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Supplementary Material

Intracellular localisation of platelet-activating factor during mammalian embryo development *in vitro*: a comparison of cattle, mouse and human

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In Figs S1 and S2, representative images of negative controls of the immunofluorescence experiments are provided. Fig. S3 contains representative images of PAF-positive bovine blastocysts used for quantitative analysis and examples of loss of PAF-staining in the nuclei during mitosis.

Supplementary Figure 1

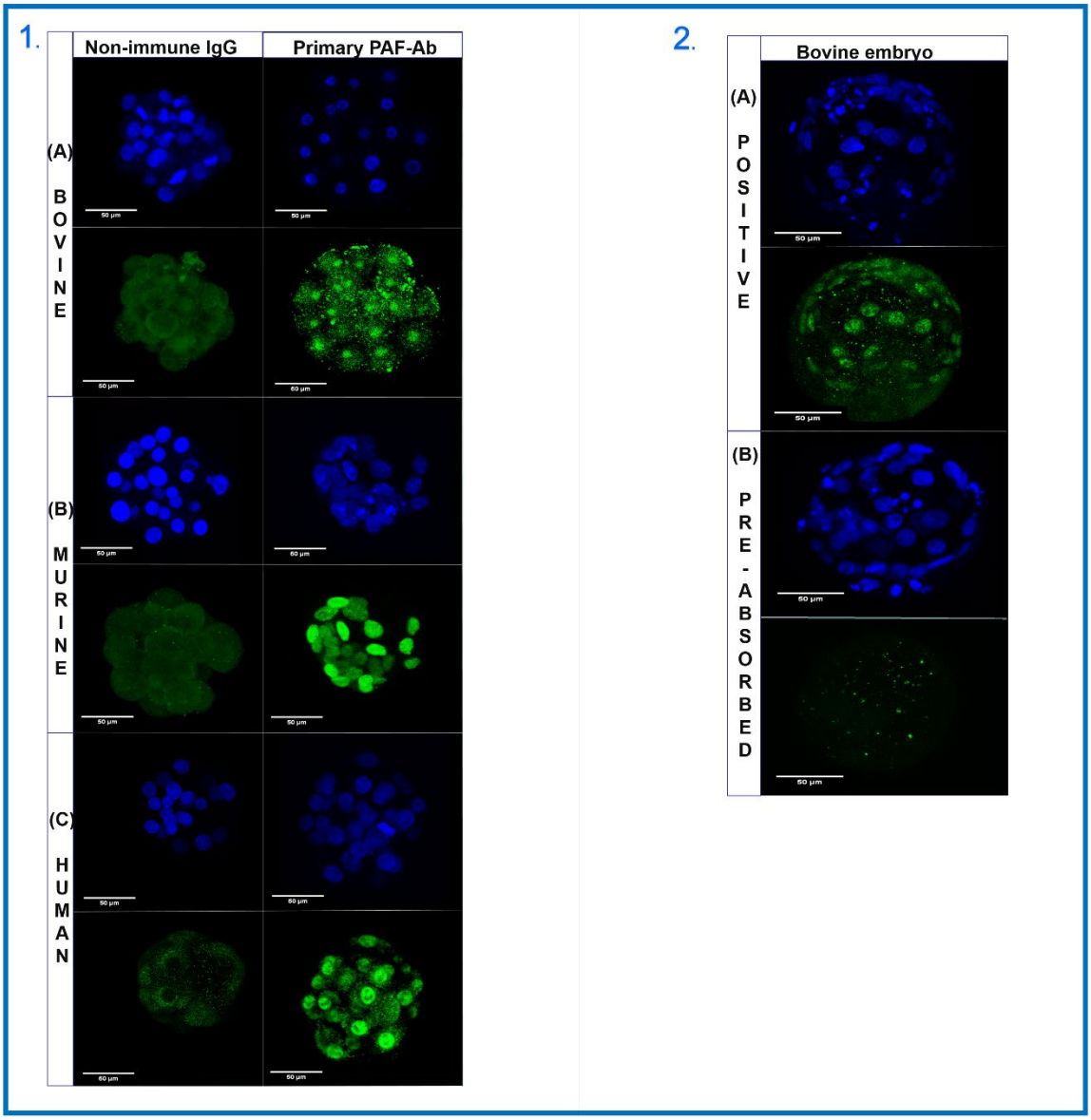


Fig. S1. Negative control images of immunofluorescence experiments in embryos. 1.1. Representative images of embryos incubated with whole molecule IgG in the corresponding dilutions (0.01 mg mL^{-1}) as the primary antibody in bovine (A), murine (B) and human (C) embryos. Corresponding images after incubation with the PAF-specific antibody are provided for each species as well. 1.2. Bovine embryos stained with anti-PAF antibodies, showed a clear signal in the nuclei (A) while embryos pre-absorbed with 100× excess carbamyl-PAF did not show a significant staining pattern (B). Z- projected sections of confocal images are shown. Chromatin is labeled with Hoechst 33342 (Blue). Subject of interest is labeled in green. Scale bars = 50 µM.

Supplementary Figure 2

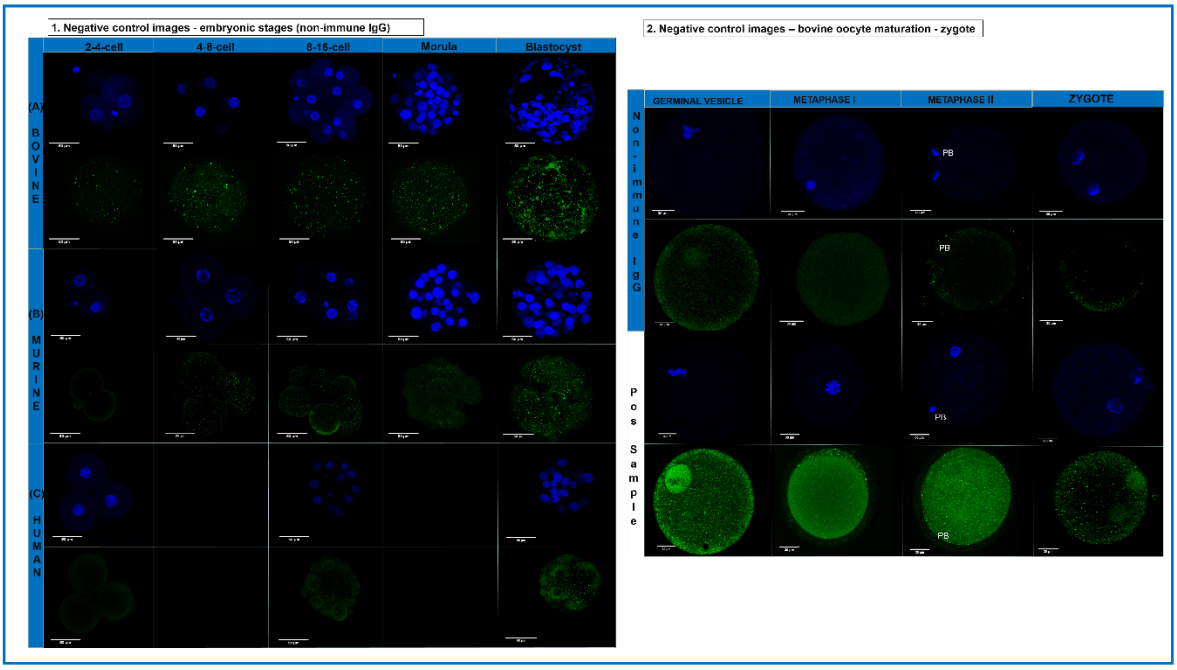
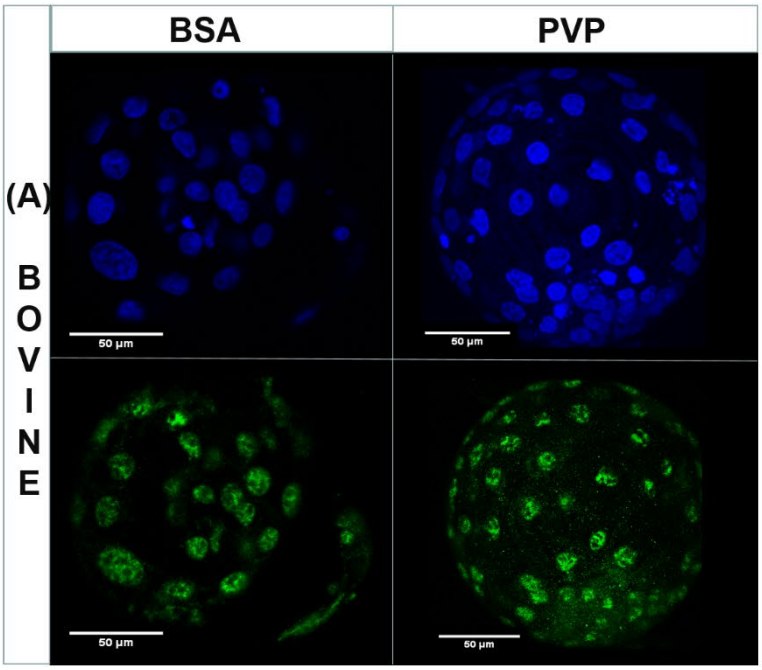


Fig. S2. Negative control images of immunofluorescence experiments in oocytes and embryos in different stages of development. 2.1. Representative images of embryos in different stages of development incubated with whole molecule IgG in the corresponding dilutions (0.01 mg mL^{-1}) as the primary antibody in bovine (A), murine (B) and human (C) embryos. 2.2. Representative images of bovine oocytes (germinal vesicle, metaphase I and metaphase II) and zygotes incubated with whole molecule IgG antibody as replacement for the specific primary antibody in the corresponding dilutions, in order to assess non-specific binding. Corresponding images after incubation with the PAF-specific antibody are provided as well (Positive sample). Z- projected sections of confocal images are shown. Chromatin is labeled with Hoechst 33342 (Blue). Subject of interest or non-immune IgG is labeled in green. Scale bars = 30 µM.

Supplementary Figure 3

1. Positive staining bovine blastocyst used for quantitative analysis



2. Examples of loss of signal during mitosis

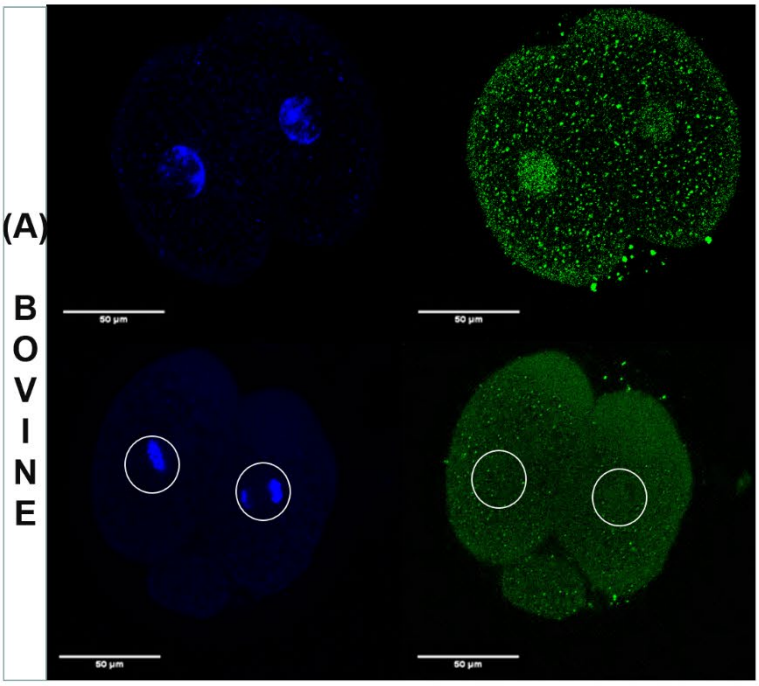


Fig. S3. Examples of bovine blastocysts with PAF-positive nuclei. 3.1. Representative images of bovine blastocysts containing PAF-positive nuclei, used for quantitative analysis. In short, we subtracted the mean intensity of the cytoplasm to correct for background. After subtracting the background, the mean fluorescence intensity was obtained by manually outlining the nuclei and multiplying by the area of the nuclei to obtain the total fluorescence intensity. To obtain a normalized value, the total fluorescence intensity was divided by the total fluorescence intensity of Hoechst 33342. ImageJ software was used to perform quantitative analysis of fluorescence intensities. 3.2. Representative images of bovine embryos, showing a clear signal in the nucleus during interphase and a loss of signal in the nucleus during mitosis. Z- projected sections of confocal images are shown. Chromatin is labeled with Hoechst 33342 (Blue). Subject of interest is labeled in green. Scale bars = 50 μM.