Research Paper

Interferon-τ increases BoLA-I for implantation during early pregnancy in dairy cows

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ABSTRACT

Interferon-τ (IFN-τ) signals pregnancy recognition in ruminants. We investigated the effects of IFN-τ produced by embryo trophoblastic cells (ETCs) on expression of bovine leukocyte antigen-I (BoLA-I), a bovine analogue of human MHC-I, in endometrial luminal epithelial cells (EECs) during early pregnancy in dairy cows. Expression of *IFN-τ* and BoLA-I was increased in endometrial tissues during early pregnancy. Expression of the anti-inflammatory cytokine IL-10 was increased in endometrial tissues, while expression of the pro-inflammatory cytokine IL-6 was decreased, indicating immunosuppression. Progesterone increased *IFN-τ* expression in EECs. IFN-τ increased p-STAT1 and p-STAT3 levels in EECs, but reduced TRAF3 levels. In addition, IFN-τ increased expression of BoLA-I and IL-10, but decreased expression of IL-6 in EECs. These results indicate that IFN-τ enables stable implantation in dairy cows by increasing expression of BoLA-I, and by immunosuppression mediated by increased IL-10 and decreased IL-6 expression.

INTRODUCTION

Interferon-τ (IFN-τ), which is a cytokine characteristic for ruminants, is an anti-luteolytic protein that is secreted by trophoblast cells during early pregnancy [1]. IFN-τ has a similar structure and function as type I IFNs; bovine IFN-τ has a molecule weight of 24 kDa and binds to the same receptor as type I IFNs (*IFNAR*)[2–4]. IFN-τ has multiple functions, including *in vitro* antiviral activity in humans, ovine and bovine [4, 5]. In addition, IFN-τ has an anti-proliferative effect on cancer cells [6], and anti-inflammatory effect during bacterial infections [7–9].

IFN-τ exerts various immunomodulatory functions, but exists only for a short time in ruminants. IFN-τ is detected from 7–8 days in pregnancy, but is quickly reduced after the conceptus is implanted in the endometrial epithelial cells [7], and its specific function during early pregnancy is not clear. In early pregnancy, estradiol and progesterone also play important roles. Estradiol appears to be essential for establishing maternal receptivity to implantation in most mammals [8]. Progesterone plays a pivotal and indisputable role in the establishment and maintenance of pregnancy in mammals and its level is predictive of subsequent pregnancy loss [9]. Estradiol and progesterone can regulate the production of IFN-τ, which can affect stable implantation.

The Major Histocompatibility Complex (MHC) class I antigen has been associated with the implantation during early pregnancy. MHC-I is designated as HLA-I in humans [10], H-2-I system in mouse [11], and BoLA-I in bovine [12]. Previous studies have indicated that MHC-I has immunosuppressive effects during early embryonic development, including inhibition of cytotoxic T cells and NK cells [13], and inducing maternal-fetal tolerance [14, 15]. The maternal-fetal tolerance in dairy cows is associated with the expression of BoLA-I [16]. Based on the important regulatory function of MHC- I in reproduction [17, 18], the IFN-τ role in regulating embryo implantation needs to be elucidated.

This study aimed to analyze the expressions of IFN-τ in trophoblasts, and to investigate the IFN-τ effect

on BoLA- I expression in endometrial luminal epithelial cells (EECs).

RESULTS

Histological changes in endometrial tissues of pregnant and non-pregnant cows

Endometrial tissues were harvested from early pregnant and non-pregnant dairy cows, sliced, and tissues slices were subjected to hematoxylin and eosin staining. As expected, endometrial tissues of early pregnant cows showed a normal structure. Early pregnant endometrial tissues showed that the luminal epithelial cells clearance was increased, and the tissue structure was loose compared with the non-pregnant cows (Figure 1), indicating that the tissue was prepared for the fetal growth. The uterus endocrine glands and the artery blood vessel were increased, preparing for compensatory nutrition for the fetal growth. Inflammatory cells, such as neutrophils and macrophages were not found, indicating a suppressed immune state in early pregnant cows.

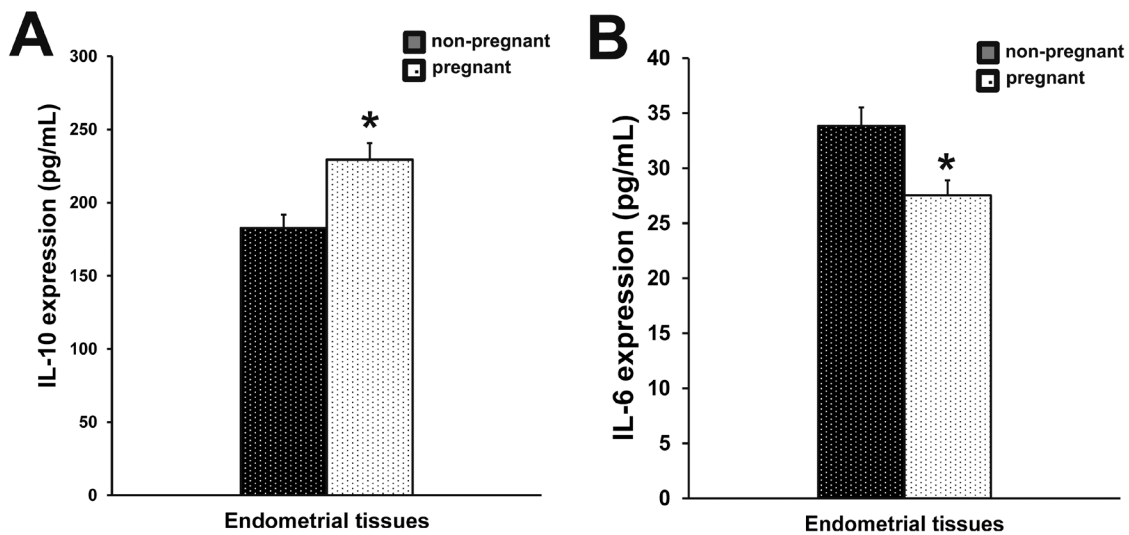### Production of IL-10 and IL-6 in endometrial tissues

To evaluate the immune status of the uterus, IL-10 and IL-6 levels were analyzed. Compared with non-pregnant cows, the IL-6 production ($P<0.05$) (Figure 2B) was decreased in early pregnant cows, while the IL-10 production was elevated, indicating an immune suppression ($P<0.05$) (Figure 2A). However, the differences in IL-6 and IL-10 production did not reach a statistical difference.
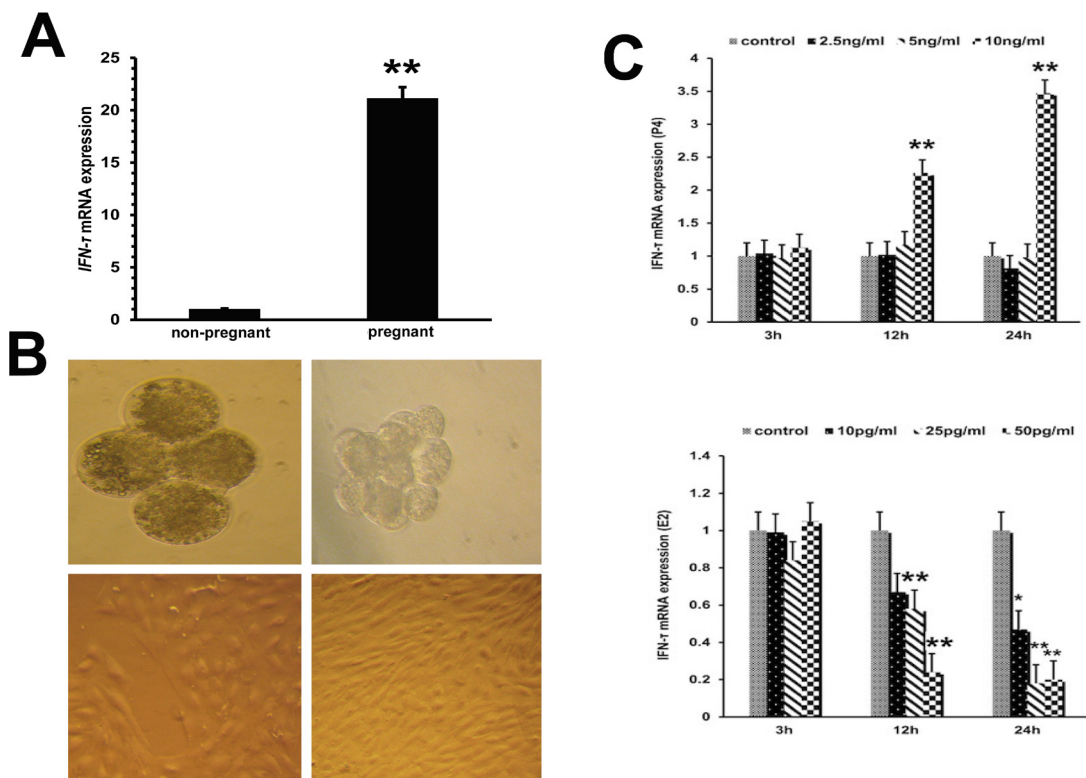
IFN-τ expression in endometrial tissues and ETCs

As shown in Figure 3A, compared with non-pregnant dairy cows, the *IFN-τ* level was significantly

Figure 1: Histopathological sections of endometrial tissues (HE,×200). Endometrial tissues from pregnant and non-pregnant cows were processed for histological evaluation.

Figure 2: Production of IL-10 and IL-6 in endometrial tissues. IL-10 **(A)** and IL-6 **(B)** expression in endometrial tissues of pregnant and non-pregnant cows was measured by ELISA. Columns represent the means. Bars represent the S.E. *P<0.05 values differ significantly from the control group; **P<0.01 values differ extremely significantly from the control group.

Figure 3: *IFN-τ* gene expression in embryo trophoblastic cells. (A) *IFN-τ* mRNA levels in endometrial tissues of pregnant and non-pregnant cows were quantified by RT-qPCR. **(B)** Parthenogenetic embryo activation and embryo trophoblastic cell culture (40×). **(C)** Effects of progesterone and estradiol on *IFN-τ* mRNA expression in ETCs. *P<0.05 values differ significantly from the control group; **P<0.01 values differ extremely significantly from the control group.

increased in early pregnant cows (*P*<0.01). To investigate the mechanisms regulating the IFN-τ expression, *IFN-τ* levels were analyzed in ETCs treated with progesterone or estradiol in order to simulate endo-environment of implantation (Figure 3B). Compared with the control group, progesterone remarkably increased the mRNA level of *IFN-τ* (*P*<0.01), whereas *IFN-τ* mRNA expression was significantly reduced (P<0.01) in estradiol-treated ETCs (Figure 3C).

BoLA-I expression in endometrial tissues

Since *BoLA-I* is a highly polymorphic gene, analyzing expression of all *BoLA-I* genes is challenging, particularly because the precise number of *BoLA-I* loci remains unknown, and the *BoLA-I* genes undergo interlocking recombination. The mRNA expression of *MIC1, BoLA-NC3* BoLA-A, BoLA-N*03101, BoLA-N*01201* and *A11* was significantly increased in early pregnant cows (*p*<0.01) (Figure 4A). In addition, the mRNA expression of *BoLA-NC1*, Heavy chain, BoLA-N*03701*, was up-regulated (P<0.05) (Figure 4A). We also measured the protein levels of BoLA-I by western

blotting. The protein expression of BoLA-I was increased in early pregnant cows (*p*<0.01) (Figure 4B).

TRAF3, STAT1, and STAT3 expression in endometrial tissues

Transcription factors regulate the implantation during early pregnancy. We analyzed protein levels of the transcription factors TRAF3, STAT1, and STAT3 in endometrial tissues by western blotting. Compared to non-pregnant cows, the phosphorylated STAT1 and STAT3 levels were increased (p < 0.01) during early pregnancy, while the expression of TRAF3 was reduced (p < 0.01) (Figure 5).

EECs identification

The EECs play a key role during pregnancy, and are the main target of IFN-τ. We found that EECs had an irregular round or oval shape, with large, round, but unclear nucleus and transparent cytoplasm. The EECs were verified by specific red staining with keratin CK18, and showed that the cytoplasm was clear and uniformly
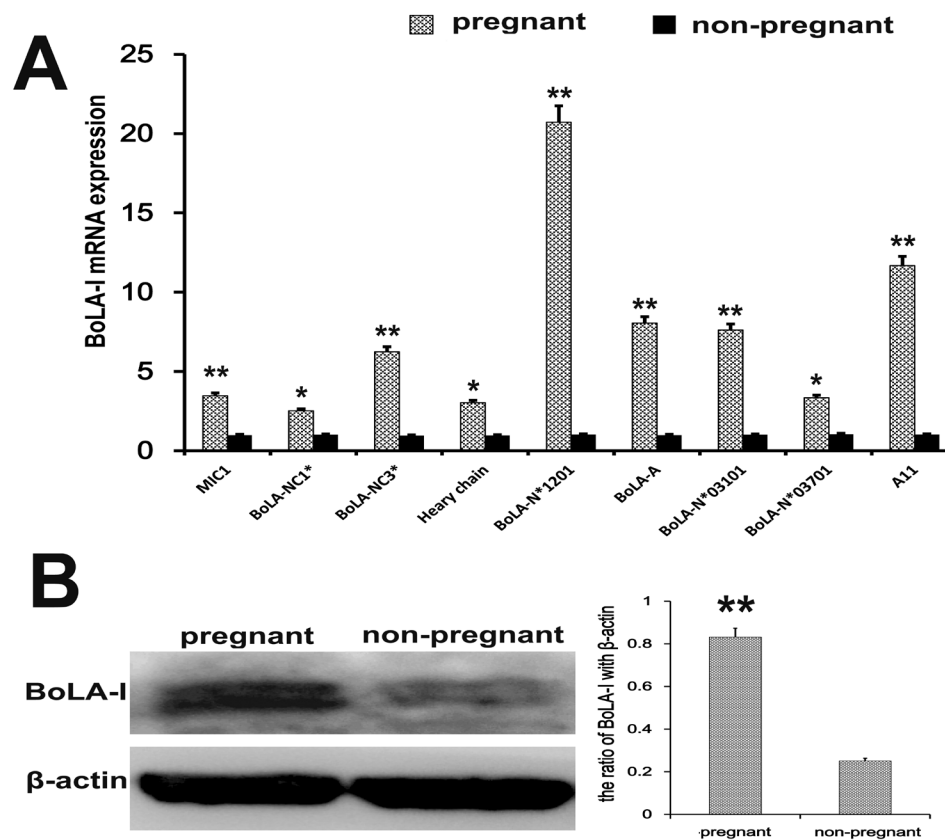
Figure 4: BoLA-I expression in endometrial tissues. (A) *MIC1, BoLA-NC1*, BoLA-NC3*, Heavy chain, BoLA-N*01201, BoLA-A, BoLA-N*03101, BoLA-N*03701* and *A11* mRNA levels in endometrial tissues of pregnant and non-pregnant cows were quantified by RT-qPCR. **(B)** Western blotting was performed to detect the BoLA-I protein levels in endometrial tissues of pregnant and non-pregnant cows. *P<0.05 values differ significantly from the control group; **P<0.01 values differ extremely significantly from the control group.

stained. Nuclei were stained blue with DAPI. Microscopic images were captured by LSCM and no other cell types were observed (Figure 6).

Effect of IFN-τ on IL-10 and IL-6 production in EECs

The EECs can produce IL-10 and IL-6 in endometrial tissues. IL-10 and IL-6 production in EECs was measured by ELISA (Figure 7). The effect was similar as in the uterus tissues. The IL-6 levels were significantly reduced in IFN-τ treated cells ($p < 0.01$). The IL-10 was slightly elevated in EECs treated by IFN-τ, but no statistical significance was found ($p > 0.05$) (Figure 7).

IFN-τ increases BoLA-I expression in EECs

BoLA-I is expressed on EECs, which are the main target of IFN-τ. The mRNA expression of *MIC1* (Figure 8A) (*P*<0.01), *BoLA-NC3** (Figure 8C) (*P*<0.01) and *BoLA-NC1** (Figure 8B) (P<0.05) were significantly up-regulated by IFN-τ. The mRNA expression of *BoLA-A* (Figure 8D) and *BoLA-N*01201* (Figure 8E) was significantly up-regulated by IFN-τ (*P*<0.01). Thus, the effect of IFN-τ on *BoLA-A* (Figure 8F), *BoLA-N*03101* (Figure 8G), *BoLA-N*03701* (Figure 8H), and *A11* (Figure 8I) mRNA expression was similar (*P*>0.05). To confirm the effect of IFN-τ on BoLA-I expression in EECs, we measured protein levels of BoLA-I by western blotting. The protein levels of BoLA-I were increased by IFN-τ in a dose-dependent manner (P<0.05) (Figure 8J).

Effect of IFN-τ on TRAF3, STAT1, and STAT3 expression in EECs

To further analyze the effect of IFN-τ on BoLA-I expression in EECs, the relevant transcription factors TRAF3, STAT1 and STAT3 were analyzed by western blot. The protein levels of phosphorylated STAT1 and STAT3 were increased ($p < 0.05$) in IFN-τ treated cells, and the effect was dose dependent (Figure 9), similarly as in the uterus tissues. In contrast, the expression of TRAF3 was reduced ($p < 0.05$) by IFN-τ treatment in a dose-dependent manner (Figure 9).

DISCUSSION

The implantation is an important event in the fetus formation during early pregnancy. Our results indicate that cell clearance is increased in endometrial tissues of pregnant cows to facilitate the fetal growth. The uterus endocrine glands and artery blood vessel are increased to enable fetal nutrition. In addition, immune modulation in the uterus plays a crucial role during early pregnancy. IL-6 is involved in immune defense in the uterus [19, 20], and IL-10 plays a major role in anti-immunological rejection [21, 22]. Our results showed that there were no appreciable differences in IL-10 and IL-6 production between early pregnant and non-pregnant cows, suggesting that the fetus was not rejected in the uterus by the immune system. Our previous studies have shown that IFN-τ can reduce the inflammatory cytokine expression [23, 24]. IFN-τ has been associated with the uterine immune regulation in early
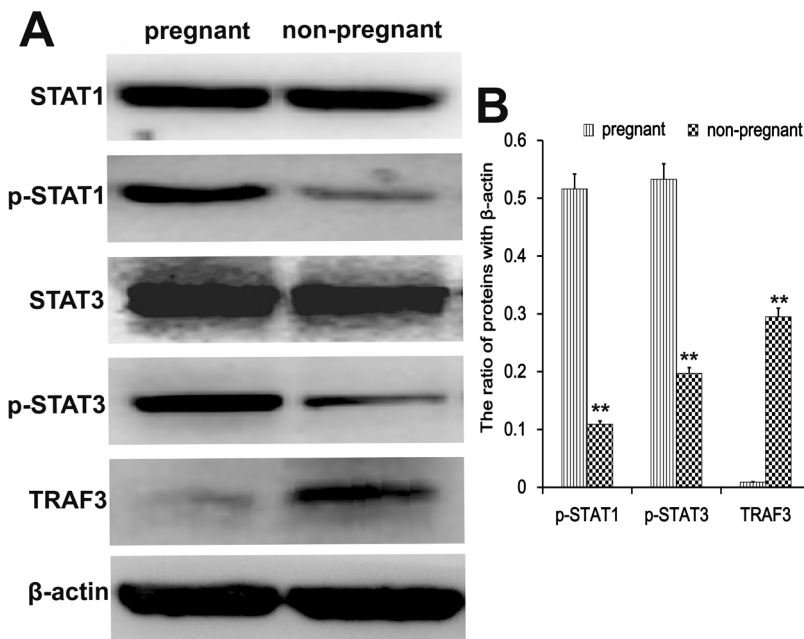

Figure 5: Transcription factor analysis in endometrial tissues. Western blots were performed to detect the TRAF3, STAT1, and STAT3 protein levels in endometrial tissues of pregnant and non-pregnant cows. *P<0.05 values differ significantly from the control group; **P<0.01 values differ extremely significantly from the control group.

pregnant cows [25]. Suggesting that it may be the key factor in developing the low immune defense for stable implantation during early pregnancy.

The proper secretion of progesterone and estradiol is vital for the establishment and maintenance of pregnancy [9]. The receptors for progesterone and estradiol are expressed on ETCs, which also express IFN-τ [26–28]. IFN-τ secretion is unique to ruminant animals during early pregnancy and might be regulated by several endometrium genes (IFN-τ stimulated genes, ISGs), the expression of which is also enhanced by IFN-τ [29]. Since the specific concentrations of progesterone and estradiol regulating ETCs are not known, we used several different concentrations of estradiol and progesterone to investigate the ETCs functions. We found that ETCs exposed to a high concentration of progesterone for 12 and 24 h

showed a significant increase in *IFN-τ* mRNA expression, while estradiol had the opposite effect. This result was in agreement with a previous study in sheep [30].

IFN-τ up-regulates the expression of MHC-I [31]. A previous study has demonstrated both classical and non-classical BoLA-I mRNA expression in bovine oocytes and developing pre-implantation embryos, suggesting that the expression is both gene- and stage-of-development specific [32]. The receptor of IFN-τ is located on ovine EECs [31], and BoLA-I is expressed on EECs [12]. Our study is the first to report that IFN-τ increases mRNA expression of non-classical *MIC1, BoLA-NC1*, BoLA-NC3* and *classical Heavy chain and BoLA-N*0120* and protein levels of BoLA-I in EECs. In addition, the results in IFN-τ treated EECs are similar to the results in endometrial tissues of early pregnancy.
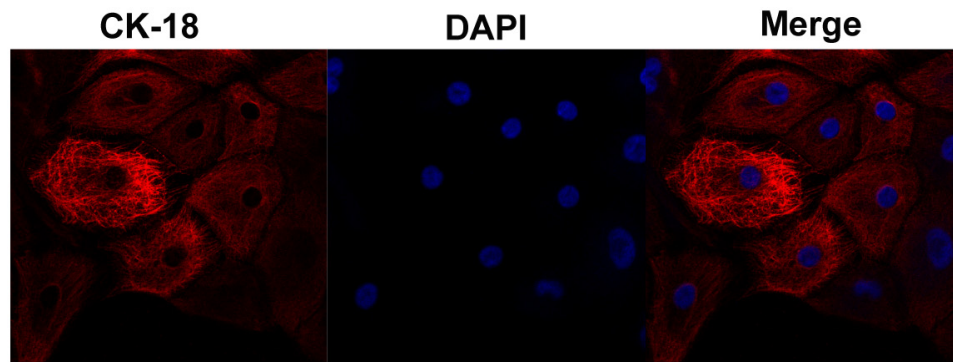
Figure 6: Endometrial epithelial cell culture and identification. Microscopic images were captured by laser scanning confocal microscope. The EECs were verified by specific red staining with keratin CK18, which merged with nuclei that were stained blue with DAPI.
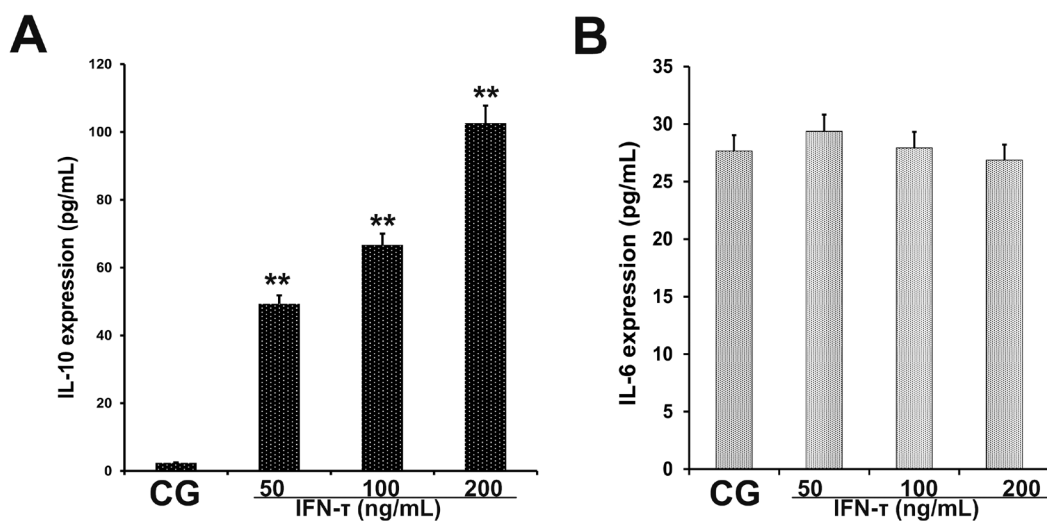
Figure 7: Effect of IFN-τ on IL-10 and IL-6 production in EECs. ELISA was performed to detect **(A)** IL-10 and **(B)** IL-6 expression in EECs treated with control DMSO (CG) or IFN-τ. *$P<0.05$ values differ significantly from the control group, **$P<0.01$ values differ extremely significantly from the control group.
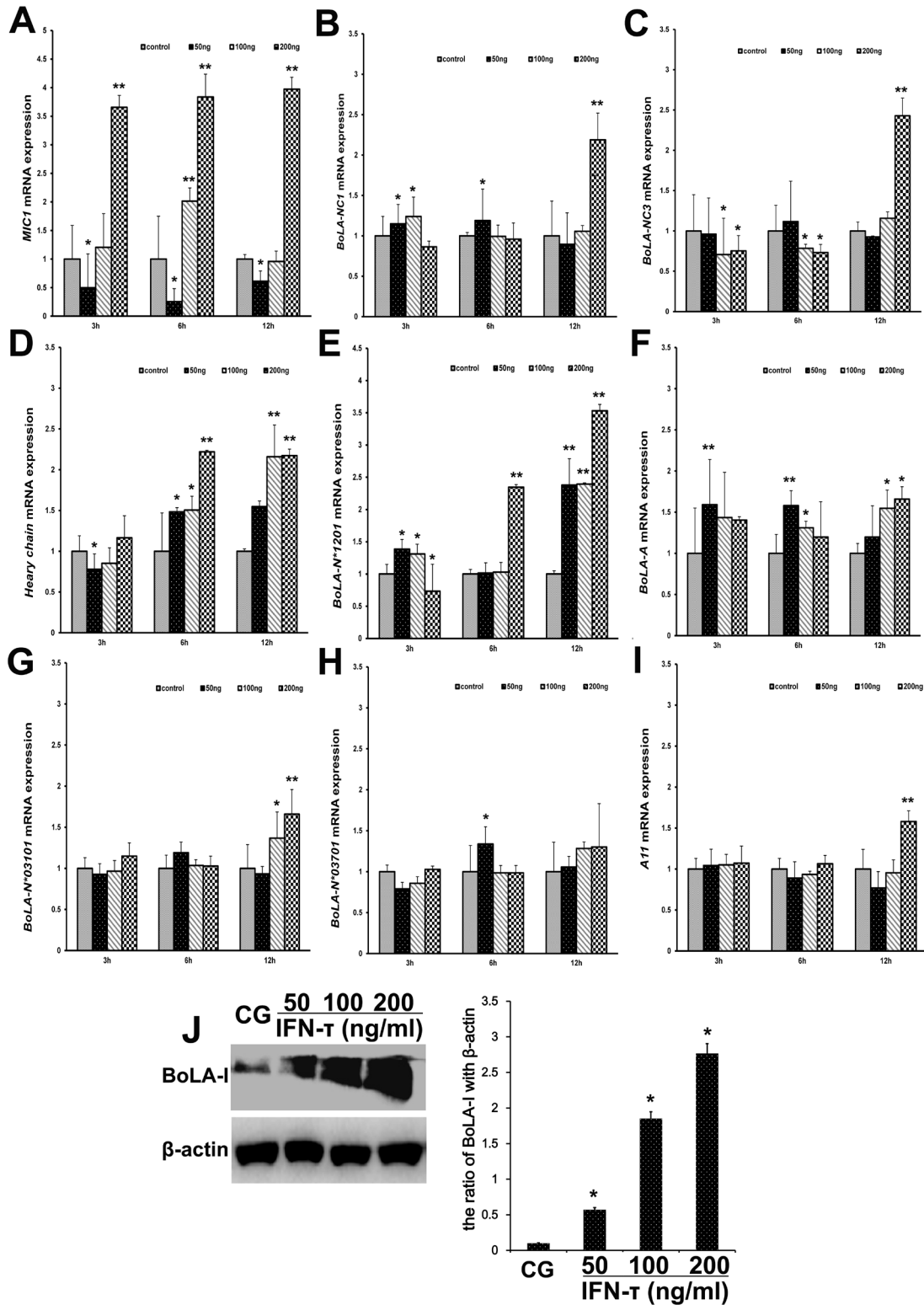
Figure 8: Effect of IFN-τ on BoLA-I expression in EECs. (A) *MIC1*, (B) *BoLA-NC1**, (C) *BoLA-NC3**, (D) *Heavy chain*, (E) *BoLA-N*01201*, (F) *BoLA-A*, (G) *BoLA-N*03101*, (H) *BoLA-N*03701* and (I) *A11* mRNA levels in EECs were quantified by RT-qPCR after IFN-τ treatment. (J) Western blots were performed to measure the BoLA-I protein levels in the control group (CG) and IFN-τ group in EECs. *P<0.05 values differ significantly from the control group, **P<0.01 values differ extremely significantly from the control group.

IFN-τ is the main messenger of communication between the ruminant female parent and the embryo, and this communication inhibits the maternal immune responses against the embryo, by adjusting cytokines and other immunomodulatory molecules [25]. Indeed, the BoLA-I promoter is responsive to IFN-τ, IFN-G, and IL4, suggesting possible roles for these cytokines in bovine pre-implantation embryo survival and/or maternal-fetal tolerance [33]. The MHC-I expression regulates the reproductive immunology in mice [11]. Our results show that IFN-τ increases *BoLA-NC1** expression in bovine endometrial cells; these results are supported by previous studies indicating that blastocyst *MHC-I* expression is a key component contributing to the establishment of pregnancy in cattle [34]. The endometrial immune profile of the estrous cycle favors a Th2 environment in anticipation of pregnancy, and the presence of an embryo acts to fine-tune this environment [35].

Our previous study has shown that IFN-τ inhibits inflammation by suppressing the activation of NF-κB and MAPKs [23, 24]. TRAF3 belongs to NF-κB non-canonical pathway involved in the regulation of type I IFN production [36]. However, we found the protein levels of TRAF3 in endometrial tissue of early pregnancy were reduced compared with non-pregnant cows. In addition, our results indicate that IFN-τ inhibits the TRAF3 expression in EECs, and during early pregnancy. IFN-τ

might provide an alternative to other type I IFNs in the treatment of human diseases, with fewer side effects and lower cytotoxicity [37].

Transporter associated with Ag processing 1 (TAP1) and low molecular mass polypeptide 2 (LMP2) are regulated by STAT1, are essential for MHC-I function, and share a common bidirectional promoter [38]. We found that IFN-τ increases the BoLA-I and phospho-STAT1/STAT3 levels in EECs. It has been shown that MIC1 belonging to the non-classical BoLA-I family binds to the receptor NKG2D on the surface of NK cells, thus stimulating the lethal effect of NK cells [39]. A previous study has shown that STAT3 ablation in tumor cells modulates NKG2D-mediated NK cell activation, and that STAT3 directly interacts with MIC1 promoter to repress MIC1 transcription [40]. The biological effects of type I IFNs are regulated by the signaling between STAT1 and STAT3 and their relative abundance [41].

In conclusion, our study demonstrates that progesterone increases the *IFN-τ* expression in ETCs of dairy cows, while estradiol inhibits it. In addition, IFN-τ increases BoLA-I expression in EECs. IFN-τ also increases IL-10, p-STAT1 and p-STAT3 levels, but decreases IL-6 and TRAF3 levels in EECs. These results also found in early pregnant endometrial tissues indicate that IFN-τ increases BoLA-I for implantation during early pregnancy in dairy cows.
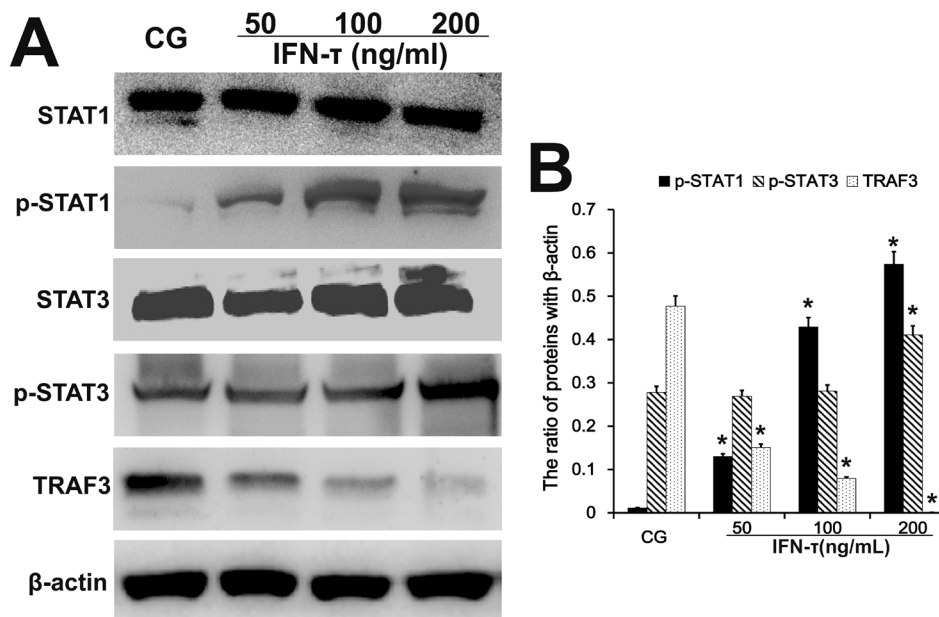
Figure 9: Effect of IFN-τ on transcription factor expression in EECs. Western blots were performed to detect the TRAF3, STAT1 and STAT3 protein levels and their phosphorylation in the control group (CG) and IFN-τ group in EECs. *P<0.05 values differ significantly from the control group, **P<0.01 values differ extremely significantly from the control group.

Table 1: Primers for RT-qPCR

Gene	Primer	Objective segment size	GenBank accession no.
β-actin	F 5'-TGGACTTCGAGCAGGAGAT-3' F 5'-CGTCACACTTCATGATGGAA-3'	194 bp	NM-173979.3
IFN-τ	F 5'-TGAACAGACTCTCCTCATCCC-3' R 5'-TGGTTGATGAAGAGAGGGCTCT-3'	151 bp	NM-001015511
BoLA-A	F 5'-GGAGACGCAGAGAACTAAGGA-3' R 5'-TCGTTCAGGGCGATGTAA-3'	194 bp	NM_001040554
Heavy chain	F 5'-TATGTGGACGACACGCAGT-3' R 5'-TCGCTCTGGTTGTAGTAGCC-3'	187 bp	NM_001193296
*BoLA-NC1**	F 5'-AGTATTGGGATCAAGAGACGC-3' R 5'-ATAGGCGTGCTGATTATACCC-3'	181 bp	NM_001105616
MIC1	F 5'-AGAAAGGAGGCTTACATTCCC-3' R 5'-GCCTGGTAATGCTTGCTTAAC-3'	199 bp	NM_001127317
*BoLA-N*01201*	F 5'-GGAGACGCGAAACTTCAAG-3' R 5'-TCGTTCAGGGCGATGTAA-3'	197 bp	DQ304655
*BoLA-NC3**	F 5'-AGATGACACGAGATGCCAAG-3' R 5'-TCGTTCAGGGCGATGTAA-3	198 bp	DQ140378
*BoLA-N*03101*	F 5'-GATGACGAGACGCGAATCT-3' R 5'-GCGATGTAATCTCTGCCGT-3'	193 bp	DQ140365
*BoLA-N*03701*	F 5'-GTATTGGGATCGGAACACG-3' R 5'-AGGTAATCTCTGCCGTCGTAG-3'	171 bp	DQ190938
A11	F 5'-GAGTATTGGGATGAGGAAACG-3' R 5'-AGGTAATCTCTGCCGTCGTAG-3'	199 bp	NM_001143743

MATERIALS AND METHODS

Reagents

Recombinant Ovine Interferon-τ (rOvIFN-τ) was purchased from Creative Bioarray (NY, USA). Fetal bovine serum (FBS) was purchased from SAFC Biosciences (Brooklyn, AUS). Bovine IL-10 and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Biolegend (San Diego, CA, USA). Rabbit TRAF3, STAT1, and STAT3 antibodies, and monoclonal antibodies against p-STAT1 and p-STAT3 were purchased from Cell Signaling Technology Inc (Beverly, MA, USA). HLA class I (ab23755, reacts with cow BoLA-I), and horseradish peroxidase conjugated goat anti-rabbit antibodies were provided by ABCAM Biotech Co., Ltd (Cambridge, UK). SYBR green Plus regent kit was purchased from Roche Applied Science (Mannheim, Germany). All other chemicals were of reagent grade.

Animal studies

The dairy cows were obtained from Animal Experiment Center of Huazhong Agricultural University (Wuhan, China). All experimental procedures involving animals and their care conformed to the Guide for the Care and Use of Laboratory Animals of National Veterinary Research. This study was approved by the Huazhong Agricultural University Animal Care and Use Committee. The dairy cows were anesthetized with sodium pentobarbital to minimize suffering. The ovaries, endometrial epithelium (for cell culture) and endometrial tissues of non-pregnant cows were collected before ovulation. Endometrial tissues of early pregnant cows were collected during the implantation (Day9 to Day25,

n = 3). Pregnancy was induced by artificial insemination (AI); the day of insemination was designated as Day 0 of pregnancy [42, 43].

Embryo trophoblastic cells (ETCs) culture and treatment

The procedures for oocyte acquisition and maturation were reported by Presicce and Yang [44]. For the maturation culture, cumulus-enclosed oocytes were cultured in 100 μL droplets of maturation medium (20–25 oocytes per droplet) covered with mineral oil at 37°C in 5% CO_2 humidified air. The maturation medium consisted of bicarbonate medium M-199 with Earle's salts, 25 mM HEPES, and 7.5% FBS, supplemented with 0.5 μg/mL ovine follicle-stimulating hormone, 5.0 μg/mL ovine luteinizing hormone, 1.0 μg/mL estradiol, and 0.25 mM pyruvate. The methods described by Susko-Parrish were employed for parthenogenetic activation [45]. The oocytes were activated by a 5 min exposure to Ca-ionophore A23187 at room temperature followed by culture in 2.5 mM 6-dimethylaminopurin, for 3.5 h [46]. After washing, the treated oocytes were cultured in KSOM with 0.1% BSA for an additional 48 h. The culture environment consisted of 5% CO_2 humidified air, or 5% O_2, 5% CO_2 and 90% N_2 at 37°C. All cleaved embryos were further cultured for 8 days in SOF-BE1 medium, which consisted of 20% FBS. The primary trophectoderm cultures were started after removing the zona pellucida of the 8-day-old parthenogenetic blastocysts with 25-gauge hypodermic needles; the punctured blastocysts helped the ETCs adhere to the wall of the culture dish. Healthy ETCs emerged from the central collapsed blastocyst mass after 2 days and grew out rapidly as a tightly knit epithelial monolayer. The ETCs were treated with progesterone (2.5 ng/mL, 5 ng/mL and 10 ng/mL) or estradiol (10 pg/mL, 25 pg/mL, and 50 pg/mL)[47]. The control group was treated with the same amount of DMSO. All experiments were performed three times, and analyzed in triplicates.

Endometrial luminal epithelial cells (EECs) culture and treatment

EECs were cultured as described previously [47]. The caruncular endometrial epithelium was mixed with sufficient 1% collagenase I, cut into 1-3 mm pieces, and digested for 1 h in a sealed container in a thermostatic shaker at 37.5°C and 88 rpm. After neutralization of collagenase I with FBS, the tissue pieces were placed on the bottom of a culture dish at 0.5 - 1 cm intervals. The dish was inverted, and the samples were incubated in 5% CO_2 at 37°C for 3 h. The EECs were cultured in DMEM/F12 supplemented with 15% FBS, 2 mM L-glutamine, 50 U/mL of penicillin and streptomycin, 100 U/mL of gentamicin, and 10 ng/mL EGF, and maintained in a 5% CO_2 humidified incubator at 37°C. The EECs were

identified by keratin 18 with Laser Scanning Confocal Microscope. The EECs were treated with 50 ng/mL, 100 ng/mL or 200 ng/mL IFN-τ [48]. The control group was treated with the same amount of DMSO. All experiments were performed three times, and analyzed in triplicates.

Quantitative real-time PCR

Total RNA was isolated by the TRIZOL Reagent, and converted into cDNA with PrimeScript 1ST Strand cDNA Synthesis Kit according to the manufacturer's instructions (Takara, Japan). The Primer Premier software (PREMIER Biosoft International, USA) was used to design specific primers (Table 1). Quantitative real-time PCR was performed on StepOne real-time PCR System (Life Technologies Corp) with the FastStart Universal SYBR Green Master (Roche, Germany) in a 25-μl reaction. Each sample was measured in triplicates. Results (fold changes) were expressed as $2^{-\Delta\Delta Ct}$. β-actin was used as a reference gene.

Western blot analysis

The total protein was extracted according to the manufacturer's recommended protocol (Vazyme, USA). The protein concentration was determined using the BCA Protein Assay Kit (Vazyme, USA). Samples with equal amounts of protein (50 μg) were fractionated on 10% SDS-PAGE, transferred to PVDF membrane, and blocked in 5% skim milk in TBST for 1.5 h. The membrane was incubated with primary antibody (1:500 dilution) at 4°C overnight. After washing with TBST, the membrane was incubated with secondary antibody (1:1,500 dilution) for 2 h. Protein expression was detected using the ECL Plus Western Blotting Detection System (Image Quant LAS4000mini, USA). β-actin was used as a loading control.

ELISA assay

The endometrial tissues were harvested and homogenized with phosphate buffered saline (PBS), centrifuged, and the supernatants were collected. The supernatants of the EECs with different treatments were also collected. The supernatants were assayed for IL-10 and IL-6 levels with enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions (BioLegend, San Diego, CA, USA).

Statistical analysis

The results were analyzed using GraphPad Prism 5 (GraphPad InStat Software, San Diego, CA, USA). Comparisons among the groups were performed with one-way and two-way ANOVA. Data were expressed as the mean ± standard error (SEM). P values <0.05 are considered to indicate a statistically significant difference.

Abbreviations

Interferon-τ (IFN-τ); Major Histocompatibility Complex I (MHC-I); bovine leukocyte antigen I (BoLA-I); embryo trophoblastic cells (ETCs); endometrial luminal epithelial cells (EECs).

Author contributions

Zhe Zhu and GanZhen Deng designed the study and participated in the data analysis and interpretation, manuscript drafting and critical revising. Zhe Zhu wrote the main manuscript text and participated in the data analysis. Zhe Zhu, Binbin Li, Yue Wu and Xiao Wang performed laboratory work and contributed to data collection. Zhe Zhu and GanZhen Deng contributed to data analysis and interpretation, and to the critical revision of the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST

The authors declare no competing financial interest.

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