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A Surgical Model of Heart Failure with Preserved Ejection Fraction in Tibetan Minipigs

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TITLE:

A Surgical Model of Heart Failure with Preserved Ejection Fraction in Tibetan Minipigs

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KEYWORDS:

Heart failure, preserved ejection fraction, descending aortic constriction, surgical model, minipig

SUMMARY:

The present protocol describes a step-by-step procedure to establish a minipig model of heart failure with preserved ejection fraction using descending aortic constriction. The methods for evaluating cardiac morphology, histology, and function of this disease model are also presented.

ABSTRACT:

More than half of heart failure (HF) cases are classified as heart failure with preserved ejection fraction (HFpEF) worldwide. Large animal models are limited for investigating the fundamental mechanisms of HFpEF and identifying potential therapeutic targets. This work provides a detailed description of the surgical procedure of descending aortic constriction (DAC) in Tibetan minipigs to establish a large animal model of HFpEF. This model used a precisely controlled constriction of

the descending aorta to induce chronic pressure overload in the left ventricle. Echocardiography was used to evaluate the morphological and functional changes in the heart. After 12 weeks of DAC stress, the ventricular septum was hypertrophic, but the thickness of the posterior wall was significantly reduced, accompanied by dilation of the left ventricle. However, the LV ejection fraction of the model hearts was maintained at >50% during the 12-week period. Furthermore, the DAC model displayed cardiac damage, including fibrosis, inflammation, and cardiomyocyte hypertrophy. Heart failure marker levels were significantly elevated in the DAC group. This DAC-induced HFpEF in minipigs is a powerful tool for investigating molecular mechanisms of this disease and for preclinical testing.

INTRODUCTION:

Heart failure with preserved ejection fraction (HFpEF) accounts for more than half of heart failure cases and has become a worldwide public health issue[1]. Clinical observations have indicated several critical features of HFpEF: (1) ventricular diastolic dysfunction, accompanied by increased systolic stiffness, (2) normal ejection fraction at rest with impaired exercise performance, and (3) cardiac remodeling[2]. The proposed mechanisms include hormonal dysregulation, systemic microvascular inflammation, metabolic disorders, and abnormalities in sarcomeric and extracellular matrix proteins[3]. However, experimental studies have shown that heart failure with reduced ejection fraction (HFrEF) causes these alterations. Clinical studies have explored the therapeutic effects of angiotensin receptor inhibitors and drugs for treating HFrEF in HFpEF[4,5]. However, unique therapeutic approaches for HFpEF are needed. Compared with understanding the clinical symptoms, the alterations in pathology, biochemistry, and molecular biology of HFpEF remain poorly defined.

Animal models of HFpEF have been developed to explore the mechanisms, diagnostic markers, and therapeutic approaches. Laboratory animals, including pigs, dogs, rats, and mice, can develop HFpEF, and diverse risk factors, including hypertension, diabetes mellitus, and aging, were selected as induction factors[6,7]. For example, deoxycorticosterone acetate alone or combined with a high fat/sugar diet induces HFpEF in pigs[8,9]. Ventricular pressure overload is another technique used to develop HFpEF in large and small animal models[10]. In addition, specific EF cut-off values to define HFpEF have been adopted across continents in recent years, as seen in the European Society of Cardiology guidelines, the American College of Cardiology Foundation/American Heart Association[11], the Japanese Circulation Society, and the Japanese Heart Failure Society[12]. Thus, many previously established models may become appropriate for HFpEF studies if the clinical criteria are adopted. For example, Youselfi et al. claimed that a genetically modified mouse strain, *Col4α3-/-*, was an effective HFpEF model. This strain developed typical HFpEF cardiac symptoms, such as diastolic dysfunction, mitochondrial dysfunction, and cardiac remodeling[13]. A previous study used a high-energy diet to induce cardiac remodeling with a mid-range of EF in aged monkeys[14], characterized by a metabolic disorder, fibrosis, and reduced actomyosin MgATPase in the myocardium. Mouse transverse aortic constriction (TAC) is one of the most widely used models to mimic hypertension-induced ventricular cardiomyopathy. The left ventricle progresses from concentric hypertrophy with increased EF to dilated remodeling with reduced EF[15,16]. The transitional phenotypes between these two typical stages suggest that the aortic constriction technique can be used to study

HFpEF.

The pathological features, cellular signaling, and mRNA profiles of a porcine HFpEF model were previously published[17]. Here, a step-by-step protocol is presented to establish this model and the approaches to evaluate the phenotypes of this model. The procedure is illustrated in **Figure 1**. Briefly, the surgical plan was made jointly by the principal investigator, surgeons, laboratory technicians, and animal care staff. The minipigs underwent health examinations, including biochemical tests and echocardiography. Following surgery, anti-inflammatory and analgesic procedures were performed. Echocardiography, histological examination, and biomarkers were used to evaluate the phenotypes.

PROTOCOL:

All animal studies were approved by the Institutional Animal Care and Use Committee of the Guangdong Laboratory Animals Monitoring Institute (approval no. IACUC2017009). All animal experiments were performed following the Guide for the Care and Use of Laboratory Animals (8th Ed., 2011, The National Academies, USA). The animals were housed in an AAALAC-accredited facility at the Guangdong Laboratory Animals Monitoring Institute (license no. SYXK (YUE) 2016-0122, China). Six male Tibetan minipigs (n = 3 each for the sham group and DAC group, 25–30 kg in weight) were used to develop the HFpEF model.

1. Animal and instrument preparation

1.1. Acclimate the animals to the facility for 14 days before surgery.

1.2. Perform health examinations, including biochemical tests and echocardiography, before the surgery. Exclude the animals with cardiac abnormalities in structure (ventricular dilation or hypertrophy) and function (EF <50%) according to the T/CALAS85-2020 Laboratory animals – Guidelines for the health assessment of major organs, such as the heart, liver, kidney, and brain of large laboratory animals (Chinese Association for Laboratory Animal Sciences, China).

1.3. Fast the animals for more than 12 h before anesthesia by not feeding on the day of the surgery.

1.4. Prepare the surgical room and devices (**Figure 2**). Check aesthesia ventilator station, veterinary and monitors, veterinary ultrasound system, aspirator, and other surgical devices. Autoclave the scissors, forceps, retractors, scalpel handles, aspirator head, surgical needles, etc. (see **Table of Materials**).

2. Sedation, tracheal intubation, and vein cannulation

2.1. Weigh the animals and calculate the anesthetic drugs. Sedate the minipigs with 1 mg/kg of zoletil injection (tiletamine and zolazepam for injection) and 0.5 mg/kg of xylazine hydrochloride injection (see **Table of Materials**).

2.2. Restrain and place the minipigs in the right lateral recumbent position on the operating surgery table. Turn on the heating system to maintain the body temperature of the animals.

2.3. Perform the echocardiography (step 5) and collect 2 mL of blood samples.

2.4. Intubate the minipigs with an endotracheal tube connected to a veterinary anesthesia ventilator station (**Figure 3A**) (see **Table of Materials**).

2.5. Initiate the ventilation at an 8 mL/kg of tidal volume and 30 breaths/min. Maintain the animals with 1.5%–2.5% of isoflurane during the surgical procedure.

2.6. Establish intravenous cannulation using a peripheral intravenous catheter (26 G) (see **Table of Materials**) from an ear vein (usually the marginal ear vein, **Figure 3B**).

2.7. Connect the animal to a veterinary monitor.

3. Surgical procedure

3.1. Shave the left thoracic area. Apply 0.7% of iodine and 75% alcohol to aseptically prepare the skin from the scapula to the diaphragm (**Figure 3C**).

3.2. Place sterile drapes over the surgical area.

3.3. Administer propofol (5 mg/kg) (see **Table of Materials**) by intravenous injection to maintain general anesthesia.

3.4. Mark the incision (~15 cm long) along the 4th intercostal space prior to skin incision with electrocautery.

3.5. Open the chest using a combination of cautery and blunt dissection of the muscle and connective tissue. Use an aspirator to remove blood during the operation.

3.6. Use a rib retractor to spread the ribs (**Figure 3D**).

3.7. Locate the thoracic descending aorta segment and determine the constriction site (**Figure 3E**). Use two 3-0 surgical sutures to loop around the segment twice (**Figure 3F**). Place three layers of medical gauze between the suture and the aorta to avoid tissue damage by sutures.

3.8. Set up pressure measurement units to determine the degree of constriction (**Figure 3F-H**).

NOTE: The unit includes a catheter that punctures the vessel wall, connection tube, pressure transducer, and a patient monitor.

3.9. Tighten the surgical suture surrounding the descending aorta segment gradually to achieve

the desired constriction degree. Allow the pressure readings to stabilize for 20 min and permanently tighten the surgical knots.

3.10. Use a drainage chest tube to evacuate the air and excess fluids in the chest cavity.

3.11. Close the chest wall in layers, reapproximate the ribs, and divide muscles with absorbable sutures.

3.12. Check for any bleeding and ensure good hemostasis.

3.13. Apply a bottle of benzylpenicillin (800,000 units) (see **Table of Materials**) to the operation area post-surgery.

3.14. Monitor the presence of eye blinking and limb movement of the animal. Disconnect the ventilator but leave the endotracheal tube. Monitor the presence of spontaneous breathing.

3.15. Return the animal to its housing room and leave it to wake up automatically.

4. Post-surgery care

4.1. Apply benzylpenicillin daily for 1 week (20,000 U/kg).

4.2. Apply 1 mg/kg of flunixin meglumine (see **Table of Materials**) daily for 1 week.

5. Transthoracic echocardiography

5.1. Sedate the animal with 1 mg/kg of zoletil.

5.2. Place the animal in a mobile restraint unit with a canvas cover.

NOTE: The mobile restraint unit (see **Table of Materials**) has four apertures designed to extend the forelimbs and hind limbs of the animal.

5.3. Shave the left chest of the animal.

5.4. Place fingers on the left-center of the chest to feel the apical pulse. Apply the ultrasonic gel to the surrounding area.

5.5. Place the ultrasound system's phased array transducer (3-8 Hz) in the third intercostal space. Move the transducer toward an anterior or posterior direction and adjust the notch angle.

5.6. Identify the atria, ventricles, and aorta. Record the B-mode and M-mode parasternal long-axis images.

NOTE: The B-mode image represents the cross-section of the left ventricle at the papillary muscle level, and the M-mode image shows the movement of the left ventricle over time.

5.7. Turn the transducer head 90° clockwise to obtain the parasternal short-axis view. Identify the left ventricle, right ventricle, and papillary muscle. Record the B-mode and M-mode images.

5.8. Use the workstation provided by the manufacturer of the ultrasound system to assess the cardiac structure and function.

REPRESENTATIVE RESULTS:

Echocardiography

Cardiac structure and function were evaluated at weeks 0, 2, 4, 6, 8, 10, and 12. The B-mode and M-mode recordings of the parasternal short-axis view are displayed in **Figure 4A**. The echocardiographic measurement included the ventricular septum thickness (VST), posterior wall thickness (PWT), and left ventricular internal dimension (LVID). The VST at end-diastole increased in the DAC hearts, whereas the PWT at end-diastole increased and then decreased during the observation period, suggesting that hypertrophic remodeling was present in the left ventricle of the DAC minipigs **(Figure 4B,C)**. The LVID at end-diastole decreased in weeks 4 and 6 and then gradually increased after week 8, suggesting that the ventricles underwent concentric hypertrophy before dilation (**Figure 4D**). The LVEF of the model hearts was maintained at >50% during the 12-week period **(Figure 4E)**.

Morphology and heart failure marker

After week 12, the hearts were harvested as previously described[17]. Compared with those of the sham hearts, enlargement of the DAC hearts was observed **(Figure 5A).** The serum concentration of cardiac troponin I (cTnI) was determined using an enzyme-linked immunosorbent assay kit at weeks 0, 4, 8, and 12 following the manufacturer's instructions (see **Table of Materials**). The optical density was measured at 450 nm using a microplate reader. The heart failure marker cTnI was significantly higher at weeks 4, 8, and 12 in the DAC group than in the sham group at the corresponding time points **(Figure 5B)**.

Histological examination

Tissues from the free walls of the left and right ventricles, ventricular septum, left and right atrium, mitral valve, and aorta were collected and fixed with 4% paraformaldehyde. The tissues were embedded, sliced into sections, and stained with hematoxylin and eosin (H & E) solution following the previous report[17]. Hypertrophic cardiomyocytes, fibrosis, inflammatory cells, pyknotic nuclei, and other structures were identified with a light microscope. Cardiomyocytes in the atria, ventricular septum, and ventricles displayed hypertrophy with pyknosis (**Figure 6A**). Muscular layers were reduced in the mitral valve (**Figure 6B**), and vascular endothelial hyperplasia was observed in the aorta (**Figure 6C**). Moreover, DAC induced extensive fibrosis in the myocardium of the minipigs (**Figure 7A**), accompanied by infiltration of inflammatory cells in the left ventricles, right atrium, and aortic walls (**Figure 7B**).

FIGURE LEGENDS:

Figure 1: Experimental design. The experimental plan was made collaboratively by the principal investigator, surgeons, laboratory technicians, and animal care staff. The minipigs underwent health examinations, including biochemical tests and echocardiography. Following the surgery, anti-inflammatory and analgesic procedures were performed. Echocardiography, histological examination, and biomarker test evaluated heart failure phenotypes. The number of animals, n = 3 each, was for the sham and DAC groups.

Figure 2. Surgical devices. The necessary devices (**A**) for the DAC surgery included aspirator (a), surgical table (b), Veterinary monitor (c), LED surgical lights (d), and aesthesia ventilator station (e). A veterinary ultrasound system was used to evaluate the structure and function of the animal hearts before and after surgery (**B**). The surgical tools included a laryngoscope (**C**) and various forceps, scalpel handles, and scissors (**D**).

Figure 3: Surgical procedure. After sedation, the animal was intubated with an endotracheal tube (**A**), and the intravenous cannulation was established through an ear vein (**B**). The surgical site was at the left chest of the animal (**C**). After exposing the descending aorta (**D,E**), the constriction site (SB) and invasive sites for pressure monitoring (SA, SC) were determined (**F,G**), and the aortic pressure was measured using a patient monitor (**H**). A cartoon displays the overview of the constriction strategy (**I**).

Figure 4: Transthoracic echocardiography evaluation. The representative B-mode and M-mode images of the pressure overload hearts from week 0 to week 12 are displayed in (**A**). The M mode images recorded for 4 s are shown. The pink scale bar indicates the record length of 1 s. The ventricular septum thickness (VST) at end-diastole increased in the DAC hearts (**B**). In contrast, the posterior wall thickness (PWT) at end-diastole gradually increased and decreased during the observation period (**C**). The left ventricular internal dimension (LVID) at end-diastole decreased in week 4 and week 6 and then gradually increased after week 8 (**D**). The LVEF of the model hearts was maintained at >50% during the 12-week period (**E**). The number of animals, n = 3 each, was for the sham and DAC groups. Unpaired t-tests were used to determine the differences between the groups. $*P < 0.05$ *vs*. the sham group.

Figure 5: Heart morphology and serum cTnI. The size of the heart appeared to increase (**A**). The heart failure marker cTnI was significantly higher at weeks 4, 8, and 12 in the DAC group than in the sham group at the corresponding time points (**B**). The number of animals, n = 3 each, was for the sham and DAC groups. Unpaired t-tests were used to determine the differences between the groups. $*P < 0.05$ *vs*. the sham group.

Figure 6: Histology of myocardium, mitral valve, and aortic wall. H & E staining was used to examine the cardiac tissue at the end of the experiment. Cardiomyocytes in atria, ventricular septum, and ventricles displayed hypertrophy (arrows in green; **A**), accompanied by pyrosis (arrows in yellow; **A**). Muscular layers are reduced in the mitral valve (arrows in blue; **B**). Vascular endothelial hyperplasia was observed in the aorta (area within the blue lines; **C**). Red asterisks:

examined tissues; L. ventricle, left ventricle; R. ventricle, right ventricle; L. atrium, left atrium; R. atrium, right atrium.

Figure 7: Fibrosis and inflammation in the DAC hearts. Histological examination showed extensive myocardial fibrosis in DAC minipigs. A fibrotic area in the left ventricle was displayed (asterisks and arrows in yellow; **A**). Infiltration of inflammatory cells was observed in the left ventricles, right atrium, and aortic walls (asterisks in green; **B**). Red asterisks: examined tissues; arrows in blue, eosinophils; L. ventricle, left ventricle; R. atrium, right atrium.

DISCUSSION:

This study used DAC techniques to develop an HFpEF model for Tibetan minipigs. A step-by-step animal and instrument preparation protocol is presented here, including sedation, tracheal intubation, vein cannulation, surgical procedure, and post-surgery care. The recording techniques for echocardiographic B-mode and M-mode heart images are also presented. After DAC, the heart underwent left ventricular hypertrophy during weeks 4 and 6 and dilation after week 8. LVEF was preserved during the 12-week period. Fibrosis and inflammation were observed in DAC hearts.

The combination of open chest operation and aortic constriction has been used to develop heart failure models in large and small animals. For example, rodent aortic constriction-induced hypertension was reported as early as the 1950s[18]. Constriction of the ascending aorta in pigs induced mild left ventricular hypertrophy in 2–4 weeks old pigs. Regarding the operation site for locating the ascending aorta, a few studies selected the third intercostal space[19,20], while another study selected the fourth intercostal space for the lateral thoracotomy[21]. It was found that constriction at the descending aorta was practical in adult Tibetan minipigs. The descending aortic segment was located right under the fourth intercostal space and surrounded by little connective tissue.

The degree of constriction can be crucial for inducing key features of HFpEF. Melleby et al. reported that a smaller ring size accelerated hypertrophy, while larger ring sizes led to preserved EF for 8–20 weeks in mice with ascending aortic constriction[22]. Massie et al. set a pressure gradient of 20 mmHg for open-chest surgery in pigs to induce ventricular hypertrophy[21]. Charles et al. adopted progressive cuff inflation to generate HFpEF in female Yorkshire-Landrace pigs[23]. In the current study, a 20% increase in pressure at the descending aorta for 12 weeks led to HFpEF. Researchers have also combined aortic constriction techniques with deoxycorticosterone acetate or Western diet to induce HFpEF in female Ossabaw swine[10,24]. The constriction degrees are typically estimated by the pressure measured using a micro manometer catheter or echocardiography. A tool had been modified to measure the aortic pressure. A catheter with disposable blood pressure transducers connected to a patient monitor was used to record the pressure at the descending aorta.

Our previous study presented typical parasternal long-axis images of the HFpEF hearts in minipigs[17]; here, representative parasternal short-axis images are added. Consistent with the earlier results, the minipig DAC model displayed two distinct stages of cardiac remodeling,

concentric hypertrophy, and dilation, during the 12-week observation period. These phenotypes are consistent with the clinical symptoms of HFpEF. New histological findings in the HFpEF model are also revealed in this work. Cardiomyocyte hypertrophy in the atria, ventricular septum, and ventricles are found. In addition, severe inflammatory cell infiltration in the left ventricle, right atrium, and aortic wall are obtained. This complements the previous findings, which demonstrated upregulation of interleukins -6 and -1β, NFκB, and cytokine production in the DAC myocardium[17]. The muscle layer disappeared in the mitral valve of the HFpEF pig, suggesting that abnormalities in the mitral valve contributed to cardiac dysfunction.

Establishing an aseptic surgical procedure is critical for obtaining successful and stable pig models. The aortic constriction surgery in pigs requires more operators than that in rodents. It usually requires an experienced surgical team of two surgeons, one anesthesiologist, two operating room nurses. These roles can be taken by veterinarians, human surgeons, and/or well-trained technicians. Compared with a rodent surgery that takes about 30 min to complete an aortic constriction procedure, it may take more than 3 h to complete a similar procedure in pigs. In practice, insufficient facilities and skilled personnel for large animal surgery limit the application of pig surgical models.

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DISCLOSURES:

The authors declare that they have no competing interests.

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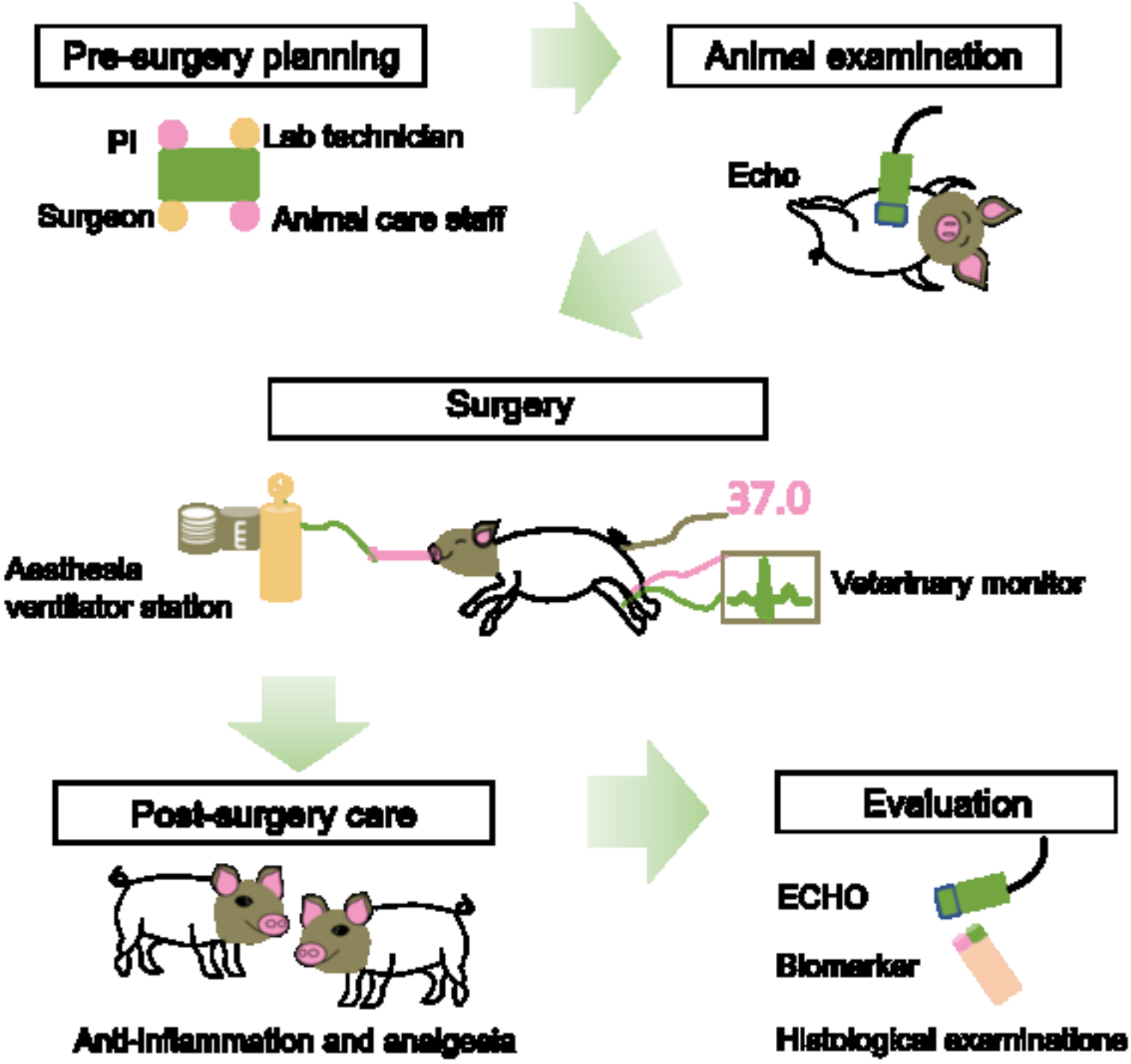
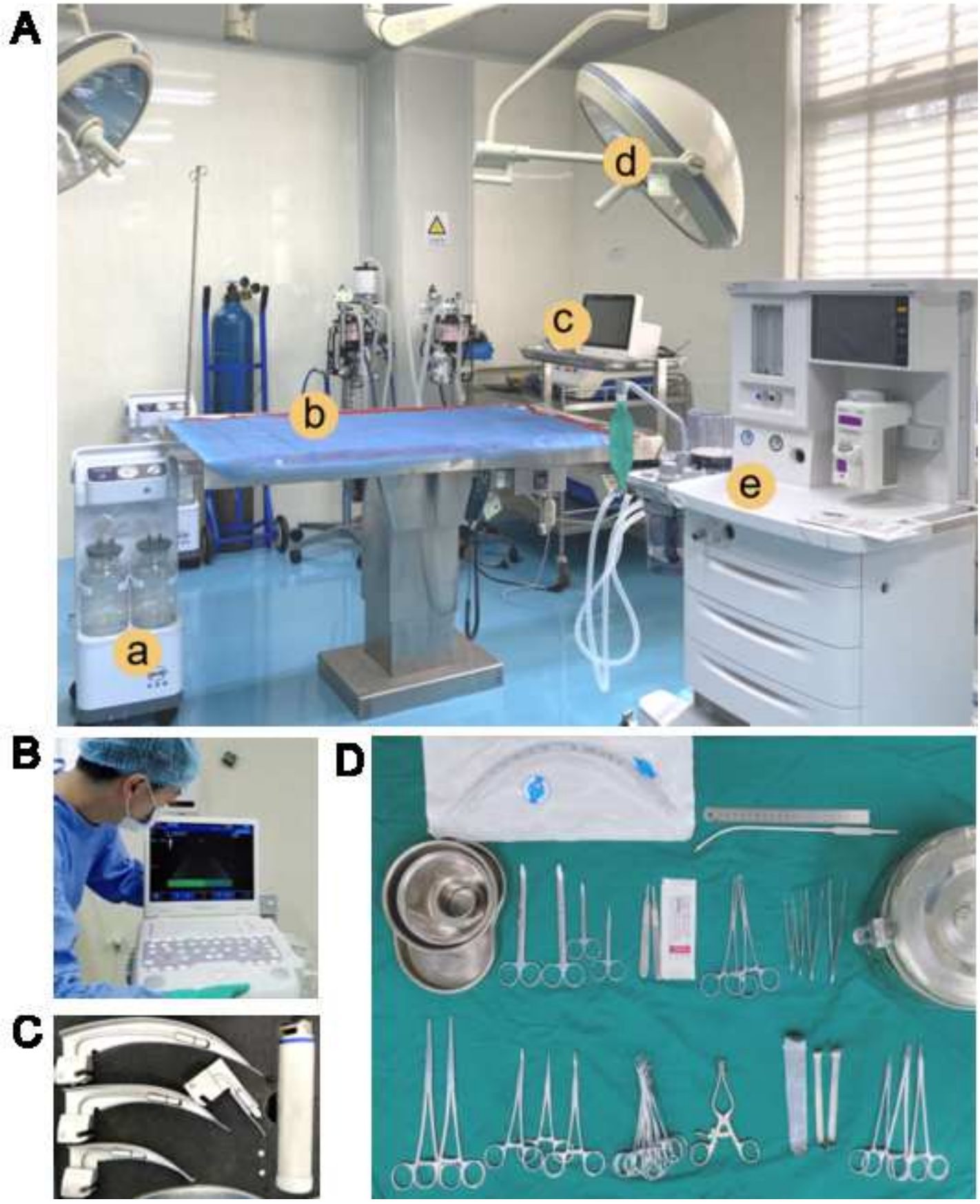
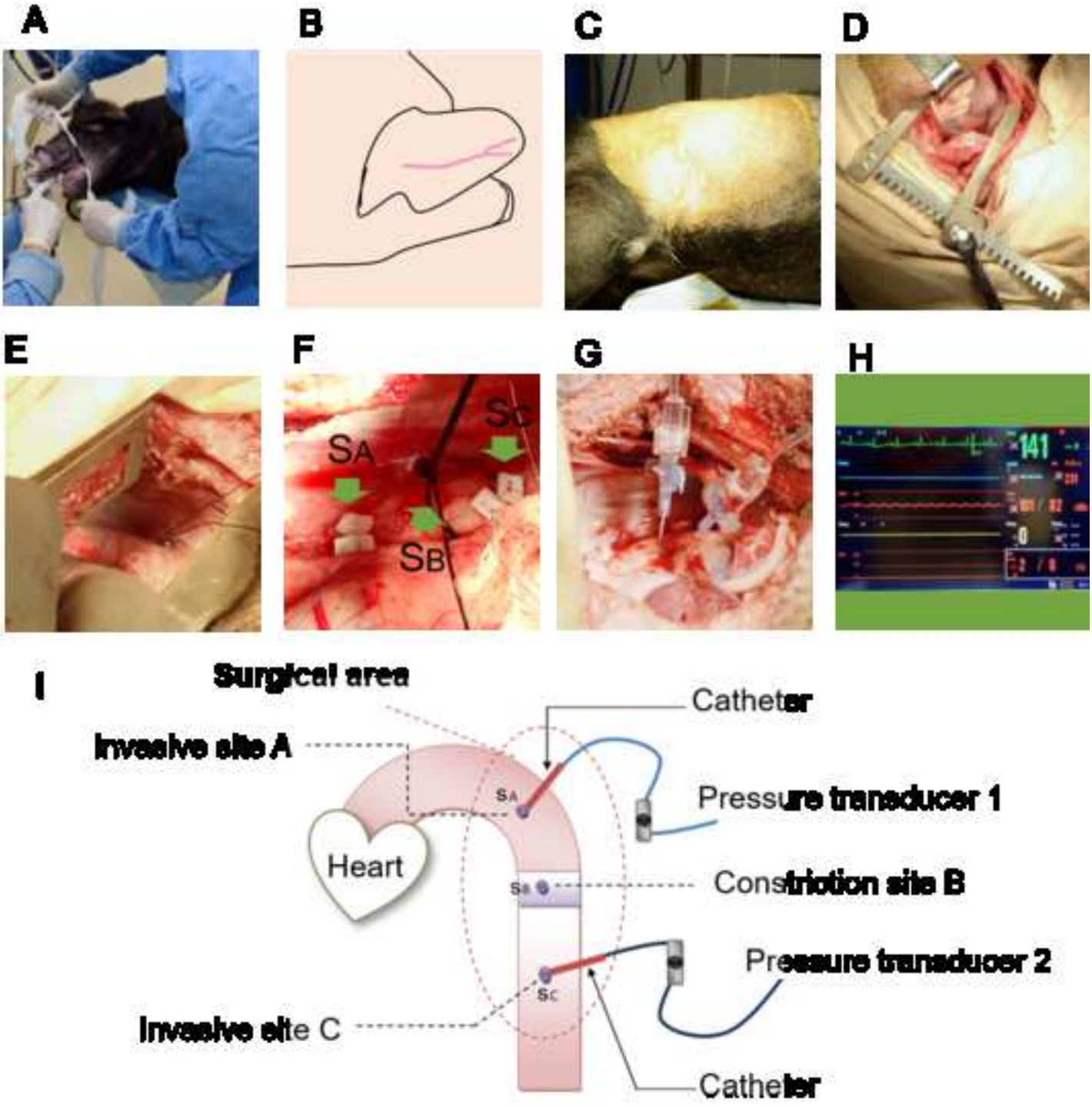
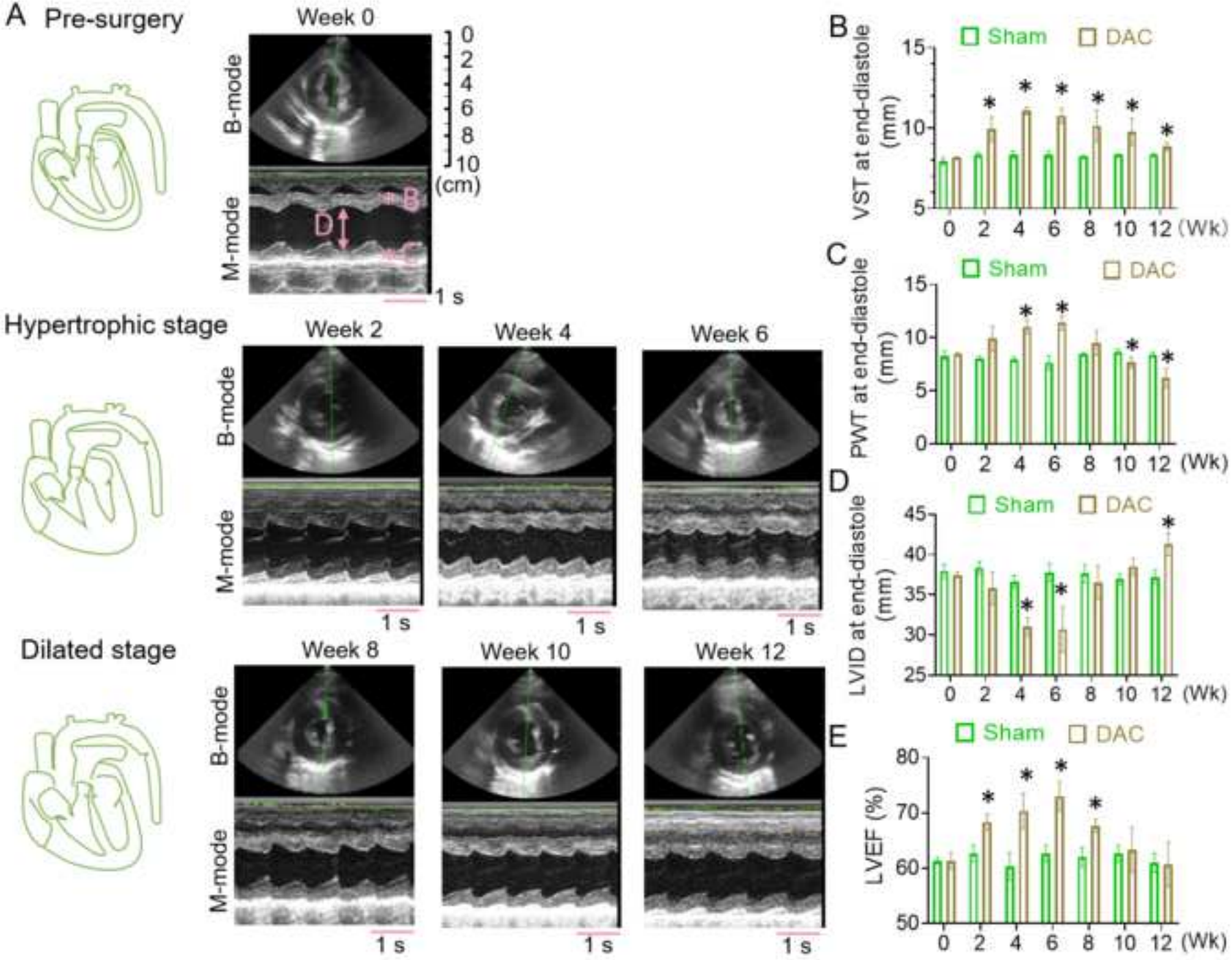
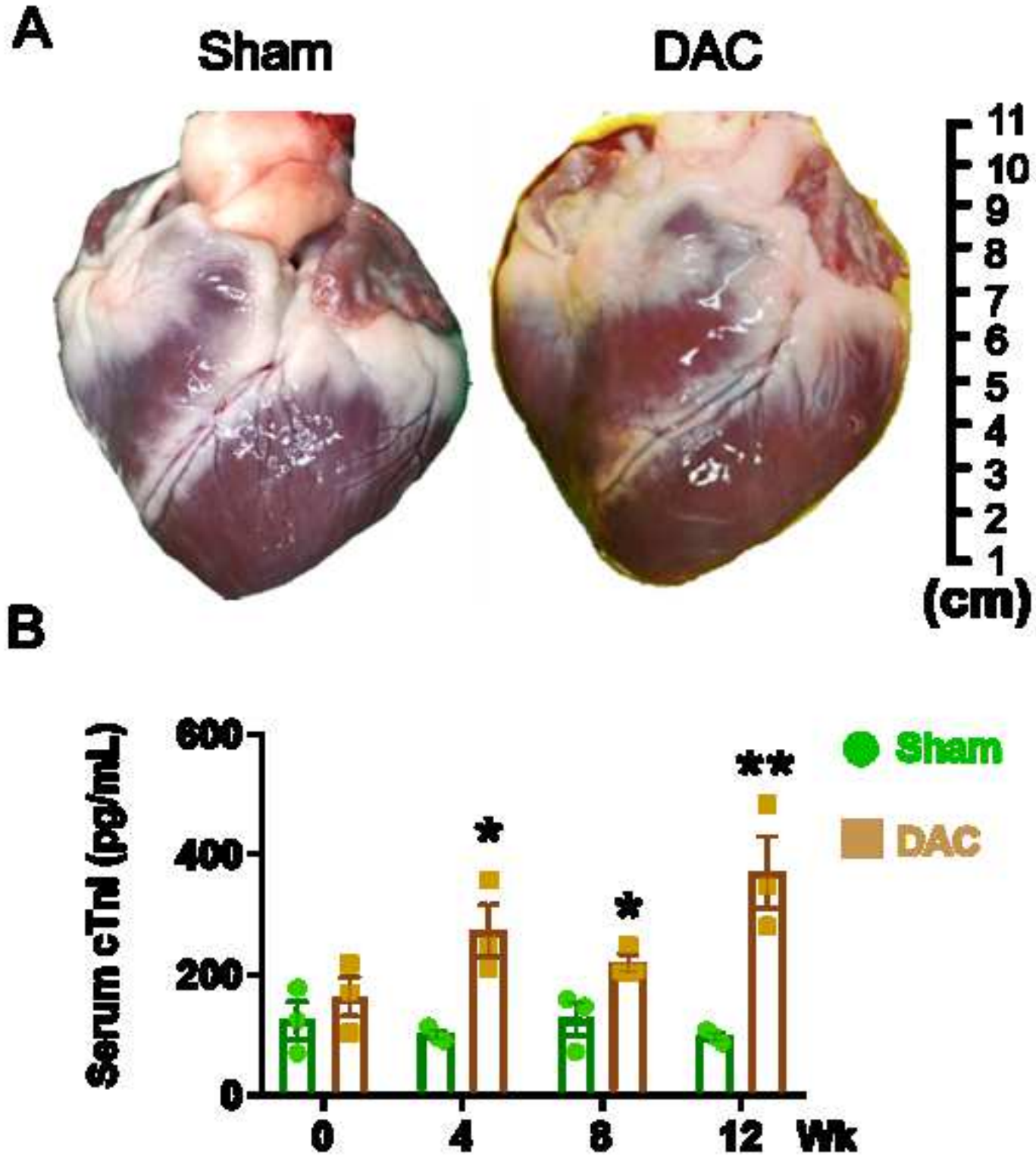
Figure1-experimental design

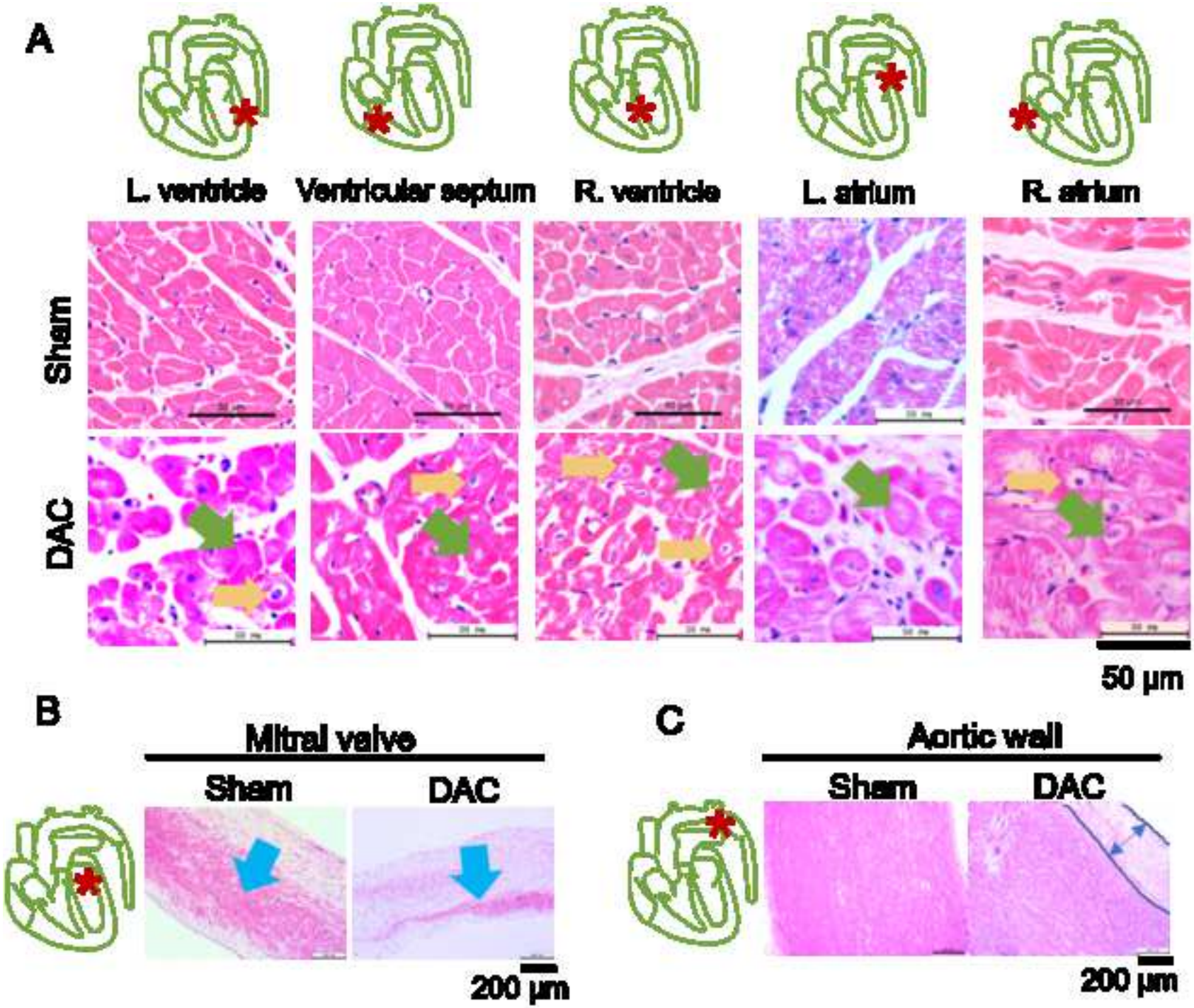


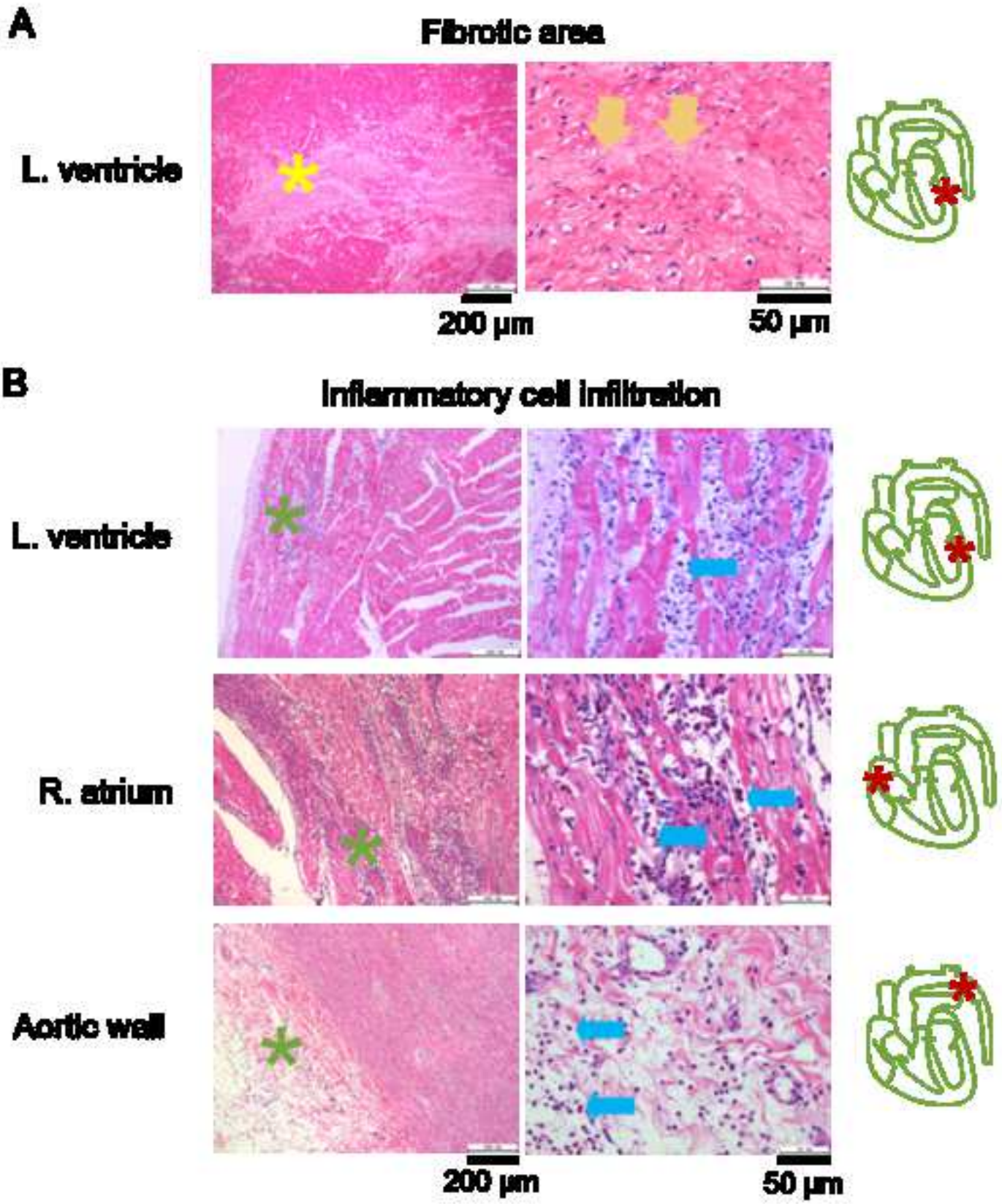












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Research Topic: Cardiovascular research - Innovative animal models of cardiac remodeling: development and evaluation

Dear Editors and Reviewers:

I wish to submit the revised manuscript titled “A surgical model of heart failure with preserved ejection fraction in Tibetan minipigs.” The paper was coauthored by Xiaohui Li, Weijiang Tan, Xiang Li, Shuang Zheng, Xiaoshen Zhang, Honghua Chen, Zhanhong Pan, Caiyi Zhu, and myself. I greatly appreciate the editors and reviewers for the thoughtful suggestions and insights. The manuscript has benefited from these insightful suggestions.

Thank you in advance for your time and consideration. Any comments regarding this manuscript will be greatly appreciated.

The responses to the editors and reviewers are attached.

Sincerely yours,

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Responses to the editors and reviewers

Editorial comments:
Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: We have edited this manuscript.

2. Please reword the following lines to avoid previously published work: 24-25, 37-38, 164-167, 177-179.

Response: We have revised these sentences.

3. Please provide an email address for each author.

Response: The email addresses are listed below.

Xiaohui Li, email: lixh1989@jnu.edu.cn
Weijiang Tan, email: twj@gdlami.com
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4. Please provide a Summary before the Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: “The present protocol describes …”

Response: The present protocol describes a step-by-step protocol to establish a minipig model of heart failure with preserved ejection fraction using descending aortic constriction, as well as the approaches to evaluate the phenotypes of this model.

5. Please ensure that abbreviations are defined at first usage.

Response: We have checked.

6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.
For example: Shenxin®

Response: Thanks. We have revised these.

7. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Response: Thanks for the information.

8. Please add more details to your protocol steps:
Step 1.4: Please ensure that the details of all the materials/reagents/equipment mentioned are included in the Table of Materials.
Step 2.3: Please include the details of the echocardiography device in the Table of Materials. Also, please mention how much blood sample was collected.
Step 2.5: Please mention how long the animals were kept under the said conditions.
Step 3.13: Is the benzylpenicillin powder the same as the benzylpenicillin injection mentioned in the Table of Materials? If not, then please include the details.
Step 4.2: Please include the details of flunixin meglumine in the Table of Materials.

Response:
Step 1.4: All have been checked.
Step 2.3: We have revised the description of this equipment.
Step 2.5: We have revised the sentence as follows: "2.5 Initiate the ventilation at tidal volume 8 ml/kg, 30 breath/minute and maintain the animals with 1.5–2.5% isoflurane during the surgical procedure"
Step 3.13: They are the same benzylpenicillin injection. We have revised that in the context and that table.
Step 4.2. We have deleted flunixin meglumine.

9. Please include one line space between the protocol steps and highlight that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Response: Yes. We have highlighted the key steps by following the instruction.

10. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next and also is in-line with the Title of the manuscript. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in the imperative tense. However, the NOTEs cannot be filmed, so please do not highlight them.

Response: Thanks for the information.

11. As we are a methods journal, please ensure that the Discussion cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) significance with respect to existing methods
e) Any future applications of the technique

Response: Thanks. In the discussion section, we added one more paragraph to indicate the limitation of this technique.

12. Please ensure that all the Figures (including the sub-panels) are referenced in the manuscript text in sequential order.

Response: Thanks for the information.

13. Figure 3I/5B: Please include the description in the figure legend.

Response: We have added the description to Figure 3I, and added "(B)" following the description in Figure 5.

14. Figure 4: For 4A-please include scale bars. 4B-E-please include the x-axis descriptions.

Response: We have added scale bars indicating the scanning depth for B-mode images and the recording time for M-mode images in Figure 4A. Descriptions of x-axis were added to Figure 4B.

15. Figure 7: Please mention in the Figure legend what the arrows in yellow and the asterisks marks indicate.

Response: We have added the description for these symbols.

16. Please do not abbreviate the journal names in the References.

Response: We have changed the reference style.

Reviewers' comments:

Reviewer #1:

Manuscript Summary

Xiaohui Li et. al. described a model of heart failure with preserved ejection fraction in Tibetan minipigs. They presented the protocol step-by-step with essential details. The paper writting is well organized.

Major Concerns:
I would like the authors to provide more specific application prospects of this model in the research field and discuss the limitation if it has.

Response: We have discussed the limitation of this protocol.

Minor Concerns:
The charts in Fig 4 missed the title of x-axis.

Response: The description of x-axis was added to Figure 4.

Reviewer #2:

Manuscript Summary:

Authors described in detail the surgical procedure of establish a large animal HFpEF model using descending aortic constriction (DAC) in Tibetan minipigs. Cardiac function, heart injury including fibrosis, inflammation, and cardiomyocyte hypertrophy, heart failure markers were fully examined. The model of HFpEF is helpful to elucidate the molecular mechanisms or preclinical findings. The manuscript was well written,
Major Concerns:
1. NT-proBNP is usually detected as a heart failure marker in patients. It is of great significance if authors provide the index.

Response: Thanks. We agree that various biomarkers are used in diagnosing heart failure. We have shown expression of ANP and BNP in the DAC hearts were significantly higher than that in the sham hearts in a previously published paper (Ref: Tan, et al., Front. Cardiovasc. Med., 2021, doi: 10.3389/fcvm.2021.677727).. Here, changes of serum cTnI at different timepoints were added to characterize the physiological condition of the HFpEF minipigs.

2. Advice authors to provide clear evidence of cardiac fibrosis using masson staining.

Response: We have previously reported that the fibrotic area, stained by Sirius Red staining, in the DAC group was significantly greater than that in the sham group. (Ref: Tan, et al., Front. Cardiovasc. Med., 2021, doi: 10.3389/fcvm.2021.677727)

Minor Concerns:
In fig 4, B-E, It is more clear that one curve represents sham group and the other curve represents DAC group. The every point indicates the value: mean ±SED

Response: Thanks. We have calculated the SEMs of each group at different timepoints and showed the graph in a summary data style of GraphPad prism 8.

In fig 5. N=3? Statistical method should be described.

Response: The statistical method was added to the legends in Figure 4 and Figure 5.