VIR006

ANALYSIS OF APOPTOSIS INDUCED BY BOVINE GAMMAHERPESVIRUS 4 IN PRIMARY CULTURE OF BOVINE ENDOMETRIAL CELLS

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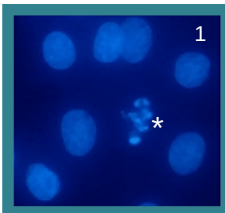
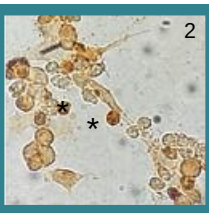
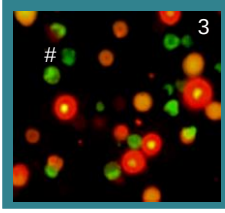
Aims

To study the apoptosis in primary culture of bovine endometrial cells (BEC) induced by BoHV-4 and lipopolysaccharide (LPS).







Representative images of DAPI (1), TUNEL (2) and Rodamine-IP (3) stain in BEC.

*apoptotic cell, #Rod+cell

Materials and methods

BoHV-4 07/435 field strain was used for *in vitro* experiments in this study (Romeo et al., 2020).

Uteri from cattle without evidence of genital disease, collected from a local abattoir immediately after slaughter, were used for the isolation and culture of BEC (Tebaldi et al., 2016).

Apoptosis induced by BoHV-4 and LPS 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 p.i., in which the condensed chromatin was evaluated, expressing the results in relative apoptosis index (RAI).

Results

It was shown that in the early stage, the permeability of the mitochondrial membrane decreases after 12 h p.i. in cells infected with BoHV-4 in absence (49% Rod+cells) and presence (45% Rod+cells) of LPS, compared to the control (90% Rod+cells) (Fig.1). 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 p.i. 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) (Fig. 2, 3). Apoptosis increased in both stages due to the interaction of BoHV-4+LPS.

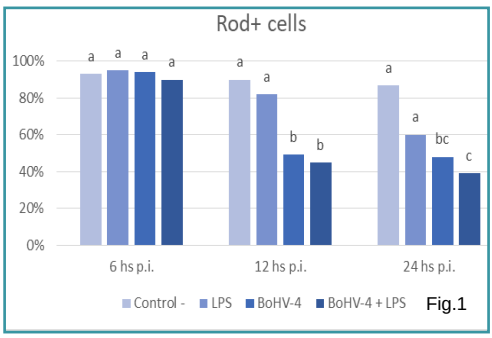


Fig.1

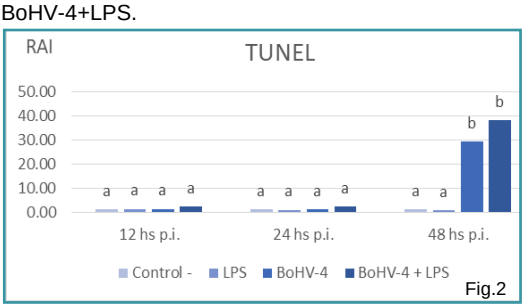


Fig.2

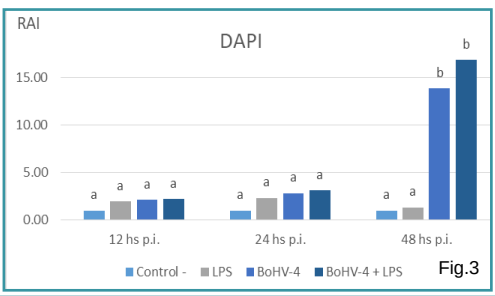


Fig.3

Conclusion

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 gram-negative bacteria in bovine uterine pathologies, since a chronic inflammatory environment is generated accompanied by endometrial tissue damage.