

| Maternal diet group | 2 mg FA | | 5 mg FA | | P-value |
|------------------------------|-----------|-----------|-----------|-----------|-------------------------|
| Postnatal diet group | 2 mg FA | 5 mg FA | 2 mg FA | 5 mg FA | |
| Epithelial proliferation (%) | 7.9 ± 0.8 | 7.4 ± 0.7 | 5.7 ± 0.6 | 5.8 ± 0.7 | 0.03* 0.08‡ 0.63§ |
| Apoptosis (%) | 3.3 ± 0.9 | 2.7 ± 0.8 | 3.6 ± 0.9 | 4.5 ± 1.3 | 0.43* 0.53§ |

Supplemental Table 1. Effect of maternal and postweaning folic acid supplementation on epithelial proliferation and apoptosis in mammary adenocarcinomas of the offspring at 21 weeks post-DMBA administration (28 weeks of age). Twenty representative mammary adenocarcinomas were selected according to the mean adenocarcinoma size from each maternal/pup diet group (total=80) for immunohistochemical analyses of epithelial proliferation and apoptosis. Epithelial proliferation was determined by staining histologic sections with monoclonal antibodies against Ki-67, a nuclear protein expressed in proliferating cells. Epithelial apoptosis was determined by Terminal uridine deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assay using the ApopTag Peroxidase In Situ Apoptosis Detection Kit S7100 (Millipore, Billerica, MA). Ki-67 and TUNEL staining was expressed as a percent of positively stained nuclei in relation to the total number of cells considered. Results are expressed as mean ± SEM. * Represents main effect due to maternal diet; § represents main effect due to pup diet; and ‡ represents multiple regression analysis adjusted for tumor latency. Maternal folic acid supplementation significantly decreased epithelial proliferation in mammary adenocarcinomas in the offspring (p=0.03). However, when tumor latency was included in the multiple regression analysis, the influence of maternal diet on mammary adenocarcinoma cell proliferation was no longer significant (p=0.08). In contrast, postweaning folic acid supplementation did not affect mammary adenocarcinoma epithelial proliferation in pups, nor was there any significant interaction between the maternal and postweaning diets. Neither maternal nor postweaning folic acid supplementation significantly influenced epithelial apoptosis in mammary adenocarcinomas in the offspring.