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Answer to the Reviewers

Name of Journal: *World Journal of Gastroenterology*
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Title: Intestinal precocious maturation can be induced in T-cell deficient athymic neonatal rats
Authors: Ester Arévalo Sureda, Catherine Gidlund, Björn Weström, Olena Prykhodko
Correspondence to: Ester Arévalo Sureda, PhD, Department of Biology, Lund University, Sölvegatan 35C, 22362 Lund, Sweden.
ester.arevalo_sureda@biol.lu.se

The authors would like to express the appreciation to all the valuable questions, comments and suggestions from the reviewers. Also we are grateful for the opportunity to respond.

(The reviewer's comments have been kept in italics and the answers can be found immediately below with a normal text in bullet points.)

Reviewer 1
The manuscript by Arévalo Sureda et al. examined gut maturation in T-cell deficient athymic neonatal rat model. The manuscript is basically well written and interesting. But, the results are interesting but a little confusing. There is room to be improved in the manuscript.

Major comments:
1. Nurturing by conventional dams increased their macromolecular absorptive capacity (BSA) and passive immunity (IgG transfer). This result indicates that nurturing by conventional dams delays gut growth and maturation, doesn't it? What causes this phenomenon?

- Nurturing by conventional dams increased macromolecular absorptive capacity (BSA), however no effects were observed in the absorption of the marker BIgG. The difference in absorption between the two marker molecules could be due to the different routes of transport, unspecific (BSA) vs. receptor-mediated endocytosis (BIgG).
- Immunocompetent dams provided with higher levels of passive immunity (IgG) simultaneously improving the unspecific intestinal absorptive capacity (BSA) of the nude pups during the suckling period, with better growth and survival. It was previously shown that immunodeficient mice fostered by wild-type dams had increased pre-weaning mRNA

expression of intestinal FcRn [28]. The importance of the absorption of immunoglobulins together with the lower availability of IgG from maternal milk of nude dams could explain why control nude pups nurtured by Nude or SD dams absorb BIgG equally.

- Consequently, differences in the absorptive capacity due to foster-nursing indicated that milk factors from conventional dams promoted gut growth and delayed gut functional maturation in nude rat pups. Thus, suggesting that the immunocompetence of the dam, maternal milk content and composition, and passive immunity transfer, participate on the timing of gut maturation even though it is a genetically programmed process. This would imply that nude dams would provide with immunodeficient milk lacking the capacity to supress gut maturation.

2. In Table 1, authors showed that one PHA or protease treatment are more effective for gut maturation than three times treatments. Discuss the reason.

- Repeated treatment with a lower dose would be an experimental simulation of the processes occurring naturally, a gradual transition from milk-based to solid-feed diet. A single administration of a higher dose would be a more suitable experimental tool, which could also be similar to an abrupt event, such as separation from the dam, with a fast compensatory mechanism of extra-accelerated maturation. Moreover, previous results showed that PHA and Protease induced precocious gut maturation were dose-dependent.

Minor comments:

Page 2, line 57. "was" reads "were".
Page 6, line 153. Check the font for "centigrade degree".
Page 8, lines 225, 233, 235, 248. Description of these p values should use "=" instead of "≤".
Page 9, line 259. "villous" reads "villus".
Page 9, line 271. "villous" reads "villi".
Page 11, line 331. "dependent" reads "depend".
Pages 11-15. References. Follow the author instruction for References format.
Table 1. Page 16, line 437. "treatments" reads "treated groups".
Page 16, line 439. Remove description for §§, §§§, §§§§.
Figures caption. Titles for Figure 1, 2, 3 seem to be too long. The detailed description should be in the legend text, not in the title. Figures of pages 18, 23 are redundant. Remove them.
Figure 1. Adult-type epithelium portion was indicated with white bars in the figure. But, the morphological difference between adult-type epithelium and immature epithelium is hard to see in the figure. The high-power picture of the portion will be helpful.

- The reviewer's comments have been highly appreciated, taken into account and applied in the text.
- Page 8, p values "≤" are kept when they include the values of more than one group.

- Figures of pages 18, 23 are redundant, however they were included due to the instructions for images containing overlapping 'arrows' to be included. We would like to apologise if it was not correct.
- We agree with the reviewer that a higher-power image would be helpful, however the villi can't be appreciated when using the 40x objective. Thus, it wouldn't be suitable to be used.

Reviewer 2

In the current manuscript, the authors reported that athymic (nude) rats gut maturation could be induced by enteral provocation of PHA and trypsin, and independence from thymus-derived T-cells. This is interesting and would gain our knowledge on intestinal maturation. The study was well designed and the manuscript was well organized. It is preferred to determine the disaccharidase activity of the small intestine for better denoting the gut maturation. A typo was present in the 7th paragraph of the Discussion section "simultaniously".

- We are aware that determination of disaccharidase activities is the method of preference for intestinal maturation assessment; however they were not included in this study. In previous studies we showed that precocious gut maturation induced by enteral PHA and protease had effects on disaccharidase with decreased lactase activity and increased sucrase and maltase activities. The shift in disaccharidase activities corresponded to the other maturational changes, which are included in this study.

- The typo has been corrected in the text.

Sincerely,

Ester Arevalo Sureda, PhD
Dept. of Biology, Lund University, Solvegatan 35C, Lund, Sweden
Telephone: +46 46 222 93 54 Fax: +46 46-222 4539
E-mail: ester.arevalo_sureda@biol.lu.se