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| Author(s) | Xu, JS; Chan, STH; Ho, PC; Yeung, WSB |
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031 HUMAN CHORIONIC GONADOTROPHIN (HCG) STIMULATES VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) PRODUCTION BY LEYDIG CELLS

AU Chak Leung, SY Chun Choi, CHAN Lai Fong
Department of Physiology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

Objective: To examine whether the Leydig cell production of a potent angiogenic factor - VEGF, is under the trophic stimulation of hCG, and if so whether the effect can be mimicked by activators of the second messenger pathways involved in mediating hCG actions. **Methods:** Percoll-purified Leydig cells (LC) and mouse Leydig cell lines (TM3 and MLTC-1) were used, and they were studied at different time intervals following the exposure to hCG, 8-bromo-cAMP, forskolin and phorbol-12-myristate-13-acetate (PMA). The culture medium was collected for the measurement of VEGF by ELISA and total RNA was extracted from cells for the quantification of VEGF mRNA levels by Northern blot analysis. **Results:** hCG (0.001-1 U/ml) produced a modest dose-dependent stimulation of VEGF secretion from rat LC and MLTC-1 cells, reaching a maximum of ~50% above the basal levels. TM3 cells failed to respond to hCG (due to the loss of LH receptors) but gave dose-dependent increase in VEGF secretion following 12 h incubation with 8-bromo-cAMP (0.1-3 mM) and PMA (0.01-1 µM). Similar results were obtained from rat LC and MLTC-1 cells which gave maximum responses of >100% increase above the control. In line with the changes in peptide secretion, VEGF mRNA levels in MLTC-1 cells were upregulated ~1.5-fold after 2 h exposure to hCG (0.01-1U/ml). For TM3 cells, their VEGF mRNA levels showed time- and dose-dependent increases in response to forskolin (0.1-10 µM) and PMA (0.01-3 µM), with the later producing a greater stimulation. **Conclusions:** VEGF peptide secretion and mRNA expression in Leydig cells are stimulated by hCG which may produce its effect through both the protein kinase A and C pathways. The hCG-induced increase in VEGF production from Leydig cells could account for the increase in endothelial cell proliferation in the testis of adult rats after receiving hCG injection. **Acknowledgement:** Supported by RGC Grant (CUHK 422/95M).

032 Human oviductal cells produces three glycoprotein fractions that stimulate mouse embryo development

Jia-Sen Xu¹, Samuel Ting-Hon Chan², Pak-Chung Ho¹, and William Shu-Biu Yeung¹
¹Departments of Obstetrics and Gynaecology, and ² Zoology, University of Hong Kong, Hong Kong SAR, China

Objective: The development of preimplantation (PI) mammalian embryos *in vitro* is inferior to that of *in vivo*. Lack of oviductal support is one of the possible reasons for the suboptimal embryo development *in vitro*. Coculture of embryos with oviductal cells suggests that paracrine pathway(s) exists between oviduct and PI embryos. We have demonstrated that coculture with human oviductal cells improves human and mouse embryo development in terms of increase in blastocyst formation, hatching and implantation rate, decrease in the incidence of cytoplasmic fragmentation and apoptosis. This study aims to study the embryotropic effects of three glycoprotein fractions from human oviductal cell conditioned medium on mouse embryo development.

Methods: Human oviductal cells were obtained from donated oviducts of hysterectomy patients and were cultured in DMEM/F12. The oviductal cell conditioned medium was fractionated by various liquid chromatographic systems. The purified fractions were tested on mouse embryos (MF1 x BALB/c) at different stages of development for different duration. Their effects on blastulation, hatching and allocation of cells to inner cell mass (ICM) and trophectoderm (TE) in the resulting blastocysts were determined.

Results: Three glycoprotein fractions named ETF-1, 2 and 3 with embryotropic effect on mouse embryo development were purified from the conditioned medium after human oviductal cells culture. ETF-1 and 2 affected the embryos at early cleavage stage and increased ICM cell number of the resulting blastocysts. ETF-3 stimulated the development of TE and had its maximal effects at around compaction. The enhanced development of TE after ETF-3 treatment led to larger expanded blastocysts with higher hatching rate.

Conclusion: Our studies showed that human oviductal cells improved mouse embryo development partly by the production of high-molecular-weight embryotropic factors. These factors had differential effects on mouse embryo development.

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