

Short note

Nuclear and cytoplasmic localization of interferon- τ in in vitro-produced bovine blastocysts

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Abstract – Experiments were conducted to detect interferon- τ in bovine in vitro-derived blastocysts by transmission electron (TEM) and confocal microscopy. TEM showed the presence of IFN- τ in the cytoplasm and the nuclei of expanded blastocysts. Confocal microscopy similarly confirmed the presence of IFN- τ in the trophectoderm of blastocysts. The distribution of IFN- τ appeared variable with some cells showing strong labeling while others appeared to be devoid of the protein.

interferon- τ / embryo / blastocyst / gene expression / IVF

1. INTRODUCTION

Interferon-tau (IFN- τ) is a protein secreted by ruminant embryos that is thought to be the primary signal for the establishment and maintenance of pregnancy. It is a type I interferon that has evolved approximately 36 MYA from an IFN- ω by acquisition of a promoter region that imparted trophectoderm-specific expression [1]. IFN- τ can be readily detected by its antiviral activity in the culture medium of in vivo- and in vitro-derived bovine blastocysts [2-4]. Moreover, IFN- τ gene transcription as well as secretion of protein have been shown to be affected by a number of genetic and environmental factors [5-11]. Previous reports have demonstrated the presence of IFN- τ protein as well as mRNA in the trophecto-

derm of ovine and bovine conceptuses that were obtained from pregnant animals [12-14]. However, nothing is known about the cellular distribution of IFN- τ during pre-elongation stages of development. The objective of this study, therefore, was to use transmission electron and confocal microscopy to localize IFN- τ in in vitro-derived bovine blastocysts.

2. MATERIALS AND METHODS

2.1. Embryo production

Oocytes from abattoir-source ovaries were matured in vitro in M199 medium supplemented with 10% defined fetal calf serum, with the addition of 2 $\mu\text{g}\cdot\text{mL}^{-1}$ -estradiol-17 β

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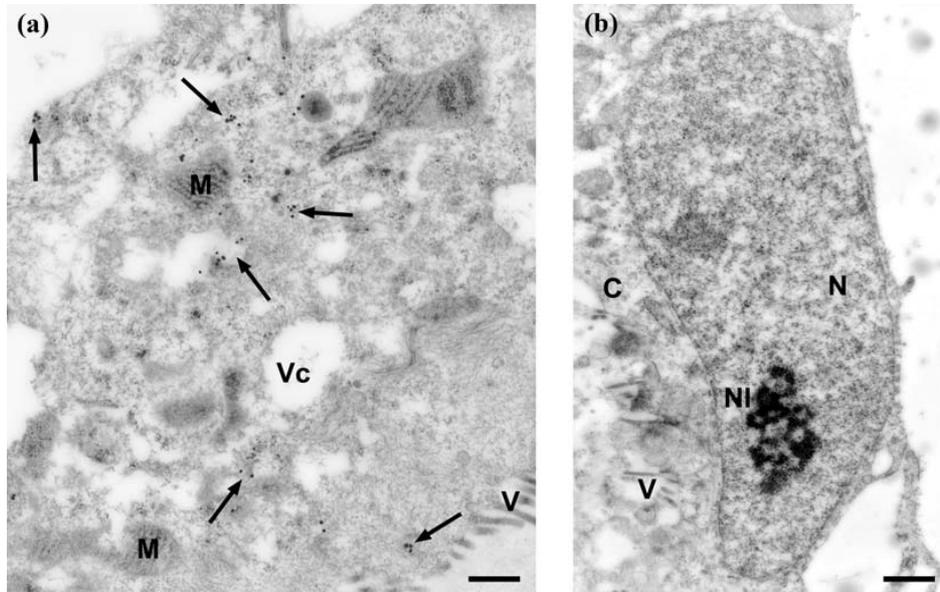


Figure 1. Transmission electron microscopy image of trophoblast cell cytoplasm. Positive image (a), negative control in which primary antibody was omitted (b). Positive signals are indicated by arrows. Vc vacuole, V villi, M mitochondrion, N nucleus, C cytoplasm, NI nucleolus. Bar: 0.5 μm .

and 2 $\mu\text{g}\cdot\text{mL}^{-1}$ FSH in 5% CO_2 at 38.5 $^\circ\text{C}$ for 24 h. Mature oocytes were fertilized, and the presumptive zygotes were then cultured in CR1aa medium with 5% defined FCS (Hyclone, Logan UT) in 5% CO_2 at 38.5 $^\circ\text{C}$. The embryos remained in culture for 7 days.

2.2. Preparation of embryos for electron microscopy

Blastocysts were embedded in 2% agarose (low melting agar) and excess agarose was trimmed off. Agarose blocks were fixed in 0.5% glutaraldehyde + 2% formaldehyde in 0.1M NaCaC buffer, pH 7.3. After dehydration in a series of ethanol dilutions, the cubes were embedded in 100% London Resin White acrylic resin (Polysciences, Warrington, PA) and polymerized overnight at 68 $^\circ\text{C}$. Thin sectioning (80 nm) was performed by an MT-XL ultratome (RMC,

Tucson, AZ) and sections were mounted on collodion (2%)-coated nickel grids.

To block non-specific binding, sections were treated with 25% nonfat milk, for 6 h in a refrigerator at 4 $^\circ\text{C}$. The grids were incubated for 2 h with a polyclonal primary antibody GTP1 (diluted 1:10) raised against a recombinant IFN- τ (a gift of Dr. Mike Roberts, University of Missouri, USA). This antibody has been shown to be specific to IFN- τ [15]. Incubation was followed by exposure to a secondary Goat-anti-rabbit (EMS, Hatfield, PA, USA) coupled to 15 nm of colloidal gold (1:10) for 1.5 h at RT. The specificity of immunolabeling was evaluated by comparing labeled profiles generated with primary antibody and normal rabbit serum at the same dilution. Sections were stained in 5% uranyl acetate, then with CNA lead. Thin sections were examined by TEM using a Zeiss10 (Zeiss, Germany).

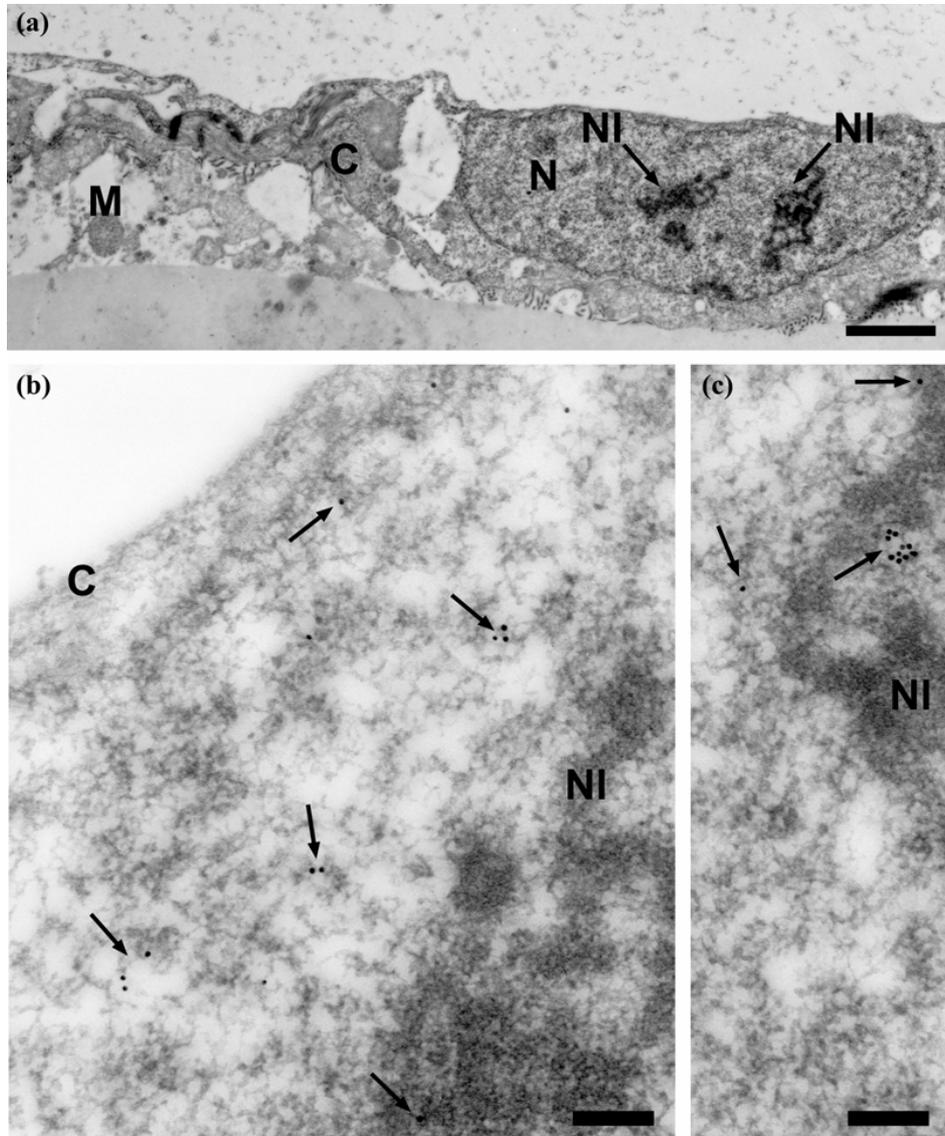


Figure 2. Transmission electron microscopy image of trophoctoderm blastomere nucleus (a) and magnification of nucleolar regions (b and c). Positive signals are indicated by arrows. N nucleus, NI nucleoli, M mitochondrion, C cytoplasm. Bars: 2.0 μm (a); 0.2 μm (b, c).

2.3. Preparation of embryos for confocal microscopy

Embryos were fixed in fresh 2% paraformaldehyde for 20 min and incubated in PBS

with 0.2% Fish Skin Gelatin (Sigma Chemical, St. Louis, MO, USA) and 0.1% Triton X-100 (Sigma Chemical, St. Louis, MO, USA) for 20 min at r.t. This was followed by incubation in 10% goat serum for 30 min

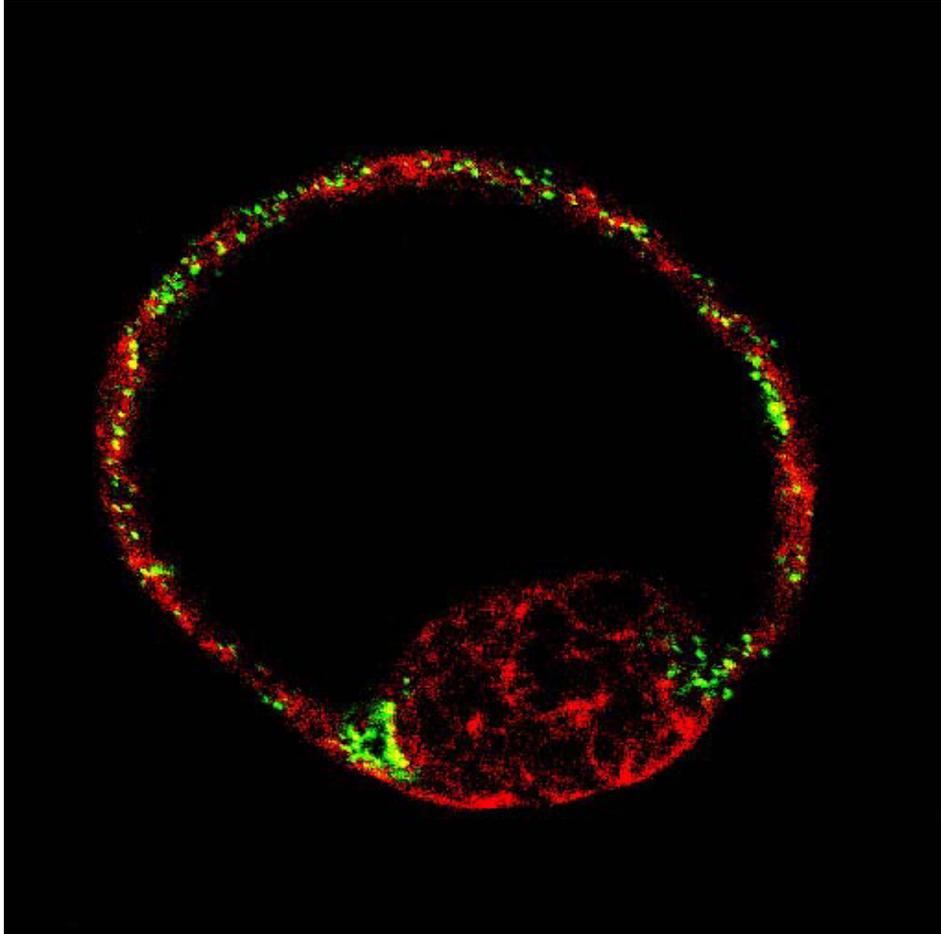


Figure 3. Confocal microscope image of a cross-sectioned non-hatched day 7 blastocyst stained for IFN- τ (green) and actin (red). The image shows the trophoctoderm with positive staining for IFN- τ and the ICM on the lower right.

and incubation in Endogenous Biotin-Blocking Kit (Molecular Probes, Eugene, OR, USA) to reduce background. The embryos were then incubated for 30 min at r.t. in 10% NSS containing the IFN- τ antibody at 1:500 dilution. Embryos were washed twice in the PBS/FSG/T \times 100, and incubated in a biotin X100-labeled goat anti-rabbit IgG secondary antibody (Molecular Probes, Eugene, OR, USA) at 1:1000

in 10% NSS for 45 min at room temperature. The embryos were washed twice before incubation in streptavidin AlexaFluor 488 (Molecular Probes, Eugene, OR, USA) at 1:1000 in PBS/FSG/T \times 100 for 10 min. The embryos were washed again in PBS/FSG/T \times 100, and then stained for actin with phalloidin AlexaFluor 568 at 1:50 dilution in 10% NSS. The embryos were washed a second time in PBS/FSG/T \times 100 and then

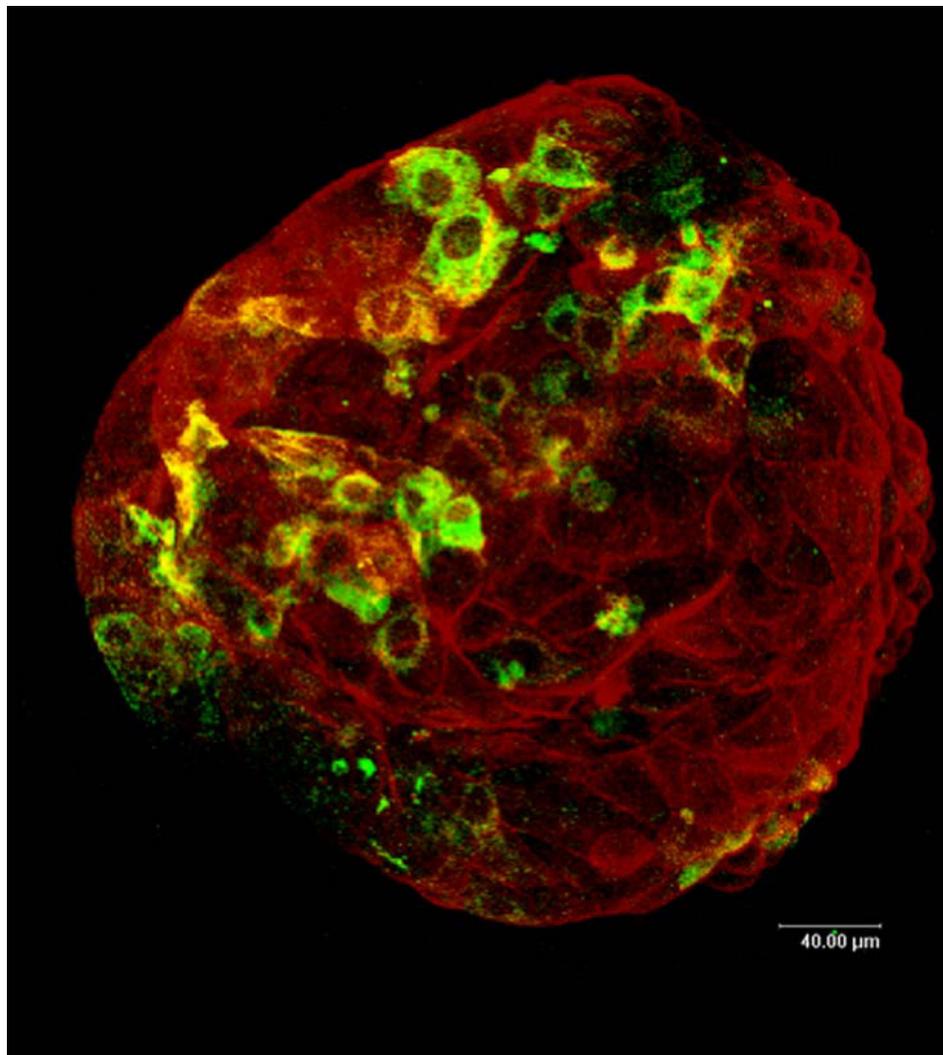


Figure 4. Confocal microscope image of a hatched blastocyst, stained for IFN- τ (green) and actin (red). The ICM can be seen on the lower left.

mounted on slides with a glycerol and DABCO anti-quenching mounting medium (non-hatched embryos) or PBS/FSG/T \times 100 (hatched embryos). The embryos were imaged on a Leica Systems laser-scanning confocal microscope with Image-Pro software (Leica Microsystems, Heidelberg, Germany).

3. RESULTS

3.1. Electron microscopy

A total of nine blastocysts were analyzed by electron microscopy each involving the examination of several cells. Analysis with gold-labeled antibody against the primary

IFN- τ antibody revealed interferon particles in the cytoplasm of all sections that were examined (Fig. 1a), while omission of the primary antibody and incubation with rabbit sera did not produce any positive labeling in any of the sections (Fig. 1b). Signals appeared to be clustered although this could not be correlated with any cellular structures. What proved most surprising was the observation that IFN- τ was also seen in the nuclei of trophoctodermal cells in hatched blastocysts, often in close proximity to the nucleolar region (Fig. 2a-2c). In several nuclei, the same positive signals could be detected in consecutive sections, precluding the likelihood that these were artifacts.

3.2. Confocal microscopy

IFN- τ was found in the trophoctoderm of all blastocysts that were examined. Cross-sectioning showed IFN- τ to be restricted to the trophoctoderm (Fig. 3). IFN- τ seemed to be concentrated in higher levels adjacent to the border with the inner cell mass. Analysis of whole blastocysts revealed that the expression was not evenly distributed throughout the trophoctoderm, with protein detectable to varying degrees in individual cells (Fig. 4). The control embryos in which the primary antibody had been omitted did not show any positive signals (data not shown).

4. DISCUSSION

The data presented here extend previous findings that IFN- τ is a protein that is produced solely by the trophoctoderm of the cattle conceptus since we did not detect any positive signals in the ICM. A striking observation in the present study is the large variation in the amount of IFN- τ that is seen between individual cells. The transcription factor Oct-4 has been shown to be one of the factors required to maintain a non-differentiated cellular state [16, 17]. Work by Ezashi et al. [18] has demonstrated that Oct-4

is capable of silencing IFN- τ expression by forming a complex with Ets-2 and one of the early events in the formation of the trophoctoderm is a down regulation of Oct-4. Analysis of Oct-4 expression has revealed a strikingly similar variability within the trophoctoderm [19]. It is conceivable that this could, in turn, account for the high variability of IFN- τ seen in this study.

One of the intriguing outcomes of the present study is that IFN- τ was detected in the nuclei of trophoblast cells. Since the same signals could often be found in consecutive sections of the same nucleus the possibility of an artifact can be safely precluded. IFN- τ has been shown to induce expression of a number of genes in endometrial cells and it appears to mediate this through signal transduction pathways such as STAT 1 and 2 [20]. Translocation of IFN- τ into the nucleus, however, would obviously point to the possible existence of other mechanisms of action.

How IFN- τ is translocated into the nucleus is unclear, although it does contain a putative nuclear localization sequence (NLS) at the C-terminus that consists of a KRLRK motif. This KRLRK motif is found in all bovine, deer and bison IFN- τ and, in a slightly changed form, in musk ox, goat and sheep. The bovine motif is homologous to what has been described as the center of nine variant nuclear localization sequences all of which contain the tetrameric consensus (R/K)_x(R/K) [21, 22]. This motif appears in this configuration or in slight variations in a number of proteins that are known to be translocated into the nucleus [23–26]. Moreover, and this is of particular interest, this NLS motif is also found in Tyk2 and JAK-1, two components of the Type 1 IFN signal transduction pathway [27, 28].

It is noteworthy that translocation into the nucleus has been demonstrated for another interferon, namely IFN- γ . Its translocation appears to involve the formation of a complex of IFN- γ with the interferon- γ receptor subunit 1 (IFNGR1) and the transcription

factor STAT1 α . IFN- γ has a nuclear localization sequence at its C terminus, which appears to facilitate nuclear translocation by binding to the nuclear importer nucleoprotein interactor 1 (NPI-1) [29, 30]. There is evidence that activated STAT1 α fails to undergo nuclear translocation in the absence of the IFN- γ nuclear localization sequence [31]. Moreover, more recent evidence has shown that an IFN- γ mimetic containing the C-terminal residues of the mouse IFN- γ including the NLS was able to act as a chaperone and facilitate nuclear translocation of STAT1 α [32].

It is at present not possible to dismiss the possibility that nuclear translocation might be an artifact of in vitro culture, particularly in light of an earlier study of in vivo-derived sheep conceptuses that failed to note nuclear localization [14]. However, that study was performed on conceptuses at much later stages of development and this discrepancy might simply reflect developmentally regulated changes in IFN- τ activities. Conversely our findings may point towards an as yet unknown function of IFN- τ in embryonic development.

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