

Video Article

Myocardial Infarction in Neonatal Mice, A Model of Cardiac Regeneration

Jessica N. Blom¹, Xiangru Lu¹, Paul Arnold¹, Qingping Feng¹¹Department of Physiology and Pharmacology, Western UniversityCorrespondence to: Qingping Feng at qingping.feng@schulich.uwo.caURL: <http://www.jove.com/video/54100>DOI: [doi:10.3791/54100](https://doi.org/10.3791/54100)

Keywords: Medicine, Issue 111, Heart, neonates, mouse model, cardiac regeneration, myocardial infarction, myocardial ischemia, coronary artery ligation

Date Published: 5/24/2016

Citation: Blom, J.N., Lu, X., Arnold, P., Feng, Q. Myocardial Infarction in Neonatal Mice, A Model of Cardiac Regeneration. *J. Vis. Exp.* (111), e54100, doi:10.3791/54100 (2016).

Abstract

Myocardial infarction induced by coronary artery ligation has been used in many animal models as a tool to study the mechanisms of cardiac repair and regeneration, and to define new targets for therapeutics. For decades, models of complete heart regeneration existed in amphibians and fish, but a mammalian counterpart was not available. The recent discovery of a postnatal window during which mice possess regenerative capabilities has led to the establishment of a mammalian model of cardiac regeneration. A surgical model of mammalian cardiac regeneration in the neonatal mouse is presented herein. Briefly, postnatal day 1 (P1) mice are anesthetized by isoflurane and placed on an ice pad to induce hypothermia. After the chest is opened, and the left anterior descending coronary artery (LAD) is visualized, a suture is placed around the LAD to inflict myocardial ischemia in the left ventricle. The surgical procedure takes 10-15 min. Visualizing the coronary artery is crucial for accurate suture placement and reproducibility. Myocardial infarction and cardiac dysfunction are confirmed by triphenyl-tetrazolium chloride (TTC) staining and echocardiography, respectively. Complete regeneration 21 days post myocardial infarction is verified by histology. This protocol can be used to as a tool to elucidate mechanisms of mammalian cardiac regeneration after myocardial infarction.

Video Link

The video component of this article can be found at <http://www.jove.com/video/54100/>

Introduction

Myocardial infarction (MI) is a leading cause of death worldwide, and remains responsible for about one third of heart failure cases¹. While the advent of percutaneous intervention and continuous optimization of the use of thrombolytics has increased reperfusion following MI, cardiomyocyte death and loss of contractile myocardium nevertheless occurs. There also remain large numbers of "no-option" patients who are not candidates for or do not see benefit from these interventions. These patients continue to experience disabling ischemia leading to scar formation and deleterious ventricular remodeling as a mechanism of infarct healing. This process ultimately results in heart failure, for which prognosis remains poor despite optimal pharmacologic management with angiotensin-converting enzyme (ACE) inhibitors and beta blockers. Unfortunately, the one-year mortality rate for patients with severely impaired left ventricular function still remains as high as 26%². Heart transplant is the final treatment option for patients with heart failure. However, the limited donor pool for heart transplantation does not make this a viable option for most patients. Thus, the discovery of novel therapeutic agents to restore the damaged myocardium remains paramount to solving the cardiac disease problem. Reliable animal models of cardiac injury are therefore required as a critical component of this process.

Traditional dogma has dictated that adult cardiomyocytes are post-mitotic, terminally differentiated cells, incapable of dividing or de-differentiating to replace the damaged myocardium³. As such, an adult mammalian heart could never completely recover from injury, and lost cardiomyocytes would be replaced with fibrous tissue. Thus, research has focused primarily on therapeutic agents to minimize infarct expansion and reduce scar formation. More recently however, a paradigm shift has occurred in the thinking surrounding cardiac healing and many research efforts have been redirected to focus on the potential for cardiac regeneration⁴.

Until recently, *in vivo* study of cardiac regeneration was restricted to non-vertebrate models, such as those in urodele amphibians and teleost fish⁵⁻⁷. However, the discovery of the capacity for cardiac regeneration in the neonatal mouse has led to the development of two surgical models of mammalian cardiac regeneration: resection of the cardiac apex and coronary artery occlusion to induce myocardial infarction^{8,9}. In 2011, a mouse apex resection model was used to demonstrate that complete cardiac regeneration is possible at postnatal day 1 (P1). However, this capacity declines rapidly after the initial neonatal period. The mammalian heart loses its regenerative potential shortly after birth at P7 as progenitor cell numbers decline, and cardiomyocytes become binucleated, lose their proliferative competency, and permanently exit the cell cycle^{10,11}. Understanding the fundamental differences between the neonatal and adult mammalian heart may lead to novel insights into cardiac regeneration.

While apex resection indeed offers insight into re-growth of contractile tissue, the model does not simulate typical human cardiac injury, and thus does not lend itself as well to the development of therapeutics. The coronary artery occlusion model, however, more directly simulates the

pathophysiologic aspects of MI pathology, and thus may provide more useful insights into mechanisms that may be applicable to therapeutic advancement for human use.

Surgical coronary ligation has been used as a useful experimental technique in many animal models¹²⁻¹⁴. In the adult coronary artery ligation model, animals are anesthetized and intubated to allow opening of the chest cavity while maintaining respiration. The heart continues to beat regularly, permitting visualization of the coronary vasculature and allowing for accurate suture placement. Furthermore, the heart remains pink as perfusion continues, and after ligation the ischemic myocardium appears pale, indicating successful coronary artery ligation. The protocol described for neonatal mice, however, is less reliable as the coronary artery is not visualized and the surgeon must estimate where to place the suture¹⁵. Although the general anatomy of the coronary vasculature is the same, individual animal variability in the direction and branching of the LAD exists¹⁶. Thus, when "going in blind," the artery could be easily missed. Other techniques such as echocardiography are then required to confirm successful induction of MI, and to ensure all surgeries result in a similar infarct size. Described here is an improvement on a recently published method¹⁵, where the position of the LAD can be established and thus LAD may be ligated to reproducibly induce MI.

This technique does not require endotracheal intubation or mechanical ventilation, as thoracotomy in a hypothermic state in the neonatal mouse does not result in lung collapse. However, in the previously described method, severe hypothermia must be induced to the point of both complete apnea and cessation of the cardiac rhythm¹⁵. The major limitation of this approach is that the coronary artery is no longer perfused and the heart appears pale even before LAD ligation. In the approach described herein, coronary artery visualization is possible at a point of torpor before deep hypothermia and cardiac rhythm cessation, with full recovery of the neonatal mouse after the surgery. This method offers a major advantage of 100% reproducibility.

Protocol

Breeding pairs of C57BL/6 and CD-1 IG-S mice were purchased from Charles River. Animals used in this study were handled in accordance with the guidelines of the Canadian Council on Animal Care, and study protocols were approved by the Animal Use Subcommittee at Western University, London, Canada.

1. Animal Care

1. After birthing is complete and pups have been initially breast-fed by their mother for a few hr, place them in a different cage with a CD-1 foster mother. CD-1 mothers display a calmer phenotype with a strong fostering instinct, and have a lower tendency to cannibalize injured pups¹⁵.

2. Surgery

1. Induce anesthesia by placing the pup in a sealed isoflurane chamber (approximately 500 μ l of 100% v/v isoflurane allowed to dissipate over 1,300 cm^3 chamber). Keep the mouse in the chamber until cessation of movement (about 30 sec).
2. Remove mouse from isoflurane chamber and induce hypothermic anesthesia by placing mouse on wet ice. To avoid frostbite, place ice inside a sterile surgical glove, wrap the mouse within the glove and cover the glove with ice OR wrap the mouse in sterile wet gauze and place it on ice.
NOTE: Mice cool faster when contact with ice is over a larger surface area, and as such melted ice can shorten hypothermia induction time.
3. Confirm anesthesia by lack of movement response to toe and tail pinch.
NOTE: Cessation of provoked movement occurs at a body temperature between 15 °C and 8 °C. (Cooling time to this temperature approximately 1 min). Complete apnea and asystole is **NOT** required for LAD ligation.
4. Move the ice bed to the surgical area equipped with an operating microscope. Ensure that the mouse pup remains on ice during the whole surgical procedure.
NOTE: The surgical area, gauze and surgical instruments should be sterilized. Maintain sterile field throughout the procedure, and wear single use, sterile surgical gloves. Ophthalmic ointment is not required as mice are born with their eyes closed¹⁷.
5. Place the mouse pup in the right lateral decubitus position. Disinfect chest by gently wiping with povidone-iodine solution followed by an ethanol swab.
6. Perform skin incision on the left chest along the mid-axillary line by cutting skin between the xyphoid and left axilla a few millimeters below the left foreleg. Also use scissors to cut through the underlying pectoral muscle layer.
7. Perform a left thoracotomy in the 4th intercostal space by separating ribs and intercostal muscles with forceps.
NOTE: Neonatal ribs are very fragile and can be easily broken. To avoid this, gently separate ribs by opening forceps along the intercostal muscle (rather than grasping ribs).
8. Visualize the left anterior descending coronary artery (LAD) emerging from the left auricle behind the pulmonary vein and descending beyond the great cardiac vein.
NOTE: In this early neonatal window, the thymus is often visualized and may cover part of the cardiac base. The LAD can be seen emerging beside the position of the thymus, depending on the angle of thoracic incision.
9. Ligate the LAD by passing an 11-0 nylon suture (0.007 mm diameter needle) below the artery through the mid ventricle below the left auricle (**Figure 1C**). Ischemia is confirmed with blanching of the myocardium below the suture site (**Figure 1G**).
10. Close the thoracic incision using 8-0 nylon sutures (0.15 mm diameter needle). Use two sutures to close the ribs. Place both rib sutures before ligating to ensure the needle does not puncture the lungs when passing through the body cavity.
11. After closing the ribs, remove the mouse from the ice bed. Keep the mouse within the surgical area and place it directly on the sterile surgical towel over a warm heating pad at 37 °C to begin warming.
12. Once on the sterile heated area, close the muscle and skin layers using 8-0 nylon sutures (0.15 mm diameter needle). Use one suture for the muscle layer and use two sutures for the skin incision.
NOTE: Muscle and skin layers may be closed on a heating pad to reduce hypothermia exposure time. Mouse temperature begins to rise once placed on the heating pad, but remains sufficiently low enough for anesthesia maintenance during muscle and skin closure.

3. Surgical Recovery

1. Continue rapid warming on a warm heating pad until return of spontaneous movement. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Remove povidone-iodine and blood by gently wiping injury site with ethanol swab.
2. After sufficient recovery from anesthesia, remove it from sterile surgical area, smear it with bedding from foster mother's cage, and return pup to foster mother. This helps prevent maternal rejection or cannibalization.
NOTE: Do not return pups to a cage with other animals until fully recovered. If an entire litter has not been used, and littermates remain with foster mother, place recovered pups in the middle of the litter. If performing more than one surgery, complete all surgeries required for an individual litter before returning mice to their foster mother.
3. Observe behavior of foster mother toward the pup every 10 - 15 min for 2 - 3 hr to ensure acceptance of the pup. If the mother displays aggression towards the injured pup, remove the pup and euthanize by isoflurane overdose (> 5%; until cessation of breathing), followed by decapitation. NOTE: Perioperative analgesia medications are not required as the centralized pain reflexes are not fully developed at this early age¹⁵.

4. Measurement of Myocardial Infarct Size 4 - 6 hr Post-MI

1. Allow pups to recover in the care of a CD-1 foster mother for a period of 4 - 6 hr. Remove the pup from cage with mother and euthanize it by isoflurane overdose followed by decapitation with large scissors.
2. Excise the heart under dissecting microscope, careful not to rip the myocardium.
 1. Cut the skin from the xyphoid process to the top of the thorax. Open abdominal wall below the ribcage. Grasp the lower rib cage and cut through the ribs and musculature longitudinally along the left mid-axillary line from the diaphragm to the axilla.
 2. Hold scissors in transverse plane and carefully cut through diaphragm from left to right side. Make sure to place scissors below the level of the heart so as to avoid any damage to the apex.
 3. Grasp rib cage and cut right side of ribs and musculature along the right mid-axillary line. Remove all vascular connections to the heart with scissors. Remove the heart from chest cavity by grasping the base.
3. Section the heart into three pieces using a surgical carbon steel razor blade. Make the first cut along the short-axis of the heart at the midpoint between the suture and the cardiac apex. Make the second cut at the level of the suture (**Figure 3A**).
NOTE: This leaves an apex section approximately 0.75 mm thick weighing 0.0018 g; a mid-base section approximately 1 mm thick, weighing 0.0065 g; and the base of the heart.
4. Place the heart sections in 1% 2,3,5-triphenyltetrazolium chloride (TTC) at room temperature for 10 - 15 min. Carefully watch specimen to avoid over-staining.
5. To increase contrast, fix stained heart slices with 4% paraformaldehyde overnight at 4 °C. To calculate % infarct area, photograph heart sections and measure infarct area on imaging software. The viable myocardium stains red while the infarct area is demarcated as white¹⁸.

5. Measurement of Cardiac Function by Echocardiography Post-MI

1. Allow pups to recover in the care of a CD-1 foster mother for a period of 24 - 48 hr. Induce anesthesia by placing the pup in a sealed isoflurane chamber (5% isoflurane). Keep the mouse in the chamber until cessation of movement (about 30 sec).
2. Secure the pup in the supine position on a heated dock (temperature 37 °C) with its nose in a cone to deliver 0.5 - 1% isoflurane (for anesthesia maintenance). Place pre-warmed echo gel on the left thoracic area.
3. Obtain a parasternal long-axis view of the left ventricle (LV). Ensure the images are obtained below the level of the suture in the LV. After acquiring the position, turn the ultrasound probe (40 MHz) 90° to obtain a parasternal short-axis view, and record M-mode echocardiographic images.
4. Measure the end diastolic and end systolic left ventricular internal diameters from the short-axis M-mode images. Calculate ejection fraction and fractional shortening.

6. Measurement of Myocardial Infarct Size 24 hr Post-MI

1. Allow pups to recover in the care of a CD-1 foster mother for a period of 24 hr. Remove the pup from cage with mother and euthanize it by isoflurane overdose followed by decapitation with large scissors.
2. Open the chest cavity as in step 4.2. Before excising the heart, carefully grasp the thoracic aorta just above the diaphragm with fine forceps. Hold fine scissors in the coronal plane, flat against the thoracic wall, and cut the thoracic aorta off the posterior thoracic wall moving scissors in the cranial direction.
3. Cut the aorta free from the thoracic wall and any other connections until it reaches the heart. Excise the heart by grasping the superior vena cava and cutting all other vascular connections to the body.
4. Rinse the heart (with aorta attached) in saline. Carefully cannulate the thoracic aorta with a 30 gauge needle and tie aorta to cannula with 8-0 nylon suture thread.
5. Perfuse the heart with 1 ml of saline through the aorta via a 30 gauge needle at a rate of 1 ml/min. Perfuse the heart with 150 µl of 2% Evans blue solution at a rate of 1 ml/min. Remove the heart from the cannula, section it into three pieces and stain it with TTC as described in section 4.
6. To calculate infarct size, photograph heart sections and measure infarct area on imaging software. The viable myocardium stains blue, the area at risk stains red, and the infarct area is demarcated as white.

Representative Results

The myocardial infarction procedure at P1 can be completed in 10 - 15 min and has a mortality rate of 7.8% (5 out of 64 pups). After surgery, mice recover from hypothermic anesthesia within the next 5 - 20 min (time of recovery depends on body temperature reached during anesthesia and speed of surgeon). When using P7 pups (for comparison with a non-regenerative myocardium), a longer period of cooling is required to reach torpor. P7 pups are much larger and have more difficulty recovering from both cardiac injury and hypothermia, resulting in a much higher mortality rate of 26.9% (14 out of 52 pups).

Results from previous studies are confirmed herein, indicating induction of hypothermic anesthesia at close to 10 °C (between 8 °C and 15 °C)¹⁹. Within this temperature window, heart rate does slow, but rhythm continues at rates of between 24 bpm and 11 bpm. The low heart rate at this level of cooling significantly reduces intraoperative bleeding. The LAD can be easily visualized and ligated at these temperatures (**Figure 1**). The LAD remains visible until body temperatures reach between 9.6 °C and 4.9 °C, causing lowering of heart rate to 2 bpm or asystole.

After LAD ligation at P1, pups recover fully and grow to a body size comparable to that of littermate controls. Once placed back with their foster mother, pups regain awareness and feeding capabilities comparable to littermates in about 10 min. Upon histological examination 3 days post-MI, there is evidence of infarction, with infiltrating inflammatory cells, a typical post-infarction response (**Figure 2**). At day 7 post-MI, left ventricular tissue appears normal. By day 21 post-MI, complete cardiac regeneration has occurred.

2,3,5-Triphenyltetrazolium chloride (TTC) staining 4 - 6 hr post-MI was used as a confirmation of consistent induction of MI. The red area represents viable myocardium and the white area represents ischemic dead tissue. In a total of 13 LAD ligation surgeries, 100% of the hearts were infarcted, with an average infarct size of 36% (**Figure 3**). TTC staining with Evans blue perfusion was also performed at 24 hours post-MI to demonstrate persistence of infarcted tissue and measure infarct size based on area at risk (**Figure 4**). In these hearts, viable myocardium stains blue, the area at risk stains red, and the infarct area is demarcated as white. Infarct size was calculated as a percent of the area at risk. In 4 LAD ligation surgeries, 100% of the hearts were infarcted, with an average infarct size of 49% of the area at risk (**Figure 4**).

To confirm infarct induction, echocardiography was performed at 24 and 48 hr post-MI. At 24 hr post-MI, ejection fraction (EF) was significantly reduced from 84% to 74%, and fractional shortening (FS) significantly reduced from 50% to 40% (**Figure 5**). By 48 hr post-MI, EF was further reduced to 46%, as compared to 80% in controls and FS was also reduced significantly to 25%, as compared to 50% in controls. Furthermore, there was a significant reduction in systolic left ventricular internal diameter at 48 hr (**Figure 6**). LV anterior wall thickness was not significantly decreased at either 24 or 48 hr post-MI (**Figures 5 and 6**).

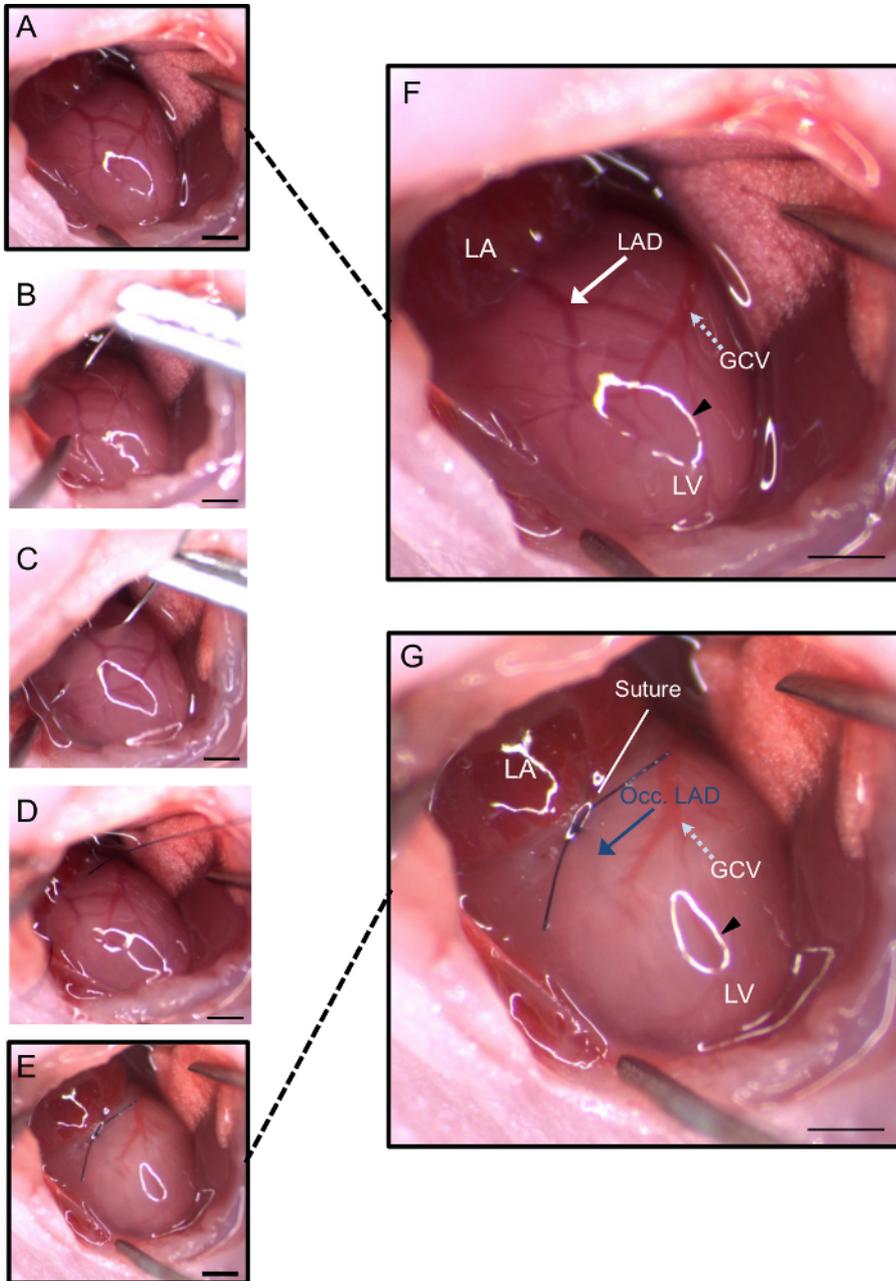


Figure 1. Coronary Artery is Visible During Neonatal LAD Procedure. P1 neonatal mouse after hypothermic anesthesia, skin incision, muscle incision and lateral thoracotomy in the fourth intercostal space. **A)** Left anterior descending coronary artery (LAD) is visible. **B-D)** 11-0 nylon suture is passed through the mid ventricle. **E)** LAD is ligated and blanching observed below the suture site. **F)** and **G)** are magnifications of **A)** and **E)** respectively. Occ. LAD, occluded left anterior descending artery. LA, left atrium. LV, left ventricle. GCV, great coronary vein. White arrow, LAD. Black arrow head, glare from lighting. Scale bars are 1 mm. [Please click here to view a larger version of this figure.](#)

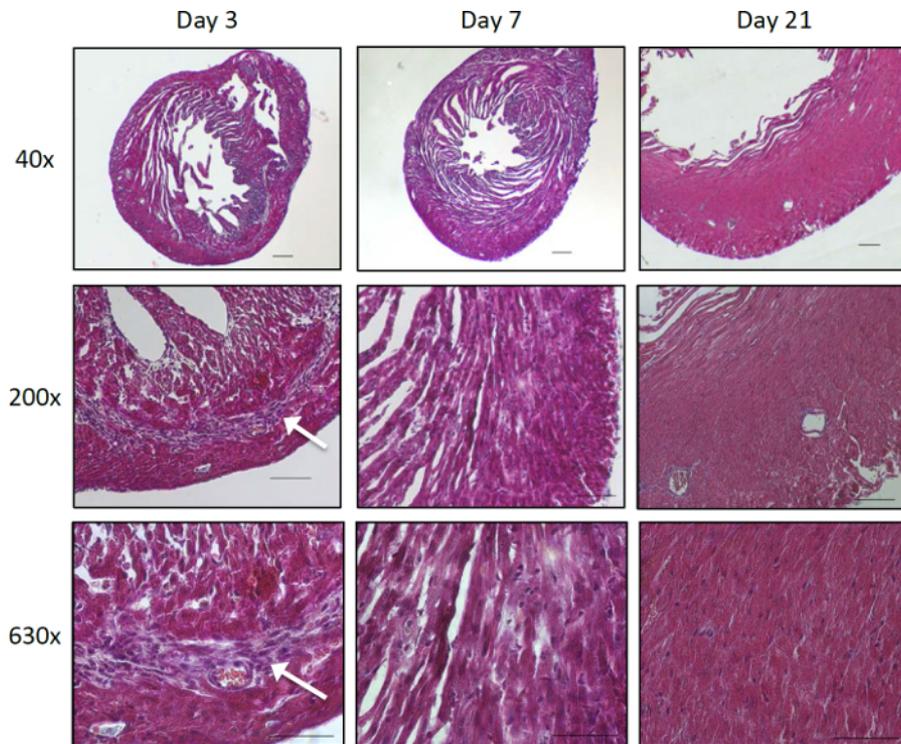


Figure 2. Evidence of Complete Cardiac Regeneration After LAD Ligation in Neonatal Mice. Masson's trichrome staining after LAD ligation in P1 mice. Whole hearts and magnifications at the infarction areas are shown on day 3, 7 and 21 after LAD ligation. Note myocyte loss and inflammatory cell infiltrate in infarct region at day 3 post-MI (white arrow). Complete infarct zone regeneration beginning at day 7 post-MI. Little evidence of fibrosis noted at day 21 post-MI. Scale bars are 200, 100 and 50 μ m for 40X, 200X, and 630X magnifications, respectively. [Please click here to view a larger version of this figure.](#)

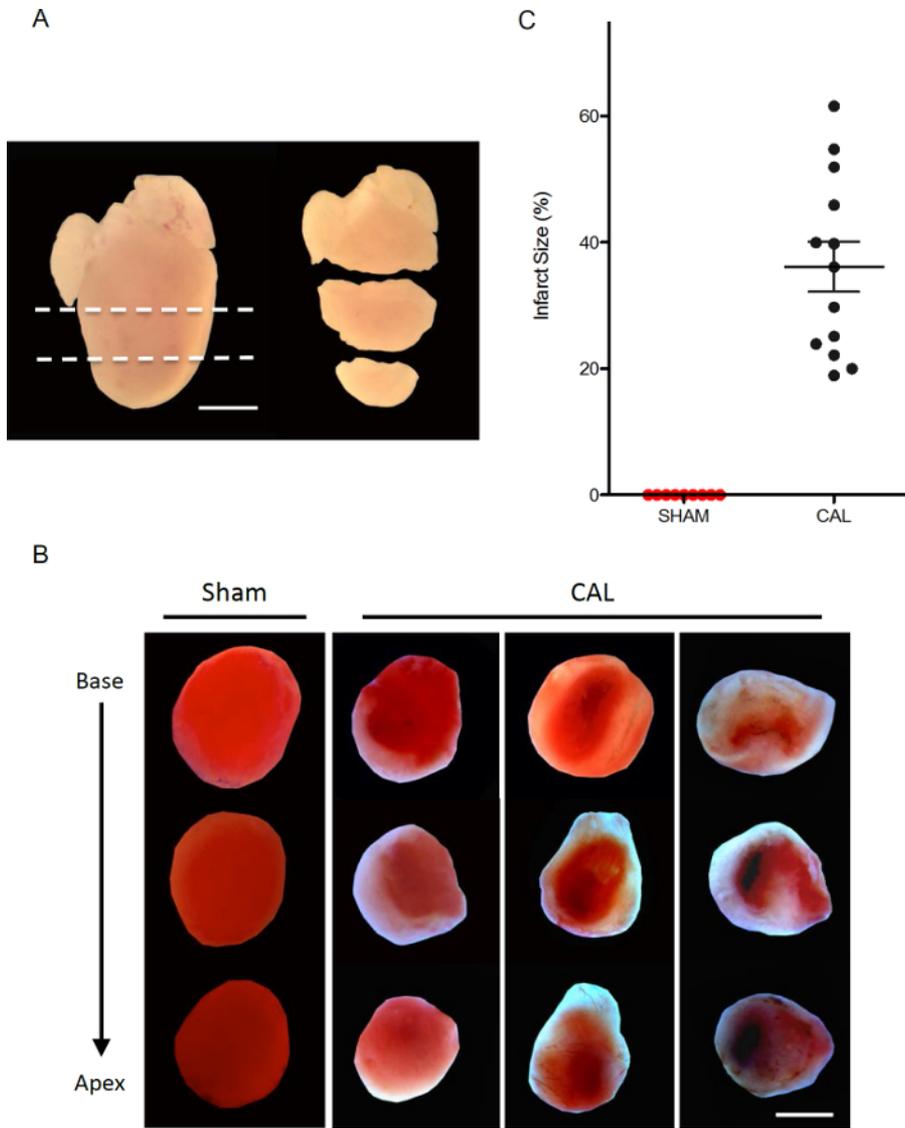


Figure 3. Neonatal LAD Ligation is 100% Reproducible. **A)** Lines of sectioning of whole heart to create a 0.75 mm apex piece and a 1 mm mid-base piece for TTC staining. **B)** Representative images of TTC stained sham control and LAD ligated hearts. Scale bars are 1 mm. **C)** Infarct size in 13 LAD ligations in P1 neonatal mice. Hearts collected 4 - 6 hr post-MI and infarct size measured after TTC staining. Red indicates viable myocardium; white indicates necrosis. Data are mean \pm SEM. [Please click here to view a larger version of this figure.](#)

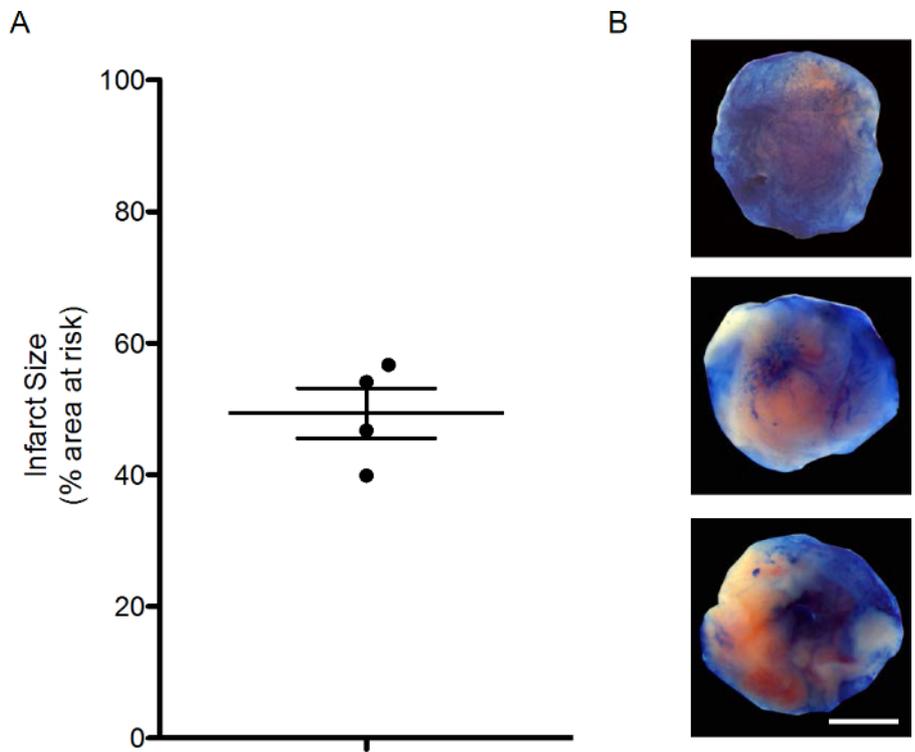
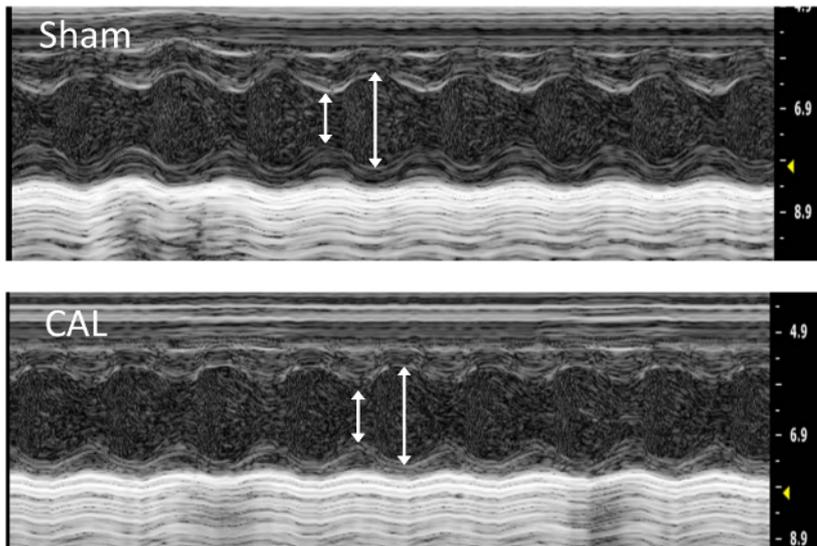


Figure 4. Neonatal LAD Ligation Results in Reliable Infarction. **A)** Infarct size as % of ischemic area after LAD ligation in P1 neonatal mice (n=4). Hearts were collected 24 hr post-MI and infarct size was measured after Evans blue and TTC staining. Blue indicates viable myocardium; red indicates area at risk; white indicates necrosis. Data are mean \pm SEM. **B)** Representative images of Evans blue and TTC stained LAD ligated hearts after LAD ligation. Scale Bar = 1 mm. [Please click here to view a larger version of this figure.](#)

A



B

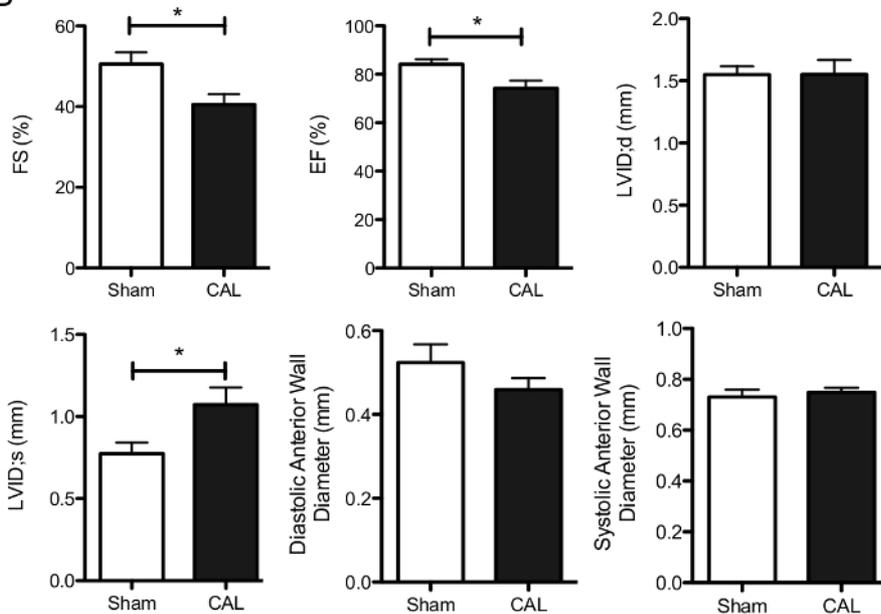
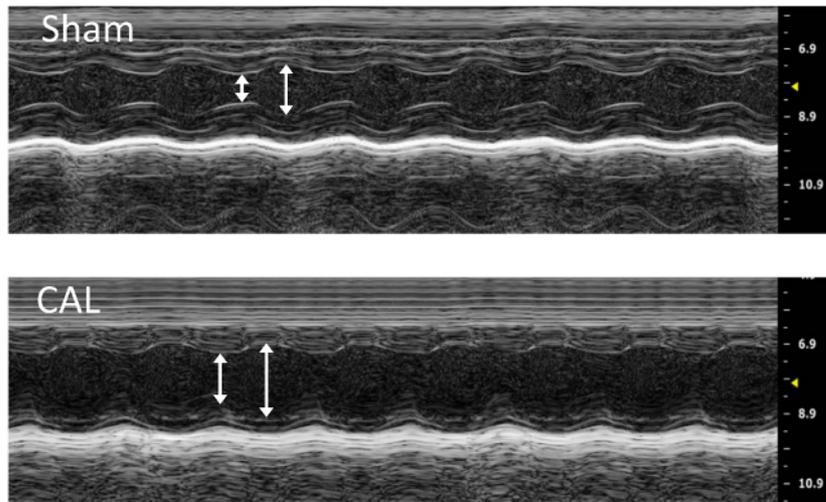


Figure 5. Neonatal LAD Ligation Results in Cardiac Dysfunction at 24 hr post-MI. Cardiac function was assessed by echocardiography. **A)** Representative M-mode images for both sham operated and LAD ligated mice. End-diastole and end-systole are indicated by arrows. **B)** Measurements of fractional shortening, ejection fraction, and left ventricular internal diameter at both diastole and systole. Data are mean ± SEM. N = 8 and 7 for sham and coronary artery ligation (CAL) respectively. * P<0.05. [Please click here to view a larger version of this figure.](#)

A



B

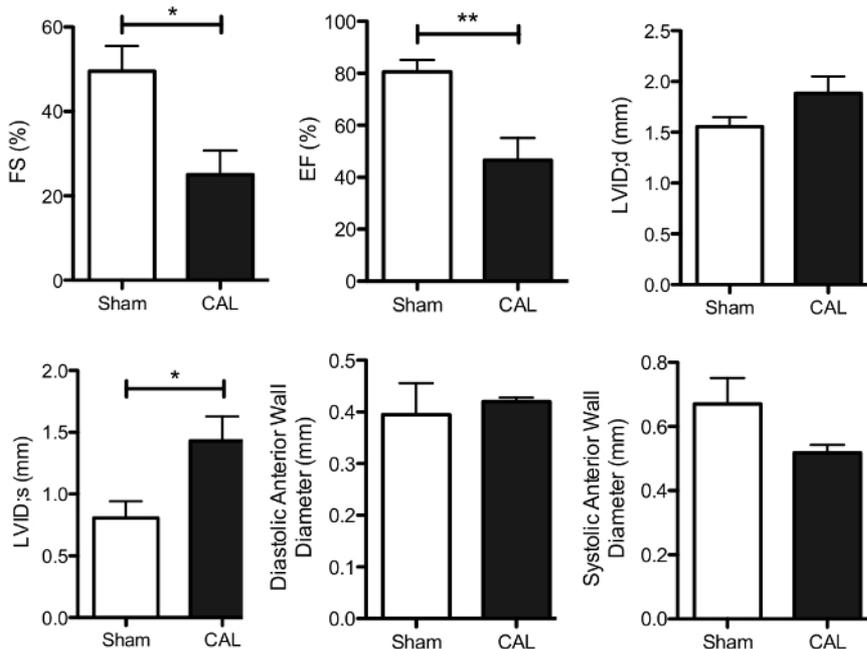


Figure 6. Neonatal LAD Ligation Results in Cardiac Dysfunction at 48 hr post-MI. Cardiac function was assessed by echocardiography. **A)** Representative M-mode images showing end-diastole (long arrows) and end-systole (short arrows) for both sham operated and LAD ligated mice. **B)** Cardiac functional measurements by echocardiography including fractional shortening, ejection fraction, and left ventricular internal diameter at both diastole and systole. Data are mean ± SEM. N = 4 and 6 for sham and coronary artery ligation (CAL), respectively. * P<0.05, ** P<0.01. [Please click here to view a larger version of this figure.](#)

Discussion

The surgical LAD ligation demonstrated herein is a reliable method to produce MI in neonatal mice. This model provides researchers with a reproducible model with which to study mammalian heart regeneration. Visualization of the coronary vasculature is a key component of this method, ensuring correct suture placement and thus guaranteeing reproducibility. While adult mice do not possess poikilothermic capabilities, the body temperature and metabolic rate of neonatal mice is closely associated with ambient temperature. Furthermore, the small size of neonatal mice makes them ideal for hypothermic induction by surface cooling. The timing of surgery and mouse body temperature are crucial for accuracy in replicating this procedure and thus must be monitored carefully. Ligation as soon as possible after torpor is reached provides the opportunity to visualize blanching of the myocardium post-ligation, confirming surgical success.

There are multiple methods that can be used to confirm MI induction. These include TTC staining, echocardiography and histological analysis. TTC staining is low cost, reproducible and reliable, and easily performed with high throughput. For these reasons, TTC staining has been widely used. Echocardiography can be used to non-invasively monitor changes in cardiac function post-MI over time. Definitive proof of MI can be

demonstrated through histological analysis. In the present study, all three of the above techniques were employed to verify successful induction of MI.

Visualization of LAD is critical to successful MI induction. If the investigator is experiencing difficulty visualizing the LAD (likely due to deep hypothermia induction), the left auricle may be lifted slightly with forceps, as the main root of the LAD is larger than the more distal segments and may be more easily visible. If the LAD cannot be visualized, the investigator should not presume artery location. This pup should not be used for experimental ligation and could rather be used as a sham operated control.

Correct entry into the chest cavity at the 4th intercostal space is important to gain optimal visualization and space for LAD ligation. This can be achieved through careful counting of the intercostal spaces. Additionally, if a minor amount of bleeding occurs during the procedure, it can be removed with sterile gauze. If bleeding is significant, however, the surgeon has likely not cooled the mouse sufficiently before beginning the procedure, and further cooling is required. Please note that the degree of cooling should be carefully controlled to ensure heart rate at around 20 bpm in order to visualize the coronary artery.

Due to the small size of neonatal mice, high resolution echocardiography is required to obtain clear M-mode images for analysis of cardiac function. For best results, ensure heart rate at about 400 bpm with light anesthesia (0.5 - 1% isoflurane inhalation) and maintain normal body temperature (heated dock and warmed echo gel). Images are best acquired if the body is tilted slightly towards the right shoulder (~5° cranially and 5° rightward). Ensure sufficient echo gel is applied to minimize noise.

Although this approach does offer 100% reproducibility, the size of the infarct may be somewhat variable depending on the coronary artery branching anatomy of the individual neonate (**Figure 3**). As a result, higher *n* numbers may be required to reach statistical significance in determining the effects of different treatments or genetic manipulations on cardiac regeneration. However, this approach holds major advantages over both neonatal apex resection and cryoinjury as the pathophysiology of these mechanisms of injury may be entirely different from MI and thus may not accurately represent human MI^{8,20}. This approach more directly mimics typical human injury, and may be used to study cardiac regeneration in the setting of mammalian MI.

This model is unique as it allows for the study of the differences in the mechanisms of recovery from cardiac injury between the non-regenerative (adult) and the regenerative (neonatal) mammalian myocardium. For example, the adult mouse MI model results in permanent scar formation. Ventricular rupture often occurs as a result of infarct expansion. In our experience with this model (>100 surgeries; unpublished data), complete regeneration is evident 21 days post-infarction and ventricular rupture has not been observed. The information accrued from use of this model may offer novel insights into mechanisms that may promote cardiac regeneration in the adult.

Recent evidence indicates cardiomyocyte proliferation is a major contributor to cardiac regeneration⁹. This is in contrast to previous studies suggesting substantial contributions from progenitor cell pools, and has directed research efforts to promote cardiomyocyte proliferation rather than progenitor cell activation. For example, Meis1 and neuregulin 1 signaling have already been identified as critical regulators of cardiomyocyte proliferation and neonatal cardiac regeneration^{4,21}. Further research using the protocol presented here may point to therapeutic targets for induction of cardiac regeneration post-MI.

Disclosures

The authors have nothing to disclose.

Acknowledgements

This study was supported by an operating grant from the Canadian Institutes of Health Research (CIHR) to Q.F. (grant #MOP-119600).

References

- Rosamond, W. *et al.* Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. **117** (4), e25-146 (2008).
- Meta-analysis Global Group in Chronic Heart Failure. The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: an individual patient data meta-analysis. *Eur Heart J*. **33** (14), 1750-1757 (2012).
- Soonpaa, M. H., & Field, L. J. Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. *Am J Physiol*. **272** (1 Pt 2), H220-226 (1997).
- D'Uva, G. *et al.* ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. *Nat Cell Biol*. **17** (5), 627-638 (2015).
- Oberpriller, J. O., & Oberpriller, J. C. Response of the adult newt ventricle to injury. *J Exp Zool*. **187** (2), 249-253 (1974).
- Poss, K. D., Wilson, L. G., & Keating, M. T. Heart regeneration in zebrafish. *Science*. **298** (5601), 2188-2190 (2002).
- Jopling, C. *et al.* Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature*. **464** (7288), 606-609 (2010).
- Porrello, E. R. *et al.* Transient regenerative potential of the neonatal mouse heart. *Science*. **331** (6020), 1078-1080 (2011).
- Haubner, B. J. *et al.* Complete cardiac regeneration in a mouse model of myocardial infarction. *Aging (Albany NY)*. **4** (12), 966-977 (2012).
- Soonpaa, M. H., Kim, K. K., Pajak, L., Franklin, M., & Field, L. J. Cardiomyocyte DNA synthesis and binucleation during murine development. *Am J Physiol*. **271** (5 Pt 2), H2183-2189 (1996).
- Li, F., Wang, X., Capasso, J. M., & Gerdes, A. M. Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. *J Mol Cell Cardiol*. **28** (8), 1737-1746 (1996).
- Feng, Q. *et al.* Elevation of an endogenous inhibitor of nitric oxide synthesis in experimental congestive heart failure. *Cardiovasc Res*. **37** (3), 667-675 (1998).

13. Xiang, F. L. *et al.* Cardiomyocyte-specific overexpression of human stem cell factor improves cardiac function and survival after myocardial infarction in mice. *Circulation*. **120** (12), 1065-1074 (2009).
14. van Kats, J. P. *et al.* Angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade prevent cardiac remodeling in pigs after myocardial infarction: role of tissue angiotensin II. *Circulation*. **102** (13), 1556-1563 (2000).
15. Mahmoud, A. I., Porrello, E. R., Kimura, W., Olson, E. N., & Sadek, H. A. Surgical models for cardiac regeneration in neonatal mice. *Nat Protoc*. **9** (2), 305-311 (2014).
16. Ahn, D. *et al.* Induction of myocardial infarcts of a predictable size and location by branch pattern probability-assisted coronary ligation in C57BL/6 mice. *Am J Physiol Heart Circ Physiol*. **286** (3), H1201-1207 (2004).
17. Kao, W. W., Xia, Y., Liu, C. Y., & Saika, S. Signaling pathways in morphogenesis of cornea and eyelid. *Ocul Surf*. **6** (1), 9-23 (2008).
18. Redfors, B., Shao, Y. Z., & Omerovic, E. Myocardial infarct size and area at risk assessment in mice. *Experimental & Clinical Cardiology*. **17** (4), 268-272 (2012).
19. Phifer, C. B., & Terry, L. M. Use of hypothermia for general anesthesia in preweanling rodents. *Physiol Behav*. **38** (6), 887-890 (1986).
20. Jesty, S. A. *et al.* c-kit+ precursors support postinfarction myogenesis in the neonatal, but not adult, heart. *Proc Natl Acad Sci U S A*. **109** (33), 13380-13385 (2012).
21. Mahmoud, A. I. *et al.* Meis1 regulates postnatal cardiomyocyte cell cycle arrest. *Nature*. **497** (7448), 249-253 (2013).