

**Abstract Guidelines for the 10<sup>th</sup> International Equine Infectious Diseases Conference (IEIDC X) are below. Abstracts should be submitted to [internationaleidc@gmail.com](mailto:internationaleidc@gmail.com) no later than October 1, 2015, for consideration.**

Please follow these style guidelines (*an abstract example is below*):

- 10-point font in Arial
- Single spaced
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- Author(s) and organization(s) centered
- Author(s) name should be First Initial, Middle Initial and Last Name/Surname
- Presenting authors should be identified with an asterisk (\*). Every word (including affiliations, author(s), references, title, figure details, acknowledgements, references, etc.) counts toward the 500 word count.
- For authors from multiple institutions, it should be listed like this:  
IG Martin<sup>1,\*</sup>, CC Macedo<sup>1</sup>, GA Monteiro<sup>1</sup>, DP Leme<sup>2</sup>, FC Landim-Alvarenga<sup>1</sup>, FO Papa<sup>1</sup>

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- Justify align abstract text
- Abstract should be one paragraph with no breaks
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- Figures and Tables should be embedded in abstract
- Abstract should not exceed 500 words.

**Immunohistochemical localization of Early Pregnancy Factor (Hsp10) in equine embryos**

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Early Pregnancy Factor (EPF) has been identified as a 10 kDa extracellular homolog of heat shock protein 10 (Hsp10). Hsp10 has been detected during early pregnancy in serum of mice, sheep, pigs, horses, cows, and humans by the rosette inhibition test. Hsp10 has also been associated with several neoplastic and autoimmune diseases. The goal of the present study was to determine if Hsp10 could be detected in the early equine embryo by immunohistochemistry. Embryos were collected from Quarter Horse mares by uterine lavage at either 8 or 25 days after ovulation. Embryos were fixed in 10% formalin solution and embedded in paraffin. Standard H&E sections were prepared along with sections for immunohistochemical (IHC) colormetric and fluorescent staining for Hsp10. Briefly, 5 µm sections of embryos, ovarian, oviductal, and uterine tissue were mounted on positively charged slides. The sections were deparaffinized and rehydrated with descending alcohol concentrations to buffer. Heat-induced epitope retrieval with citrate buffer (pH 6.0) at 125°C for 1 minute was followed by endogenous peroxidase blocking with 3% hydrogen peroxide and incubation with the primary antibody at 4°C overnight. The primary antibody was a polyclonal rabbit anti-human Hsp10 at a 1:1000 dilution. A predilute polymer based dual link HRP conjugated secondary antibody system was applied for 30 minutes (colormetric) followed by application of a DAB substrate or Texas Red for 1 hour with a mounting substrate containing DAPI, to detect the immunoreactive complexes. The colormetric slides were subsequently counterstained with Mayer's hematoxylin QS, dehydrated and mounted in xylene based medium. A total of 6 day eight and 4 day twenty-five embryos were collected for IHC evaluation. Day 8 embryos exhibited cytoplasmic localization of staining throughout the single layer of ectodermal cells forming the trophoblast. There was no nuclear localization. Day 25 embryos demonstrated intense localization focally along the apical boarder of the ectodermal cells forming

trophoblast layer of the developing chorion (Figure 1). Evaluation of other reproductive tissues revealed staining in granulosa and luteal cells of the ovary and variable staining in the oviductal epithelium. In summary, immunolocalization of Hsp10 in trophoblast cells of the extraembryonic membranes of the early equine conceptus confirms the presence and site of protein synthesis. We hypothesize that the secretion of Hsp10 by the developing embryo could have an immunomodulatory effect on the endometrium of the mare, effectively protecting the embryo from the maternal immune system. Other tissues in the reproductive tract also exhibited evidence of Hsp10. Finally, as it is unlikely that a 150 to 300  $\mu\text{m}$  morula or blastocyst stage embryo could produce sufficient Hsp10 to be detected in peripheral blood, we hypothesize that other tissues may contribute to Hsp10 production during early pregnancy.



**Figure 1.** Day 25 trophoblast extraembryonic membrane stained with H&E (left), IHC staining for Hsp10 (center), and fluorescent (right) at 40x.