Both obesity and DMBA exposure alter ovarian follicle number, ZP3 and PPP2CA in prepubertal females

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Obesity in adult females is associated with poor oocyte quality which limits female reproductive capacity. 7,12-dimethylbenz[a]anthracene (DMBA) is a polycyclic aromatic hydrocarbon which depletes the ovarian oocyte reserve through genotoxicity. Previously observed altered zona pellucida (ZP3) formation and Protein Phosphatase 2 Catalytic Subunit Alpha (PPP2CA) abundance in oocytes of adult female mice due to obesity and DMBA exposure led to the hypothesis that prepubertal obesity would alter oocyte ZP3 and PPP2CA basally and during DMBA exposure. Nine-week-old female C57BL6J mice were bred to proven males and plug checked daily. On gestational day 17, half the pregnant females were switched from a normal rodent diet containing 3.5% kCal from fat (lean) to a high fat diet comprised of 60% kCal from fat (HFD) which continued during lactation. At post-natal day (PND) 21, female offspring were weaned and received the same diet as their respective dams (lean n = 26, HFD n = 12), while the male offspring were removed from the study. All female offspring were fed *ad libitum* and puberty onset was monitored daily. At PND 35, each diet group was divided and injected intraperitoneally with corn oil as a vehicle control or 1 mg/kg DMBA for 7 d. Mice were euthanized on PND 42 and one ovary from each mouse was utilized for follicle counts and immunofluorescence staining. Body weight was increased ($P < 0.05$) by HFD but DMBA exposure had no effect. The age at puberty onset was reduced ($P < 0.05$) by the HFD but not altered by DMBA exposure. Ovarian weight tended to be increased ($P = 0.07$) in HFD mice, with no differences in DMBA-treated mice. A tendency for increased number of primary follicles ($P = 0.06$) in HFD mice was observed with no impact of DMBA exposure. Corpora lutea number tended to be increased ($P = 0.1$) by HFD DMBA exposed mice, but there was no effect of HFD alone. There was no impact of HFD or DMBA exposure on ZP3 abundance in developing follicles, however the ZP3 stain in primary follicles of HFD mice had a disconnected and underdeveloped appearance compared to lean control treated mice. PPP2CA was reduced ($P < 0.05$) in primordial follicles of HFD compared to lean mice. PPPP2CA in primary follicles was reduced ($P < 0.05$) by DMBA and HFD both alone and in combination. Preantral and antral follicles had reduced PPP2CA ($P < 0.05$) due to DMBA exposure in HFD mice, with no differences noted in lean mice. These findings support that HFD and DMBA exposure during adolescence both impact protein abundance in early folliculogenesis at puberty. Supported by 1R01ES030341-01 from NIEHS.