Peer Review File

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Reviewer A

The authors report a feasibility study on the multiple organ block for ex vivo normothermic machine perfusion technique. The focus is on the surgical and functional aspects. In my opinion, the procedure was optimized after the first two animals, perhaps including two more pigs would have been better on statistical grounds.

Comment 1： Introduction/general: the English language should be revisited, several formulations are not adequat. E.g.: line 91: thereby reducing the enzyme activity and slowing down cell metabolism. Line 125: "Be different=? Line 152: There were 5 pigs that underwent ..., I suggest to carefully look at this aspect for the whole text.

Reply 1： Thank you for your conscientious review, the English language in the part of introduction has been revisited. Several inadequate formulations have been modified in the manuscript, and the manuscript has been polished by the American journal experts (AJE) company.

Changes in the text： We have modified our text as advised (see Page 5, line 91; Page 6, line 125; Page 8, line 151-152 and so on)

Comment 2： Line 90 and 105, they use "ho" preservation, this has to be changed to normothermic.

Reply 2： Thank you for your good comment, the hot preservation used in this article is really inappropriate, and the manuscript has been revised.

Changes in the text： We have modified our text (see Page 5, line 90; Page 5, line 105).

Comment 3： Materials and methods: avoid replication of the text: lines 157 ... vs 255 ...

Reply 3： Thank you for your careful review. This was a personal mistake that led to

the duplication of the manuscript content. I apologize for this and have reworked it in the manuscript, and thank you again for your careful review.

Changes in the text： We have modified our text as advised (see Page 8, line157-159; Page 14, line284-286).

Comment 4： Line 248: two additional pigs would have been better, take out animals 1 and 2 and report on 5 animals that underwent the same procedure otherwise the statistics is of little importance.

Reply 4： Thank you for your good comment, this is indeed a limitation of this research. This research is an observational study, and it would be more convenient for statistical analysis if we could add two cases to meet 5 cases. However, due to the current staff and financial constraints, the experiment could not be completed in a short time. With sufficient funds, we will design a more rigorous controlled experiment to verify the results.

Changes in the text： No modification.

Comment 5： Table 1: please explain better: "number of hypothension.

Reply 5： Thank you for your good comment. Hypotension was defined as the mean arterial pressure (MAP) was less than 60 mmHg in this research. When the MAP is lower than 60, the organ will be in a situation of ischemia and hypoxia, which causes functional damage to the organ, clinically known as functional warm ischemic injury. Counting the number of intraoperative hypotension, as well as their duration, provides a more complete statistic of warm ischemia time and thus predicts organ damage.

Changes in the text： No modification.

Reviewer B

This is a very interesting and novel paper. The research is very topical and of significant relevance to the developments in organ preservation today. It is well written however a review of syntax and grammar would bolster its merit. There are several comments and questions on each section.

Comment 1： 1) The authors use the term ‘ hot preservation’ – this is an unusual term

that is perhaps not widely recognised – and may warrant changing to ‘warm’.

Reply 1 : Thank you for your good comment, the hot preservation used in this article is really inappropriate, and the ‘hot’ has been changed to ‘warm’ in the manuscript.

Changes in the text : We have modified our text as advised (see Page 5, line90; Page 5, line105).

Comment 2 : During NMP what was the constitution of the perfusate? Whole blood was used but what further components or agents were added to the perfusate? (I note final rehydration volume is higher than the blood collection volume (Table 1).

Reply 2 : Thank you for your comment. This was a major oversight in the writing of the manuscript, as the detailed ingredients of the perfusate were not described in detail in the previous manuscript, and a comprehensive table of perfusate ingredients has now been added to the manuscript.

Changes in the text : We added some data in the section of Results and Table 1 (see Page 14, line 275-284, and Table 1)

Comment 3 : What was the method of isolation of the ureters? – the authors state the ureters were cut – yet the bladded preserved? Was the bladder part of the AMOB? – how was urine collected? What was the protocol for urine volume replacement?

Reply 3 : Thank you for your comment. The method of isolating the ureters was not described in detail in the previous manuscript and has been added to the manuscript. The ureters were not cut in this research, the urethra was cut near the bladder (please see the page 10, line 203). We did separate the bilateral ureters intact and the bladder tissue together with the posterior peritoneum during the procurement process. It is true that the bladder is not part of the abdominal organs and we preserved the bladder for the purpose of direct bladder insertion of the catheter to easily collect urine. Because this article is a multi-organ perfusion, fluid loss is a major problem. Therefore, there is no special replacement solution for urine, but rather timely addition of supplementary fluids (such as saline solution, sodium lactate ringer's, glucose liquid, and allogeneic blood, etc.)

Changes in the text : We added some data in the section of Methods (see Page 10, line 203-205).

Comment 4 : How was rectum and esophagus ligated? Stapler device? - what was there a chance for contamination?

Reply 4 : Thank you for your comment. Rectum and esophagus were double ligated with #0 silk wire. During the operation, the principle of asepsis was strictly observed, and the severed end was cleaned three times with iodophor immediately after the separation of the esophagus and rectum, and then double ligated with silk thread to avoid leakage of the contents.

The process of placing the gastric tube may cause contamination. In addition, the ligature must be tied tightly, otherwise it may cause dislodgement over time, especially in the rectum. Due to the presence of normal intestinal peristalsis, the rectum is likely to dislodge the suture if it is not tied tightly.

Changes in the text : No modification.

Comment 5 : The authors perfused at 37.5 C° temperature - however normal temperature of pigs is closer to 39C° – can the expert authors comment on whether they think this is significant? – I appreciate the majority of ex-vivo porcine work is conducted at 37C°.

Reply 5 : Thank you for your comment, this is a very interesting issue to explore. It is indeed worth exploring whether to set 39C° will get a better perfusion effect in this research. In our study, one of the most important problems we have faced is the presence of organ temperature gradients. Since the thermostatic water bath mainly heats the perfusion fluid from the bottom, the temperature of the tissue located at the bottom is higher, while the surface tissue of multiple organs has a temperature gradient gap due to the difficulty of rapid heat transfer up from the bottom and faster heat dissipation in contact with air. If set to 39℃ in this study may cause high bottom tissue temperature, and we do not yet have a nice methodology to solve this problem. There is no uniform standard for setting the NMP temperature of porcine organs, which is mainly set at 37 ℃, 38 ℃ and 39 ℃. However, we consider that there is no difference between the temperature setting of 37℃ and 37.5℃ in this study.

Changes in the text : No modification.

Comment 6 : Why did the authors choose continuous perfusion? Vs pulsatile? Can they rationalise their choice for the reader?

Reply 6 : Thank you for your comment, we choose the continuous perfusion in this research. We also considered that pulsatile may be more physiologically appropriate, but are limited by the NMP machine and are currently unable to perform pulsatile perfusion.

Changes in the text : No modification.

Comment 7 : Was any additive required to help with acid/base balance?

Reply 7 : Thank you for your comment. In this research, sodium bicarbonate was used to regulate acid-base balance.

Changes in the text : No modification.

Comment 8 : Did the authors conduct any oxygen consumption analysis? And did hematocrit change during the course of perfusion – as this can affect oxygenation.

Reply 8 : This is a very good suggestion. In this research, we regularly tested the blood gases and found that the partial pressure of blood oxygen was high, the color of arterial and venous perfusion fluid differed significantly, and arterial blood was red in color while venous blood appeared dark black. We can monitor these indicators to predict organ function and therefore did not include indicator of oxygen consumption. This is a very good suggestion, and this important index will be added in the subsequent study. The hematocrit remained low during the experiment, and HCT values were often below 15%. We were not very active in adding allogeneic pig blood for fear of the possible adverse effects it might cause.

Changes in the text : No modification.

Comment 9 : In results they document that urine was collected and measured – however it does not mention the technique used in the methods. This should be added. They also mention that bile was collected - this was not mentioned in the Methods and the

technique should be added.

Reply 9： Thank you for your careful review, the method of collecting bile and urine has been added to the Methods section of the manuscript, thank you again for your good comment.

Changes in the text： We added some data in the Methods. (see Page 12-13, line 252-262)

Comment 10： In the results – the authors state the Median WIT was 240mins (85-600) – however in the abstract this is reported as 300sec? – please clarify this discrepancy. 240mins is excessive WIT. This is confusing as the authors then state in line 268 that the WIT is reduced to 85 seconds. Table 1 also reports WIT in seconds – the discrepancies should be clarified and uniformly corrected.

Reply 10： I apologize for making a silly mistake, which was caused entirely by my lack of seriousness. The actual median WIT is 240 sec (85-600), instead of minutes. I have double-checked the manuscript and corrected the errors.

Changes in the text： We have modified our text as advised (see Page14, line290).

Comment 11： The authors report the 'AMOB functions were damaged' relating to the 1st two cases (Line 261). Can the authors elaborate on what they mean by damage? Is this related to the biochemical functional assessment or macroscopic evaluation?

Reply 11： Thank you for your careful review. The first 2 cases of AMOB organ damage were confirmed primarily on the bases of the subsequent NMP perfusion performance. When NMP was performed in the first 2 AMOB cases, the flow rate increased slowly at the same pressure setting, requiring high doses of papaverine bases for maintenance. Blood gas analysis results also showed a rapid rise in lactate, which quickly over 20 mmol/L, and less volume of urine production during NMP. These indicators are indicative of organ damage, leading to poor subsequent NMP perfusion.

Changes in the text： No modification.

Comment 12：If the rectum was tied off – did this cause GI distension? – was it relieved?

Reply 12： Thank you for your comment, and ligation of the rectum is likely to cause

GI distention. In this research, we took an indwelling gastric tube and an indwelling rectal drainage tube to solve the GI distension in the. Only if the intestine presents good peristalsis, the rectal drainage tube can smoothly drain the intestinal contents; if the perfusion is poor, the intestinal peristalsis is not obvious and the drainage effect is limited.

Changes in the text： No modification.

Comment 13： In line 295 – the authors report that GI secretions were minimal and there was no intestinal mucosal bleeding – how was this assessed/evaluated ? endoscopically?

Reply 13： Thank you for your comment. The estimation of GI secretions was mainly based on visual observation of intestinal distension. The presence of intestinal mucosal bleeding was mainly based on visual observation of the intestinal surface color (the appearance of the intestine appears distinctly dark black in case of bleeding) and the observation of bleeding using syringe puncture to extract intestinal contents.

Changes in the text： No modification.

Comment 14：The liver receives a dual blood supply – portal and arterial. In the authors system perfusion is primarily arterial. Ex-vivo Liver NMP (ie. ORGANOX) uses both arterial and portal perfusion. Did the authors sample portal blood - lactate/oxygen as another way to assess sufficient organ perfusion? This would be interesting.

Reply 14： Thank you for your good comment, but in this research we did not retain a portal blood specimen to test for lactate/oxygen. So many thanks for giving me such a good suggestion, and we will surely include this index in the subsequent study, which may bring very interesting results.

Changes in the text： No modification.

Comment 15： The results are described for up to 10h - but the authors state that perfusion lasted up to 45h in one and in most up to 24h – can the author clarify – and is there the supplementary data for the remaining ours of perfusion?

Reply 15： Thank you for your comment. This research is our first attempt to use NMP

to maintain AMOB ex situ, so we tried to maintain it for as long as possible, with the longest case being perfused ex situ for 45 h. Since this manuscript focuses on the question of whether the en bloc procurement method without cold preservation can be perfused, not all perfusion parameters information was shown in this manuscript.

Changes in the text： No modification.

Comment 16： The authors describe their definitions of 'Hypotension' and 'WIT' on line 291. This should also be in the methods.

Reply 16： Thank you for your good comment, the definitions of 'Hypotension' and 'WIT' on line 291 have been added in section of methods.

Changes in the text： We added some data in methods (see Page11, line 217-220).

Reviewer C

I believe there is an increasing interest in understanding multiorgan en bloc perfusion and this model takes us step closer.

I have some comments which I think should be addressed:

Comment 1： The readout is extremely difficult to interpret, global assessment of lactate may not capture the differential performance of certain organs and systems while over emphasising another. So was there any histology of the various organs through the perfusion? I think some pathology would add a lot.

Reply 1： Thank you for your good comment, we also believe that it is indeed difficult to evaluate organ function, especially of certain organs, based on the results of global assessment, such as lactate. We regularly retained pathological specimens from the small intestine and liver during NMP, specimens from the spleen, kidney, and pancreatic tissue were not retained due to concerns about blood leakage, but only at the end of perfusion. Pathological data of the liver and small intestine during perfusion have been added to the manuscript.

Changes in the text： We added some data in Results and Figure 7 (see Page 17-18, line 356-372).

Comment 2 : Why was there no comparison to an en bloc procurement with cold flush and back table preparation prior to ex situ perfusion? It would have been helpful to assess the impact of a transient cold ischaemia during procurement and see if it did indeed have a detrimental effect to outcomes?

Reply 2 : Thank you for your good comment. If we want to confirm whether this en bloc procurement method without cold infusion is superior, it is indeed necessary to add the use of cold flushing as a comparison. But in this research, we do not perform cold flush mainly because the WIT is relatively short and should have little influence on the results, so we eliminated the process of cold flush to simplify the steps. At present, we cannot add more studies due to financial constraints, we will consider adding cold preservation process in future studies to observe the effect of perfusion.

Changes in the text : No modification.

Comment 3 : Please refer to the consensus paper on how to refer to different aspects of NMP please https://onlinelibrary.wiley.com/doi/10.1111/ajt.13843.

Reply 3 : Thank you for your good comment, and I have read this consensus paper thoroughly and have benefited greatly from it, thanks again.

Changes in the text : No modification.

Reviewer D

This manuscript describes a technique of normothermic perfusion of an abdominal multiorgan block (AMOB) without a period of cold ischaemia.
Five cases using a porcine model are reported in an observational manner. Difficulties with stability of blood pressure and of blood loss in the first and second case resulted in suboptimal perfusion conditions. In the last 3 cases the AMOB were perfused for longer periods (45h in case 3) with more success.

This is an extremely demanding model and the authors should be congratulated on their achievements. However, the study is limited by the small number of experiments and the observational description. Overall, more detailed methods of the perfusion conditions and analysis of function are needed to determine ‘good function’.

Comment 1 : More detail is needed about the perfusion conditions. Whole autologous

blood was used and supplemented with allogenic matched blood as necessary. Papaverine was administered to maintain blood flow. Where any other ingredients added to support metabolism?

Reply 1 : Thank you for your good comment. The ingredients of the perfusion solution were not clearly stated in the previous manuscript, which was a major shortcoming, and have been added to the manuscript, thank you for your suggestion.

Changes in the text : We added some data in Results and Table 1 (see Page 14, line 275-282, and Table 1).

Comment 2 : Organ function was assessed by measures of flow, pressure, levels of ALT, AST, creatinine, lactate and glucose. Urine output is mentioned but the amount is not reported. The authors state that in cases 3-5 AMOB demonstrated 'good function'. There are number of limitations with the reported results. Lactate levels can vary, particularly if fresh blood is added to the perfusion system. Similarly, glucose levels where not stable, perhaps due to variable amounts of glucose being added, but it may also reflect organ dysfunction.
Without additional measurements of oxygen extraction, oxygen consumption, levels of ATP/ADP, markers of gut function and histological assessment, an assumption of viability or 'good function' cannot be made. Furthermore, levels of creatinine were stable throughout perfusion. If additional creatinine is not added into the circulating blood during perfusion this suggests that creatinine clearance and renal function was suboptimal.

Reply 2 : Thank you for your good comment. This manuscript is an exploratory study, primarily to attempt whether AMOB can be maintained ex situ using en bloc procurement surgery. Therefore, the retained metrics are not very complete, which is a major shortcoming of this paper. Some pathological information on the perfusion process has been added to the manuscript. In the follow-up study, we will perform a good experimental design to better show the maintenance of AMOB by ex situ normothermic machine perfusion.

Changes in the text : We added some data in Results and Figure 7 (see Page 15 line 304-305; Page 17-18, line 355-370, and Figure 7).

Comment 3 : Data is reported on 7 h and 10 h of perfusion but the AMOBs were

perfused for much longer. Can this additional data be reported? Why were longer perfusion times used?

Reply 3： Thank you for your comment. This research is our first attempt to use NMP to maintain AMOB ex situ, so we tried to maintain it for as long as possible, with the longest case being perfused in vitro for 45 h. Since this study focuses on the question of whether the en bloc procurement method without cold preservation can be perfused, not all perfusion parameters information was shown in this manuscript.

Changes in the text： No modification.

Comment 4： There are a number of errors throughout the manuscript; eg; the warm ischaemic is reported in the methods as minutes but the results as seconds.

Reply 4： Thank you for your comment, and I apologize for making a silly mistake, which was caused entirely by my lack of seriousness. The actual median WIT is 240 sec (85-600), instead of minutes. I have double-checked the manuscript and corrected the errors.

Changes in the text： We have modified our text as advised (see Page14, line290).

Comment 5： The manuscript would benefit from proof reading for the improvement of English and grammatical errors.

Reply 5： Thank you for your comment. Since English is not my native language, I apologize for many inappropriate expressions and grammatical errors. The manuscript has been sent to AJE Company for re-touching and editing, and I hope it will be more understandable to readers after the revision.

Changes in the text： We have modified our text as advised.

Reviewer E

Comment 1： In 2017, the authors reported the first cases of ischemia-free organ transplantation for the liver, and later on also for the kidney. These cases were published without several papers explaining the preclinical work, and thereby (to my knowledge) no other group has been able to replicate this technique of ischemia-free organ transplantation, which greatly hampers the success of IFOT. To me the current

manuscript seems like highly difficult adjustment of a technique (abdominal organ block transplantation) of which the indications and outcomes are poorly described in literature.
Subsequently, the rationale of this study is not clear to me. It would be valuable to add a paragraph to the introduction explaining the indications for abdominal multiple-organ transplantation, and cite other studies on the results of this technique. If for example the outcomes are poor, that is a good rationale to study and improve the preservation techniques.

Reply 1： Thank you for your careful review. Thank you very much for your concern about the direction of our group's research and for your very good suggestions. In fact, a number of large animal experiments were performed prior to the clinical application of ischemia-free organ transplantation, and it is true that the data from these studies were not published. At present, the group believes that the clinical application of ischemia-free organ transplantation is mature, and these preclinical studies may not have much significance for publication.
I apologize for not explaining the purpose of the article clearly in the introduction of the manuscript. In this study, we first attempted to use ex situ normothermic machine perfusion to maintain abdominal multi-organ block and the current goal of this study is to try to meet the needs of teaching and scientific research. Of course, it is undeniable that the ultimate goal is to apply this technology in the clinical setting, but we have only explored it initially, and there is still a long way to run before it can be used in the clinical field.

Changes in the text： No modification.

Comment 2： Only by reading the last paragraph of the discussion, “we can use this model for scientific research and teaching, such as drug development, organ-to-organ interactions, laparoscopic live organ training, etc”, it becomes clear that perhaps this manuscript does not focus on abdominal organ block preservation to enable successful transplantation. Only there, the potential relevance of the manuscript becomes clear. However, the introduction focuses on a completely different rationale (reducing IRI, HOPE/NMP). This is very confusing, please adjust either the introduction or the discussion to provide one story line.

Reply 2： Thank you for your careful review. It is true that this manuscript does not

mention what the significance of this study is in the introduction section, and lead to a bit of confusion in the logic of the manuscript. I have made adjustments in the manuscript and thank you for such good advice and for your concern about our study.

Changes in the text : We have modified our text as advised (see Page7, line127 to 132 ;).

Comment 3 : I recommend thorough rewriting of the manuscript, as multiple linguistical errors are present, which makes it difficult to read.

Reply 3 : Thank you for your comment. Since English is not my native language, I apologize for many inappropriate expressions and grammatical errors. The manuscript has been sent to AJE Company for re-touching and editing, and I hope it will be more understandable to readers after the revision.

Changes in the text : We have modified our text as advised.

Comment 4 : Page 14, line 277-278 'which has been observed in many failed attemps'. The authors should describe all cases and the lessons learned from this, rather than only a subset of successful cases. The current manuscript inhibits reproducibility by other centers.

Reply 4 : Thank you for your comment. Because this research is indeed an exploratory research, no similar reports have been found before. The process of organ procurement, the tailoring of organs, the development and improvement of machines, the setting of organ perfusion parameters, the formulations of perfusate, and the maintain of NMP process all took a lot of time to be improved. In these processes we used pig viscera from slaughterhouses for perfusion attempts, which were less effective and data collection was not ideal and therefore not easy to analyze. Based on these perfusion experiences, we optimized the details before we conducted perfusion studies in the laboratory using the DBD model and achieved some results. These optimized protocols should be able to provide some reference for other centers to engage in similar studies. It is undeniable that although we were able to maintain AMOB ex situ, many problems still exist and there is still a long way to proceed if we really want to achieve sustained multi-organ NMP ex situ and transplantation in the future.

Changes in the text : No modification.

Comment 5： The authors describe in the discussion that a DCD model might be more 'meaningful'. I can understand this point, as long as the rationale is to study improved preservation to enable successful transplantation. However, a few lines underneath this sentence the authors write "we can use this model for scientific research and teaching, such as drug development, organ-to-organ interactions, laparoscopic live organ training, etc". I do not understand why a DCD model has any relevance for the rationales described in this sentence.

Reply 5： Thank you for your careful review. I am apologizing for the unclear description in the manuscript that caused you difficulties in understanding. The meaning of a DCD model might be more 'meaningful' in the manuscript is that we can acquire commercial pig viscera from slaughterhouses (all in DCD mode) for AMOB ex situ NMP, which is actually one of the directions of our current research. The main consideration is to significantly reduce the cost and to be more in line with animal ethical requirements.

Changes in the text： No modification.

Comment 6： According to international reporting guidelines on machine perfusion of donor organs (Karangwa et al. Am J Transpl), the term ex-vivo should not be used and replaced by ex-situ. Ex-vivo means “outside the living”, which sounds strange when talking about organs from deceased donors.

Reply 6： Thank you for your careful review, and in accordance with your suggestion, the manuscript has been revised from ex-vivo to ex-situ, thank you for your suggestion.

Changes in the text： We have modified our text as advised (see Page 1, line 1; Page 3, line 45,47,49 ; Page 5, line105; Page 6, line120; Page 8, line 150; Page 15, line 299; Page 22, line 445,451).

Comment 7： Page 6 line 110-111: “Randomized controlled clinical studies have also shown that NMP is superior to SCS in liver preservation”. I disagree with this statement. In the cited RCT, no superiority of NMP to SCS was observed on any clinically relevant endpoint (e.g. graft survival, post-transplant cholangiopathy or CCI). Naturally, as a result of the washout effect, post-transplant transaminases are

lower following ex situ NMP, and therefore this primary endpoint was not clinically relevant.

Reply 7： Thank you for your comment, I apologize for the lack of objectivity and criticism in the manuscript, and have revised it. There is no clear evidence as to whether normothermic machine perfusion is superior to traditional static cold storage methods, and there is a need for strong evidence-based medical evidence to confirm this.

Changes in the text： We have modified our text as advised (see Page 6, line 111).

Comment 8： The success study seems hampered by the use of an animal model. In a human model, one could use red blood cells from the blood bank, instead of performing exsanguination of the donor intraoperatively resulting in hypotension and hypoxia. Would the authors perform this procedure clinically indeed with red blood cells from the blood bank, or also with exsanguinated blood? And for the pig experiment, would it be possible to use washed red blood cells from another pig from the same family?

Reply 8： Thank you for give us such brilliant advice! There are still a lot of difficulties to overcome for the clinical application of this study, but if it is really used in the clinic, homozygous washed red blood cells from blood banks are definitely preferred. This problem was also encountered in preclinical studies where ischemia-free liver transplantation was performed, but relied on clinically maturated blood group identification and cross-matching, which did not a problem after ischemia-free liver transplantation was applied in the clinical setting. We believe that the blood problem can also be solved in animal experiments if washed red blood cells from another pig from the same family are used, but we are limited by the laboratory conditions to do this work for the present.

Changes in the text： No modification.