

# Evaluating the effects of Atorvastatin on cultured human endometrium in a three-dimensional fibrin matrix

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## Abstract

**Introduction:** Endometriosis, as one of the main causes of infertility in women, has devoted a lot of research to itself. Three-dimensional culture of human endometrial tissue has been known as a functional model for endometriosis. Statins have a variety of different effects on angiogenesis and they have been suggested as a candidate for the inhibition of this disease. Therefore, the aim of the present study is to determine inhibitory or promotory effects of different concentrations of Atorvastatin on the growth of cultured human endometrium.

**Materials & Methods:** 8 endometrial samples were cut into 1x1 mm pieces and were cultured in 24-well culture plates. To establish the desired three-dimensional model, 0.5 ml of fibrinogen solution in M199 medium, with a concentration of 3mg/ml and 10 $\mu$ L of thrombin were added to each well. After formation of the jelly, the endometrial fragments were placed on it and a second jelly layer was added. The endometrial tissue of each specimen was cultured in four 5-piece layers. The first row was regarded as control and to the second, third and fourth rows, 0.1, 1.0 and 10 $\mu$ M concentrations of the medication were added respectively. Every 3 days, endometrial pieces and their probable changes were observed by an invert microscope (Motic-AE31) and the culture media were changed. The tissue fragments were also photographed on days 1, 7, 14 and 21. At the end of the study, the cultures were graded according to their cellular outgrowths, invasion of deposited cells into the jelly and growth of endometrial glands and images related to the 3-D cultures were analyzed. To confirm the growth of cellular structures into the jelly, paraffin embedded cuts of the cultured specimen were immunohistochemically stained by anti-vimentin, anticytokeratin and anti-CD31. The data were analyzed by SPSS statistical software (Version 11.5). The statistical analysis related to the grading of wells was done by Freedman test and the statistical analysis of the images was performed by one-way ANOVA.

**Results:** Growth changes were 82.5% in the control group and 87.5% in the 0.1 $\mu$ M concentration group, depicting significantly higher values compared to the control group ( $p < 0.05$ ). The changes in 1 $\mu$ M and in 10 $\mu$ M groups were 50% and 5% respectively which had significant differences with that of the control group ( $p < 0.001$ ).

**Conclusion:** Different concentrations of Atorvastatin have different effects on the growth of cultured endometrial tissue, 0.1 $\mu$ M concentration increases growth of cultured human endometrium, while 1 $\mu$ M and 10 $\mu$ M can decrease growth of the tissue. Further animal studies on this subject are suggested.

**Key Words:** Atorvastatin, Endometrium, Endometriosis, 3D-culture, Tissue growth, Fibrin, Thrombin.

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