

**hCG from invasive extravillous cytotrophoblast but not from syncytiotrophoblast origin stimulates trophoblast invasion *in vitro*.**

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After implantation, human trophoblasts differentiate into two pathways: villous cytotrophoblasts (VCT) that fuse to form the syncytiotrophoblast (ST) involved in placental exchanges and endocrine function, and extravillous cytotrophoblasts (EVCT) that invade the uterus wall up to the upper third of the myometrium and the uterine arteries. It is well established that hCG is secreted by the endocrine syncytiotrophoblast (ST) into the maternal compartment. We recently reported *in situ* and *in vitro* that invasive EVCT also produce and secrete hCG suggesting an autocrine/paracrine role at the implantation site.

The aim of the study was to investigate the activity and the role in trophoblast invasion of hCG secreted *in vitro* by primary cultures of human invasive EVCT in comparison with hCG produced by *in vitro* differentiated noninvasive ST.

Strategy: Invasive EVCT and differentiated ST were obtained from first trimester chorionic villi after primary cultures of EVCT on Matrigel or fusion of VCT into ST. Invasion assays were performed on Matrigel-coated transwells. hCG were quantified in cell supernatants by immunometric assays.

Results: We first demonstrated that LH/CG receptor was expressed in EVCT *in situ* (immunohistochemistry) and *in vitro* (immunocytochemistry, Western blot, PCR) as well as in the EVCT cell line HIPEC65 that we previously established and characterized. We next showed that hCG secreted by EVCT stimulated progesterone secretion by MA10 cells in a concentration-dependent manner but to a lesser extent compared to hCG from the ST. Incubation of HIPEC65, that do not secrete hCG, with EVCT supernatants containing  $10^{-9}$  M hCG induced a 10-fold increase in cell invasion, whereas ST supernatants containing the same concentration of hCG had no effect. This stimulating effect was decreased by 85 % when hCG was depleted from EVCT supernatants by immunoprecipitation. We next quantified hyperglycosylated forms of hCG (HhCG) in each trophoblast subtype cell supernatants and found that HhCG represent 20 % of total hCG in EVCT supernatant, whereas it was almost undetectable in the ST cell cultures.

Conclusion: Our results offer strong evidence that hCG secreted *in vitro* by the invasive trophoblast, likely the hyperglycosylated form of hCG, but not by the syncytiotrophoblast, promotes trophoblast invasion and may participate to the control of the trophoblastic cell invasion process in an autocrine manner.