

*Supplemental information.*

*Nicotine and Infection Resulted in Adverse Pregnancy Outcome in Term Dams (Fig. S1).*

Initially, dams in the Control (N=1), Nic only (N=1), MP only (N=2) and Nic + MP (N=3) groups were allowed to go to term. In the Nic only, MP only, and Control groups, dams delivered normal, live born pups with no evidence of dystocia or exudative metritis. In contrast, all three dams in the Nic + MP group had severe abnormal pregnancy outcomes. Due to the severity of the pathology observed in the MP + Nic group, the term studies were immediately discontinued in consideration of the ethical concerns for the welfare of the animals involved. Therefore statistical analysis was not possible. We felt it important to include the information on term data to make other investigators aware that combining infection and this dose of nicotine could create serious animal welfare issues.

The control dam had a normal litter (12 live born pups); the Nic only dam had 5 live born pups. One MP only dam had 6 live born pups, 1 of which was runted; the other dam delivered 12 live born pups. One Nic + MP dam experienced a normal delivery of two live born pups at GD 22; an additional two retained dead, macerated fetuses were found at necropsy on GD 25. The second Nic + MP dam did not experience labor and required euthanasia at GD 25. There were 2 retained fetuses and severe granulomatous abscesses were observed in both placentas (Fig.S1). The third Nic + MP dam experienced late onset of labor at GD 25, experienced dystocia and delivered two stillborn pups. Because of the dystocia, the dam was euthanized and 2 retained fetuses were found. The retained fetuses from the latter two dams were large, had bruised and misshapen extremities consistent with dystocia, and granulomatous inflammation of the placental

tissues. Although additional implantation sites were not observed grossly, fetal resorption was suspected but could not be confirmed due to the extent of the necrosis.

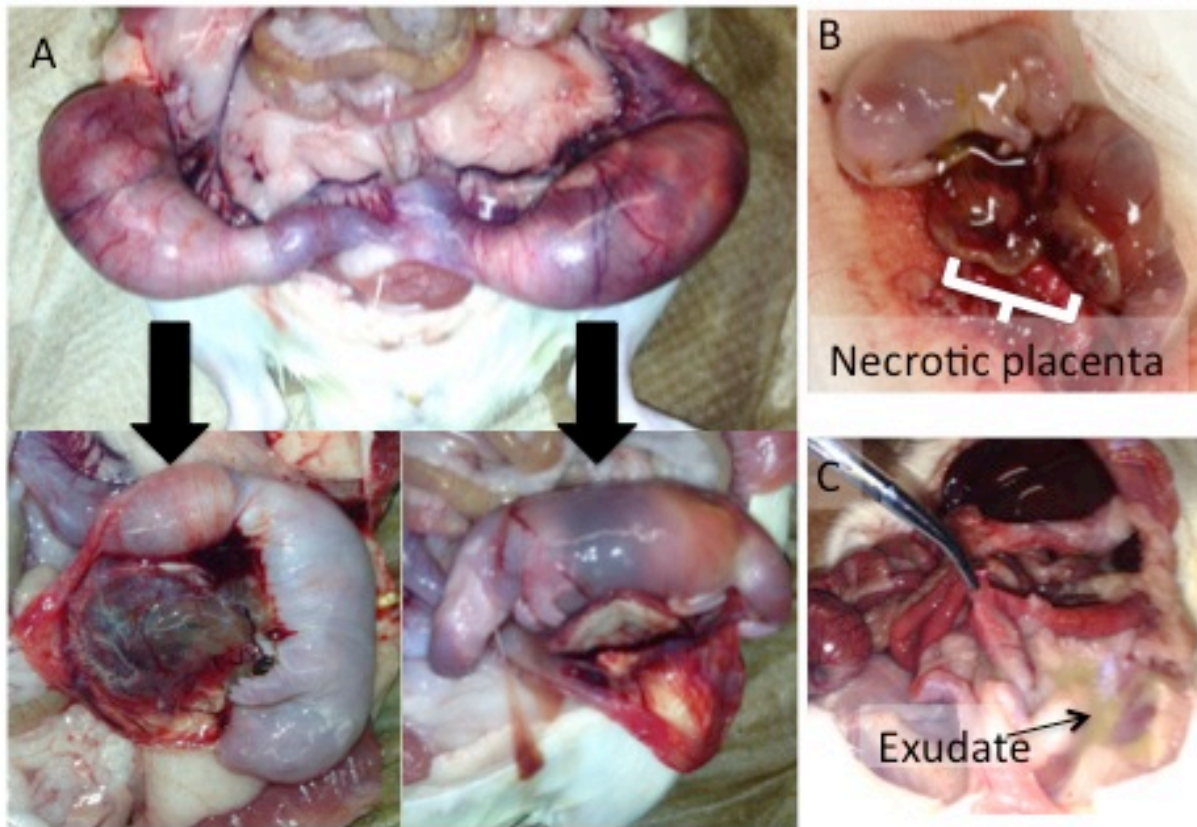


Figure S1. Adverse pregnancy outcome in term dams. A. Dam euthanized at GD 25 after failure to deliver. Arrows indicate fetuses that were removed from uterus at necropsy. Fetuses were large and misshapen, with bruising and swelling of muzzle, abdomen, and/or hind limbs. Granulomatous inflammation was widespread in placental disks. B. Dam euthanized at GD 25 after normal delivery of 2 pups at GD 22. Two macerated fetuses were retained in the uterus and were coated with viscous material. Placentas appeared necrotic. C. Upon excision of the uterus below the cervix, purulent exudate oozed from the vaginal canal.

*Detailed Scoring for Placental Lesions at GD 18 in Study Animals.* Detailed data is provided in Table S1 for the site-specific lesion scores used to obtain the maternal inflammatory response (MIR), fetal inflammatory response (FIR), or a potential mixture (MIXED) of both maternal and fetal components. A score of 0-3 was assigned for both acute and chronic inflammation at a specific site; 0 = little to no inflammation, 1 = mild inflammation, 2 = moderate inflammation, and 3 = severe inflammation, with a maximum score of 6. If tissue damage was so advanced that neutrophils could not be assessed, a score of 7 was assigned to indicate maximum lesion severity.

Supplemental Table 1. Median lesion severity scores and interquartile range for placental sites evaluated.

Site	Response <sup>a</sup> ( <i>P</i> value)	Control	MP only <sup>b</sup>	Nic only <sup>b</sup>	Nic + MP <sup>b</sup>
Choriodecidua	MIR ( $<0.0001$ )	1 (0.25 – 1.75)	<b>2 (2 – 4)<sup>A</sup></b>	2 (1 – 3)	<b>4 (2 – 5.5)<sup>AC</sup></b>
Subchorion	MIR (0.0008)	1.5 (1 – 2)	2 (1 – 3)	<b>3 (2 – 3)<sup>AB</sup></b>	2 (2 – 3)
Chorionic vessels	FIR ( $<0.0001$ )	1 (1 – 1)	<b>2 (2 – 3)<sup>A</sup></b>	<b>3 (2 – 4.3)<sup>A</sup></b>	<b>3 (2 – 3)<sup>A</sup></b>
Umbilical arteries	FIR ( $<0.0001$ )	1 (0.25 – 1)	<b>2 (1 – 2.75)<sup>A</sup></b>	<b>2 (2 – 4)<sup>A</sup></b>	<b>2.5 (1 – 4)<sup>A</sup></b>
Umbilical vein	FIR ( $<0.0004$ )	0 (0 – 1)	1 (0.75 – 2)	<b>2 (1 – 3.5)<sup>A</sup></b>	<b>2 (1 – 3)<sup>A</sup></b>
Wharton's jelly	FIR ( $<0.0006$ )	1 (0 – 2)	3 (1 – 3)	<b>3 (2 – 4)<sup>A</sup></b>	<b>3 (2 – 4)<sup>A</sup></b>
Chorionic plate	MIXED (0.0122)	1 (0.25 – 1)	<b>3 (1 – 3.5)<sup>A</sup></b>	3 (1 – 3)	2 (1 – 3)
Visceral yolk sac	MIXED ( $<0.0001$ )	0 (0 – 1)	1 (0 – 2)	<b>2.5 (1.75 – 3.25)<sup>A</sup></b>	<b>2 (1 – 4)<sup>A</sup></b>
Amnion	MIXED ( $<0.0009$ )	0 (0 – 0)	<b>1 (1 – 3)<sup>A</sup></b>	<b>2 (1 – 3)<sup>A</sup></b>	1 (0 – 2)

<sup>a</sup>P-values indicate significance of Kruskal-Wallis statistic across all treatment groups.

<sup>b</sup>Superscripted letters indicate significant differences among groups for Dunn's multiple comparison test: <sup>A</sup> significantly greater than Control; <sup>B</sup> significantly greater than MP only; <sup>C</sup> significantly greater than Nic only. Values that were statistically different are in bold.

Table S2. Culture status of placenta, amniotic fluid, and fetus for each dam and litter in the MP only and Nic + MP treatment groups. All dams in both treatment groups were culture positive for *M. pulmonis* in the uterus. In most cases, all placentas and amniotic fluids were available for culture; however, only a subset of fetuses were cultured.

<b>MP only Dams: Number Positive/Number Cultured (%)</b>			
Dams	Placenta	Amniotic Fluid	Fetus
All Dams	53/61 (87%)	43/57 (75%)	22/28 (78%)
Dam 1	8/14 (57%)	2/12 (17%)	0/4 (0%)
Dam 2	11/12 (92%)	12/12 (100%)	4/4 (100%)
Dam 3	10/11 (91%)	7/10 (70%)	3/4 (75%)
Dam 4	12/12 (100%)	12/12 (100%)	7/8 (88%)
Dam 5	12/12 (100%)	10/11 (91%)	8/8 (100%)
<b>Nic + MP Dams: Number Positive/Number Cultured (%)</b>			
Dams	Placenta	Amniotic Fluid	Fetus
All Dams	74/79 (94%)	73/80 (91%)	36/36 (100%)
Dam 1	11/16 (69%)	11/16 (69%)	4/4 (100%)
Dam 2	11/11 (100%)	9/11 (82%)	4/4 (100%)
Dam 3	5/5 (100%)	8/8 (100%)	4/4 (100%)
Dam 4	8/8 (100%)	8/8 (100%)	4/4 (100%)
Dam 5	13/13 (100%)	12/12 (100%)	4/4 (100%)
Dam 6	12/12 (100%)	11/11 (100%)	8/8 (100%)
Dam 7	14/14 (100%)	14/14 (100%)	8/8 (100%)