

Dear Editors,

Thank you for reviewing our manuscript (Submission ID 78769): Esophageal magnetic compression anastomosis in dogs.

Thank you very much for your letter and advice. We have revised the manuscript, and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point by point responses to the reviewers' comments are listed below this letter.

This manuscript has been edited and proofread by *American Journal Experts*.

We hope that the revision is acceptable for the publication in your journal.

Look forward to hearing from you soon.

With best wishes,

Yours sincerely,

Xiang-hua Xu

Reviewer #1:

1. In the aim I suggest modify it to: "Prior To study the feasibility and safety of MCA in humans, we tested MCA technology to reconstruct the esophagus in dogs."

Reply: Thank you for your advice. As we are not native English speakers, the language and presentation of our manuscript has been now polished by American Journal Experts. We have accepted your suggestion. (Page 3, line 44-45, marked in red.)

2. In the methods authors must reflect if hand-sewn anastomoses are performed with monofilament, Multifilament, Single or multiple layers and the suture material. The last sentence is also badly explained: animal weight is analysed only at one month and with the sentence it seems it is evaluated at 1, 3 and 6 months postoperatively.

Reply: Thanks for your suggestion. This is our negligence. In the hand-sewn group, we performed with 4-0 multifilament Vicryl and used interrupted single-layer inverting sutures. It has been explained in the methods of revised manuscript. We did monitor the dogs' weight at 1, 3 and 6 months postoperatively. Because the weight at 3 and 6 months between two groups didn't have statistical significance, we didn't show the medium+/-SD. We have added relevant data. (Page 3, line 49, and Page 3, line 56-59, marked in red.)

3. Results: Please add the values (E.G medium+/-SD) and if the difference in anastomoses confection time and in animal weights at one month are statistically significant. Related to X-ray examination it must be explained if are performed for both groups, day of the examination and if there is any differences between the groups. In the histological evaluation authors must explain than these differences appear only at one month and if it is significant. How were those aspects evaluated (morphometric analyses?)

Reply: Thanks for your advice. We have added the values (average +/- SD).

The difference in anastomoses confection time and in animal weights at one month are statistically significant. X-ray examination is only performed in MCA group. The purpose was to locate the magnetic device position and to observe the time when the magnetic device fell off from the esophagus. In the HE dye, we used ImageJ software to count inflammatory cells. In hand-sewn group inflammatory cells are more than that in the MCA group at 1 month. (334±37 vs. 572±65, P<0.01) We observed there were more collagenous fibers that had dyed blue in the tissue of the hand-sewn group than in the MCA group at every time point. We have added these contents in the results. (Page4, line 61-64, marked in red.)

4. Conclusion: "After the operation, the recovery of the MCA group was faster and better than that of the hand-sewn group". This can not be concluded with the information provided in the abstract and maybe neither with the whole document (no differences in morbidity and mortality between experimental groups). I suggest to rewrite the last sentence to be more cautious: "This study shows that MAYBE MCA technology can be applied to human esophageal reconstruction PROVIDED THIS FAVOURABLE RESULTS ARE CONFIRMED BY MORE PUBLICATIONS."

Reply: We appreciate this suggestion. It's true that we weren't precise enough here. We just want to show that the MCA group dogs can eat earlier than the control group dogs after operation. We have changed it. (Page 4, line 70-72, marked in red.)

5. The last commentary about the results section and the first about the conclusion section applies also for the CORE TIP.

Reply: Thank you for your advice. The CORE TIP is a summary of the document. We have modified the CORE TIP according to the revised results and conclusion. (Page 4, line 77-85, marked in red.)

6. In the INTRODUCTION section, we can mention: When authors mention published studies in other fields with MCA (gastrointestinal anastomosis,

jejunal, cholangioenteric, etc.) it could be better to mention briefly the number of treated patients and the most relevant outcomes from those mentioned publications.

7. Reply: Thanks for your suggestion. This is really good advice. We have added more details about MCA when we mention published studies in other fields. *(Page 5, line 106-113, and Page 6, line 114-119, marked in red.)*

8. In the last paragraph, line 2, I think it could be better to write “MCA COULD be a superior” than “MCA will be a superior”.

Reply: Thank you very much for your advice. This is the same language problem that we used inappropriate words. We have revised it according to your advice and the language and presentation of our manuscript has been now polished by American Journal Experts. *(Page 6, line 131, marked in red.)*

9. When they speak about “clinical reports on esophageal reconstruction using MCA”, similarly I think it could be better to mention the number of treated patients in each report and the main results.

Reply: Thanks for your suggestion. We have added some related content. There are several clinical reports on esophageal reconstruction using MCA. In this article, we mentioned there are six treated patients and all achieved satisfactory results. Detailed results have been presented in the article. *(Page6, line 133-149, marked in red.)*

10. Then, they mention the patients treated by them; some of the publications are with patients, I think authors could explain how many animals and humans have been treated in their published research and the main outcomes in a few lines.

Reply: Thank you very much for your advice. We have added detail content about our team’s work of MCA. We have done a lot of animal experiments about MCA technology. We have finished choledochojejunosomy magnamosis with 26 dogs, rectovaginal fistula repair with 12 pigs and reconstruct vessels in liver transplantation by MCA technology. We also use MCA technology in laparoscopic pancreatoduodenectomy and rectovaginal

fistula repair clinically. These have all been mentioned in the document. *(Page 7, line 153-168, marked in red.)*

11. Maybe it could be useful also to provide the quality of evidence of the published literature (are only case reports or clinical series? Is there any comparative study?).

*Reply: Thank you for your advice. Choledochojejunostomy magnamosis with 26 dogs, rectovaginal fistula repair with 12 pigs were comparative study. Using MCA technology in laparoscopic pancreatoduodenectomy is a clinical series and rectovaginal fistula repair by MCA technology clinically is a case report. These also have all been mentioned in the document. (Page 8, line 153-168, marked in red.)*

12. Nearly at the end, they mention “there is a lack of research data and animal studies”. In fact, “lack of” is not the best word, maybe “paucity of published” or similar is better to explain, as examples two publications provided also in references list treat patients with atresia: o Zaritzky M, Ben R, Johnston K. Magnetic gastrointestinal anastomosis in pediatric patients. J Pediatr Surg. 2014 Jul;49(7):1131-7. doi: 10.1016/j.jpedsurg.2013.11.002. Epub 2013 Nov 7. PMID: 24952802. o Slater BJ, Borobia P, Lovvorn HN, Raees MA, Bass KD, Almond S, Hoover JD, Kumar T, Zaritzky M. Use of Magnets as a Minimally Invasive Approach for Anastomosis in Esophageal Atresia: Long-Term Outcomes. J Laparoendosc Adv Surg Tech A. 2019 Oct;29(10):1202-1206. doi: 10.1089/lap.2019.0199. Epub 2019 Sep 16. PMID: 31524560.

*Reply: Thank you very much for your advice. This is another language problem that we used inappropriate words. We have revised it according to your advice. (Page 8, line 170, marked in red.)*

13. Talking about the METHODOLOGY: Why did the authors selected to use dogs? Which breed are the dogs employed? There are no accepted atresia animal model? Some publications refers to another models: “These include pig, rabbit, and rat. In our experience, we found the pig to be an optimal model due to the ease of handling, anatomic similarity to humans, and downward facing

snout to reduce aspiration risk. [[Bruns NE, Glenn IC, Ponsky TA. Esophageal Atresia: State of the Art in Translating Experimental Research to the Bedside. Eur J Pediatr Surg. 2019 Aug;29(4):328-335. doi: 10.1055/s-0039-1693992. Epub 2019 Aug 19. PMID: 31426114.]]

Reply: Thanks for your suggestion. In this study we select mongrel dogs, because the esophagus during neck is long enough for reconstruction. This purpose is to avoid doing surgery in the thoracic cavity and improve the survival rate. Pigs must undergo esophageal reconstruction by MCA in the thorax, which may increase the difficulty of surgery. The cost of buying and feeding pigs is more expensive than dogs. In our experience, rabbit or rat's esophagus is too small. It limits the size of magnetic ring. The diameter of the hollow pipe in the magnetic ring can only be 2 mm, even more less. It may stop the flow of saliva into stomach. This may increase aspiration risk. Rabbits and rats have a lower tolerance to surgery than dogs. In our pilot experiments, the mortality of rabbits is higher than dogs. So we selected to use mongrel dogs. *(Page 9, line 179-185, marked in red.)*

14. Newly when they describe in surgical procedure "with 4-0 absorbable sutures", they must provide the following information: suture material, Monofilament? Multifilament? Single or multiple layer anastomoses?

Reply: Thank you for your advice. We used Vicryl to finish single layer anastomosis. It's monofilament material. We selected single layer anastomosis because multiple layer anastomoses on esophagus may lead to more severe stenosis. This has been explained in the article. *(Page 9, line 191, marked in red.)*

15. Question: was any surgical drain left in place?

Reply: Thanks for your question. There was no surgical drain left in place on both groups. We used prophylactic antibiotics for 3 days postoperatively. No incisional infection occurred. *(Page 9, line 192, marked in red.)*

16. At what day was the control X-Ray for MCA group performed?

Reply: Thank you for your question. X-ray was performed every day before the magnets fall off from the esophagus. We observe the time when the magnetic

ring falls off. *(Page 11, line 230, marked in red.)*

17. Why the postoperative management was different between groups? Contro group has 7 days fasting... This may be a source of bias to compare both groups...

Reply: Thank you very much for your question. It's our wrong that we can't articulate it. After the surgery, the dogs of two groups were fasted for two days and then started on enteral nutrition. But in the hand-sewn group dogs generally showed poor appetite, even refused to eat. They started on enteral nutrition on 4 days, 5days, even more long. During this period, we can only keep them alive with parenteral nutrition. Because of this, we wrote dogs of hand-sewn group fasting for 7 days. We now know that's not appropriate and have modified. *(Page 11, line 231-236, marked in red.)*

18. Both groups received antibiotics? Which antibiotics?

Reply: Thanks for your question. All dogs of both groups were given cefazolin sodium intramuscularly for 3 days postoperatively to prevent infection. Given 0.5g intramuscular injection twice a day. *(Page 11, line 237-239, marked in red.)*

19. I suggest adding the word Thrichrome to Masson dye.

Reply: We appreciate this suggestion. It's more accurate when adding the word Thrichrome to Masson dye. We have added it according your suggestion. *(Page 11, line 253, marked in red.)*

20. In statistical analyses, authors perform parametric studies. With 18 animals per group... Did authors performed a test of normality to be able to employ parametric testing?

Reply: Thank you for your question. We had performed a test of normality. The data conformed to normal distribution and homogeneity of variance. The statistical analyses by SPSS 25.0 are listed:

#### **normality test**

group Kolmogorov-Smirnov<sup>a</sup>

Shapiro -- Wilk

		statistic	freedo	signific		freedo	significa
		s	m	ance	statistics	m	nce
time	1	.122	6	.200*	.982	6	.961
	2	.183	6	.200*	.960	6	.820
Weight0mo	1	.154	6	.200*	.961	6	.828
	2	.219	6	.200*	.968	6	.878
Weight1mo	1	.220	6	.200*	.949	6	.734
	2	.253	6	.200*	.898	6	.363
Weight3mo	1	.150	6	.200*	.957	6	.799
	2	.197	6	.200*	.945	6	.701
weight6mo	1	.172	6	.200*	.966	6	.863
	2	.266	6	.200*	.866	6	.210

\*. the lower bound of true significance.

a. Rielly's significance correction

### Independent sample test

		Levin variance isogeneity test		T test for mean isogen				
		F	signific	t	freedom	Sig.	Means	standard
			ance				differece	deviation
time	Assumed equal variance	2.500	.145	5.710	10	.000	5.0000	.8756
	No assumed equal variance			5.710	7.860	.000	5.0000	.8756

Weight 0mo	Assumed equal variance	.229	.643	.261	10	.799	.1083	.4152
	No assumed equal variance			.261	9.509	.800	.1083	.4152
Weight 1mo	Assumed equal variance	.115	.741	- 2.497	10	.032	-1.0917	.4371
	No assumed equal variance			- 2.497	9.879	.032	-1.0917	.4371
Weight 3mo	Assumed equal variance	.009	.925	-.574	10	.579	-.2750	.4794
	No assumed equal variance			-.574	9.997	.579	-.2750	.4794
Weight 6mo	Assumed equal variance	1.370	.269	- 1.341	10	.210	-.5083	.3791
	No assumed equal variance			- 1.341	8.102	.216	-.5083	.3791

21. In the RESULTS SECTION: Concerning X-ray examinations... There were any leakage in any group? Later one fistula is described... How was it diagnosed? Only X-ray or was it clinical? At what postoperative day?

Reply: Thanks for your question. We only made X-ray examinations in the MCA group to locate the magnetic device position. The dog's fistula in the hand-sewn group was found by us because it suddenly refused to eat and the incision skin was red, swollen and purulent. We performed surgical exploration and found the fistula. It's on 8 days after operation. *(Page 13, line 271-273, marked in red.)*

22. The units of the weight (I suppose kg) are not specified. Is this difference in weights between groups statistically significant? If so, p value must be added.

Reply: Thank you for your advice. The unit of the weight is kg and we have added it. Before the experiment, the weights of dogs between two groups had no statistical significance. There was statistical significance at 1 month after operation,  $P < 0.05$ . At 3 months and 6 months after the operation, the dogs' weights had no statistical significance. All weights had listed as average  $\pm$  SD. *(Page 13, line 277-284, marked in red.)*

23. Final line of "gross appearance". Concerning the term "smoother", How was this evaluation made? If it is subjective it must be mentioned...

Reply: Thanks for your suggestion. We just observed the anastomotic tissue visually and subjective judgement.

24. Was the difference in inflammatory cells number at 1 month significant statistically?

Reply: Thanks for your question. The difference in inflammatory cells number at 1 month is significant statistically. The number of inflammatory cells in two groups separately is  $334 \pm 37$  vs.  $572 \pm 65$ ,  $P < 0.01$ . *(Page 14, line 311, marked in red.)*

25. In the DISCUSSION SECTION, there are some aspects to be commented deeply: When authors hypothesize "Based on the results of the experiment, we suggest that patients should consume a liquid diet or a semiliquid diet for at least one month", they refer to both groups or only to MCA patients?

Reply: Thank you for your question. We only refer to MCA patients. In the experiment, we finished esophagus reconstruction and found the tissue at the anastomosis was very thin at 1 month after surgery. The mucous membrane was intact, but the muscularis was separated at 1 month. For security, we suggest that MCA patients consume a liquid diet or a semiliquid diet for at least one month.

26. “Second, the silk thread or absorbable thread will exist”... The suture material could play an important role... Silk is a non absorbable material and braided sutures are more prone to infection than monofilament sutures... these aspects must be mentioned in the discussion section.

Reply: Thank you very much for your advice. We really should discuss the material of the suture. The suture material could play an important role in the healing of esophagus anastomosis. Vicryl is a absorbable material but the time of absorption is more than 1 months. During the period, Vicryl maybe as a foreign body leads to foreign body granuloma or anastomosis edema. Braided sutures are more prone to infection than monofilament sutures. Vicryl is the braided suture and maybe in this study Vicryl is not the best selection. This is a limitation of this article. We have added these aspects in the article. (Page 17, line 355-359, marked in red.)

27. Concerning the commentaries about the difference in weight between groups at 1 month, The animals in hand-sewn group were maintained fasting during a week, this could be a confounding factor and must be mentioned...

Reply: Thanks for your advice. It was our fault that we didn't articulate it in the methods. After the surgery, the dogs of two groups were fasted for two days and then started on enteral nutrition. But in the hand-sewn group dogs generally showed poor appetite, even refused to eat. They started on enteral nutrition on 4 days, 5 days, even longer. During this period, we can only keep them alive with parenteral nutrition. So it was not a confounding factor. This showed the superiority of MCA technology. The dogs in the MCA group could eat earlier than the dogs in hand-sewn group.

28. "Therefore, the dogs in the MCA group could feed earlier than those in the hand-sewn group" This could be discussed. Maybe when the mucosal tissue covers the anastomotic sutures it is not important than the suture stay in place in the external layers (eg muscular)... If so, when surgeon employ silk or other non asorbable material employed sometimes in esophageal surgery patients would have a more prolonged period with eating problems...

Reply: Thank you very much for your advice. It was really worth discussing. We just observed that the dogs in the MCA group could feed earlier than those in the hand-sewn group. But we didn't study the reason. It may be that the hand-sewn group dogs endured pain longer which could affect appetite. Maybe the mucosal tissue covers the anastomotic sutures is a reason. We gave up discussing the reason in this article and we only mention what we observed. (Page 17, line 374, marked in red.)

29. Limitations of the study must be presented. Strengths and weakness of the study and the model. The possibility of a bias produced by the different postoperative management of both groups. This is a model of esophageal anastomosis, and not of anastomosis in the setting of an atresia, with the possibility of loss of esophageal tissue, etc.

Reply: Thanks for your suggestion. There were several limitations of this manuscript: 1); our surgery location is on the dog's neck, not in the thoracic cavity. This is not consistent with the clinicopathology. The position of esophageal atresia is generally flush with the bifurcation of the main bronchus and maybe have a tracheoesophageal fistula. 2). in this study, we don't consider the effect of different structural sutures on the anastomosis. Vicryl is the braided suture and perhaps in this study Vicryl is not the best selection. 3) This is a model of esophageal anastomosis, and not of anastomosis in the setting of an atresia, there are several differences. The limitations have been elaborated now. (Page 18, line 393-400, marked in red.)

30. In the CONCLUSION SECTION, I think the writing must be more cautious and suggest some modifications: MCA is an effective and safe method for

esophageal reconstruction IN DOGS. The anastomosis with MCA is faster than the hand-sewn anastomosis. Postoperatively, SOME ASPECTS OF the recovery of the MCA group WERE faster and better than that of the hand-sewn group. We provide some INFORMATION USEFUL for THE FUTURE clinical application OF THE DEVICE IN SELECTED CASES.

*Reply: Thank you very much for your suggestion. Your description is more accurate than ours. We have revised the conclusion according your suggestion. (Page 19, line 402-406, marked in red.)*

31. FIGURE 1 LEGEND: In the letter C I suggest to add “the dispositive allows food passage” and in D “and the esophagus LUMEN is COMPLETELY open”. FIGURE 4: I think there is a mistake in the fifth line of the legend; The anastomotic tissue of the hand-sewn (instead of MCA) group at 1 month, 3 months and 6 months. (D E F). FIGURE 5: if the difference in the number of inflammatory cells at 1 month is significant it could be reflected also in figure 5 legend. Newly I would like to congratulate authors for their work. Keep working in this field and keep trying to publish the results of your research.

*Reply: Thanks for your advice. Because we are not native English speakers, we didn't use words accurately enough. We accept your advice and have revised in the Figure 1 C & D. Figure 4: It is a mistake. The anastomotic tissue of the hand-sewn group, not of MCA group at 1 month, 3 months and 6 months. (D E F). Figure 5: The difference in the number of inflammatory cells at 1 month is significant and we have added it in figure 5 legend.*

Reviewer #2:

Dear editor, Thanks very much for giving me such opportunity to revise the current version of the manuscript. This is an interesting paper regarding the use of esophageal magnetic compression anastomosis in dogs.

*Reply: We appreciate your positive comments sincerely, and hope that this MCA device can be widely used in clinical promotion.*