Endometrial TGFβ signaling via TGFBR2 coordinates estrogen response during the peri-implantation window and is critical for pregnancy

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Establishment of pregnancy is a complex process that requires bidirectional communication between the maternal endometrium and the implanting embryo. Given that 1 in 4 pregnancies experience complications or result in pregnancy loss, defining the signals that coordinate endometrial receptivity is critical for improving pregnancy outcomes. Our previous work has demonstrated that the transforming growth factor β (TGFβ) pathway is critical for preparing the endometrium for implantation and for optimal pregnancy outcomes. Specifically, we showed that TGFβ signaling controls the endometrial response to estrogen and progesterone signaling. To identify the mechanisms controlling the dialog between TGFβ/estrogen/progesterone signaling during early pregnancy, we generated a new mouse model with conditional inactivation of the TGFβ type 2 receptor, TGFBR2, using progesterone receptor cre (PRcre) ("*Tgfbr2* cKO"). Compared to controls, mice with conditional TGFBR2 deletion are infertile and generated only one pup over the course of a six-month fertility trial (controls, 584 pups; *Tgfbr2* cKO, 1 pup). Mice with conditional TGBFR2 deletion also experienced high rates of mortality during the breeding trial (7/12 perished). To determine a potential role of *Tgfbr2* cKO on ovarian function, we monitored estrous cycling of control and *Tgfbr2* cKO mice for 30 days and found no statistical difference in the number of days spent in each phase or in the total number of cycles in the 30-day period between groups. Additionally, superovulation studies showed no statistically significant difference in ovulated oocytes between control and *Tgfbr2* cKO mice. Given the lack of significant ovarian defects in *Tgfbr2* cKO mice, we focused on the effect of conditional TGBFR2 deletion on endometrial function to explain the infertility phenotype. Timed mating analyses demonstrated that defects in pregnancy occurred during the peri- and post-implantation stages, where compared to controls, *Tgfbr2* cKO mice displayed fewer implantation sites at 4.5 days post coitum (dpc), 5.5 dpc, 6.5 dpc, and 8.5 dpc. Almost no implantation sites were recovered at 12.5 dpc in the *Tgfbr2* cKO mice, indicating that embryo resorption was likely completed by this point. Molecular analyses of control and *Tgfbr2* cKO implantation sites indicate a dysregulation of early pregnancy starting at implantation, that worsens throughout pregnancy and results in abnormalities in decidualization, uterine natural killer cell (uNK) invasion, and angiogenesis. At 4.5 dpc, implantation sites of *Tgfbr2* cKO mice demonstrate an increase in estrogen-response genes such as *Lifr, Lif, Lcn2,* and *Muc1*, suggesting unopposed estrogen response during early pregnancy. To further characterize the differences in hormone response between control and *Tgfbr2* cKO mice, we utilized murine endometrial epithelial organoids to define differences at the first place of maternal contact with the implanting blastocyst – the endometrial epithelium. Following estrogen and progesterone treatment, *Tgfbr2* cKO organoids displayed an upregulation of *Esr1,* as well as estrogen and progesterone-response genes, while no significant difference in *Pgr* expression was observed. These data indicate both increased endometrial epithelial estrogen response and impaired progesterone response in the epithelium of *Tgfbr2* cKO mice. Ultimately, our studies indicate that TGFβ coordinates the endometrial response to estrogen and progesterone, controls uNK cell recruitment, and that defective TGFβ signaling leads to impaired implantation and pregnancy defects during late gestation.

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