Evidence-Based View of Safety and Effectiveness of Prokineticin Receptors Antagonists during Pregnancy

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Figure S1: Effects of prokineticin antagonists’ PC7+PKRA on gestation outcomes

Panel B reports a graph that compares litter size of gravid mice treated or not with PC7+PKRA antagonists. Panel E & F report graphs that compare placenta and fetal weights of pups born from mother treated or not by PC7+PKRA, respectively. Panel D reports graph that compare placental efficiency of mice treated or not by PC7 or PKRA. Data are presented as mean ± SEM. *p < 0.05, bars with different letters are significantly different from each other.
**Figure S2.** Effects of combination concomitant treatment by prokineticin antagonists’ on the placenta proliferation, vascularization and structure.

**Panel A & B** depict comparisons of Gcm1 and Cd31 mRNA mRNA levels, respectively in placentas of CTL; PC7+PKRA. GAPDH was used to standardize mRNA expression. Data are represented as mean ± SEM. ns = not significant, *P < 0.05.

**Panel C & D** show Western blot analysis and quantification of CD31 protein levels in the placenta collected from CTL; PC7+PKRA treated mice. Standardization of immunoreactivity was performed using antibodies against β-actin.

**Panel E** shows Western blot analysis and quantification of PCNA protein levels in the placenta collected from CTL and PC7+PKRA treated mice. Panel F. Standardization of immunoreactivity was performed using antibodies against β-actin. Data are represented as mean ± SEM. *P < 0.05, ns = not significant.

**Panel G** depicts analysis of the placental zones of CTL and PC7+PKRA placentas. For each group, three placental sections/animal were analyzed. The graph shows proportions of the surface layer of 3 zones of the placenta (labyrinth, junctional zone, and decidua). Surfaces of the 3 layers were measured on parasagittal sections for each placenta. Mean values were used to calculate the mean surface proportion of the layers. Data are represented as mean ± SEM. *P < 0.05, ns = not significant.
**Figure S3:** Effect of combination treatment by prokineticin antagonists' on trophoblasts invasion and differentiation.

Panel A shows representative microphotographs of placental sections stained with Peroxide Acid Shiff at different magnifications. Placentas were collected from CTL, and PC7+PKRA treated mice at 12.5 dpc. **JZ**: Junctional Zone; **ITC**: Invasive Trophoblast Cell; **mBv**: Maternal Blood Vessel. Panel B depicts a graph that compares the number glycogenic cells in the maternal decidua. Panel C & Panel D show western blot analysis and quantification of CA9 protein levels in the placenta collected from CTL and PC7+PKRA treated mice. Standardization of immunoreactivity was performed using antibodies against β-actin. Panels E, F & G depict comparisons of **Placental lactogen**, **2 Hand1** and **Mash2** nd mRNA levels, respectively in placentas of CTL, PC7+PKRA. GAPDH was used to standardize for mRNA expression. Data are represented as mean ± SEM. ns = not significant, * p < 0.05.