***The Zinc-finger Domains of Wilms' Tumor 1 Protein are Critical for Uterine Receptivity During Pregnancy.***

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Wilms' Tumor 1 (WT1) is a zinc-finger transcription factor required for cell survival, growth and differentiation. We and others have shown that WT1 is abundantly expressed in the stromal cells of the secretory endometrium and the decidua in the first and second trimesters in humans. However, the functional roles of WT1 in the uterus during pregnancy is unknown. Mice that carry the $Wt1^{f/f}$ allele flanking exons 8 and 9 of the *Wt1* homolog gene were bred to $Pgr^{cre/+}$ mice to generate $Pgr^{cre/+}Wt1^{f/f}$ ($Wt1^{\Delta/\Delta}$) mice. The $Wt1^{\Delta/\Delta}$ mice produced a shortened WT1 protein lacking the second and third of the four DNA-binding zinc-finger domains, validated via genotyping and western blot analyses. After 6-month breeding trial, $Wt1^{\Delta/\Delta}$ female mice were infertile ($P$<0.001) as compared to the $Wt1^{f/f}$ control mice (6.0±0.4 pups per litter). Also, haploinsufficiency was spotted in which significant reductions ($P$<0.01) in pups born per litter (3.4±0.5 versus 6.0±0.4), as well as survival pups per litter at weaning (1.0±0.5 versus 5.6±0.4) were observed in $Wt1^{+/\Delta}$ mothers as compared to the $Wt1^{f/f}$ controls. The rates of stillbirth and second-day neonatal death were also high ($P$<0.05) in $Wt1^{+/\Delta}$ females. Among the stillborn pups from $Wt1^{+/\Delta}$ females were the underdeveloped fetuses with congenital limb defects. Ovarian functions were not affected by *Wt1* mutation in which no differences in the number of blastocysts at gestational day (GD) 3.5 and serum P4 levels at GD 4.5 were observed between $Wt1^{\Delta/\Delta}$ females and the $Wt1^{f/f}$ controls. Blue dye injection analyses of implantation sites showed that uteri of $Wt1^{\Delta/\Delta}$ females exhibited no implantation responses at GD 4.5 and 5.5. Histological analyses further revealed that blastocysts can only attach to the luminal epithelium but failed to invade it and initiate decidualization at GD 4.5. However, at GD 6.5 and 9.5, smaller decidual bulb with reabsorbing blastocysts was observed in $Wt1^{\Delta/\Delta}$ uteri as compared to the control, suggesting delayed and defective implantation, decidualization and placentation. $Wt1^{+/\Delta}$ females also exhibited no implantation responses at GD4.5 and 5.5 and embryos were found attached to the luminal epithelium whereas at GD6.5 and 9.5 decidual bulb were observed on $Wt1^{+/\Delta}$ mice as compared to $Wt1^{f/+}$ control mice. Immunohistochemical analyses further showed that ESR1 decreased in glandular epithelium (GE) but increased in the stroma of $Wt1^{\Delta/\Delta}$ uteri at GD 3.5 as compared to the $Wt1^{f/f}$ control. PGR was downregulated in the stromal cells of $Wt1^{\Delta/\Delta}$ uteri between pre-decidual (GD 3.5) and post-decidual stage (GD 9.5). In addition, PTGS2 was failed to express in stromal cells of implantation sites at GD 4.5 $Wt1^{\Delta/\Delta}$ uteri as compared to the $Wt1^{f/f}$ control. At decidual day -1 (DD-1; one day before artificial decidualization to mimick GD3.5), *Areg* mRNA was decreased ($P$<0.01) but *Wnt4, Ltf*, and *Bmp2* were increased in $Wt1^{\Delta/\Delta}$ uteri as compared to $Wt1^{f/f}$. Moreover, the ability of the endometrial stromal cells to undergo an artificially induced decidualization

was severely compromised ($P$<0.01) in $Wt1^{\Delta/\Delta}$ uteri, with failure of induction in *Prl, Igfbp1, Pgr* and *Areg* at DD2 and DD5. These results suggest that *Wt1,* via its second and third DNA-binding zinc-finger domains, plays critical roles in implantation and decidualization, thereby governing placentation and fetal development during pregnancy.