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Blood Circuit Reconstruction in an Abdominal Mouse Heart Transplantation Model

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TITLE:

Blood Circuit Reconstruction in an Abdominal Mouse Heart Transplantation Model

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Keywords:

Surgical technique, mouse heart transplantation, blood circuit reconstruction, intrathoracic inferior vena cava

Summary

A novel technique for blood circuit reconstruction in a heterotopic abdominal mouse heart transplantation model is demonstrated.

Abstract

The surgical technique of heterotopic abdominal heart transplantation in mice is a standard model for research in transplantation immunology. Here, the established technique for a modified blood circuit reconstruction in a heterotopic abdominal heart transplantation model is presented. This method uses the intrathoracic inferior vena cava (IIVC) instead of the pulmonary artery of the donor heart for the anastomosis to the inferior vena cava of the recipient. It is facilitating and improving success rates for abdominal heart transplantation in mice.

Introduction

The surgical technique of heterotopic abdominal heart transplantation in mice represents a

standard model for research in transplantation immunology[1-3]. However, it is very challenging to perform and this implicates a restriction to the widespread use of this model[4,5].

In traditional mouse heart transplantation (THTx), the donor aorta and the recipient abdominal aorta are anastomosed while the pulmonary artery is anastomosed to the recipient inferior vena cava[6-8].

In this modified mouse heart transplantation technique, the donor aorta is anastomosed to the recipient abdominal aorta and the donor IIVC is anastomosed to the recipient inferior vena cava[(3,4,6)] (**Figure 2 and Figure 3**).

Protocol

All animal experiments were conducted following the guidelines from the directive 2010/63/EU of the European Parliament on protection of animals used for scientific purposes (Ethic committee approved, #G1071/09).

NOTE: Preliminary preparation, anesthesia, post-operative care and monitoring work are the same as performed in traditional surgical methods[1,2,4]. BALB/c mice served as heart donors and C57BL/6J as transplant recipients. Mice were aged 8-12 weeks, weighed∼30 g at transplantation and were housed under standard conditions.

1. Preparatory steps

1.1. For anesthesia, give mice inhalative isoflurane (2%) until they fall asleep, followed by intraperitoneal injections of ketamine (100 mg/kg) + xylazine (10 mg/kg) + acepromazine (2 mg/kg). For postoperative analgesia, apply Metamizol (200 mg/kg) p.o. and Carprofen (5 mg/kg) s.c..

NOTE: The application of antibiotics was abstained on purpose as these substances may influence immunological responses.

1.2. For surgery, use a set of microscopic instruments including a micro-scissor, micro-forceps, a needle holder and micro hemostatic clamps. An electrosurgical pen is also necessary. Perform sutures using 7/0er, 10/0er or 4/0er nylon suture.

1.3. Place the mouse in a box for isoflurane inhalation (2%) for 40-60 seconds. Determine the depth of anesthesia by squeezing the paw with tweezers. If there is a complete lack of response for this stimulus, go to the next step.

1.4. Once the mouse has fallen asleep, weigh the mouse.

1.5. Apply an intraperitoneal injection of ketamine (100 mg/kg) + xylazine (10 mg/kg) + acepromazine (2 mg/kg) to the anesthetized mouse.

1.6. Clip the abdominal fur and place the mouse on the operation table. Perform disinfection using povidone iodide for 3 times, then properly drape the mouse using a

fenestrated surgical towel.

2. Donor operation procedure

2.1. Use scissors to cut the skin from the neck to the lower abdomen, and peel off the full layer of skin to the midline of both axillae.

2.2. Use scissors to cut the muscles of the abdominal wall and gently move the viscera to the left (from the operator's view). Wrap away the viscera with a saline imbibed gauze to safely expose the inferior vena cava.

2.3. Use a 1 mL syringe to inject 0.4 mL of the heparin solution (contains 500 U heparin) slowly into the lower vena cava and wait for 1 minute before pulling out the needle.

2.4. Pull out the needle and use micro scissor cut both the inferior vena cava and the abdominal aorta with scissors to accelerate exsanguination.

2.5. Use scissors to open the chest cavity by performing a u-shaped cut; completely expose the heart, lungs and all chest blood vessels.

2.5.1. Expose the thoracic aorta, cut 1/2 of the lumen, and then cut the pulmonary vein to facilitate irrigation and drainage.

2.5.2. Insert an irrigation tube into the opening of the thoracic aorta, inject at least 2 mL of 4 °C cold histidine-tryptophan-ketoglutarate cardioplegia solution (Custodiol HTK solution)[9] until the pulmonary vein outflow is completely clear and the heart completely stops beating.

2.6. Pull out the irrigation tube and detach the sternum.

2.7. Use micro scissors to remove the thymus and to slightly strip the fat around the aortic arch.

2.8. Use straight and curved forceps to expose and ligate the trunk of the arteria pulmonalis (on the right side of the aortic arch) with a 10/0 suture.

2.9. Use micro forceps to separate the fat and connective tissue attached to the IIVC, expose and ligate the superior vena cava (on the left side of the aortic arch) with 7/0 suture and use micro scissors to cut it behind the ligation.

2.10. Make a 7/0 suture around the base of the heart underneath the aortic arch, the IIVC, and both auricles. Then ligate the pulmonary artery branches and venous lung vessels.

2.11. Use micro scissors to transect the aortic arch as distal as possible, the lung vessels underneath the ligature and the IIVC near the diaphragm. Remove the heart out of the chest.

2.12. Place the explanted donor's heart into 4 °C cold HTK cardioplegia solution and preserve temporarily.

3. Recipient operation procedure

NOTE: The initial operation steps are similar to those previously shown for the donor mouse, including anesthesia and disinfection.

3.1. Perform the abdominal skin cut in a transverse manner, cover the abdominal organs with a wet gauze using saline solution.

3.2. Use micro forceps to expose the inferior vena cava and abdominal aorta and free them from surrounding fat tissue.

3.3. Use micro forceps to ligate or electrocauterize side branch vessels (lateral or under the vein/aorta) below the renal vessels.

3.4. Use clip applicator forceps to position two micro hemostatic forceps at the abdominal part of vein/aorta coming from the right leaving more than 1 cm distance for both aorta/vein to ensure space for the construction of the anastomosis in between them.

3.5. Use micro scissors to make an incision into the aorta a little nearer to the lower clamp than to the upper clamp. Alternatively, use a 30 G needle to make a small hole and open it up with micro scissors.

3.6. Position the recipient mouse so that the aorta is facing the operator with the vena cava on the other side. Then place the heart into the abdominal cavity and cover it with a small wet gauze pad.

3.7. Use a 10/0 suture to adapt and stitch the donor aorta to the recipient aorta starting caudally, make a knot and proceed with a running suture to the top of the incision (about 4-5 stitches). Next, flip the heart over to the right (from the subject’s view), cover it again and continue the suture on the left side until reaching the caudal end and knot it.

3.8. Use an irrigation tube to inject at least 0.5 mL of 4 °C HTK cardioplegia solution to flush the donor's IIVC.

3.9. Use micro-scissors to cut a round hole on the abdominal inferior vena cava of recipient, which should have the same size of the donors IIVC lumen. The incision should be located above the aortic anastomotic opening. Make the vein incision larger than the aortic incision.

3.10. Use 10/0 suture to sew the donor IIVC to the recipient vena cava starting caudally. Tie a knot and perform a running suture until the top of the incision is reached. Use five stitches and continue the suture on the left. Finally, tie a knot in the tail corner, and carefully tighten (be careful not to pull too tight).

3.11. Place the small parts of the hemostatic sponge around the vein and aortic anastomoses.

3.12. Use clip applicator forceps to remove first the lower and then the upper micro-hemostatic clamps and rinse the abdominal cavity with 38.0°C tempered 0.9% sodium chloride.

3.13. Use micro forceps to take away the hemostatic sponge.

3.14. Observe the heartbeat of the transplanted heart.

3.15. Use forceps to put the intestines back into the abdominal cavity and two-layer sutures (abdominal muscles followed by skin) to close the abdominal wound with a 4/0 suture.

3.16. Put the mice into an oxygen and temperature control workstation chamber (e.g., INVIVO2-400) to provide a warm and oxygen rich environment for the transplanted mice to recover, wait for the mice to wake up.

3.17. For postoperative analgesia, directly give Metamizol 200 mg/kg per os after operation. Four and 16 hours after operation give Metamizol 200 mg/kg per os+ Carprofen (5mg/kg) s.c. In the further followup, give Carprofen (5 mg/kg) s.c to the transplanted mice every 24 hours for three consecutive days after operation.

Representative results

Here, a modified technique of heterotopic abdominal heart transplantation in mice that has been previously developed in our laboratory and has proven useful for the last 16 years is presented. Previously, it was reported that in overall 40 cases of vena cava to vena cava (V-V group) compared to 40 cases of the traditional pulmonary artery to vena cava (P-V group) anastomosis procedure[4] (**Table 1**). The vessel anastomosis in the V-V group took 20.8±1.3 min, which was significantly shorter than in the P-V group (27.5±1.3 min, $p<0.01$). The warm ischemia time, total recipient operation time and postoperative heart repeating time, which were 25.5±1.2 min, 42.0±1.5min, and 1.1±0.2 min, respectively, were also significantly shortened than observed in the traditional P-V group (all $p<0.05$) (**Figure 1**). Even though there are no differences for longtime survival rates in this model[10,11], the modified technique facilitated abdominal heart transplantation in mice resulting both in a reduced warm ischemia and grafted heart rebeating time.

With respect to these previously published data and our experience with this model within the last 16 years, we recommend that the key operation steps should take a limited amount of time to ensure a success rate of >90%[4]. Therefore, optimal results are achieved if the harvesting of the donor heart takes no longer than 60 min, the cold ischemia time should be limited to 40 min at maximum, and the construction of the IIVC anastomosis should not take longer than 15 min as this is directly associated with a reduced warm ischemia time.

Figure 1. Comparison of procedure times between the two operation techniques (n =40 each, mean + SE)

Figure 2. Donor heart preparation using the traditional (a, upper left) and the modified (b, lower left) mouse heart transplantation model. The photographs in panels a and b depict the aorta (A), the pulmonary artery (PA), the right atrium (RA), the left atrium (LA) and the

intrathoracic inferior vena cava (IIVC) of the donor heart. Notice the difference in vessel length of the PA compared to the IIVC. Panel c shows the recipient situs prepared for the heterotopic abdominal HTX.

Figure 3. Vascular anastomosis of the modified model: aorta (A), intrathoracic inferior vena cava (IIVC), abdominal inferior vena cava (avic), abdominal aorta (aa).

Figure 4. Demonstration of vessel length for the pulmonary artery (PA) and the intrathoracic inferior vena cava (IIVC).

Figure 5. Physiological trumpet shape at the end of the intrathoracic inferior vena cava. Aorta (A), intrathoracic inferior vena cava (IIVC), right atrium (RA), left atrium (LA). Left red double arrow on the left highlights the diameter and the red circle on the right the structure of trumpet shape.

Table 1. Comparison of Time Distributions and Initial Outcomes in Two Operations (n - 40, mean + SE)

Reprinted from Wu, K., Zhang, J., Fu, J., Wu, S., Philipp, T., Uwe, H., Kribben, A. and Witzke, O. Novel technique for blood circuit reconstruction in mouse heart transplantation model. Microsurgery. 26, 594-598 (2006).

Discussion

The surgical technique of heterotopic abdominal heart transplantation in mice is very challenging and this implicates a restriction to the widespread use of this model.

One of the disadvantages of the conventional technique is the limiting length of the donor's pulmonary artery (PA). It is usually of about 2 mm of length, whereas the length of the IIVC of the donor heart used in our model is generally about 1 cm (**Figure 2**). That means that in the modified model, the IIVC anastomosis offers a clearer sight of the operation situs enabling improved surgery of anastomoses and preventing the undesired development of too strict sutures or even damaging lesions of the vessels (**Figure 4**). Both the construction of the PA as well as the IIVC anastomosis are challenging even for experienced operators. The PA is very delicate and thin-walled and the IIVC is even thinner and potentially more fragile in mice. Therefore, operators need to be aware of this restriction and should apply caution when suturing the IIVC. However, at this point it is important to emphasize that although the IIVC vessel wall is very thin, it has the great advantage that the vessel length is not associated with tension to the blood vessel connection, thereby making the application of a precise suture easier and less prone to a damage. Since the cautious and safe application of the vessel sutures are very critical for a successful operation outcome, a magnification of the situs of 10 to 20-fold is recommended.

In addition, the ending of the thoracic segment of the IIVC forms a typical trumpet shaped structure (**Figure 5**). Its larger opening diameter represents one of the important and beneficially reasons why the IIVC can be chosen. Its usage facilitates the application of a sufficient anastomosis. This reduces both the difficulty of the operation and the operation time.

A possible event that may compromise the outcome of the transplant procedure represents a thrombosis of the vessel anastomosis, often fostered by a stenosis. Although in our model the length of the vessel does increase on purpose, this was not associated with the formation of thrombi. The trumpet shaped anastomosis of the IIVC may also exert a positive effect while greatly reducing the occurence of an anastomotic stenosis. Therefore, for this procedure a posttransplant heparinization is not necessary.

A previous analysis of the IVC-IVC anastomosis method revealed several advantages and improvements when compared to the conventional technique[10]. With respect to these results and from the retrospective point of view of our longstanding experience[4,10,12,13], this technique results both in a reduced warm ischemia and rebeating time of the grafted heart. Even though there are no differences for longtime survival rates for this model[10], the modified technique presented here is facilitating the anostomotic vessel reconstruction thus reducing the difficulty of abdominal heart transplantation in mice. The training and application of this model may therefore improve the accessibility and widespread application of abdominal heart transplantation in mice for immunological as well as cardiac research purposes.

Disclosures

None.

Acknowledgments

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References

1. Corry, R., Winn, H., Russell, P. Primarily vascularized allografts of heart in mice. *Transplantation*. **16**, 343–350 (1973).

2. Habertheuer, A. et al. Donor tissue-specific exosome profiling enables noninvasive monitoring of acute rejection in mouse allogeneic heart transplantation. *Journal of Thoracic and Cardiovascular Surgery*. **155** (6), 2479-2489 (2018).

3. Plenter, R.J., Zamora, M.R., Grazia, T.J. Four decades of vascularized heterotopic cardiac transplantation in the mouse. *Journal of Investigative Surgery*. **26**, 223-228 (2013).

4. Wu, K. et al. Novel technique for blood circuit reconstruction in mouse heart transplantation model. *Microsurgery*. **26**, 594-598 (2006).

5.Alamran, F.G., Shahkolahi, M.M. Total arterial anastomosis heterotopic heart transplantation model. *Transplantation Proceedings*. **45**, 625-629 (2013).

6. Melanie, L. et al. Potential of Heterotopic Cardiac Transplantation in Mice as a Model for Elucidating Mechanisms of Graft Rejection. Cardiac Transplantation, Susan D. Moffatt-Bruce. *IntechOpen* (2012).

7. Westhofen, S. et al. The heterotopic heart transplantation in mice as a small animal model to study mechanical unloading - Establishment of the procedure, perioperative management and postoperative scoring. *PLoS One*. **14** (4), e0214513 (2019).

8. Wang, C., Wang, Z., Allen, R., Bishop, G. A., Sharland, A. F. A Modified Method for Heterotopic Mouse Heart Transplantion. *Journal of Visualized Experiments.* (88), e51423

(2014).
9. Mokbel, M. et al. Histidine-Tryptophan-Ketoglutarate Solution for Donor Heart Preservation Is Safe for Transplantation. *Annals of Thoracic Surgery*. **109** (3), 763-770 (2020).
10. Song, S. et al. Modified Suture Technique in a Mouse Heart Transplant Model. *Asian Journal of Surgery*. **34** (2), 86-91 (2011).
11. Martins, P.N. Assessment of graft function in rodent models of heart transplantation. *Microsurgery*. **28** (7),565-570 (2008).
12. Wu, K. et al. cold storage using a new histidine-tryptophan-ketoglutarate-based preservation solution in isogeneic cardiac mouse grafts. *European Heart Journal*. **32** (4), 509-16 (2011).
13. Türk, T.R. et al. Reduction of chronic graft injury with a new HTK-based preservation solution in a murine heart transplantation model. *Cryobiology*. **64** (3), 273-8 (2012).

figure1

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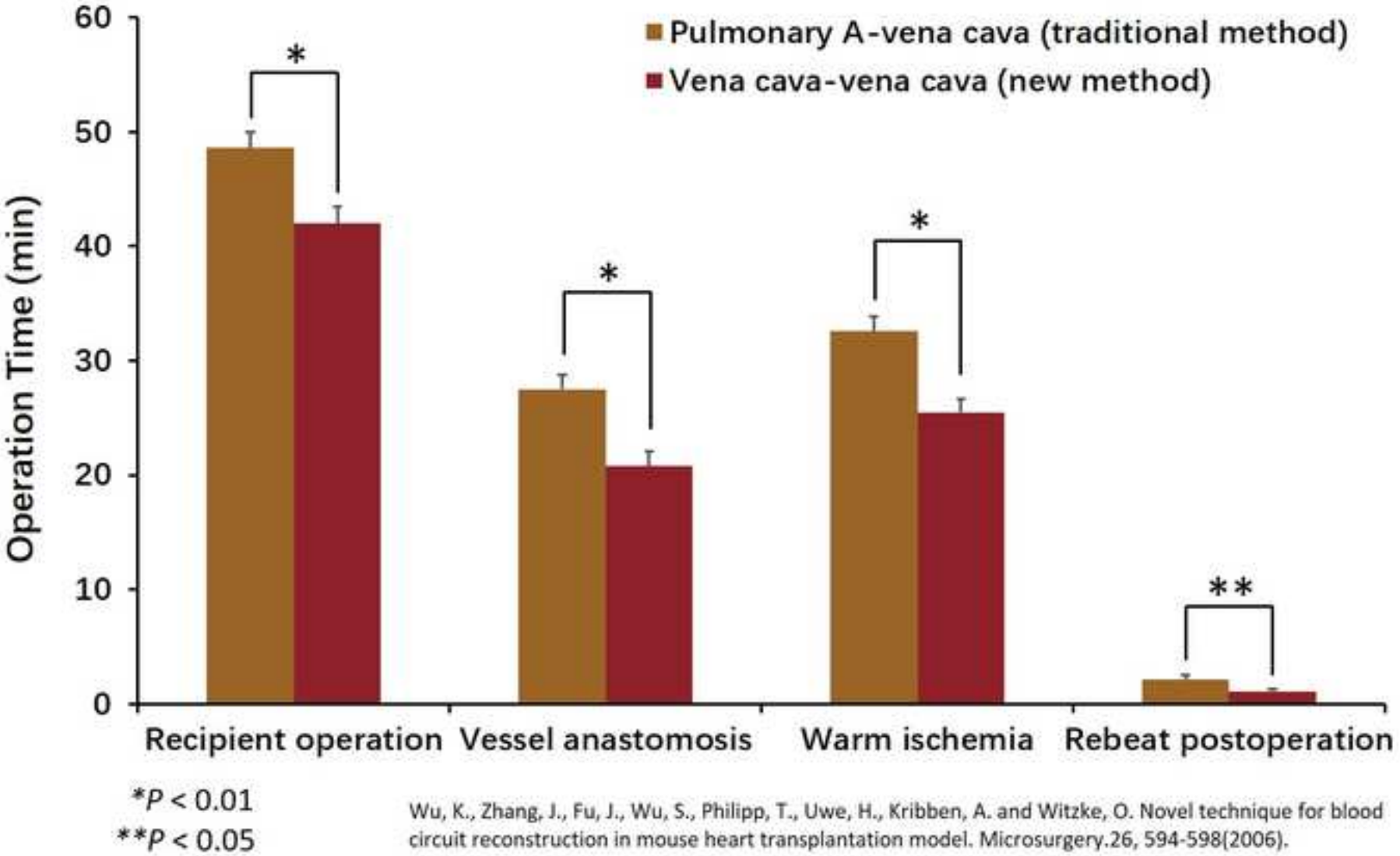

*$P < 0.01$

**$P < 0.05$

Wu, K., Zhang, J., Fu, J., Wu, S., Philipp, T., Uwe, H., Kribben, A. and Witzke, O. Novel technique for blood circuit reconstruction in mouse heart transplantation model. Microsurgery.26, 594-598(2006).

figure2

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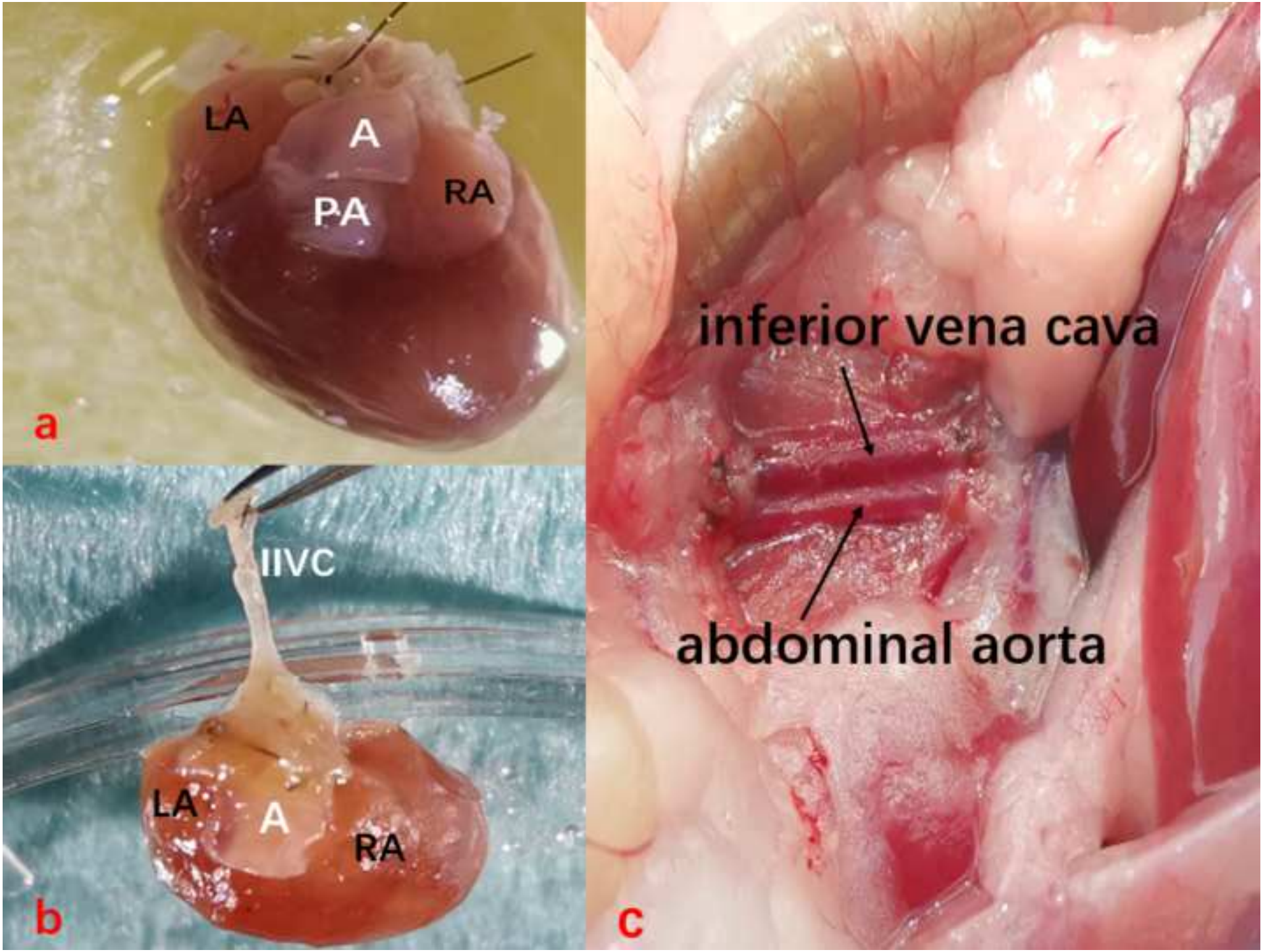

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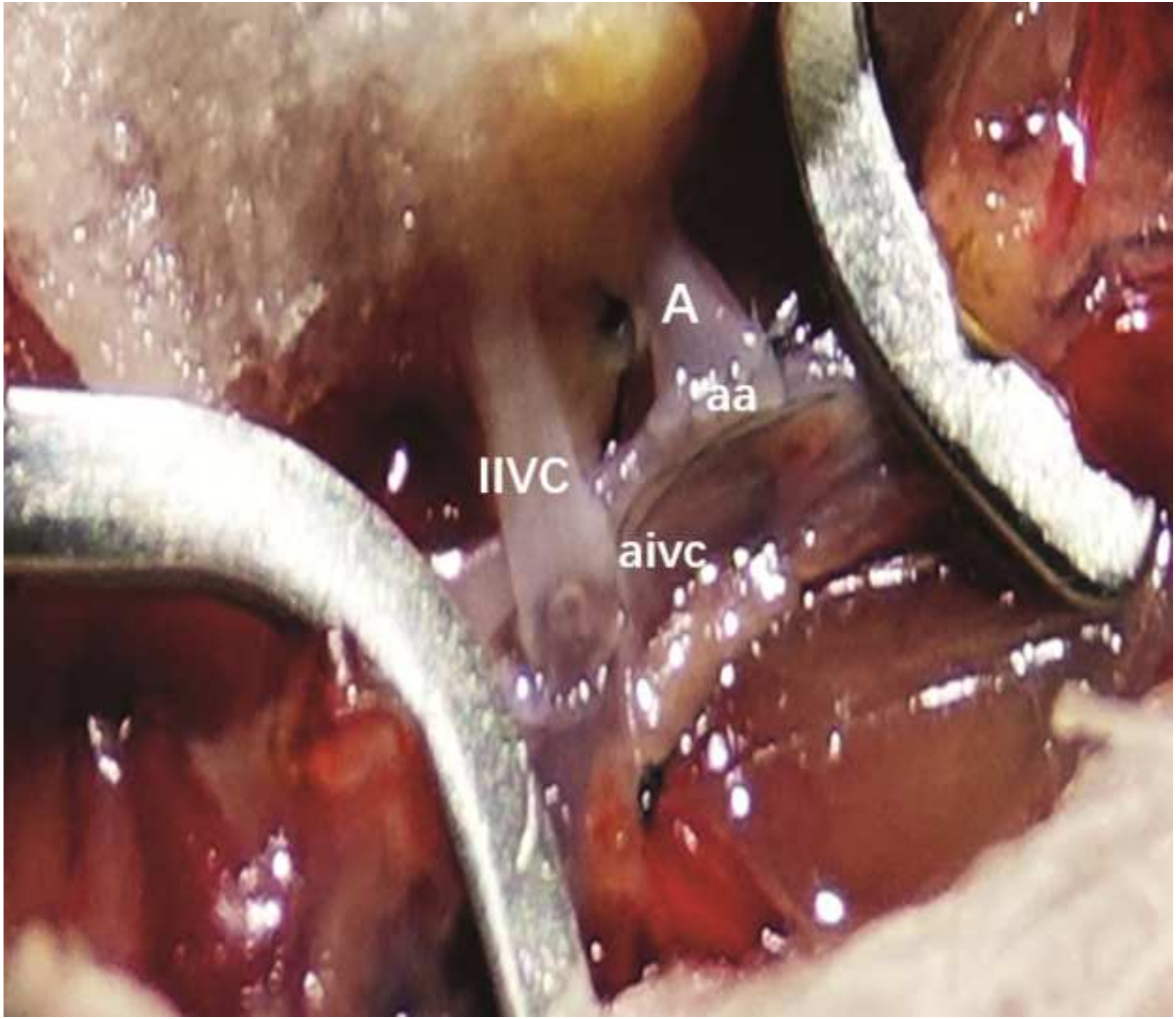

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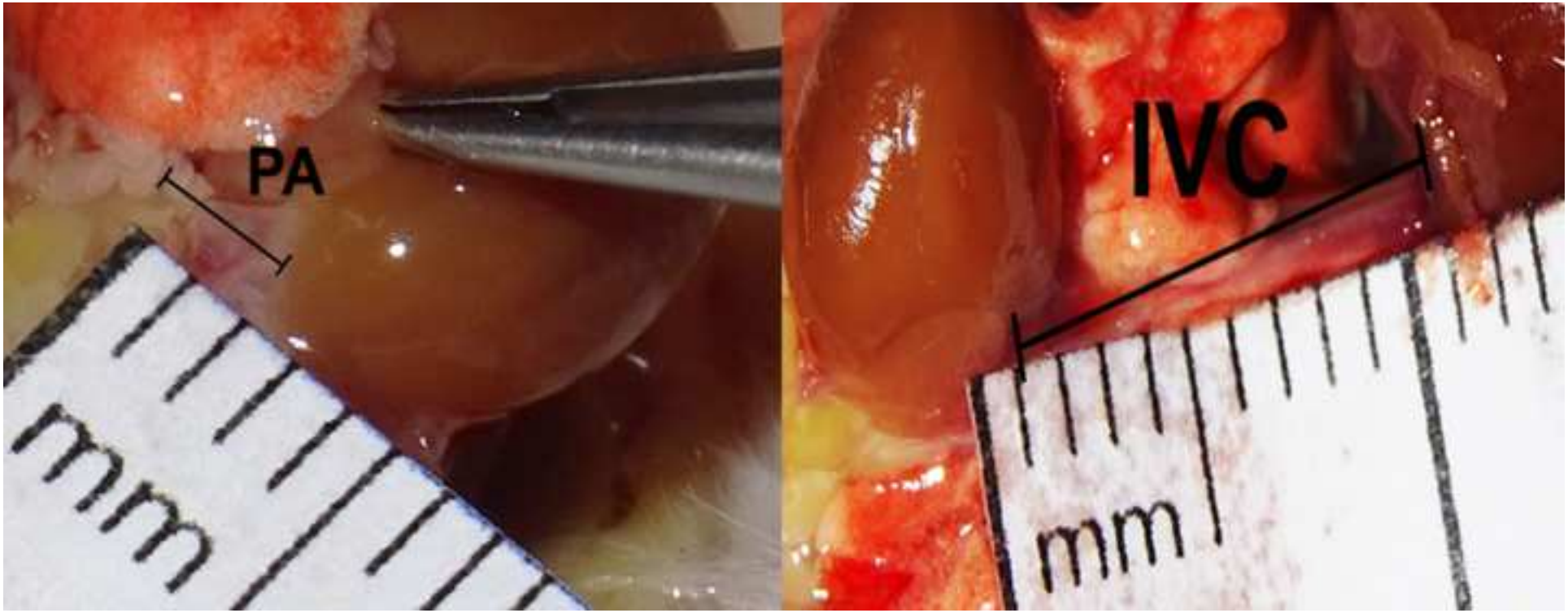

figure5

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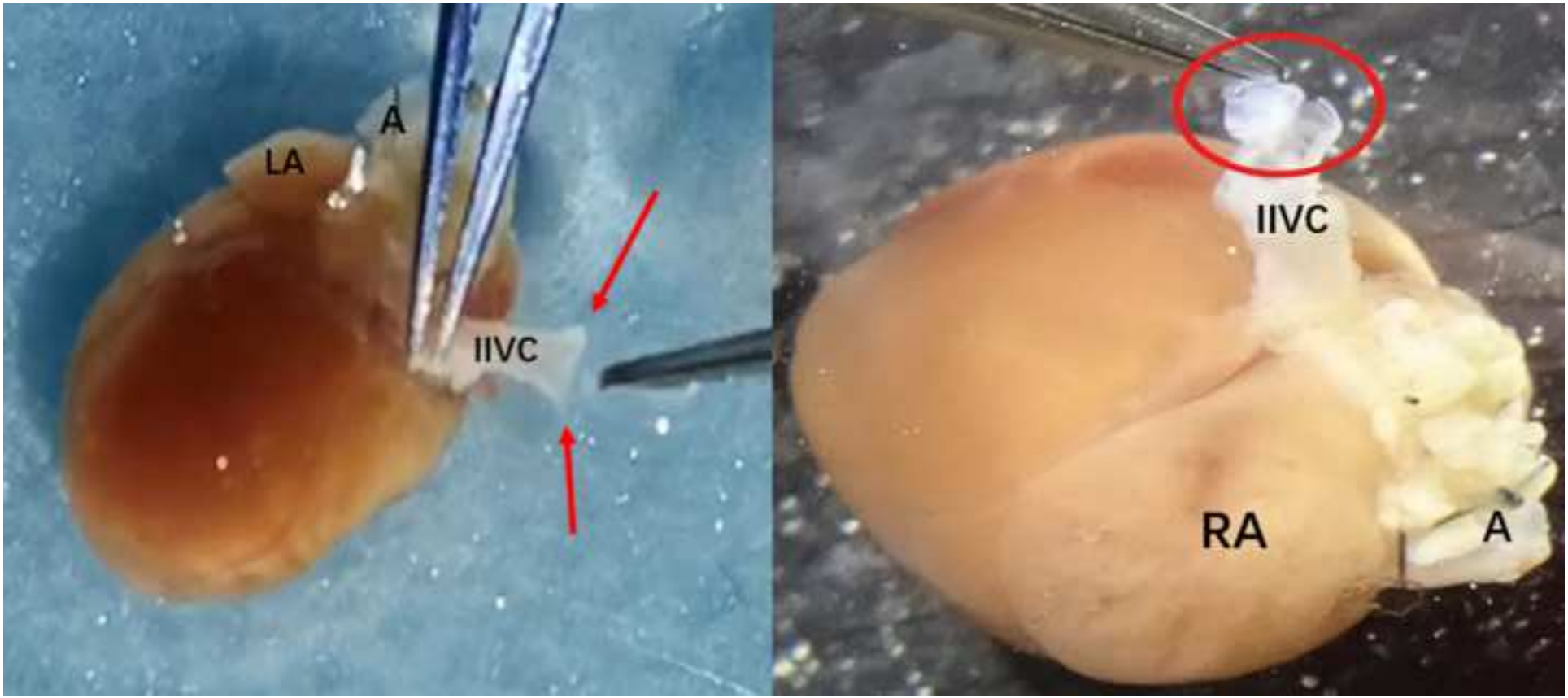

Table 1

	Vena cava to vena cava (new method)
Donor procurement (min)	12.8 + 0.3
Recipient operation (min)	42.0 + 1.5
Vessel anastomosis (min)	20.8 + 1.3
Cold ischemia (min)	32.8 + 0.6
Warm ischemia (min)	25.5 + 1.2
Rebeat postoperation (min)	1.1 + 0.2
Success rate	92.50%

***P < 0.05**

****P < 0.01.**

ɔnary A to vena cava(traditional method)
11.4 + 0.4
48.6 + 1.4*
27.5 + 1.3*
34.2 + 0.7
32.6 + 1.3*
2.1 + 0.4**
90.00%

Table of Materials

Click here to access/download;Table of Materials;Table of Materials.xlsx

Name	Company	Catalog Number
30G-needles	Braun	456300
acepromazine	CP Pharma	Tranquisol P
BALB/c AnNCrl mice	Charles River. Germany	no catalog number
Bepanthen eye ointment	Haus-Apotheke	PZN 01578675
Bonn Micro Forceps	FST	11083-07
Box for insulation and oxygen supply de	RUSKINN	INVIV
C57BL/6J mice	Charles River. Germany	no catalog number
Carprofen	Zoetis	Rimadyl 50 mg/ml
CATHETER-FEP 26G	TERUMO	Surflo-W
Clip Applicator Forceps Style	FST	18057-14
Curved forceps	WPI	14114-G
custodiol/HTK	Dr. Franz Köhler Chemie	no catalog numer
Cutasept skin disinfection	VWR	BODL980365
electrosurgical pen	Bovie	CHANGE-A-TIP
gauze pads, cotton swabs	Lohmann-Rauscher	13353
Heating mat	THERMO MAT PRO 30W	HTP-30
Hemostatic sponge	CuraSpon	J1276A
heparine-solution	Haus-Apotheke	PZN 03029820
Ice box	PETZ	No Catalog Number available
Inhalation anesthesia device	GROPPLER	BKGM 0616
insulation and oxygen supply device	RUSKINN	INVIV
isoflurane	CP Pharma	Isofluran CP 1 ml/ml
ketamine	Zoetis	no catalog numer
metamizole	WDT	no catalog numer
Micro scissors	FST	15000-00,15000-10
Micro Serrefine (Clamp) Angled / 16 m	FST	18055-06
Microscope	Leica	LEICAMZ6
Microscope light	SCHOTT	KL2500LED
Saline solution (NaCl 0.9%)	Haus-Apotheke	PZN 06178437
Scissors	Peha Instruments	991083/4
small Petri dish	Sarstedt	833900
Straight forceps	WPI	14113-G
surgical tape	BSN	4120
Suture Tying Forceps - 10 cm	FST	18025-10
Sutures(10-0)	Medtronic	N2540
Sutures(4-0)	ETHILON	V4940H
Sutures(7-0)	ETHILON	1647H
Syringe (0.3 mL)	BD	324826
Syringe (1 mL)	BD	320801
xylazine	Bayer	Rompun

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22.03.2021

Dear Dr Nguyen, dear Madams and Sirs,

Thank you for offering us the opportunity to resubmit a revised version of the video and the manuscript. We revised all the issues you have suggested. In the manuscript the modifications were highlighted accordingly.

Changes to be made by the Author(s) regarding the written manuscript:
1. Please include the Table presented in the video as an Excel file and discuss it in the results as well.
We added it into manuscript 218-226 and discuss it in the part of Discussion 290-298

Changes to be made by the Author(s) regarding the video:
1. Pacing
01:16 - 01:16 Please fade out the title, then fade up the video for every section break.

We extended the fade out in this place,and added fade out-fade up effect in every transitions.

02:37 - 02:41, 03:13 - 03:21 Please remove these long gaps of no narration.

We removed these long gaps.

07:47 - 07:49 Please remove this shot of the arms coming out of the box. It's too quick of a shot and not needed.
We removed these shots.

2. Audio
00:18 - 00:18 The narration audio is peaking real bad as well. Please lower the volume so it peaks between -12 to -6 db.

We re-recoded every audio paragraphs completely you mentioned and lower the volume to make the voice sound consistent. Please convey our apologies to the editor who is in charge of the audio.

00:27 - 00:31 This narration was re-recorded. I'm unsure why because it wasn't a note I gave. In fact most of the audio notes weren't addressed, but this random part was?

We re-recoded this paragraph completely from 00:18 - 00:48 and lower the volume to make the voice sound consistent.

00:31 - 00:31 The audio sounds like there's a duplicate track that's 1 frame off, so it sounds almost robotic/tinny. Please fix the audio so it doesn't sound like this.

We re-recoded this paragraph completely from 00:18 - 00:48 and lower the volume to make the voice sound consistent.

01:30 - 01:30 The word ""Exposed"" gets cut off. Please trim this so we can hear the full word.

We re-recoded this paragraph completely from 01:30 - 01:54 to make the voice sound consistent.

01:31 - 01:31 It sounds like the audio is being cut off at the beginning. Please either retrim the audio clip or rerecord it.

We re-recoded this paragraph completely from 01:30 - 01:54 to make the voice sound consistent.

01:43 - 01:43 The sentence sounds like it gets cut off here, please allow the sentence to end before cutting the audio.

We re-recoded this paragraph completely from 01:30 - 01:54 to make the voice sound consistent.

01:47 - 01:47 The audio before this point, and after sound completely different. Either they're recorded by different people, or in different locations, or maybe they're closer to the mic than the other because you can hear the breathing directly on the mic. I highly recommend re-recording the narration all at once by the same person in the same location at the same time.

We re-recoded this paragraph completely from 01:30 - 01:54 to make the voice sound consistent.

3. Composition
00:51 - 08:43 There's a black strip on the bottom from here until the end of the video. Please crop this out.

We remove this black strip.

08:14 - 08:14 When the figure fades out, there's a weird black border around the frame. Please fade out so there's no black border.
We cut and remove this gaps.
4. 8:03 - Please increase the resolution of the table.

We created a new table with bold font to let it look´ s clear

Kind regards, Yours

PD Dr. med. A. Hörning

Leiter der pädiatrischen Gastroenterologie und Hepatologie