Dear Dr. Bennet,

I am writing in response to the recent decision to reject our manuscript entitled: "The large-conductance voltage- and Ca2+-activated K+ channel and its 1 subunit modulate mouse uterine artery function during pregnancy". We would like to appeal the decision for two main reasons. First, Reviewer #2 recommended acceptance of our revised version of the manuscript, and we are willing to address his/her new comment. Second, although we believe that we comprehensively addressed the comments of Reviewer #1, as we detail below, we are willing to make additional changes to the paper to satisfy this reviewer.

Reviewer #1 had four criticisms in the first review, three of which he or she feels we did not adequately address in our revised manuscript.

1) "Inconsistencies and other aspects of the methods limit the strength of the study. Different buffers were used in the myograph and patch clamp studies without justification. One of the buffers contains 11 mM glucose (clearly hyperglycemic for mice). Part of the stimulations were performed with 50 mM KCl from a stock solution thereby considerably increasing not only extracellular K+, but also extracellular Cl- and osmolarity. It is not because others have been doing this as well, that this is justified." 

We addressed the question regarding the use of Krebs buffer by pointing out that the rationale for using high glucose concentrations is because it is important for *ex vivo* tissue metabolism. Hence, this buffer is widely used in physiology experiments, as described in pertinent literature, and for that reason, we did not explain these points in the manuscript. Additionally, we note that we treated vessels from all genotypes in the same conditions. Nevertheless, we would be willing to amend the text as follows (new text in italics): "UAs were dissected as described above and *cannulated onto glass cannulas filled with Krebs, a common buffer used in electrophysiological studies (Ketsawatsomkron et al. (2012); Cheranov and Jaggar (2004); Knot and Nelson (1998); Gutkowska et al. (1997)).”*

Regarding the question about adding KCl from a stock solution, we specifically performed an additional experiment to evaluate whether the contractile effect of this solution differs from that of an isosmotic high-K+ Krebs solution. In our previous response letter, we presented data showing that there was no difference between these two conditions. However, we did not include these control experiments in the manuscript because we felt it would deviate readers from the scope of our study. Nonetheless, we would be willing to mention this experiments in our manuscript since supplemental material is not permitted.

2) The reviewer commented: "The genotype of the fetuses is unclear. In the M&M section it is stated: BKCa-/- females and their WT littermates were mated to WT males, resulting in BKCa+/- and WT offspring, respectively. While in the discussion it is stated: In our study, all pups were BKCa+/- heterozygous, which may explain why fetal size did not differ from WT"

We attempted to address this concern by clarifying the sentence. We regret that this sentence was still confusing. We are completely willing to revise the Discussion again as follows:

*"All pups from BKCa+/- dams were either WT or BKCa+/- and were the same size. Conversely, Meredith et al. (2004) showed that BKCa-/- pups were smaller than their WT littermates. We conclude that loss of one copy of BKCa is insufficient to reduce pup size."*

3) The reviewer asked us to clarify the conclusions of our results. To do so and to address a concern of reviewer #2, we created a new schematic figure. This figure illustrates our main observations that BKCa channel activity increases during pregnancy in UA and this increase is, in part, through interaction with its 1 subunit. We regret that our discussion in the figure legend of possible mechanisms to explain our observations was unclear. We would be happy to revise the legend as follows (new text in italics):

“**Figure 8. Proposed model of BKCa regulation by pregnancy in murine UA.** *We propose three mechanisms by which the BKCa contribute to UA diameter under our experimental conditions.* In non-pregnant UA smooth muscle cells (SMCs), BKCa channels are primarily composed of -subunits alone.. In SMCs from pregnant UA, BKCa -subunits associate with 1- and ****1-subunits, increasing BKCa channel activity, SMC hyperpolarization, and UA dilation.. In BKCa knockout mice (BKCa-/-, middle panel), the lack of the repolarizing BKCa current leads to a a depolarized membrane, and a more contractile SMC, thus UA diameter is reduced. .In the case of 1knockout mice (1-/-, right panel), the lack of this subunit causes a similar reduction in UA diameter. However, the presence of both - and 1-subunits mitigate these reductions. EC, endothelial cells.”

4) The reviewer was satisfied with our answer to this question.

In the second review, Reviewer #2 raised an additional point about the difference in vascular tone between P14 and P18. We agree that this is an interesting finding and would be happy to add text to the Discussion to address this issue.

We hope that you will allow us another opportunity to address the remaining concerns of the two reviewers.

Best regards,