

## Abstract

One technique for the controlled delivery of ethanol to neonatal rat pups is intragastric intubation. Often, the vehicle used for delivery of ethanol is composed of a nutrient mixture to compensate for decreased suckling or other possible nutritional compromise. This study analyzed the selection of nutrient vehicle, the combination of experimental treatment groups within a litter, and the overall litter size on the growth rate of ethanol-intubated and intubated-control pups, compared with mother-raised control pups. Sprague-Dawley rat pups were raised in litters of 8 or 10, and administered ethanol by intragastric intubation with 20% (v/v) Sustacalm or 80% (v/v) Intralipid-II® nutrient vehicle. Pups were treated between postnatal days 2 and 10, and body weight was analyzed on day 10. Pups were assigned to a treatment group as either intubated ethanol, intubated control, or nonintubated mother-raised controls. Experimental comparison by statistical analyses was performed to identify the optimal treatment design (mixed treatment groups in a single litter or a single treatment group per litter), the optimal vehicle (Sustacal® or Intralipid-II®), and the optimal number of pups per litter (8 vs. 10). The analyses demonstrate that the mixing of intubated control, intubated ethanol, and nonintubated mother-raised control treatment groups within a single litter introduced an uncontrolled variable that confounded measurement of ethanol-specific alterations. The sensitivity of treatment groups to inclusion in mixed litters was dependent on the nutrient vehicle and thus nutritional adequacy. Our results suggest that an optimal design was achieved with eight pups per litter. Furthermore, ethanol intubated and intubated control pups grow at a rate identical to parallel litters of eight mother-raised control pups when Intralipid-II® is used as nutrient vehicle, and a single treatment group is present in a litter. Optimization of these experimental parameters has provided an excellent neonatal rat model for analysis of specific ethanol effects on brain development during the third trimester.