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# Organ Ischemia-Reperfusion Injury by Simulating H emodynamic Changes in a Rat Liver Transplant Model

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**TITLE:**

# Organ Ischemia-Reperfusion Injury by Simulating Hemodynamic Changes in Rat Liver Transplant Model

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**KEYWORDS**

Animal model, Anhepatic Phase, Ischemia-reperfusion injury, Orthotopic liver transplantation

**SUMMARY**:

This paper provides a detailed description of how to build an animal model of the anhepatic phase (liver ischemia) in rats to facilitate basic research into ischemia-reperfusion injury after liver transplantation.

**ABSTRACT**

Orthotopic liver transplantation (OLT) in rats is a tried and proven animal model used for preoperative, intraoperative, and postoperative studies, including ischemia-reperfusion injury (IRI) of extrahepatic organs. This model requires numerous experiments and devices. The duration of anhepatic phase is closely related to the time to develop IRI after

transplantation. In this experiment, we used hemodynamic changes to induce extrahepatic organ damage in rats and determined the maximum tolerance time. The time until the most severe organ injury varied for different organs. This method can easily be replicated and can also be used to study IRI of the extrahepatic organs after liver transplantation.

**INTRODUCTION:**

Ischemia-reperfusion injury (IRI) is a common complication after liver transplantation. Hepatic IRI is a pathological process involving ischemia-mediated cell damage and abnormal deterioration of liver reperfusion. Hepatic IRI and the local innate immune response can be divided into hot and cold IRI, according to differences in the clinical environment[1]. Hot IRI is induced by stem cell injury, usually as a result of liver transplantation, shock, and trauma[2]. Cold IRI is a complication of liver transplantation caused by endothelial cells and peripheral circulation[3]. Clinical reports have shown that hepatic IRI is associated with 10% of early organ failures and may increase the incidence of acute and chronic rejection[4,5]. In addition, hepatic IRI may also induce multiple organ dysfunction syndromes or systemic inflammatory response syndrome, with high mortality[6]. Patients with extrahepatic organ involvement tend to stay longer in the hospital, spend more money, and have a worse prognosis[7]. The development of complications is closely related to the length of the anhepatic phase of liver transplantation[8].

Orthotopic liver transplantation (OLT) in rats was first reported by the American professor Lee in 1973. The experimental operation simulated the steps of clinical liver transplantation and the anastomosis of blood vessels and the common bile duct (CBD) using the suture method. The procedure is difficult and time-consuming with a low rate of success[9]. In 1979, Kamada et al. made a significant improvement to OLT in rats by creatively using the 'two-cuff method' for anastomosis of the portal vein to control the anhepatic phase within 26 minutes[10]. In the same year, Zimmermann proposed the 'single biliary stent method.' On the basis of Lee's work, Zimmermann used polyethylene tubes to directly anastomose the CBD of the donor and recipient, simplified the reconstruction of CBD, and preserved the function of the sphincter, and this method became the standard for biliary reconstruction of OLT models[11]. In 1980, Miyata et al. proposed the 'three-cuff method' where the portal vein (PV), suprahepatic vena cava (SVC), and intrahepatic vena cava (IVC) were anastomosed by the cuff method. However, there is a risk of distortion of the cannula with this method, which can lead to the obstruction of inferior vena cava reflux[12]. In 1983, the 'two-cuff method' was proposed using the cuff method for anastomosis of the PV and IVC, but adopting the suture method for the SVC[13]. This method was adopted by scholars globally to establish OLT models. Since then, the cuff anastomosis steps have been improved to shorten the anhepatic phase and improve the survival rate of the rats[14]. Similarly, improved methods are used in clinical practice to shorten the anhepatic phase[15]. However, basic research into IRI after liver transplantation has shown that the survival rate is inversely related to the degree of injury to extrahepatic organs. Therefore, further research is required, and a simple and reproducible animal model is needed to simulate IRI after liver transplantation.

Based on the definition of the anhepatic phase, we simulated the hemodynamic changes in

liver transplantation resulting in IRI of extrahepatic organs in rats. Herein, we provide a detailed description of how to build an animal model of the anhepatic phase (liver ischemia) in rats to facilitate basic research into IRI after liver transplantation.

## PROTOCOL

The Animal Ethics Committee approved the experiment of Guangxi Medical University (No20190920). All animals were supplied by the Animal Experiment Center of Guangxi Medical University. We used SPF male Sprague Dawley rats (200-250 g, 10-12 weeks), kept under the room temperature of 25 ± 2˚C and humidity of 50 ± 10%. Feeding was stopped 24 hours before operation; however, water was provided.

NOTE: One operator can perform all operations without a microsurgery basis or surgical microscope.

### 1. Operation

1.1. After weighing, anesthetize the rats with isoflurane (5%) using an animal anesthesia machine.

1.2. After 1-2 minutes, gently clamp the toes of the rat with tweezers. If the rat does not respond after pinching, it has entered a state of anesthesia. Use vet ointment on the eyes to prevent dryness. Use animal heating lamps to keep the rats' body temperature at 37-38 °C.

1.3. Following abdominal disinfection (povidone iodine solution), fix the rat on the animal dissection table. Make a median incision of 3 cm below the xiphoid process using forceps and scissors.

1.4. Open the abdominal cavity, expose the liver using a retractor, and mobilize the hepatogastric ligament. Use cotton swabs to flip the middle lobe of the liver gently and turn it upward to expose the porta hepatis. Identify the CBD, PV, and HA.

1.5. Push the small intestine toward the left lower abdominal cavity using cotton swabs, cover it with wet gauze, and move the intrahepatic vena cava to the right renal vein.

1.6. Isolate the portal vein, hepatic artery, and the inferior vena cava above the right renal vein with an intraocular lens and forceps marked with 3-0 silk thread, each with a slip knot.

1.7. Cut open the left and right lower extremity skin and expose the femoral vein using ophthalmic forceps. Slowly inject low molecular weight heparin 625 IU/kg through the femoral vein to heparinize the whole body.

1.8. Ligate the portal vein, hepatic artery, and inferior vena cava above the right renal vein with No. 3-0 sutures, lasting 45 minutes (**Figure 1**). Replace the small intestine in the

abdominal cavity and cover it with gauze. Reduce inhalation anesthesia during these periods.

1.9. After 45 minutes, release the portal vein, hepatic artery, and the inferior vena cava above the right renal vein.

1.10. Suture the muscle and skin, layer by layer, and terminate the inhalational anesthesia. Provide postoperative analgesia using subcutaneous morphine of 5 mg/kg every 4 hours.

1.11. Observe the rat until it is awake and feed under a temperature of 25 ± 2 °C and humidity of 50 ± 10%. Animal heating lamps are necessary.

## REPRESENTATIVE RESULTS

### Rats' tolerance to liver ischemia

In this animal model, the sites at which blood vessels were ligated during operating are shown in **Figure 1**. The rats were randomly divided into 5 groups for ischemia for 15 minutes (I15 group), 30 minutes (I30 group), 45 minutes (I45 group), 60 minutes (I60), and sham group, with 10 rats in each group. The survival rate of each group was observed 14 days after the operation. All rats survived in the I15 group, I30 group, and sham group. Eight survived for 14 days in the I45 group, and only 2 survived in the I60 group. These results suggest that the rats could tolerate the anhepatic phase for a maximum of 45 minutes (**Table 1**).

### Effects of vascular ligation on circulation in rats

During the experiment, Biosystems recorded the heart rate and blood pressure (right internal carotid artery intubation) before and after the anhepatic phase. We found that the heart rate and mean arterial pressure (MAP) of rats changed dramatically after vascular ligation (**Figure 2**).

### Effects on extrahepatic organs

Hepatic ischemia congestion and edema were found in the intestines, gastric varices, and splenomegaly after ligation. Eighty rats were randomly divided into 8 groups for ischemia for 45 minutes (T0), reperfusion for 6 hours (T6), 12 hours (T12), 24 hours (T24), 48 hours (T48), 72 hours (T72), 7 days (D7), and 14 days (D14). After the rats were sacrificed, tissue from the kidney, pancreas, small intestine, heart, and lung were taken and stained with hematoxylin-eosin (HE). The whole staining process consists of five steps: dewaxing, staining, dehydration, transparency, and sealing. Except for the heart, pathological scores were assigned as previously described[16,17,18,19].

The time until maximum injury to the extrahepatic organs varied; it was 6-24 hours after the operation for the pancreas and 24-48 hours for the lungs. The intestinal tract and kidney were most severely injured after 45 minutes of ischemia. There was no obvious abnormality of the intestinal mucosa 24 hours after the operation, and the kidneys recovered after 48 hours. After reperfusion, local myocardial cell necrosis, cell fragmentation and dissolution, inflammatory cell infiltration, and local vasodilation and congestion were found in the heart by 24-48 hours after the operation (**Figure 3**).

**Lungs**

Neutrophil infiltration was found in the lung tissue after ischemia. With the increase of reperfusion time(T0,T6), mucus of the bronchial lumen could also be seen in the lung tissue. Inflammatory cell infiltration occurred in the alveolar wall, which became severely thickened. Alveolar collapse and disappearance of the alveolar cavity could also be found in some tissue. There was no significant alveolar edema or capillary congestion in the alveolar walls. They were most severely injured 24-48 hours after the operation, with some rats showing dyspnea and other manifestations 7 days post-operation. HE staining results suggested lymphadenitis in the airway, mild inflammatory cell infiltration in the alveolar wall, and local hemorrhage (**Figure 3A, Figure 4**).

**Kidneys**

A small amount of eosinophilic substance was found in renal tubules after ischemia at phase T0, but no inflammatory cell infiltration and other abnormalities were seen. However, swollen renal tubular epithelial cells, porous or vacuolated cytoplasm, necrotic cells in few lumens, karyopyknosis, fragmentation, brush border loss, and tube-shaped acidic group in many lumens were seen 6-48 hours after the operation. In addition, a small number of renal tubular epithelial cells were seen with granular degeneration, and porous and lightly stained cytoplasm seen 48 hours after the operation. Significant interstitial telangiectasia, yet no severe inflammatory cell infiltration, was found (**Figure 3B, Figure 5**).

**Small intestine**

The small intestine was most severely injured after ischemia (T0). There was severe inflammatory cell infiltration, mucosa epithelium shedding, and telangiectasia. With the increase of reperfusion time, the injury healed quickly. The mucosal epithelium was restored completely 24 hours after the operation, and only mild inflammatory cell infiltration was seen (**Figure 3C, Figure 6**).

**Pancreas**

Severe inflammatory cells infiltrated around the pancreatic tissue at phase T0. However, the pancreatic lesions were not uniform. Six out of 10 had pancreatic necrosis and inflammatory infiltration 24 hours after surgery, and the other 4 had no apparent abnormalities. Twenty-four hours after the operation, in addition to the infiltration of inflammatory cells, there was edema, widening of interlobular space, hemorrhage, necrosis of a small number of acinar cells, unclear demarcation of the cells, nuclear fragmentation and dissolution, and mild inflammatory cell infiltration in the visual field. Then, the inflammation disappeared slowly (**Figure 3D, Figure 7** ).

**Heart**

By phase T0, cardiac myocytes were arranged regularly with clear demarcation, normal cell morphology, local interstitial congestion, and mild brown-yellow pigment deposition. Moreover, inflammatory cell infiltration was observed in myocardial interstitial and perivascular regions. With the increase in reperfusion time, local myocardial cell necrosis,

cell fragmentation and dissolution, inflammatory cell infiltration, local vasodilation, and congestion were found in tissues 24-48 hours after the operation. Ventricular dilatation, porous structure, increased myocardial interstitium, and mild inflammatory cell infiltration were seen in some specimens. After 48 hours, local cardiomyocytes disappeared and were replaced by a small amount of fibrous connective tissue with mild inflammatory cell infiltration. By then, no other obvious abnormalities were seen (**Figure 8**).

**Effects of hemodynamic changes on liver, kidney, pancreas, and heart serological indexes**

Serum was collected, and the levels of alanine aminotransferase(ALT),aspartate aminotransferase(AST), creatinine, and amylase were detected by an automatic biochemical analyzer. All indicators peaked at 24–48 hours, unlike the pathological changes. Although these levels were normal 48 hours after the operation, pathological damage continued (**Figure 9**).

**Figure 1: Location of ligature: PV, HA, IVC upper of right renal vein.**

**Table 1: Rats' tolerance to liver ischemia**

**Figure 2: Hemodynamic changes in I45 min group.**

**Figure 3：Scores of organ histology.** (**A**) lung; (**B**) kidney; (**C**) intestine; (**D**) pancreas; *Statistically significant compared to the sham group ($P < 0.05$).

**Figure 4:** Pathological changes in the lungs after the operation. (**A**) Sham group; (**B**) Ischemia group (T0 group); (**C**) Reperfusion 6 hours (T6) group; (**D**) Reperfusion 12 hours (T12) group; (**E**) Reperfusion 12 hours (T12) group; (**F**) Reperfusion 24 hours (T24) group; (**G**) Reperfusion 48 hours (T48) group; (**H**) Reperfusion 7 days (D7) group; (**I**) Reperfusion 14 days (D14) group (scale 50 μm).

**Figure 5:** Pathological changes in the kidneys after the operation. (**A**) Sham group; (**B**) Ischemia group (T0 group); (**C**) Reperfusion 6 hours (T6) group; (**D**) Reperfusion 12 hours (T12) group; (**E**) Reperfusion 12 hours (T12) group; (**F**) Reperfusion 24 hours (T24) group; (**G**) Reperfusion 48 hours (T48) group; (**H**) Reperfusion 7 days (D7) group; (**I**) Reperfusion 14 days (D14) group (scale 50 μm).

**Figure 6：** Pathological changes in the small intestine after the operation. (**A**) Sham group; (**B**) Ischemia group (T0 group); (**C**) Reperfusion 6 hours (T6) group; (**D**) Reperfusion 12 hours (T12) group; (**E**) Reperfusion 12 hours (T12) group; (**F**) Reperfusion 24 hours (T24) group; (**G**) Reperfusion 48 hours (T48) group; (**H**) Reperfusion 7 days (D7) group; (**I**) Reperfusion 14 days (D14) group (scale 50 μm).

**Figure 7：** Pathological changes in the pancreas after the operation. (**A**) Sham group; (**B**) Ischemia group (T0 group); (**C**) Reperfusion 6 hours (T6) group; (**D**) Reperfusion 12 hours (T12) group; (**E**) Reperfusion 12 hours (T12) group; (**F**) Reperfusion 24 hours (T24) group; (**G**)

Reperfusion 48 hours (T48) group; (**H**) Reperfusion 7 days (D7) group; (**I**) Reperfusion 14 days (D14) group (**A** and **D** scale 50 μm; **BCEFGHI** scale 50 μm).

**Figure 8:** Pathological changes in the heart after the operation. (**A**) Sham group; (**B**) Ischemia group (T0 group); (**C**) Reperfusion 6 hours (T6) group; (**D**) Reperfusion 12 hours (T12) group; (**E**) Reperfusion 12 hours (T12) group; (**F**) Reperfusion 24 hours (T24) group; (**G**) Reperfusion 48 hours (T48) group; (**H**) Reperfusion 7 days (D7) group; (**I**) Reperfusion 14 days (D14) group (**A** scale 50 μm; **BCDEFGHI** scale 100 μm).

**Figure 9:** Changes of ALT, AST, creatinine (Cr) and amylase in each group; *Statistically significant compared to the sham group ($P<0.05$).

## DISCUSSION

OLT in rats is an ideal model for studying organ preservation in liver transplantation, IRI, transplant rejection, immune tolerance, transplantation pathology and pharmacology, homotransplantation, and xenotransplantation. At present, it is widely used in the experimental research of liver transplantation.

During pilot studies we first administered pentobarbital sodium intraperitoneal anesthesia and found that this led to high postoperative mortality and short tolerance to hemodynamic changes. Thus, we used inhalation anesthesia in subsequent trials for rapid onset of action and rapid exclusion of *in vitro* characteristics. The switch to inhalation anesthesia significantly improved the tolerance time and the postoperative survival of rats. Investigators should pay attention to the breathing and heart rates of the rat to prevent overdosing the anesthetic. Biosystems can be used to monitor the heart rate and blood pressure. We also observed the impact of surgical suture thickness on the ligation of blood vessels. Although lines smaller than 3-0 could perfectly ligate the blood vessels, they were difficult to loosen and may lead to the rupture of blood vessels. On the contrary, lines larger than 3-0 may result in incomplete vascular occlusion, which prevents hemodynamic changes. These material problems will be improved upon in future experiments. There are some limitations to our protocol. Heat lamps are not recommended for temperature maintenance due to their potential for overheating; alternate heating suggestions, such as recirculating water blankets, are recommended for the animal's benefit.

There are many reasons for distal organ injury after OLT. First, injury can be caused by cold preservation of the donor liver *in vitro*[20]. Second, IRI can occur and cause tissue damage when blood supply returns to tissue (reperfusion) after a long period of ischemia. Ischemia is the leading cause of injury, and reperfusion is the process where the injury occurs. After simultaneously blocking the IVC and PV during the anhepatic phase, a large amount of blood stasis occurred in the lower limbs and internal organs. The effective circulating volume (ECV) decreased sharply, and the MAP decreased. However, due to vagus nerve stimulation, there was no compensatory increase in heart rate in rats. In this experiment, we found that rats underwent significant hemodynamic changes within 5 minutes of ligation and vessel release, which met the definition of the ischemia-reperfusion syndrome.

Ischemia occurred in some tissues outside the liver. After the anhepatic phase, ECV increased. MAP returned to normal after the IVC and PV were unblocked, with injury occurring outside the liver after reperfusion. Furthermore, IRI of the donor's liver produced inflammatory mediators (TNF-α, interleukin-1, interleukin-6, interleukin-8) that attacked the distal organs[21]. In this experiment, the hemodynamics during the anhepatic phase were simulated, which caused the passive congestion of IVC and kidney, damage to the gastrointestinal barrier, bacterial translocation, ischemia of the organs (e.g., lung, heart, pancreas, kidney, etc.) where the SVC is located, and IRI to the extrahepatic organs.

The pathological findings showed that the peak of ischemic injury and the recovery time were different in each organ. Although cold storage and damage caused by immune factors could not be simulated in this study, anhepatic IRI can be replicated and compared with other animal models to research extrahepatic organ injury.Our model and OLT model can be compared and observed to provide a basis for the research on extrahepatic organ injury. Furthermore, our model is similar to some clinical liver operations, such as that for Hilar cholangiocarcinoma. Hilar cholangiocarcinoma is a malignant tumor that frequently invades the PV or the IVC and often requires PV clamping during surgery[22]. Hepatic portal reconstruction was performed; when the tumor invades the IVC, intraoperative clamping of the IVC is also required, and the resulting hemodynamic changes are consistent with our model.

To summarize, our model in rats is easy-to-use and straightforward, without microsurgery, and provides the basis for fundamental research into IRI of extrahepatic organs after hepatic ischemia.

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**DISCLOSURE STATEMENT**

The authors of this manuscript have no conflicts of interest to disclose.

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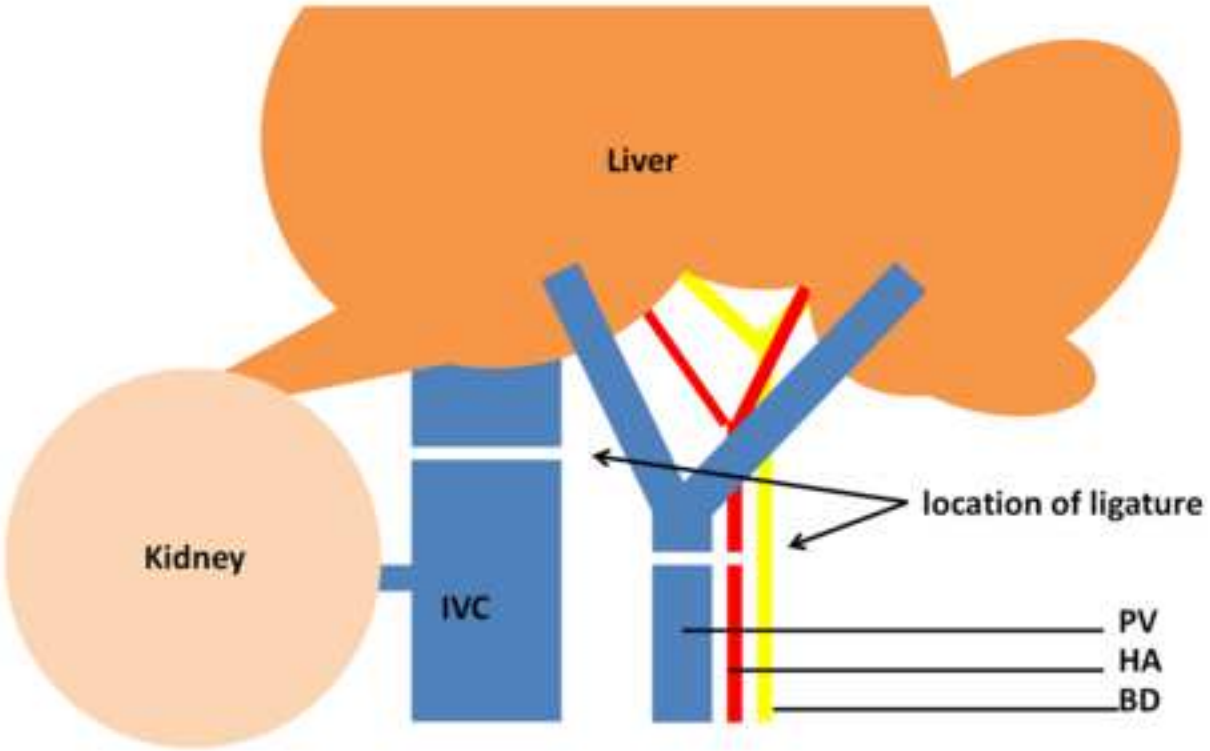


Figure 1: Location of ligature: PV, HA, IVC upper of right renal vein.

Figure 2: Hemodynamic changes in I45 min group.



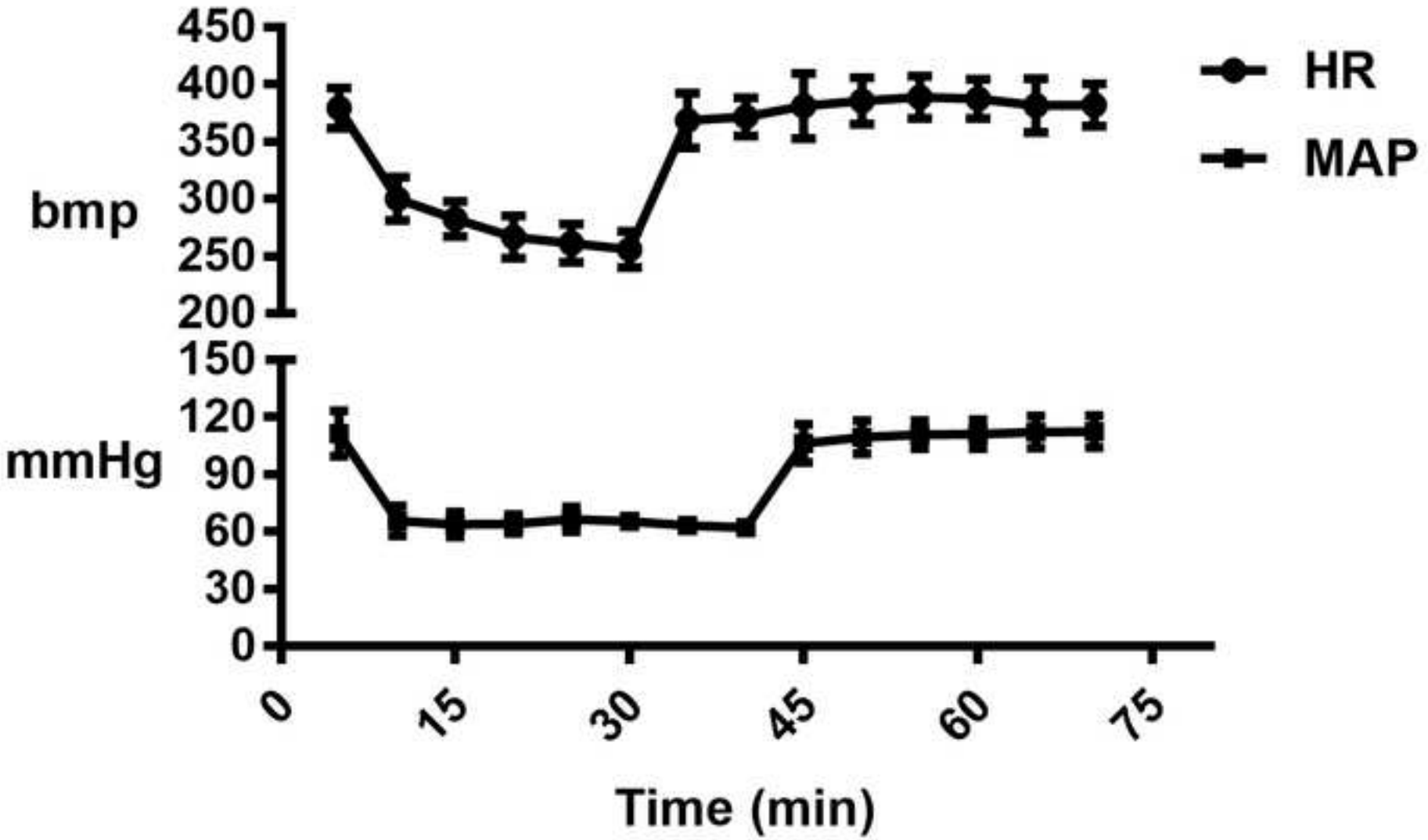

Figure 3：Scores of organ histology. A. lung; B. kidney; C. intestine; D. pancreas; *Statistically significant compared to the sham group($P < 0.05$).



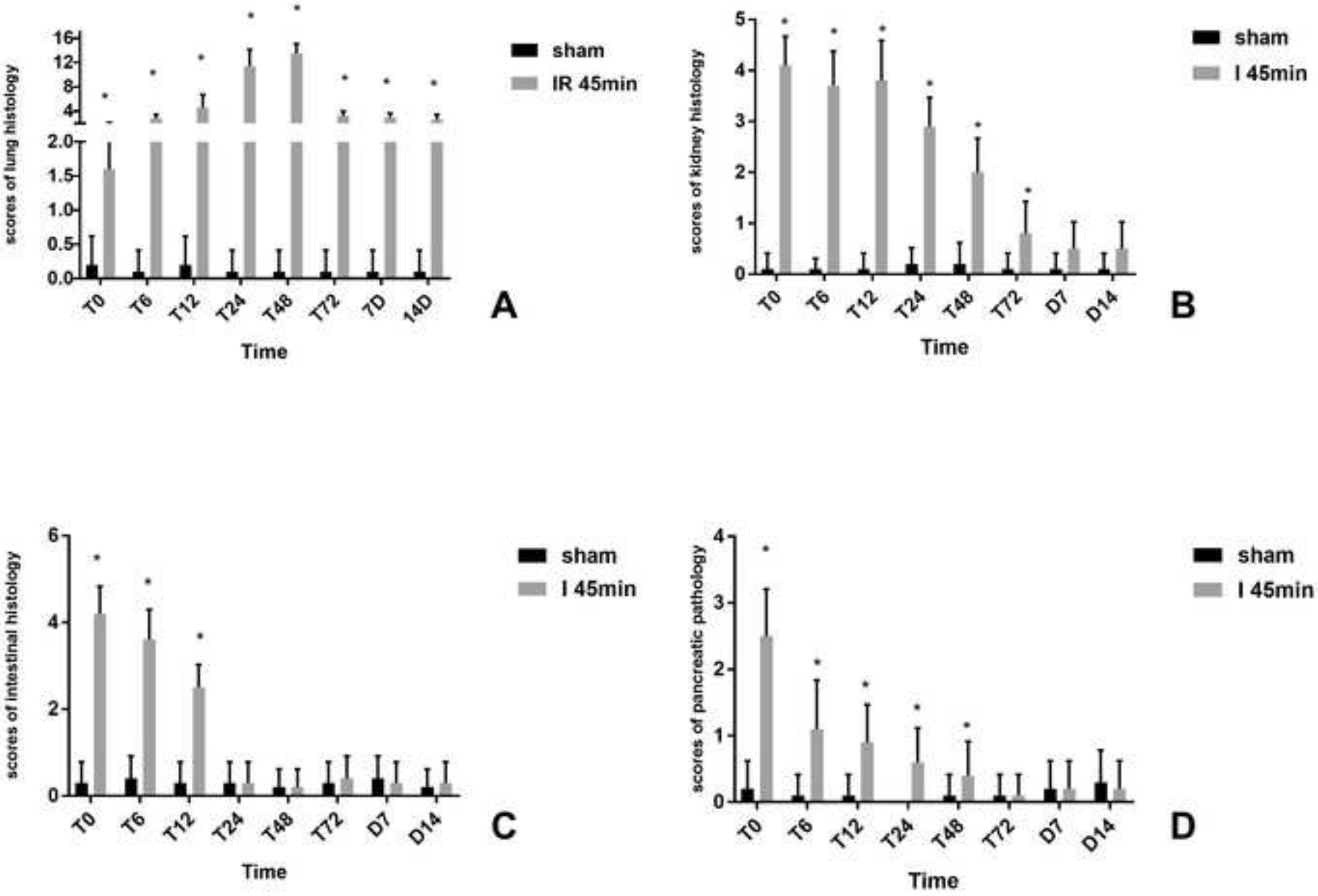

Figure 4: Pathological changes in lungs after the operation.

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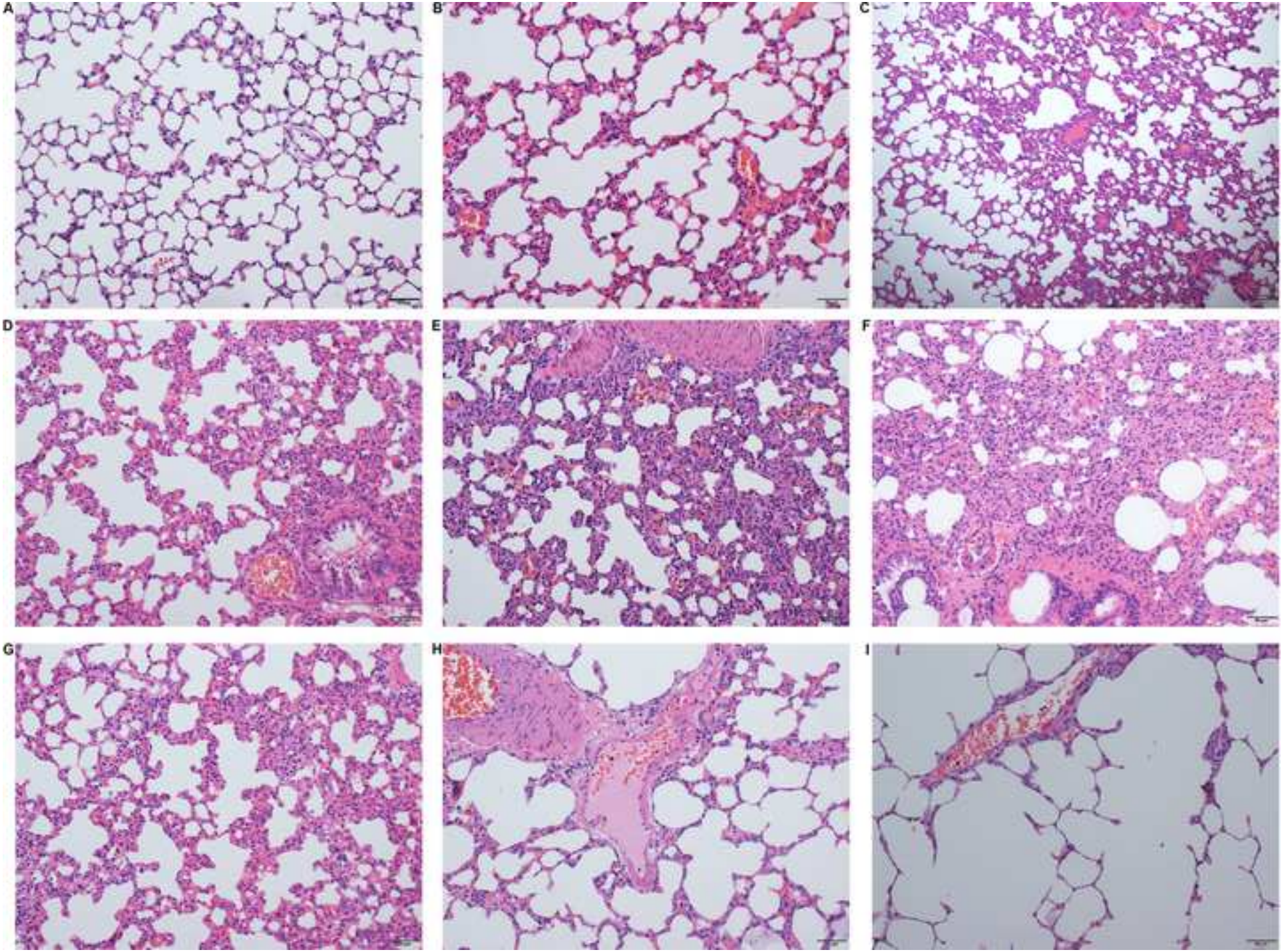

Figure 5: Pathological changes in kidneys after the operation.

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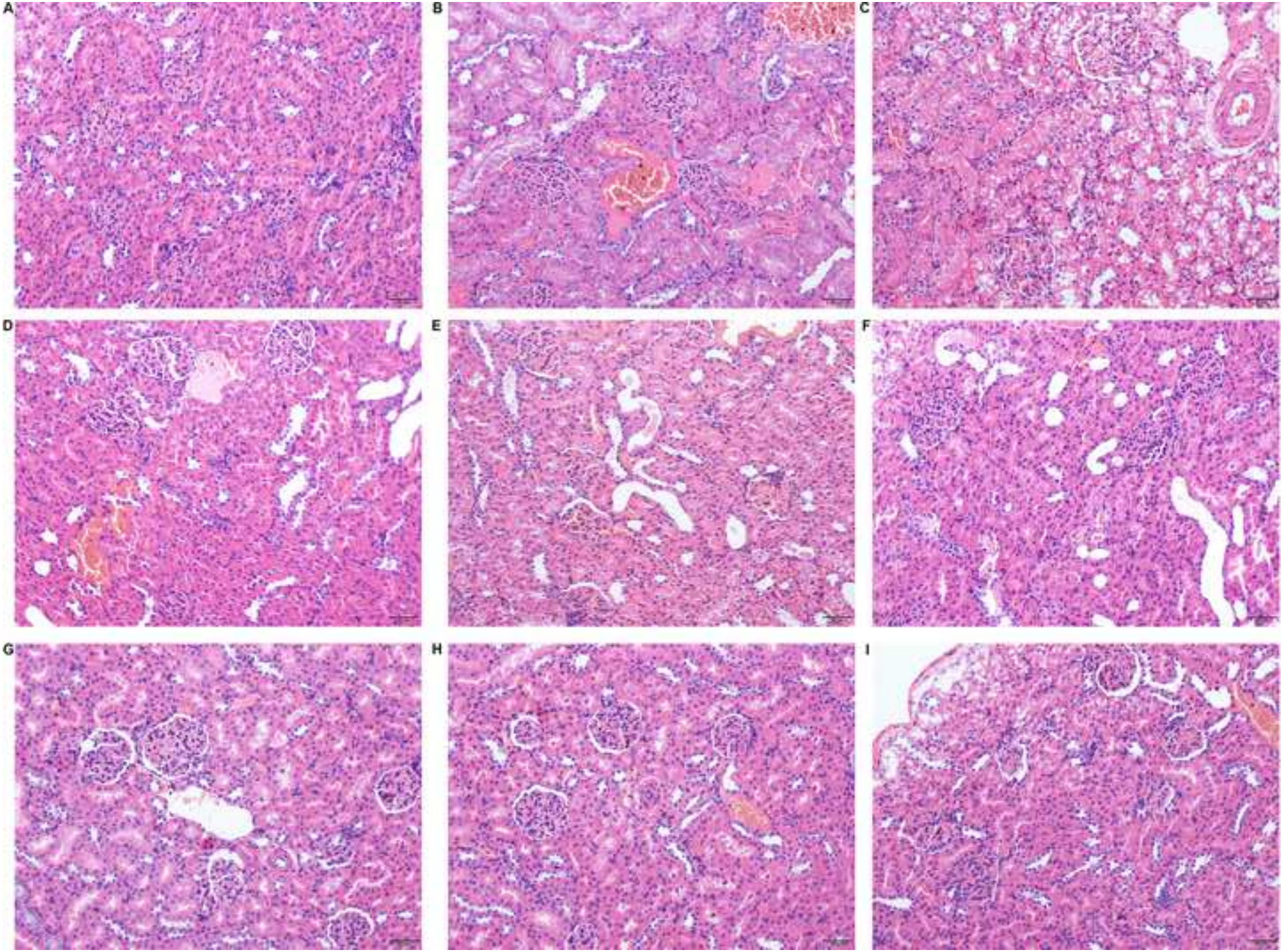

Figure 6：Pathological changes in the small intestine after the operation.

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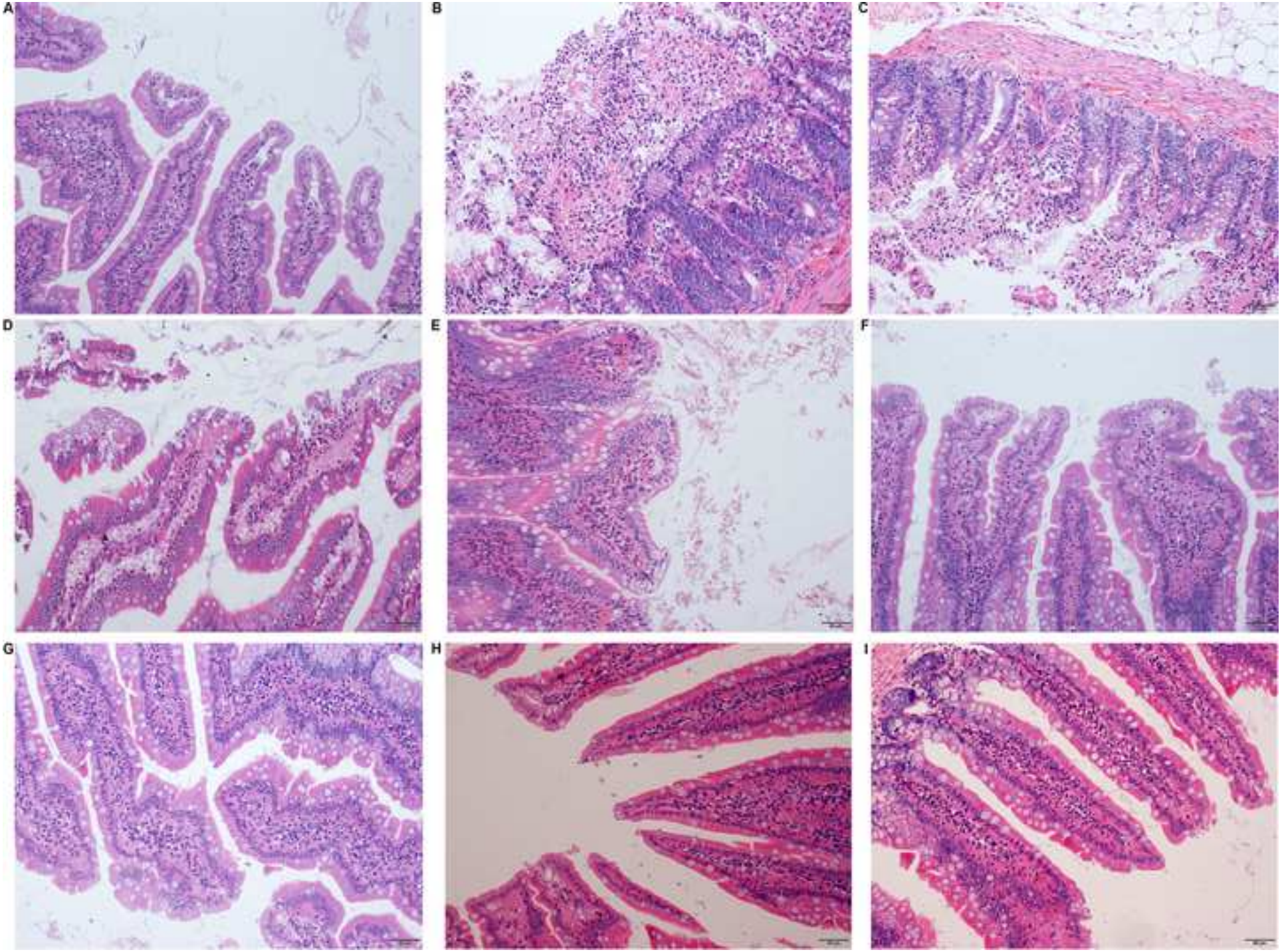

Figure 7：Pathological changes in pancreas after the operation.

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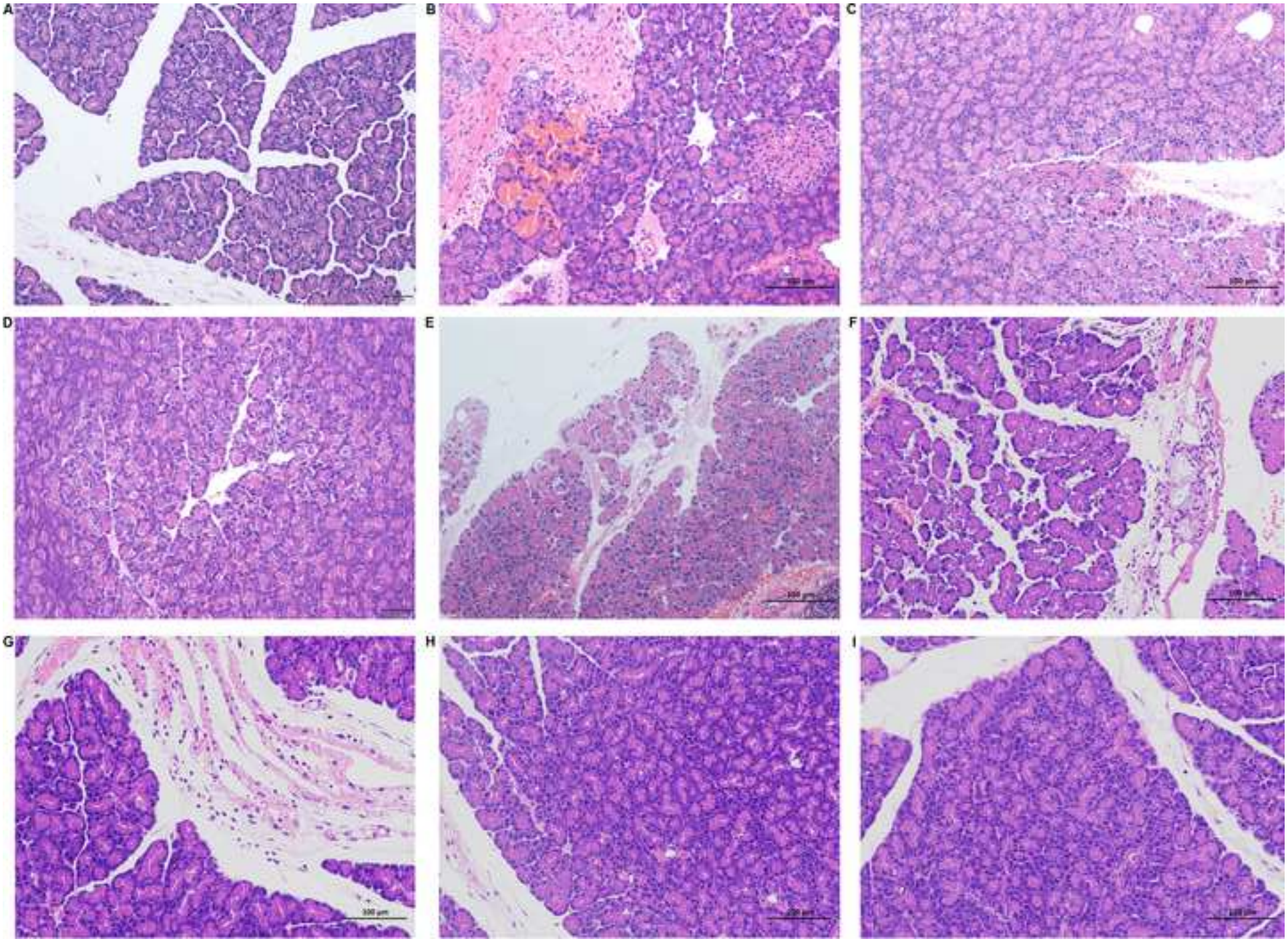


Figure 8：Pathological changes in hearts after the operation.

Click here to access/download;Figure;Revised Figure 8.jpg

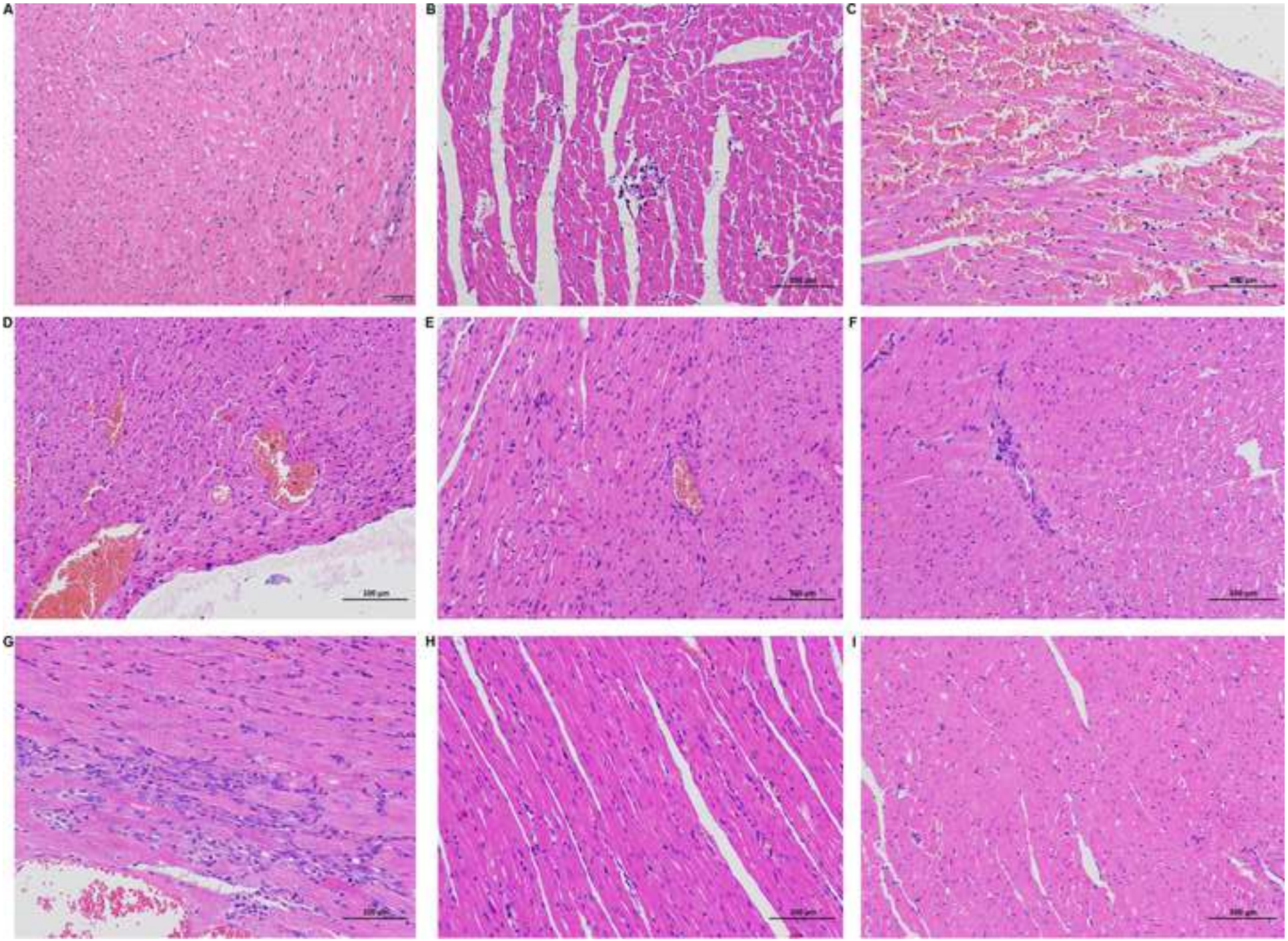
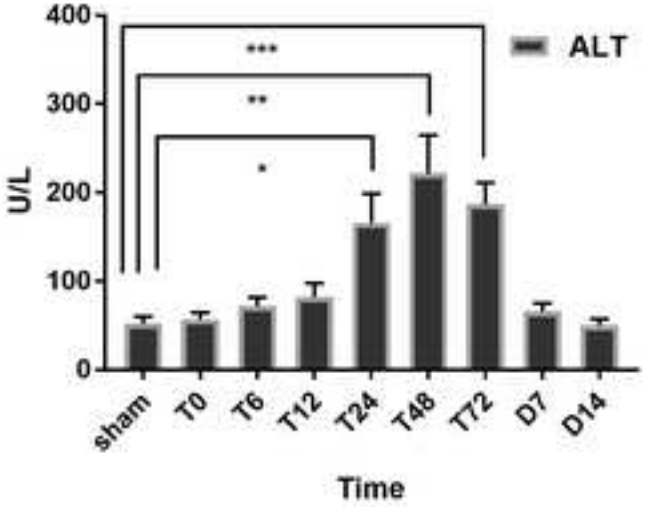
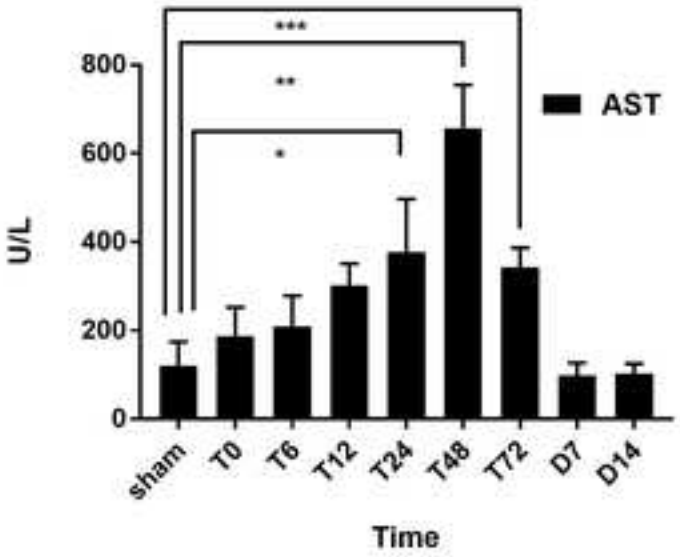
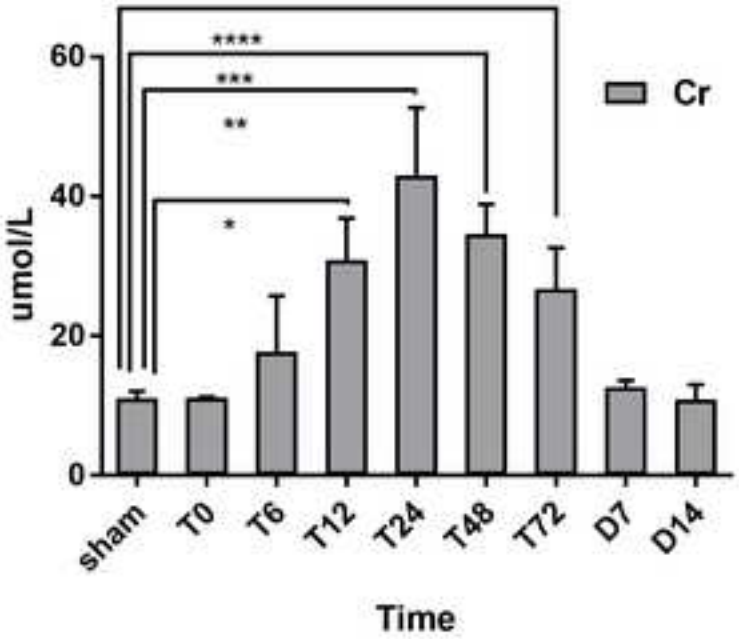


Figure 9 : Changes of ALT, AST, creatinine(Cr),amylase in each groups; *Statistically significant compared to the sham group($P < 0.05$).





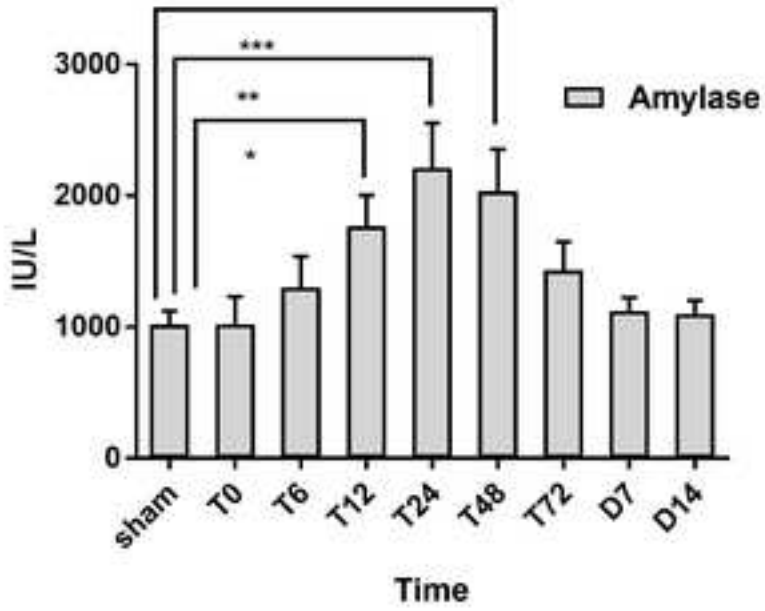



**Table 1: Rats' tolerance to liver ischemia**

| Group | n | survival at 24 h, n (%) | survival at 7 d, n (%) | survival at 14 d, n (%) |
|---|---|---|---|---|
| sham | 10 | 10 | — | — |
| I 15 min | 10 | 10/10 | 10 (100) | 10 (100) |
| I 30 min | 10 | 10/10 | 10 (100) | 10 (100) |
| I 45 min | 10 | 8/10 | 8/10 (80) | 8/10 (80) |
| I 60 min | 10 | 2/10 | 2/10 (20) | 2/10 (20) |

| Name of Material/Equipment | Company | Catalog Number |
|---|---|---|
| 4% paraformaldehyde solution | Shanghai Macklin Biochemical Co.,Ltd | P804536 |
| air drying oven | Shanghai Binglin Electronic Technology Co., Ltd. | BPG |
| Alanine aminotransferase (ALT)Kit | Elabscience Biotechnology Co.,Ltd | E-BC-K235-S |
| ammonia | Sinopharm Chemical Reagents Co. Ltd | 10002118 |
| amylase Kit | Elabscience Biotechnology Co.,Ltd | E-BC-K005-M |
| anhydrous ethanol | Sinopharm Chemical Reagents Co. Ltd | 100092183 |
| Animal anesthesia machine | Shenzhen Ruiwode Life Technology Co. Ltd | R640 |
| aspartate aminotransferase (AST)kit | Rayto Life and Analytical Sciences Co., Ltd. | S03040 |
| automatic biochemical analyzer. | SIEMENS AG FWB:SIE, NYSE:SI Co., Ltd. | 2400 |
| Biosystems（when nessary） | Chengdu Taimeng Electronics Co., Ltd. | BL-420F |
| Centrifuge | Baiyang Medical Instrument Co., Ltd. | BY-600A |
| cover glass | Jiangsu Shitai Experimental Equipment Co. Ltd | 10212432C |
| creatinine Kit | Rayto Life and Analytical Sciences Co., Ltd. | S03076 |
| dewatering machine | Hungary 3DHISTECH Co.,Ltd | Donatello Series 2 |
| embedding machine | Hubei Xiaogan Kuohai Medical Technology Co., Ltd. | KH-BL1 |
| frozen machine | Wuhan Junjie Electronics Co., Ltd | JB-L5 |
| hematoxylin-eosin dye solution | Wuhan Saiwell Biotechnology Co., Ltd | G1005 |
| high-efficiency paraffin wax | Shanghai huayong paraffin wax co., Ltd | Q/YSQN40-91 |
| hydrochloric acid | Sinopharm Chemical Reagents Co. Ltd | 10011018 |
| intraocular lens (IOL)forceps | Guangzhou Guangmei Medical Equipment Co., Ltd. | JTZRN |
| Isoflurane | Shenzhen Ruiwode Life Technology Co. Ltd | — |
| micro Scissors(when nessary) | Shanghai Surgical Instrument Factory | WA1010 |
| needle holders | Shanghai Surgical Instrument Factory | J32010 |
| neutral gum | Shanghai Huashen Healing Equipment Co.,Ltd. | — |
| normal optical microscope | Nikon Instrument Shanghai Co., Ltd | Nikon Eclipse CI |
| ophthalmic forceps | Shanghai Surgical Instrument Factory | J3CO30 |
| ophthalmic forceps | Shanghai Surgical Instrument Factory | JD1060 |
| ophthalmic Scissors | Shanghai Surgical Instrument Factory | J1E0 |
| pathological slicer | Shanghai Leica Instrument Co., Ltd | RM2016 |

| | | |
|---|---|---|
| pipettes | Dragon Laboratory Instruments Co., Ltd. | 7010101008 |
| retractors | Beijing Jinuotai Technology Development Co.,Ltd. | JNT-KXQ |
| scanner | Hungary 3DHISTECH Co.,Ltd | Pannoramic 250 |
| slide | Wuhan Saiwell Biotechnology Co., Ltd | G6004 |
| xylene | Sinopharm Chemical Reagents Co. Ltd | 1330-20-7 |

**Comments/Description**

straight
bending

Dear Nam Nguyen, Ph.D.

We wish to re-submit the manuscript titled "Organ Ischemia-Reperfusion Injury by Simulating Hemodynamic Changes in Rat Liver Transplant Model." The manuscript ID is JoVE61779R3 .

We thank you for your thoughtful suggestions and insights. The manuscript has benefited from these insightful suggestions. I look forward to working with you and the reviewers to move this manuscript closer to publication in the JoVE.

The manuscript has been rechecked and the necessary changes have been made in accordance with the reviewers' suggestions. We have had the manuscript professionally proofread for fixing English language and grammar issues.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,
Yuan Yuan

Editorial comments:

Changes to be made by the Author(s) regarding the written manuscript:
1. Please employ professional copyediting services as the language in the manuscript is not publication grade. Your Editage certification is from June 2020 and professional copyediting services are needed for the current version of the manuscript. There are many unclear phrases and sentences in the current manuscript that must be clarified.
Reply: We checked the manuscript thoroughly and revised spelling or grammar issues accordingly again.

2. Figure 2: Please provide units on the y axis.
Reply: We provided units on the y axis in Figure2.

Changes to be made by the Author(s) regarding the video:
1. Video & Audio Editing:
Please adjust all audio clips to they are roughly the same volume. Some audio clips are too loud, such as at: 02:58-3:01, and 9:09-9:31. This are only two examples, please go through the clips and adjust as needed. The target loudness should be between -6 dB and -12 dB.
• 05:14 Previous note: "Analgesia" is mispronounced here. The "g" should sound like the "g" in "gem", NOT "gum", as pronounced. "Anal-jeezea" is the phonetic pronunciation. If no re-recording is made, then the current pronunciation will stand.
• 06:11 There is a double inhalation from the narrator here that can bedeleted.
Reply:We tried our best to adjust the same volume,but we are still worried about not meeting your requirements.We apologize for our unprofessional editing techniques！

2. On-Screen Text:
• 01:34 "Protocol" Please ensure that all chapter title cards are the same video or image quality, and free from artifacts and blurriness. This card is lower quality than the other chapter title cards, and should be re-created to match the resolution and quality of the others. The quality of the chapter title card at 09:08 is a good example of what to aim for.
09:31 "Conclusion" This chapter title card also could use a quality adjustment, similar to "Protocol" @01:33.
Reply： We re-created the part of "Protocol"and "Conclusion",thank you！

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This document certifies that the paper listed below has been edited to ensure that the language is clear and free of errors. The logical presentation of ideas and the structure of the paper were also checked during the editing process. The edit was performed by professional editors at Editage, a division of Cactus Communications. The intent of the author's message was not altered in any way during the editing process. The quality of the edit has been guaranteed, with the assumption that our suggested changes have been accepted and have not been further altered without the knowledge of our editors.

## Title Of The Paper

Organ Ischemia-Reperfusion Injury by Simulating Hemodynamic Changes in Rat Liver Transplant Model

## Authors

Yuan Yuan

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Date of Issue
**January 31, 2021**

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