NOTE *Theriogenology*

Biological Activity of Recombinant Bovine Interferon τ Produced by a Silkworm-Baculovirus Gene Expression System

Hitomi TAKAHASHI[1)]*, Makoto TSUNAZAKI[1,2)], Takashi HAMANO[1,3)], Masashi TAKAHASHI[4)], Kiyoshi OKUDA[5)], Shigeki INUMARU[6)], Akira OKANO[1)], Masaya GESHI[1)] and Makoto HIRAKO[1)]

[1)]*Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, 2 Ikenodai, Tsukuba, Ibaraki 305–0901, Japan*
[2)]*Ehime Research Institute of Agriculture, Forestry an Fisheries, 7–156 Ashimo, Nomura, Iyo, Ehime 797–1211, Japan*
[3)]*Livestock Technology Promotion Center, 695 Yamamoto, Hino, Gamou, Shiga 529–1651, Japan*
[4)]*Livestock and Grassland Research Division, NARO Kyushu Okinawa Agricultural Research Center, Suya 2421, Koshi, Kumamoto 861–1192, Japan*
[5)]*Laboratory of Reproductive Endocrinology, Graduate School of Natural Science and Technology, Okayama University, 3–1–1, Tsushimanaka, Okayama, Okayama 700–8530, Japan*
[6)]*National Institute of Animal Health, Kannondai, Tsukuba, Ibaraki 305–0856, Japan*

(Received 11 September 2012/Accepted 28 October 2013/Published online in J-STAGE 11 November 2013)

ABSTRACT. Bovine interferon (bIFN) τ plays a crucial role in maternal-fetal recognition and was expressed using a *Bombyx mori* (Bm) nuclear polyhedrosis virus (silkworm baculovirus) gene expression system. The biological effects of Bm-recombinant bIFNτ (rbIFNτ) on prostaglandin (PG) $F_{2\alpha}$ synthesis were investigated in cultured bovine endometrial epithelial cells with oxytocin (OT, 100 nM) and on the *in vitro* development of bovine embryos. Bm-rbIFNτ and OT were shown to suppress $PGF_{2\alpha}$ production in a dose-dependent manner. When *in vitro* produced morula stage embryos were cultured for 72 hr in modified CR1aa medium supplemented with or without rbIFNτ, Bm-rbIFNτ (10 *n*g/m*l*) significantly promoted development to the expanded blastocyst stage. In conclusion, Bm-rbIFNτ was suggested to have the same bioactivity as native IFNτ.

KEY WORDS: bioactivity, interferon τ, maternal-fetal recognition, recombinant, silkworm baculovirus.

doi: 10.1292/jvms.12-0403; *J. Vet. Med. Sci.* 76(3): 447–451, 2014

It is well known that interferon (IFN) τ derived from trophoblastic cells plays an important role in maternal pregnancy recognition in ruminants [2, 3, 19, 29]. IFNτ expression is apparently restricted to ruminant ungulates, in which it serves as a signal for maternal recognition of conceptus before implantation. IFNτ binds to receptors (type I IFN receptor; IFNRI) on the uterine endometrium and suppresses transcription of the estrogen and oxytocin receptors genes to block pulsatile release of prostaglandin (PG) $F_{2\alpha}$. Furthermore, it has been demonstrated that IFNτ inhibits $PGF_{2\alpha}$ synthesis by cultured endometrial epithelial cells [7, 11, 33, 38]. This allows for maintenance of corpus luteum function and the continuous production of progesterone [2, 3, 20]. In addition, IFNRI expression has been found at earlier stages in ruminant conceptuses [14, 34], which suggests a possible role of IFNτ via IFNRI in an autocrine manner [34].

In recent years, recombinant IFNτ (rIFNτ) has been produced using bacteria or yeast gene expression systems [3, 30]. The baculovirus expression system is a popular and effective method for the large-scale production of vertebrate gene products, because it can express large quantities of vertebrate proteins with appropriate post-translational modifications [16]. The two common baculovirus gene expression systems use *Autographa californica* (Ac) nuclear polyhedrosis virus (NPV) with insect culture cells as the host and *Bombyx mori* (Bm) NPV with silkworm larvae as the hosts. The advantage of the AcNPV-insect cell culture system is the absence of serum protein contamination in the culture fluids. Since the cells can be cultured in serum-free media, protein purification is uncomplicated and accumulated in culture fluids. By contrast, the advantage of the silkworm-BmNPV system is its high expression efficiency and low feeding cost [18, 23].

It is reported that recombinant bovine IFNτ (rbIFNτ) can be expressed using baculovirus gene expression systems with AcNPV [33] and BmNPV [23]. Takahashi *et al.* [33] showed that Ac-rbIFNτ (derived from AcNPV-system) can suppress the synthesis of $PGF_{2\alpha}$ by bovine endometrial epithelial cells *in vitro*. Furthermore, Takahashi *et al.* [34] indicated that Ac-rbIFNτ has a growth-promoting effect on bovine embryo development *in vitro*. Nagaya *et al.* [23] established a procedure for the large-scale purification of bIFNτ using a silkworm-BmNPV gene expression system; however, the biological activity of BmNPV-rbIFNτ has not been reported. The long-term goal of these studies is to use rbIFNτ for improvement of the pregnancy rate in cows. Therefore, the

*CORRESPONDENCE TO: TAKAHASHI, H., Animal Breeding and Reproduction Research Division, NARO Institute of Livestock and Grassland Science, Tsukuba, Ibaraki 305–0901, Japan.
e-mail: orca@affrc.go.jp

present study investigated the effect of rbIFNτ derived from silkworm-Baculovirus gene expression system on the synthesis of $PGF_{2\alpha}$ in cultured endometrial epithelial cells and on the *in vitro* development of bovine embryos.

In this study, rbIFNτ was produced using the baculovirus gene expression system with BmNPV and silkworm larvae as the hosts (Bm-rbIFNτ, a gift from Dr. Hidekazu Nagaya, Sysmex Co., Ltd., Saitama, Japan) [23]. Protein purity was estimated to be>90% based on Coomassie-stained SDS-PAGE analysis. The Bm-rbIFNτ maintained a constant antiviral activity (2.62×10^9 IU/mg protein) throughout the study.

Apparently healthy uteri were obtained at a local slaughterhouse in accordance with protocols approved by the local institutional animal care and use committee. Endometrial epithelial cells were collected from bovine uteri on days 5 to 10 (day 0 =day of ovulation) of the estrous cycle. The stage of the estrous cycle was estimated by macroscopic observation of ovaries and uteri, as previously described [36]. Isolation and culture of bovine endometrial epithelial cells were carried out using the previous methods [32]. The epithelial cells were then plated at 2×10^5 cells per well in 24-well culture dishes (Becton Dickinson, Franklin Lakes, NJ, U.S.A.) coated with collagen (Cell matrix Type I-A, Nitta Gelatin Inc., Osaka, Japan) and cultured in Dulbecco's Modified Eagle's Medium and Ham's F-12, 1:1 (v:v; D 2906, Sigma, St. Louis, MO, U.S.A.), supplemented with insulin (10 μg/m*l*, Sigma), transferrin (10 μg/m*l*, Sigma), sodium selenite (25 nM, Sigma), hydrocortisone (100 *n*g/m*l*, Sigma), retinol (10 *n*g/m*l*, Sigma), L-ascorbic acid phosphate magnesium salt (100 μM, Wako, Osaka, Japan), penicillin (100 IU/m*l*, Sigma) and streptomycin (100 μg/m*l*, Sigma) at 38.5°C in a humidified atomosphere of 5% CO_2 in air. Culture media were changed every 2 to 3 days.

After reaching confluency, epithelial cells were used for experiments, at which time the medium was replaced with fresh medium. Increasing doses of Bm-rbIFNτ (0, 1, 10, 100 and 1,000 *n*g/m*l*) were added to cultured media with oxytocin (OT, 100 *n*M, Peptide Institute Inc., Osaka, Japan) to assess $PGF_{2\alpha}$ secretion from the cells. Control group was cultured without Bm-rbIFNτ nor OT. The dose of OT (100 *n*M) was chosen to ensure saturation of OT receptors [15]. After 24 hr of culture, 500 μl of each culture medium was collected into 1.5-m*l* tubes, centrifuged (130 × *g* for 10 sec) with 5 μl of stabilizer (0.3 M EDTA, 1% aspirin [Sigma]; pH 7.3) and stored at −20°C until used in the $PGF_{2\alpha}$ assay.

The concentration of $PGF_{2\alpha}$ in the culture medium was directly determined using a double-antibody enzyme immunoassay modified from a method previously described [21] using peroxidase-labeled $PGF_{2\alpha}$ as a tracer and anti-$PGF_{2\alpha}$ serum (1:15,000 final dilution; Millipore, Billerica. MA, U.S.A.). The $PGF_{2\alpha}$ standard curve ranged from 15.6 to 4,000 *p*g/m*l*, and the ED50 of the assay was 250 *p*g/m*l*. The intra- and interassay coefficients of variation were 6.2% (n=9) and 10.6% (n=9), respectively.

In vitro maturation and fertilization were performed as described by Hamano *et al.* [12]. In brief, bovine ovaries obtained at a slaughterhouse were transported to the laboratory in sterile saline at 37°C. Cumulus-oocyte complexes (COCs) were aspirated from follicles, 2 to 5 mm in diameter, with an 18-gauge needle attached to a 5-m*l* syringe. COCs were washed twice in TCM-199 (Life Technologies, Grand Island, NY, U.S.A.) containing 20 mM HEPES (HEPES M-199) supplemented with 5% (v:v) fetal calf serum (FCS, Filtron Pty. Ltd., Brooklyn, Australia) and then placed into 0.5-m*l* drops of HEPES M-199 containing 5% FCS and antibiotics in a 35-mm petri dish (Becton Dickinson). The drops were covered with liquid paraffin (Sigma) and cultured for 20 to 21 hr at 38.5°C in a humidified atmosphere of 5% CO_2 in air.

Sperm capacitation was carried out as described by Parrish *et al.* [27]. Semen taken from a Japanese Black bull previously frozen and stored in a 0.5-m*l* straw was thawed at 37°C. Semen was suspended in 10 m*l* BO solution [4] containing 5 mM caffeine (Sigma). After washing twice with centrifugation for 5 min at 800 × *g*, the concentration of spermatozoa was adjusted to 2×10^7 cells/m*l*. The sperm suspension was then diluted two-fold with BO solution containing 10 mg/m*l* BSA (Fraction V, Sigma) and 5 IU/m*l* heparin (Novo-heparin, Novo Nordisk A/S, Bagsvaerd, Denmark). After 20 to 22 hr of maturation, COCs were washed twice in BO solution and then placed into 0.5-m*l* drops of sperm suspension. Insemination was carried out for 5 hr at 38.5°C in a humidified atmosphere of 5% CO_2 in air.

After insemination, oocytes were denuded by repeated aspiration, and cumulus denuded oocytes were placed in fresh TCM-199 modified as for *in vitro* maturation. One-cell embryos were cultured in CR1 medium [31] supplemented with essential and non-essential amino acids (CR1aa; Sigma) and 3 mg/m*l* BSA at 38.5°C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2. After 5 days of culture from the day of insemination, embryos that had developed to the morula stage were collected and used for experiments.

Experiments were designed using morula stage embryos [13] before blastulation. Each morula stage embryo was cultured in 10-μl drops of CR1aa containing 3 mg/m*l* BSA supplemented with 1, 10 and 100 *n*g/m*l* or without (0 *n*g/m*l*, as a control) Bm-rbIFNτ at 38.5°C in a humidified atmosphere of 5% CO_2 in air. After 3 days of culture, the rates of embryos having developed to the expanded blastocyst stage were recorded with a stereoscopic microscope.

All data are shown as the mean ± SEM of the values obtained from five or six separate experiments. For the statistical analyses of differences in $PGF_{2\alpha}$ secretion, the percentages relative to the control were used. Statistical significance of the differences compared to treatment with 100 nM OT by ANOVA with Fisher's PLSD test (StatView; Abacus Concepts Inc., Berkeley, CA, U.S.A.).

In the present study, $PGF_{2\alpha}$ secretion by cultured epithelial cells was stimulated by OT, and the increase (to a level 2.45 times that in the control) was as great as reported in previous studies [32, 33]. Bm-rbIFNτ was shown to suppress the secretion of $PGF_{2\alpha}$ from cultured epithelial cells in a dose-dependent manner, and all concentrations (1 to 1,000 *n*g/m*l*) of Bm-rbIFNτ significantly ($P<0.05$) suppressed OT-induced secretion of $PGF_{2\alpha}$ (Fig. 1).

The effect of Bm-rbIFNτ on bovine embryonic develop-

Table 1. Effect of rbIFNτ derived from the *Bombyx mori* (Bm) nuclear polyhedrosis virus gene expression system on *in vitro* development of bovine embryos from morula stage to expanded blastocyst stage

Concentration of Bm-rbIFNτ (*n*g/m*l*)	No. of replications	No. of morulae cultured	% of expanded blastocyst (Mean ± SEM)
0	5	120	60.8 ± 4.4
1	5	126	69.0 ± 5.8
10	5	119	75.7 ± 5.6*
100	5	125	63.8 ± 4.5

Asterisk indicates a significant difference (P<0.05) compared with the control group (Bm-rbIFNτ=0).

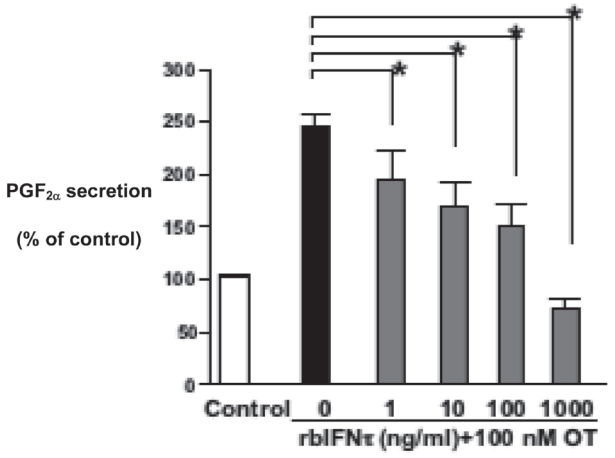

Fig. 1. Effect of rbIFNτ derived from the *Bombyx mori* nuclear polyhedrosis virus gene expression system on oxytocin (OT)-induced $PGF_{2\alpha}$ secretion from cultured bovine endometrial epithelial cells. All data are shown as the mean ± SEM of the values obtained from six separate experiments, each performed in duplicate. Asterisks indicate significant differences (P<0.05) compared to treatment with 100 nM OT only by the ANOVA-Fisher's PLSD test.

ment is shown in Table 1. Embryos that developed from the morula stage to expanded blastocyst stage were significantly promoted when embryos were cultured in CR1aa supplemented with 10 *n*g/m*l* Bm-rbIFNτ (75.7 ± 5.6%) compared with the control group (0 *n*g/m*l*, 60.8 ± 4.4%, P<0.05). Supplementation with BSA did not affect the embryonic development after the addition of Bm-rbIFNτ.

In this study, it was demonstrated that Bm-rbIFNτ derived from a silkworm- baculovirus gene expression system exhibited the characteristic bioactivity of native IFNτ [28]. The bioactivity of Bm-rbIFNτ was verified by the suppression of $PGF_{2\alpha}$ production from cultured bovine endometrial epithelial cells. This confirms that Bm-rbIFNτ is comparable to recombinant IFNτ produced in other systems [5, 6, 8, 17, 25].

It is well known that multiple forms of IFNτ are produced during early pregnancy. In bovine, 12 different polymorphic alleles (grouping to 1a-3b) exist in the genome [1, 9, 10]. Different bovine IFNτ proteins exhibit distinct differences in their ability to regulate PGs in endometrial epithelium cultures [26]. For the construction of a Bm-rbIFNτ expression in this study, bovine IFNτ cDNA originated in the identical sequence of Ac-rbIFNτ, as reported by Takahashi *et al.* [33]. The cDNA sequence can be classified into the 1a group based on phylogenetic analysis of nucleotide and amino acid differences [35]. This isoform of bovine IFNτ (1a; Ac-rbIFNτ and Bm-rbIFNτ) inhibited PG synthesis at low doses and stimulated PG synthesis concomitant with COX-2 induction at high concentrations [24, 26]. Consistent with previous reports [24, 26, 33], this study showed that low concentrations (1 to 100 *n*g/m*l*) of Bm-rbIFNτ significantly suppressed OT-induced secretion of $PGF_{2\alpha}$.

As IFNs generally possess antiproliferative activity, IFNτ may act in an autocrine manner as an antiproliferative agent to control trophoblast over-growth [14]. However, Takahashi *et al.* [33] indicated that appropriate concentration range of rbIFNτ promoted embryo development *in vitro*. Ac-rbIFNτ significantly promoted embryo development at a concentration of 100 *n*g/m*l* [33], but no significant difference was found in the growth rates between control (0 *n*g/m*l*) and high concentration groups (200 *n*g/m*l*) (our unpublished data). Similarly, in this study, Bm-rbIFNτ significantly promoted *in vitro* embryo development at a concentration of 10 *n*g/m*l*, whereas there was no significant difference in the growth rates between control and high concentration groups (100 *n*g/m*l*). These observations suggest that an appropriate concentration range of rbIFNτ acts on embryo development in an autocrine manner.

The baculovirus expression system is a suitable method for large-scale production of vertebrate gene products. Murakami *et al.* [22] and Wu *et al.* [37] reported the expression of bovine and equine IFNγ as fully functional recombinant proteins in both AcNPV and BmNPV baculovirus gene expression systems. The present study demonstrated that the bioactivity of Bm-rbIFNτ was similar to that of other rbIFNτ produced by the AcNPV baculovirus gene expression system. Interestingly, this study confirmed that Bm-rbIFNτ exerted its bioactivity at tenfold lower concentration than previously reported in Ac-rbIFNτ [33]. One possible explanation may be attributed to the different antiviral activity of these recombinant proteins. The antiviral activities of Bm-rbIFNτ and Ac-rbIFNτ are 2.62×10^9 IU/mg protein and 1.0×10^8 IU/mg protein [33], respectively. These values might reflect the bioactivities of the Bm- and Ac-rbIFNτs on the inhibition of $PGF_{2\alpha}$ secretion and the promotion of embryo

development, although the reason of the difference has not been clearly demonstrated.

In conclusion, Bm-rbIFNτ derived from a silkworm-baculovirus gene expression system possesses appropriate bioactivity for suppression of $PGF_{2\alpha}$ synthesis in cultured bovine endometrial epithelial cells and promotion of *in vitro* bovine embryo develoment. The low cost procedures and techniques for mass production of purified Bm-rbIFNτ established in the current study [23] will allow it to be readily available for *in vivo* animal experiments using cattle as a model for detailed studies on maternal pregnancy recognition. Furthermore, it should also help to improve pregnancy rates in cows.

ACKNOWLEDGMENTS. The authors thank the Ibaraki Prefectural Northern District Meat Inspection Office for their assistance. We also thank Dr. H. Nagaya of the Protein Development Center, Sysmex Co., Ltd., Saitama, Japan, for his supply of recombinant bovine IFNτ. This research was supported in part by Grants-in-Aid for Scientific Research from the Japanese Ministry of Agriculture, Forestry and Fisheries (RCP2002-4210) and JSPS (16658110) to HT.

REFERENCES

1. Alexenko, A. P., Ealy, A. D., Bixby, J. A. and Roberts, R. M. 2000. A classification for the interferon-τ. *J. Interferon Cytokine Res.* **20**: 817–822. [Medline] [CrossRef]
2. Bazer, F. W., Spencer, T. E. and Ott, T. L. 1996. Placental interferons. *Am. J. Reprod. Immunol.* **35**: 297–308. [Medline] [CrossRef]
3. Bazer, F. W., Thatcher, W. W., Hansen, P. J., Mirando, M. A., Ott, T. L. and Plante, C. 1991. Physiological mechanisms of pregnancy recognition in ruminants. *J. Reprod. Fertil. Suppl.* **43**: 39–47. [Medline]
4. Brackett, B. G. and Oliphant, G. 1975. Capacitation of rabbit spermatozoa in vitro. *Biol. Reprod.* **12**: 260–274. [Medline] [CrossRef]
5. Cerutti, M., Hue, D., Charlier, M., L'Haridon, R., Pernollet, J. C., Devauchelle, G. and Gaye, P. 1991. Expression of a biologically active ovine trophoblastic interferon using a baculovirus expression system. *Biochem. Biophys. Res. Commun.* **181**: 443–448. [Medline] [CrossRef]
6. Charlier, M., Hue, D., Martal, J. and Gaye, P. 1989. Cloning and expression of cDNA encoding ovine trophoblastin: Its identity with a class-II alpha interferon. *Gene* **77**: 341–348. [Medline] [CrossRef]
7. Danet-Desnoyers, G., Wetzels, C. and Thatcher, W. W. 1994. Natural and recombinant bovine interferon τ regulate basal and oxytocin-induced secretion of prostaglandins $F_{2\alpha}$ and E_2 by epithelial cells and stromal cells in the endometrium. *Reprod. Fertil. Dev.* **6**: 193–202. [Medline] [CrossRef]
8. Degryse, E., Dietrich, M., Nguyen, M., Archstetter, T., Charlier, M., Charpigny, G., Gaye, P. and Martal, J. 1992. Addition of dipeptide spacer significantly improves secretion of ovine trophoblast interferon in yeast. *Gene* **118**: 47–53. [Medline] [CrossRef]
9. Ealy, A. D., Pennington, K. A. and Rodina, T. M. 2006. Interferon-tau polymorphisms and their potential functions in ruminants. *Ann. Rev. Biomed. Sci.* **8**: 9–18.
10. Ealy, A. D., Wagner, S. K., Sheils, A. E., Whitley, N. C., Kiesling, D. O., Johnson, S. E. and Barbato, G. F. 2004. Identification of interferon-tau isoforms expressed by the peri-implantation goat (Capra hircus) conceptus. *Domest. Anim. Endocrinol.* **27**: 39–49. [Medline] [CrossRef]
11. Godkin, J. D., Smith, S. E., Johnson, R. D. and Dore, J. J. 1997. The role of trophoblast interferons in the maintenance of early pregnancy in ruminants. *Am. J. Reprod. Immunol.* **37**: 137–143. [Medline] [CrossRef]
12. Hamano, S., Kuwayama, M., Takahashi, M., Okamura, N., Okano, A. and Nagai, T. 1994. Effect of β-mercaptoethanol on the preimplantation development of bovine embryos fertilized in vitro. *J. Reprod. Dev.* **40**: 355–359. [CrossRef]
13. Hernandez-Ledezma, J. J., Sikes, J. D., Murphy, C. N., Watson, A. J., Schultz, G. A. and Roberts, R. M. 1992. Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. *Biol. Reprod.* **47**: 374–380. [Medline] [CrossRef]
14. Imakawa, K., Tamura, K., Lee, R. S., Ji, Y., Kogo, H., Sakai, S. and Christenson, R. K. 2002. Temporal expression of type I interferon receptor in the peri-implantation ovine extra-embryonic membranes: demonstration that human IFN α can bind to this receptor. *Endocr. J.* **49**: 195–205. [Medline] [CrossRef]
15. Kim, J. J. and Fortier, M. A. 1995. Cell type specificity and protein kinase C dependency on the stimulation of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ production by oxytocin and platelet-activating factor in bovine endometrial cells. The use of baculoviruses as expression vectors. *J. Reprod. Fertil.* **103**: 239–247. [Medline] [CrossRef]
16. Kidd, I. M. and Emery, V. C. 1993. The use of baculoviruses as expression vectors. *Appl. Biochem. Biotechnol.* **42**: 137–159. [Medline] [CrossRef]
17. Klemann, S. W., Li, J. Z., Imakawa, K., Cross, J. C., Francis, H. and Roberts, R. M. 1990. The production, purification, and bioactivity of recombinant bovine trophoblast protein-1 (bovine trophoblast interferon). *Mol. Endocrinol.* **4**: 1506–1514. [Medline] [CrossRef]
18. Kron, R., Schneider, C., Hotten, G. R., Bechotold, R. and Pohl, J. 1998. Expression of human activin C protein in insect larvae infected with a recombinant baculovirus. *J. Virol. Methods* **72**: 9–14. [Medline] [CrossRef]
19. Martal, J. L., Chene, N. M., Huynh, L. P., L'Haridon, R. M., Reinaud, P. B., Guillomot, M. W., Charlier, M. A. and Charpigny, S. Y. 1998. IFN-tau: a novel subtype I IFN1. Structural characteristics, non-ubiquitous expression, structure-function relationships, a pregnancy hormonal embryonic signal and cross-species therapeutic potentialities. *Biochimie* **80**: 755–777. [Medline] [CrossRef]
20. Meyer, M. D., Hansen, P. J., Thatcher, W. W., Drost, M. and Roberts, R. M. 1995. Effect of bovine interferon-τ on body temperature and plasma progesterone concentrations in cyclic dairy cows. *J. Dairy Sci.* **78**: 1470–1476. [Medline] [CrossRef]
21. Miyamoto, Y., Skarzynski, D. J. and Okuda, K. 2000. Is tumor necrosis factor α trigger for the initiation of endometrial prostaglandin $F_{2\alpha}$ release at luteolysis in cattle? *Biol. Reprod.* **62**: 1109–1115. [Medline] [CrossRef]
22. Murakami, K., Uchiyama, A., Kokuho, T., Mori, Y., Sentsui, H., Yada, T., Tanigawa, M., Kuwano, A., Nagaya, H., Ishiyama, S., Kaki, H., Yokomizo, Y. and Inumaru, S. 2001. Production of biologically active recombinant bovine interferon-gamma by two different baculovirus gene expression systems using insect cells and silkworm larvae. *Cytokine* **13**: 18–24. [Medline] [CrossRef]
23. Nagaya, H., Kanaya, T., Kaki, H., Tobita, Y., Takahashi, M., Takahashi, H., Yokomizo, Y. and Inumaru, S. 2004. Establishment of a large-scale purification procedure for purified recom-

binant bovine interferon-τ produced by a silkworm-baculovirus gene expression system. *J. Vet. Med. Sci.* **66**: 1395–1401. [Medline] [CrossRef]
24. Okuda, K., Kasahara, Y., Murakami, S., Takahashi, H., Woclawek-Potocka, I. and Skarzynski, D. J. 2004. Interferon-tau blocks the stimulatory effect of tumor necrosis factor-α on prostaglandin $F_{2\alpha}$ synthesis by bovine endometrial stromal cells. *Biol. Reprod.* **70**: 191–197. [Medline] [CrossRef]
25. Ott, T. L., Von Heeke, G., Jhonson, H. M. and Bazer, F. W. 1991. Cloning and expression in Saccharomyces cerevisiae of a synthetic gene for the type-I trophoblast interferon ovine trophoblast protein-1; Purification and antiviral activity. *J. Interferon Res.* **11**: 357–364. [Medline] [CrossRef]
26. Parent, J., Villeneuve, C., Alexenko, A. P., Ealy, A. D. and Fortier, M. A. 2003. Influence of different isoforms of recombinant trophoblastic interferons on prostaglandin production in cultured bovine endometrial cells. *Biol. Reprod.* **68**: 1035–1043. [Medline] [CrossRef]
27. Parrish, J. J., Susko-Parrish, J. L., Leibfried-Rutledge, M. L., Crister, E., Eystone, W. H. and First, N. L. 1986. Bovine in vitro fertilization with frozen thawed semen. *Theriogenology* **25**: 591–600. [Medline] [CrossRef]
28. Plante, C., Hansen, P. J., Thatcher, W. W., Johnson, J. W., Pollard, J. W., Mirando, M. A. and Bazer, F. W. 1990. Purification of bovine trophoblast protein-1 complex and quantification of its microheterogeneous variants as affected by culture conditions. *J. Reprod. Immunol.* **18**: 271–291. [Medline] [CrossRef]
29. Pontzer, C. H., Bazer, F. W. and Johnson, H. M. 1991. Antiproliferative activity of a pregnancy recognition hormone, ovine trophoblast protein-1. *Cancer Res.* **51**: 5304–5307. [Medline]
30. Roberts, R. M., Klemann, S. W., Leaman, D. W., Bixby, J. A., Cross, J. C., Farin, C. E., Imakawa, K. and Hansen, T. R. 1991. The polypeptides and genes for ovine and bovine trophoblast protein-1. *J. Reprod. Fertil. Suppl.* **43**: 3–12. [Medline]
31. Rosenkrans, C. F. J., Zeng, G. Q., McNamara, G. T., Schoff, P. K. and First, N. L. 1993. Development of bovine embryos in vitro as affected by energy substrates. *Biol. Reprod.* **49**: 459–462. [Medline] [CrossRef]
32. Takahashi, H., Iga, K., Sato, T., Takahashi, M. and Okano, A. 2001. Isolation and culture of bovine endometrial epithelial cells in a seurm-free culture system. *J. Reprod. Dev.* **47**: 181–187. [CrossRef]
33. Takahashi, H., Inumaru, S., Takahashi, M., Watanabe, S., Iga, K., Yokomizo, Y., Geshi, M., Okano, A. and Okuda, K. 2003. Biological activity of recombinant bovine interferon τ using an Autographa californica nuclear polyhedrosis baculovirus expression system. *J. Reprod. Dev.* **49**: 433–440. [Medline] [CrossRef]
34. Takahashi, M., Takahashi, H., Hamano, S., Watanabe, S., Inumaru, S., Geshi, M., Okuda, K., Yokomizo, Y. and Akira, O. 2003. Possible role of interferon-τ on *in vitro* development of bovine embryos. *J. Reprod. Dev.* **49**: 297–305. [Medline] [CrossRef]
35. Waterman, M. S. 1986. Multiple sequence alignment by consensus. *Nucleic Acids Res.* **14**: 9095–9102. [Medline] [CrossRef]
36. Woclawek-Potocka, I., Okuda, K., Acosta, T. J., Korzekwa, A., Pilawski, W. and Skarzynski, D. J. 2005. Phytoestrogen metabolites are much more active than phytoestrogens themselves in increasing prostaglandin $F_{2\alpha}$ synthesis via prostaglanin $F_{2\alpha}$ synthase-like 2 stimulation in bovine endometrium. *Prostaglandins Other Lipid Mediators* **78**: 202–217. [Medline] [CrossRef]
37. Wu, D., Murakami, K., Liu, N., Inoshima, Y., Yokoyama, T., Kokuho, T., Inumaru, S., Matsumura, T., Kondo, T., Nakano, K. and Sentsui, H. 2002. Expression of biologically active recombinant equine interferon-γ by two different baculovirus gene expression systems using insect cells and Silkworm Larvae. *Cytokine* **20**: 63–69. [Medline] [CrossRef]
38. Xiao, C. W., Liu, J. M., Sirois, J. and Goff, A. K. 1998. Regulation of cyclooxygenase-2 and prostaglandin F synthase gene expression by steroid hormones and interferon-τ in bovine endometrial cells. *Endocrinology* **139**: 2293–2299. [Medline] [CrossRef]