

## **Wnt Signaling: A Key Modulator of Progesterone-Dependent Synchronized Proliferation of Rat Uterine Stromal Cells**

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The female sex hormones estrogen and progesterone stimulate the proliferation and differentiation of uterine cells. Pretreatment of ovariectomized (OVX) rats with progesterone for 3 consecutive days followed by a single injection of estradiol stimulates synchronous entry of uterine stromal cells into the cell cycle. We showed previously that progesterone pretreatment down-regulates glycogen synthase kinase (GSK) 3-beta and increases the accumulation of beta-catenin in uterine stromal cells suggesting progesterone-dependent activation of the canonical Wnt signal transduction pathway. However, estrogen is necessary for stromal cell entry and transit through G1 phase of the cell cycle. Cell cycle entry correlates with the estrogen-dependent translocation of beta catenin to the nucleus. Because beta-catenin stimulates the transcription of Wnt-dependent genes, we postulated that progesterone activates Wnt signaling, in part, by increasing stromal cell expression of a Wnt receptor. The purpose of this study was to map the temporal and spatial expression of the Wnt receptor, Frizzled-2 (FZ-2), in the uterine cells of OVX, progesterone pretreated (0 hE), and progesterone pretreated plus estradiol stimulated (6 hE) uteri. Sexually mature OVX Sprague Dawley rats were injected subcutaneously (s.c.) with progesterone (2 mg) for three consecutive days. Re-entry into the cell cycle was initiated with a single s.c. injection of estradiol (0.2 micrograms). The uteri were removed under anesthesia, fixed in 4% paraformaldehyde and embedded in paraffin. Tissue was sectioned (~8 micrometers) and placed on pretreated glass slides. Uterine sections from at least three different rats at each time period were analyzed by standard immunocytochemistry using a FZ-2 antibody (Santa Cruz, sc7429) diluted 1:100. Some sections were treated without primary antibody to control for reaction specificity. FZ-2 expression was barely detected in the stromal cells of OVX rats. Expression increased following progesterone pretreatment (0 hE) primarily in the periluminal stromal cells. Estradiol further increased FZ-2 expression with strongest reactivity in the periluminal and anti-mesometrial stromal cells. This expression pattern of FZ-2 correlates with the cellular accumulation of beta-catenin after these same hormone treatments. These findings suggest that progesterone synchronizes stromal cell proliferation by activating Wnt signal transduction and up-regulating FZ-2 expression. Re-entry into the cell cycle, however, may require estrogen-dependent translocation of beta-catenin and activation of Wnt-dependent gene transcription.