INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI®

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DISSERTATION

CLONING BOVINE INTERFERON-TAU GENES AND CHARACTERIZING THEIR TRANSCRIPTIONAL EXPRESSION DURING EARLY PREGNANCY

Submitted by

Young Gie Chung

Department of Physiology

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2000

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

UMI Number: 9981326

UMI®

UMI Microform 9981326
Copyright 2000 by Bell & Howell Information and Learning Company.
All rights reserved. This microform edition is protected against unauthorized copying under Title 17, United States Code.

Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

COLORADO STATE UNIVERSITY

December 10, 1999

WE HERBY RECOMMENDED THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY YOUNG GIE CHUNG ENTITLED: "CLONING BOVINE INTERFERON-TAU GENES AND CHARACTERIZING THEIR TRANSCRIPTIONAL EXPRESSION DURING EARLY PREGNANCY" BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Committee on Graduate Work

Jonathan Carlson

R A Bowen

Colin M. Clay

D E Seidel, Jr.

Advisor

Alan Tucker

Department Head

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABSTRACT OF DISSERTATION

CLONING BOVINE INTERFERON-TAU GENES AND CHARACTERIZING THEIR TRANSCRIPTIONAL EXPRESSION DURING EARLY PREGNANCY

The interferon-tau (IFN-τ) are major cytokines that play a pivotal role in maternal recognition of pregnancy in ruminants. They block the luteolytic secretion of prostaglandin $F_2\alpha$, thereby extending the functional life span of corpus luteum, allowing pregnancy to proceed. In spite of ample evidence indicating that these bovine IFN-τ(bIFN-τ) are encoded by multicopy genes, only a few promoters had been cloned and characterized to any extent. Therefore, several different bIFN-τ genes were cloned, and their expression patterns were analyzed during early pregnancy in cattle.

Screening a bovine genomic library resulted in four putative clones: sequencing revealed that one of them was closely related to bIFN-ω, and the rest were bIFN-τ genes. All of the clones lacked premature stop codons in coding regions. The newly cloned bIFN-τ contained approximately 2.3 kilobase (kb) of promoter region, 1.0 kb of coding region, and the first putative poly (A) signal, irrespective of subtype. Substantial sequence differences were found among the subtypes, confirming the multiplicity of bIFN-τ genes. Mismatches in the coding region which lead to amino acid differences were also notable indicating that the biological properties of different subtype gene products may be different. Most severe mismatches were found in 5' ends of promoters, which had not been cloned before; thus, these regions were subjected to further study.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

To study the expression pattern of each subtype gene, RNase protection assays (RPA) were applied to various developmental stages of bovine embryos. Overall transcriptional expression dropped markedly between day 16 and 25 regardless of subtype. However, one of the new clones, IFN-τb1 showed minimal expression compared to other clones. This particular clone showed extreme mismatching in first 500 base pairs of the 5' end, suggesting that this region may be an enhancer. In addition, a polymorphism was observed in one new clone, IFN-τb2 in an RPA using different embryos. These results indicated that bIFN-τ subtype genes are not expressed equally.

The 500 bp gene fragment from a highly expressed subtype was further studied to test whether it functioned as a tissue specific enhancer. An in vitro transcription assay utilizing various cell nuclei and DNA templates revealed the 500 bp fragment increased transcription in vitro approximately 2-fold irrespective of DNA constructs and nuclei sources, suggesting that it works as a general, rather than a tissue specific enhancer. Together, these data support the hypotheses that the bIFN-τ are encoded by several subtype genes with substantial sequence differences in both promoter and coding regions, resulting in differential expression during early embryonic development, and different biological potencies of the gene products.

Young Gie Chung
Department of Physiology
Colorado State University
Fort Collins, Colorado 80523
Spring 2000

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Dr. George E. Seidel, Jr., for his patience, guidance, and support over the past several years. I will miss both his sharp wit and thorough understanding of the theories and limitations of many fields of science.

I would also like to extend my gratitude to Dr. Richard Bowen and Dr. Colin Clay. They always have welcomed me into their laboratories providing me direct inputs into the progression of my research. Appreciation is also given to Dr. Jonathan Carlson for his knowledge and input.

I would also like to acknowledge the important contributions of the members of ARBL. I cherish the friendship that that I have developed with each of them: Paul Gordy for his gracious help and advice; Scott Nelson, for his scientific insight and help; Alexandra Delille, for her help and friendship; and Zella Brink, Shari Olson, Maddy Keller, Michelle Glover, Young Ho Choi, and TaeKwang Suh for their technical assistance and providing many resources that I needed to finish this project.

I would especially like to extend my sincere gratitude to my dear wife, Eun Ja Chung. She has loved me through this endeavor. I would like to thank my family for their endless support and patience.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DEDICATION

This dissertation is dedicated to my late parents, Byung Sun Chung and Guinim Yeu. Their endless love and support have made the rough times bearable. I could not have made it without you.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE OF CONTENTS

ABSTRACT iii

CHAPTER I: REVIEW OF THE LITERATURE 1

I. INTRODUCTION 1
II. Development of the Corpus Luteum 2
III. Luteolysis 5
IV. Prostaglandin $F_2\alpha$ Synthesis 8
V. Prostaglandin $F_2\alpha$ Release 9
VI. Maternal Recognition of Pregnancy in Ruminants 11
A. Interferon-τ as an Antiluteolysin 12
B. IFN-τ Expression during Development 15
C. Mode of IFN-τ Action 17
a. IFN-τ Blocks Estrogen- and Oxytocin-Receptor Up-regulation 18
b. IFN-τ Stimulates Release of PGE_2 Preferentially to $PGF_2\alpha$ in Uterine Epithelial Cells 19
c. IFN-τ Induces Expression of Several Genes that may be Needed to Establish Pregnancy 20
VI. IFN-τ Genes 21
A. Distribution of IFN-τ Genes among Mammals 21
B. Copy Number of IFN-τ Genes 22
C. Features of IFN-τ Genes 22
1. General Features of type I IFN Genes 22
The Promoter Region of Type I IFN Genes 23
2. General Features of IFN-τ Genes 24
a. Promoter Region 25
b. Transcriptional Regulation of IFN-τ Differ from Other Type I IFNs 25
c. Why are There so Many IFN-τ Genes 29
VII. Embryonic Loss 30
A. Gross Chromosomal Abnormalities are not the Major Cause of Embryo Mortality 31
B. Asynchrony between the Embryo and Oviductal and Uterine Environment 32
C. Failure of Maternal Recognition of Pregnancy 34
VIII. Clinical Use of IFN-τ 35
IX. Conclusions 37

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER II: CLONING BOVINE INTERFERON-TAU (bIFN-τ) GENES AND CHARACTERIZING THEIR EXPRESSION PATTERN DURING EARLY PREGNANCY
Abstract.....39
Introduction.....40
Materials and Methods.....44
Preparation of Probes for Southern Blotting.....44
Preparation of Probes for Ribonuclease Protection Assays.....45
Preparation of Sense RNA of bIFN-τ Subtype Genes.....46
Screening and Sequencing of a Bovine Genomic Library.....46
RNA Preparation and Ribonuclease Protection Assay.....48
Results.....49
Structural Analysis of bIFN-τ Gene Variants.....49
mRNA Abundance of Bovine Conceptus IFN-τ Variants.....56
Discussion.....60

CHAPTER III: A DISTAL 500 BP UPSTREAM REGION OF THE BOVINE INTERFERON-TAU (bIFN-τ) GENE CONTAIN ENHANCER-LIKE ACTIVITY.....66
Abstract.....66
Introduction.....67
Materials and Methods.....70
Materials.....70
Preparation of Nuclei from Bovine Embryos.....70
Cell Culture.....71
Construction of DNA Templates.....72
In Vitro Transcription Assay.....73
Measurement of Protein in In Vitro Transcription Reactions.....73
Ribonuclease Protection Assay and Quantification of Transcripts.....74
Statistical Analysis.....75
Results.....75
The 5' Distal Promoter Fragment (500 bp) of a New bIFN-τ Clone Increased Transcription.....75
The Promoter / Reporter Gene Constructs were Transcribed In a Dose Dependent Manner and Day 20 Bovine Embryo Nuclei Induced more Transcription of the bTP-sf 2.3 kb/luc Construct than Day 45 Bovine Embryo Nuclei.....77
An upstream 500 bp 5' Region of bTP-sf promoter Increased Transcription in Cells of Trophoblast and Non-trophoblast Origin.....79
Discussion.....81

CHAPTER IV: SUMMMARY.....86

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER V: REFERENCES 91

APPENDICES

Appendix 1. Sequence Alignment of bIFN-τ variants..................118
Appendix 2. Sequence Alignment of bIFN-ω variants..................123

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES

Figure 2-1. Homologies between IFN-τ Coding Regions........................52

Figure 2-2. Homologies between IFN-τ Promoter Regions........................54

Figure 2-3. Results of RPA using Total Cellular RNA from Different Developmental Stages of Bovine Embryos........................57

Figure 2-4. Results of RPA using Total Celluar RNA from various Developmental Stages of Bovine Embryos different from Fig. 2-3........................58

Figure 2-5. Differences in Amount of mRNA for bIFN-τ Variants Relative to GAPDH mRNA During Early Conceptus Development........................59

Figure 2-6. Test of Specificity of Bovine IFN-τ Subtype Probes........................60

Figure 3-1. In Vitro Transcription Using Day 16 and 20 Bovine Embryo Cell Nuclei as Transcription Factor Sources........................76

Figure 3-2. Dose Dependent Transcriptional Activities of bTP-sf and RSV Promoters in Day 20 and 45 Bovine Embryo Nucleus Preparations........................78

Figure 3-3 In Vitro Transcription Using Various Cell Nuclei and Different Size Promoters........................80

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF TABLES

Table 2-1. Alignment of Predicted Amino Acid Sequences Of Bovine IFN-τ Subtype Genes.. 51

Table 2-2. Pairwise Comparisons of Differences in Coding Sequences of bIFN-τ Variants...53

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER I

REVIEW OF THE LITERATURE

I. INTRODUCTION

Inadequate trophoblast function is thought to be a major cause of high embryonic losses during early pregnancy in domestic animals. The consequent economic losses are enormous, estimated to be as high as 1.4 billion dollars two decades ago (Gerrits et al., 1979). Thus, major efforts have been made to understand the mechanisms involved in the early embryonic losses since Short (1969) coined the phrase " maternal recognition of pregnancy". This term can be defined as the process that allows the mother to detect the presence of an embryo in her reproductive tract.

With the advent of molecular biological techniques, it became clear that IFN-τ is a biochemical signal responsible for maintenance of the corpus luteum and its functions in ruminants. IFN-τ prevents luteolysis in pregnant animals by blocking pulsatile release of $PGF_2\alpha$ from the uterine epithelium. This unusual interferon possesses most of the common characteristics of other type I interferons, including antiviral and antiproliferative activities, in addition to antiluteolytic activity (Roberts et al., 1992). IFN-τ seems to be encoded by a multicopy gene family with substantial nucleotide differences in both promoter and coding regions (Nephew et al., 1993). Unique

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

characteristics of IFN-τ genes are multiplicity, timing, magnitude, and tissue specificity of expression. IFN-τ genes are expressed transiently, for a few weeks only in trophoblast cells. IFN-τ transcripts are composed most of poly (A)-RNA, and quantities exceed those of β-actin genes during peak expression. However, several questions remain to be answered with regard to biological potency of each IFN-τ subtype and its pattern of gene expression. Although, several IFN-τ genomic clones have been available, studies on gene expression of each subtype have been limited. Therefore, the overall objective of this study was to search for and characterize new bIFN-τ genes. In particular, it was of interest to confirm the presence of several bIFN-τ genes with different nucleotide composition in both promoter and coding regions, and whether there was differential expression. The specific aims of these studies were;

1) Clone and sequence three or more bIFN-τ genes and characterize their transcriptional expression during early gestation.
2) Identify IFN-τ promoter regions involved in transcriptional regulation in the developing bovine conceptus.

II. Development of the Corpus Luteum

To understand maternal recognition of pregnancy, one must first understand corpus luteum (CL) formation and the luteolytic process. The CL is a temporary endocrine gland that secretes progesterone (P4) to support pregnancy. It develops from the ovarian follicle after ovulation (Milvae et al., 1996). Under the influence of the preovulatory surge of LH from the anterior pituitary, the mature follicle ruptures and expels the oocyte. The wall of the follicle collapses into folds, and capillaries invade the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

developing CL, probably under the influence of angiogenic and mitogenic factors (McCracken et al., 1999). In a mature CL, 4 distinct cell types predominate: parenchymal large and small steroidogenic cells, fibroblasts, and capillary endothelial cells (Sawyer, 1995).

In preovulatory follicles, granulosal cells and thecal cells produce estradiol-17β coordinately (Bao and Garverick, 1998). Thecal cells contain enzymes necessary to convert cholesterol to androgens, and granulosa cells contain enzymes necessary to convert androgens to estradiol-17β. Thus, androgens produced by thecal cells are aromatized to estradiol by granulosa cells (Bao and Garverick, 1998). Estradiol-17β stimulates division of granulosa cells (Richards and Hedin, 1988). The preovulatory surge of luteinizing hormone (LH) induces ovulation and differentiation of these two types of cells. Theca- and granulosa-derived luteal cells give rise to two distinct types of luteal cells that differ morphologically and physiologically. The cells derived predominantly from granulosa cells have been designated as large luteal cells. They contain receptors for prostaglandin-F2α and appear to mediate the luteolytic actions of this hormone. Luteal cells derived from theca cells have been designated small luteal cells, and they respond to LH with increased secretion of progesterone. Differentiation of these two cell types into luteal cells enables production of progesterone at high rates by increased expression of enzymes necessary for conversion of cholesterol to pregnenolone, i.e. the cholesterol side chain cleavage cytochrome P450 complex, and 3β-hydroxysteroid dehydrogenase / Δ^5, Δ^4 isomerase. Concomitantly, expression of the enzymes that convert progesterone to estrogens, i.e. 17α-hydroxylase cytochrome P450 and aromatase cytochrome P450, decrease (Bao and Garverick, 1998). Although both

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cell types synthesize and secrete progesterone, and possess receptors for LH, differences in basal and LH-stimulated secretion of progesterone between small and large luteal cells are quite different. Small luteal cells isolated from ovine corpora lutea during the mid-luteal phase of estrous cycle show a marked increase (10-20 fold) in progesterone secretion when incubated in the presence of LH, whereas secretion of progesterone by large luteal cells is unaffected (Niswender and Nett, 1995).

Hormones secreted by the pituitary gland play very significant roles in normal development and function of the CL. Among these hormones, luteinizing hormone (LH) seems to play a most important role in mammals (Farin et al., 1988; Farin et al., 1990; Juengel et al., 1997). Small luteal cells respond to LH with increased secretion of progesterone. The removal of pituitary on day 5 of the estrous cycle of the ewes resulted in cessation of CL growth and low concentrations of progesterone. This loss of weight and function is associated with a decreased number of small luteal cells and fibroblasts, and decreased sizes of both small and large steroidogenic luteal cells (Farin et al., 1988; Juengel et al., 1997). The decrease of progesterone appears to be associated with reduced levels of steroidogenic proteins StAR, P450scc and 3b-HSD rather than reduced uptake of lipoproteins (Juengel et al., 1995; Juengel et al., 1997). Thus, LH seems to regulate enzymes involved in steroidogenic pathways. The overall result of the decreased number and size of the steroidogenic cells is a reduced level of secreted progesterone. Moreover, LH has to be released in a pulsatile manner for normal development and function of the CL in cows, as shown by treatment with a gonadotropin releasing hormone (GnRH) antagonist, which obliterated pulsatile, but not basal, release of LH; this impaired normal function of the CL of cows (Peters et al., 1994). In addition

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to LH, growth hormone from the pituitary is required for normal growth of the CL. Replacement of LH alone enables hypophysectomized ewes to restore normal levels of mRNA encoding enzymes, which are important for progesterone synthesis, i.e. StAR, P450scc and 3β-HSD (Ravindranath et al., 1992); however, luteal weights remain lower than in control animals (Farin et al., 1988; Juengel et al., 1995; Juengel et al., 1997). Treatment of the hypophysectomized animals with GH alone restored circulating progesterone and mRNA encoding StAR and P450sec to normal levels, but not the mRNA encoding 3β-HSD (Juengel et al., 1995; Juengel et al., 1997). The same treatment increased the weight of CL to some extent, but not to the size observed in pituitary-intact animals (Juengel et al., 1995). Only when both LH and GH were replaced in hypophysectomized ewes, were all parameters of luteal functions measured increased to levels observed in pituitary-intact animals. Thus, both LH and GH are necessary for normal luteal development and function in sheep.

III. Luteolysis

Lysis or structural demise of the corpus luteum resulting in the loss of progesterone secretion is termed luteolysis (Pate, 1994). In ruminants, normal luteolysis is dependent upon the presence of uterus. Hysterectomy (Ginther, 1974; Weems et al., 1985), destruction of uterine endothelium (Ginther, 1974; Weems et al., 1985), and autotransplantation of the uterus or ovary alone to the neck with vascular anastomoses to the jugular vein or carotid artery (Goding et al., 1972) resulted in delayed luteolysis. Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is the factor secreted from the uterus that initiates luteolysis (

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hansel et al., 1973; McCracken et al., 1970) in most species including ruminants. The decline in CL progesterone secretion is associated with luteolytic episodes of $PGF_2\alpha$ release (Hansel, 1975; Harvey et al., 1984; Parkinson and Lamming, 1990; Shemesh and Kindahl et al., 1976; Wolfenson et al., 1985).

Initiation of luteolysis by $PGF_2\alpha$ in ruminants is a local effect between each uterine horn and its ipsilateral ovary as shown by the results following the removal only of the uterine horn adjacent to the ovary with the CL (Ginther, 1974; Weems et al., 1985), or congenital absence of one uterine horn ipsilateral to ovary with the CL (McCracken and Caldwell, 1969; Warfield et al., 1986).

It appears that prostaglandin $F_2\alpha$ enters the ovarian artery from the utero-ovarian vein via a countercurrent exchange mechanism (Ginther, 1974). This allows $PGF_2\alpha$ to travel to the ovarian artery without entering the pulmonary circulation where it would be enzymatically inactivated in the lungs (Piper et al., 1970).

During luteal regression, $PGF_2\alpha$ works via two pathways: decreased blood flow to the CL and induction of apoptosis of individual luteal cells. Initially, $PGF_2\alpha$ decreases luteal blood flow and steroidogenic capacity of individual luteal cells (Braden et al., 1988; Pharriss et al., 1970). $PGF_2\alpha$ seems to work via two pathways to decrease blood flow and vascular changes during luteolysis. First is a direct effect on endothelial cells which express receptors for $PGF_2\alpha$, resulting in degeneration of luteal endothelial cells (O'Shea et al., 1977; Sawyer et al., 1990), and a marked reduction in capillary density (Azmi and O'Shea, 1984; Braden et al., 1988); this reduces blood flow to the luteal parenchyma. Second, $PGF_2\alpha$ seems to work indirectly by stimulating luteal endothelial cells to produce endothelin-1, which is a potent vasoconstrictor (Huggins et al., 1993)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and inhibitor of steroidogenic activity of luteal cells (Girsh et al., 1996). In addition, endothelin-1 may reduce blood flow during early luteolysis by causing arteriole constriction (Ohtani et al., 1998), and the resulting hypoxia may cause release of additional endothelin-1 (Rakugi et al., 1990). Thus, it is no wonder that endothelial cells exhibit dramatic morphological changes after $PGF_2\alpha$ treatment earlier than any other cells in the corpus luteum (Sawyer et al., 1990). In steroidogenic cells, conspicuous morphological changes preceded by reduced steroidogenic capacity occur 24-36 hr after $PGF_2\alpha$ treatment (Sawyer et al., 1990).

For the structural demise of luteal cells, $PGF_2\alpha$ acts by binding to specific receptors localized to large steroidogenic luteal cells (Fitz et al., 1982; Juengel et al., 1996). These receptors belong to the seven-transmembrane family of G-protein coupled receptors (Abramovitz et al., 1994; Graves et al., 1995; Sakamoto et al., 1995; Sakamoto et al., 1994; Sugimoto et al., 1994). Upon binding to receptors, $PGF_2\alpha$ activates membrane-bound phospholiphase C, which catalyzes hydrolysis of phosphatidylinositol 4,5-biphoshate (PIP_2) to inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG) (Davis et al., 1989; Berridge and Irvine, 1984). Increased cytosolic concentration of IP_3 causes release of free Ca^{++} from the smooth endoplasmic reticulum to the cytoplasmic compartment (Berridge and Irvine, 1984). Increased free Ca^{++} and DAG stimulate the catalytic activity of Ca^{++}-dependent protein kinase C (PKC) (Nishizuka, 1992). Protein kinase C is believed to mediate many of the antisteroidogenic actions of $PGF_2\alpha$ as well as induction of apoptosis that is preceded by an influx of calcium and activation of endonucleases in large luteal cells (McGuire et al., 1994; Wiltbank et al., 1991; Wiltbank et al., 1990). The characteristic features of cells

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

undergoing apoptosis include nuclear fragmentation, appearance of membrane bound vesicles of cytoplasmic contents, ladder-like fragmentation of genomic DNA into 185-bp fragments [oligonucleosomes; (Arends et al., 1990)], and changes in gene expression (Kerr et al., 1972; Sawyer et al., 1990). Most of these apoptotic features are found in luteal cells which responded to PGF2α in cattle (Juengel et al., 1993; Zheng et al., 1994), sheep (McGuire et al., 1994; Sawyer et al., 1990), and other mammals (Bacci et al., 1996; Matsuyama et al., 1996; Shikone et al., 1997). Macrophages augment the apoptotic process in populations of luteal cells by phagocytosing membrane-enclosed fragments of those cells (Hehneke et al., 1994).

One of the genes involved in apoptosis is bcl-2, which codes for membrane-associated protein that prevents cell death by regulating the maintenance of Ca^{++} homeostatic mechanisms (Baffy et al., 1993), attenuating oxidative stress (Hockenbery et al., 1993), and interacting with ras (Fernandez-Sarabia and Bischoff, 1993) and bax (Korsmeyer, 1995). Bax directly promotes apotosis by binding to, sequestering and antagonizing the cell survival function of bcl-2 (Korsmeyer, 1995; Oltvai et al., 1993). Thus, the ratio of bcl-2 and bax within a cell is related to that cell's potential to become apoptotic. During luteolysis in cattle, mRNA encoding bax is elevated, while mRNA encoding bcl-2 remains unchanged (Rueda et al., 1997), resulting in an increased ratio of bax to bcl-2, an event consistent with bax-mediated apoptosis.

IV. Prostaglandin $F_2\alpha$ Synthesis

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Prostaglandins $F_2\alpha$ and E_2 are synthesized using membrane phospholipids as substrate in a three-step series of reactions termed the cyclooxygenase pathway. Plasma membrane localized phospholiphases A_2 and C hydrolyse membrane phospholipids, liberating arachidonic acid that can be utilized as substrate for $PGF_2\alpha$ synthesis (Kawai and Clark, 1986). This is considered to be the rate-limiting step in prostaglandin (PG) synthesis (Kunze and Vogt, 1971; Lands and Samuelson, 1968; McCracken, 1997). Cyclooxygenase1 and 2 (originally known as prostaglandin H synthase 1 and 2) catalyze the first two steps in the biosynthesis of the prostaglandins from arachidonic acid. These steps are oxidation of arachidonic acid to the hydroperoxy endoperoxide PGG_2 and its subsequent reduction to the hydroxy endoperoxide PGH_2. Finally, PGH_2 is then converted to the primary prostanoids, PGD_2, PGE_2, PGF_2, PGI_2, or thromboxane A_2 by cell specific synthases and nonenzymatic mechanisms (DeWitt and Smith, 1995). $PGF_2\alpha$ is made by prostaglandin F synthase (Watanabe et al., 1985).

V. Prostaglandin $F_2\alpha$ Release

If pregnancy does not occur, the corpus luteum must regress to allow follicular growth and ovulation for a new reproductive cycle. To induce luteolysis during the mid to late luteal phase of the estrous cycle, is $PGF_2\alpha$ released from the endometrium in pulsatile manner (Silvia et al., 1991). Increases in $PGF_2\alpha$ in the endometrium (Wilson et al., 1972) and uterine venous blood (Pexton et al., 1975) are seen prior to the onset of luteolysis. Onset of $PGF_2\alpha$ secretion is probably controlled by estradiol-17β (Reynolds et al., 1983) after a period of progesterone exposure (progesterone priming).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Progesterone acts on the endometrial epithelium to increase phospholipid stores and cyclooxygenase enzyme activity necessary for conversion of arachidonic acid to $PGF_{2}\alpha$ (Boshier et al., 1987; Eggleston et al., 1990). In this regard, adequate progesterone exposure during the early to mid-luteal phase of estrous cycle is essential for initiation of endometrial $PGF_{2}\alpha$ production that leads to luteolysis (Morgan et al., 1993).

Progesterone exerts its effect through an endometrial receptor, via a ligand-inducible transcription factor to inhibit the synthesis of oxytocin receptors and estrogen receptors (Cherny et al., 1991; Findlay, 1982) in the endometrial epithelium. Recently, it was found that progesterone can bind to oxytocin receptor physically and inhibits its ligand binding and signaling function (Zingg et al., 1998). Progesterone also down regulates epithelial progesterone receptor (PR) and 2', 5'-oligo(A) synthase, which allows an increase in cellular mRNA for estrogen receptor (ER) and oxytocin receptor (OT-R) in the endothelial epithelium (Cherny et al., 1991; Miller et al., 1977; Ott et al., 1993).

Estradiol-17β, presumably acting through endometrial receptors, up-regulates endometrial oxytocin receptor formation (Burgess et al., 1990; Beard and Lamming, 1994; Beard et al., 1994; Soloff, 1975; Stevenson et al., 1994; Zingg et al., 1998). Administration of estrogen during the mid-luteal phase increases oxytocin receptor expression and causes luteolysis in cyclic ewes (Hixon and Flint, 1987a). In the absence of estrogen or estrogen receptors in the endometrial epithelium, oxytocin cannot adequately initiate endometrial $PGF_{2}\alpha$ release; destruction of follicles or treatment of heifers with the estrogen antagonist tamoxifen prolongs CL function (Fogwell et al., 1985; Jacobs et al., 1988; Villa-Godoy et al., 1985).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Oxytocin released from either the CL or posterior pituitary can then interact with its endometrial receptor to enhance $PGF_2\alpha$ synthesis and release by stimulating inositol triphosphate(IP) turnover (Tysseling et al., 1998). Endometrial content of oxytocin receptors increases during late diestrus of cows (Fuchs et al., 1990; Meyer et al., 1988; Soloff and Fields, 1989). Release of endometrial $PGF_2\alpha$ establishes a positive feedback loop with the CL, which reinforces continued oxytocin secretion for episodic secretion of $PGF_2\alpha$ (McCracken et al., 1984). Oxytocin is stored in secretory granules of large luteal cells (Fields et al., 1992; Guldenaar et al., 1984), which also express high numbers of receptors for $PGF_2\alpha$ on their plasma membranes (Fitz et al., 1982). Such a closed loop, positive feedback system continues until the $PGF_2\alpha$ receptor response system becomes desensitized, thus curtailing the supplemental release of luteal oxytocin (McCracken et al., 1995).

VI. Maternal Recognition of Pregnancy in Ruminants

Maintenance of the corpus luteum during pregnancy is referred to as "maternal recognition of pregnancy" (Short, 1969). To maintain pregnancy, the CL has to synthesize and release progesterone far beyond a normal estrous cycle. In cattle, the embryo / fetus is dependent on luteal progesterone for 200 days of gestation (Chew et al., 1979; Estergreen et al., 1967), while the shift from dependence on luteal to placental progesterone occurs after approximately 45 days of gestation in sheep (Casida, 1945; Denamur and Martinet, 1955).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

In the pregnant animal, specific factors released by the conceptus are required to block the pulsatile release of $PGF_{2}\alpha$ and allow the CL to persist beyond its normal lifespan (Bazer et al., 1986). An important result is continued production of progesterone by the corpus luteum, which provides a proper uterine milieu for implantation and maintenance of pregnancy. A conceptus factor involved in CL maintenance was inferred from extension of interestrous intervals after intrauterine infusion of total conceptus secretory protein (Vallet et al., 1988). Early investigations indicated that this conceptus signal was proteinacious, since it was heat labile and susceptible to protease inactivation (Martal et al., 1979; Rowson and Moor, 1967). In addition, prevention of luteolysis is extended only to the corpus luteum which is ipsilateral to the gravid uterine horn (Moor and Rowson, 1966a; Niswender et al. 1970), providing strong evidence that the embryonic signal acts locally rather than systemically.

A. Interferon-τ as an Antiluteolysin

Early studies indicated that the uterine luteolytic mechanism must be inhibited or overcome in some way between days 12 and 18 of pregnancy in sheep and days 17 and 22 in the cows for the pregnancy to proceed (Caldwell et al., 1969; Denamur and Maueon, 1963; Moor and Rowson, 1966abc; Moor and Rowson, 1964; Short, 1969).

Experiments using embryo transfer showed that the latest time at which embryos transferred into the uterus would maintain CL functions were day 12 of gestation in sheep and day 16 of gestation in cows, respectively (Betteridge et al., 1980; Short, 1969). Rowson and Moor (1967) showed the infusion of aqueous extracts of blastocysts mimicked the effect of transferring an embryo to a sheep uterus. The same effect was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

observed later when bovine embryo homogenates were infused into cycling cows (Northey and French, 1980). These experiments led to identification of a substance from blastocysts, and it was partially purified and identified as a heat labile protein, named trophoblastin (Martal et al., 1979). Godkin et al. (1984) found that the major secretory product of the blastocysts in vitro was identical to trophoblastin and named it ovine trophoblast protein 1 (oTP-1). Heyman et al. (1984) indicated that this substance was the product of trophoblast cells by showing extended estrous cycles after transferring trophoblastic vesicles without embryonic disc into both cows and sheep. Martal et al. (1984) suggested that the conceptus factor involved in CL maintenance was similar between the cow and ewe after observing extension of CL function following interspecies transfer of trophoblastic vesicles. Bazer et al. (1986) purified this protein from cultured trophoblastic vesicles, and found it prolonged the luteal phase of the cycle when administered to the uterus. Vallet et al. (1988) demonstrated that intrauterine infusion of purified oTP-1 extended the interestrous interval and suppressed uterine responsiveness to estradiol and oxytocin challenge comparable to that observed with infusion of total conceptus secretory protein (CSP) in cyclic ewes; however, CSP lacking oTP-1 failed to show these effects.

This protein variously termed trophoblastin, antiluteolytic protein, or ovine trophoblast protein-1, eventually was designated ovine interferon tau (oIFN-τ) based on its nucleotide and amino acid sequence similarity with type I interferon (Anthony et al., 1988; Bartol et al., 1985; Charlier et al., 1989; Charpigny et al., 1988; Godkin et al., 1988; Imakawa et al., 1989; Leaman, 1993; Stewart et al., 1987; Stewart et al., 1989). It was also shown to be secreted transiently by the sheep conceptus between days 12 and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

21 of pregnancy (Farin et al., 1989; Godkin et al., 1982) with peak expression at day 15 (Ashworth and Bazer, 1989). These observations strongly implicated oIFN-τ as the ovine antiluteolysin. It is non-glycosylated and composed of several isoforms with 172 amino acids, a relative molecular weight of about 18,000 and isoelectric points ranging from 5.3 to 5.7 (Godkin et al., 1984; Godkin et al., 1982). Shortly thereafter, it was also shown that bovine conceptuses produced a very similar protein complex [bovine interferon-tau (bIFN-τ)] with slightly higher molecular weight (Helmer et al., 1987). bIFN-τ is composed of 172 amino acids with a relative molecular weight of 22,000 to 24,000 and isoelectric points between 6.5 and 6.7. This variation is due to the degree of N-linked glycosylation and slight differences in amino acid composition (Anthony et al., 1988; Helmer et al., 1988). Initially, this protein was found in the cytoplasm of both mono- and binuclear trophectoderm cells of bovine conceptuses as shown by immunocytochemistry, indicating that it was a product of the trophoblast (Lifsey et al., 1989). However, using the more specific *in situ* hybridization techniques, IFN-τ transcripts were localized only in mononucleate cells, even within the trophoblast (Guillomot et al., 1990).

There has been substantial evidence suggesting the bIFN-τ is a unique type of interferon. The amino acid sequences of bIFN-τ and bovine interferon alpha, class II (bIFN-αII or bIFN-ω) have about 75% homology (Capon et al., 1985; Hauptmann and Swetly, 1985; Imakawa et al., 1989; Imakawa and Roberts, 1989). Computer modeling of bIFN-τ revealed that it is composed of five long alpha helices (A-E). Loop AB is believed to interact with the receptor. A peptide competition experiment with the ovine counterpart, oIFN-τ, showed that internal and carboxy terminal parts interact with a

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

common type I IFN receptor, while the amino terminus interacts with a site that elicits activity unique to INF-τ. However, the six carboxy amino acid tail which distinguish IFN-τ from other IFN-alpha and -beta has no known functional role; no effect occurred when deleted with regard to receptor binding, antiviral, and antiproliferative activities (Ealy et al., 1998).

Enriched bIFN-τ showed antiviral and physico-chemical properties similar to other bIFN-α molecules, characterized by upregulation of 2',5'-oligoadenylate synthetase, which catalyzes production of 2',5'-oligo (A), which in turn is involved in antiviral and growth inhibitory effects of IFNs. This 2',5,-oligosynthetase is present approximately 30-fold higher concentrations in uteri of pregnant compared to non-pregnant animals (Schmitt et al., 1993). In both endometrial epithelial and stromal cells, even partially purified bIFN-τ showed a comparable degree of induction of the 2',5'-oligo(A) synthetase compared to bIFN-α1 (Schmitt et al., 1993). Utilization of recombinant bIFN-τ clearly showed that 2',5' -oligoadenylate synthetase was induced by bIFN (Short et al., 1991). This protein also has antiproliferation activity as shown by inhibition of proliferation of mitogen induced lymphocytes (Skopets et al., 1992). The degree of inhibition was similar to that of bIFN-αII. However, unlike the other bIFNs, this protein has only minor antiproliferation activity in either endometrial epithelial or stromal cells, suggesting the bIFN-τ allows for growth of the endometrium during pregnancy (Davidson et al., 1994).

B. IFN-τ Expression during Development

Bovine interferon-τ is a major protein synthesized and secreted by the bovine conceptus during days 14 to 17 of pregnancy (Bartol et al., 1985; Geisert et al., 1988;

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Godkin et al., 1988; Kazemi et al., 1988). Maximal secretion occurs around days 17-19 of pregnancy, which closely corresponds to the time at which luteal regression must be prevented for pregnancy to proceed (Anthony et al., 1988). However, mRNA encoding bIFN-τ can be detected in day 12 conceptus (Farin et al., 1990) and, even earlier in in vitro derived hatched blastocysts (Hernandez-Ledezma et al., 1992; Watson et al., 1991); bIFN-τ synthesis still can be detected as late as day 36 in the chorioallantois (Godkin et al., 1988). Studies on IFN-τ expression using in vitro derived bovine embryos demonstrated that initial onset of IFN-τ expression is genetically programmed, since those embryos were not exposed to the maternal environment (Hernandez-Ledezma et al., 1992). For normal secretion of bIFN-τ, however, bovine embryos required a uterine environment as shown by an embryo transfer experiment. When morulae or blastocysts derived from in vitro maturation (IVM) and in vitro fertilization (IVF) procedures were transferred to the uteri of synchronized cows and recovered 4 days later, hatched blastocysts secreted a few times more bIFN-τ than the same aged embryos which were remained in the culture (Hernandez-Ledezma et al., 1992). Thus, although induction of bIFN-τ is regulated developmentally, amplification and sustained secretion of bIFN-τ by bovine conceptus require exposure to the uterine environment.

Expression of ovine interferon-τ (oIFN-τ) showed a very similar pattern to the bovine counterpart bIFN-τ. Large scale oIFN-τ production occurs between days 13-21 of pregnancy in sheep (Godkin et al., 1982), with peak production at day 15, when a single ovine conceptus produces 100 μg or more of oIFN-τ within 24h in culture (Ashworth and Bazer, 1989). Although oIFN-τ expression can be detected as early as day 8 of pregnancy, massive expression begins about day 13 when the conceptus

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

undergoes morphological change from a spherical to an elongated form. By day 14, when the conceptus is filamentous and may stretch into both uterine horns; oIFN-τ represents the most abundant mRNA in this tissue (Hansen et al., 1988). By day 22, when most of the trophoblast is attached to the endometrium, expression is virtually undetectable (Farin et al., 1989; Guillomot et al., 1990). Thus, contact between the trophoblast and the uterine epithelium appears to inhibit oIFN-τ gene expression by unknown mechanisms.

C. Mode of IFN-τ Action

Interferon-τ, unlike chorionic gonadotropin of primates, acts locally upon the uterus to inhibit episodic release of $PGF_{2}\alpha$ (Fincher et al., 1986; Knickerbocker et al., 1986). The exact mechanism(s) whereby the IFN-τ of sheep and cattle modulate $PGF_{2}\alpha$ production or release is not yet known. However, there is circumstantial evidence indicating that IFN-τ most probably acts through cell surface receptors found on the endometrial lining of the uterus. These molecules are thought to bind the common type I IFN receptors, since IFN-τ displaced INF-α from its receptor (Hansen et al., 1989; Li and Roberts, 1994a; Stewart et al., 1987). Furthermore, results from recent complementary DNA (cDNA) cloning of type I IFN receptors from an endometrial cDNA library support this assumption (Han et al., 1997). However, there is no clear explanation regarding how IFN-τ works differentially from other type I IFN, even though they bind to the same receptors. One possibility is that accessory proteins which affect binding affinity of various IFN may be present within that receptor complex. Because IFN-α and -β can clearly bind to at least one common receptor with apparently

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

different cell responses, this possibility is very likely (Miyajima et al., 1992; Watling et al., 1992). Furthermore, cytotoxicities of IFN-α and IFN-τ to Madin-Darby bovine kidney (MDBK) cells are dependent on the differential recognition of receptors (Subramaniam et al., 1995). In this regard, cross-linking studies with oIFN-τ using ovine endometrial membranes indicated the presence of two cross-linked products (Mr 100,000 and 70,000); both of these cross linked with oIFN-τ, but only the larger protein cross linked with IFN-α (Hansen et al., 1989). These results may also explain why bIFN-α is less effective than IFN-τ in extending CL function when infused into uteri of nonpregnant animals (Plante et al., 1988).

a. IFN-τ Blocks Estrogen- and Oxytocin-receptor Up-regulation

Given that IFN-τ binds to a common type I IFN receptor, it is assumed that the intracellular signaling pathway of IFN-τ is similar to that of other type I IFNs (Li and Roberts, 1994b). Type I interferon acts via a cytoplasmic tyrosine kinase, known as janus kinase 1 (JAK1) (Darnell et al., 1994) and *tyk2* (Valezquez et al., 1992). In response to IFN binding, constitutively expressed IFN-stimulated gene factor-3 (ISGF-3) complex is formed from three smaller molecules [STAT1 (p84), STAT1α (p91), and STAT2 (p113), where STAT is signal transducer and activator of transcription] after phosphorylation in the cytoplasm. Then the ISGF-3 binds to ISGF3, which is a DNA-binding protein, and the multimeric protein complex translocates to nucleus. This complex now binds IFN-stimulated elements present in promoter region of interferon regulatory factor-1 (IRF-1) and increases transcription. IRF-1 is a transcriptional activator (Darnell et al., 1994) and binds to IRF-element (IRF-E). One of the genes

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

containing the IRF-E in the promoter region is IRF-2. IRF-2 is a transcriptional repressor and can bind to the same motif of IRF-1(Harada et al., 1994; Taniguchi et al., 1995). Thus, the increased transcription of IRF-2 by IRF-1 binding leads to silencing of transcription of genes containing IRF-E (Kerr and Stark, 1991; Levy et al., 1988). If the estrogen receptor gene (ER) contain a functional IRF-E, production of IRF-2 in response to IFN-τ would silence transcription of the ER gene (Spencer et al., 1998). Recent genomic cloning work on ovine ERα gene promoter reveals that there is one functional IRF-E site located at −1867 nt from the putative transcription start site (Fleming et al., 1999). Furthermore, both IRF-1 and IRF-2 were found in endometrial luminal epithelium and superficial glandular epithelium of pregnant ewes or IFN-τ treated animals in appropriate time-frames. IRF-1 was detected only on days 11 and 13, and IRF-II was detected on days 13, 15,17 and 20 in the epithelia. In the IFN-τ treated animals, IRF-1 was detected in the same epithelia at 12 and 24 hr after initial injection, and IRF-II was detected at 24hr and thereafter (Spencer et al., 1998). Thus the silencing of ER gene transcription mediated by the elevated IRF-II would prevent ER-mediated increases in OTR gene transcription, preventing pulsatile release of luteolytic $PGF_2\alpha$ (Spencer et al., 1995; Spencer and Bazer, 1996).

b. IFN-τ Stimulates Release of PGE_2 Preferentially to $PGF_2\alpha$ in Uterine Epithelial Cells

Incubation of endometrial explants of day-17 cyclic cows with bIFN-τ inhibits PG-synthesis and reduced PGF secretion similarly to that of day-17 pregnant cows. Intrauterine infusion of bIFN-τ from days 15.5 to 21 extended the interestrous interval

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

from 19.5 to 26.0 days (Thatcher et al., 1989). Treatment of endometrial explants from day17 after estrus with bIFN-τ decreased the synthesis of $PGF_2\alpha$ markedly while increasing PGE_2 secretion (Helmer et al., 1989; Barros et al., 1991), which is both antiluteolytic and luteotropic at the time of pregnancy recognition. The decreased secretion of the $PGF_2\alpha$ was more prominent in oxytocin-induced endometrial epithelial cells than stromal cells after treatment of bIFN-τ, indicating differential prostaglandin responses by the two major cell types (Danet-Desnoyers et al., 1994). On some occasions, treatment of bovine endometrial cells with bIFN-τ stimulates both PGE_2 and $PGF_2\alpha$ production. However, PGE_2 synthesis is stimulated preferentially in uterine epithelial cells. Production of $PGF_2\alpha$ was stimulated 7.1 fold, and that of PGE_2, 89 fold by rbIFN-τ, which resulted in a net increase in the PGE_2:$PGF_2\alpha$ ratio of 7.7, changing the primary PG produced by these cells from $PGF_2\alpha$ to PGE2 (Asselin et al,. 1997). In addition, treatment of bovine endometrial cells with recombinant bIFN-τ upregulated the expression of the cyclooygenase-2 (COX-2) gene, which might be involved in the selective increase of PGE_2 in epithelial cells (Asselin et al., 1997), even though the pattern of COX-2 gene expression is dependent on cell type. A northern blot analysis showed that bIFN-τ decreased COX-2 messenger RNA (mRNA) levels in epithelial cells, while increasing COX-2 mRNA in stromal cells (Asselin et al., 1997; Xiao et al., 1998). Furthermore, rbIFN-τ decreased PGF synthase mRNA in both cell types, which was associated with the increase in PGE_2 : $PGF_2\alpha$ ratio (Xiao et al., 1999).

c. IFN-τ Induces Expression of Several Genes that may be Needed to Establish Pregnancy

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Upon IFN binding, *tyk2* phospholylates a variety of cytoplasmic transcription factors involved in transcription of a host of IFN responsive genes (Schindler et al., 1992; Valezquez et al., 1992). To date, several IFN stimulated genes (ISG) have been identified including 2'-5' oligoadenylate synthetase, Mx, p68 kinase, ISG6-16, ISC15, ISG54 (Borden, 1992). However, the exact roles of many of these factors in IFN responses are unclear. Among others, 2'-5' oligoadenylate synthetase seems to play a role during implantation by suppressing endometrial proliferation until the conceptus elongates, and contact between the epithelial and trophoblast is established throughout the uterine horn (Schmitt et al., 1993). In addition, bovine recombinant IFN-τ (rbIFN-τ) stimulates endometrial cells to secrete at least three different proteins (8,16, and 28Kd), two of which are granulocyte chemotactic protein-2 (8Kd protein) and ubiquitin cross-reactive protein (16Kd protein) (Austin et al., 1996; Hansen et al., 1997; Naivar et al., 1995; Rueda et al., 1993; Teixeira et al., 1997). The exact roles of these proteins are unknown, but they may are involved in the establishment of cytokine networking necessary for early pregnancy. These results suggest that many genes and their products are intermingled in a harmonious manner in the process of establishing pregnancy.

V. IFN-τ Genes

A. Distribution of IFN-τ Genes among Mammals

It appears that the trophoblast interferon (IFN-τ) genes are present only within a limited subset of mammalian species known to posses IFN-ω genes. A genomic southern blot (zoo blot) using a probe of full length oIFN-τ coding region shows the genes for the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

IFN-τ appear to be limited to ruminant species within the artiodactyls, including the goat, musk ox, gazelle and giraffe (Leaman and Roberts, 1992; Liu et al., 1996).

B. Copy number of IFN-τ genes.

Interferon-tau (IFN-τ) are encoded by multicopy genes as indicated by western blots of ovine and bovine embryo cultured media, cDNA cloning and sequencing, and southern blots of bovine and ovine genomic DNA with the specific ovine IFN-τ gene probes (Hansen et al., 1988; Imakawa et al., 1989; Klemann et al., 1990; Roberts et al., 1991) and several oIFN-τ genomic clones (Nephew et al., 1993). The estimated copy numbers of IFN-τ are 5-6 in cattle, sheep, and goats (Iannuzzi et al., 1993; Leaman, 1993; Ryan and Womack, 1993).

C. Features of IFN-τ Genes

1. General features of type I IFN genes

As mentioned earlier in this review, interferon-τ shows high homology in both nucleotide and amino acid sequences to other type I interferons. Furthermore, recombinant bovine IFN-α1 (rbIFN-α1) shows moderate levels of antiluteolytic activity when infused into uterine lumen of cyclic cows (Plante et al. 1988; Plante et al. 1989). Thus, it is conceivable that the mechanisms involved in these type I interferon genes may be applicable to study IFN-τ gene expression. The IFN are a group of related cytokines whose existence was inferred from the ability of culture medium from virus infected cells to confer resistance to cells to subsequent viral infection (Isaacs and Lindenman, 1957). However, IFN are actively involved in several other cellular processes, including

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

differentiation and proliferation of certain cells and modulating many aspects of immune responses (Pestka et al., 1987). IFN are broadly categorized into two major groups according to gene structure and nucleotide sequences, in addition to evolutionary histories. The type I IFN includes the α,β, and ω subtypes, and the type II includes only a single subtype γ. Each subtype of type I IFN possesses distinctive primary sequence, antigenicity, and relative inducibility. In addition, they are produced by specific cells preferentially, to some extent (De Maeyer and De Maeyer-Guinard, 1988).

The Promoter Region of Type I IFN Genes

Five short stretches of DNA motifs known to important for IFN-α and -β gene expression are found within 130 bases of the transcription start sites (Goodbourn et al., 1985; Harada et al., 1990; Ryals et al., 1985). In the human IFN-β gene, repeats of AARKGA, where R is G or A and K is T or G, are common (Fujita et al., 1985); tandemly repeated sequences of this 6-bp oligomer mediate viral responses when fused with a minimal promoter (Fujita et al., 1987). Two positive regulatory elements, positive regulatory domains 1 and 2 (PRD1 and PRD2) (sequences AGAAGTGAAAGT and TGGGAAATTCC respectively) and a palindromic negative regulatory domain (NRD1; TCCTCTGAATAGAGAGAGGAC) also are present in IFN-β genes (Fan and Maniatis, 1989; Goodbourn et al., 1986; Goodbourn and Maniatis, 1988). Among the PRD 1 binding factors, IFN regulatory factor-1(IRF-1) activates IFN-α and -β gene expression through an IRF-1 binding motif $(GAAAGT)_4$ in PRD1 (Fujita et al., 1989). Another PRD1 binding factor, IRF-2, binds the same motif and inhibits IRF-1 mediated expression in the absence of viral induction (Harada et al., 1989). PRD2 is a DNA motif

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to which NF-κB binds. Upon virus infection, NF-κB is released from its cytosolic inhibitor followed by translocation to the nucleus to activate IFN-β genes (Goodbourn, 1990; Hiscott et al., 1989; Leblanc et al., 1990). Treatment of cells with virus or double stranded RNA (dsRNA) can activate NF-κB, and binding of both NF-κB and IRF-1 may be required for full IFN-β induction (Lenardo et al., 1989). An AFT-2/CREBP-1 binding site that provides cAMP responsiveness to IFN-β is present in the newly found PRD IV region. In addition, the basic virus inducible element, GAAANN, is found in the human IFN-αI gene (MacDonald et al., 1990; Ryals et al., 1985).

2. General Features of IFN-τ Genes

As in other type I interferon genes, bIFN-τ genes are intronless and are found clustered on a single chromosome arm, chromosome 8 in cattle (Iannuzzi et al., 1993; Ryan and Womack, 1993). The 5'-upstream region contains a TATA box starting 28 base pairs (bp) from the putative transcription start site (Hansen et al., 1991). Sequence analysis of bovine cDNA shows that it contains an open reading frame of 195 codons. The initiation codon, ATG, is immediately preceded by the sequence TCCCC, which is not typical of the consensus sequence (CCRCC, where R is A or G) for initiation of translation in eukaryotes (Kozak, 1986). The hexanucleotide ATTAAA, which most likely works as a signal for poly (A) addition to 3' end of the mRNA is found within 20 bases of the site of polyadenylation (Imakawa et al., 1989). The entire sequence shows high homology with immunologically related ovine IFN-τ (85%) and with bovine interferon-ω (previously, it had been called IFNα_{II}) (79%). The highest conservation of sequence (> 90% across isoforms and species) is found in the 3'-untranslated region,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

whereas matching between IFN-τ genes and the IFN-ω genes in these regions is considerably less (<70%) (Imakawa et al., 1989). Thus, this region was used successfully to make a probe to differentiate IFN-τ genes from the very closely related IFN-ω genes (Hansen et al., 1991).

a. Promoter Region

Most of the regulatory domains (or similar sequences) found in IFN-α and -β genes are present in bovine and ovine IFN-τ genes. A series of GAAANN sequences which confer viral responsiveness to heterologous promoters (MacDonald et al., 1990; Raj et al., 1989) is found throughout the promoter region. In addition, two potential recognition sites for IFN regulatory factor (IRF-1)(AAGTGA and AACTGA) are present in the distal region of the gene promoters; overlapping this site is a conserved palindromic dyad resembling an extended NRD1. Several of repetitive AAATTT sites which resemble PRD1 of unknown significance are also seen in the proximal promoter region (Hansen et al., 1991). Recently, Ets-2 (a transcription factor) binding sites were recognized in proximal promoter regions of most active IFN-τ genes (Ezashi et al., 1998).

b. Transcriptional Regulation of IFN-τ Genes Differ from Other Type-I IFNs

Study of transcriptional regulation of IFN-τ has been hampered because there are currently no well defined trophoblast cell lines available from cattle or sheep. Furthermore, transfections have not been successful in primary trophoblast cell cultures (Cross and Roberts, 1991). Thus, most of the promoter analysis work has been

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

conducted using human choriocarcinoma cells despite the apparent absence of human IFN-τ genes (Leaman et al., 1992). Previous work demonstrated that Jar cells were competent to support constitutive IFN-τ promoter activity, even though activity of the transfected IFN-τ promoter by these cells was significantly lower than that observed in the developing bovine or ovine conceptus (Cross and Roberts, 1991).

As mentioned previously, onset and cessation of IFN-τ gene expression are known to be precisely timed events. To date, mechanisms of regulating IFN-τ genes have not been clearly demonstrated, although sequences associated with regulation of other Type I IFN are present within the bIFN-τ promoter. However, it is clear that these genes are developmentally regulated, and the mode of regulation is distinct from that of other IFN. These genes are only poorly inducible by dsRNA in conceptuses (Farin et al., 1991) and by viruses in leukocytes (Cross and Roberts, 1991), and there was no effect of virus on transcription in Jar cells transfected with a 1.8 kb bIFN-τ upstream sequence reporter gene plasmid (Leaman et al., 1994). A promoter deletion analysis showed that truncation of the promoter to position –450 did not affect promoter activity, whereas deletion to position –126 decreased expression up to 4-5 fold, suggesting that the sequences 5' to position –126 in the promoter may possess enhancer activity (Cross and Roberts, 1991). In addition, the promoter region between positions –280 and –400 showed general enhancer-like activity (Leaman et al., 1994). A gel mobility shift assay using nuclear extracts prepared from ovine conceptuses during the period of IFN-τ expression indicated that there are at least two transcriptionally important regions, which agreed with results from the transfection assay described. These nuclear extracts bound to at least two regions, proximal promoter region (positions –126 to–34) and distal

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

promoter region (positions –358 to –322), that are required for full constitutive expression of the bIFN-τ genes. To test the putative IRF-1 binding site (AAGTGA), IRF-1 bound and formed a complex with this region in a gel shift assay; however, it did not transactivate the bIFN-τ 1.8 kb promoter in transfected Jar cells (Leaman et al., 1994). To the contrary, another promoter deletion assay revealed that two putative inhibitory domains are located in the regions –357 to –248 and –150 to –71 bp. After conducting a series of deletion mutations of the bIFN-τ gene promoter followed by transient transfection in Jar cells, they concluded that negative regulation is predominant over positive regulation in bIFN-τ gene expression (Guesdon et al., 1996). Unlike a previous report (Cross and Roberts, 1991), when the exogenous enhancer, SV40, was provided, the same authors found that the 450 bp of bIFNτ promoter drove constitutive reporter gene expression in cells both of trophoblast and non-trophoblast origin, such as L929 cells. Without the exogenous enhancer, none of the cells expressed reporter genes. This result is consistent with a finding that nuclear extract prepared from Cos-1 cells, L929 cells, and Jar cells formed complexes with the distal promoter region in almost identical patterns with day 15 or day 21 ovine conceptus nuclear extracts (Leaman et al., 1994). This casts doubt about current understanding of tissue and\or cell specificity of the cloned bIFN-τ gene expression. In an effort to identify a putative transcription factor involved in the IFN-τ genes, Ezashi et al. (1998) found that Ets-2, a transcription factor, increased reporter gene expression up to 30 fold in transformed Jar cells. However, Ets-2 genes are known to be expressed in a wide variety cells, so that Ets-2 is not in itself sufficient for IFN-τ expression, considering IFN-τ expression is restricted to trophoblast cells (Ezashi et al., 1998).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

To determine whether selected maternally derived factors might enhance IFN-τ expression, cyclic AMP (cAMP), TPA(Phorbolester), New castle disease virus, bIFN-α1, and retinoic acid were added to the transfected Jar cells (Leaman et al., 1994). None of these stimulants affected bIFN-τ promoter activity. All of these factors are known to stimulate transcription of type I IFN genes. Unmodified activity of the 1.8 kb bIFN-τ promoter after exposure to Newcastle virus indicates that virus is not a primary inducer of these genes, at least via this part of the promoter, which is consistent with the earlier finding that the same treatment to leukocytes only induced marginal levels of expression of endogenous bIFN-τ (Cross and Roberts, 1991). However, one IFN-τ gene family member (if not all) seems to respond to external stimuli. Ko et al. (1991) described increased secretion of oIFN-τ after a combined treatment of IGF I and II to day13 ovine conceptuses in vitro. IGF-I, -II, and IGF binding protein-II were found in uteri of pregnant and non-pregnant cows. Expression of IGF-I mRNA was not affected by day of the estrous cycle or status of pregnancy. However, endometrial IGF-II mRNA was greater in pregnant than in cyclic endometrium on days 15 and 18. Levels of endometrial IGFBP-2 mRNA also increased between days 10 and 18 of estrous cycle and early pregnancy (Geisert et al., 1988).

Another factor involved in oIFN-τ gene expression is granulocyte-macrophage /colony stimulating factor (GM-CSF). At the molecular level, GM-CSF appears to act via proto-oncogenic (c-fos, c-jun) activation of the AP-1 enhancer element (Samuel et al., 1991). The accumulation of c-fos and c-jun proteins, and the expression of oIFN-τ were temporally related (days 14 and 15 of gestation) (Xavier et al., 1991), and treatment of one oIFN-τ gene, which had an AP-1 site, with GM-CSF activated expression of the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reporter gene in vitro (Imakawa et al., 1993). Thus, it is clear that IFN-τ genes are regulated in a quite different manner than other IFN genes, and more work on IFN-τ gene expression is needed.

c. Why Are There so Many IFN-τ Genes?

There are at least four distinct IFN-τ loci within the genomes of cattle, sheep, and goats (Charlier et al. 1991; Leaman, 1993; Roberts, 1991; Ryan and Womack, 1993; Stewart, 1990). However, why there are so many copies of INF-τ genes has not been answered. The proposed hypotheses are: 1) different gene products may function in slightly different manners to alter $PGF_{2}\alpha$ release, 2) the promoter regions of some genes are slightly different than others, allowing expression of certain subtype IFN-τ to occur at specific, crucial time points over the duration of IFN-τ expression, and 3) the multiplicity may contribute to the unusually high production of these proteins by the trophoblast (Leaman, 1993). Unfortunately, the available data are too scanty to confirm any of these hypotheses. Nevertheless, the accumulated data suggest that certain IFN-τ subtypes (if not all) have unique biological properties. Ealy et al. (1998b) demonstrated that three different oINF-τ variants showed different biological potencies with regard to antiproliferative activity and antiluteolytic activity. In addition, they showed that one of the oIFN-τ expressed much less than the other two variants (Ealy et al. 1998). Similar results were obtained when both type I IFNs and two oIFN-τ gene variants were tested (Nephew et al., 1993). However, the presence of bIFN-τ variants with significantly different biological potency or different patterns of gene expression has not yet been confirmed in cattle (Liu et al., 1997).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

VII. Embryonic Loss

Embryonic mortality in cattle, as well as in other domestic animals, is a major source of economic loss for livestock producers. While fertilization rates in cattle inseminated properly are close to 90%, pregnancy rates are usually between 50 and 60% (Sreenan and Diskin, 1983). The same authors showed that embryonic loss was approximately 38% (Sreenan and Diskin, 1986b).

The timing of embryonic loss has been investigated in a number of studies, and results have been inconsistent. When measured before day 25, embryonic loss varied from 8% (Boyd and Reed, 1961) to 23% (Roche et al., 1981) after artificial insemination of cows. In other studies, embryonic loss occurring before day 42 was estimated to be 20%, with most of the loss occurring between days 16 and18, or 42 % with the majority embryonic loss occurring between days 8 and 16 (Ayalon, 1978; Diskin and Sreenan, 1980; Roche et al., 1981). Thus the majority of embryonic deaths occur before day 18 of gestation.

Several factors have been implicated in embryonic mortality of domestic animals. These include genetic abnormalities of embryos (Hare et al., 1980; Iwasaki and Nakahara, 1990; King,1990), nutritional effects (Blanchard et al., 1990; Wiltbank et al., 1962), environmental influences (Ulberg and Sheean, 1973), infectious agents (Bouters, 1986; Hartigan et al., 1974), asynchrony between the embryo and oviductal and uterine environments (Diskin and Sreenan, 1980; Rowson et al., 1969), and other factors.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A. Gross Chromosomal Abnormalities are not the Major Cause of Embryo Mortality

Chromosomal abnormalities have been suggested as a main cause of embryonic mortality (Bishop, 1964). Most frequently observed are abnormalities in chromosome number and structure. In humans, a high incidence of chromosomal abnormalities is associated with spontaneous abortion in the first and second trimester (Hassold, 1980). For all age groups, clinically recognized pregnancy losses are between 15-20%, at least 50% of which are due to chromosome abnormalities. In contrast, none of 159 bovine embryos obtained 12 to 18 days after estrus from superovulated cows had gross structural chromosomal abnormalities (Hare et al., 1980). Only 1.9% (3/159) of the embryos were polyploid, which included single embryos in each of the following categories: triploid (3n), diploid-triploid (2n/3n), and diploid-hexaploid (2n/6n). However, 41.5% (66/159) of the embryos had polyploid cells, and were classified as diploid-tetraploid (2n/4n). When polyploid embryos were transferred to recipients, no pregnancy was obtained. However, pregnancies were obtained from diploid-tetraploid embryos if the number of polyploid cells was less than 25%. The high incidence of polyploidy can be explained partly by binucleate cells which are known to appear in the trophoblast by day 15 (Staples et al., 1969). King (1990) summarized the chromosomal analyses of cattle embryos produced by animals with normal karyotypes, and found from 9 studies that there was an average of 10.4 % chromosomally abnormal embryos (range 0 to 36.3%). The majority of abnormalities were found in embryos which were 7 days of age, which is the time of transition from maternal to embryonic control during development and when first differentiation occurs. This time of transition would be particularly sensitive

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

because of the inability of the embryonic genome to tolerate any gross alterations (King. 1990). Thus. the low percentage of abnormal embryos observed from later stage embryos (days 12 to18) can probably be explained on the basis that many of the abnormal embryos found in these animals had died by the time the analysis were carried out (Hare et al., 1980). Overall. these results suggest that a significant fraction of embryos is lost for reasons other than gross chromosomal abnormalities.

B. Asynchrony between the Embryo and Oviductal and Uterine Environment

As embryo transfer techniques became widely used, evidence accumulated that establishment of pregnancy depends upon close synchrony between the developmental stage of the embryo and the corresponding developmental stage of the recipient's uterus. There are several observations that ovine embryos responded to an advanced uterus by increasing cell division (Wilmut et al., 1985: Wintenberger-Torres. 1967) and slow their development after transfer to a lesser developed uterus (Wilmut and Sales. 1981; Wilmut et al., 1988). The same phenomenon was observed in cattle embryos. However. asynchronous embryos (±3 days) transferred to recipients, usually showed signs of degeneration (Albihn et al., 1991). Therefore, it is not unexpected that embryo transfer is most successful (91%) if donor and recipient are closely synchronized (Newcomb and Rowson, 1975; Rowson et al., 1969), compared to dramatic declines in pregnancy rates (52- to 57%) if the recipients are ± 1 day out of synchrony with donor animals (Rowson et al., 1972). Several factors presumably cause natural asynchrony. The timing of the first meiotic division may lead to lagging in maturation of some oocytes. This holds true in multiparous animals, such as pigs in which ovulation is not a synchronous process (Pope,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1988). A second natural cause of retardation in embryos may be related to the variable times of fertilization and completion of the second miotic division among oocytes as observed even in an inbred mouse strain (Gates. 1965). Third is variability in progesterone synthesis among animals (Ashworth and Bazer. 1984; Quirke et al.. 1979). Asynchrony was most evident in cattle and sheep during corpora lutea formation (Maurer and Echternkamp. 1982). These authors observed a lower percentage of embryonic survival in beef cows that had lower concentrations of serum progesterone shortly after CL formation, compared to more fertile cows. Similar results were obtained in dairy cows when the concentration of progesterone in milk was measured during the first 4 days after insemination (Lee and Ax. 1984), even though a number of other studies have been unable to show any definitive relationship between progesterone levels and conception rates until approximately day 16 postinsemination (Folman et al. 1973; Shemesh et al.. 1968; Sreenan and Diskin. 1983a). More direct evidence came from efforts to decrease early embryonic mortality in animals suspected of having a luteal insufficiency (Sreenan and Diskin, 1983a). Progesterone supplementation through an intra-virginal device (PRID) to lactating cows for 7 days. starting on day 5 or 10 post-insemination. increased the conception rate up to 30%. compared to control group (30% vs 60%) (Robinson et al., 1989). Similar results were obtained by Macmillan et al. (1991), who placed CIDR (controlled internal drug release) devices containing progesterone in inseminated, lactating cows, and were able to significantly increase the conception rate from 66% in control cows compared to 79% in treated cows (McMillan et al., 1991). The same effects, however, were not observed in heifers treated with same device on days 7 to 13 after insemination. suggesting that progesterone insufficiency

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

might be more problematic in cows than in heifers (Zavy, 1994). However, the increased pregnancy rate was not observed in ewes supplemented with progesterone (Diskin and Niswender, 1989). Moreover, the administration of human chorionic gonadotropin (hCG) to cows after breeding, in an effort to augment the action of a weak conceptus-derived luteotropic signal, failed to increase pregnancy rates (Diskin and Niswender, 1989; Sreenan, Diskin, 1986). Thus, supplementation of exogenous progesterone to increase pregnancy rate remains controversial.

C. Failure of Maternal Recognition of Pregnancy

The reason why asynchronous embryos without chromosomal abnormalities die has not been clearly determined. However, a couple of hypotheses are worthy of consideration. First, the uterine milieu may be only narrowly permissive and even toxic to embryos that are out of phase (Roberts et al., 1990). There are several changes in uterine secretion as the hormonal status of the mother changes (Warner et al., 1988). Furthermore, the embryos themselves can trigger secretory activity in the surrounding endometrium and thus modulate their own local environment (Geisert et al., 1982). Activation of these secretory events asynchronously may create a lethal environment, as indicated in the pig (Morgan et al., 1987). The second is that embryos fail to signal their presence adequately to the mother. As a consequence, the life span of CL is not extended, progesterone production is not maintained and the pregnant mother returns to estrus (Roberts et al., 1990). Work utilizing embryo transfer in cattle established that the period of maternal recognition of pregnancy, the phenomenon whereby the CL is rescued, is days 15 to 16 in cattle. The intrauterine infusion of days 17 to 18 conceptus

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

homogenates or trophoblastic vesicles to cycling animals on day 15 to16, which is prior to the time of maternal recognition of pregnancy, resulted in extension of the estrous cycle (Knickerbocker et al., 1986; Northey and French, 1980). Similarly, presence of the conceptus within the uterus only extended CL function when removed after, but not before, day 16 of the estrus cycle (Betteridge et al., 1980). The active component of conceptus homogenates turned out to be bIFN-τ as discussed previously. Thus, it is possible that a failure of the timing and/or intensity of the embryonic antiluteolytic signal could be implicated in causing some embryonic loss.

VIII. Clinical Uses of IFN-τ

As mentioned in this review, IFN-τ is an antiluteolytic agent in ruminants and possesses full scale leukocytic IFN activities such as antiviral, antiproliferative, and immuno-modulatory properties. Consequently, there have been several trials to use IFN-τ as a therapeutic agent in both human and veterinary medicine, as well as a fertility enhancing agent in animal agriculture. Among the factors affecting post-fertilization losses, asynchrony between the conceptus and the uterine endometrium and/or inadequate conceptus production of the antiluteolytic signal, IFN-τ are most significant (Albihn et al., 1991; Bazer et al., 1996). Therefore, supplementing domestic ruminants with exogenous IFN-τ during the period of maternal recognition of pregnancy may improve fertility by rescuing conceptuses delayed in development or producing inadequate quantities of IFN-τ. Early work using this type of strategy seemed to work. Improved survival rate of frozen cattle embryos was achieved when trophoblastic vesicles, which is

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the sole IFN-τ source (Heyman et al., 1984), were cotransferred to recipient cows (Heyman and Menezo, 1987). In sheep, recombinant bIFN-α (rbIFN-α) administered systemically resulted in a significant increase in the number of lambs born compared to control ewes (Martinod et al., 1991; Nephew et al., 1990; Schalue et al., 1991). Interestingly, enhanced fertility after supplementation of IFN-τ was observed in CBA/J x DBA/2 mice, which is an abortion-prone combination (Assal-Meliani et al., 1995; Chaouat et al., 1995). In cattle, however, results from similar experiments are rather discouraging. The administration of recombinant bovine IFN-α_1 to pregnant and non-pregnant animals induces a pyrogenic effect with peak hyperthermia 6 h after administration, which coincides with a depression in circulation levels of progesterone on day 14 (Meyer et al., 1995; Newton et al., 1990; Plante et al., 1991). Consequently, the pregnancy rate of treatment groups was lower than that of control groups (Barros et al., 1992). This kind of side effect was not observed in animals received rIFN-α or -τ by intrauterine infusion (Bleach et al., 1998). Thus, the use of rIFN-τ as an agent to manipulate luteal function in cattle requires modifications of the delivery systems and/or of the IFN molecule to eliminate aforementioned side effects.

Because of less severe cytotoxic effects without diminished antiviral or antiproliferative activities (Pontzer et al., 1988; Pontzer et al., 1991; Subramaniam et al., 1995), IFN-τ has been considered as a potent therapeutic agents in human and veterinary medicine. Indeed, ovine recombinant IFN-τ (oIFN-τ) was shown to block ovine lentivirus replication (Juste et al., 1996) and human immunodeficiency virus (HIV) replication in peripheral lymphocytes and macrophages (Dereuddre-Bosquet et al., 1996). The antiproliferative activity of IFN-τ is equivalent to or greater than that of IFN-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

α molecules with far less severe side effects in tested human amnion (WISH) and Madin-Darby bovine kidney (MDBK) cells (Pontzer et al., 1991), as well as other cells (Assal-Meliani et al., 1993; Assal-Meliani et al., 1995). The lack of severe cytotoxicity or "cell friendly" properties of IFN-τ (Bazer and Johnson, 1991) therefore holds a strong possibility to be used as a powerful therapeutic agent in the future.

IX. Conclusions

The IFN-τ are a distinctive subclass of Type I IFN that play a most important role during pregnancy establishment in ruminants. The IFN-τ seem to exert their antiluteolytic effects during maternal recognition of pregnancy by blocking pulsatile release of $PGF_2\alpha$ from the uterine epithelium, thereby allowing the CL to maintain its functional integrity throughout pregnancy.

Whereas much is known about the gene expression pattern during early development of ruminant embryos and about the properties of the gene products, the mechanisms involved in the transcriptional regulation of the bIFN-τ remain uncertain. Moreover, studies on individual bIFN-τ gene regulation have been limited to a single gene promoter in spite of the ample amount of evidence indicating that bIFN-τ are encoded by multicopy genes. In addition, most of the gene cloning work has been done with cDNA clones which do not show the promoter regions demonstrated to contain transcriptional elements allowing the unusual timing and magnitude of bINF-τ expression. Therefore, the overall goal of the experiments described herein was to clone

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and characterize several bIFN-τ genes with regard to their expression during early pregnancy of cattle.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER II

CLONING BOVINE INTERFERON-TAU (bIFN-τ) GENES AND CHARACTERIZING THEIR TRANSCRIPTIONAL EXPRESSION DURING EARLY PREGANCY

ABSTRACT

Genomic clones for bovine interferon-τ genes (bIFN-τ) were isolated by probing a bovine genomic library with both the 3' and 5'untranslated regions of a known bovine IFN-τ gene (bIFN-τ1). Of the four new clones, one was a IFN-ω variant, and 3 were IFN-τ variants. The IFN-ω variant had an extremely well conserved open reading frame compared to a known functional bIFN-ω gene. The 5' untranslated region of the gene showed only a few base pair mismatches. Among the 3 bIFN-τ variants cloned in this experiment, proximal promoter regions were well conserved: however, conspicuous base pair mismatches were found in the distal promoter regions. One of the clones (IFN-τb1), which had extreme mismatches in the distal promoter region, was transcriptionally inert, even though most known motifs for transcription of bIFN-τ, including an Ets-2 binding site, were well conserved in the proximal promoter region. The remaining 2 clones and the bIFN-τ1 gene showed minor degrees of base pair mismatches in both untranslated

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

regions and open reading frames. By using RNase protection assays (RPA), full-length protected mRNA fragments were detected for 2 of the new bIFN-τ clones (IFN-τb2 and IFN-τb3) and bIFN-τ1 in different proportions in conceptuses flushed from the uterus at days 16 and 20 of pregnancy; however, no transcripts for any of the bIFN-τ subtypes were detected in embryos older than day 25 of pregnancy. The amount of full-length protected IFN-τb3 was approximately 60% of that for IFN-τb2 and bIFN-τ1. Notably, this particular clone (IFN-τb2) showed a polymorphism because one RPA replicate had no full-length protected transcripts in a day 20 embryo preparation. These data suggest that there are at least four distinct bIFN-τ genes, or at least alleles, and they are not equally expressed in the bovine trophoblast. In addition, critical transcriptional regulatory elements of bIFN-τ genes likely are located distal to the proximal 1.8 kb of the promoter region.

INTRODUCTION

In ruminants, the functional activity of the corpus luteum (CL) is maintained by molecules secreted from live conceptuses. In cyclic cows the uterine endometrium produces prostaglandin $F_2\alpha$ ($PGF_2\alpha$), which is transported to the ovary by a counter-current transfer mechanism, and causes cessation of progesterone production and structural demise of the CL (Hixon and Hansel, 1974; Lukaszewska and Hansel, 1980). In pregnant cows, protein products from developing embryos prolong the functional lifespan of CL and production of progesterone far beyond the normal estrous cycle (Betteridge et al., 1980; Northey and French, 1980). Daily infusion of conceptus

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

homogenates or transfer of trophoblastic vesicles to cyclic cows or ewes successfully extended the estrous cycle (Heyman et al., 1984; Northey and French, 1980; Rowson and Moor, 1967). The active component was a heat-labile, species-specific, protease-sensitive Type-I interferon structurally related to the IFN-ω subtype (Charlier et al., 1989; Charpigny et al., 1988; Imakawa et al., 1989; Stewart, 1990; Stewart et al., 1989; Stewart et al., 1990). These interferons, which are 175 amino acids in length, possess full antiviral (Short et al., 1991) and antiproliferative (Davidson et al., 1994; Skopets et al., 1992) activities. These proteins, now termed bovine interferon-tau (bIFN-τ), appear to be encoded by multiple genes (Flint et al., 1991; Hansen et al., 1991; Imakawa et al., 1989; Roberts et al., 1991; Ryan and Womack, 1993) with distinct 5'-promoter and 3'-flanking sequences, which led to assigning them as another subtype of type I IFN (Hansen et al., 1991).

IFN-τ acts on the endometrial luminal epithelium to prevent the rapid increase in oxytocin receptors (OTR) that occurs just before luteolysis in cyclic ruminants (Ayad and Wathes, 1989; Ayad et al., 1991; Horn et al., 1998; Lamming et al., 1995; Wathes and Hamon, 1993; Wathes et al., 1996). The physiological mechanism by which IFN-τ interacts with the endometrial epithelium to prevent OTR formation is not well defined, but involves prevention of up-regulation of endometrial receptors for estrogen (ER) (Spencer et al., 1995; Spencer and Bazer, 1996; Spencer et al., 1996). Estradiol-17β, presumably acting through endometrial ER, up-regulates endometrial OTR formation (Pimentel et al., 1986; Spencer and Bazer, 1996), which leads to episodic release of prostaglandin $F_{2}\alpha$ ($PGF_{2}\alpha$) (Flint et al., 1990; Hixon and Flint, 1987b; Xiao and Goff, 1999). In addition, IFN-τ may act on the enzymes involved in prostaglandin synthesis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

IFN-τ stimulates endometrial production of an intercellular, PG-synthesis inhibitor (Thatcher et al., 1989; Thatcher et al., 1992), and in addition, it inhibits prostaglandin synthetase or cyclooxygenase-2 (COX-2) directly (Hixon and Flint, 1987b; Xiao et al., 1998).

IFN-τ genes are intronless, like other Type I IFN. The promoter regions, up to 100 bases upstream from the transcription start site, are almost completely conserved across different IFN-τ genes and across species (Hansen et al., 1991). There are several putative regulatory elements in the proximal promoter region. Among them, multiple copies of the viral response element, GAAANN, are scattered broadly in the region (MacDonald et al., 1990). A similar site to the positive regulatory domain 2 (PRD 2), TGGGAAATTCC, is also represented in this region. Other putative regulatory elements include the10 base Ets motif (core: GGAA) and a palindromic negative regulatory domain (NRD1) which overlaps the PRD2 site. Recently, it was found that both Ets-2 and NRD1 sites are functionally active (Ezashi et al., 1998; Guesdon et al., 1996). Conspicuously, most of these motifs are found further upstream than in human INF-α and -β genes, which function as strong promoters with only 120 bp beyond the site of transcription initiation (Goodbourn and Maniatis, 1988; Ragg and Weissmann, 1983). In the 3' untranslated region (3'UTR), the extreme conservation of sequences found across subtypes and across species made it possible to distinguish the IFN-τ genes from structurally similar IFN-ω genes (Hansen et al., 1991; Stewart et al., 1990).

Despite the apparent presence of viral response elements, this promoter showed limited response to virus in leukocytes (Cross and Roberts, 1991) and to double stranded RNA in trophectoderm (Farin et al., 1991), and there was no effect of virus nor poly

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(I)(C) on human choriocarcinoma cells (Jar) transfected with a bIFN-τ promoter-reporter gene plasmid (Guesdon et al., 1996b; Leaman et al., 1994). Thus, it is clear that virus is not a primary inducer of these genes. Earlier experiments utilizing in vitro-produced bovine embryos indicated that maternally-derived factors enhance IFN-τ expression once it has been initiated (Hernandez-Ledezma et al., 1992b). However, several factors including cAMP, TPA (phorbolester), bIFN-α_1, and retinoic acid did not enhance IFN-τ promoter (1.8 kb) / reporter plasmid expression in stably transfected Jar cells (Leaman et al., 1994). Thus, it is not yet clear which external stimuli are required for the full transcription of these genes. In addition, the cloned bIFN-τ gene promoter (bIFN-τ1) is so weak that it is not expressed in the Jar cells unless an exogenous enhancer is provided (Guesdon et al., 1996).

Studies to measure the copy number of these genes suggest that there are at least four subtype genes (Anthony et al., 1988; Helmer et al., 1987; Iannuzzi et al., 1993; Imakawa et al., 1989; Ryan and Womack, 1993). However, why so many copies of the gene have arisen is not clear. One possibility is that the different gene products possess different biological potency with regard to blocking $PGF_{2}\alpha$ release. Another possibility is that promoter regions of some genes are different than others so that expression of certain IFN-τ occur at different time points to ensure unusually high production of these proteins over the period of maternal recognition of pregnancy. Some reports support these hypotheses by showing differential expression of distinct IFN-τ genes and different biological activities of their products in sheep (Ealy et al., 1998; Nephew et al., 1993). However, mechanisms involved in gene expression have not been illustrated clearly, partly due to lack of well defined trophoblast cell lines from cattle or sheep, and partly

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

due to lack of available different bIFN-τ promoters. Unfortunately, few promoters have been cloned and studied (Hansen et al., 1991; Stewart et al., 1990) despite strong indications of the presence of multiple copies of these genes. Therefore, the aims of this experiment were firstly to clone and sequence several bIFN-τ genes, and secondly to verify whether differential transcriptions of the cloned bIFN-τ genes are present during early gestation in cows.

MATERIALS AND METHODS

Preparation of Probes for Southern Blotting

To clone full length bIFN-τ genes, two ^{32}P-labelled probes were prepared. To identify bIFN-τ genes selectively, a specific probe was generated from the 3'-UTR of a known bIFN-τ gene (designated as bIFN-τ1 herein; Hansen et al., 1991). This 250 base probe was generated using asymmetric polymerase chain reaction (PCR) amplification. To prepare template DNA, a plasmid vector containing both the coding region and 3'UTR (pBTPLacZTP; a generous gift from Dr. M Roberts, University of Missouri-Columbia) was cut with BglII (New England Biolabs, Beverely, MA). The BglII recognition site was located adjacent to the translation stop codon, so enzymatic digestion allowed the Taq polymerase to run off before amplification of coding sequences. The primer was designed to anneal to the first putative poly (A) signal allowing amplification toward the 5' end of this DNA construct. The primer sequence was: 5'-GATGTACAAATGAAATGT-3'. The reaction volume was 15 μl consisting of PCR buffer [(50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.001% gelatin (w/v), 1.5 mM of $MgCl_2$

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

], 2.5 U of Taq polymerase (Perkin-Elmer Cetus, Norwell, CT), 50 µM of each dATP, dGTP, dTTP, 3.85 µM unlabelled dCTP (Amersham Pharmacia Biotech, Piscataway, NJ) and 5.0 µl of α-^{32}P-dCTP (12.5 pM; ICN, Cleveland, OH), 2 µg of template DNA, and 100 ng of primer. The PCR amplification was carried out using a Perkin-Elmer thermocycler (DNA Thermal cycler, Perkin-Elmer Cetus, Norwalle, CT). A total of 30 cycles of amplification were performed with denaturation (95°C, 30 sec), annealing (53°C, 30sec), and primer extension (72°C, 3 min).

To generate the second probe, a 1.8 kb bIFN-τ1 promoter fragment was isolated from pBTP-CAT plasmid (a generous gift from Dr. RM Roberts, University of Missouri-Columbia) after digestion with BamH1 and NcoI (New England Biolabs, Beverely, MA) and was ^{32}P-labelled using a random priming kit according to the manufacturer's instructions (Gibco BRL, Cat # 18187-013, Grand Island, NY). The reaction was conducted in a total 50 µl of volume with 25 ng of template DNA, nucleotides (0.05 mM of each dATP, dGTP, and TTP), random primer mix, α-^{32}P-dCTP (12.5 pM; ICN, OH), and 4 U of Klenow fragment DNA polymerase I for 2 h at room temperature. The probe was purified by passing through a sephadex G-50 column.

Preparation of Probes for Ribonuclease Protection Assay

To prepare antisense RNA probes, all of the newly cloned bIFN-τ gene coding regions (590 bp) were subcloned into pBluescript II KS$^-$ vector (Strategene, La Jolla, CA) after isolation by cutting with Nco1 and BglII (New England BioLabs, Beverly, MA). The bIFN-τ1 gene coding region was also subcloned in the same vector to serve as a reference. Upon subcloning, their orientation and nucleotide sequences were determined at Macromolecular Resources at Colorado State University using an Applied Biosystems

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Model 377 DNA sequencer. All of the insert DNAs were in the T3 promoter to T7 promoter direction. Antisense strand RNA probes were made from these constructs by incorporating ^{32}P-UTP using a Maxiscript in vitro transcription kit (Ambion, Austin, TX). The reaction volume was 20 μl containing transcription buffer (Ambion's *in vitro* transcription buffer), 10mM of each ATP, CTP, GTP, 0.1mM of UTP, 5 μl of α-^{32}P-UTP (16.5 pM; ICN, Cleveland, OH), 2 μg of linearized template DNA, and 10 U of T7 RNA polymerase plus 10 U of RNase inhibitor. The reaction was carried out at 4°C overnight.

Preparation of Sense RNA of bIFN-τ Subtype Gene

Template DNAs were linearized by enzymatic digestion with SacI (New England BioLabs, Beverly, MA) whose recognition site was located close to T7 promoter of the plasmid vector. T3 RNA polymerase, therefore, transcribed sense RNA. Reaction conditions were the same as for anti-sense probe generation, but equimolar (10mM) amounts of unlabelled UTP replaced the ^{32}P-labelled UTP, and T3 RNA polymerase was used instead of T7 polymerase. After overnight incubation at 4°C, RNase free DNase (10 U; Ambion, Austin, TX) was added to the reaction to remove DNA templates. The concentration of each RNA in each reaction was measured after purification with a G-25 spin column (Amersham Pharmacia Biotech, Piscataway, NJ). RNAs were kept at –80°C until use.

Screening and Sequencing of a Bovine Genomic Library

A bovine genomic library generated from an adult male liver was purchased from Clontech (Cat # BL1015j, Palo Alto, CA). Approximately twenty-million plaque-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

forming units (pfu) were plated with *Escherichia coli* K802 cells (Clontech, Palo Alto, CA) on Luria broth agar plates and incubated overnight at 37°C. Duplicate Hybond N membranes (Amersham Phamarcia Biotech, Piscataway, NJ) were overlaid on the plates, prehybridized for 2 h at 42°C in 5 X SSC (1xSSC = 0.15 M NaCl and 0.015 M sodium citrate), 50 % (v/v) deionized formamide, 10 X Denhardt's [100 X Denhardt's = 2% bovine serum albumin, 2 % (w/v) sodium dodecylsulphate (SDS)], and 100 µg denatured herring sperm DNA/ml. Hybridization was performed in the same buffer after adding ^{32}P-labelled bIFN-τ 3' UTR probe (10^6 c.p.m. / ml; 1.5×10^9 c.p.m / µg) at 42°C for overnight. Filters were washed twice with 2X SSC and 0.1% (w/v) SDS for 10 min at room temperature, washed twice with 1 X SSC and 0.1% SSC (w/v) for 15 min at 65°C, and then exposed to x-ray film (XAR; Eastman Kodak, Rochester, NY) plus an intensifying screen for 18 h at –80°C. Filters were then stripped to hybridize with the second probe, the 1.8kb bINF-τ1 promoter region. Briefly, the membranes were incubated in 0.4M NaOH solution at 42°C for 30 min with agitation, incubated in neutralization solution [0.1x SSC, 0.1% SDS, 0.2 M Tris HCl (pH7.5)] at 42°C for 30 min with gentle agitation, and dried at room temperature. Rehybridization of the membranes was carried out as described previously. Only plaques that hybridized with both probes were isolated and subjected to secondary screening. Procedures used for secondary screening were the same as for primary screening, except one more washing step (0.1x SSC plus 0.1% SDS for 10 min at 65°C) was added to selectively isolate plaques which bound to probes with high stringency. Positive clones (N=24) were identified, of which 21 plaques were strongly labeled. These 21 plaques were amplified, and their DNA was isolated using a polyethylene glycol (PEG) precipitation method

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(Sambrook et al., 1989). The insert DNAs were released by means of restriction endonuclease enzyme digestion. Among the restriction enzymes, only EcoR1 gave DNA fragments which were reasonably easy to subclone into sequencing vector [pBluscript II KS^- (Strategene, La Jolla, CA)]. Of the initial 21 clones, 4 clones yielded DNA fragments which hybridized with the both probes and were longer than 2.5 kilobases (kb). These 4 DNA fragments were subcloned into pBluescript KS^- vector (Stratagene, La Jolla, CA) and transformed in *E.Coli* 5αDH cells (Gibco BRL, Grand Island, NY). DNA sequencing in both direction was conducted by Macromolecular Resources at Colorado State University using an Applied Biosystems Model 377 DNA sequencer. Sequence comparisons were made using GeneWorks software (Intelligenetics, Mountain View, CA).

RNA Preparation and Ribonuclease Protection Assays

Bovine conceptuses of beef breeds were collected nonsurgically at days 16, 20, 25, 27, 30, 45, and 60. All conceptuses were obtained after conventional artificial insemination, except day 16 conceptuses, which were obtained after superovulation of cows. Up to 20 days of pregnancy, a regular Foley catheter (17G, TFX Medicals, Wycombe, England) was used to collect embryos. For conceptuses older than day 20, a metal tube of 6 mm inside diameter connected with a 50 ml syringe was inserted through the cervix around a stainless steel cervical expander; with this system the conceptus broke into pieces if it was too big to fit through the lumen. Flushing was performed by filling the uterus with modified Dulbecco's phosphate-buffered saline (PBS) (Elsden and Seidel, 1995) containing 0.1% BSA with a 50 ml syringe. Upon collection, all tissues

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

were washed in Mg^{++} and Ca^{++} -free PBS 3 times, and frozen and stored in liquid nitrogen until analyzed. Total cellular RNA was isolated by the guanidium thiocyanate method (Chomczynski and Sadler, 1987). All RNA preparations were treated with RNase-free DNase (20 U, Ambion, Austin, Texas) for 15 min at 37°C.

For the ribonuclease protection assay (RPA) with total cellular RNA, specificity of each probe was tested by hybridization with all of the other bIFN-τ sense RNA constructs. Ribonuclease protection assays were conducted using an RPA III kit (Ambion, Austin, TX), according to the manufacturer's instructions, by using 5.0 μg total cellular RNA from bovine conceptuses. RNA was hybridized with 10^5 counts per minute (c.p.m.) of each anti-sense RNA probe (1.4×10^8 c.p.m. / μg) and 10^5 c.p.m. of bovine GAPDH anti-sense RNA probe (1.4×10^8 c.p.m. / μg) at 45°C overnight. RNase (10μg/ml RNase A, 200 U/ml RNase T1) then was added to the mixture for 30 min at 37°C. Protected fragments were separated by electrophoresis in 5% (w/v) acrylamide gels containing 8 M urea. Dried gels were then exposed to XAR-5 film (Eastman Kodak, Rochester, NY) on intensifying screens for 1-2 h. Gels from three different assays, each representing distinct pools of conceptus RNA, were scanned, and optical density units for full- length bIFN-τ subtype open reading frames and GAPDH fragments were determined using Phosphoimager and Image Quant software (Molecular Dynamics, Sunnyvak, CA).

Results

Structural Analysis of bIFN-τ Gene Variants

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Of the 20 million plaques screened from the genomic library, 21 positive clones were initially detected, and 4 (designated IFN-ωb1, IFN-τb1, IFN-τb2, and IFN-τb3) were chosen for sequencing on the basis of restriction enzyme digestion and ease of subcloning. Between 2.9 kb and 3.7 kb were sequenced, depending on the individual gene. On the basis of the nucleotide sequence similarity to other bIFN-τ (Stewart et al., 1990) in the both coding region and untranslated regions, it was concluded that three clones were bIFN-τ variants with 2.3 kb of putative promoter region, and one was a bIFN-ω gene. No premature terminations were present in any of the bIFN-τ genes or the bIFN-ω clone. The three new bIFN-τ variants have 585 nucleotides of open-reading frames encoding a 195 amino acid protein that contained a putative 23 amino acid signal peptide which has been inferred by Imakawa et al., (1989) followed by a 172 amino acid mature protein (Figure 2-1 and Table 2-1). The bIFN-ω variant (IFN-ωb1) had 585 nucleotides of open-reading frame that encodes a 195 amino acid protein (Appendix 2). Sequence comparisons revealed that the coding region of this clone matched a bIFN-ω gene previously cloned (Stewart et al., 1990) with 100 % homology (Appendix 2); however, there were a number of base pair differences in the putative promoter regions (Appendix 2).

Overall sequence differences were highly variable among the bIFN-τ subtypes: 0.9-14 % in the promoter region, 0.0 –7.0 % in the coding region, and 0.0 –9.8 % in deduced amino acid sequences (Figure 2-2 and Table 2-2). Of the three bIFN-τ gene clones, one (IFN-τb1) showed highly variable nucleotide sequences, particularly in the 5' distal promoter region. Of the remaining 2 clones, nucleotide sequences of one clone (IFN-τb2) perfectly matched a previously cloned bIFN-τ variant (bIFN-τ2) (Stewart et

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 2-1. Alignment of Predicted Amino Acid Sequence of Bovine IFN-τ Subtype Genes. Asterisks mark amino acid sequence differences between subtypes. bIFN-τ1 and bIFN-τ2 were cloned by Hansen et al., (1991) and Stewart et al., (1990).

INF-τb1	MAFVLSLLMA	LVLVSYSPGR	SLGCYLSENH	MLGARENLRL	LAQMNRLSTH	50
bIFN-τ2	MAFVLSLLMA	LVLVSYGPGR	SLGCYLSEDH	MLGARENLRL	LARMNRLSPH	50
INF-τb3	MAFVLSLLMA	LVLVSYGPGR	SLGCYLSEDH	MLGARENLRL	LARMNRLSPH	50
bIFN-τ1	MAFVLSLLMA	LVLVSYGPGR	SLGCYLSEDH	MLGARENLRL	LARMNRLSPH	50
INF-τb3	MAFVLSLLMA	LVLVSYGPGR	SLGCYLSEDH	MLGARENLRL	LARMNRLSPH	50
Consensus	MAFVLSLLMA	LVLVSYGPGR	SLGCYLSEDH	MLGARENLRL	LARMNRLSPH	50
		*	*		* *	
INF-τb1	SCLQDRKDFG	LPWEMVEGDQ	LQKDQAISVL	HEMLQQCFNL	FHTEHSSAAW	100
bIFN-τ2	PCLQDRKDFG	LPQEMVEGNQ	LQKDQAISVL	HEMLQQCFNL	FYTEHSSAAW	100
INF-τb2	PCLQDRKDFG	LPQEMVEGNQ	LQKDQAISVL	HEMLQQCFNL	FYTEHSSAAW	100
bIFN-τ1	PCLQDRKDFG	LPQEMVEGNQ	LQKDQAISVL	HEMLQQCLNL	FYTEHSSAAW	100
INF-τb3	PCLQDRKDFG	LPQEMVEGSQ	LQKDQAISVL	HEMLQQCFNL	FHIEHSSAAW	100
Consensus	PCLQDRKDFG	LPQEMVEGNQ	LQKDQAISVL	HEMLQQCFNL	FYTEHSSAAW	100
	*	* *		*	*	
INF-τb1	NTTLLEQLCT	GLHQQLDDLD	ACLGQVMEEK	DSALGRMGPI	LTVKKYFQGI	150
bIFN-τ2	NTTLLEQLCT	GLQQQLEDLD	ACLGPVMGEK	DSDMGRMGPI	LTVKKYFQGI	150
INF-τb2	NTTLLEQLCT	GLQQQLEDLD	ACLGPVMGEK	DSDMGRMGPI	LTVKKYFQGI	150
bIFN-τ1	NTTLLEQLCT	GLQQQLEDLD	ACLGPVMGEK	DSDMGRMGPI	LTVKKYFQGI	150
INF-τb3	NTTLLEQLCT	GLQQQLEDLD	ACLGPVMGEK	DSDMGRMGPI	LTVKKYFHDI	150
Consensus	NTTLLEQLCT	GLQQQLEDLD	ACLGPVMGEK	DSDMGRMGPI	LTVKKYFQGI	150
		* *	* *	**	**	
INF-τb1	HVYLKKKEYS	DCAWEIIRVE	MMRALSSSTS	LQERLRKIGG	DLNSS	195
bIFN-τ2	HVYLKEKEYS	DCAWEIIRME	MMRALSSSTT	LQKRLRKMGG	DLNSL	195
INF-τb2	HVYLKEKEYS	DCAWEIIRME	MMRALSSSTT	LQKRLRKMGG	DLNSL	195
bIFN-τ1	HVYLKEKEYS	DCAWEIIRVE	MMRALSSSTT	LQKRLRKMGG	DLNSL	195
INF-τb3	HVYLKEKEYS	DCAWEIIRVE	MMRALSSSTT	LQKRLRKMGG	DLNSL	195
Consensus	HVYLKEKEYS	DCAWEIIRVE	MMRALSSSTT	LQKRLRKMGG	DLNSL	195
	*	*	*	* *	*	

```
IFN-τb1     .....T..T. .......... .......... T......... ........A. .......... .......... .....  75
IFN-τb2     .....C..C. .......... .......... C......... ........G. .......... .......... .....  75
bIFN-τ2     .....C..C. .......... .......... C......... ........G. .......... .......... .....  75
bIFN-τ1     .....C..C. .......... .......... C......... ........G. .......... .......... .....  75
IFN-τb3     .....C..C. .......... .......... C......... ........G. .......... .......... .....  75
Consensus   ATGGCYTTYG TGCTCTCTCT ACTGATGGCC YTGGTGCTGG TCAGCTACRG CCCGGGACGA TCTCTGGGTT GTTAC  75

IFN-τb1     .........A .......... .......... .......... .......... ..A....... ........CA .....  150
IFN-τb2     .........G .......... .......... .......... .......... ..G....... ........TC .....  150
bIFN-τ2     .........G .......... .......... .......... .......... ..G....... ........TC .....  150
bIFN-τ1     .........G .......... .......... .......... .......... ..G....... ........TC .....  150
IFN-τb3     .........G .......... .......... .......... .......... ..G....... ........TC .....  150
Consensus   CTGTCTGAGR ACCACATGCT AGGTGCCAGG GAGAACCTCA GGCTCCTGGC CCRAATGAAC AGACTCTCYM CTCAT  150

IFN-τb1     .......... .A........ .......... .....CTG.. .......... ...TGA.... .......... .C...  225
IFN-τb2     .......... .G........ .......... .....TCA.. .......... ...CAA.... .......... .T...  225
bIFN-τ2     .......... .G........ .......... .....TCA.. .......... ...CAA.... .......... .T...  225
bIFN-τ1     .......... .G........ .......... .....TCA.. .......... ...CAA.... .......... .T...  225
IFN-τb3     .......... .G........ .......... .....TCA.. .......... ...CAG.... .......... .T...  225
Consensus   YCCTGTCTGC ARGACAGAAA AGACTTTGGT CTTCCYYRGG AGATGGTGGA GGGYRRCCAG CTCCAGAAGG AYCAG  225

IFN-τb1     .......... .......C.. .......... ......T... ........C. ..C....... .......... .C...  300
IFN-τb2     .......... .......T.. .......... ......T... ........T. ..C....... .......... .C...  300
bIFN-τ2     .......... .......T.. .......... ......T... ........T. ..C....... .......... .C...  300
bIFN-τ1     .......... .......C.. .......... ......C... ........T. ..C....... .......... .C...  300
IFN-τb3     .......... .......C.. .......... ......T... ........C. ..T....... .......... .T...  300
Consensus   GCTATCTCTG TGCTCCAYGA GATGCTCCAG CAGTGCYTCA ACCTCTTCYA CAYAGAGCAC TCGTCTGCTG CYTGG  300

IFN-τb1     .......... .......... .......... ..A.....T. .......... T........T .......... .G.AG  375
IFN-τb2     .......... .......... .......... ..G.....A. .......... G........C .......... .C.CA  375
bIFN-τ2     .......... .......... .......... ..G.....A. .......... G........C .......... .C.CA  375
bIFN-τ1     .......... .......... .......... ..G.....A. .......... G........C .......... .C.CA  375
IFN-τb3     .......... .......... .......... ..G.....A. .......... G........C .......... .C.CA  375
Consensus   AACACCACCC TCCTGGAGCA GCTCTGCACT GGRCTCCAWC AGCAGCTGGA KGACCTGGAY GCCTGCCTGG GSCMR  375

IFN-τb1     .......A.. ....A..... ..C.C..... .......... .......... C......... ........G. GC...  450
IFN-τb2     .......G.. ....A..... ..A.A..... .......... .......... T......... ........G. GC...  450
bIFN-τ2     .......G.. ....A..... ..A.A..... .......... .......... T......... ........G. GC...  450
bIFN-τ1     .......G.. ....A..... ..A.A..... .......... .......... T......... ........G. GT...  450
IFN-τb3     .......G.. ....G..... ..A.A..... .......... .......... T......... ........T. AC...  450
Consensus   GTGATGGRAG AGAARGACTC TGMCMTGGGA AGGATGGGCC CCATTCTGAC YGTGAAGAAG TACTTCCAKG RYATC  450

IFN-τb1     .....G.... .....A.G.. G........C .......... .......... ....G..... .......... .....  525
IFN-τb2     .....C.... .....G.A.. A........T .......... .......... ....A..... .......... .....  525
bIFN-τ2     .....C.... .....G.A.. A........T .......... .......... ....A..... .......... .....  525
bIFN-τ1     .....C.... .....G.A.. A........T .......... .......... ....G..... .......... .....  525
IFN-τb3     .....C.... .....G.A.. G........T .......... .......... ....G..... .......... .....  525
Consensus   CATGTSTACC TGAAARARAA RGAATACAGY GACTGCGCCT GGGAAATCAT CAGARTGGAG ATGATGAGAG CCCTC  525

IFN-τb1     .......... ...G...... .G........ ........A. .......... .......TC. ...        588
IFN-τb2     .......... ...C...... .A........ ........G. .......... .......CT. ...        588
bIFN-τ2     .......... ...C...... .A........ ........G. .......... .......CT. ...        588
bIFN-τ1     .......... ...C...... .A........ ........G. .......... .......CT. ...        588
IFN-τb3     .......... ...C...... .A........ ........G. .......... .......CT. ...        588
Consensus   TCTTCATCAA CCASCTTGCA ARAAAGGTTA AGAAAGATRG GTGGAGATCT GAACTCAYYT TGA        588
```

Figure 2-1. Homologies between IFN-tau Coding Regions. bIFN-τ1 was cloned by Hansen et al., (1991) and bIFN-τ2 was cloned by Stewart et al., (1990) respectively. Only mismatches are shown.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 2-2. Pairwise Comparisons of Differences in Coding Sequences of bIFN-τ Variants

	IFN-τb1	IFN-τb2	IFN-τb3	bIFN-τ1*	bIFN-τ2**
IFN-τb1		9.8	9.8	9.8	9.8
IFN-τb2	6.8		2.6	1.2	0
IFN-τb3	7.0	1.8		2.6	2.6
bIFN-τ1	7.0	0.7	1.8		2.6
bIFN-τ2	6.8	0	1.8	0.7	

Upper right: **Amino Acid Sequence Difference (%)**

Lower left: **Nucleotide Sequence Difference (%)**

* bIFN-τ1 – Cloned by Hansen et al., (1991)
** bIFN-τ2 – Cloned by Stewart et al., (1990)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

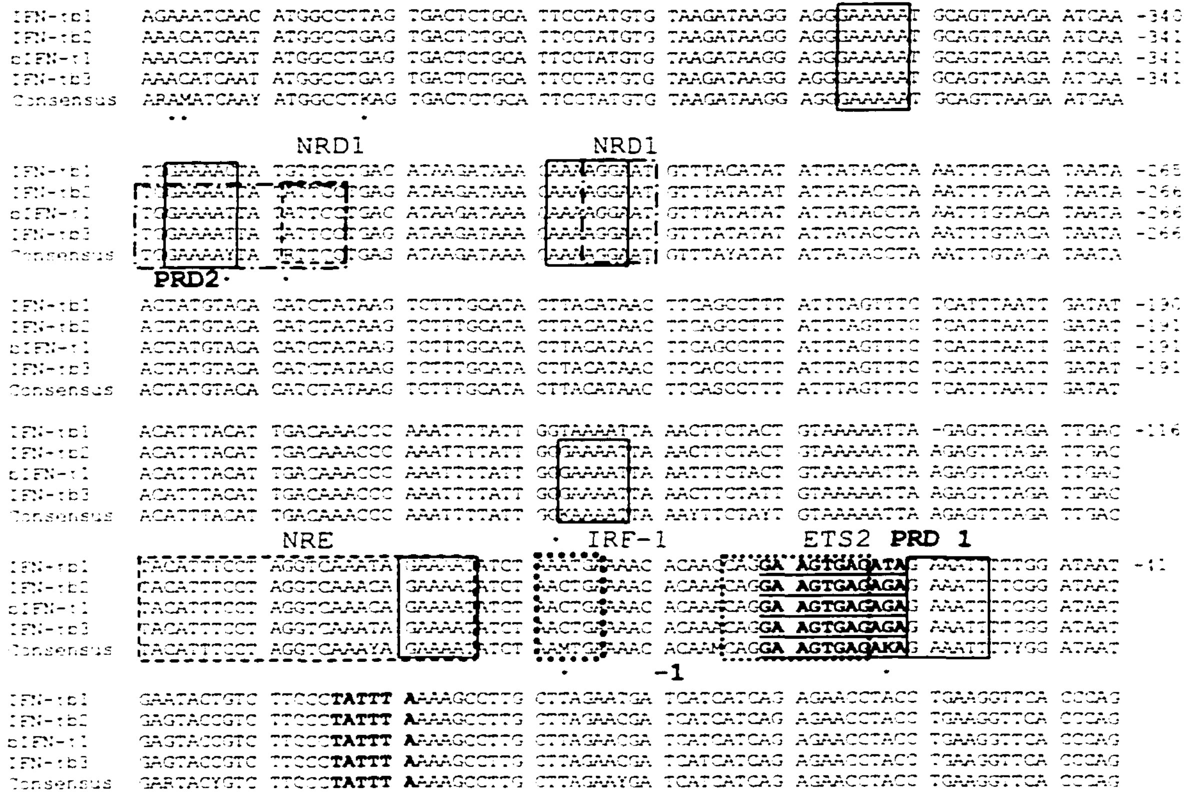


Figure 2-2. Homologies between IFN-tau Promoter Regions.
Probable regulatory elements are indicated as follows: viral regulatory elements boxed in solid lines; negative regulatory domain-1 (NRD 1) sites are boxed in broken lines and marked; interferon responding factor-1 binding site (IRF-1), positive regulatory domain-1 and –2 (PRD 1 and 2) sites are also boxed in broken lines and marked; NRE site is the negative regulatory element found in renin and other genes (Barret et al., 1992). TATA boxes and transcription start sites are indicated with bold characters. An ETS-2 site is boxed in a broken line. bIFN-τ1 was cloned by Hansen et al., (1991). * Asterisks mark sequence differences between subtypes.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

al., 1990). All of the known putative regulatory elements including several virus inducible elements (GAAANN), a couple of interferon responding factor-1 (IRF-1) binding sites (AAGTGA or AACTGA), and an Ets-2 binding site (CAGGAAGTG) were remarkably well conserved across the clones (Figure 2-2) except one of new clones, IFN-τb1, was missing one IRF-1 binding site (mutation from AACTGA to AAATGA) and one NRD-1 site (mutation from GTTCC to ATTCC). A putative TATA box (TATTTAA) starting 33 bases upstream from the transcription start site and 100 bases from the ATG start codon was present in all clones.

The IFN-τb1 clone showed a base mismatch at position –79 where an A residue mutated to C, compared with rest of clones. This position may be important for transcriptional expression of bIFN-τ genes by Ets-2 factor as indicated by Ezashi et al.(1998). In addition, there was a single base pair mismatch in the negative regulatory element, located in the region –95 to –116, of IFN-τb3 and IFN-τb1 clones (from C to T residue transition). In the distal silencer element, located in the region –302 to –338, three base pair mismatches were found among the bIFN-τ clones. However, the roles of these mismatches remain uncertain. In the distal promoter region that extended approximately 500 bp upstream from the cloned bIFN-τ1 gene (Hansen et al., 1991), several putative transcriptional elements were found. Among them are a couple of Ets-1 binding sites and CEBP binding sites (Appendix 1); however, their functionalities remain to be elucidated.

Differences in the inferred amino acid sequences for the bIFN-τ genes are illustrated in Table 2-1. Clone IFN-τb1 displayed a total of 40 nucleotide changes accounting for 20 aa substitutions compared to bIFN-τ1. The remaining 2 clones showed

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

minor variability: 4 nucleotide changes accounting for 2 aa substitutions for IFN-τb2 and 10 nucleotide changes accounting for 5 aa substitution for IFN-τb3. The nucleotide and aa sequences of IFN-τb2 was perfectly matched to those of bIFN-τ2 (Figure 2-1, Table 2-1). These various aa substitutions resulted in different bIFN-τ proteins differing in charge and isoelectric point.

mRNA Abundance of Bovine Conceptus IFN-τ Variants

The relative abundance of mRNA for IFN-τb1, IFN-τb2, IFN-τb3 and bIFN-τ1 variants were assessed at different developmental stages of bovine embryos. As shown on representative autoradiographs (Figures 2-3 and 2-4), mRNA was detected for the IFN-τb2, IFN-τb3, and bIFN-τ1 variants up to day 25 conceptuses, as reflected by the presence of full-length protected riboprobe fragments after RNase digestion. However, one of new clones (IFN-τb1) had no detectable transcripts at any stages of pregnancy examined even though this subtype gene had a well conserved proximal promoter region. Quantities of bIFN-τ1 and IFN-τb2 mRNA were similar within each stage of development and were higher ($P < .01$) than that of IFN-τb3 over most of developmental stages and replicates tested (Figure 2-5). A possible polymorphism was noted in the IFN-τb3 variant, which was barely expressed on day 20 in one embryo (Figure 2-4). This was confirmed by protected fragments of smaller size which represent the presence of bIFN-τ transcripts that contained stretches of sequences that were identical to parts of these probes were abundant up to day 25 embryos, but ceased to appear later, indicating that after day 25 of pregnancy, all of the bIFN-τ genes, regardless of subtype, shut down their transcriptional activity.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

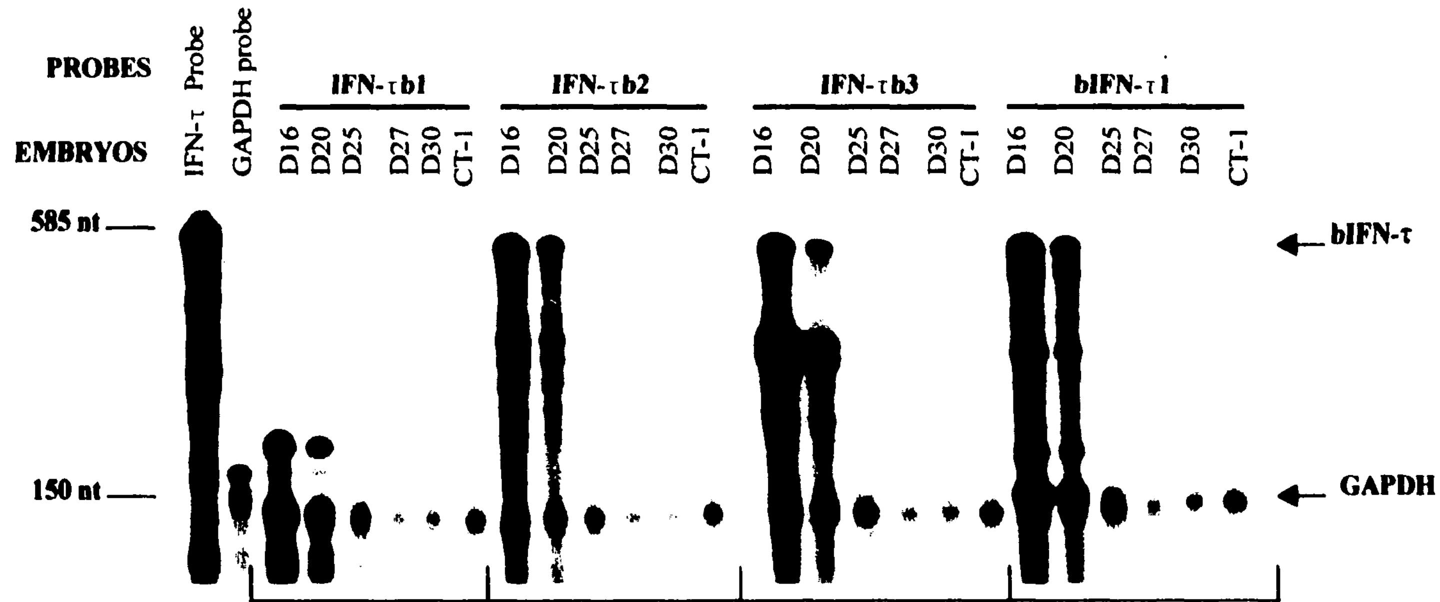
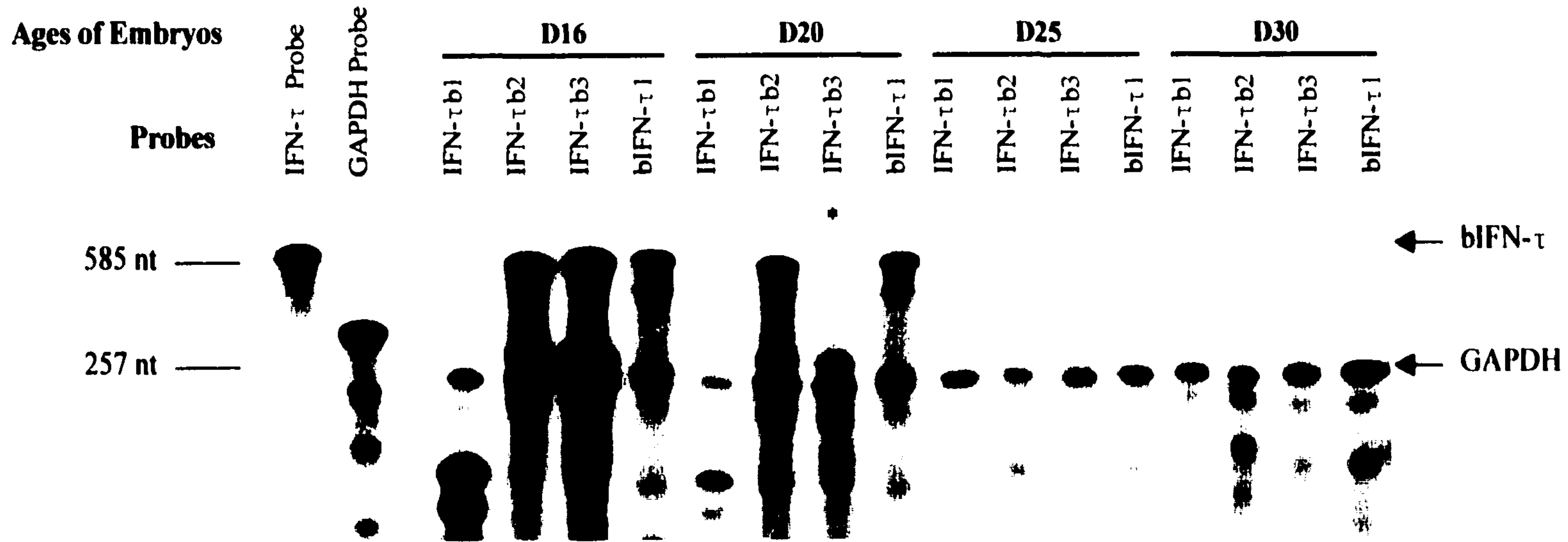


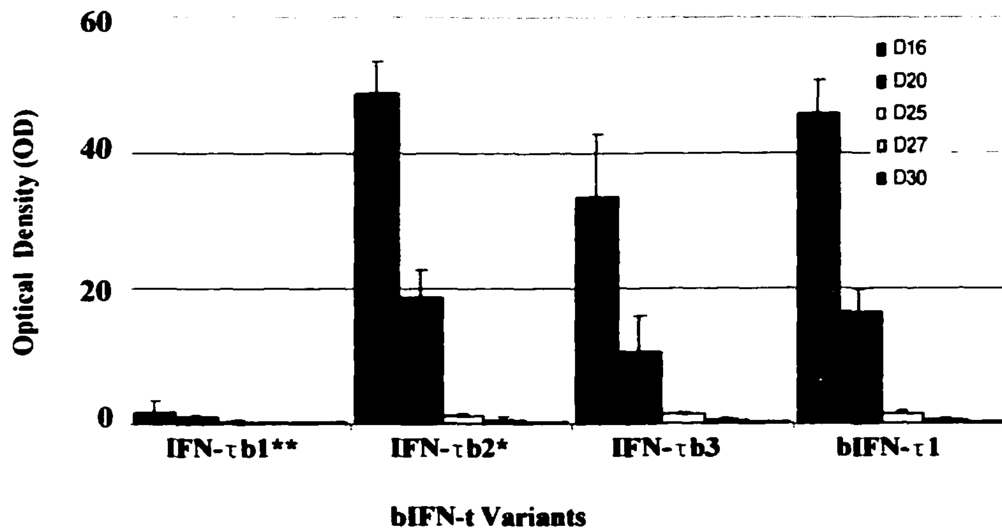
Figure 2-3. Results of RNase Proctection Assay Using Total Cellular RNA from Different Developmental Stages of Bovine Embryos. RPA were performed on total cellular RNA (5 μg) from bovine conceptuses by using ^{32}P-labeled riboprobes for bIFN-τ variants and for bovine GAPDH as internal control. The sizes of riboprobes for bIFN-τ are 585 nt regardless of subtypes, so only a single probe is shown here. The protected fragments were 585 nt for all bIFN-τ variants and 150 nt for GAPDH. Notice that mRNA representing each bIFN-τ subtypes ceased to appear from day 27 of gestation. CT-1 is a bovine embryo trophoblast cell line. In these cells, none of bIFN-τ transcripts was detected.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



* Notice that IFN-τb2 transcripts are missing in this particular day 20 embryo, possibly due to allelic differences.

Figure 2-4. Results of RPA using Total Cellular RNA from Various Developmental Stages of Bovine Embryos different from Fig 2-3. All procedures for RPA were same as in Fig 2-4, however, different pools of embryos were used in this replicate.



* Relative amounts of IFN-τb3 were lower than IFN-τb2 within and between stages of development up to day 20 ($P<.01$).

** The amount of IFN-τb1 mRNA was lower ($P<.01$) than that of other variants up to day 25, at which time the transcripts of bIFN-t were barely detectable.

Figure 2-5. Differences in Amount of mRNA for bIFN-τ Variants During Early Conceptus Development. Optical densities (OD) for each mRNA were normalized by OD of GAPDH in each given reaction. There were 5 replicates in this experiments using different embryos or pools of embryos, and error bars represent standard deviations. OD for each mRNA within and between stages of development were analyzed using ANOVA plus Tukey's multiple comparison test.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Discussion

The present studies confirm that bIFN-τ is encoded by multiple, distinct genes in cattle. However, there are no available data indicating exactly how many genes exist; some of the constructs cloned could be allelic. The functional significance of multiple genes and proteins for bIFN-τ remain to be elucidated. However, they might play a distinct role in the process of maternal recognition of pregnancy as suggested by work using ovine counterparts oIFN-τ variants (Ealy et al., 1998). In addition, there is a suggestion that individual bIFN-τ subtype proteins exert different degrees of antiviral and antiproliferative activity (Alexenko et al., 1997).

Current studies focused on the finding new bIFN-τ variants with sequence differences in both promoter and coding regions with an objective of finding new insights into mechanisms involved in transcriptional regulation of this gene. In contrast to a previous report (Cross and Roberts, 1991), we found the 1.8 kb bIFN-τ1 promoter would not drive reporter gene expression in human chorionic carcinoma cells (Jar cells) (not presented). Jar cells tend to drift in genetic makeup in culture, which might explain this discrepancy. Furthermore, endogenous IFN-τ is not found in these cells, and they may be missing some critical components for activation of this gene promoter. In another experiment (not presented), the CT-1 cell line, which originated from bovine trophectoderm, did not support any detectable levels of expression of the promoter / reporter gene construct (bIFN-τ1 1.8kb- luc or gal). Later, it was found that the CT-1 cells did not transcribe any bIFN-τ variant mRNAs to any extent. Thus, it is not yet clear if the lack of activity of the 1.8 kb bIFN-τ1 promoter is due to an incomplete, short

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

promoter or absence of cell lines that can support this promoter. In any case, the cloned bIFN-τ 1 gene promoter seems so weak that unless an external enhancer element or stimulus is provided, little or no expression is induced in transfected cell lines (Ezashi et al., 1998; Guesdon et al., 1996).

A subtle nucleotide change in a transcriptional regulatory motifs might affect promoter activity greatly as shown by one promoter induction assay (Ezashi et al., 1998). This group found a 2 base deletion and lack of the core GGAA motif in Ets-2 binding site of IFN-τ gene promoters abolished expression of the reporter gene (luciferase) in Jar cells co-transfected with an Ets-2 expression vector (CMV-Ets-2). We found one of our bIFN-τ clones, IFN-τb1, did not express any detectable levels of mRNA in early bovine embryos, even though the putative Ets-2 binding site was well conserved with intact core motif. Notably, this subtype showed extreme sequence differences in the distal promoter region compared to the other subtypes, which showed minor degrees of base substitutions (14% vs 2%) (Appendix I). This may indicate that sustaining the high degree of bIFN-τ gene expression is regulated by an element(s) located farther upstream than in known sequences. All of the new bIFN-τ gene clones have 2.3 kb putative promoter regions which are approximately 500 bp longer than previously cloned bIFN-τ 1 promoter (approximately 1.8 kb). In the distal 5' region, several transcriptional regulatory elements including Ets-1 binding sites and CEBP binding sites were found. However, the functionality of those motifs has not been tested .

As indicated by studies using the closely related oIFN-τ isoforms, multiple bovine IFN-τ gene products may exert different roles at the level of the feto-maternal interface with different biological activities. In structure-function studies (Klemann et

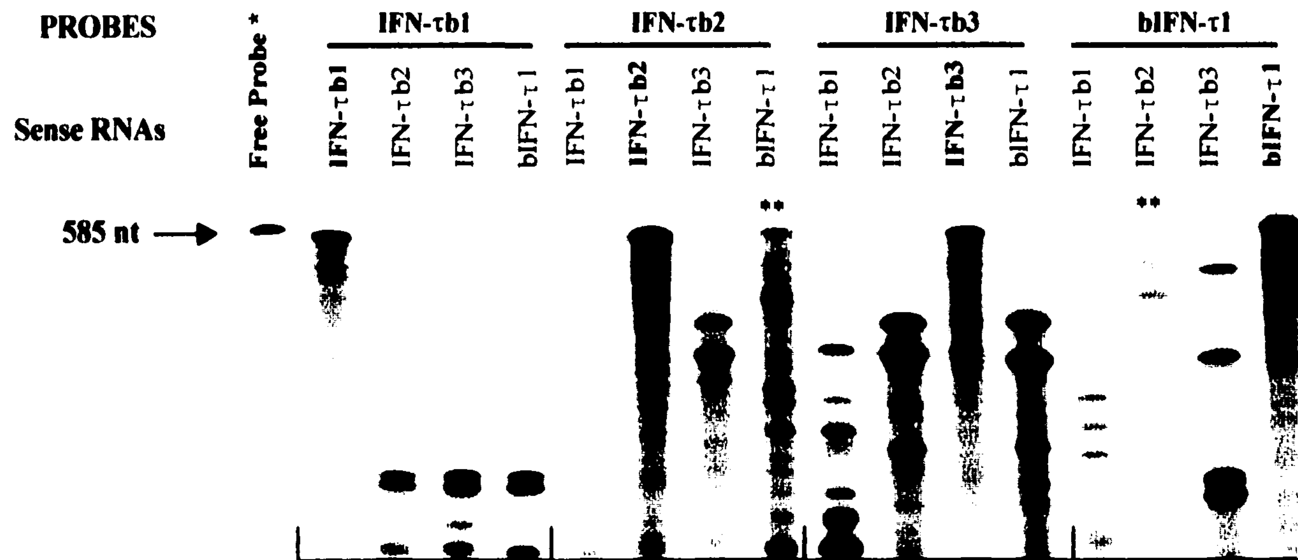
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

al., 1990; Li and Roberts, 1994b; Niswender et al., 1997; Senda et al., 1995), mutation of amino acid residue(s) in the carboxy terminal of oIFN-τ affected receptor binding, antiviral, and antiproliferative activities significantly. The same effect also was observed in the human IFN-α4 gene (Cheetham et al., 1991). Thus, the various substitutions found in bIFN-τ genes in the present study could alter the biologically active domains as well as other regions of bIFN-τ, resulting in proteins that differ in biological function. This may be true for clone IFN-τb1 if it were expressed, in which Lys 160 was substituted with Glu 160. The deletion of this position reduced antiviral activity to 14 % of control, removed antiproliferative activity completely, and had little effect on receptor binding (Li and Roberts, 1994b). Moreover, another clone, IFN-τb3, showed an aa substitution at position 126 from Gly to Asp. Glycine at this position is known to confer flexibility for receptor binding of IFN-τ molecules (Senda et al., 1995).

The ribonuclease protection assay is a sensitive, quantitative means of distinguishing mRNA populations that differ from each other in as little as a single base. In our experiments, each IFN-τ anti-sense probe differentiated from the others, even if there were only 4 base differences (IFN-τb2 vs bIFN-τ1 in Figure 2-6). Notably, several smaller bands appeared if sense RNA and anti-sense probe were not matched perfectly. The smaller bands arose because of the considerable degree of sequence identity present among the bIFN-τ subtype genes tested. The same features were observed when total cellular mRNA from various developmental stages of bovine embryos was used. However, this may partially be due to the presence of allelic variants of the genes in addition to sequence similarity. By using highly specific probes, amounts of particular subtype gene transcripts were measured successfully.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



* The probes had different sequences representing individual bIFN-τ subtypes. However, the size was 585 nt regardless of subtype, thus only a single probe is shown here.

** IFN-τb2 and bIFN-τ1 shared more than 99 % homologies in their coding regions (4 nucleotide differences out of 585 nucleotides), therefore the background signals due to incomplete digestion of probes by RNases was relatively high compared to other combinations. However, the amount of signal were less than 5 % of perfectly matched combinations (i.e., IFN-τb2 vs IFN-τb2 and bIFN-τ1 vs bIFN-τ1).

Figure 2-6. Test of Specificity of Bovine IFN-τ Subtype Probes. RNase protection assays were performed using *in vitro transcribed* sense RNAs (10 ng) of different bIFN-τ subtypes plus anti-sense riboprobes (10^5 cpm /probe: 1.4 x 10^8 cpm / μg). Full length protected fragments were 585 nt for all IFN-τ riboprobes. Perfectly matched sequenced are represented in bold characters.

On the basis of the amount of mRNA corresponding to the bIFN-τ subtype genes, it appears that these genes are differentially transcribed during pregnancy (Figures 2-4 and 2-5). Transcripts for IFN-τb2 and bIFN-τ1 predominated, compared to those for IFN-τb3, on day 20 of gestation. In contrast to IFN-τb2 and bIFN-τ1 mRNA, IFN-τb1 transcripts were not detected in any stage embryos or in the different pools of embryos tested. Obviously, there were several smaller protected bands were present, indicating that very similar but not quite identical transcripts are abundant in given samples of mRNA. Furthermore, amounts of transcripts for IFN-τb3 were quite variable from one embryo to another. On one occasion, they were totally missing in day 20 bovine embryo preparations, indicating that this subtype gene might be a polymorphic allelic gene.

Overall transcriptional expression of bIFN-τ lasted up to day 25 of pregnancy at which time most of subtype gene transcripts were barely detectable. These results are not in agreement with a previous report that bIFN-τ protein could be detected up to day 36 of pregnancy (Godkin et al., 1988). However, considering that IFN-τ gene expression nearly ceased at the time that the trophoblast and the uterine epithelium make contact as indicated by studies using oIFN-τ genes, the complete turn off timing of these genes seems to be between day 25 and day 27 of pregnancy. Furthermore, recent reports utilizing the same technique support our observations (Liu et al., 1997; Winkelman et al., 1999). The observed pattern of transcripts further suggests that expression of these genes peaked around day 16 of pregnancy and then declined gradually up to day 25 of pregnancy at which time transcripts are hardly detectable. Except IFN-τb1 which showed no transcription, this phenomenon was universally observed regardless of subtypes of the gene.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

In summary, we have confirmed the existence of at least three different functional bIFN-τ genes and their differential expression patterns during early pregnancy of cattle. A major function of multiple copies may simply be to increase the amounts of this protein produced, which after all is needed for perpetuation of the species. This gene multiplicity suggests that bIFN-τ may be functionally heterogeneous. The differences in amino acid sequences of at least some of the IFN-τ genes are certainly substantial enough to allow for proteins with quite different properties. This appears to be the first documentation of a possible polymorphic bIFN-τ variant (IFN-τb3), and a transcriptionally inert bIFN-τ variant (IFN-τb1) showing extreme sequence variation in the distal 5' upstream region but little variation proximally, suggesting that regulation of expression of these genes may be controlled by the putative regulatory elements located in the far upstream region of the promoters.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER III

A DISTAL 500 BP UPSTREAM REGION OF THE BOVINE INTERFERON-TAU (bIFN-τ) GENE CONTAINS ENHANCER-LIKE ACTIVITY

ABSTRACT

Transcriptionally active nuclei were prepared from day 20 and 45 bovine embryos. Jar cells. Hela cells. and STO cells. Rheus monkey simian virus promoter directed efficient transcription by RNA polymerase in all of these nuclei, whereas bIFN-τ promoters directed more active transcription in day 20 bovine embryo nuclei than other cell nuclei. A 500 bp DNA fragment from the 5' end of a new bIFN-τ clone. IFN-τb2. functioned as a general enhancer. Insertion of this sequence into both tyrosine kinase and prolactin minimal promoters increased activities of these promoters ($P<.05$) in all cell nuclei tested. Deletion of these sequences from the original promoter reduced transcriptional activity significantly in all of the cell nuclei tested. However, the enhancer effect of the 500 bp portion of the bIFN-τ gene promoter on transcription was not tissue-specific.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

INTRODUCTION

Massive amounts of interferon tau (IFN-τ) are secreted by the developing conceptus during maternal recognition of pregnancy in ruminants. These small proteins are known as antiluteolysins, and act directly on the endometrium of pregnant animals to block episodic release of prostagladin-$F_2\alpha$. As a consequence, progesterone production is maintained, and pregnancy can proceed (Bazer et al., 1991; Helmer et al., 1989; Roberts, 1989; Stewart, 1990; Thatcher et al., 1989). Similarly to their close relatives, type I IFNs, interferon-τ are encoded by multiple subtype genes with substantial sequence variation (Capon et al., 1985; Charlier et al., 1989; Charlier et al., 1991; Imakawa et al., 1989; Lund et al., 1984; Roberts et al., 1991; Streuli et al., 1980; Weissmann et al., 1982). Sequence differences are found both in coding regions and putative promoter regions among the bIFN-τ variants; this could give rise to differential expression of these genes during early embryonic development in cows and sheep (Ealy et al., 1998; Nephew et al., 1993) (also see chapter II). However, general features of these variants are very similar.

Conspicuously, all known IFN-τ gene variants posses a very well conserved 3'-untranslated region (3'UTR) which is an important parameter used to assign IFN-τ as a unique member of IFN-α gene family (Hansen et al., 1991; Stewart et al., 1990). In proximal promoter regions, there are several transcriptional regulatory motifs which also are well conserved across subtypes and across species (Guesdon et al., 1996; Hansen et al., 1991). Among them, however, only the Ets-2 site and a couple of negative regulatory domains seem to be functional regulatory elements. Other motifs found are

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

virus response elements, IFN regulatory factor binding sites, activating transcription factor (ATF)/cAMP response elements, and Oct-like factor binding sites. All of these elements are found in Type I IFN genes and play very important roles in regulation of gene expression. However, none of them seems to function in IFN-τ genes as shown by stably transfecting Jar cells with 1.8 Kb bIFN-τ 1-reporter gene constructs (Leaman et al., 1994). Virus is a primary inducer of type I IFN genes, in which only 120 bp from the putative transcription start site are required for full scale transcription (Goodbourn and Maniatis, 1988; MacDonald et al., 1990; Ragg and Weissmann, 1983); however, IFN-τ genes respond very poorly to virus (Guesdon et al., 1996; Leaman et al., 1994). Therefore, it is clear that the mechanism(s) underlying regulation of IFN-τ gene expression is quite different from other common type I interferon genes.

There has been a suggestion that initial onset of bIFN-τ gene expression is a genetically programmed event, but maternally-derived factors enhance expression of these genes (Hernandez-Ledezma et al., 1992a). Supporting evidence was found in one ovine IFN-τ variant in which the promoter region contained a putative AP-1 binding site which responded to granulocyte macrophage-colony stimulating factor (GM-CSF) (Imakawa et al., 1993). Insulin-like growth factor-I and –II (IGF-I and –II) also enhanced expression of the oIFN-τ gene when added simultaneously in day 13 ovine embryo culture medium. Those cytokines were found in uterine fluids regardless of pregnancy status, but the amount of IGF-II was higher in pregnant animals (Ko et al., 1991). However, it is not clear whether the same regulatory scheme can be applied to bovine IFN-τ gene expression. For instance, GM-CSF did not enhance expression of endogenous bovine nor ovine IFN-τ genes when added to culture media (De Moraes et

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

al., 1997). Therefore, it still remains to be elucidated whether maternally-derived factor(s) are required for full transcription of IFN-τ.

Among the factors hampering studies on regulation of IFN-τ gene expression, the lack of apropriate cell lines derived from bovine or ovine trophoblast cells is most problematic, since IFN-τ genes are expressed only in trophoblast cells (Guillomot et al., 1990; Lifsey et al., 1989) and during limited times (Helmer et al., 1987). Finding or making such cells would greatly facilitate IFN-τ promoter analysis. Another issue is that limited numbers of bIFN-τ promoters have been available. So far, only a single bIFN-τ gene promoter (bIFN-τ1; Hansen et al., 1991) has been studied to any extent. As indicated by several studies, there must be several copies of these genes, and their promoters may have different arrangements of transcriptional regulatory elements, leading to differential expression.

Recently we cloned several bIFN-τ gene variants with considerable sequence variations in both coding and 5' non-coding regions. Among them, the largest variation was found in first 500 bp upstream region of a transcriptionally inert variant (IFN-τb1). RNase protection assay (RPA) results showed that this bIFN-τ subtype gene was barely expressed regardless of pools or ages of embryos (see chapter II). Unexpectedly, however, this variant showed remarkable conservation of proximal promoter sequences (>98 %). Thus, we postulated that the core regulatory elements of bIFN-τ genes might be located in distal promoter regions instead of proximal promoter regions. To test this hypothesis, the first 500 bp distal promoter region from the 5' end of a functional bIFN-τ gene was subcloned and tested using an in vitro transcription system. Here, we show that this fragment of DNA behaves like a general enhancer.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

MATERIALS AND METHODS

Materials

Restriction enzymes were purchased from New England Biolabs (Beverly, MA). [α-^{32}P] UTP was obtained from ICN Biomedicals (Cleveland, OH). RNase protection assay kits and in vitro transcription kits were obtained from Ambion (Austin, TX). dNTPs were purchased from Amersharm Pharmacia Biotech (Piscatawa, NJ). Specific and non-specific protease inhibitors were purchased from Sigma (St. Louis, MO), and the following final concentrations were used: 0.5 mM phenylmethyl-sulphonyl fluoride (PMSF), 10 μM N-tosyl-L-phenylalanine chloromethyl ketone (TPCK), 10 μM Nα-p-tosyl-L-lysine chloromethyl ketone (TLCK), 8 μl/ml of aprotinin, and 0.5 μg/ml each of pepstatin, antipain and leupeptin. All other chemicals were purchased from Sigma.

Preparation of Nuclei from Bovine Embryos

Nuclei from day 16, 20, and 45 bovine conceptuses were prepared. To obtain enough day 16 embryonic tissue, cows were superovulated and embryos were collected nonsurgically using standard methods; approximately 4 embryos from the same cow were used to make a batch of nuclei. For the other stages, single embryos were collected nonsurgically from 3 cows at each stages of development after artificial insemination as described in Chapter II. Upon collection, the conceptus was washed three times in Ca^{++} and Mg^{++} -free phosphate-buffered saline (PBS), and trophectoderm was isolated after removing fetal tissues. All manipulations were carried out at 4°C from this stage on by using solutions, tubes and centrifuge rotors that were prechilled to 4°C. Procedures for

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

purification of nuclei were as previously described with slight modifications (Hagenbuchle and Wellauer, 1992). Briefly, embryonic tissues were suspended in 10 ml of 0.3M sucrose in buffer A (60 ml KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 15 mM Hepes (pH 7.8), 14mM mercaptoethanol, 0.5% Nonidet P-40 and protease inhibitors as described above). Cell disruption was performed by stroking an all glass Dounce homogenizer (B-type pestle) until more than 95% of cells were lysed as judged by light microscopy. For the rigid day 45 embryos, a motor driven tissue homogenizer (Polytron, Brinkman Instruments, NY) was used initially, and then the cell suspension was filtered through 100 μm mesh gauze before lysing using the Dounce homogenizer. Homogenates were then layered on top of a 10 ml cushion of 0.9 M sucrose in buffer A without Nonidet P-40 and centrifuged for 10 min at 750 X g in a Beckman 7.8 rotor. The nuclear pellet was resuspended in 10 ml of 0.3 M sucrose in buffer A and 0.2% Nonidet P-40 by homogenization in a Dounce homogenizer (3 strokes, B-type pestle) and recentrifuged over 10 ml 0.9 M of sucrose. The nuclear pellet was resuspended in 1ml of buffer B [75 mM Nacl, 0.5 mM EDTA, 20 mM Tris.KCl (pH 7.9), 0.8 mM dithiothreitol (DTT), 0.1 mM PMSF and 50% glycerol] by repeated passage through a micropipette tip. The concentration of suspension was adjusted to 5 X 10^5 nuclei/μl by removing extra buffer after centrifugation at 14000 rpm at 4°C for 2 min in a microcentrifuge. This nuclear suspension was used directly or stored at –70°C prior to use.

Cell Culture

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The Hela, STO, and Jar cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum at 37°C in 5% CO_2 in air. For homogenization, approximately 10^9 cells were resuspended in 10 ml of 0.3 M sucrose in buffer A containing 0.5% Nonidet P-40 and disrupted using the same procedures described above.

Construction of DNA Templates

Subcloning of 500bp Distal Promoter from a bIFN-τ Subtype Gene

Previous ribonuclease protection assays (RPA) showed no indication of polymorphism of one of the new bIFN-τ subtype genes (IFN-τb2). Thus, we chose this particular cloned promoter fragment for testing. To obtain the distal 500 bp fragment, a KpnI to XbaI fragment of the IFN-τb2 5' region was isolated, subcloned into pBluescript II KS$^-$ cloning vector (Stratagene, La Jolla, CA) and amplified. This fragment was later isolated and ligated to the upstream of a bIFN-τ1 1.8 kb-luc reporter construct (bIFN-τ1 1.8luc) to extend promoter size to 2.3 kb (bIFN-τ1 2.3luc). The same fragment (KpnI to XbaI) was also inserted in front of a tyrosine kinase-pGL2 vector (TK-pGL2) and a prolactin-pGL2 vector (PRL-pGL2) (generous gifts from Dr. C Clay, Colorado State University, CO) both of which contained only a minimal promoter region. Other IFN-τ subtype gene promoters (IFN-τb1, IFN-τb2, IFN-τb3: all are about 2.3 kb in length) and the bIFN-τ1 1.8 kb region were inserted into KpnI and NheI sites of pGL2-basic vectors (IFN-τb1 / luc, IFN-τb2 / luc, IFN-τb3 / luc, and bTP-1.8 / luc respectively). To generate truncated 1.8 kb IFN-τb2 / luc, the first 500 bp from 5'end of bTP-sf was removed by digestion with Xba I and KpnI and religated after blunting the 5' and 3'

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

overhangs with T4 DNA polymerase. Before in vitro transcription, all of the templates were linearized with SmaI whose recognition site was located a few base pairs upstream from the 5' end of the subcloned promoters.

To generate antisense probes for bIFN-τ / luc templates, this luciferase coding region from Hind III to Spl1 sites (231 bp) was isolated from pGL2-Basic vector (Strategene, La Jolla, CA) and religated into pBluscript II KS^- vector (Strategene, La Jolla, CA). To make antisense probes for RSV/ luc, the luciferase coding region from HindIII to ScaI sites (200 bp) was isolated from pGL3-basic vector (Strategene, La Jolla, CA) and ligated to HindIII and SmaI sites of pBluscript II KS^- vector.

In Vitro Transcription Assay

Transcription reactions (50 μl) contained 2 μg of linear DNA template and various numbers of cell nuclei in a buffer containing 15 mM Hepes (pH 7.6), 80 mM KCl, 6 mM $MgCl_2$, 25 mM each of NTPs, 14% glycerol, 2 mM creatinine phosphate, 0.04 μg/μl poly dI:dC, 2 mM DTT, and 0.1 mM EDTA. After 1 hr of incubation at 30°C, the reaction was terminated. Approximately 20 μl of reactions were coprecipitated with 0.5×10^6 count per minute (cpm) of ^{32}P-labelled luciferase antisense probe (1.4×10^8 c.p.m. / μg) for the RPA.

Measurement of Protein in In Vitro Transcription Reactions

Because of the difficulty of counting precise numbers of nuclei in a given sample, protein content was measured using the BCA protein assay reagent kit (Pierce, Cat #. 23223, Rockford, IL). All procedures were essentially the same as the manufacturer's

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

instructions. However, trichloroacetic acid (TCA) protein precipitation was performed prior to the protein assay to eliminate possible interference by several in vitro transcription buffer components. Briefly, a 10 µl mixture of each in vitro transcription reaction was mixed with 5 µl of 100 % TCA, vortexed thoroughly and placed in –20°C freezer 15 min before centrifugation at maximal speed of a microcentrifuge for 10-15 min. The supernatant was removed, and the pellet was resuspended in 100 µl of 0.1M NaOH solution. Then 10 µl of the suspension was used for the BCA protein assay. The amount of protein was measured using the microtiter plate protocol at a wavelength of 562 nm in a Labsystems multiskan RC scanner (Fisher Scientific, Houston, TX). The protein concentration for each sample was determined using a standard curve generated with bovine serum albumin. The calculated concentration of protein in a given sample was then used to normalize optical density of the same sample.

Ribonuclease Protection Assay (RPA) and Quantification of Transcripts

Generation of antisense RNA probes was performed using an in vitro transcription kit (Ambion, Austin, Texas) with 2 µg of template DNA according to the manufacturer's instructions. RPA was carried out using an RPA III kit (Ambion, Austin, Texas) according to the manufacturer's instructions using 20 µl of each transcription reaction and approximately 0.5 x 10^6 cpm of probe. After overnight hybridization at 45°C, RNase (10 µg/ml RNase A, 200 U/ml RNase T1) was added to the mixture and incubated for 30 min at 37°C. Protected fragments were separated by electrophoresis in 5% (w/v) polyacrylamide gels containing 8 M urea. Dried gels were then exposed to XAR-5 film (Eastman Kodak, Rochester, NY) on intensifying screens for 2 h to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

overnight. Optical densities of bands representing specific transcripts were determined using Phosphoimager and Image Quant software (Molecular Dynamics, Sunnyvak, CA). Data were normalized based on the amount of protein in a given reaction.

Statistical Analysis

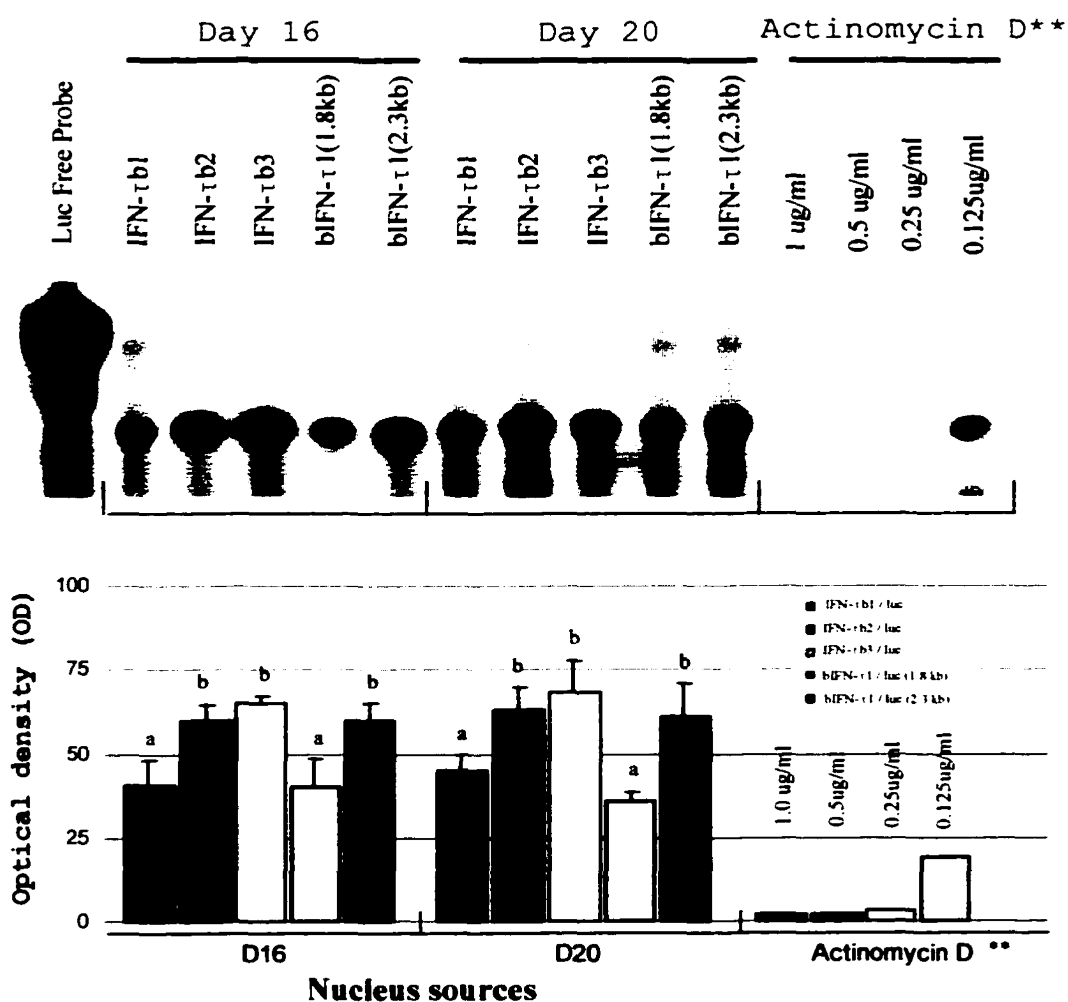
Each experiment was replicated 3 times using different batches of cells, different embryos from day 20 and older, and pools of embryos for day 16. Analysis of variance was used to compare effects of DNA constructs and various kinds of cell nuclei. Tukey's multiple comparison test was used to compare means. Regression analysis was used to compare slopes of dose response reactions in experiment 2. All analyses were performed using SAS (SAS, 1989, NC).

RESULTS

The 5' Distal Promoter Fragment (500 bp) of a New bIFN-τ Clone Increased Transcription

All of the new bIFN-τ promoters including 1.8 kb bIFN-τ 1 yielded detectable amounts of transcripts in reactions containing 1 x 10^6 nuclei. Notably addition of the 500 bp DNA fragment to 1.8 kb bIFN-τ promoter increased transcription approximately 2-fold in both day 16 and 20 embryo preparations (Figure 3-1). This experiment was replicated 3 times using different pools of embryos with similar results. When actinomycin D (0.125, 0.25, 0.5 and 1.0 μg /ml) was added to the reaction containing 1 x 10^6 day 16 embryo nuclei and IFN-τb2 (2.3kb) / luc, it inhibited transcription effectively

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



* a,b Values with different superscripts differ (P<.05).

** All actinomycin D treated reactions contained 2.3 kb bTP-sf/luc and 1 million cell nuclei from day 16 bovine embryos.

Figure 3-1. *In vitro* Transcription Using day 16 and 20 Bovine Embryo Cell Nuclei as Transcription Factor Sources. Addition of 500 bp bTP-sf 5' to 1.8 kb bIFN-τ1 nearly doubled transcription. Addition of 0.25 ug/ml or higher actinomycin D obliterated transcription.

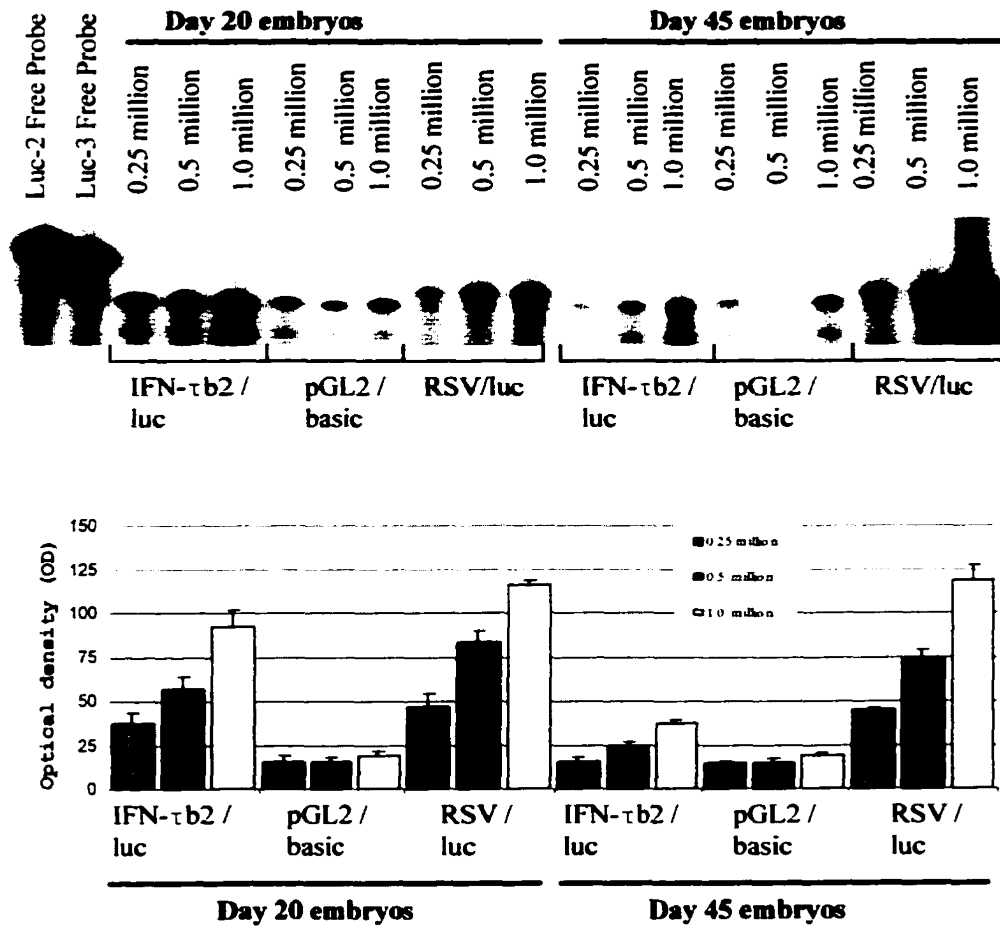
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

at 0.25μg/ml or higher, indicating that transcription was RNA polymerase-specific (Figure 3-1).

Promoter /reporter Gene Constructs Were Transcribed in a Dose Dependent Manner and Day 20 Bovine Embryo Nuclei Induced more Transcription of the IFN-τb2 (2.3kb) / luc Construct than Day 45 Bovine Embryo Nuclei.

In a preliminary experiment, extra nuclear extract drove non-specific transcription; the promoterless pGL2 vector yielded detectable transcripts when more than 8 units of HeLa nuclear extract were used (1 unit is defined as the amount of nuclear extract needed for incorporation of 50 fmol of nucleotides into 363 nucleotide transcripts generated from the CMV immediate early promoter in 1 h at 30°C). The nuclei used in our experiments are relatively crude, so direct comparison with HeLa cell nuclear extract was impossible in terms of actual amounts of transcription factors. Thus, to determine optimum numbers of nuclei for specific transcription, a second experiment was performed using different numbers of nuclei (0.25, 1.0, and 1.0 x 10^6 respectively). As a negative control, day 45 bovine embryo cell nuclei were used. The yields of transcripts were dose-dependent (Figure 3-2). IFN-τb2 (2.3kb) / luc yield depended on dosages of cell nuclei and the developmental stage of embryos ($P < .01$). In the day 20 embryo preparation, even 0.25 x 10^6 cell nuclei generated substantial transcription with IFN-τb2 (2.3kb) / luc and RSV / luc constructs. The pGL-2 basic vector did not showed dose-dependent transcription activity in either day 20 or day 45 embryo preparations. The RSV/luc construct clearly showed the dose-dependent transcriptional activity, regardless of developmental stages of embryos. These results indicated that approximately 0.25 x

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



* IFN-τb2 / luc was transcribed significantly more in the day 20 than in day 45 embryo preparations ($P<.01$), and the slope of increment of transcripts differed between the two developmental stage embryo preparations ($P<.01$).

Figure 3-2. Dose Dependent Transcriptional Activities of IFN-τb2 and RSV Promoters in day 20 and 45 Bovine Embryo Nucleus Preparations. Notice that the promoterless pGL2-basic vector did not respond to different amounts of nuclei.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

10^6 nuclei were sufficient for monitoring in vitro transcription. This number of nuclei was used for subsequent experiments unless otherwise specified

An Upstream 500 bp 5' Region of IFN-τb2 Increased Transcription in Cells of Trophoblast and Non-trophoblast Origin

The third experiment was performed to determine whether the most 5' end 500 bp fragment functioned as a tissue-specific enhancer. For this experiment, nuclei from day 20 embryos, Jar cells, Hela cells, and STO cells (mouse embryonic fibroblast cells) were prepared as described above. DNA templates were RSV/luc, TK/luc, TK500/luc, PRL/luc, PRL 500/luc, IFN-τb2 (2.3kb) / luc, and IFN-τb2 (1.8kb) / luc. The TK/luc and PRL/luc constructs contain only minimal promoters, so they are not transcriptionally active unless an enhancer motif is provided. Unexpectedly, the small DNA fragment increased transcription of all the DNA constructs irrespective of cell type ($P < .01$) (Figure 3-3). The increased percentage transcription of TK500 /luc and PRL500/luc constructs was virtually the same in all cell nuclei tested, suggesting that the 500 bp DNA fragment may contain a general enhancer motif. However, day 20 bovine embryo cell nucleus preparations supported greater ($P < .01$) transcription of IFN-τb2 / luc constructs than other cell preparations, regardless of promoter size. Nevertheless, the 2.3kb IFN-τb2 / luc construct showed significantly higher transcription in every cell nuclei preparation than 1.8kb IFN-τb2 / luc construct ($P < .01$). The 1.8 kb IFN-τb2 / luc construct supported basal transcription in non-bovine embryo cell nuclei, confirming that this 5' end 500 bp region contains a common enhancer or enhancer-like motif. The reduced activity of 1.8kb bIFN-τ promoter in non-bovine embryo cell preparations indicates that

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

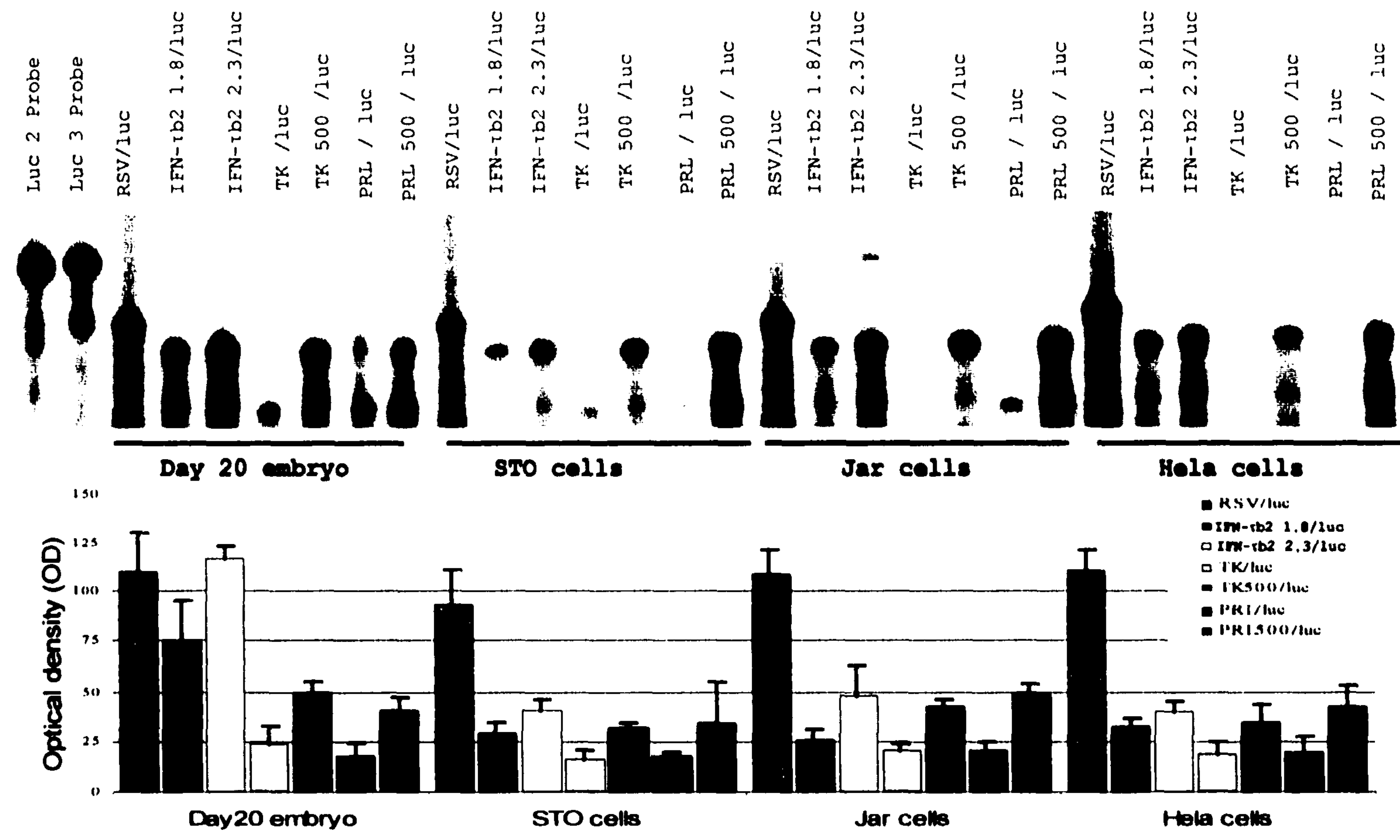


Figure 3-3. *In vitro* Transcription Using Various Cell Nuclei and Different Size Promoters. The 1.8 kb IFN-τb2 was generated by truncating 500 bp fragment from 5'end of 2.3 kb IFN-τb2 . The same fragment of DNA was added to TK and PRL minimal promoters to generate TK500 /luc and PRL 500/ luc constructs. Addition of this DNA fragment increased transcription of nuclei from all sources tested.

cell or tissue specific regulatory motifs reside within 1.8 kb from the putative transcription start site.

DISCUSSION

Previous studies indicated that bIFN-τ gene expression is restricted to trophoblast cells in ruminants (Farin et al., 1989; Farin et al., 1990; Morgan et al., 1993). Moreover regulation of these gene is primarily transcriptional. Like the common IFN-α and -β genes, their transcripts have an AU-rich region in their 3' noncoding region, which works as a recognition signal for rapid mRNA degradation (Caput et al., 1986; Shaw and Kaman, 1986). However the AU-rich region is interspersed in G and C rich regions in IFN-τ genes (Imakawa et al., 1989), which may contribute to a slightly longer mRNA half-life (Hansen et al., 1991) than other INF transcripts.

One of the difficulties in studying the IFN-τ promoter is lack of an appropriate cell line derived from bovine or ovine trophoblast. Primary bovine embryo trophoblast cells in culture failed to support endogenous bIFN-τ gene expression for more than 1 day (Hansen et al., 1991), and the transfection efficiency of primary bovine trophoblast cells by any means was too low to be utilized in our laboratory. Recently a bovine trophoblast cell line (CT-1 cells, a generous gift from Dr. Neil Talbot, USDA, Maryland) has been established from in-vitro produced bovine blastocysts. However CT-1 cells did not express detectable levels of bIFN-τ transcripts in our previous experiments (see chapter II). Moreover, cells of trophoblast origin such as Jar or Jeg 3 do not contain the endogenous IFN-τ genes, which may explain why transcriptional activation of bIFN-τ /

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reporter gene constructs required exogenous enhancer motifs (Guesdon et al., 1996) in these cells. To surmount all of the problems that may be encountered when improper cells are used, we utilized an in vitro transcription assay using several kinds of cell nuclei as transcription factor sources. The in vitro transcription systems have been used to decipher the precise molecular mechanisms underlying selective transcription, and to test tissue-specific transcription of several cellular and viral genes (Gorski et al., 1986; Miyamoto et al., 1984; Sawadogo and Roeder, 1985).

In experiment I, we tested whether day 16 and 20 bovine embryo-derived cell nuclei can support expression of various bIFN-τ / luc constructs in vitro. At the same time, the 500 bp fragment of 5'end of IFN-τb2 subtype was tested. This region had not been cloned and tested before, so it may provide useful information with regard to the bIFN-τ gene expression. Unexpectedly, addition of this small piece of DNA to the 1.8 Kb bIFN-τ promoter increased transcription of a reporter gene (luciferase) approximately 2 fold in the in vitro transcription reactions. There is no obvious explanation for this increased transcription, but this region might have enhancer(s) like motifs. Sequence analysis using the MatInspector program (MatInspector Release professional 3.3, Genomatix Software GmBH, München, Germany) showed several putative transcriptional regulatory domains. Among them, two Ets-1 sites, a CEBP binding site, and a STAT site were prominent, with well conserved homologous sequences. However, the functionality of these motifs remain to be elucidated. Addition of Actinomycin D verified that the transcription was RNA polymerase- specific.

Experiment 2 was designed to optimize the number of nuclei for cell- or tissue-specific transcription. Among the factors affecting in vitro transcription, number of cell

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

nuclei (or amount of nuclear extract) is the most important parameter to be optimized. We observed non-specific transcription, even from a promoterless pGL2-basic vector when extra Hela cell nuclear extract was added to the reaction. To monitor transcription capacity of different preparations of cell nuclei at different concentrations, the three templates IFN-τb2, pGL2-basic, RSV/ luc were used. Transcription of the tissue-specific IFN-τb2 / luc, and RSV/ luc gene constructs showed dose dependent transcriptional activity in both day 20 and 45 bovine embryo nuclei preparations, although the degree of enhancement of transcription for IFN-τb2 (2.3kb) / luc in day 45 embryo preparations was lower ($P < .01$) than in day 20 embryo preparations. The pGL2-basic vector did not show enhanced transcription as numbers of cell nuclei increased, indicating that the transcription was promoter dependent. In day 20 bovine embryo preparations, even 0.25 million cell nuclei induced IFN-τb2 / luc gene transcription conspicuously. The amount of transcripts was about the same as that of RSV / luc in the reaction containing the same number of nuclei. These results indicated that if the optimal numbers of nuclei are used, in vitro transcription can be used to measure the activities of certain gene promoters.

The tissue specificities of IFN-τb2 promoters and the 500 bp 5'end region of IFN-τb2 gene were tested in experiment 3. For these, day 20 bovine embryo cell nuclei, Jar cell nuclei, STO cell nuclei, and Hela cell nuclei were used with several gene promoter / luc constructs. Both TK / luc and PRL / luc constructs showed basal transcriptions in all cell nuclei preparations. However, addition of the 500 bp fragment increased transcription significantly regardless of cell type. Similarly, the 2.3 kb IFN-τb2 promoter showed approximately 2 times more transcriptional activity than 5' truncated 1.8 kb IFN-τb2 promoter, indicating that the 5' end region works as general

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

enhancer rather than a tissue-specific enhancer. Since both TK / luc and PRL / luc constructs contain only minimal promoters, the increased transcription was notable. The IFN-τb2 / luc constructs also showed detectable levels of transcription in both trophoblast cells and non-trophoblastic cell nuclei preparations, so this particular subtype gene promoter is not entirely tissue-specific. The previously reported 1.8 kb bIFN-τ1 promoter also showed substantial transcription in non-trophoblast cells including L929 murine fibroblasts, CHO (Chinese hamster ovary cells), and murine L6 myoblasts (Cross and Roberts, 1991; Guesdon et al., 1996; Leaman et al., 1994). Thus, these results confirm earlier findings indicating that the isolated bIFN-τ promoters used to date are not totally tissue specific. These cells may contain a common transcription factor that is needed for IFN-τ gene expression as indicated by formation of specific complexes of the –358 to –322 region of the bIFN-τ1 promoter with nuclear proteins from Jar, COS-1 and L929 cells (Leaman et al., 1994). In a preliminary experiment, a 500 bp 3' untranslated region of the bIFN-τ which showed extreme sequence homology across subtypes and species (Hansen et al., 1991; Stewart et al., 1990) was tested to determine whether this region increased transcription. Disappointingly, addition of this fragment to the 1.8 kb bIFN-τ / luc or gal constructs yielded no effect on transfected Jar cells. Thus, a particular regulatory region rendering tissue-specific expression of the bIFN-τ genes still remains elusive.

In conclusion, we found that a newly cloned 5' fragment of the bIFN-τ promoter works as a general enhancer, and an in vitro transcription assay can be used to measure bIFN-τ gene promoter activity as an alternative for transfection studies if several parameters of the in vitro transcription assay are optimized. Possibly, cloning of longer

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

bIFN-tau gene flanking regions, combined with more rigorous studies on promoters, will provide clearer insight into the mechanisms involved in regulating bIFN-τ gene expression.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER IV

GENERAL SUMMARY AND CONCLUSION

Early embryonic mortality is very expensive for cattle producers. The majority of embryonic loss occurs between days 15 and 18 of gestation (Sreenan and Diskin, 1986), when several critical cellular and molecular events occur. One of the major factors contributing to embryonic loss is failure of maternal recognition of pregnancy.

Molecular factors are secreted by the peri-implantation conceptus of ruminants to effect the process of "maternal recognition of pregnancy". These factors block secretion of luteolytic levels of prostaglandin $F_2\alpha$ ($PGF_2\alpha$), thereby allowing the functional lifespan of corpus luteum (CL) to be extended beyond the normal estrous cycle. Structurally, these factors resemble most closely interferon-ω (IFN-ω), and have equipotent anti-viral, anti-proliferative, and immuno-modulatory activities to type I interferons (Roberts et al., 1992). The factors are now designated as interferon-tau (IFN-τ) based on the origin of tissue, gene structure, and biological activities (Roberts et al., 1992).

Almost a decade ago the first genomic clone of bIFN-τ was isolated (Stewart et al., 1990). However, little progress has been made since then with regard to the mechanisms involved in regulation of this gene. Factors hampering study of bIFN-τ

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

gene regulation include lack of a useful cell line, and a limited number of genomic clones (Leaman et al., 1994). Thus, this study focused on finding new bIFN-τ subtype genes with significant variation in nucleotide sequences in the putative promoter regions; we also needed alternatives to the normal transfection procedures used to characterize most of gene promoters because of difficulty in transfecting trophoblast cells.

In the first study, 21 positive clones were initially detected after screening a bovine genomic library with IFN-τ gene-specific probes. Four clones were chosen for further studies, based on restriction enzyme site mapping and ease of subcloning. Sequencing revealed that 3 were bIFN-τ variants, and 1 was a bIFN-ω variant. All new clones were potentially functional, since there were no premature stop codons found in the coding regions. The putative promoter regions showed considerable nucleotide mismatches (up to 7 %) compared to a previously cloned bIFN-τ variant [bIFN-τ1: (Hansen et al. 1991)]. This variation may give rise to different promoter activity. In addition, the new bIFN-τ clones possessed ≅500 bp longer putative promoter regions than bIFN-τ1. One of the new clones designated as IFN-τb1 showed extreme sequence variation from other clones in this region. Interestingly, this clone turned out to be transcriptionally inert as revealed by RNase protection assays. Thus, we studied this region of transcriptionally active clones further.

Deduced amino acid sequences of individual bIFN-τ clones differed sufficiently potentially to alter biological activity; even a single amino acid substitution in a critical position of mature IFN-τ can affect receptor binding and bioactivity conspicuously (Li and Roberts, 1994b; Niswender et al., 1997). Thus, studies on bioactivities of newly

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cloned bIFN-τ may provide new insight into questions regarding multiple variation of this gene family.

To characterize transcriptional expression patterns of individual bIFN-τ variants during early pregnancy, ribonuclease protection assays (RPA) were used at different developmental stages of bovine embryos. In preliminary studies, no bIFN-τ subtype mRNA was detected from embryos older than day 30. These results were observed repeatedly throughout our experiments, casting doubt on a previous report that bIFN-τ can be detected up to day 38 of pregnancy (Godkin et al., 1988). However, considering the critical time point that demise of the CL must be blocked for pregnancy to proceed, our observation is not surprising. Individual bIFN-τ variants including bIFN-τ1 showed differential expression patterns during early pregnancy. There has been a lingering question with regard to multiplicity of this gene. One attractive postulate was that individual subtype genes might have different patterns of expression, and perhaps different biological activities. RPA results confirmed that bIFN-τ genes are not expressed equally. A new clone, IFN-τb2, showed extreme sequence mismatches in first 500 bp 5' region and was transcriptionally inert regardless of pools or batches of embryos. Another clone, IFN-τb3, showed reduced transcriptional activity through day 20 of pregnancy. Interestingly, this particular clone showed no transcript in one pool of embryos, suggesting that this gene (or allele) might be polymorphic. However, both of these clones showed well conserved proximal promoter regions with most of the known regulatory elements intact. Thus, we postulated that the critical regulatory elements might be located further upstream in the proximal promoter region. This region had not been cloned before, so its function was worthy of study.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Experiment II concerned characterization of a sequence between 1.8 to 2.3 kb upstream of the transcription start site of a bIFN-τ. This was tested via an in vitro transcription system as an alternative to transfection. After optimization of amounts of nuclei and $MgCl_2$ concentrations (data not shown). this system seemed to work well. In the promoter analysis. the 500 bp fragment functioned as a general enhancer; it even increased transcription of minimal promoters (Tyrosine Kinase and Prolactin). When this portion of the promoter was deleted from the original construct. transcription was reduced on a similar scale. This phenomenon was observed in nuclei of both trophoblast and non-trophoblast origin. suggesting that this region did not confer tissue specificity. The truncated 1.8 kb bTP-sf promoter showed basal transcription in most cell nuclei tested. with strong expression in nuclei from day 20 bovine embryos. strongly implicating that tissue-specific regulation resides within the 1.8 kb promoter region. Unfortunately. there is no simple way to verify these results. A transgenic approach might be effective in facilitating bIFN-τ promoter analyses.

Recombinant bovine or ovine IFN-τ has been tested extensively for increasing pregnancy rates in cattle. However. results have been disappointing. contrary to initial success in sheep (Martinod et al., 1991). The most severe side effect was hyperthermia (Newton et al., 1990) which might lead to embryonic death after intramuscular injection. In another study, hyperthermia was not observed in cows receiving IFN-τ via intrauterine infusion (Plante et al., 1989). To date, no practical way has been developed to deliver IFN-τ to uteri of pregnant animals at critical time points. Thus, utilization of IFN-τ as a pregnancy-enhancing agent in cattle still remains problematic. Alternatives may be 1) modification of the bIFN-τ protein, 2) searching for subtypes that do not show severe

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

side effects, and 3) searching for a functionally intact bIFN-τ promoter. As mentioned earlier, IFN-τ are present in multiple forms with different bioactivities. Thus, it might be possible to find an IFN-τ variant with fewer side effects. Now there are additional IFN-τs that can be tested. Understanding mechanisms involved in bIFN-τ expression may lead to practical ways to modify such expression.

In conclusion, we cloned new bIFN-τ variants with considerable nucleotide variations in both coding and promoter regions. These variants gave rise to transcriptionally different expression. Thus, the recent sequence data can be used for future promoter analysis, which would be greatly aided by having a suitable cell line. More rigorous studies on regulations of these genes may provide new ways to increase pregnancy rates in domestic ruminants.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER IV

REFERENCES

Abramovitz M, Boie Y, Nguyen T, Rushmore TH, Bayne MA, Metters KM, Slipetz DM, and Grygorczyk R, 1994. Cloning and expression of a cDNA for the human prostanoid FP receptor. J Biol Chem 269:2632-2636

Albihn A, Gustafsson H, and Rodriguez-Martinez H, 1991. Maternal influence on the early development of asynchronously transferred bovine embryos. Anim Reprod Sci 24:25-35

Alexenko AP, Leaman DW, Li J, and Roberts RM, 1997. The antiproliferative and antiviral activities of IFN-τ variants in human cells. J Interferon Cytokine Res 17:769-779

Anthony RV, Helmer SD, Sharif SF, Roberts RM, Hansen PJ, Thatcher WW, and Bazer FW, 1988. Synthesis and processing of ovine trophoblast protein-1 and bovine trophoblast protein-1, conceptus secretory proteins involved in the maternal recognition of pregnancy. Endocrinology 123:1274-1280

Arends MJ, Morris RG, and Wyllie AH, 1990. Apoptosis. The role of the endonuclease. Am J Pathol 136:593-608

Ashworth CJ and Bazer FW, 1984. Patterns of progesterone secretion and embryonic survival during repeated pregnancies in Manline ewes. Proc 10th Int Congr Anim Reprod AI. 2:74.

Ashworth CJ and Bazer FW, 1989. Changes in ovine conceptus and endometrial function following asynchronous embryo transfer or administration of progesterone. Biol Reprod 40:425-433

Assal-Meliani A, Charpigny G, Reinaud P, Martal J, and Chaouat G, 1993. Recombinant ovine trophoblastin (roTP) inhibits ovine, murine and human lymphocyte proliferation. J Reprod Immunol 25:149-165

Assal-Meliani A, Kinsky R, Martal J, and Chaouat G, 1995. In vivo immunosuppressive effects of recombinant ovine interferon-tau (trophoblastin): r.oTP (r.oIFN-tau) inhibits local GVH reaction in mice (PLN assay), prevents fetal resorptions, and favors embryo survival and implantation in the CBA/J x DBA/2 mice combination. Am J Reprod Immunol 33:267-275

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Asselin E, Bazer FW, and Fortier MA, 1997. Recombinant ovine and bovine interferon-τ regulate prostaglandin production and oxytocin response in cultured bovine endometrial cells. Biol Reprod 56:402-408

Asselin E, Drolet P, and Fortier MA, 1997. Cellular mechanisms involved during oxytocin-induced prostaglandin F2α production in endometrial epithelial cells in vitro: role of cyclooxygenase-2. Endocrinology 138:4798-4805

Asselin E, Lacroix D, and Fortier MA, 1997. IFN-τ increases PGE2 production and COX-2 gene expression in the bovine endometrium in vitro. Mol Cell Endocrinol 132:117-126

Austin KJ, Ward SK, Teixeira MG, Dean VC, Moore DW, and Hansen TR, 1996. Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. Biol Reprod 54:600-606

Ayad VJ, Matthews EL, Wathes DC, Parkinson TJ, and Wild ML, 1991. Autoradiographic localization of oxytocin receptors in the endometrium during the oestrous cycle of the ewe. J Endocrinol 130:199-206

Ayad VJ and Wathes DC, 1989. Characterization of endometrial and myometrial oxytocin receptors in the non-pregnant ewe. J Endocrinol 123:11-18

Ayalon N, 1978. A review of embryonic mortality in cattle. J Reprod Fertil 54:483-493

Azmi TI and O'Shea JD, 1984. Mechanism of deletion of endothelial cells during regression of the corpus luteum. Lab Invest 51:206-217

Bacci ML, Barazzoni AM, Forni M, and Costerbosa GL, 1996. In situ detection of apoptosis in regressing corpus luteum of pregnant sow: evidence of an early presence of DNA fragmentation. Domest Anim Endocrinol 13:361-372

Baffy G, Miyashita T, Williamson JR, and Reed JC, 1993. Apoptosis induced by withdrawal of interleukin-3 (IL-3) from an IL-3- dependent hematopoietic cell line is associated with repartitioning of intracellular calcium and is blocked by enforced Bcl-2 oncoprotein production. J Biol Chem 268:6511-6519

Bao B and Garverick HA, 1998. Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: a review. J Anim Sci 76:1903-1921

Barret G, Horriuchi M, Paul M, Pratt RE, Nakamura N, and Dzau VJ, 1992. Identification of a negative regulatory element involved in tissue-specific expression of mouse renin genes. Proc Natl Acad Sci U S A 89: 885-889

Barros CM, Newton GR, Thatcher WW, Drost M, Plante C, and Hansen PJ, 1992. The effect of bovine interferon-αII on pregnancy rate in heifers. J Anim Sci 70:1471-1477

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Barros CM, Plante C, Thatcher WW, and Hansen PJ, 1991. Regulation of bovine endometrial secretion of prostaglandins and synthesis of 2',5'-oligoadenylate synthetase by interferon-α molecules. Am J Reprod Immunol 25:146-152

Bartol FF, Roberts RM, Bazer FW, Lewis GS, Godkin JD, and Thatcher WW, 1985. Characterization of proteins produced in vitro by periattachment bovine conceptuses. Biol Reprod 32:681-693

Bazer FW and Johnson HM, 1991. Type I conceptus interferons: maternal recognition of pregnancy signals and potential therapeutic agents [editorial]. Am J Reprod Immunol 26:19-22

Bazer FW, Spencer TE, and Ott TL, 1996. Placental interferons. Am J Reprod Immunol 35:297-308

Bazer FW, Thatcher WW, Hansen PJ, Mirando MA, Ott TL, and Plante C, 1991. Physiological mechanisms of pregnancy recognition in ruminants. J Reprod Fertil Suppl 43:39-47

Bazer FW, Vallet JL, Roberts RM, Sharp DC, and Thatcher WW, 1986. Role of conceptus secretory products in establishment of pregnancy. J Reprod Fertil 76:841-850

Beard AP, Hunter MG, and Lamming GE, 1994. Quantitative control of oxytocin-induced PGF2α release by progesterone and oestradiol in ewes. J Reprod Fertil 100:143-150

Beard AP and Lamming GE, 1994. Oestradiol concentration and the development of the uterine oxytocin receptor and oxytocin-induced PGF2α release in ewes. J Reprod Fertil 100:469-475

Berridge MJ and Irvine RF, 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312:315-321

Betteridge KJ, Eaglesome MD, Randall GC, and Mitchell D, 1980. Collection, description and transfer of embryos from cattle 10-16 days after oestrus. J Reprod Fertil 59:205-216

Bishop M, 1964. Paternal contribution to embryonic death. J Reprod Fertil 7:383-389

Blanchard T, Ferguson J, Love L, Takeda T, Henderson B, Hasler J, and Chalupa W, 1990. Effect of dietary crude-protein type on fertilization and embryo quality in dairy cattle. Am J Vet Res 51:905-908

Bleach EC, Peiris ID, Grewal TS, Shepherd DA, and Savva D, 1998. Effect of administration of a novel recombinant bovine interferon on length of oestrous cycle in cattle. Res Vet Sci 64:73-77

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Borden EC, 1992. Interferons: pleiotropic cellular modulators. Clin Immunol Immunopathol 62:S18-S24

Boshier DP, Fairclough RJ, and Holloway H, 1987. Assessment of sheep blastocyst effects on neutral lipids in the uterine caruncular epithelium. J Reprod Fertil 79:569-573

Bouters R, 1986. Uterine pathogens and embryonic mortality. In: Sreenan JM and Diskin MJ (eds) *Embryonic Mortality in Farm Animals*. Boston: Martinus Nijihoff, 249-259.

Boyd J and Reed HCB, 1961. Investigation into the incidence and causes of infertility in dairy cattle. Influence of some management factors affecting the semen and insemination condition. Br Vet J 117:74-86

Braden TD, Gamboni F, and Niswender GD, 1988. Effects of prostaglandin $F_2\alpha$-induced luteolysis on the populations of cells in the ovine corpus luteum. Biol Reprod 39:245-253

Burgess KM, Ralph MM, Jenkin G, and Thorburn GD, 1990. Effect of oxytocin and estradiol on uterine prostaglandin release in nonpregnant and early-pregnant ewes. Biol Reprod 42:822-833

Caldwell BV, Rowson LE, Moor RM, and Hay MF, 1969. The uterus-ovarian relationship and its possible role in fertility. J Reprod Fertil Suppl 8:59-76

Capon DJ, Shepard HM, and Goeddel DV, 1985. Two distinct families of human and bovine interferon-α genes are coordinately expressed and encode functional polypeptides. Mol Cell Biol 5:768-779

Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, and Cerami A, 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. Proc Natl Acad Sci U S A 83:1670-1674

Casida LE, 1945. The necessity of the corpus luteum for maintenance of pregnancy in the ewe. J Anim Sci 4:34-36

Channing CP, 1969. Tissue culture of equine ovarian cell types: culture methods and morphology. J Endocrinol 43:381-390

Chaouat G, Assal-Meliani A, Martal J, Raghupathy R, Elliot J, Mosman T, and Wegmann TG, 1995. IL-10 prevents naturally occuring fetal loss in the CBA x DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by in vivo injection of IFN-τ. J Immunol 154:4261-4267

Charlier M, Hue D, Boisnard M, Martal J, and Gaye P, 1991. Cloning and structural analysis of two distinct families of ovine interferon-α genes encoding functional class II and trophoblast (oTP) α-interferons. Mol Cell Endocrinol 76:161-171

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

secretion of interferon-τ, other proteins, and prostaglandin E2 by the bovine and ovine conceptus. Domest Anim Endocrinol 14:193-197

Denamur R and Martinet J. 1955. Effects de l' ovariectome chez la brebis pendant la gestation. C R Seanc Soc Biol 149:2105-2107

Denamur R and Maueon P. 1963. Endocrine control of persistence of the corpus luteum in sheep. C R Acad Sci Hebd Seances Acad Sci D 257:527-530

Dereuddre-Bosquet N, Clayette P, Martin M, Mabondzo A, Fretier P, Gras G, Martal J, and Dormont D. 1996. Anti-HIV potential of a new interferon, interferon- τ (trophoblastin). J Acquir Immune Defic Syndr Hum Retrovirol 11:241-246

DeWitt D and Smith WL. 1995. Yes, but do they still get headaches?. Cell 83:345-348

Diskin MG and Niswender GD. 1989. Effect of progesterone supplementation on pregnancy and embryo survival in ewes. J Anim Sci 67:1559-1563

Diskin MG and Sreenan JM. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J Reprod Fertil 59:463-468

Ealy AD, Alexenko AP, Keisler DH, and Roberts RM. 1998. Loss of the signature six carboxyl amino acid tail from ovine interferon-τ does not affect biological activity. Biol Reprod 58:1463-1468

Ealy AD, Green JA, Alexenko AP, Keisler DH, and Roberts RM. 1998. Different ovine interferon-τ genes are not expressed identically and their protein products display different activities. Biol Reprod 58:566-573

Eggleston DL, Wilken C, Van Kirk EA, Slaughter RG, Ji TH, and Murdoch WJ. 1990. Progesterone induces expression of endometrial messenger RNA encoding for cyclooxygenase (sheep). Prostaglandins 39:675-683

Elsden RP and Seidel Jr. GE. 1995. Procedures for recovery, bisection, freezing and transfer of bovine embryos. In: Elsden RP and Seidel Jr. GE (eds) *Animal Reproduction and Biotechnology Laboratory Bulletin No. 09 (6 th eds)*. Fort Collins: Colorado State University Press, 12

Estergreen VL, Frost OL, Gomes WR, Erb RE, and Bullard JF. 1967. Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. J Dairy Sci 50:1293-1295

Ezashi T, Ealy AD, Ostrowski MC, and Roberts RM. 1998. Control of interferon-τ gene expression by Ets-2. Proc Natl Acad Sci U S A 95:7882-7887

Fan CM and Maniatis T. 1989. Two different virus-inducible elements are required for human β-interferon gene regulation. EMBO J 8:101-110

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Farin CE, Cross JC, Tindle NA, Murphy CN, Farin PW, and Roberts RM, 1991. Induction of trophoblastic interferon expression in ovine blastocysts after treatment with double-stranded RNA. J Interferon Res 11:151-157

Farin CE, Imakawa K, Hansen TR, McDonnell JJ, Murphy CN, Farin PW, and Roberts RM, 1990. Expression of trophoblastic interferon genes in sheep and cattle. Biol Reprod 43:210-218

Farin CE, Imakawa K, and Roberts RM, 1989. In situ localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. Mol Endocrinol 3:1099-1107

Farin CE, Moeller CL, Mayan H, Gamboni F, Sawyer HR, and Niswender GD, 1988. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. Biol Reprod 38:413-421

Farin CE, Nett TM, and Niswender GD, 1990. Effects of luteinizing hormone on luteal cell populations in hypophysectomized ewes. J Reprod Fertil 88:61-70

Fernandez-Sarabia MJ and Bischoff JR, 1993. Bcl-2 associates with the ras-related protein R-ras p23. Nature 366:274-275

Fields MJ, Barros CM, and Watkins WB, and Fields PA, 1992. Characterization of large luteal cells and their secretory granules during the estrous cycle of the cow. Biol Reprod 46:535-545

Fincher KB, Bazer FW, Hansen PJ, Thatcher WW, and Roberts RM, 1986. Proteins secreted by the sheep conceptus suppress induction of uterine prostaglandin F2α release by oestradiol and oxytocin. J Reprod Fertil 76:425-433

Findlay JK, 1982. Oestrogen receptors and protein synthesis in caruncular and intercaruncular endometrium of sheep before implantation. J Reprod Fertil 64:329-339

Fitz TA, Mayan MH, Sawyer HR, and Niswender GD, 1982. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol Reprod 27:703-711

Fleming JG, Spencer TE, and Bazer FW, 1999. Analysis of the upstream sequences of the ovine estrogen receptor α gene. Biol Reprod 60 (suppl 1):55

Flint AP, Parkinson TJ, Stewart HJ, Vallet JL, and Lamming GE, 1991. Molecular biology of trophoblast interferons and studies of their effects in vivo. J Reprod Fertil (suppl 43):13-25

Flint AP, Sheldrick EL, McCann TJ, and Jones DS, 1990. Luteal oxytocin: characteristics and control of synchronous episodes of oxytocin and PGF2 α secretion at luteolysis in ruminants. Domest Anim Endocrinol 7:111-124

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Fogwell RL, Cowley JL, and Wartman JA. 1985. Luteal function in cows following destruction of ovarian follicles at mid cycle. Theriogenology 23:389-398

Folman Y, Rosenberg M, Herz Z, and Davidson M, 1973. The relationship between plasma progesterone concentration and conception in post-partum dairy cows maintained on two levels of nutrition. J Reprod Fertil 34:267-278

Fuchs AF, Behrens O, Helmer H, Liu CH, Barros CM, and Fields MJ, 1990. Oxytocin and vasopressin receptors in bovine endometrium and myometrium during the estrus cycle and eraly pregnancy. Endocrinology 127:629-636

Fujita T, Kimura Y, Miyamoto M, Barsoumian EL, and Taniguchi T, 1989. Induction of endogenous IFN-α and IFN-β genes by a regulatory transcription factor, IRF-1. Nature 337:270-272

Fujita T, Ohno S, Yasumitsu H, and Taniguchi T, 1985. Delimitation and properties of DNA sequences required for the regulated expression of human interferon-β gene. Cell 41:489-496

Fujita T, Shibuya H, Hotta H, Yamanish K, and Taniguchi T, 1987. Interferon-β gene regulation: tandemly repeated sequences of a synthetic 6 bp oligomer function as a virus-inducible enhancer. Cell 49:357-367

Gates AH, 1965. Rate of ovular development as a factor in embryonic survival. In: Wolstenholme G and O'Connor M (eds) *Preimplantation Stages of Pregancy*. Churchil, Livingstone, London, Ciba Foundation Symposium, pp. 270-293.

Geisert RD, Renegar RH, Thatcher WW, Roberts RM, and Bazer FW, 1982. Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol Reprod 27:925-939

Geisert RD, Zavy MT, Biggers BG, Garrett JE, and Wettemann RP, 1988. Characterization of the uterine invironment during early conceptus expansion in the bovine. Anim Reprod Sci 16:11-25

Gerrits RJ, BLosser TH, Purchase HG, Terell CE, and Warwick EJ, 1979. Economics of improving reproductive efficiency in farm animals. In: Hawk HW (eds) *Beltsville Symposia in Agricultural Research*. New York: John Wiley&Sons, pp 413-421.

Ginther OJ, 1974. Internal regulation of physiological processes through local venoarterial pathways. J Anim Sci 39:550-564

Girsh E, Wang W, Mamluk R, Arditi F, Friedman A, Milvae RA, and Meidan R, 1996. Regulation of endothelin-1 expression in the bovine corpus luteum: elevation by prostaglandin F2α. Endocrinol 137:5191-5196

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Goding J, Baird D, Cumming I, and McCracken JA, 1972. The functional assessment of autotransplanted endocrine glands. Acta Endocrinol Suppl (Copenh) 158:169-199

Godkin JD, Bazer FW, Moffatt J, Sessions F, and Roberts RM, 1982. Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at day 13-21. J Reprod Fertil 65:141-150

Godkin JD, Bazer FW, and Roberts RM, 1984. Ovine trophoblast protein 1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein synthesis. Endocrinol 114:120-130

Godkin JD, Lifsey BJJ, and Gillespie BE, 1988. Characterization of bovine conceptus proteins produced during the peri- and postattachment periods of early pregnancy. Biol Reprod 38:703-711

Goodbourn S, 1990. The regulation of beta-interferon gene expression. Semin Cancer Biol 1:89-95

Goodbourn S, Burstein H, and Maniatis T, 1986. The human β-interferon gene enhancer is under negative control. Cell 45:601-610

Goodbourn S and Maniatis T, 1988. Overlapping positive and negative regulatory domains of the human β-interferon gene. Proc Natl Acad Sci U S A 85:1447-1451

Goodbourn S, Zinn K, and Maniatis T, 1985. Human β-interferon gene expression is regulated by an inducible enhancer element. Cell 41:509-520

Gorski K, Carneiro M, and Schibler U, 1986. Tissue-specific in vitro transcription from the mouse albumin promoter. Cell 47:767-776

Graves PE, Pierce KL, Bailey TJ, Rueda BR, Gil DW, Woodward DF, Yool AJ, Hoyer PB, and Regan JW, 1995. Cloning of a receptor for prostaglandin F2α from the ovine corpus luteum. Endocrinology 136:3430-3436

Guesdon FM, Stewart HJ, and Flint AP, 1996. Negative regulatory domains in a trophoblast interferon promoter. J Mol Endocrinol 16:99-106

Guillomot M, Michel C, Gaye P, Charlier N, Trojan J, and Martal J, 1990. Cellular localization of an embryonic interferon, ovine trophoblastin and its mRNA in sheep embryos during early pregnancy. Biol Cell 68:205-211

Guldenaar SE, Wathes DC, and Pickering BT, 1984. Immunocytochemical evidence for the presence of oxytocin and neurophysin in the large cells of the bovine corpus luteum. Cell Tissue Res 237:349-352

Hagenbuchle O and Wellauer PK, 1992. A rapid method for the isolation of DNA-binding proteins from purified nuclei of tissues and cells in culture. Nucleic Acids Res 20:3555-3559

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Han CS, Mathialagan N, Klemann SW, and Roberts RM, 1997. Molecular cloning of ovine and bovine type I interferon receptor subunits from uteri, and endometrial expression of messenger ribonucleic acid for ovine receptors during the estrous cycle and pregnancy. Endocrinology 138:4757-4767

Hansel W, Concannon PW, and Lukaszewska J, 1973. Corpora lutea of the large domestic ruminants. Biol Reprod 8:222-245

Hansen TR, Austin KJ, and Johnson GA, 1997. Transient ubiquitin cross-reactive protein gene expression in the bovine endometrium. Endocrinol 138:5079-5082

Hansen TR, Cross JC, Farin CE, Imakawa K, and Roberts RM, 1991. Slowed transcription and rapid messenger RNA turnover contribute to a decline in synthesis of ovine trophoblast protein-1 during in vitro culture. Biol Reprod 45:94-100

Hansen TR, Imakawa K, Polites HG, Marotti KR, Anthony RV, and Roberts RM, 1988. Interferon RNA of embryonic origin is expressed transiently during early pregnancy in the ewe. J Biol Chem 263:12801-12804

Hansen TR, Kazemi M, Keisler DH, Malathy PV, Imakawa K, and Roberts RM, 1989. Complex binding of the embryonic interferon, ovine trophoblast protein- 1, to endometrial receptors. J Interferon Res 9:215-225

Hansen TR, Leaman DW, Cross JC, Mathialagan N, Bixby JA, and Roberts RM, 1991. The genes for the trophoblast interferons and the related interferon-αII possess distinct 5'-promoter and 3'-flanking sequences. J Biol Chem 266:3060-3067

Harada H, Fujita T, Miyamoto M, Kimura Y, Maruyama M, Furia A, Miyata T, and Taniguchi T, 1989. Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. Cell 58:729-739

Harada H, Takahashi E, Itoh S, Harada K, Hori TA, and Taniguchi T, 1994. Structure and regulation of the human interferon regulatory factor 1 (IRF-1) and IRF-2 genes: implications for a gene network in the interferon system. Mol Cell Biol 14:1500-1509

Harada H, Willison K, Sakakibara J, Miyamoto M, Fujita T, and Taniguchi T, 1990. Absence of the type I IFN system in EC cells: transcriptional activator (IRF-1) and repressor (IRF-2) genes are developmentally regulated. Cell 63:303-312

Hare WC, Singh EL, Betteridge KJ, Eaglesome MD, Randall GC, Mitchell D, Bilton RJ, and Trounson AO, 1980. Chromosomal analysis of 159 bovine embryos collected 12 to 18 days after estrus. Can J Genet Cytol 22:615-626

Hartigan PJ, Griffin JF, and Nunn WR, 1974. Some observations on Corynebacterium pyogenes infection of the bovine uterus. Theriogenology 1:153-167

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Harvey D, Basu S, Betteridge KJ, Goff AK, and Kindahl H, 1984. The influence of pregnancy on PGF2-alpha secretion in cattle.II. Urinary levels of 11-ketotetranor PGF metabolites and plasma progesterone concentrations during oestrus cycle and early pregnancy. Anim Reprod Sci 7:217-234

Hassold TJ, 1980. A cytogenetic study of repeated spontaneous abortions. Am J Hum Genet 32:723-730

Hauptmann R and Swetly P, 1985. A novel class of human type I interferons. Nucleic Acids Res 13:4739-4749

Hehneke KE, Christenson LK, Ford SP, and Taylor M, 1994. Macrophage infiltration into the porcine corpus luteum during prostaglandin F2α induced luteolysis. Biol Reprod 50:10-15

Helmer SD, Gross TS, Newton GR, Hansen PJ, and Thatcher WW, 1989. Bovine trophoblast protein-1 complex alters endometrial protein and prostaglandin secretion and induces an intracellular inhibitor of prostaglandin synthesis in vitro. J Reprod Fertil 87:421-430

Helmer SD, Hansen PJ, Anthony RV, Thatcher WW, Bazer FW, and Roberts RM, 1987. Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1. J Reprod Fertil 79:83-91

Helmer SD, Hansen PJ, and Thatcher WW, 1988. Differential glycosylation of the components of the bovine trophoblast protein-1 complex. Mol Cell Endocrinol 58:103-107

Helmer SD, Hansen PJ, Thatcher WW, Johnson JW, and Bazer FW, 1989. Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend corpus luteum lifespan in cyclic cattle. J Reprod Fertil 87:89-101

Hernandez-Ledezma JJ, Sikes JD, Murphy CN, Watson AJ, Schultz GA, and Roberts RM, 1992. Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. Biol Reprod 47:374-380

Heyman Y, Camous S, Fevre J, Meziou W, and Martal J, 1984. Maintenance of the corpus luteum after uterine transfer of trophoblastic vesicles to cyclic cows and ewes. J Reprod Fertil 70:533-540

Heyman Y and MenezoY, 1987. Interaction of trophoblastic vesicles with bovine embryos developing in vitro. In: Bavister BD (eds) *The Mammalian Preimplantation Embryo. Regulation of Growth and Differentiation in vitro*. New York, London: Plenum Press, pp. 175-191

Hiscott J, Alper D, Cohen L, Leblanc JF, Sportza L, Wong A, and Xanthoudakis S, 1989. Induction of human interferon gene expression is associated with a nuclear factor that

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

interacts with the NF-κB site of the human immunodeficiency virus enhancer. J Virol 63:2557-2566

Hixon JE and Flint AP, 1987. Effects of a luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinositide turnover and prostaglandin F-2α secretion in sheep. J Reprod Fertil 79:457-467

Hixon JE and Hansel W, 1974. Evidence for preferential transfer of prostaglandin F2α to the ovarian artery following intrauterine administration in cattle. Biol Reprod 11:543-552

Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, and Korsmeyer SJ, 1993. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell 75:241-251

Horn S, Bathgate R, Lioutas C, Bracken K, and Ivell R, 1998. Bovine endometrial epithelial cells as a model system to study oxytocin receptor regulation. Hum Reprod Update 4:605-614

Huggins JP, Pelton JT, and Miller RC, 1993. The structure and specificity of endothelin receptors: their importance in physiology and medicine. Pharmacol Ther 59:55-123

Iannuzzi L, Di Meo GP, Gallagher DS, Ryan AM, Ferrara L, and Womack JE, 1993. Chromosomal localization of omega and trophoblast interferon genes in goat and sheep by fluorescent in situ hybridization. J Hered 84:301-304

Iannuzzi L, Gallagher DS, Ryan AM, Di Meo GP, and Womack JE, 1993. Chromosomal localization of omega and trophoblast interferon genes in cattle and river buffalo by sequential R-banding and fluorescent in situ hybridization. Cytogenet Cell Genet 62:224-227

Imakawa K, Hansen TR, Malathy PV, Anthony RV, Polites HG, Marotti KR, and Roberts RM, 1989. Molecular cloning and characterization of complementary deoxyribonucleic acids corresponding to bovine trophoblast protein-1: a comparison with ovine trophoblast protein-1 and bovine interferon-αII. Mol Endocrinol 3:127-139

Imakawa K, Helmer SD, Nephew KP, Meka CSR, and Christenson RK, 1993. A Novel Role for GM-CSF: Enhancement of Pregnancy Specific Interferon Production, Ovine Trophoblast Protein-1. Endocrinology 132:1869-1871

Imakawa K and Roberts RM, 1989. Interferons and maternal recognition of pregnancy. Prog Clin Biol Res 294:347-358

Isaacs A and Lindenman J, 1957. Virus interference.I. The interferon. Proc R Soc Lond (Biol) 147:258-267

Iwasaki S and Nakahara T, 1990. Incidence of embryos with chromosomal anomalies in the inner cell mass among bovine blastocysts fertilized in vitro. Theriogenology 34:683-690

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Jacobs AL. Edgerton LA, Silvia WJ. and Schillo KK. 1988. Effect of an estrogen antagonist (tamoxifen) on cloprostenol-induced luteolysis in heifers. J Anim Sci 66:735-742

Juengel JL. Garverick HA. Johnson AL. Youngquist RS. and Smith MF. 1993. Apoptosis during luteal regression in cattle. Endocrinology 132:249-254

Juengel JL. Meberg BM. Turzillo AM. Nett TM. and Niswender GD. 1995. Hormonal regulation of messenger ribonucleic acid encoding steroidogenic acute regulatory protein in ovine corpora lutea. Endocrinology 136:5423-5429

Juengel JL. Nett TM. Anthony RV. and Niswender GD. 1997. Effects of luteotrophic and luteolytic hormones on expression of mRNA encoding insulin-like growth factor I and growth hormone receptor in the ovine corpus luteum. J Reprod Fertil 110:291-298

Juengel JL. Wiltbank MC. Meberg BM. and Niswender GD. 1996. Regulation of steady-state concentrations of messenger ribonucleic acid encoding prostaglandin F2α receptor in ovine corpus luteum. Biol Reprod 54:1096-1102

Juste RA. Ott TL. Kwang J. Bazer FW. and Concha-Bermejillo A. 1996. Effects of recombinant interferon-τ on ovine lentivirus replication. J Interferon Cytokine Res 16:989-994

Kawai Y and Clark MR. 1986. Mechanisms of action of gonadotropin releasing hormone on rat granulosa cells. Endocr Res 12:195-209

Kazemi M. Malathy PV. Keisler DH. and Roberts RM. 1988. Ovine trophoblast protein-1 and bovine trophoblast protein-1 are present as specific components of uterine flushings of pregnant ewes and cows. Biol Reprod 39:457-463

Kerr IM and Stark GR. 1991. The control of interferon-inducible gene expression. FEBS Lett 285:194-198

Kerr JF. Wyllie AH. and Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239-257

Kindahl H. Edquist LE. Bane A. and Granstrom E, 1976. Blood levels of progesterone and 15-keto-13.14-dihydro-prostaglandin F2alpha during the normal oestrous cycle and early pregnancy in heifers. Acta Endocrinol (Copenh) 82:134-149

King WA. 1990. Chromosome abnormalities and pregnancy failure in domestic animals. Adv Vet Sci Comp.Med. 34:229-250

Klemann SW, Imakawa K. and Roberts RM. 1990. Sequence variability among ovine trophoblast interferon cDNA. Nucleic Acids Res 18:6724

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Klemann SW, Li JZ, Imakawa K, Cross JC, Francis H, and Roberts RM, 1990. The production, purification, and bioactivity of recombinant bovine trophoblast protein-1 (bovine trophoblast interferon). Mol Endocrinol 4:1506-1514

Knickerbocker JJ, Thatcher WW, Bazer FW, Barron DH, and Roberts RM, 1986. Inhibition of uterine prostaglandin-F2α production by bovine conceptus secretory proteins. Prostaglandins 31:777-793

Knickerbocker JJ, Thatcher WW, Bazer FW, Drost M, Barron DH, Fincher KB, and Roberts RM, 1986. Proteins secreted by day-16 to -18 bovine conceptuses extend corpus luteum function in cows. J Reprod Fertil 77:381-391

Ko Y, Lee CY, Ott TL, Davis MA, Simmen RC, Bazer FW, and Simmen FA, 1991. Insulin-like growth factors in sheep uterine fluids: concentrations and relationship to ovine trophoblast protein-1 production during early pregnancy. Biol Reprod 45:135-142

Korsmeyer SJ, 1995. Regulators of cell death. Trends Genet 11:101-105

Kozak M, 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell 44:283-292

Kunze H and Vogt W, 1971. Significance of phospholipase A for prostaglandin formation. Ann N Y Acad Sci 180:123-125

Lamming GE, Wathes DC, Flint AP, Payne JH, Stevenson KR, and Vallet JL, 1995. Local action of trophoblast interferons in suppression of the development of oxytocin and oestradiol receptors in ovine endometrium. J Reprod Fertil 105:165-175

Lands WEM and Samuelson B, 1968. Phospholipid precursors of prostaglandins. Biochim Biophys Acta 164:426-429

Leaman DW, 1993. The evolution and transcriptional regulation of the trophoblast interferon genes. University of Missouri-Columbia. Ph D Thesis.

Leaman DW, Cross JC, and Roberts RM, 1992. Genes for the trophoblast interferons and their distribution among mammals. Reprod Fertil Dev 4:349-353

Leaman DW, Cross JC, and Roberts RM, 1994. Multiple regulatory elements are required to direct trophoblast interferon gene expression in choriocarcinoma cells and trophectoderm. Mol Endocrinol 8:456-468

Leaman DW and Roberts RM, 1992. Genes for the trophoblast interferons in sheep, goat, and musk ox and distribution of related genes among mammals. J Interferon Res 12:1-11

Leblanc JF, Cohen L, Rodrigues M, and Hiscott J, 1990. Synergism between distinct enhansor domains in viral induction of the human-β interferon gene. Mol Cell Biol 10:3987-3993

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Lee CN and Ax RL. 1984. Milk progesterone of dairy cows injected with gonadotropin-releasing hormone at the 1st post partum breeding. In: Proc 10th Int Cong Anim Reprod and AI. 2: 401-401.

Lenardo MJ, Fan CM, Maniatis T. and Baltimore D. 1989. The involvement of NF-κβ in β-interferon gene regulation reveals its role as widely inducible mediator of signal transduction. Cell 57:287-294

Levy DE. Kessler DS. Pine R. Reich N. and Darnell JEJ. 1988. Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. Genes Dev 2:383-393

Li J and Roberts RM. 1994a. Interferon-τ and interferon-α interact with the same receptors in bovine endometrium. Use of a readily iodinatable form of recombinant interferon-τ for binding studies. J Biol Chem 269:13544-13550

Li J and Roberts RM. 1994b. Structure-function relationships in the interferon-τ (IFN-τ). Changes in receptor binding and in antiviral and antiproliferative activities resulting from site-directed mutagenesis performed near the carboxyl terminus. J Biol Chem 269:24826-24833

Lifsey BJ. Baumbach GA. and Godkin JD. 1989. Isolation. characterization and immunocytochemical localization of bovine trophoblast protein-1. Biol Reprod 40:343-352

Liu L. Larson SF. Kubisch HM. and Roberts RM. 1997. Definition and expression of the major interferon-τ transcripts in bovine conceptuses. Biol Reprod 56:185-185

Liu L. Leaman DW. and Roberts RM. 1996. The interferon-τ genes of the giraffe. a nonbovid species. J Interferon Cytokine Res 16:949-951

Lukaszewska J. and Hansel W, 1980. Corpus luteum maintenance during early pregnancy in the cow. J Reprod Fertil 59:485-493

Lund B. Edlund T, Lindenmaier W, Ny T, Collins J, Lundgren E, and von Gabain A. 1984. Novel cluster of α-interferon gene sequences in a placental cosmid DNA library. Proc Natl Acad Sci U S A 81:2435-2439

MacDonald NJ. Kuhl D. Maguire D. Naf D. Gallant P. Goswamy A. Hug H. Bueler H. Chaturvedi M. de la Fuente J. Ruffner H. Meyer F. and Weismann C. 1990. Different pathways mediate virus inducibility of the human IFN-α1 and IFN-β genes. Cell 60:767-779

Martal J. Lacroix MC, Loudes C, Saunier M, and Wintenberger-Torres S. 1979. Trophoblastin. an antiluteolytic protein present in early pregnancy in sheep. J Reprod Fertil 56:63-73

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Martal J. Camous S. Fevre J. Charlier M. and Heyman Y. 1984. Specificity of embryonic signals maintaining corpus luteum function in early pregnancy in ruminants. Proc 10th Int Congr Anim Reprod AI. 3: 510

Martinod S. Maurer RR. Siegenthaler B. Gerber C. and Hansen PJ. 1991. The effect of recombinant bovine interferon-alpha on fertility on ewes. Theriogenology 36:231-239

Matsuyama S. Chang KT. Kanuka H. Ohnishi M. Ikeda A. Nishihara M. and Takahashi M. 1996. Occurrence of deoxyribonucleic acid fragmentation during prolactin- induced structural luteolysis in cycling rats. Biol Reprod 54:1245-1251

Maurer RR and Echternkamp.S.E. 1982. Hormonal asynchrony and embryonic developmet. Theriogenology 17:11-22

McCracken JA. 1997. Prostaglandins and leukotrienes. In: Conn PM. and Melmeds (eds) Endocrinology: Basic and Clinical Principles. Clifton: Humana Press. pp. 101-104

McCracken JA and Caldwell BV. 1969. Corpus luteum maintenance in a ewe with one congenitally absent uterine horn. J Reprod Fertil 20:139-141

McCracken JA. Custer EE. and Lamsa JC. 1999. Luteolysis: Neuroendocrine-Mediated event. Physiological Reviews 79:263-323

McCracken JA. Custer EE. Lamsa JC. and Robinson AG. 1995. The central oxytocin pulse generator: a pacemaker for luteolysis. Adv Exp Med Biol 395:133-154

McCracken JA. Glew ME. and Scaramuzzi RJ. 1970. Corpus luteum regression induced by prostagland in F2α. J Clin Endocrinol Metab 30:544-546

McCracken JA. Schramm G. and Okulicz WC. 1984. Hormone receptor control of pulsatile secretion of PGF2α from the ovine uterus during luteolysis and its abrogation in early pregnancy. In: Edquist LE and Kindahl H (eds) *Prostaglandins in Animal Reproductions II*. Amsterdam: Elsevier, pp. 31-56

McGuire WJ. Juengel JL. and Niswender GD. 1994. Protein kinase C second messenger system mediates the antisteroidogenic effects of prostaglandin F2α in the ovine corpus luteum in vivo. Biol Reprod 51:800-806

McMillan KL. Taufa VK. Day AM. and Peterson AJ. 1991. Effect of supplemental progesterone on pregnancy rates in cattle. J Reprod Fertil Suppl 43 (Abstr):304

Meyer MD. Hansen PJ. Thatcher WW. Drost M. and Roberts RM. 1995. Effect of bovine interferon-τ on body temperature and plasma progesterone concentrations in cyclic dairy cows. J Dairy Sci 78:1470-1476

Meyer HD. Mittermeier TH. and Schams D. 1988. Dynamics of oxytocin. estrogen and progestin receptors in the bovine endometrium during the estrus cycle. Acta Endocrinol (Copenh) 118:96-104

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Miller BG, Moore NW, Murphy L, and Stone GM, 1977. Early pregnancy in the ewe: effects of oestradiol and progesterone on uterine metabolism and on embryo survival. Aust J Biol Sci 30:279-288

Milvae RA, Hinckley ST, and Carlson JC, 1996. Luteotropic and luteolytic mechanisms in the bovine corpus luteum. Theriogenology 45:1327-1349

Miyajima A, Kitamura T, Harada N, Yokota T, and Arai K, 1992. Cytokine receptors and signal transduction. Annu Rev Immunol 10:295-331

Miyamoto NJ, Moncollin V, Wintzerith M, Hen R, Egly JM, and Chambon P, 1984. Stimulation of *in vitro* transcription by the upstream element of the adenovirus-2 major late promoter involves a specific factor. Nucleic Acids Res 12:8779-8799

Moor RM and Rowson LEA, 1964. Influence of the embryo and uterus on luteal function in the sheep. Nature 201:522-523

Moor RM and Rowson LEA, 1966a. Local maintenance of the corpus luteum in sheep with embryos transferred to various isolated portions of the uterus. J Reprod Fertil 12:539-550

Moor RM and Rowson LEA, 1966b. Local uterine mechanisms affecting luteal function in the sheep. J Reprod Fertil 11:307-310

Moor RM and Rowson LEA, 1966c. The corpus luteum of the sheep: functional relationship between the embryo and the corpus luteum. J Endocrinol 34:233-239

Morgan G, Wooding FB, and Godkin JD, 1993. Localization of bovine trophoblast protein-1 in the cow blastocyst during implantation: an immunological cryoultrastructural study. Placenta 14:641-649

Morgan GL, Geisert RD, McCann JP, Bazer FW, Ott TL, Mirando MA, and Stewart M, 1993. Failure of luteolysis and extension of the interoestrous interval in sheep treated with the progesterone antagonist mifepristone (RU 486). J Reprod Fertil 98:451-457

Morgan GL, Geisert RD, Zavy MT, Shawley RV, and Fazleabas AT, 1987. Development of pig blastocysts in a uterine environment advanced by exogenous oestrogen. J Reprod Fertil 80:125-131

Naivar KA, Ward SK, Austin KJ, Moore DW, and Hansen TR, 1995. Secretion of bovine uterine proteins in response to type I interferons. Biol Reprod 52:848-854

Nephew KP, McClure KE, Day ML, Xie S, Roberts RM, and Pope WF, 1990. Effects of intramuscular administration of recombinant bovine interferon-αII during the period of materal recognition of pregnancy. J Anim Sci 68:2766-2770

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Nephew KP. Whaley AE. Christenson RK. and Imakawa K. 1993. Differential expression of distinct mRNAs for ovine trophoblast protein-1 and related sheep type I interferons. Biol Reprod 48:768-778

Newcomb R and Rowson LEA 1975. Conception rate after uterine transfer of cow eggs. in relation to synchronization of oestrus and age of eggs. J Reprod Fertil 43:539-541

Newton GR. Martinod S. Hansen PJ, Thatcher WW. Siegenthaler B. Gerber C. and Voirol MJ. 1990. Effect of bovine interferon on acute changes in body temperature and serum progesterone concentrations in heifers. J Dairy Sci 73:3439-3448

Nishizuka Y. 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607-614

Niswender GD. Dziuk PJ. Graber J. and Kaltenbach CC. 1970. Function of the corpus luteum in the ewe following relocation of the uterus or embryo. J Anim Sci 30:935-940

Niswender GD and Nett TM. 1995. Corpus luteum and its control in infraprimate species. In: Knobil E.and Neil JD (eds) *The Physiology of Reproduction.* New York: Raven Press. pp. 781-816.

Niswender KD. Li J. Powell MR. Loos KR. Roberts RM. Keisler DH. and Smith MF. 1997. Effect of variants of interferon-tau with mutations near the carboxyl terminus on luteal life span in sheep. Biol Reprod 56:214-220

Northey DL and French LR. 1980. Effect of embryo removal and intrauterine infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. J Anim Sci 50:298-302

O'Shea JD. Nightingale MG. and Chamley WA. 1977. Changes in small blood vessels during cyclical luteal regression in sheep. Biol Reprod 17:162-177

Ohtani M. Kobayashi S. Miyamoto A. Hayashi K. and Fukui Y. 1998. Real-time relationships between intraluteal and plasma concentrations of endothelin. oxytocin. and progesterone during prostaglandin F2α-induced luteolysis in the cow. Biol Reprod 58:103-108

Oltvai ZN. Milliman CL. and Korsmeyer SJ. 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog. Bax. that accelerated programmed cell death. Cell 74:609-619

Ott TL. Zhou Y. Mirando MA. Stevens MG. Harney JP. Ogle TF. and Bazer FW. 1993. Changes in progesterone and estrogen receptor mRNA and protein during maternal recognition and luteolysis in ewes. J Mol Endocrinol 10:757-769

Parkinson TJ and Lamming GE. 1990. Interrelationships between progesterone. 13.14-dihydro-15-keto PGF-2α (PGFM) and LH in cyclic and early pregnant cows. J Reprod Fertil 90:221-233

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Pate JL. 1994. Cellular components involved in luteolysis. J Anim Sci 72:1884-1890

Pestka S. Langer JA. Zoon KC and Samuel CE. 1987. Interferons and their actions. Annu Rev Biochem 56:727-777

Peters KE. Bergfeld EG, Cupp AS, Kojima FN, Mariscal V, Sanchez T, Wehrman ME. Grotjan HE. Hamernik DL. and Kittok RJ. 1994. Luteinizing hormone has a role in development of fully functional corpora lutea (CL) but is not required to maintain CL function in heifers. Biol Reprod 51:1248-1254

Pexton JE. Weems CW. and Inskeep EK. 1975. Prostaglandin F in uterine and ovarian venous plasma from nonpregnant and pregnant ewes collected by cannulation. Prostaglandins 9:501-509

Pharriss BB. Cornette JC. and Gutknecht GD. 1970. Vascular control of luteal steroidogenesis. J Reprod Fertil Suppl 10:97-103

Pimentel CA. Vijayakumar R. Weston PG. Pimentel SM. Wagner WC. and Hixon JE. 1986. Effects of estrone infusion on secretion of F series prostaglandins and on function of the corpus luteum during late gestation in cattle. Am J Vet Res 47:1972-1977

Piper PJ. Vane JR. and Wyllie JH. 1970. Inactivation of prostaglandins by the lungs. Nature 225:600-604

Plante C. Hansen PJ. Martinod S. Siegenthaler B. Thatcher WW. Pollard JW. and Leslie MV. 1989. Effect of intrauterine and intramuscular administration of recombinant bovine interferon-α1 on luteal lifespan in cattle. J Dairy Sci 72:1859-1865

Plante C. Hansen PJ. and Thatcher WW. 1988. Prolongation of luteal lifespan in cows by intrauterine infusion of recombinant bovine alpha-interferon. Endocrinology 122:2342-2344

Plante C, Thatcher WW. and Hansen PJ. 1991. Alteration of oestrous cycle length. ovarian function and oxytocin-induced release of prostaglandin F2α by intrauterine and intramuscular administration of recombinant bovine interferon-α to cows. J Reprod Fertil 93:375-384

Pontzer CH. Bazer FW. and Johnson HM. 1991. Antiproliferative activity of a pregnancy recognition hormone. ovine trophoblast protein-1. Cancer Res 51:5304-5307

Pontzer CH. Torres BA. Vallet JL. Bazer FW. and Johnson HM. 1988. Antiviral activity of the pregnancy recognition hormone ovine trophoblast protein-1. Biochem Biophys Res Commun 152:801-807

Pope WF. 1988. Uterine asynchrony: a cause of embryonic loss. Biol Reprod 39:999-1003

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Quirke JF. Hanrahan JP. and Gosling JP. 1979. Plasma progesterone levels throughout the oestrous cycle and release of LH at oestrus in sheep with different ovulation rates. J Reprod Fertil 55:37-44

Ragg H and Weissmann C. 1983. Not more than 117 base pairs of 5'-flanking sequence are required for inducible expression of a human IFN-α gene. Nature 303:439-442

Raj NB. Israeli R. Kellum M. and Pitha PM. 1989. Upstream regulatory elements of murine alpha 4-interferon gene confer inducibility and cell type-restricted expression. J Biol Chem 264:11149-11157

Rakugi H. Tabuchi Y. Nakamaru M. Nagano M. Higashimori K. Mikami H. Ogihara T. and Suzuki N. 1990. Evidence for endothelin-1 release from resistance vessels of rats in response to hypoxia. Biochem Biophys Res Commun 169:973-977

Ravindranath N. Little-Ihrig L. Benyo DF. and Zeleznik AJ. 1992. Role of luteinizing hormone in the expression of cholesterol side-chain cleavage cytochrome P450 and 3 beta-hydroxysteroid dehydrogenase, delta 5-4 isomerase messenger ribonucleic acids in the primate corpus luteum. Endocrinology 131:2065-2070

Reynolds LP. Robertson DA. and Ford SP. 1983. Effects of intrauterine infusion of oestradiol-17β and prostaglandin E2 on luteal function in non-pregnant heifers. J Reprod Fertil 69:703-709

Richards JS and Hedin L. 1988. Molecular aspects of hormone action in ovarian follicular development. ovulation. and luteinization. Annu Rev Physiol 50:441-463

Roberts RM. 1989. Conceptus interferons and maternal recognition of pregnancy. Biol Reprod 40:449-452

Roberts RM. 1991. A role for interferons in early pregnancy. Bioessays 13:121-126

Roberts RM. Cross JC. Farin CE. Hansen TR. Klemann SW. and Imakawa K. 1990. Interferons at the placental interface. J Reprod Fertil Suppl 41:63-74

Roberts RM. Cross JC. and Leaman DW. 1992. Interferons as hormones of pregnancy. Endocrine Review 13:432-452

Roberts RM. Farin CE. and Cross JC. 1990. Trophoblast proteins and maternal recognition of pregnancy. Oxf Rev Reprod Biol 12:147-180

Roberts RM. Klemann SW. Leaman DW. Bixby JA, Cross JC. Farin CE. Imakawa K. and Hansen TR. 1991. The polypeptides and genes for ovine and bovine trophoblast protein-1. J Reprod Fertil Suppl 43:3-12

Roberts RM. Leaman DW. and Cross JC. 1992. Role of interferons in maternal recognition of pregnancy in ruminants. Proc Soc Exp Biol Med 200:7-18

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Robinson NA. Leslie KE. and Walton JS. 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J Dairy Sci 72:202-207

Roche JF. Bolandl MP. and McGeady TA. 1981. Reproductive wastage following artificial insemination of heifers. Vet Rec 109:401-404

Rowson LEA. Lawson RA. Moor RM. and Baker AA. 1972. Egg transfer in the cow: synchronization requirements. J Reprod Fertil 28:427-431

Rowson LEA and Moor RM. 1967. The influence of embryonic tissue homogenate infused into the uterus. on the life-span of the corpus luteum in the sheep. J Reprod Fertil 13:511-516

Rowson LE. Moor RM. and Lawson RA. 1969. Fertility following egg transfer in the cow: effect of method. medium and synchronization of oestrus. J Reprod Fertil 18:517-523

Rueda BR. Naivar KA. George EM. Austin KJ. Francis H. and Hansen TR. 1993. Recombinant interferon-τ regulates secretion of two bovine endometrial proteins. J Interferon Res 13:303-309

Rueda BR. Tilly KI. Botros IW. Jolly PD. Hansen TR. Hoyer PB. and Tilly JL. 1997. Increased bax and interleukin-1β-converting enzyme messenger ribonucleic acid levels coincide with apoptosis in the bovine corpus luteum during structural regression. Biol Reprod 56:186-193

Ryals J. Dierks P. Ragg H. and Weissmann C. 1985. A 46-nucleotide promoter segment from an IFN-α gene renders an unrelated promoter inducible by virus. Cell 41:497-507

Ryan AM and Womack JE. 1993. Type I interferon genes in cattle: restriction fragment length polymorphisms. gene numbers and physical organization on bovine chromosome 8. Anim Genet 24:9-16

Sakamoto K. Ezashi T. Miwa K. Okuda-Ashitaka E. Houtani T. Sugimoto T. Ito S. and Hayaishi O. 1994. Molecular cloning and expression of a cDNA of the bovine prostaglandin F2α receptor. J Biol Chem 269:3881-3886

Sakamoto K. Miwa K. Ezashi T. Okuda-Ashitaka E. Okuda K. Houtani T. Sugimoto T. Ito S. and Hayaishi O. 1995. Expression of mRNA encoding the prostaglandin F2α receptor in bovine corpora lutea throughout the oestrous cycle and pregnancy. J Reprod Fertil 103:99-105

Sambrook J. Fritsch EF. and Maniatis T. 1989. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor. NY: Cold Spring Harbor Press

SAS. 1989. SAS/STAT User's guide.. Cary, NC: Statistical Analysis System Institute Inc.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Sawadogo M and Roeder RG. 1985. Factors involved in specific transcription by human RNA polymerase II: Analysis by a rapid and quantitative *in vitro* assay. Proc Natl Acad Sci U S A 82:4394-4398

Sawyer HR. 1995. Structural and functional properties of the corpus luteum of pregnancy. J Reprod Fertil Suppl 49:97-110

Sawyer HR, Niswender KD, Braden TD, and Niswender GD. 1990. Nuclear changes in ovine luteal cells in response to PGF2α. Domest Anim Endocrinol 7:229-237

Schalue TK, Farin PW, Cross JC, and Roberts RM. 1991. Effect of injected bovine interferon-α1 on oestrus cycle length and pregnacy success in sheep. J Reprod Fertil 91:356

Schindler C, Shuai K, Prezioso VR, and Darnell JEJ. 1992. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 257:809-813

Schmitt RA, Geisert RD, Zavy MT, Short EC, and Blair RM. 1993. Uterine cellular changes in 2',5'-oligoadenylate synthetase during the bovine estrous cycle and early pregnancy. Biol Reprod 48:460-466

Senda T, Saitoh SI, Mitsui Y, Li J, and Roberts RM. 1995. A three-dimensional model of interferon-τ. J Interferon Cytokine Res 15:1053-1060

Shaw G and Kaman R. 1986. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA-degradation. Cell 46:659-667

Shemesh M, Ayalon N, and Lindner HR. 1968. Early effects of conceptus on plasma progesterone levels in the cow. J Reprod Fertil 15:161-164

Shemesh M and Hansel W. 1975. Levels of prostaglandin F (PGF) in bovine endometrium uterine venous, ovarian arterial and jugular plasma during the estrous cycle. Proc Soc Exp Biol Med 148:123-126

Shikone T, Kokawa K, Yamoto M, and Nakano R. 1997. Apoptosis of human ovary and uterine endometrium during the menstrual cycle. Horm Res 48 Suppl 3:27-34

Short ECJ, Geisert RD, Helmer SD, Zavy MT, and Fulton RW. 1991. Expression of antiviral activity and induction of 2',5'-oligoadenylate synthetase by conceptus secretory proteins enriched in bovine trophoblast protein-1. Biol Reprod 44:261-268

Short RV. 1969. Implantation and the maternal recognition of pregnancy. In: Wolstenholme G and O'Connor M (eds) *Foetal Autonomy*. Churchill, London: Ciba Foundation, pp. 2-26

Silvia WJ, Lewis GS, McCracken JA, Thatcher WW, and Wilson LJ. 1991. Hormonal regulation of uterine secretion of prostaglandin F2α during luteolysis in ruminants. Biol Reprod 45:655-663

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Skopets B, Li J, Thatcher WW, Roberts RM, and Hansen PJ, 1992. Inhibition of lymphocyte proliferation by bovine trophoblast protein-1 (type I trophoblast interferon) and bovine interferon-αII. Vet Immunol Immunopathol 34:81-96

Soloff MS, 1975. Uterine receptor for oxytocin: Effects of estrogen. Biochem Biophy Res Com 65:205-212

Soloff MS and Fields MJ, 1989. Changes in uterine oxytocin receptor concentrations throughout the estrus cycle of cow. Biol Reprod 40:283-287

Spencer TE and Bazer FW, 1996. Ovine interferon-τ suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. Endocrinology 137:1144-1147

Spencer TE, Becker WC, George P, Mirando MA, Ogle TF, and Bazer FW, 1995. Ovine interferon-τ regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. Biol Reprod 53:732-745

Spencer TE, Mirando MA, Mayes JS, Watson GH, Ott TL, and Bazer FW, 1996. Effects of interferon-τ and progesterone on oestrogen-stimulated expression of receptors for oestrogen, progesterone and oxytocin in the endometrium of ovariectomized ewes. Reprod Fertil Dev 8:843-853

Spencer TE, Ott TL, and Bazer FW, 1998. Expression of interferon regulatory factors one and two in the ovine endometrium: effects of pregnancy and ovine interferon-τ. Biol Reprod 58:1154-1162

Sreenan JM and Diskin MG, 1983. Early embryonic mortality in the cow: its relationship with progesterone concentration. Vet Rec 112:517-521

Sreenan JM and Diskin MG, 1986a. Progesterone and embryo survival in the cow. In: Sreenan JM, and Diskin MG (eds) *Embryonic Mortality in Farm Animals*. Boston: Martinus Nijhoff, pp. 142-158

Sreenan JM and Diskin MG, 1986b. The extent and timing of embryonic mortality in the cow. In: Sreenan JM, Diskin MG (eds) *Embryonic mortality in Farm Animals*. Boston: Martinus Nijhoff. pp. 1-9

Staples RE, McEntee K, and Hansel W, 1969. Luteal function as related to pituitary and ovarian cytology and embryo development in the bovine. J Dairy Sci 44:2049-2057

Stevenson KR, Riley PR, Stewart HJ, Flint AP, and Wathes DC, 1994. Localization of oxytocin receptor mRNA in the ovine uterus during the oestrous cycle and early pregnancy. J Mol Endocrinol 12:93-105

Stewart HJ, 1990. Trophoblastic factors and the maternal recognition of pregnancy in sheep and cattle. J Dev Physiol 14:115-123

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Stewart HJ, Flint AP, Lamming GE, McCann SH, and Parkinson TJ, 1989. Antiluteolytic effects of blastocyst-secreted interferon investigated in vitro and in vivo in the sheep. J Reprod Fertil Suppl 37:127-138

Stewart HJ, McCann SH, Barker PJ, Lee KE, Lamming GE, and Flint AP, 1987. Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein. J Endocrinol 115:R13-R15

Stewart HJ, McCann SH, and Flint AP, 1990. Structure of an interferon-alpha 2 gene expressed in the bovine conceptus early in gestation. J Mol Endocrinol 4:275-282

Stewart HJ, McCann SH, Northrop AJ, Lamming GE, and Flint AP, 1989. Sheep antiluteolytic interferon: cDNA sequence and analysis of mRNA levels. J Mol Endocrinol 2:65-70

Streuli M, Nagata S, and Weissmann C, 1980. At least three human type alpha-interferons: structure of alpha 2. Science 209:1343-1347

Subramaniam PS, Khan SA, Pontzer CH, and Johnson HM, 1995. Differential recognition of the type I interferon receptor by interferons-τ and -α is responsible for their disparate cytotoxicities. Proc Natl Acad Sci U S A 92:12270-12274

Sugimoto Y, Hasumoto K, Namba T, Irie A, Katsuyama M, Negishi M, Kakizuka A, Narumiya S, and Ichikawa A, 1994. Cloning and expression of a cDNA for mouse prostaglandin F receptor. J Biol Chem 269:1356-1360

Taniguchi T, Harada H, and Lamphier M, 1995. Regulation of the interferon system and cell growth by the IRF transcription factors. J Cancer Res Clin Oncol 121:516-520

Teixeira MG, Austin KJ, Perry DJ, Dooley VD, Johnson GA, Francis BR, and Hansen TR, 1997. Bovine granulocyte chemotactic protein-2 is secreted by the endometrium in response to interferon-τ (IFN-τ). Endocrine 6:31-37

Thatcher WW, Danet-Desnoyers G, and Wetzels C, 1992. Regulation of bovine endometrial prostaglandin secretion and the role of bovine trophoblast protein-1 complex. Reprod Fertil Dev 4:329-334

Thatcher WW, Hansen PJ, Gross TS, Helmer SD, Plante C, and Bazer FW, 1989. Antiluteolytic effects of bovine trophoblast protein-1. J Reprod Fertil Suppl 37:91-99

Tysseling KA, Thatcher WW, Bazer FW, Hansen PJ, and Mirando MA, 1998. Mechanisms regulating prostandin F2α secretion from the bovine endometrium. J Dairy Sci 81:382-389

Ulberg LC and Sheean LA, 1973. Early development of mammalian embryos in elevated ambient temperatures. J Reprod Fertil Suppl 19:155-161

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Valezquez L, Fellous M, Stark GR, and Pellegrini S, 1992. A protein tyrosine kinase in the interferon-α / -β signaling pathway. Cell 70:313-322

Vallet JL, Bazer FW, Fliss MF, and Thatcher WW, 1988. Effect of ovine conceptus secretory proteins and purified ovine trophoblast protein-1 on interoestrous interval and plasma concentrations of prostaglandins F2α and E and of 13,14-dihydro- 15-keto prostaglandin F2α in cyclic ewes. J Reprod Fertil 84:493-504

Villa-Godoy A, Ireland JJ, Wortman JA, Ames NK, Hughes TL, and Fogwell RL, 1985. Effect of ovarian follicles on luteal regression in heifers. J Anim Sci 60:519-527

Warfield SJ, Seidel GEJr, and Farrand GD, 1986. Lack of natural luteolysis associated with uterine horn aplasia in a heifer. J Am Vet Med Assoc 189:1586-1586

Warner CM, Brownell MS, and Ewoldsen MA, 1988. Why aren't embryos immunologically rejected by their mothers? Biol Reprod 38:17-29

Watanabe K, Yoshida R, Shimizu T, and Hayaishi O, 1985. Enzymatic formation of prostaglandin F2α from prostaglandin H2 and D2. Purification and properties of prostaglandin F synthetase from bovine lung. J Biol Chem 260:7035-7041

Wathes DC and Hamon M, 1993. Localization of oestradiol, progesterone and oxytocin receptors in the uterus during the oestrous cycle and early pregnancy of the ewe. J Endocrinol 138:479-492

Wathes DC, Mann GE, Payne JH, Riley PR, Stevenson KR, and Lamming GE, 1996. Regulation of oxytocin, oestradiol and progesterone receptor concentrations in different uterine regions by oestradiol, progesterone and oxytocin in ovariectomized ewes. J Endocrinol 151:375-393

Watling D, Holland SJ, Pellegrini S, Stark GR, and Kerr IM, 1992. A human cell line responsive to beta but not alpha interferons. J Interferon Res 12 (suppl 1):s91

Watson AJ, Hogan A, Hahnel A, Weimer D, and Schultz GA, 1991. Expression of growth factor ligand and receptor genes in the preimplantation bovine embryos. Mol Reprod Dev 31:87-95

Weems CW, Huie JM, Hoyer G, Magness RR, and Whysong GL, 1985. Maternal recognition of pregnancy in ruminants. In: Davis WC, Shelton JN, and Weems CW (eds) *Characterization of the Bovine Immune System and the Genes Regulating Expression of Immunity with Particular Reference to their Role in Disease Resistance*. Pullman: Washington State University Press, pp. 193-210

Weissmann C, Nagata S, Boll W, Fountoulakis M, Fujisawa A, Fujisawa JI, Haynes J, Henco K, Mantei N, Ragg H, Schein C, Schmid J, Shaw G, Streuli M, Taira H, Todokoro K, and Weidle U, 1982. Structure and expression of human IFN-α genes. Phil Trans R Soc Lond B Biol Sci 299:7-28

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Wilmut I. Ashworth CJ. Springbett AJ. and Sales DI. 1988. Effect of variation in embryo stage on the establishment of pregnancy. and embryo survival and growth in ewes with two embryos. J Reprod Fertil 83:233-237

Wilmut I and Sales DI. 1981. Effect of an asynchronous environment on embryonic development in sheep. J Reprod Fertil 61:179-184

Wilmut I. Sales DI. and Ashworth CJ. 1985. Physiological criteria for embryo mortality- is asynchrony between embryo and ewe a significant factor. In: Land RB and Robinson D (eds) *The Genetics of Reproduction in Sheep*. London: Butterworths. pp. 275-287

Wilson L. Cenedella RJ. Butcher RL. and Inskeep EK. 1972. Levels of prostaglandins in the uterine endometrium during the ovine estrus cycle. J Anim Sci 34:93-101

Wiltbank JN. Rowden WW. Ingalls JE. Gregory KE. and Koch RM. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. J Anim Sci 21:219-225

Wiltbank MC. Diskin MG. Flores JA. and Niswender GD. 1990. Regulation of the corpus luteum by protein kinase C. II. Inhibition of lipoprotein-stimulated steroidogenesis by prostaglandin F2α. Biol Reprod 42:239-245

Wiltbank MC. Diskin MG. and Niswender GD. 1991. Differential actions of second messenger systems in the corpus luteum. J Reprod Fertil Suppl 43:65-75

Winkelman GL. Larson SF. Roberts RM. and Ealy AD. 1999. Selected interferon-τ variants are expressed by the ovine and bovine conceptus. Biol Reprod 60 (abstr):115

Wintenberger-Torres S. 1967. Action de la progesterone et des steroids ovarians sur la segmentation des oeufs chez la brebis. Anns Biol Anim Biochim Biophys 7:391-406

Wolfenson D. Thatcher WW. Drost M. Caton D. Foster DB. and LeBlanc MM. 1985. Characteristics of prostaglandin F measurements in the ovarian circulation during the oestrous cycle and early pregnancy in the cow. J Reprod Fertil 75:491-499

Xavier F. Guillomot M. Charlier M. Martal J. and Gaye P. 1991. Co-expression of the proto-oncogene fos (c-fos) and an embryonic interferon (ovine trophoblastin) by sheep conceptuses during implantation. Biol Cell 73:27-33

Xiao CW and Goff AK. 1999. Hormonal regulation of oestrogen and progesterone receptors in cultured bovine endometrial cells. J Reprod Fertil 115:101-109

Xiao CW. Liu JM. Sirois J. and Goff AK. 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

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Xiao CW, Murphy BD, Sirois J, and Goff AK. 1998. Down-regulation of oxytocin-induced cyclooxygenase-2 and prostaglandin F synthase expression by interferon-τ in bovine endometrial cells. Biol Reprod 60:656-663

Zavy MT. 1994. Embryonic mortality in cattle. In: Zavy MT and Geisert RD (eds) *Embryonic Mortality in Domestic Species*. Ann Arbor: CRC, pp. 99-140

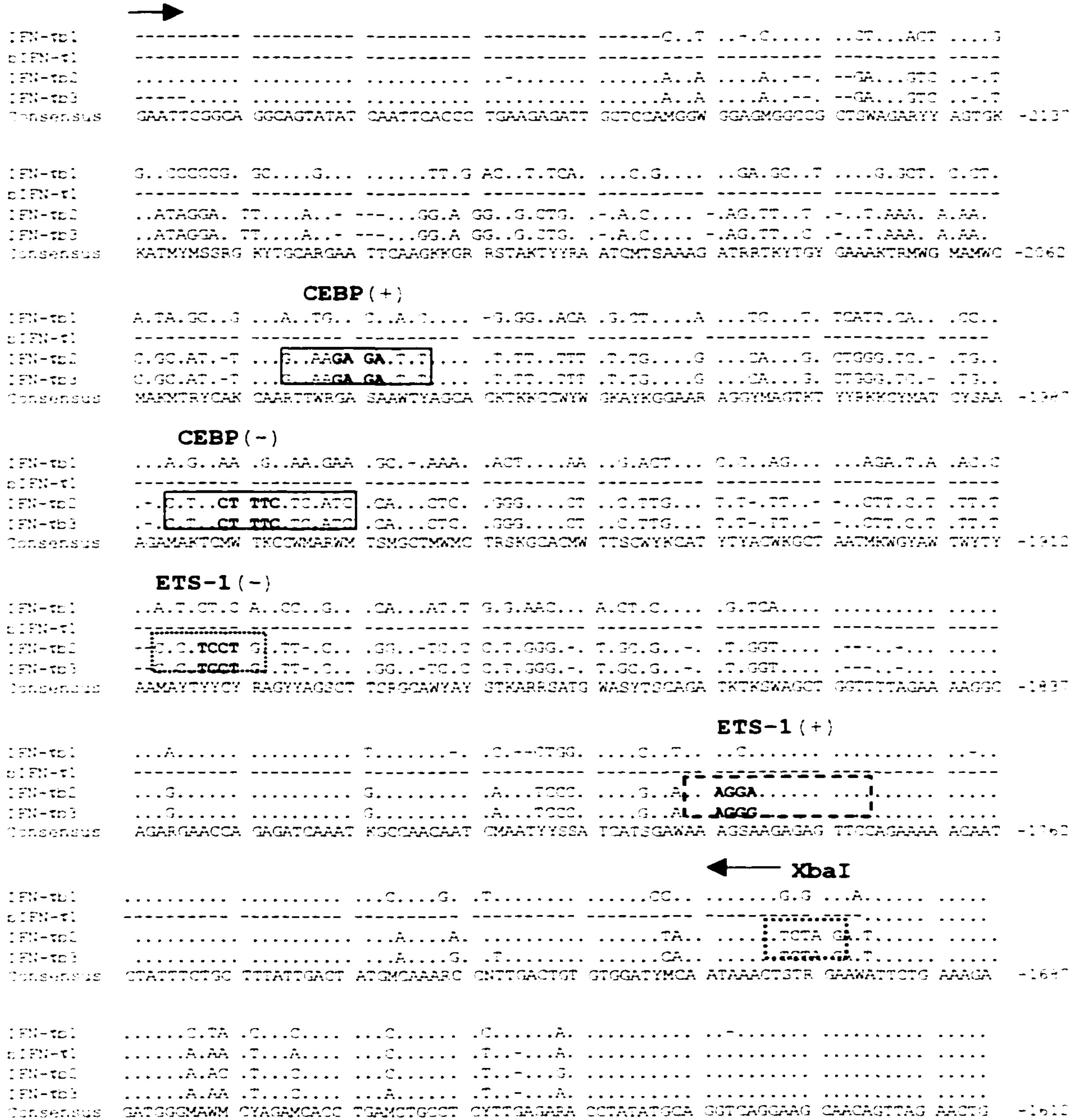
Zheng J, Fricke PM, Reynolds LP, and Redmer DA. 1994. Evaluation of growth, cell proliferation, and cell death in bovine corpora lutea throughout the estrous cycle. Biol Reprod 51:623-632

Zingg HH, Grazzini E, Breton C, Larcher A, Rozen F, Russo C, Guillon G, and Mouillac B. 1998. Genomic and non-genomic mechanisms of oxytocin receptor regulation. Adv Exp Med Biol 449:287-295

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

APPENDIX 1

Sequence Alignment of bIFN-τ Variants.



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

```
IFN-τb1     .......... .......... .......... .......... .......... .....A...- .......... .....
bIFN-τ1     .......... .......... .......... .......... .......... .....G.... .......-.. .....
IFN-τb2     .......... .......... .......... .......... .......... .....G...- .......... .....
IFN-τb3     .......... .......... .......... .......... .......... .....G...- .......... .....
Consensus   GACATGGAAC AACAGACTGG TTCCAAATAG GAAAAGGAGT ACATCAAGGC TGTATRTTGA TGACCCTGCT TATTT   -1537

IFN-τb1     .A........ .....T...T .A.......A ........A. .G........ .C.....AC. ....T.G.C. .....
bIFN-τ1     .T........ .....G...G .G.......T ........G. .A........ .G.....TT. ....A.C.T. .....
IFN-τb2     .T........ .....G...G .G.......T ........G. .A........ .G.....TT. ....A.C.T. .....
IFN-τb3     .T........ .....G...G .G.......T ........G. .A........ .G.....TT. ....A.C.T. .....
Consensus   AWCTTATATG CAGAGKACAK CRTGAGAAAW GCTGGGCTRG ARGAAGCACA ASCTGGAWYC AAGAWTSCYG GGAGA   -1462

IFN-τb1     A......... .......... .......... ....-..... .....-T... .......... .......... .....
bIFN-τ1     A......... .......... .......... .......... .....-.... .......... .......... .....
IFN-τb2     A......... .......... .......... .......... .....-.... .......... .......... .....
IFN-τb3     G......... .......... .......... .......... ......A... .......... .......... .....
Consensus   RATATCAATA ACCTCAGATA TGCAGATGAC ACCAACCCTT ATGYCAWGAA AGTGAAGAGG AACTAAAAAG CCTCT   -1387

IFN-τb1     .......... .......A.. .....C..A. .......... .......... ........A. .........A .....
bIFN-τ1     .......... ...-...G.. .....A..C. .......... .......... .......-G. .........C ....-
IFN-τb2     .......... ...-...G.. .....A..C. .......... .......... ........G. .........C .....
IFN-τb3     .......... ...-...G.. .....A..G. .......... .......... ........G. .........T ....-
Consensus   TGATGAAAGT GAAAGAGRAG AGTGAMAAVG TTGGCTTAAA GCTGAACATT GAGAAAACRA AGATCATGGM ATCTG   -1312

IFN-τb1     .-........ .......... ....A..... .......... .......... .......... ...G...... .....
bIFN-τ1     .......... .......... ....G....- --------.. .......... .......... ...T...... .....
IFN-τb2     .......... .......... ....G....- --------.. .......... .......... ...T...... .....
IFN-τb3     .......... .......... ....G....- --------.. .......... .......... ...T...... .....
Consensus   GGTCCCATCA CTTCATGGGA AATARATGGG GAAACAGTGG AAACAGTGTC AGACTTTATT TTTKGGGGCT GGAAA   -1237

IFN-τb1     .......... .......... .......... .......... ......T... .......... .......... ...T.
bIFN-τ1     .......... .......... .......... .......... ......G... .......... .......... ...C.
IFN-τb2     .......... .......... .......... .......... ......T... .......... .......... ...C.
IFN-τb3     .......... .......... .......... .......... ......T... .......... .......... ...C.
Consensus   ATCACTGCAG ATGGTGATTG CAGCCATGAA ATTAAAAGAC GCTTACKCCT TGGAAGGAAA GTTATGACCA ACCYA   -1162

IFN-τb1     .........T .C...G.A.. .......... .T.....C.. .G........ .......... .........A .A...
bIFN-τ1     .........G .A...A.C.. .......... .G.....T.. .A........ .......... .........C .T...
IFN-τb2     .........G .A...A.C.. .......... .G.....T.. .A........ .......... .........C .T...
IFN-τb3     .........G .A...A.C.. .......... .G.....T.. .A........ .......... .........C .T...
Consensus   KATAGCATAK TMAAARCMAG AGACATTACT TKGCCAAYAA ARGTCCGTCT AGTCAAGGCT ATGGTTTTTM CWGTG   -1087

IFN-τb1     .......... ...GG...AG .....C.... .......... ..T....... ...C...... ........AT ..T..
bIFN-τ1     .......... ...TT...TA .....T.... .......... ..C....... ...T...... ........GC ..T..
IFN-τ2      .......... ...TT...TG .....T.... .......... ..C....... ...T...... ........GC ..C..
IFN-τ3      .......... ...TT...TG .....T.... .......... ..C....... ...T...... ........GC ..T..
Consensus   TTCATGTATG GATKKGAGWR TTGGAYTGTG AAGAAAGCTG AGYGCCAAAG AATYGATGCT TTTGAAYTRY GGYGT   -1012

IFN-τb1     .......... .......... CC........ G....G.... .......... .......... .......... C....
bIFN-τ1     .......... .......... TT........ A....A.... ....---... .......... .......... T....
IFN-τb2     .......... .......... TT........ A....A.... ....---... .......... .......... T....
IFN-τb3     .......... .......... TT........ A....A.... ....---... .......... .......... T....
Consensus   TGGAGAAGAC TCTTGAGAGT YYCTTGGACT RCAAGGRGAT CCAACCAGTC CATCCTAAAG GAGATCAGTC YTGGG   -937

IFN-τb1     .......... .....A.... G.T....... .......... -TC....... ..T..C.... .......... ..T..
bIFN-τ1     .......... .....C.... A.C....... .......... .CT....... ..A..T.... .......... ..C..
IFN-τb2     .......... .....C.... G.C....... .......... .CT....... ..A..T.... .......... ..C..
IFN-τb3     .......... .....C.... G.C....... .......... .CT....... ..A..T.... .......... ..C..
Consensus   TGTTCTTTGG AAGGAMTGAT RCYAAAGCTG AAACTCCAAT AYYTTGGCCA CCWCAYGTGA AGAGTTGACT CAYTG   -862

IFN-τb1     .......G.. .........A .......... G......... G........G .......... .......... .....
bIFN-τ1     .......C.. .........G .......... A......... A........A .......... .......... .....
IFN-τb2     .......C.. .........G .......... A......... A........A .......... .......... .....
IFN-τb3     .......C.. .........G .......... A......... A........A .......... .......... .....
Consensus   GAAAAGASTC TGATGCTGGR AGGGATTGGG RGCAGGAGGA RAAGGGGACR ACAGAGGATG AGATGGCTGG ATGGC   -787
```


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

```
IFN-tb1     ......-... .CT...G..A ...--.T... ......A..C A..G...... ........G. .......GA. G....
oIFN-t1     ........-. .TG...C..G ...--.G... ......C..T G..A...... ........A. .......AA. A....
IFN-tb2     ........-. .TG...C..G ...--.G... ......C..T G..A...... ........A. .......AG. G....
IFN-tb3     .......... .TG...C..G ......G... ......C..T G..A...... ........A. .......AG. G....
Consensus   ATCACCGAAC TYKATGSACR TGAAGGKTTG GGTGAAMTCY RGGRGTTGGT GATGGACARG GAGGCCTRRT RTGCT   -712

IFN-tb1     .CG.....T. .......... ..G.C...TG .....C..C. ......A.C. ...A...... .......... .....
oIFN-t1     .CA.....T. .......... -.T.-...CA .....A..A. ......C.G. ...G...... .......... .....
IFN-tb2     .AA.....C. .......... -.T.G...CA .....G..A. ......C.G. ...G...... .......... .....
IFN-tb3     .CA.....C. .......... -.T.G...CA .....G..A. ......C.G. ...G...... .......... .....
Consensus   CMRATTCAYG GGGTCACAAA GAKTCRMAYR CGACTVAGMG ACTGAAMTSA ACTRAGCCCA CAGGTGTATT CCCCT   -637

IFN-tb1     ........C. .......... .......... ......T..C .......... .......... .......... .....
oIFN-t1     ........G. .......... .......... ......A..G .......... .......... .......... .....
IFN-tb2     ........C. .......... .......... ......A..G .......... .......... .......... .....
IFN-tb3     ........C. .......... .......... ......A..G .......... .......... .......... .....
Consensus   TCCCTGAGGG CCCTGGAGGG CTGGAAACTC TTGATGWCTS TGTCATCCTG GTTTACTGAT ATGGGGGAA ATACT   -562

IFN-tb1     C...TT..AT G.C...C... .......... .......... .......... .......... .......... .....
oIFN-t1     C...TC..GT G.T...G... .......... .......... .......... .......... .......... .....
IFN-tb2     C...TC..GT G.T...G... .......... .......... .......... .......... .......... .....
IFN-tb3     T...CC..GG C.T...G... .......... .......... .......... .......... .......... .....
Consensus   YCACYYTCRK SAYCTTSTGG CCCCAGTGAA ATGTAATCTC AGGAACCTGA ATGAATGTTC TCAAAAGAAC AGAAA   -487

IFN-tb1     .......... G......... ........A. .......... .......... .......... ..GT.G.A.. ...C.
oIFN-t1     .......... C......... ........A. .......... .......... .......... ..AC.A.C.. ...T.
IFN-tb2     .......... G......... ........A. .......... .......... .......... ..AC.A.C.. ...T.
IFN-tb3     .......... G......... ........G. .......... .......... .......... ..AC.A.C.. ...T.
Consensus   TAACTGAGTA STTCATTCAC TCTTAATTRC AAGCTTATCC TCTGTGAGAC AGAAAGAAA ATRYARAMAT CAAYA   -412

IFN-tb1     ......T... .......... .......... .......... .......... .......... .......... .C...
oIFN-t1     ......G... .......... .......... .......... .......... .......... .......... .T...
IFN-tb2     ......G... .......... .......... .......... .......... .......... .......... .T...
IFN-tb3     ......G... .......... .......... .......... .......... .......... .......... .T...
Consensus   TGGCCTKAGT GACTCTGCAT TCCTATGTGT AAGATAAGGA GGGAAAAATG CAGTTAAGAA TGAATGGAAA AYTAT   -337

IFN-tb1     G.......C. .......... .......... ....C..... .......... .......... .......... .....
oIFN-t1     A.......C. .......... .......... ....T..... .......... .......... .......... .....
IFN-tb2     A.......G. .......... .......... ....T..... .......... .......... .......... .....
IFN-tb3     A.......G. .......... .......... ....T..... .......... .......... .......... .....
Consensus   RTTCCTGAGA TAAGATAAAC AAAAGAATG TTTAYATATA TTATACCTAA ATTTGTACAT AATAACTATG TACAT   -262

IFN-tb1     .......... .......... .......... ...G...... .......... .......... .......... .....
oIFN-t1     .......... .......... .......... ...G...... .......... .......... .......... .....
IFN-tb2     .......... .......... .......... ...G...... .......... .......... .......... .....
IFN-tb3     .......... .......... .......... ...C...... .......... .......... .......... .....
Consensus   ATCTATAAGT CTTTGCATAC TTACATAACT TCASCCTTTA TTTAGTTTCT CATTTAATTG ATATACATTT ACATT   -187

IFN-tb1     .......... .......... .T........ .C.....C.. .........- .......... .......... .....
oIFN-t1     .......... .......... .G........ .T.....C.. .......... .......... .......... .....
IFN-tb2     .......... .......... .G........ .C.....C.. .......... .......... .......... .....
IFN-tb3     .......... .......... .G........ .C.....T.. .......... .......... .......... .....
Consensus   GACAAACCCA AATTTTATTG GKAAAATTAA AYTTCTAYTG TAAAAATTAA GAGTTTAGAT TGACTACATT TCCTA   -112

IFN-tb1     .......T.. .......... .A........ ...C...... ......T... ......T... ......A... T....
oIFN-t1     .......C.. .......... .C........ ...A...... ......G... ......C... ......G... C....
IFN-tb2     .......C.. .......... .C........ ...A...... ......G... ......C... ......G... C....
IFN-tb3     .......T.. .......... .C........ ...A...... ......G... ......C... ......G... T....
Consensus   GYTCAAAYAG AAAATATCTA AMTGAAAACA CAAMCAGGAA GTGAGAKAGA AATTTTYGGA TAATGARTAC YGTCT   -37
```


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TATA box

+1

ATG

TGA

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

```
IFN-tb1    .......... ...C...... .....T...T .-...--.C. .--..--.C. .--.----C. .A..----.- -...-
bIFN-t1    .......... ...T...... .....C...C ........T. ........T. ........T. .G........ .....
IFN-tb2    .......... ...T...... .....C...C ...------- ---------- ---------. .C-------- -----
IFN-tb3    .......... ...T...... .....C...C ...------- ---------- ---------- ---------- -----
Consensus  CAGAAGACTC TGAYTTCTGC TTCAGYCACY GAAATCATYG AATTACTTYA ACTGATACYT TYTCAGCAGT AATAA   799
```

Poly A

```
IFN-tb1    -.-C..C.-- -------... ..----.-.. ...------- ------.C-- ---------- ---------- -----
bIFN-t1    ...A..A... .......... .......... .......... .......T.. .......... .......... .....
IFN-tb2    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-tb3    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
Consensus  [illegible] [illegible]GTAC TGAGCT[illegible]TAG GGGCATGAGT CCTTAAGYGA TGCCTGCCCT [illegible] [illegible]   864
```

Homologies between bIFN-τ distal promoter regions. Putative CEBP binding sites are boxed in solid lines, and putative ETS-1 binding sites are boxed in broken lines, with core sequences marked with bold characters. Orientations of the motifs are also indicated. The cloning site (XbaI) is also indicated. Arrowheads represent the segment of DNA subcloned into the TK- and PRL-luc vectors. Note that none of these elements is present in INF-τb1 subtype. Notice that most severe mismatches are found in the distal 5' ends of these genes. Putative TATA box, transcription start site, translation start site, translation stop site, and polyadenylation signal are indicated. bIFN-τ 1 was cloned by Hansen et al., (1991).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

APPENDIX 2

Sequence Alignment of bIFN-αII Variants

```
bIFN-αII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    GAATTCTTGG TAGGGTTAAT GGCCGGGCCA GCAATGGACA ATAGGTCTCC AGTACGAAAA TGGTGAAAGC AAGCG
Consensus  GAATTCTTGG TAGGGTTAAT GGCCGGGCCA GCAATGGACA ATAGGTCTCC AGTACGAAAA TGGTGAAAGC AAGCG   [illegible]

bIFN-αII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    TGATGTCTGT AATAGTCCTG ACTTCGTGGT TCCCTGAAGC ACAACCACAT GGACCTGCAG GAACGTGTTT TAGAA
Consensus  TGATGTCTGT AATAGTCCTG ACTTCGTGGT TCCCTGAAGC ACAACCACAT GGACCTGCAG GAACGTGTTT TAGAA   [illegible]

bIFN-αII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    TGAAAAGAAA CATGGGAGGA GCTCATGGAC TCACAATAAT CATCCTCTTC TCTAAATCAA ACACTCAAGA AAACC
Consensus  TGAAAAGAAA CATGGGAGGA GCTCATGGAC TCACAATAAT CATCCTCTTC TCTAAATCAA ACACTCAAGA AAACC   -440

bIFN-αII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    CATCCTTTGT TTCAAACAGT TTCTTTGCTA GAGTGACCTG GTAAATGCCT GATAAACACA GAGCCTGCCT TCCTA
Consensus  CATCCTTTGT TTCAAACAGT TTCTTTGCTA GAGTGACCTG GTAAATGCCT GATAAACACA GAGCCTGCCT TCCTA   -365

bIFN-αII   ---------- ---------- ---------- GATCA-GGCC --AAATGTGT TCATG--ACA G-AAAGAGCC ACA-T
IFN-ωb1    AGTAAAGTAA GAAACAGAAA TTAAGCCTAG GATCAGGGCC AAAAATGTGT TCATGGAACA GAAAAGAGCC ACATT
Consensus  AGTAAAGTAA GAAACAGAAA TTAAGCCTAG GATCAGGGCC AAAAATGTGT TCATGGAACA GAAAAGAGCC ACATT   [illegible]

bIFN-αII   TACACATGAA AGAGAAAGAG TGATAATGTT -GTAC-TAGA AACCTACATC ATTTCTAATG TAAGACAAAG ACTTT
IFN-ωb1    TACACATGAA AGAGAAAGAG TGATAATGTT TGTACTTAGA AACCTACATC ATTTCTAATG TAAGACAAAG ACTTT
Consensus  TACACATGAA AGAGAAAGAG TGATAATGTT TGTACTTAGA AACCTACATC ATTTCTAATG TAAGACAAAG ACTTT   [illegible]

bIFN-αII   TTT-ATTTGA TTGATAAATA TCCATTTGGA TGGGTACATT TGAAGTTATT GTGAAATAGA TAAAAGTAAT AGTTT
IFN-ωb1    TTTCATTTGA TTGATAAATA TCCATTTGGA TGGGTACATT TGAAGTTATT GTGAAATAGA TAAAAGTAAT AGTTT
Consensus  TTTCATTTGA TTGATAAATA TCCATTTGGA TGGGTACATT TGAAGTTATT GTGAAATAGA TAAAAGTAAT AGTTT   -140

                                               IRF-1
bIFN-αII   AGACTGATGA CAATTTCTTT GACCATATTC GGAATACATA AATGAAAATC AAAAAAGGAA GTACAAGTAC ACAGA
IFN-ωb1    AGACTGATGA CAATTTCTTT GACCATATTC AGAATACATA AATGAAAATC AAAAAAGGAA GTACAAGTAC ACAGA
Consensus  AGACTGATGA CAATTTCTTT GACCATATTC AGAATACATA AATGAAAATC AAAAAAGGAA GTACAAGTAC ACAGA   [illegible]

           TBB         IRF-1                         TATA box                 +1
bIFN-αII   AATGACTAGA AAATGAAAAT TACTGTGTTC CCTATTTAAT -GGCCTTGCT TAGAAAGCAT GGCATCAGAG AACCT
IFN-ωb1    AATGACTAGA AAATGAAAAT TACTGTGTTC CCTATTTAAT GGGCCTTGCT TAAAAAGCAT GGCATCAGAG AACCT
Consensus  AATGACTAGA AAATGAAAAT TACTGTGTTC CCTATTTAAT GGGCCTTGCT TAAAAAGCAT GGCATCAGAG AACCT   [illegible]

                                                                    ATG
bIFN-αII   ACCTCAAGGT TCCACCAGAC GCTGTCTCAG CCAGCCCAGC AGCAGCCTCA TCTTCCCATG GCCTTCGTGC TTCTC
IFN-ωb1    ACCTCAAGGT TCCACCAGAC GCTGTCTCAA CCAGCCCAGC AGCAGCCTCA TCTTCCCATG GCCTTCGTGC TTCTC
Consensus  ACCTCAAGGT TCCACCAGAC GCTGTCTCAR CCAGCCCAGC AGCAGCCTCA TCTTCCCATG GCCTTCGTGC TTCTC   [illegible]

bIFN-αII   TCTACTGATG GCCCTGGTGC TGGTCAGCTA TGGCCCGGGA GGATCCCTGG GCTGTGACTT GTCTCCGAAC CACGT
IFN-ωb1    TCTACTGATG GCCCTGGTGC TGGTCAGCTA TGGCCCGGGA GGATCCCTGG GCTGTGACTT GTCTCCGAAC CACTT
Consensus  TCTACTGATG GCCCTGGTGC TGGTCAGCTA TGGCCCGGGA GGATCCCTGG GCTGTGACTT GTCTCCGAAC CACGT   160

bIFN-αII   GCTGGTTGGC AGGCAGAACC TCAGGCTCCT GGGCCAAATG AGGAGACTCT CCCCTCGCTT CTGTCTGCAG GACAG
IFN-ωb1    GCTGGTTGGC AGGCAGAACC TCAGGCTCCT GGGCCAAATG AGGAGACTCT CCCCTCGCTT CTGTCTGCAG GACAG
Consensus  GCTGGTTGGC AGGCAGAACC TCAGGCTCCT GGGCCAAATG AGGAGACTCT CCCCTCGCTT CTGTCTGCAG GACAG   235
```


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cIFN-αII AAAAGACTTT GCTTTCCCCC AGGAGATGGT GGAGGTCAGT CAGTTCCAGG AGGCCCAGGC CATTTCTGTG CTCCA
IFN-αI AAAAGACTTT GCTTTCCCCC AGGAGATGGT GGAGGTCAGC CAGTTCCAGG AGGCCCAGGC CATTTCTGTG CTCCA
Consensus AAAAGACTTT GCTTTCCCCC AGGAGATGGT GGAGGTCAGC CAGTTCCAGG AGGCCCAGGC CATTTCTGTG CTCCA 310

cIFN-αII TGAGATGCTC CAGCAGAGCT TCAACCTCTT CCACAAAGAG CGCTCCTCTG CTGCCTGGGA CACTACCCTC CTGGA
IFN-αI TGAGATGCTC CAGCAGAGCT TCAACCTCTT CCACAAAGAG CTCTCCTCTG CTGCCTGGGA CACTACCCTC CTGGA
Consensus TGAGATGCTC CAGCAGAGCT TCAACCTCTT CCACAAAGAG CGCTCCTCTG CTGCCTGGGA CACTACCCTC CTGGA 385

cIFN-αII GCAGCTCCTC ACTGGACTCC ATCAGCAGCT GGATGACCTG GATGCCTGTC TGGGCCTTTT GACTGGAGAG GAAGA
IFN-αI GCAGCTCCTC ACTGGACTCC ATCAGCAGCT GGATGACCTG GATGTCTGTC TGGGCCTGTT GACTGGAGAG GAAGA
Consensus GCAGCTCCTC ACTGGACTCC ATCAGCAGCT GGATGACCTG GATGCCTGTC TGGGCCTGTT GACTGGAGAG GAAGA 460

cIFN-αII CTCTGCCCTG GGAAGGACGG GCCCCACACT GGCCATGAAG AGGTACTTCC AGGGCATCCA TGTCTACCTG CAAGA
IFN-αI CTCTGCCCTG GGAAGGACGG GCCCCACACT GGCCATGAAG AGGTACTTCC AGGGCATCCA TGTCTACCTG CAAGA
Consensus CTCTGCCCTG GGAAGGACGG GCCCCACACT GGCCATGAAG AGGTACTTCC AGGGCATCCA TGTCTACCTG CAAGA 535

cIFN-αII GAAGGGATAC AGCGACTGTG CCTGGGAAAT CGTCAGACTG GAAATCATGA GATCCTTGTC TTCATCAACC AGCTT
IFN-αI GAAGGGATAC AGCGACTGTG CCTGGGAAAT TGTCAGACTG GAAATCATGA GATCCTTGTC TTCATCAACC AGCTT
Consensus GAAGGGATAC AGCGACTGTG CCTGGGAAAT TGTCAGACTG GAAATCATGA GATCCTTGTC TTCATCAACC AGCTT 610

TGA

cIFN-αII GCAAGAAAGG TTAAGAATGA TGGATGGAGA CCTGAAATCA CCTTGACATG ACTCTCACTG ACTAAGATGC CTCAT
IFN-αI GCAAGAAAGG TTAAGAATGA TGGATGGAGA CCTGAAATCA CCTTGACATG ACTCTCACTG ACTAAGATGC CTCAT
Consensus GCAAGAAAGG TTAAGAATGA TGGATGGAGA CCTGAAATCA CCTTGACATG ACTCTCACTG ACTAAGATGC CTCAT 685

cIFN-αII CATCTTTGCA CACTCATCTG TGGCCATTTC AAAAGACTC**T GA**TTTCTGTT GTAGCCACAA AATTTATTGA ATTAC
IFN-αI CATCTTTGCA CACTCATCTG TGGCCATTTC AAAAGACTC**T GA**TTTCTGTT GTAGCCACAA AATTTATTGA ATTAC
Consensus CATCTTTGCA CACTCATCTG TGGCCATTTC AAAAGACTC**T GA**TTTCTGTT GTAGCCACAA AATTTATTGA ATTAC 760

cIFN-αII TTCAGCCAAT ACTTTGTCAG TAGTAAATGA ATATACATAA ATTTTTTTGG CTGCAGGTGC ATCAGTCCCG AAGTG
IFN-αI TTCAGCCAAT ACTTTGTCAG TAGTAAATGA ATATACATAA ATTTTTTTGG CTGCAGGTGC ATCAGTCCCG AAGTG
Consensus TTCAGCCAAT ACTTTGTCAG TAGTAAATGA ATATACATAA ATTTTTTTGG CTGCAGGTGC ATCAGTCCCG AAGTG 835

cIFN-αII AAGACTGCCC TTATTTTATT GTTTGCTTAT TTATTTTGTT ATATTTATTC TTTTATTTCC TCATATTTAT TTTTC
IFN-αI AAGACTGCCC TTATTTTATT GTTTGCTTAT TTATTTTGTT ATATTTATTC TTTTATTTCC TCATATTTAT TTTTC
Consensus AAGACTGCCC TTATTTTATT GTTTGCTTAT TTATTTTGTT ATATTTATTC TTTTATTTCC TCATATTTAT TTTTC 910

Poly A

cIFN-αII CATATAAAAT ATTTTTGTTT ACATTG**TATT AAA**ATTTAAC AAATACATTA ACATTTTTAT TTCATTATAT TTGTA
IFN-αI CATATAAAAT ATTTTTGTTT ACATTG**TATT AAA**ATTTAAC AAATACATTA ACATTTTTAT TTCATTATAT TTGTA
Consensus CATATAAAAT ATTTTTGTTT ACATTG**TATT AAA**ATTTAAC AAATACATTA ACATTTTTAT TTCATTATAT TTGTA 985

cIFN-αII ATTTGTTTTA TTTATTAAAT ATTGTCAAGG TGAACTTCTT GAATT----- ---------- ---------- -----
IFN-αI ATTTGTTTTA TTTATTAAAT ATTGTCAAGG TGAACTTCTT GAATTTTTTT ACCGTTTTAT GTTTAGTTGC CAGGT
Consensus ATTTGTTTTA TTTATTAAAT ATTGTCAAGG TGAACTTCTT GAATTTTTTT ACCGTTTTAT GTTTAGTTGC CAGGT 1060

cIFN-αII ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-αI AAAGTCTGAT TCTTTTGTGA CCCCATAGAC TGTAGCCCAC CAGGCTCCTC TGTCCATGGG ATGATCCAGG CAAGA
Consensus AAAGTCTGAT TCTTTTGTGA CCCCATAGAC TGTAGCCCAC CAGGCTCCTC TGTCCATGGG ATGATCCAGG CAAGA 1135

cIFN-αII ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-αI CTACTGGAGT ATGTTGCCAT TTACAGGGGA TCTTCCCAAC CAAGGATGGA ATCTGGAATC TGCATTTTAG GCAGA
Consensus CTACTGGAGT ATGTTGCCAT TTACAGGGGA TCTTCCCAAC CAAGGATGGA ATCTGGAATC TGCATTTTAG GCAGA 1210

cIFN-αII ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-αI TTCTTTACCA TCTGAGCCAC AAGGGAAGAC TCTTGTTTGA AAGAAATGAG AGAAAAAGGG ATTTTTTAAA CTGAA
Consensus TTCTTTACCA TCTGAGCCAC AAGGGAAGAC TCTTGTTTGA AAGAAATGAG AGAAAAAGGG ATTTTTTAAA CTGAA 1285

cIFN-αII ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-αI ATTAATAATT TACGATAGGT ACTGGGTCAT GAGTTCAGTT CAATTCATTC ACTCAGTCGT TTCTGACTCC TTGAG
Consensus ATTAATAATT TACGATAGGT ACTGGGTCAT GAGTTCAGTT CAATTCATTC ACTCAGTCGT TTCTGACTCC TTGAG 1360

cIFN-αII ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-αI ACCCCACGAA CTGCAGAATG CCAGGCCTCC CAAGTCCATC ACCAACTCCC AGAGTCCACC CAAACTAACG TCCAA
Consensus ACCCCACGAA CTGCAGAATG CCAGGCCTCC CAAGTCCATC ACCAACTCCC AGAGTCCACC CAAACTAACG TCCAA 1435

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

```
cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     TGATTCAGTG ATGCCATCCA AGCCATCTCA TCCTCTGTTG TCCCCTTCTC CTCCTGCCCT CAATCTTTCC CAGCA
Consensus   TGATTCAGTG ATGCCATCCA AGCCATCTCA TCCTCTGTTG TCCCCTTCTC CTCCTGCCCT CAATCTTTCC CAGCA    1510

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     TCAGGGTCTT TTCAAATGAG TCAGCTTTTC CCATTAGGTA GCCAAAATAT TGGAGTTTCA GCTTCAACAT CAGTC
Consensus   TCAGGGTCTT TTCAAATGAG TCAGCTTTTC CCATTAGGTA GCCAAAATAT TGGAGTTTCA GCTTCAACAT CAGTC    1585

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     CCTTCAAAGA ACACCCAGGA CTGATCTCCT TTAGGATGGA CTGCTTGGAT CTCCCTGTAG TCCAAGGACT CTAAG
Consensus   CCTTCAAAGA ACACCCAGGA CTGATCTCCT TTAGGATGGA CTGCTTGGAT CTCCCTGTAG TCCAAGGACT CTAAG    1660

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     AGTCTTCTCC AACACCACAG TTGAAAAGCA TCAATTCTTT GGTGCTCGGC TTTCTTTATA GTCTCAGATC CATAC
Consensus   AGTCTTCTCC AACACCACAG TTGAAAAGCA TCAATTCTTT GGTGCTCGGC TTTCTTTATA GTCTCAGATC CATAC    1735

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     ATGACCACTG GAAAAACCAT AGTCTTTTCT AGATGGACCT CTGTTGACAA AGTAACGTCT CTACTTTTTA ATATG
Consensus   ATGACCACTG GAAAAACCAT AGTCTTTTCT AGATGGACCT CTGTTGACAA AGTAACGTCT CTACTTTTTA ATATG    1810

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     CTGTCTAGGT TCATCATAAC TTTCCTTCCA AGTAGTATGC TTCTTTCAAT TTCAAGGCTG CAATCAACAA CTGCA
Consensus   CTGTCTAGGT TCATCATAAC TTTCCTTCCA AGTAGTATGC TTCTTTCAAT TTCAAGGCTG CAATCAACAA CTGCA    1885

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     ATTGATTTTG GGGCCCAGAA AATAAAGTCA GCCACTGTTT CCACTGTTTC CCCATCTATT GGCCATGAAG TGATG
Consensus   ATTGATTTTG GGGCCCAGAA AATAAAGTCA GCCACTGTTT CCACTGTTTC CCCATCTATT GGCCATGAAG TGATG    1960

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     GGAACAGATG CCATGATCTT AGTTTTCTGA AAGTTGATCT TTAAGCCACC TTTTTCACTC TCCTCTTTCA CTTTC
Consensus   GGAACAGATG CCATGATCTT AGTTTTCTGA AAGTTGATCT TTAAGCCACC TTTTTCACTC TCCTCTTTCA CTTTC    2035

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     ATCAAGAGGC CCTTTAGTTC TTCTTCACTT TTCTGCCCTA AGGGTGGTAT CTTCTGCATA TCTGAGGGTA TCGAT
Consensus   ATCAAGAGGC CCTTTAGTTC TTCTTCACTT TTCTGCCCTA AGGGTGGTAT CTTCTGCATA TCTGAGGGTA TCGAT    2110

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     ATTTCTCCCC ACAATCTTCA TTCCAGCTTG TGTTTTCTCC AGCCCAGTGT TTCTCATGAT GTACTCTGCA TATAA
Consensus   ATTTCTCCCC ACAATCTTCA TTCCAGCTTG TGTTTTCTCC AGCCCAGTGT TTCTCATGAT GTACTCTGCA TATAA    2185

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     GTTAAATAAG CAGGGTGACA AAATACAGCC TTGACAGACT CCTTTTCCTA TTTGGAACCA TTCTGTTGTT CCATG
Consensus   GTTAAATAAG CAGGGTGACA AAATACAGCC TTGACAGACT CCTTTTCCTA TTTGGAACCA TTCTGTTGTT CCATG    2260

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     TCCAGTTGTA CCTGTTGCTT CCTGACCTGC ATACAGATTT CTCAAAGGCG GGTAGGTGAT CTGGTGTGCC CATCT
Consensus   TCCAGTTGTA CCTGTTGCTT CCTGACCTGC ATACAGATTT CTCAAAGGCG GGTAGGTGAT CTGGTGTGCC CATCT    2335

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     CCTGAAGAAT TTTCCACAGT TTATTCTGAT TCACCCAGTC AAAGGCTTTG GCATAGTCAA TAAAGCAGAA ACAGA
Consensus   CCTGAAGAAT TTTCCACAGT TTATTCTGAT TCACCCAGTC AAAGGCTTTG GCATAGTCAA TAAAGCAGAA ACAGA    2410

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     CGTTTTTCTG GGAACTCTCT TCTTTTTTGA TGATCTAGCA GATGTTGGCA ATTTGATCTC TGGCTCCTCT GTCTT
Consensus   CGTTTTTCTG GGAACTCTCT TCTTTTTTGA TGATCTAGCA GATGTTGGCA ATTTGATCTC TGGCTCCTCT GTCTT    2485

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     TTCTAAAACC AACTTGAACA TCTGGAAGTT CATGGTTCAC GTATTGCTGA AGCCTTGCTT GGACAATTTT GAGCA
Consensus   TTCTAAAACC AACTTGAACA TCTGGAAGTT CATGGTTCAC GTATTGCTGA AGCCTTGCTT GGACAATTTT GAGCA    2560

cIFN-aII    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1     TTACTTTACT AGCCTGTGAG ATGAGTGCAA TTGTGAGGTA GTTTTAGCAT CCTTTGGCAT TGTTAGTCAA ATACG
Consensus   TTACTTTACT AGCCTGTGAG ATGAGTGCAA TTGTGAGGTA GTTTTAGCAT CCTTTGGCAT TGTTAGTCAA ATACG    2635
```


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

```
bIFN-aII     ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1      GCATTCATGC AATGAAGTGA GATAAACTGG GAAGCAATTT TGCAAACATT ACTGATAATT GTGTTTCCAC TGAAG
Consensus    GCATTCATGC AATGAAGTGA GATAAACTGG GAAGCAATTT TGCAAACATT ACTGATAATT GTGTTTCCAC TGAAG   [illegible]

bIFN-aII     ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1      GCAAGAACTT AAAATTTTGT TTGGTCTTAG AGATAATATC TTAATATTTA AGACTTATAA AGTTTAAGTT ATTTA
Consensus    GCAAGAACTT AAAATTTTGT TTGGTCTTAG AGATAATATC TTAATATTTA AGACTTATAA AGTTTAAGTT ATTTA   [illegible]

bIFN-aII     ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1      TATTTACTTA TAAATTATAA GAATCCACCT GCAATGCAGG GGTCCACAGG TCGATGAGTG GGTCAGGAAG ATTCC
Consensus    TATTTACTTA TAAATTATAA GAATCCACCT GCAATGCAGG GGTCCACAGG TCGATGAGTG GGTCAGGAAG ATTCC   [illegible]

bIFN-aII     ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1      CTTGGAGAAA GGATAAGGTA ACCCACTCCA ATATTCTTGA GTTTCCCTAA TGGCTCATCT GGTAAAGTAT CTGCC
Consensus    CTTGGAGAAA GGATAAGGTA ACCCACTCCA ATATTCTTGA GTTTCCCTAA TGGCTCATCT GGTAAAGTAT CTGCC   [illegible]

bIFN-aII     ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1      TGCAATGTGG GAAACCTGGG TTCGATTCCT GGGTTGGGAA GTTCCCCTGG AGAAGGGAGT GGCTAACCAC TCTAG
Consensus    TGCAATGTGG GAAACCTGGG TTCGATTCCT GGGTTGGGAA GTTCCCCTGG AGAAGGGAGT GGCTAACCAC TCTAG   [illegible]

bIFN-aII     ---------- -----
IFN-ωb1      TAATCTGGCC TGGAG
Consensus    TAATCTGGCC TGGAG                                                                     [illegible]
```

Boxes indicate the conserved motif of viral response element (GAAANN). Also shown are interferon response element (IRF-1), a TG-protein binding site (TBB), putative TATA box, transcription start site, and translation start site. TG-protein is a novel factor mediating viral inducibility (MacDonald et al. 1990). bIFN-aII was cloned by Capon et al., (1985). Notice the extremely well conserved sequences particularly in the coding region, which is identical. Putative translation stop site and polyadenylation signal are marked.
* Asterisks mark sequence differences between subtypes.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

```
bIFN-aII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    GCATTCATGC AATGAAGTGA GATAAACTGG GAAGCAATTT TGCAAACATT ACTGATAATT GTGTTTCCAC TGAAG
Consensus  GCATTCATGC AATGAAGTGA GATAAACTGG GAAGCAATTT TGCAAACATT ACTGATAATT GTGTTTCCAC TGAAG   2710

bIFN-aII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    TCAAGAACTT AAAATTTTGT TTGGTCTTAG AGATAATATC TTAATATTTA AGACTTATAA AGTTTAAGTT ATTTA
Consensus  TCAAGAACTT AAAATTTTGT TTGGTCTTAG AGATAATATC TTAATATTTA AGACTTATAA AGTTTAAGTT ATTTA   2785

bIFN-aII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    TATTTACTTA TAAATTATAA GAATCCACCT GCAATGCAGG GGTCCACAGG TCGATGAGTG GGTCAGGAAG ATTCC
Consensus  TATTTACTTA TAAATTATAA GAATCCACCT GCAATGCAGG GGTCCACAGG TCGATGAGTG GGTCAGGAAG ATTCC   2860

bIFN-aII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    CTTGGAGAAA GGATAAGGTA ACCCACTCCA ATATTCCTGA GTTTCCCTAA TGGCTCATCT GGTAAAGTAT CTGCC
Consensus  CTTGGAGAAA GGATAAGGTA ACCCACTCCA ATATTCCTGA GTTTCCCTAA TGGCTCATCT GGTAAAGTAT CTGCC   2935

bIFN-aII   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
IFN-ωb1    TGCAATGTGG GAAACCTGGG TTCGATTCCT GGGTTGGGAA GTTCCCCTGG AGAAGGGAGT GCCTAACCAC TCTAG
Consensus  TGCAATGTGG GAAACCTGGG TTCGATTCCT GGGTTGGGAA GTTCCCCTGG AGAAGGGAGT GCCTAACCAC TCTAG   3010

bIFN-aII   ---------- -----
IFN-ωb1    TAATCTGGCC TGGAG
Consensus  TAATCTGGCC TGGAG                                                                    3025
```

Boxes indicate the conserved motif of viral response element (GAAANN). Also shown are interferon response element (IRF-1), a TG-protein binding site (TBB), putative TATA box, transcription start site, and translation start site. TG-protein is a novel factor mediating viral inducibility (MacDonald et al, 1990). bIFN-aII was cloned by Capon et al., (1985). Notice the extremely well conserved sequences particularly in the coding region, which is identical. Putative translation stop site and polyadenylation signal are marked.

* Asterisks mark sequence differences between subtypes.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.