

Filis et al: Supplemental Figure 2.

Expression of PLC- β 1 protein in the uterus during pre- and peri-implantation period.

Expression of PLC- β 1 protein in the uteri of WT females during embryonic days 0.5 to 4.5 (E0.5 – E4.5) of pregnancy is shown in Figure 2A-E (PLC- β 1 expression is in red; nuclei are stained with Hoechst stain, shown in blue). No staining is seen in the absence of PLC- β 1 primary antibody (Figure 2F). PLC- β 1 is localised mainly to the membrane of luminal and glandular epithelium throughout the first days of pregnancy. Expression is increased at E1.5, at which time there is also expression in uterine stromal cells (Figure 2B). This increase in uterine expression at E1.5 is prior to the arrival of the embryo at the uterus. At the time of implantation (E4.5), PLC- β 1 is expressed in the luminal epithelial cells adjacent to the embryo (Figure 2E, with blastocyst and surrounding uterine epithelial cells shown in magnified inset). This localisation pattern is consistent with a role of PLC- β 1 in preparation of the uterus for implantation, and in implantation itself.

Materials and Methods.

Uterine tissues were fixed overnight in 4% paraformaldehyde, embedded in paraffin and sectioned at 5 μ m. Sections were dewaxed in xylene and gradually rehydrated through ethanol. Epitope retrieval was performed by boiling sections 4 x 5min in sodium citrate buffer. Sections were incubated in blocking buffer (PBS with 20% goat serum, 5% BSA and 0.1% Triton X-100) for 30min at room temperature. Rabbit polyclonal PLC- β 1 antibody (Santa Cruz, Biotechnology, #sc205) was diluted 1 in 100 in the blocking buffer, applied on the sections and left to incubated overnight at 4°C. Sections were washed the following day with PBS containing 0.1% Triton, probed with a biotinylated polyclonal goat anti-rabbit (Dako, #E0432) for 1 hour at room temperature, washed again and incubated with streptavidin conjugated to Alexa Fluor 568 (Molecular Probes, Invitrogen #811226). The sections were washed again, stained with Hoechst 33342 nuclear counterstain (Invitrogen, #H3570) diluted in PBS at 1 μ g/ml for 10min and mounted in Vectashield (Vector Laboratories #H-1000).

