During morphogenesis, 2D epithelial tissue undergoes architectural changes to form 3D structures called folds. Folding is a key phenomenon during embryogenesis and organogenesis and is essential for several physiological functions. For example, folds in the stomach (rugae) and intestine (crypts) increase surface area for nutrient absorption and in the brain (gyri) increase cortical surface area for neural processing. The uterine luminal epithelium in mammals, including humans, horses, and rodents, undergoes structural changes to form folds. Although improper uterine folding in horses results in pregnancy failure, the precise role of folds in embryo implantation remains unknown. Using 3D imaging and 3D reconstruction of the mouse uterus, we uncover dynamic changes in the luminal folding pattern. We show that in a healthy pregnancy, the uterus forms transverse folds prior to embryo implantation. Using models of aberrant uterine folding, we show that longitudinal folds lead to embryo-uterine axes misalignment and abnormal chamber formation. Further, we show that increased estrogen signaling and reduced progesterone signaling lead to aberrant longitudinal folds. Finally, we extend our findings to examine the effects of excess estrogen signaling on folding during hyperstimulation – a clinical procedure performed during In Vitro Fertilization (IVF) to increase egg numbers for higher success rate of implantation and pregnancy. In women, pregnancies following hyperstimulation often lead to preterm birth, placental abnormalities, and other complications. Our findings suggest that hyperstimulation in mice leads to pregnancy loss due to aberrant folding. Our research can be potentially used to improve pregnancy outcomes following IVF and fresh embryo transfer. In addition to fueling future research on endometrial folds in humans, our research will open up new avenues for the treatment of infertility and provide new targets for diagnosis based on uterine 3D structure.