

AVEN and BCL-xL expression pattern and protein-protein interaction assessment through bovine early embryo development

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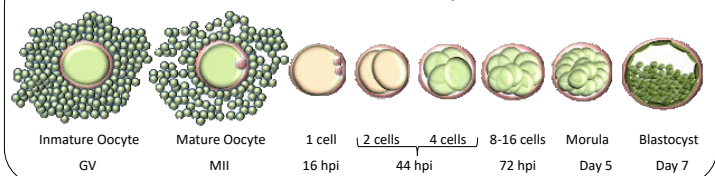
Introduction

Apoptosis in embryonic cells is important for embryo development; stabilizing cell numbers and playing a role in cell quality control (1). However, it is also associated with embryonic loss and cellular response to suboptimal developmental conditions and stress (2). AVEN, a novel P4-regulated protein, inhibits the mitochondrial apoptosis pathway by binding to and enhancing anti-apoptotic BCL-xL activity (3). The objective of this study was evaluate the protein expression profile and protein-protein interaction of AVEN and BCL-xL during early embryo development in cattle employing whole-mount fluorescent immunocytochemistry (WM) and Proximity Ligation Assay (PLA).

Material and Methods

In Vitro Embryo Production

Fix with PAF 4% at 7 time-points:



Stain

WM

PLA

Analysis

Epifluorescence microscopy

Confocal microscopy

Nº of foci (PLA)

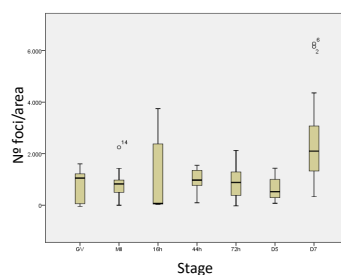
Co-localization (WM)

Expression pattern (PLA)

Results

1 Nº of foci (PLA)

The highest amount of signal was detected at the blastocysts stage (Day 7, $p < 0.05$). The lowest number of foci was detected on Day 5 embryos being significantly different from MII, 16 hpi and Day 7.

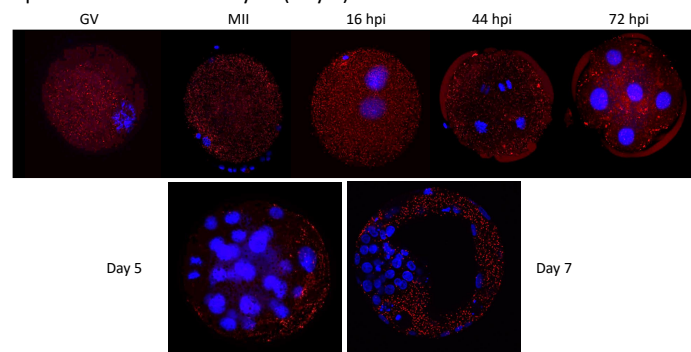


	GV	MI	16hpi	44hpi	72hpi	D5	D7
GV							***
MI						***	***
16hpi	**					***	*
44hpi			**				***
72hpi							***
D5		***	***				***
D7	***	***	*	***	***	***	***

*, **, *** Indicate significant differences $p \leq 0.05, 0.01, 0.001$ respectively

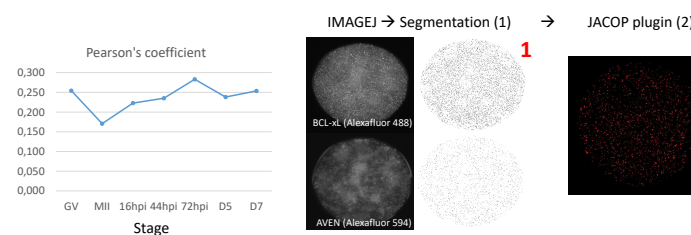
2 Expression pattern (PLA)

The PLA signal was evenly distributed in the cytoplasm and nucleus of the oocytes (GV and MII) and embryos until the 72 hpi. The signal was restricted mainly to the cells on the periphery of Day 5 embryos and to the trophoblast of the blastocysts (Day 7)



3 Co-localization (WM)

A positive Pearson's correlation coefficient indicates the existence of co-localization between AVEN and BCL-xL in the WM samples.



Conclusions

- A distinct labelling pattern was observed for AVEN-BCL-xL interaction during embryo development.
- The PLA was corroborated by the co-localization pattern of AVEN and BCL-xL in the whole-mount samples.
- PLA can be used to assess many other protein-protein interactions during early embryo development.

References

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2. Gjørret, Jakob O, Hiemke M Knijn, Steph J Dieleman, Birthe Avery, Lars-Inge Larsson, and Poul Maddox-Hyttel. 2003. "Chronology of Apoptosis in Bovine Embryos Produced in Vivo and in Vitro." *Biology of Reproduction* 69 (4): 1193–1200. doi:10.1095/biolreprod.102.013243.
3. O'Shea, Lynne C, Carmel Hensley, and Trudee Fair. 2013. "Progesterone Regulation of AVEN Protects Bovine Oocytes from Apoptosis during Meiotic Maturation." *Biology of Reproduction* 89 (6): 146. doi:10.1095/biolreprod.113.111880.