

## **Analysis of apoptosis induced by bovine gammaherpesvirus 4 in primary culture of bovine endometrial cells**

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BoHV-4 is tropic for bovine endometrium being increasingly considered as responsible for reproductive tract problems during postpartum period in concomitance with gramnegative bacteria. Some gammaherpesviruses, including BoHV-4, carry genes that can inhibit or induce apoptosis. BoHV-4 has two genes that encode proteins (v-Bcl2 and v-Flip) with anti-apoptotic functions. However it was shown that, the induction of apoptosis *in vitro* by BoHV-4 depends not only on the dose of the viral inoculum and the time of infection, but also on the nature of the infected cells. The objective of this work was to study apoptosis induced by BoHV4 and lipopolysaccharide (LPS) in primary culture of bovine endometrial

cells. Apoptosis was evaluated in two stages: a) early stage (reversible moment), by staining with rhodamine and propidium iodide at 6, 12 and 24 h post infection (pi), in which mitochondrial permeability was studied, expressed in % of positive rhodamine cells (Rod+cells); b) late stage (irreversible moment), using TUNEL and DAPI after 12, 24 and 48 h pi, which the condensed chromatin was evaluated, expressing the results in relative apoptosis index (RAI). It was shown that in the early stage, the permeability of the mitochondrial membrane decreases after 12 h pi in cells infected with BoHV-4 (49 % Rod+cells) and BoHV-4 +LPS (45 % Rod+cells) compared to the control (90 % Rod+cells). While in the late stage a progressive increase in RAI is found in cells treated with BoHV-4 and/or LPS, being remarkable at 48 h pi both in TUNEL (control = 1.00; LPS = 0.76; BoHV-4 = 29.50; BoHV-4 + LPS = 38.40) and DAPI (control = 1.00; LPS = 1.34; BoHV-4 = 13.90; BoHV4 + LPS = 16.91). Apoptosis increased in both stages due to the interaction of BoHV-4+LPS. Likewise, the induction of apoptosis in bovine endometrial cells infected with BoHV-4 was shown to be time dependent, being further increased in the presence of bacterial LPS. This finding reaffirms the synergy effect of BoHV-4 and gramnegative bacteria in bovine uterine pathologies, since a chronic inflammatory environment is generated accompanied by endometrial tissue damage.

**Keywords:** BoHV-4, LPS, apoptosis, primary culture, bovine endometrium.