

Distribution of Ovarian Steroid Hormones Across the Oviductal Epithelium: Insights from a Compartmentalized in Vitro Model

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The oviduct epithelium undergoes profound changes driven by variations in ovarian steroid hormone levels throughout the mammalian estrous cycle. However, due to the inherent challenges of conducting in vivo studies, the dynamic distribution profile of these crucial hormones within the oviduct microenvironment remains poorly understood. Therefore, we employed a compartmentalized in vitro model of porcine oviduct epithelial cells (POEC), to elucidate the basal-apical distribution of progesterone (P4) and 17 β -estradiol (E2) across the oviduct epithelium.

Primary POEC were plated into 24-well hanging inserts (0.4 μ m pore size) and cultured with medium in both compartments until day 6. Subsequently, the medium in the apical compartment was removed, and the cells were sustained at the air-liquid interface until day 21 using a serum-free medium in the basal compartment. Oviduct fluid surrogates generated on the apical cell side, basal medium, and cellular layers were collected, extracted and subsequently steroids were measured via enzyme immunoassay (EIA).

In experiment 1, POEC (2 donors/treatment/duration) were stimulated basolaterally with a single dose of either P4 or E2 representing serum concentrations during diestrus (30 ng P4 /mL medium) or estrus (60 pg E2/mL medium) for 0.5, 1, 3, 6, 12, and 24 hours (h). Basolateral P4 levels exhibited a rapid decrease to 56.01% after 3h, with only 5.19% of the initial P4 content remaining after 24h. Apical P4 levels peaked between 0.5 and 1h (3.19 - 4.13 ng/mL), and then declined to 2.00 ng/mL at 3h and 0.27 ng/mL at 24h, remaining lower than basal levels. Additionally, the total intracellular P4 also reached its peak level between 0.5 and 1h (0.79-1.02 ng), then declined to 0.4 ng by 3h, and further decreased to 0.06 ng, equivalent to 5.9% of the peak level, by 24h. Contrarily, the E2 levels in the basolateral side showed a gradual decline to 59.65% after 6h, with 11.43% of the initial E2 content still observed after 24h. Meanwhile, the total intracellular E2 content peaked between 1 to 3h (11.30-10.40 pg), followed by a gentle descent to 6.15 pg, equivalent to 54.42% of the peak level, by 24h.

In experiment 2, POEC (6 donors/treatment/duration) were exposed to either a single dose of 30 ng/mL P4 or 60 pg/mL E2 for 12 and 72h, or subjected to repeated treatment for 72h (hormone refreshment every 12h). In the P4 group, 12h after single treatment, the basal side exhibited a P4 level of 2.86 ng/mL, significantly higher than the apical side (0.62 ng/mL, $p < 0.001$). Comparable levels were observed in the repeated-treatment group after 72h (basal: 3.71 ng/mL; apical: 0.51 ng/mL). 72h after single treatment, the P4 level in the basal side decreased to 0.15 ng/mL, representing only 0.55% of the initial P4 content. In the E2 group, repeated treatment for 72h resulted in an E2 level of 25.05 pg/mL, significantly higher than after single

treatment for 12h (16.65 pg/mL, $p < 0.001$), as well as higher than 72h after single treatment (6.42 pg/mL, $p < 0.001$).

In summary, E2 and P4 at physiological concentrations exhibit dynamic and divergent distribution patterns in the oviductal epithelium. These results build a basis for further studies on the effect and conversion of steroids in the oviduct epithelium and will thus help to better understand the regulation of oviduct functions.

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