Reproductive Biology and Endocrinology

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The effects of estrogen, its antagonist ICI 182, 780, and interferon-tau on the expression of estrogen receptors and integrin alphaV beta 3 on cycle day 16 in bovine endometrium

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Published: 24 April 2003

Reproductive Biology and Endocrinology 2003, **1**:38

Received: 5 February 2003
Accepted: 24 April 2003

This article is available from: http://www.RBEj.com/content/1/1/38

Abstract

We have shown previously that downregulation of intercaruncular stromal integrin $\alpha_v\beta_3$ in bovine endometrium on day 16 of the estrous cycle coincided with the antibody recognition of estrogen receptors (ER) in the luminal epithelium. In pregnancy, these changes were not observed. Our hypothesis was that on day 16 of the estrous cycle, estrogen from the dominant follicle causes a reduction in integrin $\alpha_v\beta_3$ and affects ERα in the luminal epithelium. The pregnancy recognition protein, interferon-τ (IFN-τ), may prevent downregulation of integrin $\alpha_v\beta_3$ and suppress ERα expression in the luminal epithelium. On days 14 to 16, heifers received uterine infusions of the anti-estrogen ICI 182, 780, estradiol 17β, IFN-τ or the saline control. On day 16, reproductive tracts were collected for analysis of integrin $\alpha_v\beta_3$ and ERα. Estrogen receptor α immunoreactivity was largely restricted to the luminal epithelium in control animals. Using anti-ERα recognizing the amino terminus, estrogen-treated animals showed reactivity in the stroma, shallow and deep glands and myometrium as is typical of estrus, whereas ICI 182, 870 treated heifers showed little or no reactivity. In contrast, carboxyl terminus-directed antibodies showed a widespread distribution of ERα with reactivity detected in the uterine epithelium, stroma and myometrium of both estrogen and ICI 182, 780 treated animals. Heifers treated with IFN-τ had low ERα reactivity overall. Control and IFN-τ treated heifers had lower intercaruncular stromal expression of integrin $\alpha_v\beta_3$ in comparison to estrogen and ICI 182, 780 treatments. Overall, the results suggest that on day 16 of the estrous cycle, estrogen effects on integrin $\alpha_v\beta_3$ are indirect and do not directly involve ERα in the luminal epithelium. During pregnancy, interferon-tau may block ERα in the luminal epithelium but likely does not rescue integrin $\alpha_v\beta_3$ expression.

Introduction

Day 16 of the bovine estrous cycle is critical as it is the last day for embryo transfer and marks the generation of the luteolytic signal in the absence of a viable conceptus [1,2].

We have identified two potential molecular markers of the day 16 uterine environment: the adhesion and signalling molecule, integrin $\alpha_v\beta_3$ and the estrogen receptor (ER) [3,4]. Integrins are transmembrane heterodimers that facilitate cell-cell and cell-extracellular matrix attachment. In doing so, integrins influence differentiation states of the cells on which they are expressed and adjacent cells through bi-directional signalling to and from the cell and its environment [5]. In many species including cattle, integrin $\alpha_v\beta_3$ is present at the fetomaternal interface during embryo attachment and implantation [6–10].

In comparison to other domestic animals [7,9], preferential expression of integrin $\alpha_v\beta_3$ in intercaruncular stromal endometrium is unique to cattle [3]. There is little expression in luminal epithelium and the stroma of the caruncles, the endometrial sites where the maternal component of the placenta will develop. Its expression is strongest in the periluminal stroma in cells in contact with the basal lamina of luminal epithelium. Downregulation of integrin $\alpha_v\beta_3$ in subepithelial stroma occurs on day 16 of the estrous cycle, but not pregnancy [3,10]. This downregulation coincides with a transient change in the ER in the luminal epithelium and the onset of luteolysis [4]. In support of a role for estrogens in regulating integrin $\alpha_v\beta_3$ expression in cyclic endometrium, downregulation of the rate limiting integrin subunit β_3 expression by estrogen is reported in cultured endometrial cells [11–13].

The proposed sequence of hormonal and molecular events leading to luteolysis involves positive feedback between the endometrium and ovary [2,14]. The role of estrogens and their receptors in initiation of luteolysis is not yet clear, but exogenous estrogen stimulates luteal regression [15,16]. Studies in sheep suggest that upregulated ER and oxytocin receptor expression in the luminal epithelium are essential for initiation of luteolysis [17,18]. During ovine pregnancy, conceptus secreted interferon tau (IFN-τ) is thought to block upregulation of ERα and oxytocin receptor expression in the luminal epithelium, which in turn suppresses luteolysis [18,19]. In cattle, the effects of IFN-τ on ERα in the luminal epithelium at maternal recognition of pregnancy are unknown. There are reported differences in the uterine location and timing of ERα expression between sheep and cows, and among research groups [4,17,20]. There are no reports of ERβ localization in mature bovine endometrium, although our preliminary unpublished observations using anti-human ERβ indicate that it is present in association with blood vessel walls but is not detectable in other endometrial cells. Using an antibody that localized ERα (clone ID5), Robinson et al. [20] detected ERα protein in the bovine uterine luminal epithelium at levels ranging from low to undetectable throughout the estrous cycle and pregnancy. Using a different ERα antibody (clone AER314) we detected strong staining for ERα in the uterine luminal epithelium only on day 16 of the estrous cycle and not in endometrium from pregnant cows [4]. The antibodies used in these bovine studies recognise different domains of the ERα, which have been shown previously to affect immunohistochemical reactivity in the luminal epithelium [21].

The anti-estrogen ICI 182, 780 is classified as a pure anti-estrogen due to its lack of estrogen-like activity [22,23]. ICI 182,780 acts by binding ER, which causes disassociation of receptor associated proteins and results in impaired receptor dimerisation and increased receptor degradation [22,23]. In utero ICI 182, 780 blocks the trophic action of estrogen in rodents, women and pigs [23,24]. The effects of ICI 182, 780 in bovine endometrium have not yet been tested.

Our objectives were to investigate the effects of estrogen, the antiestrogen ICI 182, 780 and IFN-τ on the expression of ERα and integrin $\alpha_v\beta_3$ in bovine endometrium at the time of maternal recognition of pregnancy. We hypothesized that 1) IFN-τ alters expression of ERα in the luminal epithelium and prevents downregulation of integrin $\alpha_v\beta_3$ on day 16 of pregnancy and, 2) estrogen acting via ERα in the luminal epithelium causes downregulation of integrin $\alpha_v\beta_3$ in the subepithelial stroma on day 16 of the estrous cycle.

Materials and Methods

Animals

This study was performed using 12 beef heifers of mixed breeds of similar age and weight (1.5–2 years; 520 ± 31 kg) and exhibiting estrous cycles of 18–20 days. Heifers were checked for estrus twice daily throughout the experiment and trained for tie stalls one month prior to uterine infusions to adjust them to handling and confinement. Animals were synchronized to estrus using Estrumate (500 μg cloprostenol, Schering Canada Inc., QC, Canada), according to the synchronization schedule shown in Figure 1. All procedures performed were in accordance with the guidelines of the Canadian Council on Animal Care and were reviewed and approved by the Nova Scotia Agricultural College Animal Care and Use Committee.

Treatments

Following induced estrus, 3 heifers per treatment were randomly assigned to receive uterine infusion of a control solution (0.1% BSA in saline), or estradiol-17β (117 ng/dose; Sigma, Saint Louis, MO), or ICI 182, 780 (3 mg/dose, Tocris, Avonmouth, Bristol, United Kingdom) or recombinant ovine IFN-τ (0.25 mg/dose = biological activity of 5×10^7 antiviral units/day; kindly provided by Drs. T. E. Spencer and F.W. Bazer, Texas A & M University, College Station, TX), from days 14 to 16 of the estrous cycle.

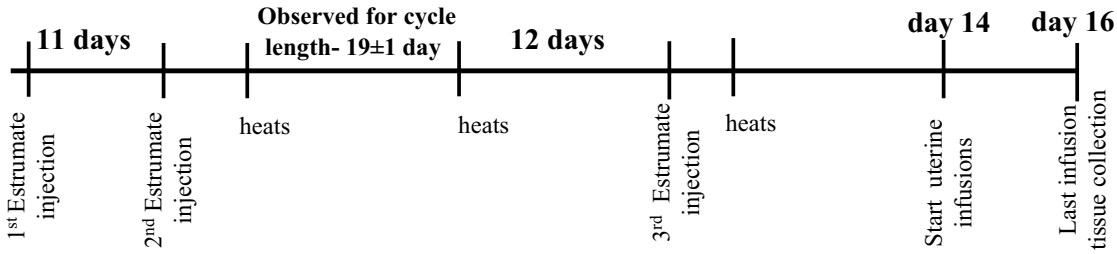

Figure 1
Synchronization schedule, heat detection and treatment regimes for heifers used in the study. Heifers were injected with Estrumate® 11 days apart and observed for heats after the second injection. All heifers were observed in standing heat within the same twelve hour period then observed for one cycle length of 18–20 days. Twelve days after non-induced estrus, heifers were injected a third time and observed for heats. At 6 pm, fourteen days following standing heat treatment began. Heifers received four uterine infusions 12 hr apart of either saline control, IFN-τ, estrogen or ICI 182,780. At 2 pm on day 16 of the estrous cycle, heifers were slaughtered and tissues were collected for analysis.

The dose of estradiol was based on physiological levels, where the maximal venous-arterial difference in estradiol-17β was multiplied by the average daily uterine arterial blood flow measured for cows on days 13–17 of the estrous cycle [25]. The dose used for ICI 182, 780 was based on an effective mg/kg dose administered to sheep via intrauterine infusion [26]. The dose for IFN-τ was selected based on previous studies in sheep [18] and increased to account for the greater uterine size of cows. Recombinant ovine IFN-τ has been shown to be as effective as recombinant bovine IFN-τ in preventing luteolysis [27]. Treatments were delivered every 12 h beginning at 6 pm on cycle day 14 via intrauterine infusion of both horns using 12 gauge Foley® catheters (Agtech, Manhattan, KS), which were positioned immediately anterior to the cervix prior to delivery of the 5 ml treatment volume. Treatment schedules are shown in Figure 1. At the time of treatment, the horns were gently massaged to ensure even distribution throughout the uterus. Volume and delivery mechanism were optimized prior to the experiment using reproductive tracts obtained from the local abattoir and in vivo using cull heifers.

Tissue Collection

Uteri were collected within 30 min postmortem and morphological observations were made on the ovaries and reproductive tract. Data on ovarian morphology according to the methods of Ireland [28] were recorded to confirm cycle stage. For each heifer uterine, cervical and corpus luteum weight and length were recorded. Transverse sections 1 cm^3 were dissected from the uterine horn ipsilateral to the corpus luteum and snap frozen in liquid nitrogen and stored at -80°C prior to preparation of cryostat cross-sections for immunohistochemical analysis. Intercaruncular endometrium was dissected from the ipsilateral horn, and snap frozen in liquid nitrogen for use in mRNA analysis. Caruncular tissue was not used for analysis of integrin $\alpha_v\beta_3$ since our previous work showed low expression in the caruncles regardless of the day of the cycle or pregnancy state [3,10].

Immunohistochemistry

Integrin $\alpha_v\beta_3$ and ERα protein were localized as previously described [3,4]. Briefly, 5–10 μm cryostat cross sections of endometrium were placed on Superfrost Plus slides (Fisher Scientific, Whitby, ON, Canada), air dried for 30 minutes, then fixed in acetone for 10 min and allowed to air-dry overnight before long-term storage at -80 C. Sections were rehydrated in PBS, blocked in 2% BSA in PBS and incubated with the monoclonal anti-$\alpha_v\beta_3$ from Novus Biologicals (clone BV4, Littleton, CO), a monclonal antibody to the amino terminus of the ERα (ER Ab-1, clone AER314 Neomarkers Inc., Union city, CA, USA) or to the carboxy terminus of the ERα (ER Ab-8, clone

AER314), or the control, purified mouse IgG (Chemicon) for 2 h. Sections were then washed and incubated with biotinylated horse-anti-mouse secondary (Vector Laboratories, Burlingame, CA) for 30 min. Immunoreactivity was visualized using a fluorochrome conjugated streptavidin probe (Alexa-488, Molecular Probes, Inc., Eugene, OR, USA). Immunohistochemistry experiments were repeated on different days using tissue sections from all animals on each day. To determine effects of treatment on levels of integrin $\alpha_v\beta_3$ protein in uterine tissue, signal intensity of the intercaruncular subepithelial stroma was scored by two observers on a six-point scale (0-negative, 1-very weak, 2-weak but clearly positive, 3-intermediate, 4-high, and 5-very high). Four slides from each heifer were scored.

Northern Blotting

Total RNA was extracted from intercaruncular endometrium using Trizol™ reagent (Life Technologies, Burlington, ON, Canada). Thirty five μg of total RNA was electrophoresed in denaturing 1.2% agarose, 2.2 M formaldehyde gels in 1X morpholinepropanesulfonic acid (MOPS, 0.02 M, pH 7.0, 2 mM sodium acetate, 1 mM EDTA pH 8.0) with appropriate markers for 2 h at 8 V/cm. Gels were blotted onto a positively charged nylon membrane (Roche Molecular Biochemicals, Laval, QC, Canada) using downward capillary transfer in alkaline buffer for 1 h. Two probing methods were used for two separate northern blots each with samples from each of the 12 animals. For the first blot, biotin labeled riboprobes were prepared via *in vitro* transcription (Roche). Blots were prehybridized in Ultrahyb™ (Ambion Inc, Austin, TX, USA) at 65 C for 30 min prior to the addition of the riboprobe. The blot was probed overnight at 65 C, and then washed at 65 C, 3 times for 20 min in 2X SSC/0.1% SDS (1X SSC = 150 mM sodium chloride and 15 mM sodium citrate pH 7.0). Hybridized probe was detected using chemiluminescence's performed according to the North2South™ kit protocol (Pierce Chemical Company, Rockford, IL). For the second blot, ^{32}P-labeled riboprobes were prepared via *in vitro* transcription and membrane was prehybridized at 68 C for 30 min, followed by overnight hybridization in Ultrahyb™ at 68 C in the presence of 1×10^6 cpm probe/ml. After hybridization the membrane was washed at room temperature in 2X SSC/0.1% SDS (1X SSC = 150 mM sodium chloride and 15 mM sodium citrate pH 7.0) two times for 5 min each, followed by 2 washes at 68 C in 0.25X SSC/0.1% SDS for 15 min each. After washing the membrane was exposed to x-ray film using an intensifying screen at -80 C for three days. Intensity of integrin subunit $\alpha_v\beta_3$ mRNA and 18S rRNA bands were measured using the Gene Genius Bioimaging System (Fisher Scientific). As indicated above, Northern blots were repeated once so there were six observations per treatment (3 animals × 2 blots).

Data Expression and Statistical Analysis

Data were checked for normality using the univariate procedure in SAS™ (Statistical Analysis Software Version 8, SAS Institute Inc., Cary, NC) and analyzed using the general linear models procedure to examine the effect of treatment on reproductive tract measurements, integrin $\alpha_v\beta_3$ protein immunohistochemical score, and integrin subunit $\alpha_v\beta_3$ mRNA. Day of experiment and day by treatment interactions were included in the immunohistochemistry and Northern blot analyses, although interactions were not significant. For northern blots, intensity of the 18S band within blot was included as a covariate to account for loading differences. Least squares means were compared using the Tukey-Kramer test. The selected α error rate was $P \leq 0.05$.

Results

Effects of Treatment on Uterine Characteristics

From visual examination all uteri were similar among treatments in terms of size, tone or color. Although not statistically significant there was a tendency for heavier uterine weights from estrogen-treated heifers ($P = 0.10$). No differences were observed between uterine, cervical or corpus luteum measurements of weight and length ($P > 0.05$). Gross uterine observations are summarized in Table 1.

Table 1: Reproductive tract characteristics of heifers treated with interferon-tau (IFN-τ), estrogen or ICI 182, 780.

Treatment	Uterine Weight (g)	Uterine Length (cm)	Cervix Length (cm)	Corpus Luteum Weight (g)
Control	171 ± 6	23 ± 3	9 ± 3	5 ± 0.4
IFN-τ	187 ± 8	23 ± 4	8 ± 1	5 ± 0.4
Estrogen	275 ± 19	25 ± 7	8 ± 1	4 ± 1
ICI 182,780	189 ± 43	22 ± 3	7 ± 1	5 ± 1

Mean ± standard error

Effect of Treatment on Uterine Distribution of the Estrogen Receptor-α

Reactivity to ERα antibodies (AER314 and AER 311) was used to measure the effects of treatments. Immunofluorescent reactivity in the luminal epithelium was present in sections from control heifers using both AER314 (Fig. 2A) and AER311 (Fig. 2B), and a small proportion of stromal cells showed nuclear reactivity (Fig. 2A,2B, arrows). In some animals, there was reactivity in the glands (data not shown). In sections from heifers infused with IFN-τ, reactivities to AER314 and AER311 were low in comparison to control animals (Fig. 2C,2D). There was no reactivity in the luminal epithelium. In some animals, there was nuclear staining in the glandular epithelium of some sections (data not shown). In sections from ICI 182, 780 treated animals, there was little or no specific immunofluorescence was detected throughout the endometrium using antibody AER314 (Fig. 2G). In contrast, strong immunofluorescent signals were present in the nuclei of glands, stroma and luminal epithelium in sections from ICI 182, 780 treated animals using antibody AER311 (Fig. 2H). Estrogen treated heifers showed strong reactivity to both antibodies AER314 and AER311 in the nuclei of stroma, glandular epithelium and myometrium (Fig. 2E,2F), but only in the luminal epithelium using antibody AER311 (Fig. 2F).

Effect of Treatment on Uterine Distribution of Integrin $\alpha_v\beta_3$

For all treatments, integrin $\alpha_v\beta_3$ expression was detected in the stroma (Fig. 3A), myometrium and arteriolar smooth muscle (data not shown). In tissue sections from all animals, reactivity was low in the caruncles (eg., ICI 182, 780 panel of Fig. 3). Intense reactivity was observed in sections from estrogen and ICI 182, 780 treated heifers on stromal cells in close proximity to the basement membrane of the luminal epithelium of intercaruncular endometrium (Fig 3A, arrows). Accordingly, sections from ICI 182,780 and estrogen treated heifers had higher scores for subepithelial stromal cell staining than control or IFN-τ treated animals ($P < 0.05$) (Fig. 3B).

Effect of Treatment on Expression of Integrin Subunit β_3 mRNA

Northern blot analysis revealed a single 2.4 kb transcript for bovine integrin subunit β_3 corresponding to the published coding sequence of 2.364 kb [29]. In intercaruncular tissue samples levels of integrin subunit β_3 transcript were lowest from heifers infused with control solution and IFN-τ in comparison to tissue from estrogen and ICI 182,780 treated heifers ($P < 0.05$) (Fig. 4).

Discussion

This is the first study to test the anti-estrogen ICI 182, 780 in the bovine uterus. No differences in uterine morphology were observed between treatments, which is not unexpected given the short exposure period, although estrogen but not ICI 182, 780 treated heifers tended to have heavier uterine weights. Unexpectedly, expression of ERα was readily detected in the luminal epithelium, stroma and glandular epithelium of sections from ICI 182, 780 treated heifers using antibody AER311. The distribution was similar to that observed in the estrogen-treated heifers with this antibody. Previous studies have shown that high levels of estrogen, such as those observed at estrus, are associated with this pattern of ERα expression [4,20,30]. Since ERα distribution did not differ between estrogen and ICI 182, 780 treated heifers and did differ from controls, ICI 182, 780 showed agonist, rather than antagonist, activity towards the bovine estrogen receptor. Interestingly, the reactivity to antibody AER314 was reduced in ICI 182, 780 but not estrogen treated animals, indicating that the effects of the two compounds are distinct. This may explain the lack of ICI 182, 780 effect on uterine morphology. In bovine endometrial explants, ICI 182, 780 was antagonistic to estrogen [31]. The reported mode of action of ICI 182, 780 is to disrupt translocation of receptors to the nucleus and increase receptor degradation [22,23], so a decline in ERα reactivity was expected regardless of antibody used. In the ewe, endometrial expression of ERα was lower in ICI 182, 780 treated than estrogen treated animals [26]. However similar to results presented here, in that species the ICI 182, 780 also showed partial agonist effects as evidenced by increased uterine weights, and GAPDH mRNA expression that did not differ from estrogen treated ewes [26].

Schuler et al. [21] localized ERα in bovine placentomes using four antibodies to ERα. Two of the antibodies recognized the carboxy terminus and positively reacted with maternal luminal epithelium, while the two amino terminal targeting antibodies did not. The current study is consistent with this pattern. The amino terminus of the ERα contains the AF-1 domain that undergoes a conformational change when the receptor is bound by estrogen and its activity is regulated by growth factors [22,32]. It is interesting that this antibody recognizes ERα in the luminal epithelium only in endometrium from calves [21], day 16 of the estrous cycle but not in tissues from other days of the estrous cycle [4], from pregnant cattle [4,21], or in endometrium from ICI 182,780 treated animals. The changes in AER314 binding may reflect changes in epitope accessibility related to a different functional conformation of the ERα. Possible causes include changes in receptor phosphorylation, estrogen binding, and/or in ERα cofactor association [22,32]. Impaired cofactor association is a reported mechanism of action of ICI 182,780 [22,23], thus it is conceivable that there was some antagonistic activity of the compound in the current study, which resulted in reduced recognition of the ERα by antibody

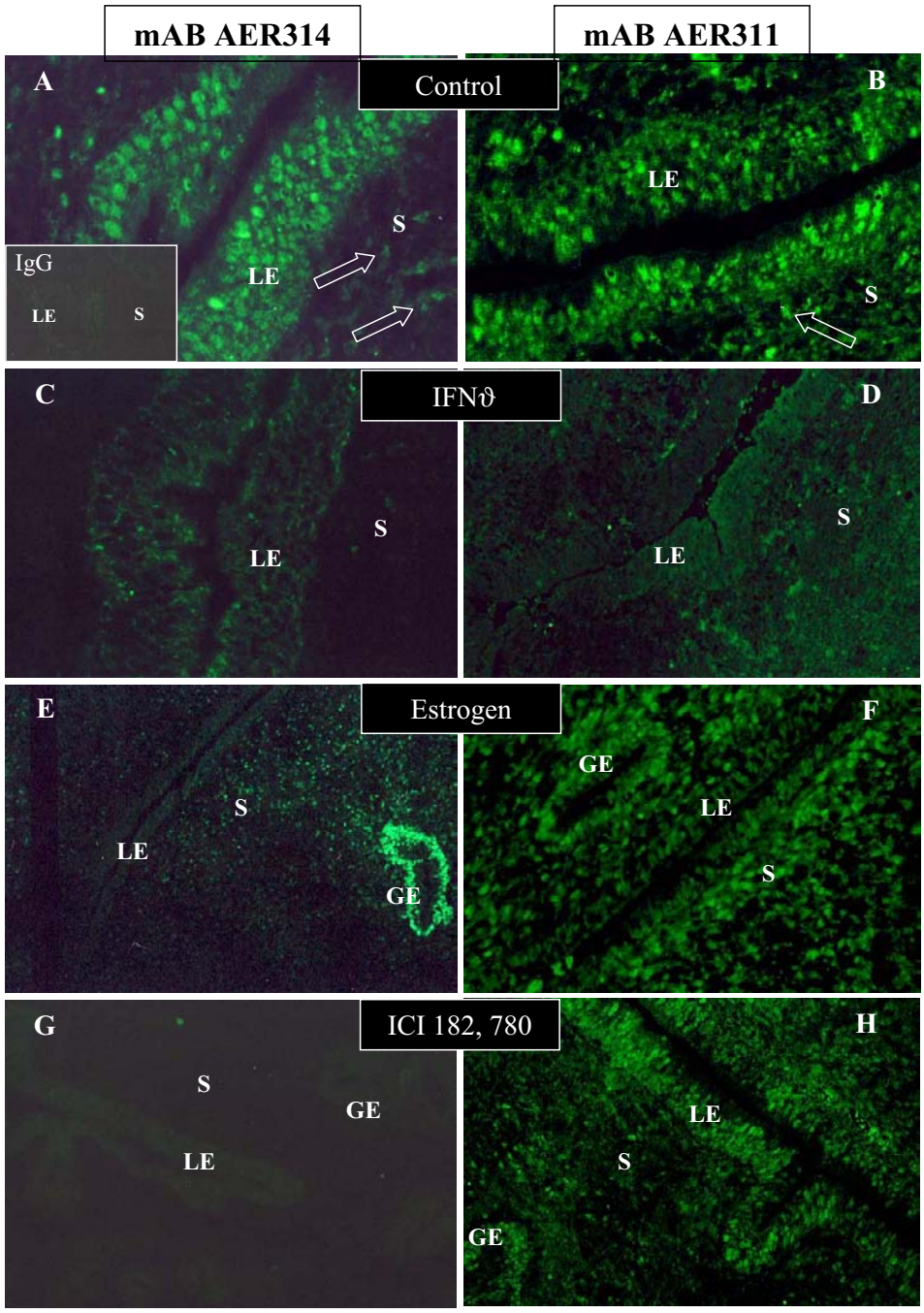

Figure 2
Immunofluorescent localization of the estrogen receptor using antibodies AER314 and AER311 in cyrostat cross-sections of endometrium obtained on day 16 of the estrous cycle after uterine infusion with a BSA-saline control solution (**A-B**), interferon-tau (**C-D**), ICI 182, 780 (**E-F**) or estrogen (**G-H**). A tissue section treated with mouse IgG in place of primary antibody is shown inset in A. Specific nuclear reactivity was present in some cells of the stroma (arrows) in control animals. LE – luminal epithelium, S – stroma, GE – glandular epithelium. Magnification for A-C was 400X, D,F,H was 200X and E,G 100X.

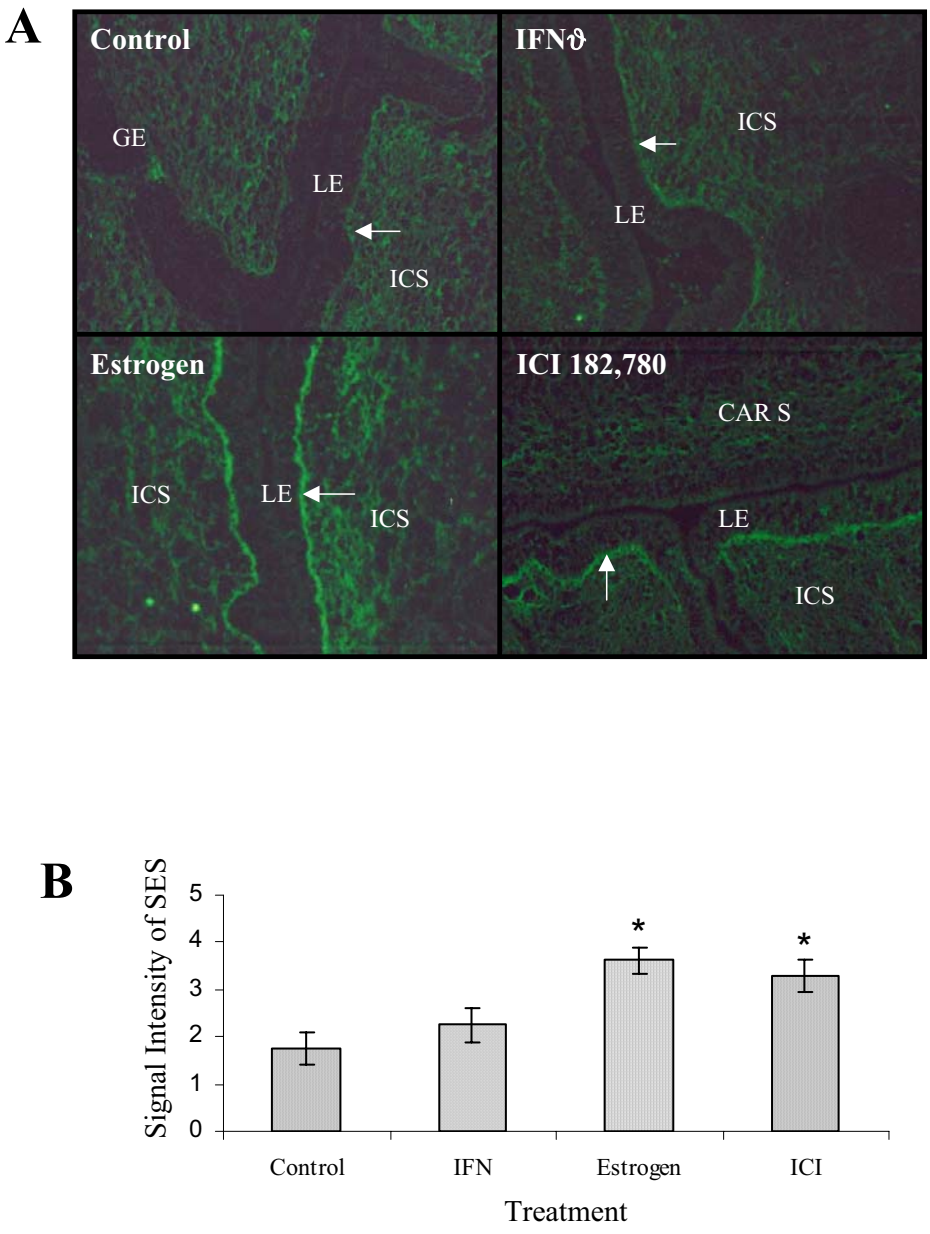

Figure 3
Immunofluorescent analysis of integrin $\alpha_v\beta_3$ in cryostat cross-sections of endometrium. (**A**) Immunoreactivity in sections from heifers treated with control solution, interferon-tau (IFN-τ), estrogen or ICI 182, 780. Diffuse staining was present in the dense stroma, low reactivity was observed in the glands (GE) and luminal epithelium (LE). Note the strong reactivity of intercaruncular subepithelial stromal cells (arrows) in sections from estrogen and ICI 182, 780 treated animals. CARS – caruncular stroma; ICS – intercaruncular stroma. Magnification was 100X. **B**) Least squares means ± standard error of signal intensity of integrin $\alpha_v\beta_3$ in the subepithelial stroma (SES) by treatment (3 animals × 4 replicate experiments per treatment). Intensity was scored on a six point scale (0-negative, 1-very weak, 2-weak but clearly positive, 3-intermediate, 4-high, and 5-very high) and the data were subjected to ANOVA and the Tukey-Kramer multiple comparison of means to determine effects of treatment in comparison to the control. Asterisks indicate means that are significantly different from the control ($P < 0.05$).

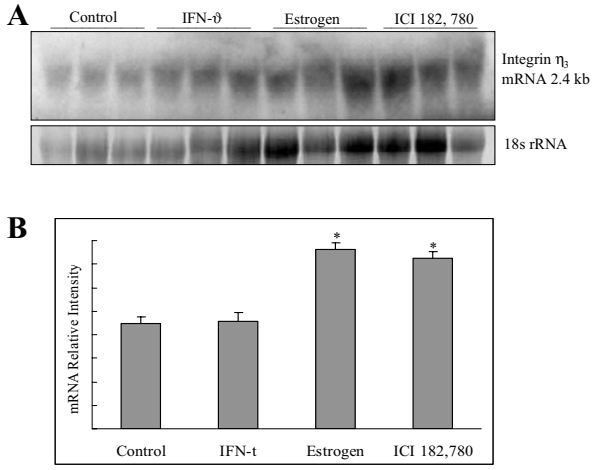

Figure 4
Effect of treatment onexpression of integrin subunit β_3 as detected by Northern blot analysis of intercaruncular endometrium. **A**) Three animals were used in each treatment group and received uterine infusions of either the saline control, interferon tau (IFN-τ), estrogen or ICI 182, 780. Thirty-five micrograms of total RNA from each animal were loaded per lane and biotin labeled riboprobes specific to integrin subunit β_3 were used for hybridization to the membrane. A single 2.4 kb transcript was detected. **B**) Least squares means ± standard error for integrin subunit β_3 mRNA band intensity by treatment (3 animals/treatment × 2 replicate blots) were calculated using 18S as a covariate. Asterisks indicate means that are significantly different from the Control and IFN-τ ($P < 0.05$).

AER314. Early postnatal calf uterine epithelium would be in a continued state of differentiation that is likely to require functional ERα [34], consistent with AER314 recognition of a functional form of the receptor. To summarize, the results indicate that the AER314 antibody recognizes a temporary change in the ERα, and may be used to detect altered ERα conformation as an early marker of the onset of luteolysis.

In sheep, IFN-τ blocks ERα transcription in the luminal epithelium by stimulating binding of IFN regulatory factor 1 to interferon stimulated response elements on the ERα gene [19,35,36]. This action of IFN-τ is thought to prevent ERα expression and the onset of luteolysis. Estrogen receptor mRNA and protein are present in the luminal epithelium on day 16 of bovine pregnancy, although levels may be reduced [20,37]. As was observed in pregnancy [4], in the current study ERα were not detected by the AER314 or AER311 antibody in the luminal epithelium of heifers infused with IFN-τ. Together, these results suggest that in the cow, like the ewe, IFN-τ may affect ERα transcription.

Integrin $\alpha_v\beta_3$ mRNA and protein expression were high in heifers receiving intrauterine infusions of estradiol. These results confirm what we have observed in cycling animals at estrus and suggest that at high levels following progesterone exposure, estrogen is a positive regulator of integrin $\alpha_v\beta_3$. Heifers infused with ICI 182, 870 also highly expressed integrin $\alpha_v\beta_3$ on day 16. As an antagonist, ICI 182, 780 would prevent the characteristic downregulation of integrin $\alpha_v\beta_3$ on cycle day 16 if endogenous follicular estrogen was the mediator of the event. As an agonist, it would upregulate endometrial ERα producing an estrus-like uterine environment, which also would be associated with high levels of integrin β_3 gene expression. Whether ICI 182, 780 acted as an antagonist or agonist with respect to the integrin β_3 gene thus cannot be distinguished. It is clear that AER314-reactive ERα expression in the luminal epithelium is not necessary for the cycle day 16 downregulation of the integrin, since both IFN-τ and ICI-182, 780 treated animals lacked this reactivity but only the former showed the integrin downregulation. The role of estrogen in this process remains to be determined. The promoter region for bovine integrin subunit β_3 has not been sequenced. While there are no estrogen response elements in the avian, murine and human integrin subunit β_3 promoters, both have AP-1 and SP-1 binding sites [11,38,39]. Many examples exist of ER interacting with the AP-1 and SP-1 transcription factors to confer estrogen responsiveness [40–42]. Alternatively, the transcription factor HOXA10 has been shown to be upregulated in cultured human endometrial cells in response to estrogen and progesterone and to regulate expression integrin subunit β_3 mRNA [43,44]. Integrin $\alpha_v\beta_3$ regulation in the subepithelial stroma is likely to involve an epithelial-stromal dialogue given that the cells of interest are in contact with the basal lamina. In other species it has been shown that the steroid responsiveness of epithelium and stroma is co-dependent in endometrium [45].

Uterine infusion with IFN-τ did not prevent downregulation of integrin $\alpha_v\beta_3$ in intercaruncular subepithelial stroma on day 16 of the estrous cycle but did influence ERα in the luminal epithelium. Clearly downregulation of integrin $\alpha_v\beta_3$ in the subepithelial stroma of cyclic animals is not a necessary consequence of ERα changes in the luminal epithelium. Pregnancy does prevent downregulation of integrin $\alpha_v\beta_3$ [10]. It is possible that the exposure period in this experiment was too brief to elicit expression of epithelial or stromal interferon stimulated genes required for maintenance of integrin $\alpha_v\beta_3$ in the subepithelial stroma. Endogenous IFN-τ begins to be produced by

trophoblast around day 12 [46], so pregnant animals would have two additional days to respond to the compound. After four days exposure to IFN-τ, the IFN-τ signaling molecules, signal transduction and activation of transcription 1 and 2, were observed in the subepithelial stroma of cyclic ewes [47]. On the other hand, other trophoblast factors or endometrial dialogue not induced by treatment with IFN-τ may be necessary for integrin $\alpha_v\beta_3$ regulation.

To summarize, on day 16 of the estrous cycle both estrogen and ICI 182, 780 induced expression of ERα in the endometrium and blocked the transient downregulation of integrin $\alpha_v\beta_3$ on cycle day 16. Intrauterine infusion of IFN-τ suppressed changes in ERα protein reactivity in the luminal epithelium but did not affect integrin $\alpha_v\beta_3$ expression, suggesting IFN-τ may not prevent integrin $\alpha_v\beta_3$ downregulation during early pregnancy. We conclude that on day 16 of the estrous cycle, estrogen effects on integrin $\alpha_v\beta_3$ are indirect and do not directly involve ERα in the luminal epithelium.

Acknowledgements

The authors are grateful to Drs. Ted Semple, Joe Arosh and the staff at NSAC, Westmorland Institute and HUB meat packers for assistance with the animals. We thank Dr. Nancy H. Ing for her advice regarding ICI 182, 780 dose and Dr. Michel A. Fortier for critical reading of the manuscript. The Natural Sciences and Engineering Research Council of Canada supported this research through a postgraduate scholarship to S. K. and an individual research grant to L. A. M.

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