MINIREVIEW

τ-Interferon: Pregnancy Recognition Signal in Ruminants (44053)

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Introduction

Maternal recognition of pregnancy results from biochemical signaling between the conceptus (embryo and its associated membranes) and the maternal system. Pregnancy recognition signals ensure maintenance of structural and functional integrity of the corpus luteum (CL), which would otherwise regress at the end of the estrous or menstrual cycle. The CL produces progesterone, the hormone of pregnancy, which is responsible for maintaining endometrial functions that permit early embryonic development, implantation, placentation, and successful fetal/placental development.

The estrous cycle of ruminants is uterine dependent, and the luteolytic signal responsible for structural and functional demise of the CL, or luteolysis, is prostaglandin $F_{2\alpha}$ (PGF). The uterine endometrium, primarily lumenal epithelium and perhaps superficial glandular epithelium, is influenced by progesterone, estrogen, and oxytocin, through their cognate receptors, to release pulses of PGF required for luteolysis. The antiluteolytic signal for pregnancy recognition in ruminants (i.e., sheep, cattle, goats) is a novel Type I interferon named τ-interferon (IFN-τ). IFN-τ is secreted by trophectoderm of sheep conceptuses between Days 10 and 21 of pregnancy and exerts a paracrine effect on the uterine endometrium to abrogate the luteolytic mechanism. The antiluteolytic mechanism(s) induced by ovine IFN-τ (oIFN-τ) has been studied in greatest detail and is the primary focus of this review.

Estrous Cycle and Luteolytic Mechanism in Ruminants

Sheep (*Ovis aries*) are seasonally polyestrous, and ewes exhibit recurring estrous cycles of 16.5–17.5 days during late summer and mid-winter. Estrus, the period of sexual receptivity, marks the beginning of each estrous cycle and lasts about 30 hr. Ewes ovulate spontaneously 24–27 hr after the onset of estrus in response to an estrogen-induced discharge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. Following ovulation and during metestrus, Days 1–4 after onset of estrus, theca and granulosa cells of the follicle differentiate into luteal cells under the influence of LH, a process termed luteinization, and give rise to the corpus luteum (CL). The CL begins to secrete progesterone on about Day 4, which marks the beginning of diestrus. The CL is at its maximum size and functional activity between Days 7 and 14 (1).

Progesterone suppresses behavioral estrus, GnRH pulse frequency and circulating levels of LH and FSH; therefore, Graafian follicles which form during diestrus do not ovulate. Progesterone acts on the uterus to: (i) provide an endometrial environment conducive to conceptus growth and development; and (ii) maintain the myometrium in a quiescent state. Ovarian follicles grow and regress during the estrous cycle, a phenomenon termed "follicular waves," but progesterone from the CL suppresses growth of an ovulatory

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Graafian follicle and ovulation until after luteolysis has occurred during proestrus. Increases in estradiol-17β (E_2) in ovarian venous blood on Days, 3, 9, and 14 correspond to successive nonovulatory and ovulatory follicular waves (2). In pregnant ewes, these nonovulatory waves of follicular growth and atresia continue until about Day 60. Changes in circulating estrogens associated with nonovulatory follicular waves may affect endometrial function, but such effects have not been characterized.

Luteolysis and Prostaglandin $F_{2\alpha}$

The CL regresses between Days 15 and 16 postestrus in response to pulsatile release of prostaglandin $F_{2\alpha}$ (PGF) by the uterus. Thus, the estrous cycle of ewes is uterine dependent. If ewes are hysterectomized during the active life of the CL, luteolysis does not occur, and CL life span is prolonged to about 5 months, the duration of normal pregnancy (3, 4). In ruminants an intimate anatomical relationship between the uterine branch of the ovarian vein and the ovarian artery is required for countercurrent exchange of luteolytic PGF from the uterine venous drainage to the ovarian artery (5). McCracken *et al.* (6) demonstrated that [^{3}H]PGF is transferred from the utero-ovarian vein to the ovarian artery and, therefore, to the CL by a local route. Uterine PGF is also transported from the lymphatic drainage to the ovarian artery (7, 8). Injection of ewes with exoogenous PGF causes premature luteolysis (5) and immunization of ewes against PGF blocks luteolysis (9, 10). Available evidence clearly indicates that PGF is the uterine-derived luteolytic hormone in sheep (5).

Uterine-derived PGF binds to receptors on luteal cells and initiates intracellular events which terminate production of progesterone and initiate cell death (5, 7, 8). Luteolytic effects of PGF may be due to (i) a decrease in luteal blood flow; (ii) a reduction in LH receptors; (iii) uncoupling of the LH receptor from adenylate cyclase; (iv) activation of protein kinase C; (v) influx of high levels of calcium; and/or (vi) cytotoxic effects (11). Luteolysis does not require a decrease in LH receptors or withdrawal of basal LH support. Rather, PGF may activate protein kinase C (PKC) in large luteal cells to inhibit progesterone production (12) and cause luteolysis (13). Treatment of large luteal cells with PGF increases intracellular free calcium (14), which may activate an apoptotic cascade (15) as well as decrease expression of mRNA for 3β-hydroxysteroid dehydrogenase (16).

Endometrial Production of Prostaglandin $F_{2\alpha}$

Progesterone exposure during the early to mid-luteal phase of the estrous cycle is essential for initiation of endometrical PGF production and luteolysis. Progesterone increases phospholipid stores (17) and prostaglandin synthase activity necessary for the conversion of arachidonic acid to PGF (18). Administration of exogeneous progesterone to cyclic ewes during metestrus decreases interestrous intervals, whereas administration of the progesterone receptor (PR) antagonist mifepristone (RU486) during the early luteal phase delays onset of endometrial PGF production and luteolysis (19). Therefore, exposure of the endometrium to progesterone for 10 to 12 days, as occurs during diestrus, permits activation of mechanisms for endometrial production of luteolytic PGF.

During the peri-luteolytic period, arachidonic acid is released from phospholipid stores in endometrial epithelium by phospholipase A_2 and converted to PGF by prostaglandin synthase in those cells (20). Endometrial expression of prostaglandin synthase is highest by Days 12–13 postestrus in both cyclic and pregnant ewes (21, 22) and, in response to progesterone, is located principally in lumenal epithelial cells responsible for pulsatile release of PGF. However, glandular epithelial and stromal cells may also produce pulses of $PGF_{2\alpha}$ in response to oxytocin during proestrus (Days 15–17).

Concentrations of PGF in the uterine vein increase during luteolysis associated with pulsatile secretion of PGF by the endometrium (5). McCracken *et al.* (5) demonstrated that CL must be exposed to approximately five pulses of PGF over a 25-hr period to undergo complete luteolysis. Pre-luteolytic pulses of PGF are released into the utero-ovarian vein on Days 13–14 in both cyclic and pregnant ewes; however, the luteolytic pulses of PGF on Days 15 and 16 occur only in cyclic ewes (5). In pregnant ewes, the antiluteolytic signal from the conceptus abrogates the luteolytic mechanism to prevent pulsatile release of PGF (5, 23, 24).

Oxytocin

Pulsatile release of PGF by the ruminant uterus occurs in response to oxytocin binding to its receptors (OTR) on uterine endometrial cells and activation of the phosphatidyl inositol-diacylglycerol-protein kinase C second messenger system (PKC) (20, 25). Oxytocin is synthesized and secreted by large luteal cells (26), and released from the posterior pituitary (27). In large luteal cells, the oxytocin gene is transcribed on Days 0–4 (28), oxytocin mRNA is translated to oxytocin to about Day 7, and stores of oxytocin and its neurophysin in luteal cells are highest on Days 10–12 (20, 29). Thus, CL contain finite stores of oxytocin, which are not replenished later in the estrous cycle or during pregnancy.

Secretion of luteal oxytocin can be stimulated by a PGF agonist (20). During the peri-luteolytic period greater than 95% of PGF pulses coincide with pulses of oxytocin (27). Low amplitude pulses of PGF from the uterus stimulate large luteal cells to release oxytocin from secretory granules which then induces pulsatile release of luteolytic PGF (20). Major pulses of PGF occur at 4- to 5-hr intervals on Days 14–16, and uterine release of about five pulses of PGF per 25 hr results in complete luteolysis (5). An unknown mechanism results in synchronous pulsatile release of oxytocin from CL on each ovary and from the posterior pituitary in ewes (27).

Immunization of ewes against oxytocin extends CL lifespan and interestrous interval (20, 30), as does the administration of an OTR antagonist (31). Likewise, chronic system infusion of oxytocin prevents OTR formation in the endometrium during late diestrus and blocks luteal regression in cyclic ewes (20). Luteal oxytocin is released before development of endometrial responsiveness to oxytocin, and temporal release of oxytocin from CL and posterior pituitary does not differ between cyclic and pregnant ewes (29). Therefore, oxytocin-induced release of luteolytic pulses of PGF is dependent on the presence of endometrial OTR.

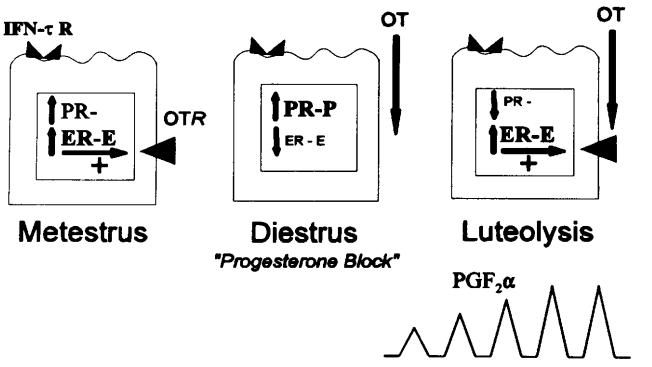

Figure 1. Schematic illustrating regulation of hormone receptor expression in the ovine uterus during the estrous cycle. During metestrus, OTR are present on the uterine epithelium because estrogen levels and ER expression are high. High levels of PR are also present, but low circulating levels of progesterone result in insufficient numbers of occupied PR to suppress ER and OTR synthesis. During diestrus, endometrial ER and estradiol in plasma are low and progesterone levels begin to increase with formation of the CL. Progesterone acts through its receptor to maintain the "progesterone block" to ER and OTR synthesis for 10–12 days. During late diestrus, progesterone negatively autoregulates PR expression to allow upregulation of ER and OTR synthesis, an event that is facilitated by increasing secretion of estrogen by ovarian follicles. The pulsatile release of oxytocin from the CL and posterior pituitary induces release of luteolytic pulses of $PGF_{2\alpha}$ from the endometrium to regress the CL. E, estrogen; ER, estrogen receptor; IFN-τ R, τ-interferon receptor; OT, oxytocin; OTR, oxytocin receptor; P, progesterone; PR, progesterone receptor; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$. (Adapted from Ref. 23.)

Regulation of Endometrial Oxytocin Receptor Gene Expression

McCracken *et al.* (5) proposed that progesterone inhibits endometrial OTR synthesis for 10–12 days; a phenomenon termed the "progesterone block" to endometrial OTR formation (Fig. 1). However, progesterone suppresses expression of PR to end the "progesterone block," and the endometrium expresses estrogen receptor (ER), responds to estrogen, and expresses OTR. The initial increases in OTR occur in cyclic ewes as ER increase and PR decrease in endometrial epithelium, and the rapid increases in OTR occurs when circulating progesterone decreases and follicular estrogens increase during late diestrus, proestrus, and estrus (5, 32, 33).

Expression of OTR mRNA increases between Days 13 and 14 in the endometrial epithelium (34), and OTR protein, which is low or undetectable between Days 4 and 13, increases rapidly between Day 14 and estrus (35, 36). The OTR are expressed initially on lumenal and shallow glandular epithelia on Day 14, but expression extends to caruncular stroma and deep glandular epithelium by Day 16 and is abundant in essentially all endometrial cells during estrus (32, 34, 36, 37). Expression of OTR on the lumenal and superficial glandular epithelium appears responsible for pulsatile release of PGF and luteolysis. Regulation of OTR gene expression appears to be at the level of transcription and influenced primarily by estrogen and progesterone (30, 34, 38).

Progesterone and Oxytocin Receptors. Progesterone blocks increases in ER and OTR during the early to mid-luteal phase of the estrous cycle of ewes (5), but the molecular mechanism is not known. Progesterone may (i) induce expression of a factor that affects OTR gene expression; (ii) directly suppress transcription of the OTR gene; or (iii) suppress ER gene expression to prevent estrogen from increasing OTR gene expression. When ovariectomized ewes are treated with progesterone for 10–12 days, ER and OTR gene expression increase and oxytocin-induced PGF release occurs (39–43). This loss of the "progesterone block" to ER and OTR formation following chronic exposure to progesterone in cyclic ewes may result from progesterone-induced negative autoregulation of endometrial PR gene expression in the epithelium which would permit estrogen to increase OTR expression (43). Alternatively, increases in OTR expression may occur with loss of PR expression and independent of effects of estrogen.

Ovariectomized ewes, with basal concentrations of both progesterone (<1 ng/ml) and E_2 (1 pg/ml) were used to study factors regulating OTR formation (39, 40). In those ewes, expression of endometrial OTR (100–1000 fmol/mg protein) is high and apparently con-

stitutive in lumenal and superficial glandular epithelium and treatment with progesterone initially caused complete loss of OTR expression (30, 44, 45). However, chronic progesterone treatment for 12 days results in increased endometrial phospholipid stores, OTR expression, prostaglandin synthase activity, and oxytocin-induced pulsatile release of PGF (22, 43, 44). The OTR that develop as progesterone downregulates PR are localized exclusively to lumenal and superficial glandular epithelium, which is essentially identical to the spatial expression of OTR during luteolysis (30).

Estrogen and Oxytocin Receptors. The role of estradiol in regulating OTR gene expression during development of the endometrial luteolytic mechanism remains a contentious topic. Administration of E_2 on Days 11 or 12 of the estrous cycle causes luteolysis in sheep and cattle (46, 47); an effect mediated through induction of endometrial ER and OTR expression. Administration of a pharmacological dose of E_2-benzoate (750 μg, im) to cyclic ewes on Days 9 or 10 of the estrous cycle results in (i) increases in OTR synthesis in both caruncular and intercaruncular endometrium by 24 hr; (ii) increases in oxytocin-induced phosphoinositide turnover at 24 hr in intercaruncular endometrium; (iii) episodic PGF secretion at 35 ± 3 hr; (iv) decreasing concentrations of progesterone in plasma by 42 ± 3 hr; and (v) behavioral estrus at 67 ± 9 hr (47). This sequence of events is very similar to that responsible for luteolysis in intact cyclic ewes (5, 23, 24); however, changes in expression of ER were not reported (47). Effects of estrogen on the timing, magnitude, and pattern of PGF response to oxytocin are probably mediated through increases in OTR gene expression (5, 39), enhanced coupling of OTR to its second messenger system (48), and increased activity of the prostaglandin synthase system (18, 49).

Destruction of developing follicles and immunization of ewes against estrogen delays luteolysis, suggesting a crucial role for estrogen in luteolysis (50). In the absence of estrogen, oxytocin cannot stimulate uterine PGF release adequately to induce luteolysis (51). Moreover, endometrial expression of OTR increases when progesterone is low relative to E_2 in ovariectomized ewes (39, 40).

Treatment of anestrus ewes with estrogen increases OTR in both epithelium and stroma (52), and treatment with E_2 following progesterone withdrawal results in greater OTR expression than that resulting from progesterone withdrawal alone (44). However, OTR on lumenal epithelium increase prior to progesterone withdrawal in intact cyclic ewes. The E_2-induced increases in OTR during progesterone withdrawal are primarily in uterine glandular epithelium and caruncular stroma, as occurs during proestrus in cyclic ewes (30). Expression of OTR in ovariectomized ewes treated with E_2 alone is intermediate to that for ewes treated with E_2 following progesterone withdrawal (44, 45). Moreover, Hazard and Stormshak (53) found that daily treatment of cyclic ewes with 500 μg E_2 beginning on Day 4 prolonged luteal function and reduced endometrial expression of OTR. In this case, continuous exposure of the endometrium to estrogen may have suppressed expression of ER and OTR or increased expression of PR to block expression of OTR.

Oxytocin and Oxytocin Receptors. In addition to effects of sex steroids, oxytocin can regulate its own receptor, but the mechanism(s) is not known (30). Systemic infusion of oxytocin beginning in the mid-luteal phase prevents luteolysis and extends the interestrous interval in cyclic ewes (54–56). Ayad *et al.* (56) determined that ORT expression was absent on the lumenal epithelium if oxytocin was infused systemically, but not when oxytocin was infused into the uterine lumen. Thus, OTR may be localized to the basolateral domain of epithelial cells so that intraluminal oxytocin cannot access those receptors. Oxytocin treatment of endometrium *in vitro* also reduces OTR expression (57). Wathes and Lamming (30) suggested that oxytocin increases OTR gene transcription, since OTR mRNA increased but OTR protein did not; however, this has not been confirmed. Whether or not the amount of oxytocin released from CL affects endometrial OTR expression in cyclic ewes is not known.

Results from *in vivo* studies clearly indicate that progesterone progressively suppresses expression of PR and, therefore, loses its ability to suppress endometrial expression of ER and OTR during mid- to late diestrus. Moreover, there is strong evidence that estrogen induces expression of OTR in the lumenal epithelium, which is responsible for production and release of luteolytic PGF. Because hormone-responsive organs, tissues, and cells are governed by the presence of hormone receptors, a review of ER and PR gene expression in the ovine uterus is important.

Estrogen Receptor and Progesterone Receptor Gene Expression in Endometrium

There are few reports of temporal and spatial changes in ER and PR mRNAs in endometrium during the estrous cycle or pregnancy in ewes (32, 33, 58). In endometrium of ewes, estrogen increases expression of both ER and PR protein (59–62) and mRNA (43, 46, 63), whereas progesterone inhibits ER and PR protein expression (60–62, 64).

Estrogen Receptor. Ott *et al.* (58) reported that ER mRNA increased in endometrial homogenates between Days 10 and 16 of the estrous cycle, whereas ER protein was low between Days 10 and 14, and increased 4-fold between Days 14 and 16. Cherny *et al.* (65) and Wathes and Hamon (32) used immunocytochemistry to demonstrate that expression of ER pro-

tein was maximal in lumenal epithelium, glandular epithelium and stroma on Days 0 to 2, declined to low levels by Day 5, and was low or undetectable during mid- to late diestrus in most cells of the uterus. However, immunoreactive ER remained abundant in deep glandular epithelium throughout the cycle. Staining intensity for ER increased between Days 14 and 15 in the lumenal epithelium and Days 12 to 15 in the stroma (i.e., in advance of and during luteolysis). Therefore, these authors questioned the role of estrogen in initiating OTR expression and regulating secretion of luteolytic pulses of PGF. However, Spencer *et al.* (33, 46) demonstrated that increases in ER gene expression precede OTR formation in cyclic ewes.

Spencer *et al.* (33) used *in situ* hybridization and immunocytochemical analysis to determine that ER mRNA and protein were low on Days 6 and 11 of the estrous cycle of ewes, increased between Days 11 and 15 in lumenal and shallow glandular epithelium, and were expressed at low levels in stroma and deep glandular epithelium on Days 11 to 15 (33). Available results indicate that increases in expression of ER and OTR genes are coordinate during development of the luteolytic mechanism. Moreover, it is clear that estrogen stimulates increases in expression of both ER and OTR genes in the endometrium.

Progesterone Receptor. Steady-state levels of endometrial PR mRNA in endometrial homogenates increase from Days 10 to 14 of the estrous cycle, whereas PR protein decreases from Days 10 to 12 and then increases between Days 14 and 16 of the cycle (58). The increase in PR protein during proestrus is probably in response to increases in endometrial expression of ER and circulating estrogens from maturing ovarian follicles.

Expression of PR mRNA in endometrium of cyclic ewes is highest on Day 1, decreases between Days 1 and 11, and increases again between Days 13 and 15 (33). Based on results from *in situ* hybridization and immunocytochemical analyses, PR mRNA and protein are present on Days 1 and 6 in the lumenal and shallow glandular epithelium, absent on Days 11 to 15 of the estrous cycle, but present at low levels in stroma and deep glandular epithelium on Days 11 to 15 (33). In lumenal and superficial glandular epithelium of cyclic ewes, which is responible for release of pulsatile PGF, PR protein expression is absent during estrus, detectable on Days 1 to 2, maximal on Days 5–7 and low or undetectable between Days 9 and 15 (32). However, PR is present throughout the cycle in stromal and myometrial cells, but highest on Days 1–2 and Days 9–14 of the estrous cycle (32). Thus, the "progesterone block" to ER and OTR expression in the lumenal epithelium is clearly associated with loss of PR expression which occurs prior to the decline in circulating progesterone. Consequently, there is coordinate loss of PR expression and activation of the luteolytic mechanism in the lumenal and superficial glandular epithelia (32, 33).

Working Hypothesis on Regulation of Endometrial Hormone Receptor Expression during the Estrous Cycle

Available results on tissue- and cell-type specific expression of ER, PR, and OTR in ovine endometrium support the McCracken (5) model of regulation of hormone receptor gene expression during the estrous cycle (Fig. 1). In cyclic ewes, endometrial ER expression is maximal during estrus and metestrus (Days 0–5), due to high levels of estrogen secreted by Graafian follicles and very low levels of circulating progesterone. Interactions of estrogen and ER, in the absence of occupied PR, allow estrogen to induce expression of OTR in all cell types of the endometrium. Although OTR expression is high, circulating oxytocin is low or absent and pulsatile secretion of PGF by the endometrium is absent. During early diestrus (Days 6–13), the "progesterone block" prevents expression of endometrial ER and OTR. However, chronic progesterone downregulates expression of PR in lumenal and superficial glandular epithelium by Days 12–13 to permit increases in ER and OTR gene expression and activation of the luteolytic mechanism. During luteolysis (Days 14–17) estrogen from maturing ovarian follicles further stimulates ER and OTR expression to ensure that oxytocin from the CL and posterior pituitary stimulates pulsatile release of luteolytic PGF by the endometrium to terminate CL function which allows onset of estrus and another opportunity for the establishment of pregnancy.

Maternal Recognition of Pregnancy in Ruminants

The ovarian cycle of subprimate mammals, including ruminants, is uterine dependent. Therefore, pregnancy recognition signals from the conceptus are antiluteolytic and act in a paracrine manner on the endometrium to prevent production of luteolytic pulses of PGF, but do not act directly on the CL. The antiluteolytic pregnancy recognition signal in ruminants is interferon-τ (IFN-τ) (23, 24).

Moor and Rowson (66, 67) demonstrated that a conceptus must be present in the uterus by Days 12 or 13 of the cycle for establishment of pregnancy following embryo transfer. Removal of conceptuses from uteri of ewes before Day 13 of pregnancy had no effect on CL life span, whereas removal of the conceptus after Day 13 extended CL life span to 25–35 days (66–68). Thus, maternal recognition of pregnancy in the ewe occurs on Days 12–13. A functional CL is required until Days 50–60 of gestation, after which time the placenta secretes sufficient progesterone to maintain pregnancy (69, 70).

Ovine τ-Interferon. Homogenates of sheep con-

ceptuses from Days 14–15, but not Days 21–25, extend CL life span and the interestrous interval when infused into the uterus of cyclic ewes (71, 72). However, conceptus homogenates had no effect on CL life span when injected intravenously. This suggests that the pregnancy recognition signal in ewes acts locally on the uterine endometrium. The antiluteolytic molecule produced by the conceptus between Days 11 and 20 of pregnancy was an acidic, 19.5-kDa protein first named "Protein X," then renamed ovine trophoblast protein–1 (oTP-1), and finally officially designated ovine τ-interferon (oIFN-τ) (23, 24, 73).

Ovine IFN-τ was determined to be a member of the IFN-ω subclass of the Type I IFN family of proteins based on protein and DNA sequencing (73). The oTP-1 gene possesses a 585-bp open reading frame that encodes for an 195–amino acid polypeptide from which a 23–amino acid signal peptide is cleaved to yield a 172–amino acid mature protein (73). Homology between oTP-1 and the 165–amino acid bovine IFN-α1 nucleotide and amino acid sequence was determined to be 63% and 50%, respectively (73). Homology with the 172–amino acid bovine IFN-ω1 at the nucleotide and amino acid levels was 85% and 72%, respectively (73). However, the highest homology was between oTP-1 and bovine trophoblast protein one (bTP-1); 90% and 80% at the nucleotide and amino acid sequence levels, respectively (73). Thus, oTP-1 and bTP-1 are Type I trophoblast interferons that constitute a distinct subgroup of the IFN-α family. Because of the unique developmental expression of oTP-1 and its relatedness to other Type I IFNs (α, β, and ω), oTP-1 or Type I trophoblast IFN was classified as IFN-τ by the committee on IFN nomenclature of the International Interferon Society (73).

Expression of IFN-τ. The ovine trophoblast produces IFN-τ between Days 13 and 21 of pregnancy with maximal production per cell on about Day 15 (23, 24, 73). On Day 15, ovine conceptuses in culture secrete 100 μg of oIFN-τ in 24 hr, but low amounts of IFN-τ secretion can be detected in culture medium as early as Days 8 and 10 of pregnancy (74). Concentrations of IFN-τ mRNA in conceptuses were highest around Day 14 (75, 76) and decreased to 10% peak values by Day 20 (75). *In situ* hybridization analyses detected oIFN-τ mRNA as early as Days 10 and 11 with maximum expression after Day 13 and declining values after Day 17 (73, 77). Secretion of IFN-τ by the ovine conceptus (ng/uterine flushing) coincides with the morphological transition of the conceptus from a spherical (312 ng), to tubular (1380 ng) and filamentous (4455 ng) form on Days 12 to 13 (78), and the reduction in IFN-τ gene expression occurs during the initial stages of placentation. Thus, IFN-τ is produced by the conceptus prior to the expected time of formation of ER and OTR in endometrial epithelium.

Biological Properties of IFN-τ. The antiluteolytic properties of IFN-τ are unique, but IFN-τ has antiviral, antiproliferative and immunomodulatory activities comparable to other Type I interferons (73, 79–81; unpublished observations). τ-Interferon inhibits proliferation of lymphocytes after exposure to mitogens and mixed lymphocyte cultures (73, 82, 83). In addition, oIFN-τ is as potent as rhIFN-α1 in activating natural killer (NK) cells *in vitro* (84).

Several reports indicate that oIFN-τ is less cytotoxic than human IFN-α (80, 85–87). Because exogenous IFN-α can induce pyrogenicity, vomiting, diarrhea, and malaise due to its cytotoxic nature (88), IFN-τ may be a useful therapeutic agent which can be used at higher doses, for longer periods of time, or as an alternative of IFN-α for treatment of viral diseases and cancers in veterinary and human medicine. Since IFN-τ exerts negative regulatory effects on ER and OTR gene transcription (38), it may be also be a useful therapeutic agent for treatment of estrogen-dependent diseases of mammary and reproductive tissues.

Antiluteolytic Effects o IFN-τ. The unique biological effect of IFN-τ is its antiluteolytic activity in ruminants. Intrauterine injections of ovine IFN-τ into cyclic sheep (89), cattle (90), and goats (91) abrogate development of the luteolytic mechanism and extend CL life span and interestrous interval. τ-Interferon is not detectable in the uterine venous or lymphatic drainage (23). Therefore, antiluteolytic effects of IFN-τ are assumed to be limited to the uterine endometrium. The antiluteolytic effects of IFN-τ *in uteri* of ewes, cows, and goats may differ slightly (92), but primary mechanism(s) of action is very similar.

Effects of oIFN-τ on Endometrial OTR and Prostaglandin Synthesis. During the luteolytic period endometrial expression of OTR is high, exogenous oIFN-τ cannot prevent binding of oxytocin to its receptor, inhibit oxytocin stimulation of endometrial inositol phosphate metabolism, or inhibit oxytocin stimulation of endometrial PGF secretion (23). Rather, the endometrium of cyclic ewes must be exposed to IFN-τ from Days 12 to 14 (i.e., 2–3 days before OTR is expected to increase), to block oxytocin-induced inositol phospholipid metabolism and PGF secretion (23). Intrauterine injections of IFN-τ on Days 11–15 prevent increases in endometrial ER and estrogen-dependent increases in OTR gene expression (93, 94), an effect which requires progesterone (95, 96). Thus, it appears that IFN-τ cannot modify OTR response to oxytocin after OTR is formed but IFN-τ can block expression of OTR and luteolysis.

Effects of oIFN-τ on Steroid Hormone Receptor Expression. Estrogen and progesterone play major roles in expression of the OTR gene in ovine endometrium. Therefore, it is reasonable to anticipate that IFN-τ regulates endometrial ER and PR gene expres-

sion to control OTR formation. However, a direct effect of IFN-τ on OTR gene expression cannot be excluded at present (38). During early pregnancy, endometrial OTR are very low and oxytocin can not elicit pulsatile release of PGF by the uterus (23). Although basal secretion of PGF is higher during early pregnancy than the cycle (23), the uterus does not release luteolytic pulses of PGF because oIFN-τ prevents OTR expression.

In pregnant ewes, endometrial PR mRNA levels decline between Days 10 to 12 and Day 16, whereas PR protein is low and does not change between Days 10 and 16 (58). Endometrial ER mRNA and protein decrease from Days 10 to 16 in pregnant ewes but increase during this period in cyclic ewes (58). Immunoreactive ER are absent in luminal and superficial glandular epithelium of pregnant ewes; however, ER expression in deep glandular epithelium, stroma or myometrium is similar for Day 15 pregnant and cyclic ewes (32, 33, 65). Thus, products of the conceptus may either stabilize endometrial PR and/or inhibit ER expression in the luminal epithelium and superficial glandular epithelium during pregnancy recognition (58); however, oIFN-τ does not increase expression of PR in the endometrium (96). These results were confirmed in ewes in which one uterine horn contains a conceptus and the other does not, but both uterine horns are exposed to the same endocrine environment provided by the ewe. In this model, there is expression of ER and OTR in endometrium of the nonpregnant uterine horn but not the pregnant uterine horn (97). The cellular and molecular mechanism(s) by which oIFN-τ prevents ER and OTR formation on endometrial cells has not been established.

Working Hypothesis on IFN-τ Action on the Endometrium to Regulate Hormone Receptor Expression

A model for pregnancy recognition in ruminants (Fig. 2) was used to formulate working hypotheses which have now been tested. It was proposed (23) that IFN-τ acts on endometrial steroid hormone receptors during pregnancy recognition to inhibit formation of OTR and pulsatile release of luteolytic PGF by extending the "progesterone block" to ER and OTR formation. By acting through Type I IFN receptors present on the luminal and superficial glandular epithelium and, through an underlined signal transduction system, IFN-τ was hypothesized to (i) stabilize or upregulate expression of PR to extend the "progesterone block" and prevent expression of ER and OTR; (ii) directly inhibit expression of OTR; (iii) inhibit ER gene expression to prevent estrogen-induced increases in OTR; and/or (iv) inhibit post-OTR mechanisms and oxytocin-induced pulsatile release of PGF.

In order to establish the mechanism of IFN-τ regulation of expression of ER, PR, and OTR in the uterine endometrium of ewes, it was first important to establish the temporal and cell-specific regulation of those receptors. Previous studies used whole endometrial homogenates (58, 65, 93) or examined only protein levels (65) and not mRNA levels. Therefore, we undertook a series of experiments using the sensitive techniques of *in situ* hybridization, ribonuclease protection assay, and nuclear runoff transcription assays with homologous probes in combination with immunohistochemical analyses of these same tissues. These techniques were applied to a well-characterized model, the catheterized ovine uterus, that was developed for study of luteolysis and maternal recognition of pregnancy in our laboratory (23) and utilized highly purified recombinant oIFN-τ (roIFN-τ).

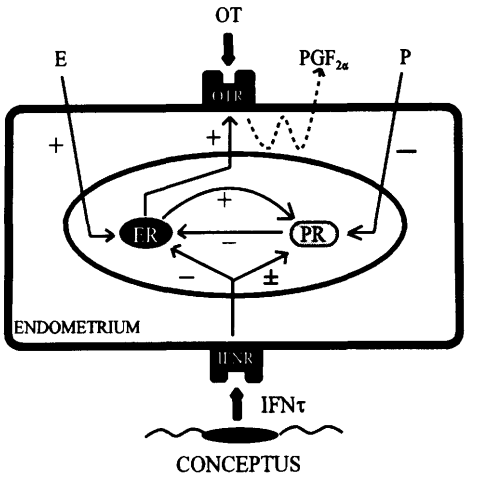

Figure 2. Schematic illustrating a working hypothesis of IFN-τ action to regulate oxytocin receptor gene expression during maternal recognition of pregnancy in sheep. The conceptus produces IFN-τ between Days 10 and 21 of early pregnancy. IFN-τ binds to Type I IFN receptors on the endometrial epithelium and, through an unknown signal transduction pathway, inhibits estrogen-induced increases in ER and OTR expression and production of luteolytic pulses of $PGF_{2\alpha}$. IFN-τ may block ER and thus OTR formation (i) indirectly, by stabilizing or preventing downregulation of PR gene expression by progesterone; and/or (ii) directly, by inhibiting ER gene expression. Available evidence indicates that IFN-τ blocks transcription of ER and OTR genes but has no detectable effect on expression of PR. E, estrogen; ER, estrogen receptor; IFNτ interferron tau; IFNR, Type I IFN receptor; OT, oxytocin; OTR, oxytocin receptor; P, progesterone; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

Differences in hormone receptor expression between cyclic and pregnant ewes provide critical information about the mechanism of pregnancy recognition. For example, endometrium of cyclic ewes expresses ER mRNA and protein at high levels on Day 1 which decline to low or undetectable levels between Days 6 and 10 and then increase between Days 11 and 15 (33). Similarly, PR mRNA and protein are highest on Day 1, decrease to low or undetectable levels between Days 6 and 11, and then increase between Days 13 and 15. The PR mRNA and protein were undetectable in luminal and shallow glandular epithelium be-

tween Days 11 and 13, and ER mRNA and protein were low on Days 6 and 11, after which time ER mRNA and protein increased to Day 15 in the luminal and shallow glandular epithelium. Low levels of both ER and PR gene expression were detected in deep glandular epithelium and stroma in both cyclic (Days 11 to 15) and pregnant (Days 11 to 25) ewes. Whereas for pregnant ewes, endometrial ER mRNA and protein decreased between Days 11 and 15 and remained low to Day 25, and PR mRNA and protein were low and not different from cyclic ewes between Days 11 and 15. Expression of both ER and PR mRNAs and proteins were low or absent in the luminal and shallow glandular epithelium between Days 13 and 25 of pregnancy, but consistently detectable at low levels in stroma and deep glandular epithelium between Days 11 to 25 of pregnancy. These results suggest that endometrial ER and PR gene expression is regulated in a tissue and cell-type–specific manner which differs between the estrous cycle and early pregnancy. The absence of detectable ER and PR (33), and OTR (30) in lumenal and glandular epithelium of endometrium of pregnant ewes between Days 11 and 25 suggests negative regulation of expression of ER and OTR by oIFN-τ, since expression of PR did not differ between pregnant and cyclic ewes.

Therefore, effects of roIFN-τ on PR and ER gene expression were examined (43). Ovariectomized ewes received intrauterine injections of roIFN-τ from Days 11 to 15 and either progesterone alone (P) or progesterone + estradiol (P + E) intramuscularly. Endometrial PR mRNA and protein were higher on Day 16 in ewes receiving P + E compared with P alone; however, increases in PR mRNA and protein were less in endometrium of roIFN-τ–treated ewes. Interestingly, PR expression was limited to stroma and deep glandular epithelium in ewes treated with P alone. Endometrial ER expression was suppressed by roIFN-τ in ewes treated with P and P + E. Only in control ewes could ER mRNA and protein be detected in the lumenal and superficial glandular epithelium on Day 16. Endometrial OTR expression was also suppressed in roIFN-τ–treated ewes regardless of steroid treatment. There was no evidence that roIFN-τ stabilized or prevented downregulation of PR expression in the endometrium. However, undefined interactions between signal transduction pathways activated by P and IFN-τ may be of importance. Clearly, roIFN-τ suppressed endometrial expression of both ER and OTR.

Interactions between oIFN-τ and progesterone to inhibit endometrial expression of ER and OTR following treatment with E_2 were examined (96). Ewes were ovariectomized on Day 4 and treated with P from Days 4 to 10 or from Days 4 to 15 postestrus and then with P + E on Days 13–15, and they received intrauterine injections of either roIFN-τ or control proteins on Days 11–15 (96). The roIFN-τ suppressed E_2-induced increases in ER and OTR regardless of steroid treatment, but did not affect expression of PR. Immunoreactive ER and PR were absent in lumenal and superficial glandular epithelium of roIFN-τ–treated ewes, but present in deep glandular epithelium and stroma regardless of steroid or protein treatment (96). Thus, P probably inhibits E_2-induced increases in ER, PR, and OTR expression in PR-positive deep glandular epithelium and stromal cells, whereas roIFN-τ blocked E_2-induced increases in ER, PR, and OTR expression in a PR-negative luminal and superficial glandular epithelium. Thus, the combined actions of oIFN-τ and progesterone may be required to suppress E_2-induced increases in ER and OTR in all endometrial cell types to prevent luteolysis. These results support those of Ott *et al.* (95).

In a subsequent study (94) using cyclic ewes that received intrauterine injections of either roIFN-τ or control proteins on Days 11–14, it was confirmed that (i) ER mRNA and protein were abundant in luminal and glandular epithelium of control ewes, but very low or undetectable in roIFN-τ–treated ewes; (ii) PR mRNA was equally low in endometrial epithelium and stroma of control and roIFN-τ–treated ewes; (iii) PR protein was present in stromal and epithelial cells of control ewes, but only in stromal cells of roIFN-τ–treated ewes; (iv) OTR were lower in endometrium of roIFN-τ–treated than control ewes; and (v) only control ewes responded to exogenous oxytocin with increased plasma concentrations of PGFM (94). These results confirm that antiluteolytic effects of roIFN-τ are to prevent increases in endometrial ER and OTR.

The E_2-induced events leading to luteolysis have been described by Hixon and Flint (47). Therefore, their model was used to determine if intrauterine injections of roIFN-τ can block E_2-induced luteolysis in cyclic ewes (46). In response to an intramuscular dose of 750 μg E_2 on Day 12, endometrial ER mRNA and PR mRNA increased after 12 hr, ER protein and PR protein increased after 24 hr, OTR began to increase after 12 hr with the greatest increase was between 36 and 48 hr, and luteal regression was almost complete by 48 hr post-E_2 for control ewes. *In situ* hybridization and immunohistochemical analyses indicated that ER increased in the epithelium at 12 hr and stroma by 48 hr, whereas PR gene expression increased in the stroma at 12 hr and in the epithelium at 48 hr in control ewes. In roIFN-τ–treated ewes, E_2 was unable to induce expression of endometrial ER mRNA, ER protein, or OTR, and CIL function was maintained. However, E_2 did induce a transient increase in PR mRNA between 12 and 36 hr which decreased by 48 hr (46). Interestingly, the E_2-induced increase in PR mRNA was limited to stromal cells and was not accompanied by a detectable increase in PR protein in roIFN-τ–

treated ewes. These results strongly support the conclusion that roIFN-τ prevents E_2-induced endometrial expression of ER and OTR to prevent luteolysis (46).

Given the strong evidence that roIFN-τ prevents expression of endometrial ER and OTR genes, levels of ER mRNA and transcription rates of ER and OTR genes in cyclic and pregnant ewes were compared on Day 15 postestrus in one experiment (38). Results indicated that transcription rates for ER and OTR genes and ER mRNA expression were 2-fold lower in pregnant than cyclic ewes. In the second experiment, cyclic ewes received intrauterine injections of either roIFN-τ or control proteins on Days 11–14 and were hysterectomized on Day 15 postestrus. Both ER mRNA and ER and OTR gene transcription rates were 2-fold lower for roIFN-τ–treated compared with control ewes. Therefore,, oIFN-τ inhibits ER gene transcription and this may block estrogen-dependent increases in OTR gene transcription; however, a direct effect of oIFN-τ on OTR gene transcription is also possible (38).

What Intracellular Mechanisms Activated by IFN-τ Suppress Transcription of the Ovine ER Gene? Molecular mechanisms whereby IFN-τ suppresses ER gene transcription have not been defined, but they likely involve the Type I IFN receptor signal transduction system and several members of the Type I IFN-induced transcription factor family (Fig. 3). The Type I IFN transcription factor family includes interferon stimulated gene factor–3 (ISGF3), interferon regulatory factor–1 (IRF-1), IRF-2, interferon consensus sequence–binding protein (ICSBP), and lymphoid-specific IRF (LSIRF) (98). The ICSBP and LSIRF are unique to cells of lymphoid lineage. In contrast, ISGF3, IRF-1, and IRF-2 represent a transcription factor network that regulates expression of IFN-inducible genes in several cell types (99). The ISGF3 transcription factor complex is composed of four proteins which are dissociated and normally located in the cytoplasm. As reviewed by Darnell *et al.* (99), binding of a Type I IFN to its receptor immediately activates latent tyrosine kinases, JAK1 and tyk2, which phosphorylate tyrosine residues of STAT1 (p84), STAT1a (p91) and STAT2 (p113). These three phosphoproteins then bind a fourth DNA-binding protein, p48, and the multimeric protein complex is transported to the nucleus. This ISGF3 transcription factor complex binds to an IFN-stimulated responsive element (ISRE) present in the promoter/enhancer region of IFN-responsive genes and increases rates of gene transcription. The interferon regulatory factor (IRF)–1 gene contains an ISRE and is upregulated by Type 1 IFNs IRF-1 is a positive-acting transcription factor which binds to an interferon regulatory factor element (IRF-E) and increases gene transcription. The IRF-E is often contained within the larger ISRE. Although the IRF-1 protein can bind to an ISRE containing an IRF-E and activate transcription, the ISGF3 transcrip-

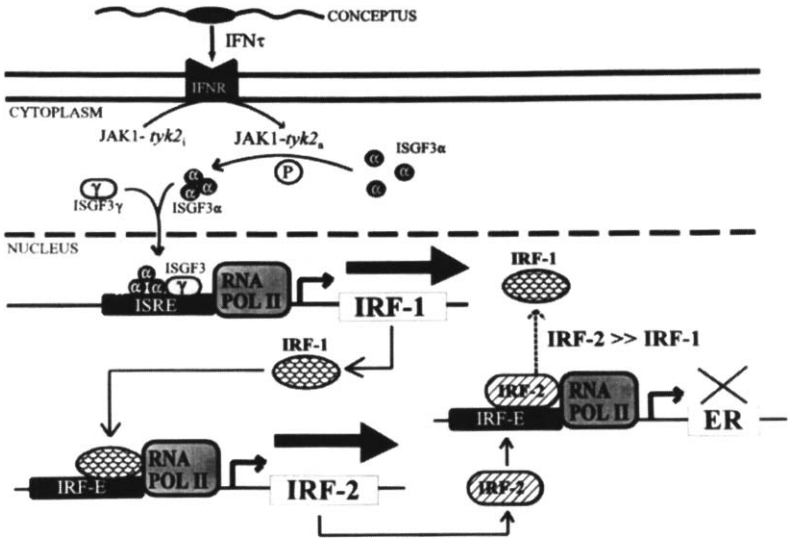

Figure 3. Proposed model for the intracellular actions of IFN-τ to suppress transcription of the ovine ER gene in the endometrial epithelium. Trophoblast IFN-τ binds to Type I IFN receptors on the apical surface of the endometrial luminal and superficial glandular epithelium. This activates a signal transduction system similar to that for other Type I IFNs. Changes in conformation of the receptor activate two latent cytoplasmic tyrosine kinases, JAK1 and tyk2. These then phosphorylate STAT1, STAT1a, and STAT2. These three phosphoproteins then bind a fourth DNA-binding protein, ISGF3γ, which forms the ISGF3 transcription factor complex. Formation of this multimeric complex allows ISGF3 to translocate to the nucleus and bind to an ISRE in the IRF-1 gene. The IRF-1 gene is transcribed and then translated. This positive-acting transcription factor for IFN-responsive genes then binds to an IRF-E and increases IRF-2 gene expression. The IRF-2 gene is transcribed and translated. This transcription factor is a negative-acting transcription factor which silences transcription of genes containing an IRF-E. IRF-2 has greater affinity for IRF-Es and will displace IRF-1 binding. If the ovine ER gene contains a functional IRF-E, production of IRF-2 in response to IFN-τ may silence transcription of the ER gene.

tion factor complex can only bind to an ISRE. Transcription of the IRF-2 gene is increased by IRF-1 and IRF-2 then acts as a negative-acting transcription factor which binds to IRF-E to silence gene transcription. The IRF-2 can displace IRF-1 binding, and it can silence activities of other transcription factors such as Spl (99). This yin-yang interaction between IRF-1 and IRF-2 regulates induction and repression of Type I IFN-responsive gene expression.

τ-Interferon is assumed to activate the Type I IFN signal transduction system in the ovine endometrium. Since production of oIFN-τ is developmentally regulated and it is produced in massive quantities for an extended period (i.e., much longer than the period of IFN-α induced by a viral infection), one may hypothesize that IFN-τ activates expression of large amounts of IRF-2. If the ovine ER gene contains a functional IRF-E, IRF-2 could silence transcription of the ER gene. Silencing the ER gene would be expected as long as a high IRF-2:IRF-1 ratio was maintained. Silencing ER gene transcription in lumenal and superficial glandular epithelium of the ovine endometrium may block ER and OTR gene transcription and aborgate the luteolytic mechanism. Progesterone from the CL could continue to act on PR positive stroma and deep glandular epithelium to suppress ER and OTR expression in those cells to complete induction of the antiluteolytic mechanism.

The hypothesis that IRF-2 negatively regulates ER and OTR gene transcription is supported by the fact that the trophectoderm must produce IFN-τ prior to development of the endometrial luteolytic mechanism. That is, IFN-τ would first activate transcription of IRF-1 and then IRF-2. This would require sequential IRF-1 gene activation and translation followed by another round of transcriptional and translational activity to produce IRF-2. This process may require 24 to 48 hr. Recent results (Spencer TE, Bazer FW, unpublished data) are consistent with this hypothesis in that endometrium exposed to serum proteins (control) expressed low levels of IRF-1 (compact stroma and mid-glandular epithelium) and IRF-2 (mid-glandular epithelium), but not in the lumenal or superficial glandular epithelium at either 1, 3, 6, 12, 24, 48, 72, 96 or 120 hr post-treatment on Day 11. However, endometrium exposed to roIFN-τ expressed abundant IRF-1 in lumenal and superficial glandular epithelium at 12 and 24 hr post-injection, and abundant IRF-2 in those same cells between 24 and 120 hr. Collectively, these results indicate that roIFN-τ induces transient expression of IRF-1 which induces expression of IRF-2. Furthermore, IRF-2 may then suppress expression of IRF-1, as well as ER and OTR. The length of time required for induction of expression of IRF-1 and then IRF-2 is consistent with results from early embryo transfer experiments which demonstrated that the conceptus must be present in the uterus 48–72 hr prior to development of the luteolytic mechanism in order to block expression of ER and OTR by lumenal and superficial glandular epithelium.

Summary and Future Directions

There are distinct temporal and spatial, tissue and cell-type alterations in ER, PR, and OTR during the estrous cycle and pregnancy. Expression of PR in luminal and superficial glandular epithelium is undetectable between Days 11 and 13. Expression of ER is low in these same cells between Days 6 and 11, and then increases along with OTR between Days 11 and 15 in preparation for luteolysis in cyclic ewes. The coordinated increase in ER and OTR gene expression between Days 13 and 17 in the luminal and superficial glandular epithelium appears to be necessary for establishing pulsatile release of luteolytic PGF (32–34, 56). In response to oIFN-τ, expression of ER, PR, and OTR remains low or undetectable in lumenal and shallow glandular epithelium between Days 13 and 25 of pregnancy. In pregnant and cyclic ewes, both ER and PR are expressed in low amounts by stromal cells and deep glandular epithelium.

Antiluteolytic effects of oIFN-τ are assumed to be limited to the uterine lumen since there is no evidence that it is released into either the uterine venous or lymphatic drainage (23). As noted previously, oIFN-τ does not prevent increased expression of ER by cells of the stroma and deep glandualr epithelium. However, there is evidence that intrauterine injections of roIFN-τ induce expression of Mx mRNA throughout the entire uterine wall (epithelium, stroma, and myometrium) and that there is coordinate expression of the Mx gene in uterine epithelial cells that express PR (100). Therefore, IFN-τ–stimulated epithelial cells may produce "interferomedins" that act as paracrine signals to activate Mx gene expression in stroma, deep glands, and myometrium. Alternatively, IFN-τ may have access to the entire uterine wall through an as yet undefined mechanism. Further, the mechanism whereby persistent expression of PR is maintained in the stroma also remains undefined. Reciprocal interactions between epithelial and stromal cells in complex heterogenous epitheliomesenchymal organs (101) may account for requirements for both IFN-τ actions on epithelium and progesterone effects on stroma to abrogate completely the luteolytic mechanism.

The antiluteolytic effects of IFN-τ involve suppression of transcription rates for ER and OTR genes in endometrium of pregnant ewes or cyclic ewes given intrauterine injections of roIFN-τ. However, neither pregnancy nor intrauterine injections of roIFN-τ gene transcription. The antiluteolytic action of IFN-τ may, therefore, suppress ER and OTR gene expression by inducing a negative-acting transcriptional factor(s),

such as IRF-2, to prevent OTR formation on the endometrial luminal and superficial glandular epithelium.

What Are the Conceptus-Endometrial and Epithelial-Stromal Interactions That Maintain the Endometrium under Progesterone Dominance Necessary to Establish Pregnancy? A model for conceptus-endometrial and epithelial-stromal interactions that may occur during maternal recognition of pregnancy is illustrated in Figure 4. There is evidence that (i) progesterone negatively autoregulates expression of the PR gene, particularly in the endometrial luminal and superficial glandular epithelium; (ii) endometrial expression of ER, PR, and OTR is undetectable in lumenal and superficial glandular epithelium during the period of pregnancy recognition; and (iii) low levels of PR and ER are detectable only in the stroma and deep glandular epithelium. Administration of a PR antagonist, ZK2.993 (Schering, Berlin, Germany), to mated ewes between Days 4 and 15 postestrus (Spencer TE, Bazer FW, unpublished observations) prevented the establishment of pregnancy. These results indicate that progesterone is essential for the maintenance of pregnancy. However, the mechanism by which progesterone maintains the endometrial epithelium in a progestational state when PR are low or undetectable is not known.

Eley *et al.* (102) demonstrated that 5α-reductase is present in bovine and ovine endometrium and that a primary metabolite of progesterone in sheep and cow endometrium is 5α-dihydroprogesterone (5α-DHP). The 5α-DHP cannot be converted to testosterone or estrogen. Unpublished results (Nobelius A, Short R) suggest that 5α-DHP binds to androgen receptors (AR) with an affinity similar to that for testosterone and that it may also bind to PR with an affinity about 50% that for progesterone. Immunoreactive 5α-reductase and AR are relatively abundant in stromal and glandular epithelial cells of the ovine endometrium on Days 11, 13, 15, and 17 of both the estrous cycle and pregnancy (Wiley AA, Bazer FW, unpublished observations). It was of interest to know whether progesterone effects are limited to its ability to bind available PR or if progesterone is converted to 5α-DHP so that it can exert its effect by binding both PR and AR. In Hela cells transfected with either the full-length human AR or PRb, 5α-DHP and 5β-DHP did not stimulate an AR promoter-reporter construct; however, both forms of DHP stimulated the PRb promoter-reporter construct as effectively as R5020, a PR agonist (Spencer TE, Bazer FW, unpublished results). Also of interest is the report that DHT antagonizes effects of estrogen in the immature rat uterus, perhaps by decreasing estrogen-induced gene expression at a point subsequent to ER binding (103). In the rat anterior pituitary and uterus 5α-DHP causes acute tissue-specific decrease in ER,

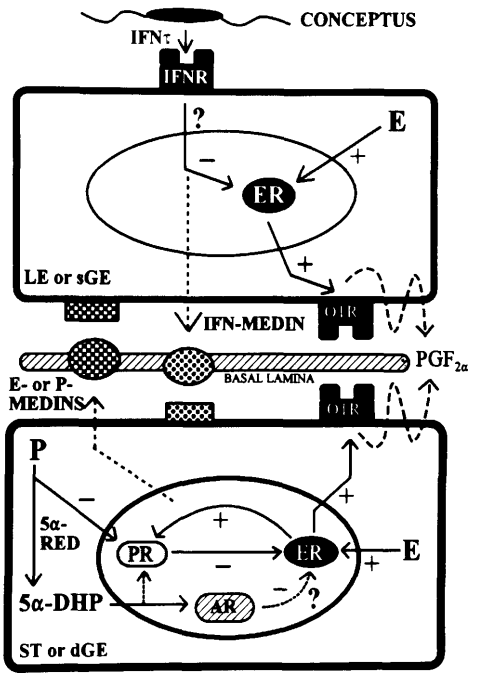

Figure 4. Proposed model of conceptus-endometrial interactions and epithelial-stromal interactions that occur during maternal recognition of pregnancy in sheep. In cyclic ewes during late diestrus, PR is undetectable in the luminal (LE) and superficial glandular (sGE) epithelium. In the absence of suppression by progesterone, ER gene transcription increases in the epithelium which allows for ER-mediated increases in OTR formation. However, in pregnant ewes trophoblast IFN-τ binds to Type I IFN receptors and, through an undefined signal transduction pathway, initiates intracellular events that suppress transcription of the ER gene, possibly through a negative-acting transcription factor such as IRF-2. In this model, actions of IFN-τ would prevent OTR formation in the PR-negative LE and sGE, abrogate pulsatile production of luteolytic $PGF_{2\alpha}$ and maintain CL production of progesterone, which in turn acts on the PR-positive stroma (ST) and deep glandular epithelium (dGE) to suppress estrogen-induced increases in ER and OTR gene expression. These actions prevent pulsatile production of $PGF_{2\alpha}$ by the cells of the ST and dGE. Thus the combination of IFN-τ and progesterone suppress OTR formation in the entire endometrium.

Although progesterone production is maintained, the LE and the sGE lack detectable levels of ER and PR. How does the epithelium remain responsive to hormones? Recent evidence indicates that the stroma mediates receptor-negative epithelial cell functions *via* hormone-induced growth factors. Therefore, cells of the stroma could produce either "estromedins" or "progestomedins," such as HGF and KGF, which bind to receptors expressed only by epithelium. Moreover, IFN-τ binding to epithelial cells may cause the production of "interferonmedins," which could relay signals from the epithelium to the stroma. Reciprocal interactions between te epithelium and stroma may be required for the integration and coordination of endometrial responses to conceptus signals.

Progesterone may also be metabolized to 5α-DHP by the actions of 5α-reductase. Evidence indicates that 5α-DHP is nonmetabolizable and has greater affinity for the AR than the PR. Activation of the AR by 5α-DHP may antagonize effects of estrogen on gene expression or regulate expression of androgen-responsive genes encoding growth factors or other factors important for endometrial function and conceptus growth and development. PR, progesterone receptor; ER, estrogen receptor; OTR, oxytocin receptor; IFNτ, tau-interferon; CL, corpus luteum; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; HGF, hepatocyte growth factor; KGF, keritinocyte growth factor; 5α-DHP, 5α-dihydro progesterone; AR, androgen receptor.

similar to effects of progesterone (104). The role(s) of 5α-DHP and AR in regulation of ovine endometrial physiology remains unknown.

In many epitheliomesenchymal organs, epithelial function is affected by products of the mesenchymal or stromal cells. Progesterone and androgen action on receptor-negative epithelial cells can be mediated by stromal cell–derived factors such as keratinocyte growth factor (KGF or FGF-7) which act as "progestomedins" (105). The KGF receptors appear to be expressed exclusively on epithelial cells. In explant cultures of undifferential seminal vesicle, KGF can replace the effects of androgen to promote tubule morphogenesis (106). Hepatocyte growth factor (HGF) or scatter factor acts as an "estromedin" and binds to its high-affinity receptor, *c*-met, to induce mitogenesis, cell motility and morphogenesis in renal epithelial cells (107). Although KGF and HGF have not been identified and characterized in the ovine uterus, their functions in other model systems suggest that they may allow PR and/or ER positive stromal cells to regulate functions of ER- and/or PR-negative epithelial cells in primates (108) and pigs (109). It is known that IFN-τ stimulates secretion of at least 11 proteins by the endometrium (110). One or more of these proteins could act as "interferomedins" on stromal cells to stimulate production of "estromedins" or "progestomedins."

Summary

The ovine uterus provides an excellent *in vivo* model system for the study of the cellular and molecular mechanisms of Type I IFN and steroid hormone regulation of hormone receptor gene expression in an epitheliomesenchymal organ. Future experiments will be directed toward determining the signal transduction pathway activated by IFN-τ and mechanisms involved in regulation of gene expression. These experiments include (i) cloning the Type I IFN receptor found on the endometrial luminal and superficial glandular epithelium; (ii) eluciding the signal transduction pathway activated by IFN-τ binding to the Type I IFN receptor; (iii) cloning and structural analysis of the ovine ER and OTR genes; (iv) determining mechanisms regulating transcription of the ovine ER and OTR genes, particularly those involving both IFN-τ and progesterone; (v) determining the role(s) of IFN-τ–induced proteins (e.g., Mx protein) in establishment of pregnancy; and (vi) investigating conceptus-endometrial and epithelial-stromal interactions that integrate and coordinate endometrial physiology during the estrous cycle and pregnancy.

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