# MITOGENIC EFFECT OF INSULIN-LIKE GROWTH FACTOR-II ON VELVET ANTLER CELLS *IN VITRO*

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The effect of human recombinant IGFII on DNA synthesis was studied in primary culture of fibroblast- and osteoblast-like cells. Primary culture of fibroblast- and osteoblast-like cells were successfully prepared from adult red deer (*Cervus elaphus*) stags whose antlers were harvested after 60 days of growth. Tissues were dissected from each of the zones and dispersed with collagenase and then the cells were grown in 45% Fitton-Jackson modification media ($BGJ_b$), 45% $F_{12}$ nutrient, 10% fetal bovine serum (FBS), Penicillin (100 U/ml) and streptomycin (100 µg/ml) and streptomycin (100 µg/ml). $2x10^4$ cells/$cm^2$ were seeded in 24 well plates and incubated in a humidified 5% $CO_2$ at 37° C. After 48 hr the media was changed to serum-free media (SFM) and incubated for a further 24 hr, followed by a 24 hr incubation in either SFM or 10 nM IGFII or 10 nM IGFI or both 10 nM IGFII and 10 nM IGFI. After 22 hr 2.5 µc $^3$H-Thymidine were added to each well for two hours. The reaction was terminated with 10% TCA. $^3$H-Thymidine was counted. Results are the mean of triplicate experiments and log (counts) were analysed by ANOVA. The Table shows the incorporation of $^3$H-Thymidine (DNA synthesis) into fibroblast- and osteoblast-like cells.

| 1st 24 hr | 2nd 24 hr | Fibroblast-like cells | Osteoblast-like cells |
|---|---|---|---|
| SFM | SFM | 1,552 | 2,128 |
| SFM | 10 nM IGFI | 10,495 | 4,207 |
| SFM | 10 nM IGFII | 9,441 | 14,060 |
| SFM | 10 nM (IGFI, IGFII) | 8,750 | - |
| Least significant ratio (LSR) | | 1.40 | 1.37 |

Table 1. Geometric mean $^3$H-Thymidine (DNA synthesis) uptake into fibroblast- and osteoblast-like cells DPM.

IGFII significantly increased the $^3$H-Thymidine incorporation in both cell types compared to SFM. Previously the mitogenicity of IGFI was shown on these cells. There was no synergistic effect of IGFII and IGFI compared with either growth factor alone which is possibly due to their exerting their mitogenicity through one receptor. The results indicate that both IGFs could be mitogenic for deer antler cells and open up a series of challenging studies to determine the precise effects of each growth factor.