doi: http://dx.doi.org/10.5016/1806-8774.2006v8p9

ARBS Annual Review of Biomedical Sciences

pdf freely available at http://arbs.biblioteca.unesp.br
2006;8:9-18

Interferon-tau Polymorphisms and Their Potential Functions in Ruminants

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Received: 30 June 2006; accepted 10 August 2006

Abstract

Ealy AD, Pennington KA, Rodina TM. Interferon-tau Polymorphisms and Their Potential Functions in Ruminants. ARBS Annu Rev Biomed Sci 2006;8:9-18. In ruminants, the establishment and maintenance of pregnancy requires production of a Type I interferon, termed IFN-τ. This protein is synthesized by the developing conceptus and interacts with the uterus to promote continued secretion of progesterone. Multiple genes encode IFN-τ, and a majority of these genes are transcribed during early pregnancy. The proteins possess divergent biological activities, including the ability to prevent the corpus luteum from regressing at the end of a normal estrous cycle. In all likelihood multiple IFN-τ isoforms are produced during early pregnancy to ensure that sufficient quantities of bioactive IFN-τ are present to modulate uterine biology during early pregnancy. Although IFN-τ has evolved to serve as the pregnancy recognition hormone in ruminants, other Type I IFNs, such as IFN-α and IFN-ω, are capable of producing a uterine response similar to that of IFN-τ. Hence, the polymorphic nature of IFN-τ genes appear to have generated new and potentially more active forms of the hormone, but the unique expression pattern for IFN-τ is likely the preeminent feature ensuring its use as the maternal recognition of pregnancy factor in ruminants.

Keywords: interferon-tau, embryo, conceptus, endometrium, placenta, trophectoderm

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1. Introduction

Maternal recognition of pregnancy is the term used to describe how the conceptus plays an active role in modifying the maternal environment to promote the establishment and persistence of pregnancy (Short, 1969). In most eutherian mammals, one important aspect of maternal recognition of pregnancy is the ability of the conceptus to extend the duration of progesterone production from the corpus luteum (CL). Prolonged progesterone synthesis and secretion creates a pregnancy-receptive uterus capable of permitting continued conceptus development. Different mechanisms are used to prevent CL regression in various species. In cattle, sheep, goats, red deer and potentially all related pecoran ruminants (antelope, musk ox, giraffe), a conceptus-derived factor now known as interferon-tau (IFN-τ) is responsible for extending the functional lifespan of the CL (Demmers *et al*., 2000; Ealy *et al*., 1998b; Homeida & al Afaleq, 1994; Kiesling *et al*., 2000; Meyer *et al*., 1995).

The discovery of maternal recognition of pregnancy in ruminants occurred in the 1960's when Moor and Rowson used embryo collection and transfer studies to establish that extensions in CL function require the presence of a viable conceptus at day 12 post-estrus in sheep and at day 16 post-estrus in cattle (Moor & Rowson, 1966a; Moor & Rowson, 1966b; Moor *et al*., 1969; Rowson *et al*., 1972; Rowson *et al*., 1969). In 1979, a proteinaceous compound termed trophoblastin was found to represent this conceptus-derived factor (Martal *et al*. 1979). Soon thereafter, Bazer, Roberts, Thatcher and colleagues identified a series of related low molecular weight proteins (19 to 26 kDa) produced by ovine and bovine conceptuses during the time of sustained CL function (Bartol *et al*., 1985; Godkin *et al*., 1982; Godkin *et al*., 1984a). A purified preparation of these proteins, which collectively were termed ovine trophoblast protein-1 (oTP-1), prolonged luteal actions upon injection into the uterine lumen of non-pregnant ewes (Godkin *et al*., 1984b). Soon thereafter, an ovine conceptus cDNA expression library was screened with antiserum directed against oTP-1, and the resulting oTP-1 cDNA was structurally similar to Type I IFNs, a family of proteins that include the alpha (α), beta (β), omega (ω), and delta (δ) IFNs (Imakawa *et al*., 1987). Hence, the name oTP-1and bTP-1 (for the bovine counterpart) was changed to IFN-τ to reflect their designation as a trophectoderm-derived IFN.

In this review, we have attempted to provide a perspective on the evolution and actions of IFN-τ in ruminants. Our review of the literature explores how genes for IFN-τ are thought to have evolved to serve as the maternal recognition of pregnancy factor in ruminants and provides possible explanations for why multiple forms of IFN-τ are produced during early pregnancy. Detail on the mechanisms controlling biological activity of IFN-τ are presented briefly because recent reviews already exist for this topic (Demmers *et al*., 2001; Spencer *et al*., 2004).

2. Evolution of IFN-τ

By analyzing base substitution rates, Roberts *et al.* (1997, 1998) estimated that the present day IFN-τ genes (denoted as *IFNT*) arose approximately 36 million years ago in ancestors of the pecoran ruminants (Ruminantia suborder) by the duplication of an IFN-ω gene (*IFNW*). The timing of this event follows the predicted separation of pecoran ruminants (Ruminantia suborder) from other Artiodactyls (Tylopoda suborder: camels, llamas; Suidae suborder: pigs) (Roberts *et al*., 2003). Genes for IFN-τ have been identified by Southern blotting in species within the Bovidae (cattle, sheep, goats, musk ox and gazelle), Cervidae (various species of deer), and Giraffidae (giraffe) families of Artiodactyls but are not evident in other mammals (Leaman & Roberts, 1992; Roberts *et al*., 2003).

Present day *IFNT* and *IFNW* are ~80% identical in nucleotide sequence within their respective coding regions (Ealy *et al*., 1998b; Ealy *et al*., 2001; Ealy *et al*., 2004), but sequence similarities vanish ~130 bases upstream of their transcription start site and ~120 bases downstream of their stop codons (Ealy *et al*., 2001; Leaman & Roberts, 1992). Presumably during or shortly after the initial *IFNW* duplication event took place, a series of insertion events occurred to create an ancestral *IFNT* that contained new 5' and 3' untranslated sequences. Regulatory regions within these sequences provides *IFNT* with its unique ability to be expressed constitutively by the developing conceptus during early pregnancy, whereas the expression of most other Type I IFN genes are tissue restricted and induced in response to viruses and pathogen exposure (Cross & Roberts, 1991). Expression of *IFNT* is confined to the trophectoderm, the tissue that gives rise to the outermost layer of the placenta (Farin *et al*., 1990; Roberts *et al*., 1997). In the cow, IFN-τ protein is first detected at the late morula and early blastocyst stage (day 6-7 of pregnancy) when trophectoderm is first evident (Hernandez-Ledezma *et al*., 1992).

The production of bovine (bo) IFN-τ mRNA and protein increases dramatically from day 14 to 19 of pregnancy and decreases thereafter coincident with attachment to endometrium (Ealy *et al*., 1998b; Ealy *et al*., 2001; Farin *et al*., 1990).

Although the control of *IFNT* expression is not fully understood, comparisons of promoter and enhancer regions for various placental-specific genes with the 5' regulatory regions of *IFNT* reveals several common DNA elements. A core transcriptional regulatory circuit is predicted to enable efficient transcription of genes recruited for maternal recognition of pregnancy and placental development. One component of this circuit is Ets2. An Ets2 DNA binding site is located within the proximal *ovine (ov)* and *boIFNT* promoter (Ealy *et al*., 2001; Ezashi *et al*., 1998; Ezashi & Roberts, 2004). Mutation of this site prevents Ets2 binding and subsequent *IFNT* transcription (Ezashi *et al*., 1998; Ezashi & Roberts, 2004). A functional Ets site also is essential for placental lactogen-II expression in the mouse (Sun & Duckworth, 1999) and for hCGβ subunit expression in human placenta (Ghosh *et al*., 2003; Johnson & Jameson, 2000).

It remains uncertain why an IFN was chosen as the maternal recognition of pregnancy signal in ruminants. Luteal function is not prolonged by IFNs in mammals outside of the Ruminantia suborder, but various IFNs are expressed by placental tissues in other mammals. Antiviral activity associated with IFN production is present in the mouse placenta (Fowler *et al*., 1980). In the human and mouse, IFN-α is expressed by the placenta throughout pregnancy and is thought to play a facilitative role in regulating uterine gene expression and immune responses to pathogens (Bennett *et al*., 1996; Duc-Goiran *et al*., 1994; Fink *et al*., 2001; Jokhi *et al*., 1997). In the pig, IFN-δ and Type II IFN, or IFN-γ, is produced by the trophectoderm during peri-implantation period (Lefevre *et al*., 1990; Lefevre & Boulay, 1993; Niu *et al*., 1995). Neither IFN has been found to sustain corpus luteum function beyond the length of a normal estrous cycle when provided into the uterine lumen of non-pregnant gilts (Lefevre *et al*., 1998). Therefore, it seems probable that a facilitative IFN system present in several mammals was converted into a required component of pregnancy recognition in an ancestor to present day ruminants shortly after they diverged from other Artiodactyls.

3. Multiplicity of IFN-τ Genes

Like the IFN-α, - β and – ω genes, multiple copies of *IFNT* exist within the genome of cattle, sheep and goats (see Fig. 1). Presently, 18 distinct ovine, 12 different bovine, and at least 9 caprine polymorphic alleles exist in their respective genomes (Alexenko *et al*., 2000; Ealy *et al*., 2004). By using multiple IFN-τ- riboprobes and ribonuclease protection, several IFN-τ mRNA populations are present during early pregnancy in sheep and cattle (Ealy *et al*., 1998b; Ealy *et al*., 2001; Winkelman *et al*., 1999). In addition to the full-length IFN-τ mRNA, truncated versions also are evident in ovine and bovine conceptuses. These smaller fragments likely represent allelic variants of the same gene or transcripts from different *IFNT*. In a corresponding fashion, multiple IFN-τ protein isoforms are evident in ovine and bovine conceptuses. Two-dimensional SDS-PAGE indicates that at least four isoelectric variants are secreted by ovine and bovine conceptuses (Anthony *et al*., 1988; Bartol *et al*., 1985; Godkin *et al*., 1982). Post-transcriptional modifications to IFN-τ are evident in some species. Most notably, the bovine and caprine conceptus, but not the ovine conceptus secretes IFN-τ that exhibit differential glycosylation (Anthony *et al*., 1988; Baumbach *et al*., 1990; Helmer *et al*., 1988). Glycosylation of IFN-τ may impact the stability of these proteins but does not appear to affect its biological activity since recombinant forms of boIFN-τ contain potent antiluteolytic and antiviral activities (Ealy *et al*., 2001; Meyer *et al*., 1995).

The necessity of multiple expressed forms of *IFNT* during the establishment of pregnancy remains elusive. A long-held suspicion is that various IFN-τ isoforms contain distinct activities during the pregnancy recognition process. In this scenario, a subset of IFN-τ proteins may be required for extending CL life-span whereas others act in other fashions to ensure that the uterus is prepared to receive the pregnancy. An attractive alternative hypothesis argues that the expression of multiple *IFNT* is necessary to ensure that sufficient quantities of bioactive protein are available during maternal recognition of pregnancy. Support for this explanation is demonstrated in the sheep. Large quantities of ovIFN-τ are secreted from conceptuses at day 15-16 of pregnancy (20-200 μg/day) but very little ovIFN-τ, by comparison, is secreted by conceptuses on day 12 (1-2 μg/day) when the pregnancy recognition signal must first be realized (Ashworth & Bazer, 1989).

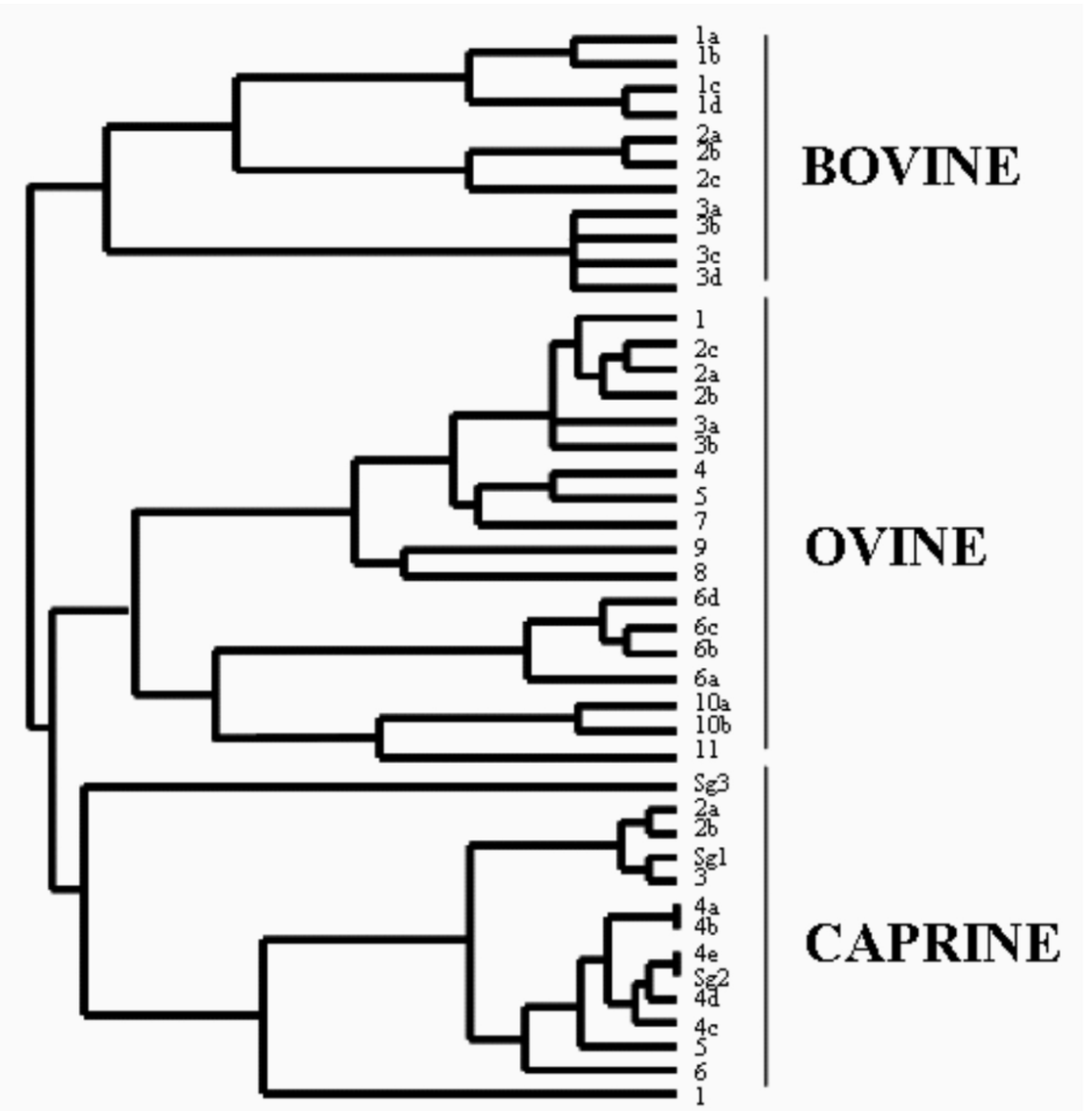

Figure 1. Cladograph of nucleotide sequence diversity among bovine, ovine and caprine *IFNT* sequences. The phylogenetic analysis is based on differences in nucleotide sequences within coding regions of all known bovine, ovine, and caprine *IFNT*. The nomenclature used for identifying each *IFNT* is based on the scheme described previously (Alexenko *et al*., 2000; Ealy *et al*., 2004).

4. Biological Activities of IFN-τ Polymorphs

Ruminants are spontaneous ovulators that utilize uterine-dependent systems to regulate estrous cycles. The basic events of luteal regression and subsequent return to estrus have been described eloquently by others in recent years (Demmers *et al*., 2001; Spencer *et al*., 2004). During late diestrus (day 13-15 post-estrus in the sheep; day 17-20 post-estrus in the cow), oxytocin-dependent pulses of prostaglandin F2α (PGF2α) are released from the endometrium and travel to the ovaries through a local counter-current exchange mechanism to induce the functional and structural regression of the corpus luteum (Flint *et al*., 1994). Oxytocin is produced and stored in the corpus luteum and is released in response to PGF2α stimulation, which then causes subsequent pulses of uterine PGF2α release. The luminal and superficial glandular epithelium are primary sources of PGF2α during luteolysis, and oxytocin acts on these target tissues by binding to its plasma membrane-associated receptor, which is expressed late in diestrus coincident with the initiation of luteolysis (Jenner *et al*., 1991; Spencer *et al*., 1995).

The primary action of IFN-τ during early pregnancy is prevention of the oxytocin-mediated release of endometrial PGF2α. In a pregnant state, oxytocin receptors are not expressed on the luminal and glandular epithelial endometrium at the time of normal luteolysis (Jenner *et al*., 1991; Spencer *et al*., 1995), and the magnitude and frequency of PGF2α pulses is greatly diminished, if not totally ablated. In the sheep, IFN-τ controls oxytocin receptor expression indirectly by limiting the expression of estrogen receptors (Spencer *et al*., 2004). A similar event is operational in cattle, although the antiluteolytic mechanism appears more complex. In pregnant cattle, IFN-τ-induced down-regulation of the oxytocin receptor precedes any changes in estrogen receptor abundance (Robinson *et al*., 1999; Robinson *et al*., 2001). Therefore, it is likely that IFN-τ is able to affect estrogen receptor activity prior to its down regulation in bovine endometrium.

Luteal activity and estrous cycle duration can be extended in non-pregnant sheep and cattle by providing exogenous IFN-τ into the uterine lumen via indwelling catheters or systemically by subcutaneous or intramuscular IFN-τ injections (Ealy *et al*., 1998b; Godkin *et al*., 1984b; Meyer *et al*., 1995; Niswender *et al*., 1997; Ott *et al*., 1993). The induction of this pseudopregnant state initially was used to establish how IFN-τ acts to prevent CL regression. More recently, this approach has been used as a tool to assess the antiluteolytic activity of specific IFN-τ isoforms. Interestingly, different recombinant ovIFN-τ proteins display different abilities to induce a pseudopregnant state (Ealy *et al*., 1998b; Winkelman *et al*., 1999). Four different ovIFN-τ protein variants have been compared with each other over the years (Ealy *et al*., 1998b; Winkelman *et al*., 1999). The two most effective ovIFN-τ proteins were derived from the τ4 and τ2c cDNAs (formerly known as p3 and p8, respectively) and are able to extend luteal function when provided at a dose of 10 to 50 μg/day. Another protein, termed ovIFN-τ6d (formerly known as p6), exhibited an intermediate activity (minimum effective dose of 100-250 μg/d) and the remaining protein, now termed ovIFN-τ11 (formerly known as s4), was only effective when provided at ≥1 mg/d. These proteins differ by as much as 13% in primary sequence, and this undoubtedly accounts for differences in their biological activities. However, it remains unknown which amino acid substitutions are responsible for improving antiluteolytic activity.

A second means by which IFN-τ promotes continued luteal function is by directly regulating the metabolism of prostaglandins in the endometrium. Concentrations of prostaglandin E2 (PGE2) increase dramatically in the uterine vasculature during pregnancy (Pratt *et al*., 1977). This prostaglandin is produced by the conceptus and endometrium and possesses luteotrophic and antiluteolytic activities (Henderson *et al*., 1977; Pratt *et al*., 1977; Pratt *et al*., 1979). Hence, it is reasonable to implicate this prostaglandin in serving at least a facilitative function in maintaining luteal activity during early pregnancy.

When using endometrial culture systems, IFN-τ directly modifies PGF2α and PGE2 production in endometrial epithelial cells. Interestingly, a biphasic dose effect of IFN-τ on prostaglandin metabolism exists. Exposing primary bovine endometrial epithelium or a bovine endometrial cell line (BEND cells) to low IFN-τ concentrations (< 1μg/ml) decreases basal and phorbol ester-induced production of PGF2α and PGE2 and decreases cyclooxygenase-2 (COX-2) mRNA concentrations, the rate limiting enzyme in prostaglandin metabolism (Binelli *et al*., 2001; Guzeloglu *et al*., 2004; Parent *et al*., 2003). By contrast, exposing endometrial cells to high levels of IFN-τ (> 1 μg/ml) increases basal and phorbol ester-induced production of PGF2α and PGE2 and increases COX-2 mRNA abundance (Guzeloglu *et al*., 2004; Parent *et al*., 2003). Moreover, in the high dose IFN-τ treatment, endometrial production of PGE2 is ten-fold greater than that of PGF2α (Guzeloglu *et al*., 2004; Parent *et al*., 2003). These observations support our current understanding of how prostaglandin metabolism is controlled during early pregnancy. The low level of IFN-τ treatment likely simulates IFN-τ levels during the initiation of pregnancy recognition when PGF2α metabolism and release must be diminished. Later in pregnancy, as IFN-τ secretion increases dramatically, prostaglandin production increases and vast amounts of PGE2 is generated by the endometrial epithelium.

Different ov and boIFN-τ proteins possess distinct differences in their ability to regulate prostaglandins in endometrial epithelium cultures (Parent *et al*., 2003). All IFN-τ protein isoforms, including the ovIFN-τ4 and –τ11 isoforms are able to diminish prostaglandin production in primary bovine endometrial epithelium when provided at low doses. However, only a select few IFN-τ isoforms stimulate prostaglandin production when provided at high concentrations. The ovIFN-τ4 isoform inhibited PGF2α and PGE2 production at both low and high doses whereas ovIFN-τ11 inhibited the production of both prostaglandins at low doses and stimulated prostaglandin production at high doses.

Recent discoveries of new functions for IFN-τ and detailed evaluations of how different IFN-τ protein isoforms function to promote a pregnant state have not provided a conclusive explanation for why multiple IFN-τ proteins are produced in early pregnancy. Current findings do support the concept that IFN-τ variants act in different capacities during the establishment and maintenance of pregnancy in ruminants. Certainly a subset of these proteins contains substantial antiluteolytic activities. For the sheep, the most active antiluteolytic isoforms (τ4 and τ2c) are part of a cluster of highly polymorphic *ovIFNT* (see Fig. 1). This subset of *ovIFNT* currently contains nine different coding sequences whose protein products differ in one to seven amino acids (95.9 to 99.4% identical in amino acid sequence). Perhaps this expansive cluster of *ovIFNT* has been introducing new and potentially more active *IFNT* isoforms into the sheep genome, and these isoforms are being 'evaluated' for their ability to act as antiluteolytic factors. This concept is supported further by examining the polymorphic nature of the remaining *ovIFNT* clusters. The *ovIFNT* cluster that includes the τ6d product, an intermediate antiluteolysin, contains only four genes that encode proteins differing by one or two amino acids (98.8 to 99.4% amino acid sequence identity). At least some if not all of these genes most certainly represent allelic variants. Similarly, the *ovIFNT* cluster containing the poor antiluteolysin τ11 contains only three variants. Genes in this cluster probably are not normally produced during early pregnancy. OvIFN-τ11 mRNA cannot be detected throughout early pregnancy in ovine conceptuses and all three genes lack a functional Ets-2 DNA binding domain (Ealy *et al*., 1998b; Ezashi *et al*., 1998). It remains uncertain if these presumptive pseudogenes lost their ability to be expressed because their protein products were no longer required for pregnancy recognition or if the depression in biological activity of these protein products occurred after the genes were no longer expressed.

5. Is IFN-τ Unique Among IFNs for Serving as the MRP Signal?

When researchers first began evaluating the actions of IFN-τ, recombinant IFN-α preparations were used in place of IFN-τ because recombinant forms of this protein did not exist (Barros *et al*., 1991; Martal *et al*., 1990; Vallet & Lamming, 1991). During these initial studies, researchers realized that supra-physiological quantities of IFN-α were required to mimic the events of early pregnancy and induce a pseudopregnant state in non-pregnant animals. This observation spurred a debate over whether IFN-τ possesses unique activities that make it better able to serve as a pregnancy recognition hormone than other Type I IFNs. This dilemma has been studied extensively over the past several years, and several key findings provide evidence that IFN-τ is no better than other Type I IFNs in generating an antiluteolytic response in the uterus.

One unique feature of IFN-τ, which also is present in their structural relative, IFN-ω, is the presence of a six amino acid extension at the carboxyl terminus that results in proteins that are 172 amino acids in length rather than the standard 166 amino acid length for IFN-α. Since this 'tail' may be sufficiently long to interact with putative receptor binding regions of IFN-τ or with the domains of the Type I IFN receptor complex, a carboxyl six amino acid truncated ovIFN-τ was generated and tested for its biological activity (Ealy *et al*., 1998a). Antiviral, antiproliferative, and antiluteolytic activities of this truncated IFN-τ were not different from its full length counterpart, indicating that this structural motif does not provide a benefit for IFN-τ acting as a maternal recognition of pregnancy hormone.

The uterine receptors that interact with IFN-τ also have been evaluated to determine if they possess unique features that may make them better able to interact with IFN-τ than other Type I IFNs. To date, however, there is no indication that uterine receptors differ from IFN receptors found in other tissues. The Type I IFN receptors, which bind IFN-τ and other Type I IFNs, are comprised of at least two subunits, termed IFN-α receptor 1 and 2 (IFNAR1 and 2) (Uze *et al*., 1995). Complementary DNA for both receptor subtypes have been cloned from the bovine and ovine endometrium (Han *et al*., 1997), and these receptors are identical to receptors present in other bovine and ovine tissues. Moreover, the uterine Type I IFN receptor complex is able to interact with other Type I IFNs. Recombinant ovIFN-α is able to generate an antiviral response in ovine uterine epithelial cells (Green *et al*., 2005).

Definitive evidence that IFN-τ does not contain a unique activity that makes it a superior antiluteolytic factor was reported recently. A pseudopregnant state could be induced in non-pregnant ewes following intrauterine injections of either ovIFN-τ or an equivalent amount of bioactive ovIFN-α (assessed by antiviral activity) (Green *et al*., 2005). Similarly, in unpublished work, recombinant ovIFN-ω was able to extend luteal function in non-pregnant ewes when provided into the uterine lumen at an

antiviral activity dose that is equivalent to ovIFN-τ (Ealy, Green and Roberts; unpublished observations). Based on these findings, any Type I IFN can extend the life span of the CL if a minimum biological active amount of protein is provided. Such findings do not dismiss the possibility that IFN-τ contains a unique ability to act on other aspects of pregnancy recognition, such as modulating the local immune system or controlling uterine protein production. In fact, uterine secretions are slightly different following treatment with IFN-τ and IFN-α (Naivar *et al*., 1995). The production of at least one uterine protein, granulocyte chemotactic protein-2, is stimulated by IFN-τ but not by IFN-α in bovine endometrium (Staggs *et al*., 1998). The role this and other IFN-τ-induced uterine proteins play in the establishment and maintenance of pregnancy remains unresolved.

6. Concluding Remarks

The IFN-τ observed in today's ruminant species evolved from other Type I IFNs and is serving as a crucial component of pregnancy recognition. Since all Type I IFNs appear to contain antiluteolytic activity in ruminants, IFN-τ likely evolved to serve as the maternal recognition of pregnancy factor because it gained the ability to be produced in the right place and right time and in sufficient quantities to serve this function. The polymorphic nature of *IFNT* appears to have generated new and potentially more active forms of IFN-τ.

7. Acknowledgments

Authors thank Dr. Sally Johnson (University of Florida) for reviewing this manuscript. Authors are grateful to the National Research Initiative Competitive Grants Program for support of this research topic over the years. These projects were supported by National Research Initiative Competitive Grant numbers 1997-35203-4767 and 2003-35203-15382 from the USDA Cooperative State Research, Education, and Extension Service and by the USDA-Special Grants Program in Tropical/Subtropical Agriculture Research-Caribbean Grant number 2005-34135-995.

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