Animal Reproduction

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Embryology, developmental biology, and physiology of reproduction

Effects of endocrine disruptors ketoconazole and diethylstilbestrol on BOEC air-liquid interface monolayer culture

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Reproductive disorders, a worldwide public health concern, have been associated with exposure to endocrine disrupting chemicals (EDCs). Two well-known EDCs to influence female reproductive health are diethylstilbestrol (DES; a synthetic estrogen agonist) and ketoconazole (KTZ; a CYP450 steroidogenesis enzyme inhibitor). Here, the effects of DES and KTZ on a bovine oviduct epithelial cell (BOEC) culture, an *in vitro* animal model of the first embryo-maternal contact site (i.e. the oviductal epithelium), are explored. To establish a BOEC monolayer an air-liquid interface (ALI) culture approach was adopted, which supports cell differentiation (Chen et al., Sc. Reports, 7, 2017). Reported KTZ and DES effects include ALI-BOEC monolayer permeability, confluency, and actin organisation.

BOECs were mechanically isolated from the lumen of oviducts, obtained from slaughterhouse cows post-mortem. BOECs ($5x10^5$ cells) were seeded and cultured for 8 days in Transwell® cell culture inserts (Corning, USA, NY, CLS3413, 6.5mm). To introduce an ALI apical media was removed, while basolateral media was maintained. At day 14 of ALI, BOEC monolayers were exposed for 4 days to DES (10^{-9} M, 10^{-7} M, 10^{-5} M) or KTZ (10^{-8} M, 10^{-7} M, 10^{-6} M), or 0.01% v/v DMSO (vehicle). Effects of DES and KTZ on the permeability of the BOEC monolayer were assessed by transepithelial electrical resistance (TEER) measurement and paracellular tracer flux assay. TEER measurements confirmed confluency in all BOEC monolayers, with no significant difference between DES and KTZ treated vs DMSO treated monolayer (one-way ANOVA). The cell-impermeable tracer fluorescein disodium salt (12 µg/mL, 0.4kDa) was used for the tracer flux assay. Apical media was supplemented with the tracer and, after a 2h incubation, the basolateral media was collected to measure fluorescence. There was low percentage (<1.2%) of total tracer transferred from the apical to the basolateral side, and not significantly different between DES- or KTZ-exposed vs vehicle treated monolayers (one-way ANOVA). Consistent with TEER and tracer flux data, confocal microscopy of stained (phalloidin, acetylated α tubulin, Hoechst 33342) BOEC monolayers further supported confluency after DES and KTZ exposure. Clear basolateral cell-cell and cell-membrane adhesion was observed for all monolayers. In contrast to the membrane-adhering side of DES- or KTZ-treated BOECs, lateral cell-cell connections were scarce towards the apical side of the cells. This effect was similar in all doses of DES or KTZ and evidenced by gaps between phalloidin staining of individual cells, and it was not observed in DMSO treated BOECs.

In conclusion, DES and KTZ induced abnormalities in BOEC cytoskeletal organisation. We hypothesize that cell-cell junctional contacts in BOECs are disturbed by these EDCs. This distortion has an effect on cell polarity, and may also change epithelial cell binding and secretion properties. The possibility that this may indirectly cause aberrant early embryo-development, which could differ from direct exposure of these EDCs to the embryo cultures, is under current investigation.

Keywords: oviduct epithelium, endocrine disruptor, cytoskeleton abnormalities

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