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Effects of Injectable Anesthetic Combinations on Left Ventricular Function and Cardiac Morphology in Sprague–Dawley Rats

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Novel anesthetic agents or combinations may provide superior general anesthesia for echocardiography in rodents with the potential for reduced adverse effects. This study sought to characterize the effects of 3 injectable anesthetics on left ventricular (LV) systolic function and cardiac morphology in healthy male and female rats. Rats underwent echocardiographic assessment after general anesthesia via pentobarbital or combinations of ketamine and medetomidine (KME) and ketamine and midazolam (KMI) according to a crossover Latin-square design. Blood samples for serum estradiol measurements were obtained from all females after echocardiography with each anesthetic. Rats given KMI showed superior LV systolic function with the highest values for fractional shortening (FS), ejection fraction (EF) and stroke volume, whereas heart rate was greatest with pentobarbital, followed by KMI and then KME. KME produced the greatest effects on cardiac morphology, most notably during systole, including reduced septal and posterior wall thickness and increased LV chamber dimensions and volumes. In addition, KME had the greatest cardiac-depressing effects on LV systolic function, including reduced FS, EF, and heart rate values. Compared with male rats, female rats had superior LV function with greater EF and FS values, whereas male rats showed higher heart rate. Significant negative correlations were noted between serum estradiol levels and FS and EF values in female rats receiving KME. We conclude that the combination of KMI may be a superior anesthetic for use in male and female rats undergoing echocardiography.

Abbreviations: CO, cardiac output; d, diastole; EF, ejection fraction; FS, fractional shortening; HR, heart rate; IVS, interventricular septal thickness; KME, ketamine and medetomidine; KMI, ketamine and midazolam; KX, ketamine and xylazine; LV, left ventricular; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVPW, left ventricular posterior wall thickness; SV, stroke volume; s, systole.

The rat is a popular experimental animal to study physiologic and pathologic interventions on cardiac function; these studies often use echocardiography to assess phenotypic changes to these models. Historically, studies of this nature have seldom included female rats in addition to male rats.[15] However, sex-associated differences are important in heart disease in human subjects and in experimental animal models. As such, rat echocardiography studies involving only males lack comparisons to females and are subsequently limited with regard to human comparisons as well. Therefore, an increasing number of studies using rat models of cardiovascular disease are including both males and females in their design. A prudent approach to studying sex-related differences in cardiac responses to experimental interventions such as surgery and drug treatments is to first evaluate sex-associated differences in wildtype (normal) rats under control conditions of optimal health and to determine the effect of sex on echocardiographic assessment with different anesthetics. The current literature lacks such echocardiographic studies in rats.

The vast majority of investigators use general anesthesia when performing echocardiography in rodents to keep animals stationary and to reduce stress resulting from handling and restraint. In addition, general anesthesia is preferable to sedation because the same level of unconsciousness is easier to reproduce with anesthesia, and ultrasound image artifacts are minimized with no movement of the animal. However, performing echocardiography under general anesthesia does have disadvantages. General anesthetics can alter cardiovascular function through direct effects on the heart, indirectly by modulating the sympathetic and parasympathetic nervous systems or by affecting peripheral vasculature function and their contributions to cardiac afterload and preload.[29] Pentobarbital, isoflurane, and the combination of ketamine and xylazine (KX) have been among the most popular general anesthetics used when performing echocardiography in rats and mice. Although the depth of anesthesia is more easily controlled with inhalant than injectable anesthetics, an appropriate delivery system is required, and biosafety can be a concern. An ideal injectable anesthetic could avoid these issues as well as offer convenience for the investigator.

General anesthesia with pentobarbital can depress the respiratory center; however, it has been associated with less adverse effects on the rat cardiovascular system, compared with other anesthetics such as KX and isoflurane.[13,27] Ketamine is a poor muscle relaxant, and as a result, it is most often used in conjunction with α2-adrenergic agonists (such as xylazine) to enhance muscle relaxation and to provide sedation and analgesia. Medetomidine is a newer and more selective α2-adrenergic agonist that has largely replaced xylazine for routine use in

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veterinary anesthesia of cats and dogs, because of its perceived superior safety profile. To our knowledge, the combination of ketamine and medetomidine (KME) has not been evaluated for use in rat echocardiography. Alternatively, ketamine may be combined with a sedative–hypnotic agent, such as diazepam or midazolam, which provides sedation and muscle relaxation. Midazolam reportedly produces minimal effects on the cardiovascular and respiratory systems and, when given with ketamine, may offer an ideal anesthesia combination for rat echocardiography.[33] Echocardiographic studies in normal mice have shown that the combination of ketamine and midazolam (KMI) produces less cardiodepressant effects than does KX, as assessed through heart rate (HR) and fractional shortening (FS) values.[22,25] Rat echocardiographic studies using midazolam are absent from the literature. In the current study, we used in vivo echocardiography to evaluate the effects of 3 injectable anesthetic agents—pentobarbital and the novel combinations of KMI and KME—on echocardiographic assessment of cardiac morphology and left ventricular (LV) function in healthy wildtype rats. We also determined whether sex-dependent differences in echocardiographic assessment parameters are evident in wildtype male and female rats. We hypothesized that the combination of KMI would be least cardiodepressant, thereby best preserving LV function in both male and female rats when compared with pentobarbital and KME. We also hypothesized that sex would have a differential effect on echocardiographic assessments with the various anesthetic combinations. The results of the current study likely provide valuable information regarding novel injectable anesthetic combinations that may optimize rat echocardiography and reveal key sex-associated differences with echocardiographic assessments that will improve the design and interpretation of future rat cardiovascular studies.

Materials and Methods

Study design. Male (n = 15; weight, 275 to 300 g) and female (n = 15; weight, 200 to 225 g), age-matched (9 to 10 wk old), Sprague–Dawley rats were obtained from Charles River (Saint Constance, Canada) for this study. Rats were housed individually and maintained on a soy-free rat maintenance chow (2014 Teklad Global, Harlan, Indianapolis, IN) and tap water ad libitum on a 12:12-h light:dark cycle. Rats were acclimated to these conditions for at least 72 h before the study commenced. All protocols were approved by the University of Guelph Animal Care Committee and conducted within the guidelines of the Canadian Council on Animal Care.[3]

Rats were anesthetized by using 3 different regimens: pentobarbital (Ceva Sante Animale, Libourne, France), a combination of ketamine (Vetalar, Bioniche, Belleville, Ontario, Canada) and medetomidine (Dormitor, Pfizer, Kirkland, Quebec, Canada), and a combination of ketamine and midazolam (Sandoz Canada, Boucherville, Quebec, Canada). Male and female rats were randomly allocated to receive each of the 3 anesthetic regimens in a randomized crossover order by using a Latin square study design. A washout period of at least 7 d between anesthetic regimens was observed. The dosages of anesthetics used were based on current cited recommendations for producing general anesthesia in veterinary laboratory animals and previous echocardiographic studies using rodents: pentobarbital (40 mg/kg), ketamine (75.0 mg/kg) and midazolam (7.5 mg/kg), and ketamine (75.0 mg/kg) and medetomidine (0.5 mg/kg).[10,13,21,27,33] Prior to each anesthetic regimen, rats were weighed. All drugs were administered via intraperitoneal injection. After drug administration, induction of general anesthesia was determined in all rats by absence of a pedal withdrawal response following moderate hindlimb toe pinch with maintenance of spontaneous normal breathing;[20,24] time to induction of general anesthesia was recorded. The time required to complete an echocardiographic assessment after induction of general anesthesia was recorded for each animal and anesthetic regimen.

Echocardiography protocol. After general anesthesia was achieved, rats were placed in the supine position on heating pads and the thorax shaved. Core body temperature was monitored with a rectal probe before and after imaging (average initial temperature, 37.6 ± 0.40 °C; average final temperature, 37.1 ± 0.65 °C). Electrocardiograms were collected during all echocardiographic assessments. Prewarmed ultrasonography gel was applied to the thorax prior to application of the imaging probe. A single imager (CFS) used the Sequoia C512 ultrasonography system (Siemens Medical Solutions USA, Mountain View, CA) and the 15L8 linear-array transducer (14 MHz) to obtain 2D and M-mode images of the parasternal short axis of the heart at the midlevel of the papillary muscles. Sweep speed of M-mode recordings was 200 mm/s (Figure 1). Image quality was excellent in all rats. After echocardiography, 10 mL 0.9% NaCl was given subcutaneously to all rats to facilitate recovery from anesthesia. Atipamezole (0.5 mg/kg IP; Antisedan, Pfizer), an α2-adrenoreceptor antagonist, was administered to reverse the effects of medetomidine in rats receiving KME.[21]

Echocardiographic image analysis. Blinded observers (CFS and RJJ) evaluated the 2D guided M-mode images. M-mode measurements of LV end-diastolic and end-systolic dimensions (LVEDD and LVESD, respectively) and interventricular septal thickness (IVS) and LV posterior wall thickness (LVPW) in systole (s) and diastole (d), were performed by using the leading-edge method of the American Society of Echocardiography;[26] other echocardiographic parameters were calculated by using standard equations.[7,27,30] For estimation of each parameter, the average of at least 3 measurements from 3 different cycles in an image was obtained. LV mass (mg) was estimated as $0.80 \times (1.04 \times [(\text{LVEDD} + \text{LVPWd} + \text{IVSd})^3 - \text{LVEDD}^3]) + 0.6$; LV volume (μL) at end-diastole (LVEDV) was estimated as $(7.0/2.4 + \text{LVEDD}) \times \text{LVEDD}^3$ and at end-systole (LVESV) as $(7.0/2.4 + \text{LVESD}) \times \text{LVESD}^3$; change (%) in IVS thickness between systole and diastole was estimated by using (IVSs – IVSd/IVSd), and change (%) in LVPW thickness was calculated as (LVPWs – LVPWd/LVPWd). Functional parameters were estimated as follows: cardiac output (CO; mL/min) was determined by multiplying HR by stroke volume (SV); SV (μL) was estimated by using LVEDV – LVESV; EF (%) was determined by using (LVEDV – LVESV/LVEDV) × 100%; FS (%) was calculated as (LVEDD – LVESD/LVEDD) × 100%. Values for HR were determined by taking the average of 3 R–R intervals from the electrocardiogram of each image analyzed.

Observer agreement. Observer agreement with M-mode measurements was evaluated. Blinded observers (CFS and RJJ) each independently analyzed a set of 15 male and 15 female echocardiographic exams that were randomly selected to provide an estimation of interobserver agreement (SAS version 9.1.3, SAS Institute, Cary, NC). Intraobserver agreement[14] was tested by having the same observer (RJJ) repeat the echocardiographic image analysis of the randomly selected echocardiographic exams on different days. Lin's concordance correlation was calculated to measure interobserver agreement, including approximate 95% confidence intervals, based on Z-transformation.[16] Bland–Altman statistical methods for assessing agreement between 2 methods of clinical measurement

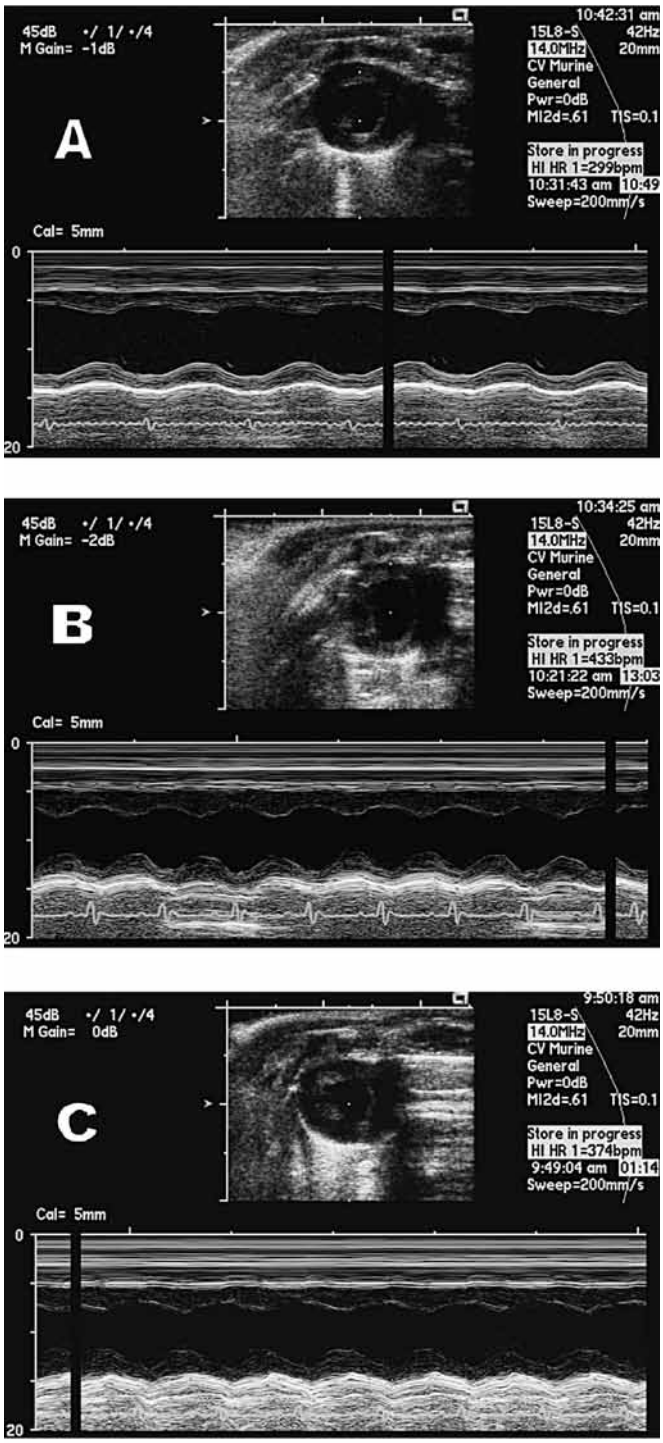

Figure 1. 2D and M-mode images of the parasternal short axis at the level of the papillary muscles from the same rat given (A) KMI, (B) pentobarbital, or (C) KME.

were used to evaluate agreement, including a Bland–Altman plot of pairwise differences of ratings compared with the average of the pair (SAS, SAS Institute). However, instead of plotting the typical mean difference ± 2 SD (the so-called 'limits of agreement'), we computed approximate 95% tolerance intervals that included 95% of population values.

Serum estradiol measurements. After completion of echocardiography for each anesthetic tested, an arterial blood sample (1.0 mL) was collected from the tail artery of each female rat, and serum was harvested immediately after clotting on ice and centrifugation (3000 × *g* for 10 min). All samples were immediately stored at −80 °C until assayed for estradiol levels by using a commercial competitive E_2 EIA Kit (catalog number 582251, Cayman Chemical, Ann Arbor, MI) according to manufacturer recommendations and previous methods.[35] The accuracy of the assay was within 15% of the true value for the range of reference estradiol standards used (6.25 to 400 pg/mL). The precision of the assay was calculated to be less than 10% coefficient of variation for all reference standards, with the 400 pg/mL estradiol standard having a precision of less than 15% coefficient of variation. We followed the manufacturer's instructions to determine the reference curve from a 4-parameter logistic fit of the $\%B/B_0$ values (ratio of the absorbance of the reference standard well to that of the maximally binding well) compared with the log reference standard concentrations. According to manufacturer recommendations, the limit of quantitation of each assay plate was set as the 80% B/B_0 value (linear portion of the sigmoidal fit). An overall limit of quantification of 19.52 pg/mL was determined from the average of 4 different assay plates with each reference standard run in triplicate.

Statistical analysis. All echocardiographic variables and intraobserver agreement were analyzed by using the Proc Mixed procedure in SAS (version 9.1.3; SAS Institute, Cary, NC). Bland–Altman plots were created by using SAS (SAS Institute). Anesthetic, sex, and anesthetic×sex interaction terms were included as fixed effects in the model. In addition, body weight and HR were included as covariates, because they have been shown to effect estimates of cardiac function and morphology.[1,28,32] Inclusion of HR and body weight in the model allowed evaluation of anesthetic- and sex-associated effects on echocardiographic parameters after adjusting for the effect of HR or body weight. To examine the ANOVA assumptions, residuals were evaluated for normality, presence of outliers, or evidence of unequal variances. Results are expressed as least-square means and associated 95% confidence intervals. Posthoc comparisons were performed by using a least significance difference test. Differences with *P* values less than 0.05 were considered significant. Statistical analysis revealed limited anesthetic×sex interactions. Therefore, main effects of anesthetic and sex are reported. In addition, to simplify the presentation of effects of the covariates HR and body weight on echocardiographic parameters, we restricted our reporting and discussions to significant effects between HR or body weight and anesthetic or sex. To examine the relationships between echocardiographic parameters and serum estradiol levels for each anesthetic, Spearman correlations were computed. In addition to separate correlations for each treatment, an overall correlation (ignoring anesthetic) was computed, and two-tailed *P* values were computed for each correlation. The assumptions of the correlations were addressed in the computed ANOVA via their residual analyses.

Results

Rats were weighed prior to each anesthetic regimen. Overall, body weights (mean and 95% confidence intervals) for male rats (0.32 kg, 0.30 to 0.34 kg) were significantly ($P < 0.0001$) greater than female body weights (0.24 kg, 0.21 to 0.26 kg), but no weight differences were noted between female rats among anesthetic groups or between male rats among anesthetic groups (data not shown). All rats breathed spontaneously with each of the anesthetic regimens.

Once general anesthesia was obtained, an echocardiographic assessment was completed within approximately 4 to 6 min, with no significant time differences between anesthetic regimens

or sexes (data not shown). Although, there were no significant sex-associated differences in the time to onset of anesthesia (data not shown), the median time (mean [95% confidence interval]) to attainment of general anesthesia was greatest in the pentobarbital group (6.4 [5.1 to 8.2] min) compared with the KMI group (3.4 [2.7 to 4.3] min; $P = 0.0002$) and the KME group (2.1 [1.6 to 2.8] min; $P < 0.0001$). In addition, the KMI group times were greater ($P = 0.0097$) than those of the KME group. Results of the intraobserver agreement values were excellent for most parameter estimates, and interobserver agreement was substantial to excellent across most parameter estimates (Table 1). 2D and M-mode images of the parasternal short axis of the heart at the midlevel of the papillary muscles from the same rat given each anesthetic regimen are shown in Figure 1. Image quality was excellent in most rats, as was acquisition of the electrocardiogram tracing.

Mortality. Five female rats that received KME died during the recovery period after their echocardiographic examinations. Subsequently, KME use was discontinued in the remaining female rats. The female rats that died appeared to be recovering appropriately after echocardiographic assessment, showing no abnormalities in breathing or heart rate or rhythm during the assessment, only to deteriorate and die 2 to 6 h after echocardiography. All of these female rats had received an injection of atipamezole to reverse the effects of medetomidine, as per protocol. In addition, one female rat that received pentobarbital was not anesthetized sufficiently for echocardiography and recovered to an awake standing condition but subsequently died 7 h later. Attempts to resuscitate all affected female rats were unsuccessful. A veterinarian board-certified in laboratory animal medicine performed postmortems on all female rats that died after administration of KME; significant respiratory depression was reported as the tentative cause of death in all cases.

In total, echocardiographic data from 15 male rats given all 3 anesthetic regimens, 12 female rats given KMI, 11 female rats given pentobarbital, and 8 female rats given KME were included in the results analysis. Incomplete female data sets for KMI and pentobarbital are the result of deaths after KME in these rats; these deaths occurred before the rats had received all 3 anesthetic regimens as per our Latin-square design.

LV morphology. Comparisons of echocardiographic estimates of morphologic parameters across anesthetics are shown in Table 2; results of comparisons of morphologic parameters between male and female rats are shown in Table 3 Estimates of IVSs were lowest in the KME group compared with the KMI group ($P = 0.0005$) and pentobarbital group ($P = 0.0004$), with no difference noted between the KMI and pentobarbital groups ($P = 0.9219$). There was a significant sex-associated difference for estimates of IVSs, with male rats having greater values than female rats ($P = 0.0441$). No significant overall differences were noted in IVSd values across anesthetics ($P = 0.8711$) or between sexes ($P = 0.5498$). Comparison of results of anesthetic effects on estimates of LVPWs showed the lowest values in rats given KME compared with the KMI group ($P < 0.0001$) and pentobarbital group ($P = 0.0004$), with no differences noted between the pentobarbital and KMI groups ($P = 0.2188$). No significant sex-associated differences were noted with estimates of LVPWs ($P = 0.8066$) or with estimates of LVPWd ($P = 0.3050$). Analysis of LVPWd data showed no significant anesthetic effects ($P = 0.8782$). IVS thickness in the KME group was significantly less than that in the KMI group ($P = 0.0006$) and pentobarbital group ($P = 0.0363$), but no differences were found between the pentobarbital and KMI groups ($P = 0.1199$). Similarly, LVPW thickness in the KMI group was greater than that of the KME group ($P < 0.0001$) and pentobarbital group ($P = 0.0131$), with no differences between the KME and pentobarbital groups ($P = 0.0693$). No sex-associated differences were noted for either IVS thickness in male rats (49.8% [45.5% to 54.1%] compared with female rats (55.3% [49.9% to 60.8%]; $P = 0.1183$) or LVPW thickness in male rats (63.1% [55.0% to 71.2%]) compared with female rats (70.0% [60.7% to 79.4%]; $P = 0.1382$; data not shown).

Table 1. Observer agreement associated with various echocardiographic parameters

	Intraobserver ρ (95% confidence interval)	Interobserver ρ (95% confidence interval)
IVSs	0.993 (0.987-0.997)	0.946 (0.901-0.971)
LVPWs	0.995 (0.989-0.998)	0.935 (0.87-0.868)
LVESD	0.998 (0.996-0.999)	0.989 (0.978-0.995)
IVSd	0.983 (0.965-0.992)	0.853 (0.738-0.920)
LVPWd	0.956 (0.908-0.980)	0.813 (0.639-0.908)
LVEDD	0.993 (0.985-0.997)	0.911 (0.825-0.956)
HR	0.999 (0.997-0.999)	0.998 (0.996-0.999)
FS	0.994 (0.988-0.997)	0.901 (0.798-0.953)
EF	0.994 (0.987-0.997)	0.862 (0.724-0.934)
SV	0.975 (0.949-0.988)	0.795 (0.615-0.896)

ρ indicates Lin's concordance coefficient of agreement (0, no agreement; 0.1–0.2, poor; 0.21–0.40, slight; 0.41–0.60, fair; 0.61–0.80, moderate; 0.81–0.90, substantial; 0.91–1.0, excellent).[14]

Estimates for LVEDD differed across anesthetics. Anesthesia with KME resulted in larger LVEDD values compared with pentobarbital ($P = 0.0072$), but not compared with KMI ($P = 0.4034$). LVEDD values in the KMI group were larger than the pentobarbital group ($P = 0.0063$). Male estimates of LVEDD were greater than female values, but these differences were not significant ($P = 0.6636$). Anesthesia with KME also resulted in the greatest LVESD values compared with pentobarbital ($P < 0.0001$) and KMI ($P < 0.0001$). No differences were detected between pentobarbital and KMI groups ($P = 0.6516$). Estimates of LVESD were greater in males compared with females ($P = 0.0039$).

LV volume and mass. Anesthetic and sex had significant effects on LV volume estimates (Tables 3 and 4). Anesthesia with pentobarbital produced the lowest LV volumes in diastole compared with those associated with KMI ($P = 0.0173$) or KME ($P = 0.0002$). Estimates of LVEDV were greater with KME compared with KMI ($P = 0.0168$). LVESV was greatest in the KME group compared with the pentobarbital group ($P < 0.0001$) and KMI group ($P < 0.0001$), with no differences between the KMI and pentobarbital groups ($P = 0.6316$). Overall, male values of LVEDV were greater than female LVEDV values, as were male values of LVESV compared with female values. Echocardiographic estimates of LV mass in the KME group were greater than those of the pentobarbital group ($P = 0.0444$) but not the KMI group ($P = 0.1621$); neither did LV mass differ between the KMI and pentobarbital groups ($P = 0.2475$). Overall estimates of LV mass were not different in males compared with females ($P = 0.8664$).

LV systolic function. Comparisons of estimates of the echocardiographic functional parameters SV, CO, EF, and FS across anesthetics are shown in Table 5 and across sexes in Table 3. Estimates of EF for the KMI group were greater than for the pentobarbital group ($P = 0.0247$) and KME group ($P < 0.0001$). Similarly EF values for the pentobarbital group were greater than for the KME group ($P < 0.0001$). There was a significant sex-associated difference in EF, with higher EF values in female rats compared with male rats ($P = 0.0244$). FS similarly showed

Table 2. Left ventricular morphologic parameters by anesthetic

	KMI (n = 27)	Pentobarbital (n = 26)	KME (n = 23)
IVSd (mm)	2.06 (1.95–2.17)	2.09 (1.97–2.20)	2.04 (1.90–2.18)
IVSs (mm)	3.19 (3.04–3.34)	3.20 (3.05–3.36)[a]	2.81 (2.64–2.99)[b]
LVPWd (mm)	1.64 (1.56–1.72)	1.64 (1.55–1.74)	1.61 (1.50–1.72)
LVPWs (mm)	2.91 (2.79–3.03)	2.81 (2.66–2.96)[a]	2.29 (2.05–2.54)[b]
IVS (% thickness)	60.0 (54.3–65.6)	53.6 (47.8–59.4)[a]	44.2 (37.5–50.8)[b]
LVPW (% thickness)	79.1 (69.7–88.5)[c]	66.5 (56.1–75.2)	54.9 (44.4–65.5)[b]
LVEDD (mm)	6.42 (6.14–6.70)[c]	5.93 (5.64–6.22)[a]	6.61 (6.20–7.02)
LVESD (mm)	3.31 (3.06–3.56)	3.38 (3.09–3.66)[a]	4.90 (4.46–5.33)[b]

Data are given as mean (95% confidence interval) of pooled male and female data for each anesthetic.
[a]Significant ($P < 0.05$) difference between pentobarbital and KME values.
[b]Significant ($P < 0.05$) difference between KMI and KME values.
[c]Significant ($P < 0.05$) difference between KMI and pentobarbital values.

Table 3. Comparison of echocardiographic measures by sex

	Female (n = 31)	Male (n = 45)
LV morphology		
IVSd (mm)	2.10 (1.93–2.28)	2.02 (1.90–2.14)
IVSs (mm)	2.95 (2.78–3.12)[a]	3.19 (3.03–3.34)
LVPWd (mm)	1.68 (1.56–1.81)	1.58 (1.50–1.67)
LVPWs (mm)	2.66 (2.51–2.81)	2.68 (2.54–2.82)
LVEDD (mm)	6.25 (5.81-6.69)	6.39 (6.04-6.74)
LVESD (mm)	3.54 (3.22–3.87)[a]	4.18 (3.88–4.48)
LV volumes and mass		
LVEDV (μL)	191.2 (168.1–214.3)[a]	221.8 201.4–242.1)
LVESV (μL)	59.5 (46.3–72.7)[a]	82.8 (70.5–95.0)
LV mass (mg)	631.1 (551.7–710.4)	640.5 (575.7–705.2)
LV systolic functional parameters		
CO (mL/min)	66.8 (45.7–88.0)	60.0 (50.2–69.8)
SV (μL)	137.2 (118.0–156.4)	142.3 (125.0–159.6)
EF (%)	71.7 (67.5–76.0)[a]	65.2 (61.5–68.9)
FS (%)	43.2 (39.2–47.1)[a]	36.1 (32.6–39.5)
HR (bpm)	347.2 (331.8–362.7)[a]	371.3 (359.0–383.5)

Data are given as mean (95% confidence interval) of pooled anesthetic data for each sex.
[a]Significant ($P < 0.05$) difference between female and male rats.

a sex-associated effect, again with higher values in female rats compared with male rats ($P = 0.0030$). Estimates of FS were lowest in the KME group compared with the pentobarbital group ($P = 0.0021$) and KMI group ($P < 0.0001$), with FS values in the KMI group also being greater than values in the pentobarbital group ($P = 0.0185$). Rats anesthetized with pentobarbital had significantly lower SV values compared with those given KMI ($P = 0.0014$) but not KME ($P = 0.1133$); SV did not differ significantly between the KMI and KME groups ($P = 0.1550$). There was no sex-associated difference noted in SV values ($P = 0.5450$). No differences in CO values between anesthetic groups were noted ($P = 0.1288$) or between sexes ($P = 0.4479$). HR values for animals anesthetized with pentobarbital were highest compared with KMI values ($P < 0.0001$) and KME values ($P < 0.0001$). HR

Table 4. Left ventricular volumes and mass by anesthetic

	KMI (n = 27)	pentobarbital (n = 26)	KME (n = 23)
LVEDV (μL)	202.1 (182.9–221.2)[a]	171.6 (150.6–192.6)[b]	245.8 (213.7–277.9)[c]
LVESV (μL)	48.5 (38.4–58.6)	51.2 (40.0–62.4)[b]	113.7 (96.6–130.8)[c]
LV mass (mg)	623.3 (576.5–670.2)	587.8 (534.3–641.3)[b]	696.1 (599.5–792.8)

Data are given as mean (95% confidence interval) of pooled male and female data for each anesthetic.
[a]Significant ($P < 0.05$) difference between KMI and pentobarbital values.
[b]Significant ($P < 0.05$) difference between pentobarbital and KME values.
[c]Significant ($P < 0.05$) difference between KMI and KME values.

Table 5. Left ventricular systolic functional parameters by anesthetic

	KMI (n = 27)	pentobarbital (n = 26)	KME (n = 23)
CO (mL/min)	67.5 (53.4–81.6)	64.5 (50.4–78.5)	58.3 (42.2–74.3)
SV (μL)	155.2 (135.9–174.5)[a]	123.7 (104.1–143.2)	140.3 (119.1–161.5)
EF (%)	75.9 (72.1–79.7)[a]	70.5 (66.7–74.4)[b]	59.0 (54.6–63.4)[c]
FS (%)	47.4 (44.4–50.5)[a]	41.8 (37.9–45.8)[b]	29.6 (23.0–36.2)[c]
HR (bpm)	350.2 (334.3–366.1)[a]	417.8 (401.4–434.1)[b]	309.7 (290.9–328.6)[c]

Data are given as mean (95% confidence interval) of pooled male and female data for each anesthetic.
[a]Significant ($P < 0.05$) difference between KMI and pentobarbital values.
[b]Significant ($P < 0.05$) difference between pentobarbital and KME values.
[c]Significant ($P < 0.05$) difference between KMI and KME values.

values ($P = 0.0017$) for the KMI group were greater than those for the KME group. Overall HR values in male rats were greater than female rats ($P = 0.0176$).

Serum estradiol levels. Serum estradiol levels measured ranged from 25.4 to 171.1 pg/mL, with no samples below the limit of quantification of the assay. Few significant overall correlations between estradiol concentrations and echocardiographic parameters were noted. Overall HR showed significant ($P = 0.007$) negative correlation with estradiol concentrations ($r_S = -0.49836$, $n = 28$), whereas significant ($P = 0.0312$) positive correlation was noted for LV mass estimates compared with estradiol concentrations ($r_S = 0.40777$, $n = 28$; Figure 2). Significant correlations between estradiol concentrations and echocardiographic parameters for individual anesthetics were noted primarily with the KME group (Figure 3). Significant negative correlations occurred between estradiol concentrations and FS ($r_S = -0.82857$, $P = 0.0416$, $n = 6$), EF ($r_S = -0.94286$, $P = 0.0048$, $n = 6$), and LVPWs ($r_S = -0.88571$, $P = 0.0188$, $n = 6$) when female rats were anesthetized with KME. Significant positive correlations between estradiol concentrations and estimates of LVESV ($r_S = 0.94286$, $P = 0.0048$, $n = 6$) and LVESD ($r_S = 0.94286$, $P = 0.0048$, $n = 6$) were present in female rats that received KME (data not shown). Significant ($P = 0.0092$) negative correlation was noted between estradiol concentrations and estimates of SV in female rats given pentobarbital ($r_S = -0.76970$, $n = 10$; Figure 3).

Discussion

The current study characterized the effects of novel anesthetic combinations on the echocardiographic assessment of healthy male and female Sprague–Dawley rats and evaluated the relationship between endogenous estradiol levels and echocardiographic parameters in female rats across anesthetics. To our knowledge this study is the first to identify key differences in LV systolic function and cardiac morphology after use of the novel anesthetic combinations KMI and KME in healthy male and female rats.

Our study revealed a profound effect of anesthetic regimen on echocardiographic assessment in healthy rats. Our results showed that HR values were lowest in the KME group and highest in the pentobarbital group. Ketamine administration alone typically induces sympathoexcitation and cardiovascular activation by increasing systemic vascular resistance, blood pressure, and HR.[20,24] Marked bradycardia after the use of selective α2 adrenergic agonists including xylazine and medetomidine is well documented and can override the cardiostimulatory effects of ketamine.[6,19,20,27] Medetomidine is an molar-equal mixture of 2 optical isomers, levomedetomidine (considered to be pharmacologically inactive)[18] and dexmedetomidine. The activation of peripheral postsynaptic vascular α2 receptors by medetomidine causes vasoconstriction leading to reflex increased cardiac parasympathetic tone, whereas activation of central and peripheral prejunctional α2 receptors causes decreases in sympathetic outflow to the heart, with both effects reducing HR.[6,19] Rats that received KMI in the current study had HR values that were intermediate to those recorded for rats given pentobarbital or KME. When ketamine was combined with midazolam in mice[22,25] or diazepam in rats,[33] effects on cardiac function and HR were much less than the cardiodepression and HR reduction due to KX. In the current study, rats anesthetized with pentobarbital maintained HR values closest to cited mean HR values of 402 ± 9 bpm[4] and 421 ± 26 bpm[27] in conscious restrained male Wistar rats and male F344 rats, respectively. However, elevation of HR secondary to handling stress is likely in those previous studies, in light of the HR values of 338 ± 18 bpm in conscious unrestrained male Wistar rats implanted with radiotelemetric devices.[12] Therefore, according to the HR values obtained in conscious unrestrained rats, KMI appears to maintain physiologic HR.

Cardiac function in rodents is affected by HR.[13,34] Although rats that received pentobarbital in our current study yielded the highest HR values, they also produced the lowest SV, LVEDV, and LVEDD, which support a reduced diastolic filling time for the ventricles. In contrast, rats that received KME showed the lowest HR and greatest LVEDD and LVEDV values compared with those of rats given pentobarbital and KMI but higher SV values than the pentobarbital group. Therefore, the decreased HR in the KME group may have facilitated chamber filling and maintained SV in the face of reduced LV systolic function in this

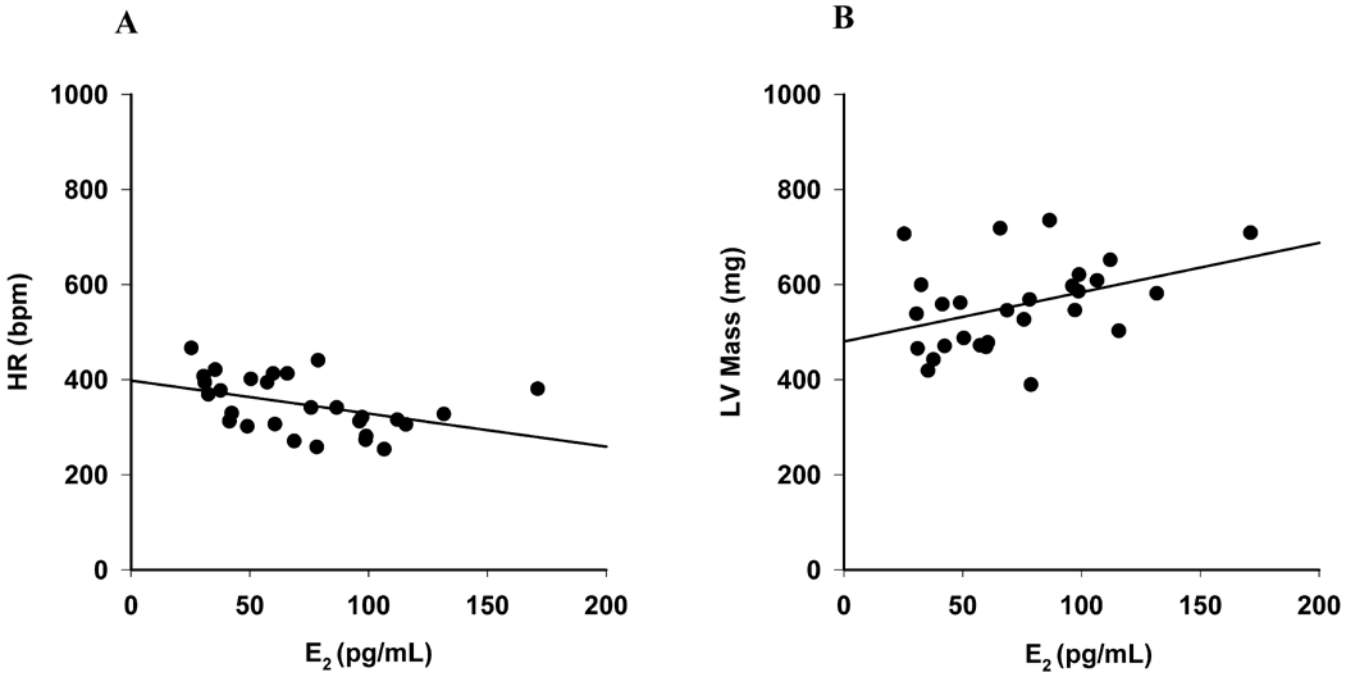

Figure 2. Serum estradiol concentrations compared with (A) HR and (B) LV mass in female rats (n = 28).

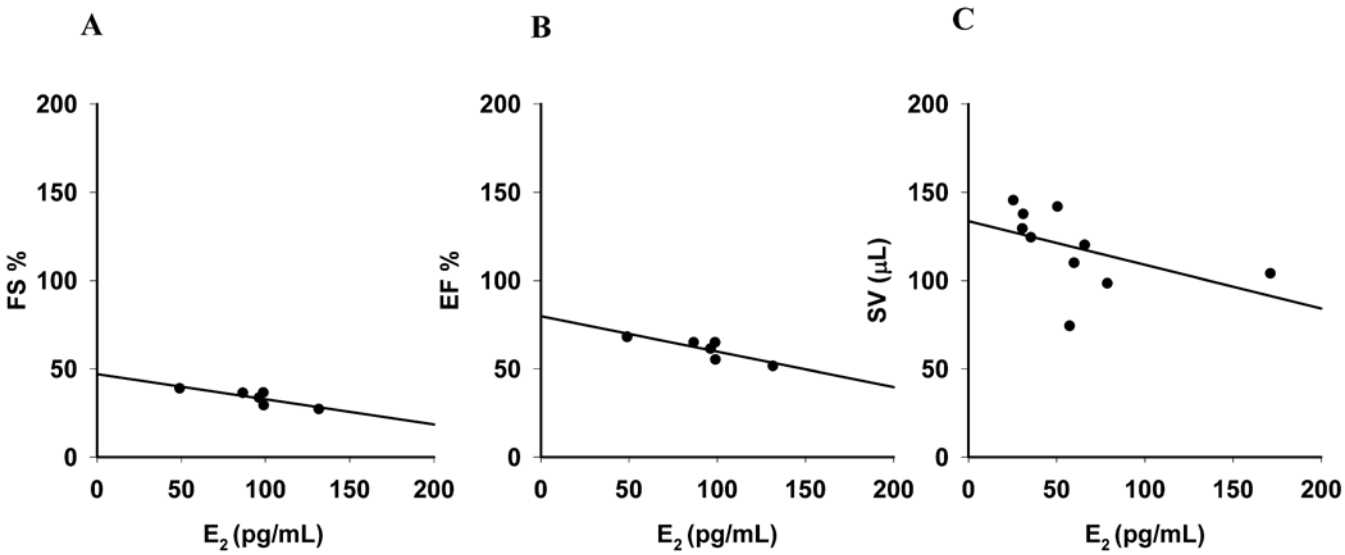

Figure 3. Serum estradiol concentrations compared with (A) FS% (n = 6) and (B) EF% (n = 6) in female rats receiving ketamine–medetomidine and compared with (C) SV in female rats receiving pentobarbital (n = 10).

group. The effects of pentobarbital on the cardiovascular system in rats have varied between studies. Comparisons with other rat studies using pentobarbital at an equivalent dose to that in our study show reductions in mean arterial blood pressure.[2,33] As such, perhaps HR increases in rats that receive pentobarbital, to compensate for reductions in peripheral vascular resistance and blood pressure, or loss of venomotor tone and reduced preload and SV, or both. However, other rat studies similarly using pentobarbital at 40 mg/kg have reported minimal effects on HR, CO, and mean arterial blood pressure. In addition, general anesthesia with pentobarbital may directly reduce myocardial contractility.[17]

In the current study, rats given KMI showed superior LV systolic function, with the greatest FS and EF values. Rats that received KME had significantly lower EF and FS values and lower CO values than did the other anesthetic groups. Reduced systolic function in the KME group is supported further by the findings of greater LVESD and LVESV values and reduced IVSs and LVPWs dimensions compared with those of the KMI and pentobarbital groups. Although our results are in line with other studies showing greater FS and EF values when rats receive pentobarbital compared with KX, our values for FS and EF in rats given KME are lower.[13,27] These studies were conducted in different strains of rats, using xylazine instead of medetomidine, and at a differed dosing rate from that in our study. CO values were not significantly different between groups. This result is not surprising given that CO is a derived echocardiographic parameter, and we obtained marked differences in HR and SV values with the different anesthetics. Estimation of CO in the current study showed the greatest variability, when compared

with other parameters. Echocardiographic determination of CO generally is considered to be challenging compared with the estimation of other echocardiographic parameters. Estimates of CO are calculated from other echocardiographic parameters, each with its own inherent variability, and therefore may incur more total variability.[31] In comparison, calculation of EF, representing both volumes of the cardiac cycle, is more reliable and easier and therefore may be more recommended as an index of LV systolic function.[31]

In the present study, the inclusion of HR and body weight in the statistical model allowed evaluation of the anesthetic effects on outcome measures of LV systolic function and cardiac morphology after adjusting for the effects of HR and body weight. Body weight had significant effects on anesthetic-associated differences in LVEDD and LV mass but not on LV systolic functional parameters. HR had significant effects on anesthetic-related differences in FS and several cardiac morphologic parameters, namely LVESV, LVEDV, LVPWs, LVESD, and LV mass. We also found a significant ($P = 0.0038$) positive relationship between FS and HR in the KMI group only. This finding supports a force–frequency relationship between HR and myocardial contractility that is best observed over physiologic ranges of heart rates, as were found in the KMI group. A similar finding has been described previously in mice given pentobarbital, KX, or halothane[4,23,34] but not in rats given pentobarbital, KX, or isoflurane anesthetic.[27]

Overall comparisons of sex-associated differences with echocardiographic assessment of LV systolic function and cardiac morphology yielded significant findings that warrant discussion. Regarding comparisons of LV systolic function, we found that overall estimates of EF and FS were greater in female rats compared with male rats, whereas male rats showed overall greater HR values. Echocardiographic estimations of SV and CO were not significantly different between sexes. Inclusion of HR and body weight in the statistical model again allowed for evaluation of sex effects on outcome measures of LV systolic function and morphology after adjusting for the effect of HR and body weight. We found that only body weight produced significant effects on sex-associated differences in LV systolic function, and these were limited to estimates of CO in the present study. Because HR was included in the derivation formula for CO and therefore expected to affect this parameter, we did not include HR as a covariate in the statistical model for analysis of CO.

Review of the literature shows no comparable studies in healthy wildtype male and female rats of similar age to the animals in the present study. This finding is somewhat surprising given that 8- to 10-wk-old rats appear to be group most often used for cardiovascular study. However, our results are in agreement with those obtained from 24- to 25-mo-old male and female Fischer-344 rats, in which FS values were greater in females than males, HR tended to be higher in males, and weight-adjusted CO values tended to be higher in females.[8] Similar findings of increased FS in control female Wistar rats compared with control males of approximately 9 wk of age were noted in a study evaluating hypertrophic remodeling after LV pressure overload subsequent to aortic banding.[5] Echocardiographic studies in humans have shown that young females have greater diastolic function than do age-matched males; that, with age, females maintain systolic function compared with males;[9] and that females are better able to preserve LV systolic function in the face of diastolic dysfunction and heart failure.[1,11] Taken together, these previous findings and those in the current study suggest that female rats and humans may have greater intrinsic cardiac function than do males.

Inclusion of body weight—but not HR—in the statistical model affected the estimation of LV mass in the present study, such that LV mass values in male rats were no longer significantly greater than those from than female rats. Most studies have reported sex-dependent effects on echocardiographic estimates of rat cardiac morphology including LV mass when absolute values were compared between male and female rats.[8,32] Body weight, as a covariate, also affected sex-related differences of other cardiac morphologic parameters in the present study, specifically IVSd, LVPWd, and LVEDD. Similar findings have been noted in other rat studies, with male estimates of cardiac morphology in diastole (IVSd, LVPWd, LVEDD) generally being greater than female values, but when adjusted for body weight, the trend switched to greater female values.[5,8] In the present study, we found that estimates of LVESD, LVESV, and LVEDV were greater in male rats compared with female rats and that body weight did not affect these outcome measures. These morphologic differences appear to support our other results suggesting reduced intrinsic myocardial function in male compared with female rats. However, not all findings were supportive, in that IVSs was greater in male than female rats. In light of findings from our current study, we conclude that comparisons of morphologic parameters between sexes should include consideration of body weight as a covariate, whereas intersex comparisons of most LV systolic functional parameters are affected negligibly by body weight.

Sex is a variable that must be considered in basic science and clinical research.[15] Most studies have evaluated the effects of estrogens on cardiovascular function and morphology by comparing results obtained from intact females to those obtained from ovariectomized females with and without estrogen supplementation. Estrogen supplementation usually is delivered continuously at pharmacologic doses by surgical implants, a regimen that differs substantially from conditions in intact cycling females. Given the potential for interactive effects between anesthetics and endogenous estradiol on cardiac function and morphology, it is important to understand the effects of these variables on echocardiographic assessments in animal models using intact females. We are unaware of any studies that have examined the relationship between endogenous serum estradiol concentrations and echocardiographic parameters in healthy intact female rats. In our current study, we found significant negative correlations with both FS and EF values and endogenous estradiol levels in female rats that received KME. These findings suggest that increases in serum estradiol levels were associated with reduced LV systolic functional parameters in the presence of KME but not other anesthetics. Given that these effects were not noted in female rats that received KMI, medetomidine likely is important in regard to the relationship between serum estradiol concentrations and FS and EF in the KME group.

Presynaptic α2-adrenergic inhibition of myocardial norepinephrine release by the selective α2-adrenergic agonist clonidine is greater in female compared with male Sprague–Dawley rats; ovariectomy attenuated this finding.[6] These same investigators showed that antagonism of presynaptic α2-adrenergic receptors by rauwolscine enhanced norepinephrine overflow more in hearts from female compared with male rats and that this enhanced release of norepinephrine was associated with increased chronotropic and inotropic effects.[5] The authors concluded that estradiol may increase presynaptic α2-adrenergic receptor density and activity in the heart. Our findings showing

a significant negative correlation between endogenous estradiol levels and LV systolic function in females that received medetomidine, a selective α2-adrenergic agonist, strongly supports these previous findings.[6] Also in our current study, we found significant negative correlation between overall HR values and estradiol levels in female rats, thus supporting our findings of overall higher heart rates in male compared with female rats. These findings in healthy intact female rats highlight the need to consider the effects of endogenous estradiol levels when echocardiography is used in rat cardiovascular models to assess cardiac function and morphology. The use of selective α2-adrenergic agonists such as medetomidine and the more commonly used agent xylazine can affect LV systolic function that appears to be modulated by endogenous estradiol levels in intact female rats and thus warrant additional caution with interpreting echocardiographic findings.

Due to the design and length of our study, which was comprised of multiple general anesthetics and recoveries requiring approximately 3 wk to complete for each rat, it was not feasible to instrument and reliably maintain these animals for direct blood pressure determination. However, we recognize that direct blood pressure measures would have provided additional valuable information and that this lack is a limitation of the current study.

In summary, echocardiography offers an excellent noninvasive research tool to assess cardiac function and morphology in rats. The use of echocardiography in rat cardiovascular research requires consideration of anesthetic selection and sex-dependent differences across anesthetics. Evaluation of novel anesthetic combinations that can offer superior reproducible quality of anesthesia and enhanced safety profiles for rodent cardiovascular research is crucial. The increasing trends in research to include females in studies using rat models of cardiovascular disease also underscores the need to understand sex-associated differences in echocardiographic assessment of healthy wildtype rats with different anesthetics so that rat models of cardiovascular disease can be effectively evaluated by using research tools such as echocardiography. Our results show that the combination of KMI produced minimal cardiodepressant effects in rats, as evidenced by superior LV systolic function compared with that after use of the other anesthetics. Anesthesia with KME produced the greatest cardiodepressant effects in both sexes and was associated with significant negative correlation between endogenous estradiol levels and LV systolic function in female rats, as well as some mortality. Overall, female rats showed superior LV systolic function compared with that of male rats, whereas males maintained higher heart rates. Clearly anesthetics can have adverse effects on cardiac function. As shown in the current study, KMI holds promise as a superior injectable anesthetic for routine echocardiographic assessment in rats, whereas the use of KME warrants further study and more extensive dosage optimization between sexes.

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