Characterization of a novel suspension cell culture system for bovine oviduct epithelial cells

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Introduction

The oviduct is responsible for the accommodation of the gametes and the early embryo by providing an optimal environment for successful fertilization. To examine early embryo-maternal communication *in vitro*, a cell culture system mimicking the oviduct with its peculiar physiological characteristics is a prerequisite. Bovine oviduct epithelial cells (BOEC) are known to be difficult to culture without de-differentiation. Therefore, a novel short-term BOEC suspension culture system was established and validated by ultrastructural and gene expression analyses using qRT-PCR.

Materials and Methods

Simmental heifers were synchronized and slaughtered on day 3.5 after standing heat. BOEC from the ampulla of ipsi- and contralateral oviducts were isolated seperately by squeezing along the oviduct with forceps. This procedure yielded sheets of epithelial cells that were further separated by pipetting and passing through syringes before being recovered by sedimentation.The purity of epithelial cell culture was tested by immunocytochemistry against cytokeratin filaments and vimentin (Fig.1). Cells were cultured in TCM-199 with 2% estrous cow serum. Cultures were sampled for light microscopy, transmission electron microscopy, scanning electron microscopy, qRT-PCR and Western blotting at 0, 6, 12 and 24 hours after seeding (Fig.2). To study effects of hormone supplementation (10 ng/mL P4 or 10 pg/mL E2), cultures were sampled after 6 and 18 hours of stimulation (Fig. 3).

Results and Discussion

Maintenance of the ciliated epithelial cell phenotype and ultrastructural characteristics of cultured cells (Fig.1) indicates that the culture system is able to provide cells which closely resemble cells *in situ*. The mRNA levels of several marker genes varied after seeding but stabilized thereafter, indicating a period of adaption. The majority of the investigated genes showed a relatively stable mRNA expression level after 6 hours in culture (Fig. 2). qRT-PCR results showed no significant difference in gene expression between cultured BOEC obtained from ipsi- or contralateral oviducts (Fig. 2). Differences monitored *in vivo* [1] are therefore likely to result from countercurrent blood flow between ovary and corresponding oviduct and are negligible during the time course of the culture. The BOEC responded to a stimulation with physiological doses of steroids (Fig.3), similarly to the *in vivo* situation [2].

Conclusion

This *in vitro* model is suitable to investigate the effects of various stimulants and can be employed for co-culture experiments with embryos to unravel details of the contribution of the bovine oviduct in embryo-m[aternal communication].

[1] Bauersachs et al. Biol Reprod 2003, 68:1170-77

[2] Ulbrich et al. J Steroid Biochem Mol Biol 2003, 84:279-89

Fig.1: morphological examination of BOEC during cultivation

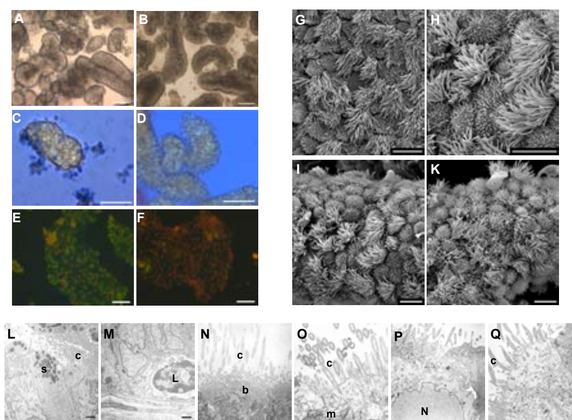

Fig. 1: Light microscopy showed vigorously beating cilia and rapidly moving aggregates with unchanged morphology of BOEC during a culture period of 24 hours (A,B). Trypan blue staining showed that cells contained in aggregates were viable while single cells stained positive (C,D). Immunocytochemistry against cytokeratin filaments and vimentin demonstrated that BOEC cultures contained > 95% epithelial cells (E,F). Scanning electron microscopy of BOEC *ex vivo* (G), and after 0 hours (H) and 24 hours (I,K) in culture. Ultrastructural examinations revealed that the morphology of BOEC was very similar to cells analyzed ex vivo (L-Q). Both secretory cells with numerous secretory granules (s) and mitochondria (m), and ciliated cells with long, well-developed and actively moving kinocilia (c) were visible.

Fig.2: mRNA and protein expression in BOEC during cultivation

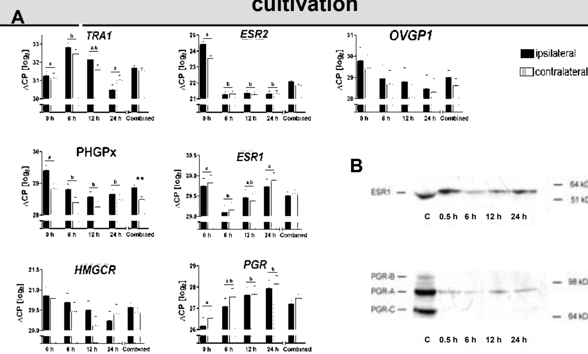

Fig. 2 BOEC derived from ipsi- or contralateral oviducts analyzed during cultivation using (A) qRT-PCR for several candidate genes and (B) Western blot analysis of ESR1 and PGR protein expresision with endometrium as positive control (c). Both proteins (B) were stably expressed during 24h of cultivation. mRNA data are presented as relative expression of Crossing Points (Δ CP) ± SEM. One ΔCP signifies a doubling of mRNA. * indicates significant differences ($p<0{,}05$).

Fig 3: mRNA expression in BOEC after steroid stimulation

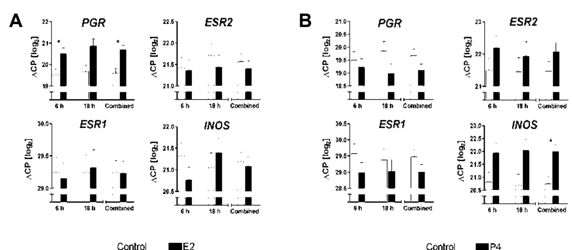

Fig. 3 BOEC stimulated with physiological doses of estradiol-17β (A) or progesterone (B). The mRNA expression of progesterone receptor (PGR) was significantly elevated after E2 stimualtion, whereas iNOS expression responded to P4. Data are presented as relative expression of Crossing Points (Δ CP) ± SEM. One ΔCP signifies a doubling of mRNA. * indicates significant differences ($p<0{,}05$).

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