surgery in which their endogenous ovaries were removed, placed in cold saline and returned to the original bursae. Intact transplant animals underwent a bilateral ovariectomy and subsequent ovarian transplantation and received a pair of 60-day-old ovaries from a donor mouse of the same strain. The OX-TX cohort underwent ovarian transplantation in which the glass beads placed at 21 days of age were removed and replaced with a pair of ovaries from a 60-day-old donor mouse of the same strain. Data on vaginal cytology were collected

for at least 10 consecutive days pre- and post-surgery to ensure: 1) complete removal of the ovarian tissue, and 2) success of the ovarian transplantation procedure. Vaginal smears were taken daily beginning 10–14 days after surgery. One estrous cycle was defined as the period from the day nucleated epithelial cells first appeared (i.e., proestrus) to the day preceding the next appearance of nucleated epithelial cells in the vaginal smear, provided there was a period of leukocytic presence (i.e., diestrus) in between. Estrus was determined by the presence of large, squamous epithelial cells, with or without nuclei. After surgery, each female was housed individually in a 26 × 17 × 13 cm shoebox cage. Additionally, all serum samples submitted from mice at the time of necropsy were negative for parvovirus.

Exclusion Criteria

Presumptive ovariectomized mice that displayed signs of gonadal input prior to surgery at 11 months were excluded from analysis. Gonadal input was defined as cyclic changes within the population of vaginal cells, presumably due to cyclic influence of ovarian hormones. No gonadal input was defined as the lack of cyclic changes within the vaginal cell population. Ovarian transplant recipients that failed to display evidence of gonadal input after transplant surgery were also excluded from analysis. Gonadal input was determined by vaginal cytology analysis, as described in *Surgical Procedures*. Mice that displayed no cyclic activity for a 10-day period before and/or after surgery were determined to have no gonadal input for said period. Mice that displayed at least one full estrous cycle in a 10-day period before and/or after surgery were determined to have gonadal input for said period. Mice that fit these criteria were the only mice used for analysis throughout this study.

Sample Collection

Mice in all groups were submitted for necropsy at death. Aged mice found with overt clinical signs were euthanized by inhalation of carbon dioxide. Gross necropsy with tissue collection in 10% neutral buffered formalin was performed. Mice that were found dead had major body cavities opened and were placed in 10% neutral buffered formalin. Tissues were fixed for at least 24 hours before processing. Samples from at least 20 organ/tissue categories, including, but not limited to, brain, liver, spleen, kidneys, pancreas, heart, lungs, thymus, lymph nodes, distal reproductive tract region, urinary bladder, stomach, cecum, small intestine, colon, pituitary gland, adrenal glands, ovaries, uterus and mammary gland were examined in each mouse microscopically. Supplementary histology was conducted on any additional tissues with significant findings (in addition to those listed). Samples were processed routinely to paraffin blocks. Five- μ m sections were made and stained with hematoxylin and eosin (H&E). Mineralization was initially determined through routine evaluation of H&E-stained sections with confirmation by Von Kossa staining where necessary.

Histopathologic examination of representative slides was performed blindly by pathologists at the Comparative Pathology Laboratory at the University of California, Davis. Organs or tissues with changes were reviewed a second time and categorized for group comparisons. Histopathological analysis of the selected treatment groups suggested the presence of a cardioprotective effect associated with the transplantation of a new ovary. To determine the long-term effects of ovarian influence on the level of cardioprotective effort, cardioprotective effects were ascribed to the number of animals that succumbed to death with evidence of significant cardiomyopathy, compared with all non-CVD-related pathologies. A diagnosis of Myocardial Fibrosis was determined by the presence of excess fibrous connective tissue as a presumptive reparative or reactive process. A diagnosis of Atrial Thrombosis was determined by the formation of white thrombi in the atrial lumen composed of fibrin, platelets, and mixed inflammatory cells. A diagnosis of Cardiomyopathy included, but was not limited to quantity and severity of cardiovascular-

related lesions and sphere of lesion influence, including myocardial degeneration, mineralization and necrosis, atrial and ventricular thrombosis, myocardial fibrosis and other organ involvement, with particular focus on compromised pulmonary function. Death with evidence of significant cardiomyopathy was determined where 1) severe cardiovascular lesions were determined to be probable or contributory to the cause of death and 2) these lesions were present in the absence of other neoplastic or non-neoplastic contributing lesions that would affect the moribund state or, 3) these lesions were present in the presence of incidental minor lesions that would not have contributed to the animal's moribund state. When death occurs at an extreme age, cause of death is often difficult to determine and can be ascribable to multiple concurrent conditions. As many of the mice in this study died at an extreme age, we chose to describe the conditions present at death rather than guess at one conclusive cause of death. This approach provides the reader with additional information for making a determination regarding the level of influence of potential causes of death.

Statistical analysis

Experimental data were analyzed preliminarily by one-way ANOVA and linear regression analysis. Significance tests for each predictor in the multiple linear regression model were implemented with the Student's t-test, two-tailed, unequal distribution of variance assumed and Chi-square (χ^2) analysis where appropriate. Test results were considered significant for P-values $p < 0.05$.

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Figure 1.

Representative H&E-stained fields from control (IT-S) mice with and without significant cardiomyopathy at death. **A)** Cardiac tissue with no significant lesions. **B)** Regional myocardial degeneration and loss with replacement fibrosis (n = normal tissue, F = replacement fibrosis). **C)** Myocardiocyte vacuolar degeneration (V) and cytoplasmic hypereosinophilia with nuclear pyknosis (N) [necrosis]. **D)** Large atrial thrombus (lamellations of fibrin and cellular debris, T), atrial myocardium (M) and atrial chamber lumen (L).

Figure 2.

Evidence of significant cardiomyopathy (CM) at death. Ovarian transplantation significantly affected evidence of cardiomyopathy at death. Intact mice (IT) that received ovarian transplants (IT-TX) had less evidence of cardiomyopathy at death than intact mice subjected to only a sham (IT-S) surgery (IT-TX=36.0%, IT-S=73.3%, $P=0.005$). Ovariectomized mice (OX) that received new ovaries (OX-TX) were no different from ovariectomized mice subjected to only a sham surgery (OX-S) with respect to cardiomyopathy at death (OX-TX=59.1%, OX-S=58.3%, $P=0.96$). Significant cardiomyopathy was determined where severe cardiovascular lesions were determined to be probable or contributory to the cause of death in the absence of other contributing lesions that would affect the moribund state. Error bars represent SE. (* $P<0.05$, ** $P<0.005$).

Figure 3.

Life span with and without evidence of significant cardiomyopathy (CM) at death. **A)** Intact mice that received ovarian transplants lived significantly longer than sham transplanted mice (Table 1, $P=0.015$). Mean age at death in mice with evidence of significant cardiomyopathy was not different between all groups. **B)** Mice not cycling at transplant lived significantly longer than cycling mice (Table 1, $P=0.048$), but mean age at death in mice with significant cardiomyopathy was not different between these groups. Error bars represent SE. (* $P<0.05$).

Figure 4.

Evidence of atrial thrombosis at death. Evidence of atrial thrombosis at death was influenced by ovarian transplantation (TX=32.1%, Sham=40.7%). Intact mice that had stopped cycling at transplant displayed the greatest effect (IT- TX_{cycling} =37.5%, IT-TX_{not-cycling} =14.3%). Ovariectomy also influenced thrombus formation (OVX=41.7%, Intact=32.8%). Ovarian transplantation partially buffered this negative effect (OX-TX=39.1%, OX-S=44.0%). Error bars represent SE.

Figure 5. Evidence of myocardial fibrosis at death. The incidence of myocardial fibrosis at death was increased by ovarian transplantation ($P=0.023$) and ovariectomy ($P=0.004$). Error bars represent SE. (** $P<0.005$).

Figure 6. Experimental design. IT (intact), OX (ovariectomized), S (sham), and TX (ovarian transplant).

Table 1

Effects on disease state at death between treatments.

Treatment group	OX-S	OX-TX	IT-S	IT-TX	IT-TX (cycling)	IT-TX (acyclic)
Mean age at death	715	757	728	780	754	811
Mean age at death with CM	746	756	753	759	772	748
CM at death (%)	14/24 (58.3)	13/22 (59.1)	22/30 (73.3)	9/25 (36.0)	4/15 (26.7)	5/10 (50.0)
Fibrosis at death (%)	21/25 (84.0)	19/23 (82.6)	14/34 (41.2)	23/30 (76.7)	11/16 (68.8)	12/14 (85.7)
Thrombrosis at death (%)	11/25 (44.0)	9/23 (39.1)	13/34 (38.2)	8/30 (26.7)	6/16 (37.5)	2/14 (14.3)
Mammary Lesion at death (%)	0/25 (0.0)	0/23 (0.0)	2/34 (5.9)	2/30 (6.7)	1/16 (6.3)	1/14 (7.1)

(Cardiovascular pathology at death was inconclusive for some mice; Cardiomyopathy [CM]).