PROLIFERATING CELL NUCLEAR ANTGEN IN COUMESTROL TREATED HUMAN ENDOMETRIAL STROMAL CELLS CULTURE

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Estrogens are acknowledged as the most important proliferative agents for human uterine cells. Vegetables and legumes containing non-steroidal coumestans which can be converted by intestinal flora to biologically active substances with estrogen-like activity such as coumestrol and 4'-metoksycouemestrol. Coumestans act via estrogen receptor and mimic steroid function in target organs, although their influence on target tissues seems to be weaker (0.03%) comparing to diethylstilboestrol effect. Coumestrol has been observed to possess tumour-promoting activity similar to that of E₂ and hypothetically can inducing proliferation in estrogen-dependent tissues. Proliferating cell nuclear antigen (PCNA) is synthesized in early G1 and S phases of the cell cycle and serve as a cell proliferation marker. This study was designed to investigate E₂ and coumestrol action on human endometrial stromal cells proliferation in vitro. Normal proliferative endometrium taken from 7 women underwent laparotomy for non-malignant uterine disease. The endometrium was cut into small pieces and digested by collagenase in MEM1959 supplemented with 10% FCS. Finally, 0.5-1 x 10⁵ isolated stromal cells were placed in 16 box tissue culture chambers slide (Nagle Nunc Inc.). After 24 hrs of preincubation, the medium was replaced by a new one containing E₂ (0,50, 250 and 500 ng/ml). Supernatant was discharged after 48 and 96 hrs and cultured cells were fixed with ethanol at room temperature for 10 min. PCNA was localized in the fixed endometrial stromal cells immunohistochemically (SIGMA) and counted semiquantitatively by H-score method, according to Nyholm procedure. We observed PCNA antigen only in endometrial stromal cells culture treated higher E₂ concentrations (H-score 0.4; control 0.0). PCNA antigen was not detected in lower E₂ concentration (50 ng/ml) as well as for all coumestrol treated cells (H-score 0.0). The present study indicates that coumestrol, contrary to estradiol, did not induce endometrial stromal cells proliferation in vitro. However, it is possible that coumestrol requires much higher concentration or longer exposure time to produce similar effect to E₂.