

Age-related changes in the uterine environment during the peri-implantation period of pregnancy.

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Decidualization and placentation defects are a major cause of age-related reproductive decline. This study tested the hypothesis that age-related changes in the uterine environment begin during the window of receptivity (WOR) in which suboptimal priming of endometrial stromal cells for decidualization may create a cascading effect, leading to compromised decidualization and placentation. To excluding ovarian aging factors, we used an artificial decidualization protocol in this study. After ovariectomy and two weeks of rest to eliminate endogenous ovarian steroids, aged (45-48 weeks of age) and young (6-8 weeks of age) female mice (n=21-24) were administered daily E2 (100 ng per mouse) injections for three days. After two days of rest, mice were treated with E2 (6.7 ng per mouse) and P4 (1 mg per mouse) for three days. On the second day [DD-1; mimicking gestational day (GD) 3.5], a subset of both aged and young mice (n=7) was euthanized 6 hours after E2 and P4 administration. On the third day (DD0; mimicking GD 4.5), the remaining mice were administered a single injection of 0.05 ml of sesame oil to the right uterine horn, and the uterine tissues (n=8) were collected 6 hours later. The rest of the aged and young mice (n=7) continued receiving E2 and P4 for one additional day and were then euthanized 6 hours after the injection (DD1; mimicking GD 5.5). Initial qPCR analyses showed that both the P4- and E2-mediated pathways were downregulated ($P<0.01$) as well as methylcytosine dioxygenase family (*Tet1*, *Tet2* and *Tet3*) in DD-1 aged uteri. Immunohistochemical analyses revealed decreases of PGR expression in the uterine stromal cells as well as increases of proliferation marker Ki67 in the uterine luminal epithelium cells of aged uteri at DD-1. Furthermore, there were increases in 5-methylcytosine (5mC) and decreases in 5-hydroxymethylcytosine (5-hmC) in aged uteri as compared to young uteri at DD-1. At DD0, qPCR analyses showed that aged female mice exhibited decreases ($P<0.01$) in mRNA expression of *Ptgs2* and *Foxo1* when compared to the young controls. At DD1, aged mice exhibited decreases ($P<0.01$) in the ratio of decidual to control horn weight compared to young female mice. There were also decreases ($P<0.01$) in decidualization markers *Igf1*, *Bmp2*, and *Prl8a2* in aged decidual uteri as compared to young controls at DD1. DD-1 uteri was further subjected to RNA-seq analyses. A total of 1,432 differentially expressed genes (DEG; 913 up and 519 down) were identified between young and aged uteri at DD-1. Canonical pathway analyses identified the top enriched pathways in the aged uteri, including activation of extracellular matrix organization, cellular stress and injury, and inhibition of cell cycle. We further identified 599 upstream regulators enriched by aging, including activation of TP53, IL6, IL4, AR, MUC1 and BCL2; and inhibition of MYC, SIRT1, SIX1, HAND2, PGR and

ESR1. This study confirms that alterations in uterine pathways during WOR impact implantation and decidualization and provides insights into understanding how aging affects the uterine function independently of the ovary.