**Background and Hypothesis:** Coordinated changes in protein expression govern progression from pregnancy to lactation during mammary gland (MG) postnatal development. In published profiling of miRNA expression in whole mouse MGs, many miRNAs declined precipitously between pregnancy and lactation. We postulated that the decreased expression of miRNAs in the MG during this stage of development allows translation of genes critical for milk production.

**Methods:** To test this hypothesis, we isolated mammary epithelial cells (MECs) and performed simultaneous expression profiling of miRNA and mRNA. We identified microRNA-150 (miR-150) as having the highest fold decrease between pregnancy day 14 (P14) and lactation day 2 (L2). This was verified by qRT-PCR and *in situ* hybridization (ISH). Pathway analysis of miR-150 predicted targets significantly increasing in MECs between P14 and L2 suggested that miR-150 might play a regulatory role in lipid and cholesterol biosynthesis. By crossing WAP-Cre with ROSA26-lox-STOP-lox-miR-150 transgenic mice, we forced expression of miR-150 in the mouse mammary epithelium from late pregnancy throughout lactation to override the natural decrease.

**Results:** Compared to offspring nursed by litter mate controls, 3-day old pups nursed by the bi-transgenic dams had approximately 80% decrease in survival ($p<0.0001$) and surviving pups had smaller milk spots. Foster litters and surviving biological pups nursed by bi-transgenic dams showed failure to thrive. Compared to L2 MGs from normal dams, forced expression of miR-150 resulted in a decrease in alveolar density, which along with increase cleaved caspase 3 activity, suggested premature involution. Microarray performed on L2 RNA samples from MECs of both genotypes revealed that the majority of genes downregulated after forced expression of miR-150 were involved in lipid synthesis more than any other pathway critical to lactation. Suppression (~30-70%) of four predicted targets at the protein level was confirmed by western blot: FASN ($p=0.0001$), ACACA ($p=0.01$), OLAH ($p=0.006$), and STAT5B ($p=0.0005$). Mammary epithelial localization of FASN and ACACA suppression was confirmed by immunohistochemistry. Quantitative gas chromatography mass spectrometry revealed a significant reduction in medium-chain fatty acids, including the sum of all *de novo* synthesized fatty acids ($p=0.003$) along with 16:0 ($p=0.02$) and 18:0 ($p=0.03$), in MECs from mice with constitutive expression of miR-150 compared to control mice.

**Conclusions:** These results strongly suggest that the decline in miRNAs, such as miR-150, are necessary for successful lactation by allowing translation of critical genes including *Fasn*, *Olah*, *Acaca* and *Stat5b*.