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Ovine Model of Ischemic Mitral Regurgitation

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Abstract

Ischemic mitral regurgitation (IMR) is a common complication of ischemic heart disease that doubles mortality after myocardial infarction and is a major driving factor increasing heart failure. IMR is caused by left ventricular (LV) remodeling which displaces the papillary muscles that tether the mitral valve leaflets and restrict their closure. IMR frequently recurs even after surgical treatment. Failed repair associates with lack of reduction or increase in LV remodeling, and increased heart failure and related readmissions. Understanding mechanistic and molecular mechanisms of IMR has largely attributed to the development of large animal models. Newly developed therapeutic interventions targeted to the primary causes can also be tested in these models. The sheep is one of the most suitable models for the development of IMR. In this chapter, we describe the protocols for inducing IMR in sheep using surgical ligation of obtuse marginal branches. After successful posterior myocardial infarction involving posterior papillary muscle, animals develop significant mitral regurgitation around 2 months after the surgery.

Keywords

Mitral regurgitation; myocardial infarction; sheep; echocardiography; tethering; papillary muscle

1. Introduction

Ischemic mitral regurgitation (IMR) is a common complication of ischemic heart disease that doubles mortality after myocardial infarction (MI) and is a major driving factor

increasing heart failure [1,2]. Moderate or greater IMR occurs in ~500,000 patients/year in the U.S. – 20% of those with new MI and 50% with systolic left ventricle (LV) failure [3–5]. Experimental and human studies have revealed a wide range of attributable factors, including mitral annular dilatation [6,7], leaflet tethering [8,9], altered LV geometry [10] and insufficient leaflet adaptations [11,12]. IMR is caused by ischemic LV distortion: inferior wall bulging displaces the papillary muscle (PM) to which the leaflets are anchored [13–19,10,20–24,9,8]. This tethers the leaflets into the LV cavity and restricts their closure [25,26]. IMR reflects a deficiency in mitral valve (MV) leaflet tissue relative to the dilating ventricle [12]. Surgery for ischemic MR remains challenging; standard surgical therapy includes annular ring reduction (improving leaflet apposition by correction of posterior annular dilatation). Operative mortality is higher than in organic MR and the long-term prognosis is worse. The NHLBI-sponsored CTSN (Cardiothoracic Surgical Trials Network) has shown that annular ring reduction for IMR often fails: persistent MV tethering causes recurrent MR[27–32] in 33% of patients at 1 year and 59% at 2 years.[33–36] Failed repair associates with lack of reduction or increase in LV remodeling, with increased heart failure and related readmissions[34,37].

Understanding mechanistic and molecular mechanisms of IMR has attributed to the development of large animal models close to the human. Newly developed therapies targeted to the primary causes can also be tested in these models. Sheep and swine resemble the coronary anatomy of humans closely, whereas dogs have an extensive coronary collateral supply and much faster heart rates [38]. The relatively comparable body size of sheep and swine and similar coronary anatomy and vasomotor responsiveness to human make them relevant for utilization of multiple diagnostic and therapeutic strategies. The most popular and classic ovine or swine model of chronic IMR can be made by ligating the obtuse marginal branches to induce a posterolateral infarct with PM involvement, which results in significant (moderate or greater) IMR. These highly reproducible models require a thoracotomy to occlude the target vessels [21]. Although the percutaneous techniques were developed, open chest models are usually preferred because they can allow more comprehensive echo views and better resolution.

Advantage of sheep animal model

The sheep is one of the most suitable models for cardiovascular research because it can be handled easily. The ovine model is currently accepted as the gold standard for mitral and aortic valve replacement. The size of the heart and chest cavity and vascular anatomy resemble those of the human. At cellular and molecular levels, the predominant (~100%) myosin heavy chain isoform in the sheep heart is similar to humans (~95%). The resting heart rate (60–120 bpm), systolic (~90–115 mmHg) and diastolic (~100 mmHg) pressure in sheep are akin to humans and so are the hemodynamic responses. However, sheep contractile and relaxation kinetics are slightly faster than humans [39]. Sheep do not have an abundant network of coronary collaterals like dogs and have a left-dominant coronary system, and the left and right coronary arteries communicate with only minor overlap [40]; occlusion of a coronary artery can make distinct ischemic injuries with sharp border zone regions accordingly.

Mitral valve anatomy

Sheep heart has four cardiac valves with principally similar structures and locations to human. The gross morphology of the chordae attaching at the valve leaflet and the PM is very similar; on average, there are 12 chordae tendineae in each of the MV leaflet [41]. The anterior-posterior diameter of the mitral annulus is significantly smaller than that of human (25.8 ± 6.3 vs. 32.5 ± 5.6 mm), while the intercommissural diameters are similar. Notably, the fibrous continuity between the two fibrous trigones, termed the membranous septum, is completely absent in sheep [42].

Echocardiography

Echocardiography in the non open-chest model is challenging. Keel-shaped chest with narrow intercostal spaces makes difficult to locate the ultrasound transducer and limits the acoustic window. In addition, the presence of gas in the reticulo-rumen hampers the acquisition of subcostal and apical views [45]. Transducers with frequencies up to 5.0 MHz are generally preferred. During general anesthesia, sheep usually lie on its right decubitus position for left lateral thoracotomy. In this position, it is difficult to acquire apical images of the heart including apical 4, 3, 2 chamber views. Only parasternal short and long axis views are available so the parameters to be acquired are limited. Placing the probe on the left 4th intercostal space, cranio-dorsally oriented with 0° to 20° rotation, the left parasternal long axis view showing the LV outflow tract can be obtained. To acquire the short axis images of the above areas, the probe needs to be rotated perpendicular to the long axis plane, scanning from apex to base. Placing the transducer on the 4th or 5th inter-costal space, just dorsal to the sternum and aiming dorsally and to the right, left parasternal four chamber or five chamber views can be obtained [46]. Even after left thoracotomy, although the resolution and image qualities are improved indeed (Figure 1), making a pericardial cradle to suspend the heart [47,11,48] is essential to get apical images (See Note 1).

All parameters can be indexed to the body surface area (BSA) using the following equation.

$$BSA (m^2) = 0.84 \times \text{body weight (Kg)}^{0.66}$$

Several studies have proposed reference values for echocardiographic parameters in healthy sheep [46,49–51]. However, care should be taken for interpretation of the results because the parameters will be affected by using sedatives or anesthetic agents.

Optimal occlusion site for inducing IMR

Posterior myocardial infarction produces MR more often than anterior infarction [52]. The roles of papillary muscle infarction and annular dilatation in the pathogenesis of IMR are crucial. In previous studies, large acute posterior infarctions (32 to 35% of total LV, occlusion of OM1,2,3), involving the posterior PM, produce a moderate degree of acute MR [53,54] and severe MR at several weeks later. After larger infarction with posterior PM

¹-Forming a pericardial cradle allows suitable fields for operation, an exposure of apex, which facilitates apical window of echocardiography and the insertion of high-fidelity catheter used for pressure-volume loop. It can be created by suturing multiple single stitches longitudinally along the open edges and attaching them to the chest retractor to keep the heart suspended throughout the procedure.

infarction by occlusion of OM2, 3 and PDA (around 40% of total LV), severe MR developed in all sheep immediately after infarction [21]. However, two branch occlusion (usually OM2,3) model is usually preferred, because long-term survival is threatened in large infarction models. In one study, ligation of OM2 and OM3 infarcted $21.4 \pm 4.0\%$ (moderate infarct) of the LV with complete infarcts of the posterior PM and 11 out of 11 sheep developed a moderate degree of MR by six weeks after MI creation. Keep in mind that only 1+ MR develops immediately after MI creation when you ligate OM2 and OM3 [21] (Figure 2). In our lab, after successful induction of moderate posterior infarctions (OM 2 and 3 occlusion), 2-month survival rate was 92% and that of 6-month was 83%.

2. Materials

1. Adult Dorsett hybrid Sheep (42–46kg) (See Note 2)
2. Heated surgical table to maintain body temperature, table pad
3. Mechanical ventilator and respiration hose for large animals
4. Anesthesia machine with isoflurane vaporizer
5. Vital monitors including pulse oximeter, blood pressure monitor, capnograph, rectal temperature monitor, ECG monitor
6. Portable warming lamp
7. Defibrillator with internal paddles
8. Centrifuge
9. -80°C freezer
10. Liquid nitrogen
11. Vacutainers for blood sampling and cryogenic vials
12. Induction: Intravenous propofol
13. Alcohol swabs
14. Endotracheal tube (7.5–8.0mm)
15. Laryngoscope
16. Anesthesia: isoflurane gas inhalant
17. Artificial tears to prevent drying of eyes
18. Tube gauze
19. Suction canister, suction tubing and suction tip
20. Sterile syringes, needles, etc.

²There is no data regarding gender difference in sheep after IMR model development. However, it is well recognized that there are distinct gender differences in epidemiology, pathophysiology, clinical manifestations, and outcomes of human MV and AV disease [43]. Life expectancy of a sheep is about 10 to 12 years. The use of sheep around 12 months of age, 40–45 kg allows for the testing of valve replacement, ring annuloplasty, because their valve size is comparable to that of humans [44]. The use of animals of similar age, weight and same gender is recommended for a good achievement.

21. 70% isopropyl alcohol
22. Povidone iodine
23. Hydrogen peroxide
24. Hair clippers (Oster size 40)
25. 18G angiocatheter for intravenous access
26. Fabric tape to secure IV lines
27. IV fluids (sodium chloride and lactated ringer's solution)
28. Amiodarone (50mg/mL)
29. Analgesic: buprenorphine (0.3mg/mL)
30. Antibiotic: cefazolin (1g/vial)
31. Glycopyrrolate (0.2–0.4mg)
32. Standard emergency drugs
33. Ground plate
34. Cautery
35. #15 scalpel blade
36. Basic surgical pack containing sterile drapes
37. Sterile towels
38. Sterile surgical instruments and chest retractor
39. 0.7% Iodine povacrylex
40. Echocardiography machine
41. Sterile echo transducer covers
42. 5Fr high-fidelity conductance catheter for the acquisition of pressure-volume loops
43. Control unit and connected laptop for the pressure-volume loops acquisition
44. Sutures: Silk and Prolene and Vicryl
45. 11 blade
46. 18Fr chest tube
47. Bupivacaine 0.5% (5mg/mL)
48. Lidocaine (20mg/mL)
49. Heparin sodium (1,000 USP units/mL)
50. Furosemide (10mg/mL)
51. Flunixin meglumine (50mg/mL)

52. Triple antibiotic ointment

3. Methods

3.1. IMR induction

1. Administer amiodarone 200mg PO once daily for 2–3 days prior to surgery to prevent arrhythmia during surgery.
2. NPO the animal overnight prior to surgery.
3. After induction with propofol 0.5–1.5mg/kg IV, shave the left jugular vein site with clippers and clean area with povidone-iodine and 70% isopropyl alcohol.
4. Cannulate the left jugular vein using an 18 G angiocatheter.
5. Intubate the animal with a 7.5–8.0mm endotracheal tube (depending on size of animal) with the aid of a laryngoscope.
6. Secure endotracheal tube with tube gauze.
7. Place animal on its right side down on heated surgical table and secure legs.
8. Ventilate animal at 15mL/kg with 2–4% isoflurane and oxygen 3–4L/min.
9. Place the vital monitors and begin monitoring and documenting isoflurane level and vital signs including respiration rate, heart rate, SpO₂, ETCO₂, blood pressure, muscle tone, and body temperature.
10. Apply artificial tears to both eyes to prevent drying.
11. Shave and clean left saphenous intravenous access site with 70% isopropyl alcohol and povidone iodine.
12. Obtain intravenous access in the left saphenous vein using an 18 G angiocatheter.
13. Begin administration of IV fluids: lactated Ringer's solution and sodium chloride solution containing amiodarone 50mg to prevent arrhythmia.
14. Administer analgesic buprenorphine 0.008–0.01mg/kg, glycopyrrolate 0.4mg to limit perioperative secretions of tracheal and bronchial secretions, and antibiotic cefazolin 1g intravenously at least 15 minutes prior to chest wall incision.
15. Prepare surgical site using aseptic technique by shaving skin and cleaning site with 70% isopropyl alcohol, povidone iodine.
16. Cover the animal with sterile drapes.
17. Clean surgical site with 0.7% Iodine povacrylex solution applicator to ensure asepsis of the skin.
18. Using a sterile #15 blade scalpel, make an approximately 13cm long skin incision between and parallel to the 4th and 5th ribs (Figure 4A).
19. Divide intercostal muscles and cauterize small bleedings.
20. Administer intercostal nerve block with 0.5% bupivacaine 0.5–1.0mg/kg IM.

21. Place chest retractors and gently separate ribs being careful to avoid rib damage.
22. Open the pericardium to allow for coronary interventions and imaging. Following opening the pericardium from the apex to the base, a cradle is created (**Pericardial cradle creation, see Note 1**).
23. Before myocardial infarction creation, acquire baseline two and three-dimensional echocardiography images using sterile echo transducer covers (**see Note 3**) and record baseline hemodynamic parameters (Pressure-volume loop acquisition) using a 5F high fidelity conductance catheter placed in the left ventricle via the apex of the heart. Close the insertion site using purse string suture with Prolene (4–0).
24. Using Prolene (4–0) suture, permanently ligate the second and third obtuse marginal branches of the left circumflex coronary artery at their origins for myocardial infarction creation (Fig 4B, **see Note 4**).
25. Repeat echocardiography and hemodynamic data acquisition as described in step 3.1 (**See Note 5**).
26. Following completion of data collection and confirmation of stable vital sign and no recurrent ventricular arrhythmias, remove chest retractors.
27. Using an 11 blade, make a small (6–7mm) incision between the 5th and 6th intercostal spaces and insert a 18Fr chest tube.
28. Begin closing the chest by approximating the ribs using Vicryl sutures.
29. Close the muscle in three layers using Vicryl antibacterial sutures and close the skin using Vicryl (3–0) sutures.
30. Administer 0.5% bupivacaine (0.5–1.0mg/kg) intrapleurally via the chest tube for additional analgesia.
31. Evacuate the chest and remove the chest tube under negative pressure.
32. Administer furosemide 20–40mg and cefazolin 1g intravenously.
33. Wean animal off isoflurane general anesthesia in decrements of 0.5% until 0% is reached.
34. Remove left saphenous IV access and apply manual pressure to site for approximately 10–15 minutes until hemostasis is achieved.
35. Clean surgical sites and IV access sites with hydrogen peroxide and apply triple antibiotic ointment.

³For epicardial echocardiography, a sterile surgical probe cover (commercially available) can be used. A sterile latex glove soaked with ultrasound gel is a good alternative for the probe cover. Enough ultrasonic gel has to be applied for reducing near field artifact.

⁴Using Prolene 4–0 (BB needle) suture, permanently ligate the second and third obtuse marginal branches of the left circumflex coronary artery at their origin for myocardial infarction creation. The Prolene suture should be placed around the OM2 and OM3 branches approximately 3–4mm deep and 4mm wide.

⁵In our lab, considering the coronary artery anatomic variation, we ligate OM2 first. After carefully reviewing the extent of myocardial discoloration under direct visualization and echo images (regional wall motion abnormalities (RMWs) and papillary muscle involvement) (Figure 3), we decide whether we ligate OM3 additionally. See movie clips of echocardiographic images of normal and PM dysfunction after infarction (Movie 1A–E).

36. Move animal to transport pen for recovery from anesthesia before returning to animal facility.
37. Monitor all vital signs during recovery period including heart rate, SpO₂, blood pressure, and respiratory rate.
38. Once the animal begins to breathe on its own, decrease mechanical ventilator support until off and allow the animal to breathe room air while continuously monitoring SpO₂.
39. Extubate the animal when alert, swallowing, moving its head, and able to breathe normally on its own.
40. Continuously observe and monitor animal to ensure smooth and comfortable recovery while minimizing stress and discomfort.
41. Give additional buprenorphine 0.008–0.01mg/kg IM or flunixin meglumine 1–2mg/kg IM if pain is evident (fast heart rate, fast respiration rate, shaking, high blood pressure, teeth grinding).
42. Once conscious of environment, responding to external stimuli, and recovered from anesthesia, transport the animal back to the animal facility and continue to monitor post-operatively.
43. Provide post-operative analgesia with buprenorphine 0.008–0.01mg/kg IM every 8–12 hours for 72–96 hours plus as needed and flunixin meglumine 1–2mg/kg IM once every 24 hours for 72–96 hours post-operatively.
44. Observe and monitor animals 3–4 times daily for 3 days following surgery to ensure smooth and successful recovery.
45. Observe, interact with, and encourage socialization and activity during the post-operative period and daily thereafter to provide environmental enrichment and comfort.
46. For post-mortem assessment of infarct size and MV apparatus, see Note 6.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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⁶.After eviscerating the heart, we usually open the left atrium first, and dissect the LV wall from the anterolateral commissure thorough anterior-lateral PM (Fig 4C-D). The photos with a ruler can be used for the further image analyses including infract area and mitral leaflet area. To establish a standard dissection method (Fig 4C), communication with other researchers is encouraged (especially for histological analyses).

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Figure 1. Representative echocardiographic images available without pericardial cradle creation (A-G: routine parasternal views, H and I: lower parasternal views).

An RV focused view. **B** LV focused view. **C** Parasternal short axis view of the LV at MV level. **D** parasternal short axis view of the LV at PM level. **E** M-mode tracing of the LV at the mid-ventricular short axis. **F** short axis image of the LV at PM base (more apically displaced). **G**. Lower parasternal MV focused view. **H** lower parasternal 3 chamber view. **I** lower parasternal 5 chamber view.

Figure 2. Development of MR after MI creation.

A no MR at baseline. **B and C** Trivial MR developed after MR creation. Even after OM2 and OM3 occlusion, only trivial MR develops immediately after MI creation. **D** Significant MR developed 2 months after MI creation.

Figure 3. Identification of PM dysfunction and regional wall motion abnormalities.
A Modified parasternal long axis image showing PM and attached chordae. **B** Short axis image. You can see the dysfunctional PM (yellow arrow) by infarction and non-contracting myocardium (white arrows). See also movies 1A-E.

Figure 4.

A Incision site of lateral thoracotomy. **B** Occlusion of OM2 and OM3. **C** Illustration of the LV and MV dissections. **D** Real photo of dissected LV. The left atrium was opened and cut, and the LV wall was dissected from the anterolateral commissure thorough anterior PM.