Estrous cycle stage-dependent manner of type I interferon-stimulated genes induction in the bovine endometrium

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Abstract. Interferon tau (IFN-τ) is a ruminant-specific type I IFN secreted by a conceptus before its attachment to the uterus. IFN-τ induces the expression of IFN-stimulated genes (ISGs) via the type I IFN receptor (IFNAR), which is composed of IFNAR1 and IFNAR2 subunits in the endometrium. However, expression patterns of IFNARs during the estrous cycle have not been reported. We hypothesized that the response to a type I IFN changes along with IFNARs and the IFN-regulatory factors (IRFs) driving transcription of IFN signal-related genes and modulating a type I IFN signal during the estrous cycle. We investigated the estrous cycle stage-dependent type I IFN induction of ISGs and expression patterns of IFN signal-related genes in bovine endometrial tissues. Endometrial tissue pieces collected from bovine uteri at each estrous stage (early, mid, and late) were cultured with or without recombinant bovine IFN-α or concentrated pregnant uterine flushing (PUF) on day 18 after confirming the presence of a conceptus. IFN-α and PUF each significantly increased the expression of ISGs in endometrial tissues. The induction levels of the typical ISGs (MX1-a and ISG15) were significantly higher at the mid stage and correlated with high expression of IRFs at the mid stage. The immunostaining of IFNARs showed strong fluorescence intensities in luminal and glandular epithelia at the early and mid stages. Collectively, these results suggest that the endometrium exhibits estrous cycle stage-dependent responsiveness to type I IFN that may be associated with the expression of IFNARs and IRFs for pregnancy recognition.

Key words: Cow, Interferon-stimulated gene (ISG), Type I Interferon (IFN), Type I interferon receptor (IFNAR), Uterus

Interferon tau (IFN-τ) is secreted by the conceptus of ruminants at the preimplantation stage [1–4]. IFN-τ is essential for pregnancy recognition in order to maintain the corpus luteum (CL). The CL is necessary for continuous production of progesterone during pregnancy. In the estrous cycle, the CL regresses, and this is followed by the next follicular phase because the CL secretes oxytocin, which binds to the oxytocin receptor expressed in the uterus and induces the CL regression factor: prostaglandin (PG) F2α [4, 5]. On the other hand, in the presence of a conceptus in the uterus, IFN-τ secreted by the conceptus inhibits the expression of oxytocin receptors. The synthesis of PGF2α is consequently inhibited, which prevents regression of the CL [4, 6]. Interferon-stimulated genes (ISGs) are strongly expressed in the uterus at this time [7–9]. Because the production of IFN-τ is limited to days 14–21 of pregnancy in cows [10], the expression of bovine Myxovirus resistance (MX) genes, typical ISGs, decreases immediately after implantation is completed, on days 25–40 [11]. The expression of ISGs is induced by the ISGF3 complex, which consists of phosphorylated and dimerized signal transducer and activator of transcription (STAT) 1/2 and IFN-regulatory factor (IRF) 9 in the IFN-stimulated Janus kinase (JAK)-STAT signaling pathway [12–14]. IFN-τ belongs to the type I IFN family, which includes...
hypothesized that the estrous cycle stage-dependent responsiveness to type I IFN in the bovine endometrium is accompanied by a change in the expression of IFNARs and IRFs. In the present study, we investigated type I IFN-mediated induction of ISGs and expression patterns of IFN signal-related genes in bovine endometrial tissue at each estrous stage: early, mid, and late.

Materials and Methods

Collection of samples of endometrial tissues

This study was conducted in accordance with the Hokkaido University guidelines for the care and use of animals. Uteri collected from abattoirs were subdivided into three stages: early (days 4–9), mid (days 10–15), and late (days 16–21) according to the luteal stages of the ovary [39] as well as the status and electrical impedance of uterine mucus [40]. Intercaruncular endometrial tissues were collected from the uterine horn ipsilateral to the CL. The collected tissues were embedded in Optimal Cutting Temperature compound (Sakura Finetek, Tokyo, Japan) in liquid nitrogen, and stored in a freezer at –80°C until section preparation. Moreover, the collected tissues were used in expression analyses or explant cultures. Noncultured tissue samples were stored in a freezer at –80°C until RNA extraction. All the experiments were conducted on the tissue samples of at least three cows at each stage.

Uterine flushing and bovine recombinant IFN-α

Pregnant uterine flushing (PUF) was collected from a cow, confirming the existence of a conceptus on day 18 of pregnancy after embryo transfer on day 7 of the estrous cycle. Nonpregnant uterine flushing (NPUF) was collected from a cow confirmed to not have a conceptus after embryo transfer on day 7 of the estrous cycle. Approximately 2 ml of each collected uterine flushing was filtered through gauze to remove cell debris. Then, the flushing was concentrated to 20 ml by means of a dialysis membrane (Size 36, Wako, Osaka, Japan) and dehydrated using polyethylene glycol 20,000 (Merck Schuchardt OHG, Hohenbrunn, Germany) at 4°C overnight. Concentrated uterine flushing was finally filtered through a 0.45-μm membrane, and then stored in a freezer at –80°C. Bovine recombinant IFN-α was kindly provided by Novartis (Novartis Animal Health, Basel, Switzerland).

The IFN-τ protein was detected in PUF by a western blot analysis. The antiviral activities of PUF and IFN-α were measured by a viral resistance assay using Madin-Darby bovine kidney cells and were found to be 82,620 IU/ml and 602,290 IU/ml, respectively. Five microliters of PUF or 0.5 μl of IFN-α was added to 1 ml of the medium, which was adjusted to approximately 400 IU/ml or 300 IU/ml. Five microliters of NPUF (the volume equivalent to that of PUF, 5 μl) was also added to the medium as a negative control.

Explant culture conditions

Collected tissues were placed in calcium- and magnesium-free Hanks’ balanced salt solution (HBSS) (–) and cut into small pieces (5 × 5 mm). These pieces were preincubated in Dulbecco’s Modified Eagle’s medium—high glucose (DMEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 0.06 g/l penicillin G potassium (Nacalai Tesque, Kyoto, Japan) and 0.1 g/l streptomycin sulfate (Nacalai Tesque) at 38.5°C [41, 42] for 1 h at 5% of CO2. The medium volume was 2 ml in Falcon 35-mm Easy Grip Style Not Treated Bacteriological Petri Dishes, Sterile (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). After the preincubation, endometrial tissue pieces were cultured with nonsupplemented DMEM containing 10% (v/v) of fetal bovine serum (FBS, ICN Bio-Source International, Camarillo, CA, USA) (Cont.) or DMEM containing 10% of FBS and supplemented with IFN-α (300 IU/ml), PUF (400 IU/ml), or
NPUF (5 μl/ml) for 12 or 24 h at 38.5°C and 5% CO2 in duplicate. The medium volume was 0.5 ml/well in Thermo Scientific™ Nunc™ Cell-Culture Treated Multidishes with 24 round wells (Thermo Fisher Scientific, Roskilde, Denmark). Cultured tissues were stored in a freezer at –80°C until RNA extraction.

RNA extraction and quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissues homogenized using BioMasher® (Nippi, Tokyo, Japan) by NucleoSpin® RNA II (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer’s protocol. All RNA samples were stored in a freezer at –80°C. Each RNA sample’s concentration was measured by spectrophotometry (NanoDrop ND-2000, Thermo Scientific, Wilmington, DE, USA), and cDNA was synthesized from 0.2 μg of total RNA by reverse transcription using the ReverTra Ace® qPCR RT Master Mix (Toyobo Life Science, Osaka, Japan) according to the manufacturer’s protocol. PCR was run on an Astec Program Temp Control System (PC-815 or 816, Astec, Fukuoka, Japan). All cDNA samples were stored in a freezer at –30°C. Specific primers for MX1-a, MX1B, MX2, ISG15, IDO1, IFNAR1, COPS5, IRF1, IRF2, IRF3, IFNAR2, and H2AFZ were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primer details are shown in Table 1. The relative expression levels of MX1-a, MX1B, MX2, ISG15, IFNAR1, IFNAR2, COPS5, IRF1, IRF2, IRF3, and IRF9 were assessed by qRT-PCR using a LightCycler® 480 System II (Roche Diagnostics, Basel, Switzerland) and THUNDERBIRD™ SYBR® qPCR Mix (Toyobo Life Science) added at the final concentration of each primer 0.5 μM. Thermal cycling conditions were 1 cycle at 95°C for 30 sec (denaturation), followed by 50 cycles at 95°C for 10 sec (denaturation), 55°C for 15 sec (primer annealing), and 72°C for 30 sec (extension). Relative mRNA abundance was calculated by the ΔΔCt method using the expression of H2AFZ as a reference gene.

Immunohistochemical analysis of frozen tissue slices

Frozen tissue slices were prepared and mounted on slides (Kenis, Osaka, Japan) using a Leica CM3050 S Research Cryostat (Leica Biosystems, Nussloch, Germany) and fixed in 4% (v/v) paraformaldehyde in calcium- and magnesium-free phosphate-buffered saline (PBS) (–) for 15 min. The samples were then washed three times with PBS (–) for 5 min and permeabilized by incubation with PBS (–) containing 0.2% (v/v) Triton X-100 (PBS-T) for 10 min. After three washes with PBS (–) for 5 min, the samples were blocked with 1% (w/v) BSA (Sigma) in PBS-T for 1 h. After three washes with PBS (–) for 5 min, the samples were incubated with a primary rabbit polyclonal antibody to IFNAR1 (sc-845, Santa Cruz Biotechnology, Dallas, TX, USA) or IFNAR2 (GTX105770, GeneTex, CA, USA) diluted 1:500 with Solution A of Can Get Signal Immunostain Immunoreaction Enhance (Toyobo Life Science) at 4°C overnight. The samples were washed three times with PBS (–) for 5 min, and incubated for 1 h.

Table 1. Information on primer sequences used for qRT-PCR

| Gene  | Sequence (5’-3’) | Accession No. | Product length (bp) |
|-------|-----------------|---------------|--------------------|
| MX1-a | GCCAACTAGTCAGCAGCTACATGGTC  
|       | GCTCTTGGAGGACTCCATGTC | NM_173940.2 | 139 |
| MX1B  | GTGATACTCTCAACAGTGAAGC  
|       | AAATGATGGAGGAAGCCGAC | AB_060169.1 | 94 |
| MX2   | CAGAGACGCTTCGTCGAAAG  
|       | GAGACTGTGTCTGGTTCGAC | NM_173941.2 | 113 |
| ISG15 | TGAGGAGTCTCATGGAGTCAAG  
|       | GCGTGAAGAGCCAGGACATTG | NM_174366.1 | 72 |
| IFNAR1| GGCCAAGGATTCGCAACAG  
|       | TCAAGGCGAGTCAAGGAC | NM_174552.2 | 275 |
| IFNAR2| TCATGTATCTGCGCCTGTCT  
|       | GTCGCTGCTTTACCCACAA | NM_174553.2 | 231 |
| NCPS5 | GCTCCTGACTAAGGATACACC  
|       | TTTCAGTGGCTTCGATG | NM_001192139.1 | 100 |
| IRF1  | CACTGTTGCAGCTACATGG  
|       | AAGTTGTCAGGCTCTGGT | NM_001192161.2 | 147 |
| IRF2  | GGAGCGGTGTAAGGCAAACAG  
|       | AGGAGCTGTGGTAAGGACC | NM_001205793.1 | 150 |
| IRF3  | GCTCAACGTACGGGAGTGG  
|       | TGCCCTGCTAATGGTGG | NM_001029845.3 | 116 |
| IRF9  | CAGTTCCAGGATGTGCTG  
|       | TATTACGCCAGCGCTGGA | NM_001024506.1 | 125 |
| H2AFZ | AGAGCCGTTTGCAGTTCCCG  
|       | TACTCCAGGAGTGCTGGCTG | NM_174809.2 | 116 |
with a fluorescent-conjugated secondary antibody (Alexa Fluor® 568-conjugated donkey anti-rabbit IgG antibody, A10042, Thermo Fisher Scientific, CA, USA) diluted 1:200 with Solution A of Can Get Signal Immunostain Immunoreaction Enhance (Toyobo Life Science). The samples were washed three times with PBS (–) for 5 min, covered with 10 μl of the mounting solution, Vectashield with DAPI (Vector Laboratories, Burlingame, CA, USA), and examined under a confocal microscope (Leica TCS SP5 II, Leica Biosystems).

**Statistical analysis**

All data are shown as the mean ± standard error of the mean (SEM). The significance of differences was assessed by analysis of variance (ANOVA) followed by Fisher’s protected least-significant difference (PLSD) procedure as a multiple-comparison test in the StatView statistical analysis software (version 5; SAS Institute, Cary, NC, USA). Data with P values of < 0.05 were considered significant. P values of < 0.1 were assumed to indicate a slight difference.

**Results**

Induction of ISG mRNAs in mid-stage endometrial tissues cultured with IFN-α or PUF

PUF stimulated the expression of ISGs as well as IFN-α did. The expression of MX1-a and ISG15 significantly increased in tissues cultured for 12 and 24 h with IFN-α or PUF (Fig. 1A and D; P < 0.05). The expression of MX2 significantly increased in tissues cultured for 12 and 24 h with IFN-α or for 12 h with PUF (Fig. 1C; P < 0.05). The expression of MX1B significantly increased in tissues cultured for 12 h with IFN-α or PUF (Fig. 1B; P < 0.05). The expression of all ISGs was sufficiently induced in tissues cultured for 12 h with IFN-α or PUF, not with NPUS for 12 or 24 h.

Expression of ISG mRNAs stimulated by IFN-α or PUF in endometrial tissues at each stage

In tissues cultured for 12 h, the magnitude of induction of MX1-a by PUF was significantly greater at the mid stage than at the early and late stages (Fig. 2A; P < 0.05). Additionally, the induction of MX1B by PUF and ISG15 by IFN-α or PUF was significantly stronger at the mid stage than at the late stage (Fig. 2B and D; P < 0.05). In spite of the absence of a significant difference, the expression of MX1-a under the influence of IFN-α from the mid to late stage and MX1B under the influence of PUF from the mid to early stage had a tendency to be high (Fig. 2A; P = 0.079, B; P = 0.085). No significant difference was observed in the expression of MX2 among tissues of all stages cultured for 12 h with IFN-α or PUF.
Changes in the expression of IFNAR1, IFNAR2, COPS5, IRF1, IRF2, IRF3, and IRF9 mRNAs during the estrous cycle

In noncultured tissues, IFNAR1, IFNAR2, and COPS5 mRNAs were strongly expressed at the early stage. The expression of IFNAR1 mRNA was slightly stronger at the early stage than at the mid stage (Fig. 3A; P = 0.057). The expression of IFNAR2 mRNA was significantly stronger at the early stage than at other stages (Fig. 3B; P < 0.05). The expression of COPS5 mRNA was slightly stronger at the early stage than at the late stage (Fig. 3C; P = 0.093). IRF1, IRF2, IRF3, and IRF9 mRNAs were strongly expressed at the mid stage. The expression of IRF1 and IRF2 mRNAs was slightly stronger at the mid stage than at the late stage (Fig. 3D and E; P = 0.065 and 0.055). The expression of IRF2 and IRF3 mRNAs was significantly stronger at the mid stage than at the late stage (Fig. 3E and F; P < 0.05). The expression of IRF9 mRNA was significantly stronger at the mid stage than at the early and late stages (Fig. 3G; P < 0.05).

Expression and localization of IFNAR1 and IFNAR2 proteins in endometrial tissues at each stage

IFNAR1 and IFNAR2 proteins were detected in the LE and GE in each tissue slice (Fig. 4A and Fig. 5A). The fluorescence intensities of IFNAR1 and IFNAR2 were strong at the early and mid stages and weak at the late stage. The localization of IFNAR1 in GE was clearer at the mid stage. The relative fluorescence intensities of IFNAR1 in LE and GE significantly decreased from the early to late stage (Fig. 4B and Fig. 5B; P < 0.05), whereas those of IFNAR2 in LE and GE remained stable from the early to mid stage and were slightly weaker at the late stage (Fig. 4C and Fig. 5C; P < 0.05 and P = 0.087).

Discussion

The present study demonstrated that the type I IFN response is strong at the mid stage of the estrous cycle according to the induction levels of ISG mRNAs in endometrial tissues cultured with a type I IFN. Additionally, this result was associated with the expression of IFNARs and IRFs in the endometrium. These data suggest that the endometrium at the mid stage of the estrous cycle requires specific conditions to respond to IFN-τ for pregnancy recognition between a mother and fetus in cows.

In other studies, IFN-τ has been detected in the PUF of cows, whereas other type I IFNs were not [43, 44]. Sakurai et al. [45] applied PUF as an alternative to IFN-τ and reported promotion of the processes of attachment of bovine trophoblast cells to endometrial epithelial cells, thereby mimicking the uterine environment at the peri-implantation stage in vivo. In the present study, the bovine endometrial reaction to a type I IFN was investigated by the experimental method of an explant culture with the PUF.
We initially attempted the explant culture at IFN-α concentrations of 300 IU/ml and 3000 IU/ml at the mid stage (when pregnancy recognition occurs), as reported previously for a bovine embryo culture [46], and evaluated the relative expression of ISGs (MX1-a and MX1B) in cultured tissues. Both concentrations similarly induced MX1-a and MX1B mRNA expression (Supplementary Fig. 1: online only). These results indicate the absence of significant differences in type I IFN induction levels within an antiviral activity range between 300 IU/ml and 3000 IU/ml. PUF and IFN-α both sufficiently stimulated the expression of ISGs in the tissues cultured for 12 h (Fig. 1). Accordingly, we compared the expression of ISGs in tissues cultured for 12 h with PUF or IFN-α at each stage: the early, mid, and late stages.

In tissues cultured with PUF or IFN-α, the expression of MX1-a, MX1B, and ISG15 was high at the mid stage (Fig. 2A, B and D). MX and ISG genes have been shown to suppress viral proliferation in the innate immune system [14, 47, 48]. When progesterone is dominantly produced in livestock, the suppression of immune functions in the uterus enhances the risk of uterine infection [49]. In humans, progesterone was found to suppress IFN-α-induced MxA expression in peripheral blood mononuclear cells infected with hepatitis C virus [50]. ISGs may prevent infection at the preimplantation stage.

The expression of IFNAR1 and IFNAR2 mRNAs was strong at the early stage (Fig. 3A and B). A small amount of IFN-τ is known to be produced in vitro by hatched blastocysts (days 8–10) [51]. IFN-τ-mediated signal transduction prior to the peak in IFN-τ production may be necessary for achieving a successful pregnancy. The results of the immunohistochemical analysis showed strong fluorescence intensities of IFNAR1 and IFNAR2 from the early to mid stage and their weak intensities at the late stage (Figs. 4 and 5). Furthermore, the IFNAR1 protein was clearly localized to the cytoplasm or cell membrane in GE at the mid stage; therefore, the IFNAR1 protein may be more functional at the mid stage. In addition, the expression of COPS5 decreased slightly from the early to late stage (Fig. 3C). COPS5 has been shown to stabilize IFNAR1 protein levels by regulating ubiquitination and degradation [19]. These findings suggest that IFNAR is strongly expressed at the early stage because the uterus needs to prepare to respond to IFN-τ for pregnancy recognition at the mid stage. On the other hand, in the absence of a conceptus, the IFNAR protein is degraded from the mid to late stage.

All IRF genes were strongly expressed at the mid stage (Fig. 3D–G).
IRF1 regulates DNA damage and apoptosis in addition to activating immune responses including type I IFN signaling [13, 52]. One study suggested that IFN-τ induces apoptosis in bovine endometrial cells [53]. IRF1 activates the induction of IFN-τ-stimulated genes and may regulate the apoptosis in the endometrium for uterine tissue remodeling at the peri-implantation stage. IRF2 has been shown to inhibit the type I IFN signaling pathway [13, 54]. It may also suppress excessive responses to a type I IFN in the endometrium because a successful pregnancy requires immune tolerance. IRF3 is a transcriptional regulator of IFN-α and -β in the innate immune system [13, 14]. This observation suggests that not only the production of IFN-τ but also the expression of type I IFNs such as IFN-α and -β in maternal uterine tissues is necessary for pregnancy recognition. IRF9 drives the transcription of ISGs [13, 14]. The strong expression of IRF9 at the mid stage may enable endometrial tissues to positively respond to IFN-τ, and consequently, the expression of MX1, MX1B, and ISG15 is strong at the mid stage. As described above, IRF genes may be involved in the type I IFN response for pregnancy recognition; in particular, the expression of IRF1 and IRF9 is more important than that of IFNAR because they serve as the activator and transcription factor in type I IFN signaling after binding of IFN-τ to IFNAR.

In conclusion, herein we demonstrated that the endometrium...
responds to a type I IFN specifically at the mid stage of the estrous cycle. To the best of our knowledge, this is the first study to examine stage-dependent differences in the induction of ISGs by a type I IFN in bovine endometrial tissue. The expression of IFNARs and IRFs is key to the type I IFN response in the bovine endometrium during the estrous cycle. These results suggest that the bovine endometrium prepares for pregnancy recognition via IFN-τ and easily responds to IFN-τ at the mid stage. ISGs induced by IFN-τ may play important roles in the endometrium for successful implantation, for example, protection from viral and other infections.

Fig. 5. Localization and relative fluorescence intensities of IFNAR1 and IFNAR2 in the glandular epithelium of the bovine endometrium at different estrous stages. Immunostaining images are shown at magnification ×20. The white scale bar is 250 μm. (A) The localization of IFNAR1 and IFNAR2 and merged images of DNA and each target protein in the glandular epithelium (GE) (A). NC means incubation with PBS (−) instead of the primary antibody. (B and C) The relative fluorescence intensity of the target protein toward each fluorescence intensity at the early stage, (B) IFNAR1 in GE, (C) IFNAR2 in GE. E: early stage, M: mid stage, and L: late stage. Graph data are shown as the mean ± standard error of the mean (SEM). Letters (P < 0.05) indicate significant differences among stages according to ANOVA followed by Fisher’s PLSD procedure as a multiple-comparison test. The number sign (#) indicates the tendency (P < 0.1).

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References

1. Imakawa K, Anthony RV, Kazemi M, Marotti KR, Politis HG, Roberts RM. Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophoectoderm. Nature 1987; 330: 377–379. [Medline] [CrossRef]

2. Roberts RM, Cross JC, Leaman DW. Interferons as hormones of pregnancy. Endocr Rev 1992; 13: 432–452. [Medline]

3. Roberts RM, Leaman DW, Cross JC. Role of interferons in maternal recognition of pregnancy in ruminants. Proc Soc Exp Biol Med 1992; 200: 7–18. [Medline] [CrossRef]

4. Demmers KJ, Derecka K, Flint A. Trophoblast interferon and pregnancy. Reproduction 2001; 121: 41–49. [Medline] [CrossRef]

5. Flint AP, Sheldrick EL, McCann TJ, Jones DS. Ovine oxytocin: characteristics and control of synchronous episodes of oxytocin and PGI2 alpha secretion at luteolysis in ruminants. Domest Anim Endocrinol 1990; 7: 111–124. [Medline] [CrossRef]

6. Flint AP, Lamming GE, Stewart HL, Abayasekara DR. The role of the endometrial oxytocin receptor in determining the length of the sterile oestrous cycle and ensuring maintenance of luteal function in early pregnancy in ruminants. Philos Trans R Soc Lond B Biol Sci 1994; 349: 291–304. [Medline] [CrossRef]

7. Ott TL, Yin J, Wiley AA, Kim HT, Germai-Naini B, Spencer TE, Bartol FF, Burghardt RC, Bazer FW. Effects of the estrus cycle and early pregnancy on uterine expression of Mx protein in sheep (Ovis aries). Biol Reprod 1989; 49: 764–794. [Medline] [CrossRef]

8. Hicks BA, Ettar Sj, Carnahan KG, Joyce MM, Assisi AA, Carling SJ, Kodali K, Johnson GA, Hansen TR, Miranda MA, Woods GL, Vanderwall DK, Ott TL. Expression of the uterine Mx protein in cyclic and pregnant cows, gilts, and mares. J Anim Sci 2003; 81: 1552–1561. [Medline] [CrossRef]

9. Mansouri-Attia N, Aubert J, Reinaud P, Giraud-Delville C, Taghouri G, Gallo L, Everts RE, Degrelle T, Richard C, Hue I, Yang X, Tian XC, Lewin HA, Renard JP, Sandra O. Gene expression profiles of bovine caruncular and intercaruncular endometrium at implantation. Physiol Genomics 2009; 39: 14–27. [Medline] [CrossRef]

10. Everts RE, Degrelle T, Sandra O, Delvin B, Everts RE, Degrelle T, Sandra O, Delvin B. Expression dynamics of bovine Mx genes in the endometrium and placenta during early to mid pregnancy. J Reprod Dev 2016; 62: 29–35. [Medline] [CrossRef]

11. Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994; 264: 1415–1421. [Medline] [CrossRef]

12. Taniguchi T, Ogasawara K, Takasaki A, Tanaka N, IBE family of transcription factors as regulators of host defense. Ann Rev Immunol 2001; 19: 623–655. [Medline] [CrossRef]

13. Schneider WM, Chevirote MB, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Ann Rev Immunol 2014; 32: 513–545. [Medline] [CrossRef]

14. Li J, Roberts RM. Interferon-tau and interferon-alpha interact with the same receptors in bovine endometrium. Use of a readily iodimatable form of recombinant interferon-tau for binding studies. J Biol Chem 1994; 269: 13544–13550. [Medline] [CrossRef]

15. Binelli M, Subramaniam P, Diaz T, Johnson GA, Hansen TR, Bradling L, Thatcher WW. Bovine interferon-tau stimulates the Janus kinase-signal transducer and activator of transcription pathway in bovine endometrial epithelial cells. Biol Reprod 2001; 64: 654–665. [Medline] [CrossRef]

16. Spencer TE, Sandra O, Wolf E. Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Reproduction 2008; 135: 165–179. [Medline] [CrossRef]

17. Schneider WM, Chevirote MB, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Ann Rev Immunol 2014; 32: 513–545. [Medline] [CrossRef]

18. Li J, Roberts RM. Interferon-tau and interferon-alpha interact with the same receptors in bovine endometrium. Use of a readily iodimatable form of recombinant interferon-tau for binding studies. J Biol Chem 1994; 269: 13544–13550. [Medline] [CrossRef]

19. Samuel EE, Takahashi K. Identification of the first member of a novel type I interferon family. Circulation 2000; 102: 155–160. [Medline] [CrossRef]

20. Bierzychudek P, Biery LB, Everts RE, Degrelle T, Sandra O, Delvin B, Everts RE, Degrelle T, Sandra O, Delvin B. Expression dynamics of bovine Mx genes in the endometrium and placenta during early to mid pregnancy. J Reprod Dev 2016; 62: 29–35. [Medline] [CrossRef]

21. Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994; 264: 1415–1421. [Medline] [CrossRef]

22. Taniguchi T, Ogasawara K, Takasaki A, Tanaka N, IBE family of transcription factors as regulators of host defense. Ann Rev Immunol 2001; 19: 623–655. [Medline] [CrossRef]

23. Schneider WM, Chevirote MB, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Ann Rev Immunol 2014; 32: 513–545. [Medline] [CrossRef]

24. Li J, Roberts RM. Interferon-tau and interferon-alpha interact with the same receptors in bovine endometrium. Use of a readily iodimatable form of recombinant interferon-tau for binding studies. J Biol Chem 1994; 269: 13544–13550. [Medline] [CrossRef]

25. Samuel EE, Takahashi K. Identification of the first member of a novel type I interferon family. Circulation 2000; 102: 155–160. [Medline] [CrossRef]

26. Bierzychudek P, Biery LB, Everts RE, Degrelle T, Sandra O, Delvin B, Everts RE, Degrelle T, Sandra O, Delvin B. Expression dynamics of bovine Mx genes in the endometrium and placenta during early to mid pregnancy. J Reprod Dev 2016; 62: 29–35. [Medline] [CrossRef]
44. Forde N, McGettigan PA, Mehta JP, OHara L, Mamo S, Bazer FW, Spencer TE, Lonergan P. Proteinomic analysis of uterine fluid during the pre-implantation period of pregnancy in cattle. Reproduction 2014; 147: 575–587. [Medline] [CrossRef]
45. Sakurai T, Bai H, Bai R, Arai M, Iwazawa M, Zhang J, Konno T, Godkin JD, Okuda K, Imakawa K. Coculture system that mimics in vivo attachment processes in bovine trophoblast cells. Biol Reprod 2012; 87: 60. [Medline] [CrossRef]
46. Takahashi M, Takahashi H, Hamano S, Watanabe S, Inumaru S, Geshi M, Okuda K, Yokomizo Y, Okano A. Possible role of interferon-tau on in vitro development of bovine embryos. J Reprod Dev 2003; 49: 297–305. [Medline] [CrossRef]
47. Lenschow DJ, Giannakopoulos NY, Gunn LJ, Johnston C, O’Guin AK, Schmidt RE, Levine B, Virgin HW 4th. Identification of interferon-stimulated gene 15 as an antiviral molecule during Sindbis virus infection in vivo. J Virol 2005; 79: 13974–13983. [Medline] [CrossRef]
48. Staeheli P, Horisberger MA, Haller O. Mx-dependent resistance to influenza viruses is induced by mouse interferons alpha and beta but not gamma. Virology 1984; 132: 456–461. [Medline] [CrossRef]
49. Lewis GS. Steroidal regulation of uterine resistance to bacterial infection in livestock. Reprod Biol Endocrinol 2003; 1: 117. [Medline] [CrossRef]
50. Tayel SS, Helmy AA, Ahmed R, Esmat G, Hamdi N, Abdelaziz AI. Progesterone suppresses interferon signaling by repressing TLR-7 and MxA expression in peripheral blood mononuclear cells of patients infected with hepatitis C virus. Arch Virol 2013; 158: 1755–1764. [Medline] [CrossRef]
51. Hernandez-Ledezma JJ, Sikes JD, Murphy CN, Watson AJ, Schultz GA, Roberts RM. Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. Biol Reprod 1992; 47: 374–380. [Medline] [CrossRef]
52. Tanaka N, Ishihara M, Kitagawa M, Harada H, Kimura T, Matsuyama T, Lamphier MS, Aizawa S, Mak TW, Taniguchi T. Cellular commitment to oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF-1. Cell 1994; 77: 829–839. [Medline] [CrossRef]
53. Wang B, Xiao C, Goff AK. Progesterone-modulated induction of apoptosis by interferon-tau in cultured epithelial cells of bovine endometrium. Biol Reprod 2003; 68: 673–679. [Medline] [CrossRef]
54. Whiteside ST, King P, Goodbourn S. A truncated form of the IRF-2 transcription factor has the properties of a postinduction repressor of interferon-beta gene expression. J Biol Chem 1994; 269: 27059–27065. [Medline]