Reproductive Biology and Endocrinology

BioMed Central

Review

Steroidal regulation of uterine resistance to bacterial infection in livestock

Gregory S Lewis*

Address: USDA, Agricultural Research Service, U.S. Sheep Experiment Station, Dubois, Idaho, USA

Email: Gregory S Lewis* - glewis@pw.ars.usda.gov

* Corresponding author

Published: 28 November 2003

Reproductive Biology and Endocrinology 2003, **1**:117

Received: 08 July 2003

Accepted: 28 November 2003

This article is available from: http://www.rbej.com/content/1/1/117

Abstract

Postpartum uterine infections reduce reproductive efficiency and have significant animal welfare and economic consequences. Postpartum uterine infections are classified as nonspecific, but *Arcanobacterium pyogenes* and *Escherichia coli* are usually associated with them in cattle and sheep. Pyometra is the most common type of uterine infection in dairy cattle, and it is detected almost exclusively in cows with active corpora lutea. Luteal progesterone typically down-regulates uterine immune functions and prevents the uterus from resisting infections. Progesterone also can down-regulate uterine eicosanoid synthesis. This seems to be a critical event in the onset of uterine infections, because eicosanoids can up-regulate immune cell functions in vitro. In addition, exogenous prostaglandin F2 alpha stimulates uterine secretion of prostaglandin F2 alpha and enhances immune functions in vivo. Thus, one may hypothesize that eicosanoids can override the negative effects of progesterone and that the up-regulatory effects of exogenous prostaglandin F2 alpha allow the uterus to resolve an infection, regardless of progesterone concentrations. Based on the results of studies to test that hypothesis, cows, sheep, and pigs in various physiological statuses are resistant to intrauterine infusions of *Arcanobacterium pyogenes* and *Escherichia coli*, unless progesterone concentrations are increased. In sheep and pigs, exogenous prostaglandin F2 alpha stimulates uterine production of prostaglandin F2 alpha and allows the uterus to resolve *Arcanobacterium pyogenes-Escherichia coli*-induced infections, even when progesterone is maintained at luteal phase concentrations before and after treatment. Prostaglandin F2 alpha is a proinflammatory molecule that stimulates the production of various proinflammatory cytokines, and it may enhance uterine production of leukotriene B_4. Proinflammatory cytokines and leukotriene B_4 enhance phagocytosis and lymphocyte functions. Even though there are clear associations among prostaglandin F2 alpha, leukotriene B4, proinflammatory cytokines, phagocytosis, and lymphocyte functions, the mechanism of action of exogenous prostaglandin F2 alpha in overriding the down-regulatory effects of progesterone and resolving uterine infections has not been elucidated. Defining this mechanism should yield new prevention and treatment strategies for uterine infections that do not rely on antibiotic and antimicrobial compounds.

Review

Background

Nonspecific uterine infections reduce the reproductive efficiency of livestock [1-3]. They are called nonspecific because the initial colonizing bacterium is not known, and the specific bacteria causing the signs of infection are

not known [2,4-6]. Even though numerous bacteria, in a variety of combinations, have been isolated from infected uteri, *Arcanobacterium pyogenes* and *Escherichia coli* are usually associated with uterine infections in cattle and sheep [3-6].

Estimates of the annual incidence of uterine infections in postpartum animals in herds and flocks range from 10 to 50% of the dairy cattle [1,2], 20 to 75% of the dairy buffaloes [7], and 5 to 10% of the dairy sheep [8]. Published estimates of the incidence of analogous uterine infections, as opposed to the mastitis, metritis, agalactica syndrome, in postpartum pigs are not available. However, swine herd managers and veterinarians often state that they are "common" (personal communications). Despite the published estimates for cattle, buffaloes, and sheep, the "true" incidence of uterine infections is not known for any livestock species because detection and diagnosis are often inaccurate and reporting is not mandatory.

In the United States, intramuscular (i.m.) injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have become the preferred treatment for pyometra in cattle [2]. Pyometra is the most common type of uterine infection in dairy cattle, and it is the one most often associated with reduced reproductive performance [2]. Pyometra typically develops just after the onset of luteal function during the postpartum period [2]. Apparently, bacteria that are normally found in livestock environments are introduced during or after calving, particularly when cows with dystocia are assisted and when retained fetal membranes are removed manually [2]. In cows that later develop uterine infections, the bacteria seem to reside in the uterus, without proliferating into an infection, until luteal progesterone down-regulates immune functions. Then the bacteria proliferate and create a pathological condition. The infections usually persist until luteolysis. The resultant reduction in progesterone concentrations seems to permit the uterine immune system to up-regulate and resolve the infection. Thus, exogenous $PGF_{2\alpha}$ is used to induce luteolysis and clear uterine infections, although the true mechanism of action of $PGF_{2\alpha}$ in eliminating uterine infections is not known. In fact, $PGF_{2\alpha}$ seems to have effects that are independent of its effects on corpus luteum function.

Even though exogenous $PGF_{2\alpha}$ has become the preferred treatment, intrauterine and systemic antibiotic treatments are common in the United States and in other countries [2,9]. General concerns about the relationship between antibiotic use in livestock and the potential for accelerating the evolution of antibiotic-resistant strains of bacteria have aimed our research at determining whether nonantibiotic, native compounds can be used to enhance host immunity and prevent or resolve uterine infections. Because of the pivotal role of progesterone in converting the uterus from resistant to susceptible to infections, understanding the mechanism of action of progesterone is essential for developing methods for enhancing the ability of the uterus to manage pathogenic bacteria. Thus, the purpose of this article is to briefly review the role of progesterone in making the uterus susceptible to infections and describe how eicosanoids may be used to override the immunosuppressive effects of progesterone.

Progesterone

Data from numerous studies that have been published over the last 50 years indicate that cattle, sheep, and pigs are resistant to uterine infections when progesterone concentrations are basal, and they are susceptible when progesterone concentrations are increased [10-15]. For example, spontaneous uterine infections in dairy cows do not usually develop until after formation of the first postpartum corpus luteum, although bacterial contamination can be sufficient to induce the onset of puerperal metritis very soon after calving when progesterone concentrations are basal [1,2,15]. Postpartum beef cows that received intrauterine infusions of *A. pyogenes* and *E. coli* when progesterone concentrations were basal did not develop uterine infections, whereas all cows developed uterine infections when the bacteria were infused after the onset of luteal function and progesterone concentrations had begun to increase [6]. The same was true for postpartum ewes (i.e., female sheep) with spontaneous onset of luteal function and for postpartum ewes that had been ovariectomized and treated i.m. with progesterone [16,17]. In addition, none of the ewes or gilts (i.e., nulliparous pigs) that received intrauterine infusions of *A. pyogenes* and *E. coli* during estrus developed uterine infections, but all of the ewes and gilts that received *A. pyogenes* and *E. coli* infusions during the luteal phase of the estrous cycle developed uterine infections [18-20].

Lymphocyte proliferation in vitro has been used as a general measure of immune responsiveness, even though neutrophils, which are much more difficult to evaluate, probably have a greater role in the initial response to bacteria that enter the uterus [21-25]. Unstimulated, concanavalin A (Con A)-stimulated (stimulates T-cells), and lipopolysaccharide (LPS)-stimulated (stimulates B-cells) proliferation were greater for lymphocytes collected from postpartum ewes that had been ovariectomized before the onset of luteal function than was proliferation of lymphocytes collected from ewes with spontaneous luteal function and increased progesterone [17]. (In contexts, such as in the previous sentence, greater, increased, decreased, etc. refers to an event with $P < 0.05$). Unstimulated and Con-A stimulated lymphocyte proliferation were greater for sesame oil-treated than for progesterone-treated postpartum ewes [17]. Unstimulated, Con A-stimulated, and LPS-stimulated proliferation were greater for

lymphocytes collected from estrous ewes than from luteal phase ewes, and unstimulated and Con A-stimulated lymphocyte proliferation were greater for estrous gilts than for luteal phase gilts [18,20]. Indeed, several authors have reported that progesterone suppresses lymphocyte proliferation [26-29], and this effect of progesterone was associated with the inability of the uterus to prevent the development of infections.

The previous examples clearly support the idea that progesterone converts the uterus from an organ that is resistant to one that is susceptible to infections. Data from the studies selected for the examples, as well as reports from other authors, also support the idea that, when progesterone concentrations are basal and bacterial contamination is not severe enough to overwhelm uterine defenses (i.e., puerperal metritis is not an issue), the uterus "defaults" to resistant to infections. We have referred to this as a "protected period," although we acknowledge that some threshold, albeit unknown, load of bacteria will overwhelm uterine defenses. Basal progesterone and basal estradiol concentrations are two characteristics of the protected period, and we assume that "basal" immune responses are usually able to prevent bacteria from proliferating into a pathological condition. We have used postpartum and seasonally anestrous ewes to determine whether the uterus is, in fact, protected.

In two studies, none of the autumn-lambing ewes that were ovariectomized on day 9 or day 14 postpartum, before spontaneous onset of luteal function and increases in progesterone concentrations, and treated i.m. with canola oil or safflower oil developed infections after intrauterine infusion of *A. pyogenes* and *E. coli* [16,17]. By contrast, all of the ewes in the two studies with spontaneous luteal function and all of the ewes treated i.m. with progesterone in canola oil or safflower oil developed infections after intrauterine infusion of *A. pyogenes* and *E. coli* [16,17]. For the ewes ovariectomized on day 14, $PGF_{2\alpha}$ concentrations in vena caval blood were greatest in ewes with the least vena caval progesterone, and $PGF_{2\alpha}$ was least in the ewes with the greatest progesterone [17]. Vena caval blood was collected through catheters that were positioned just cranial to the entry of uteroovarian blood [30]. Indeed, 6α-methyl-17α-hydroxyprogesterone acetate (a progestogen commonly used to control the estrous cycle) reduced uterine secretion of $PGF_{2\alpha}$ [31]. Ovariectomy increased and exogenous progesterone decreased Con A-stimulated lymphocyte proliferation, and ovariectomy increased LPS-stimulated lymphocyte proliferation [17]. The results were less clear for the ewes ovariectomized on day 9, but exogenous progesterone seemed to have some suppressive effects on lymphocyte proliferation [16]. Overall, suppressed $PGF_{2\alpha}$ secretion, suppressed lymphocyte proliferation in vitro, and increased progesterone were associated with the inability of the uterus to prevent infections. When progesterone concentrations were basal, the "default" immune status of the ewes was adequate to prevent uterine infections.

In another study, seasonally anestrous ewes were used to determine whether, in the long-term absence of ovarian progesterone, the uterus was resistant to infections [32]. The ewes in this study had not been detected in estrus for at least three months, had basal endogenous progesterone concentrations, and had no ovarian follicles large enough to be estrogen active. None of the control ewes developed infections after intrauterine infusion of *A. pyogenes* and *E. coli*, whereas all of the progesterone-treated ewes developed infections after intrauterine *A. pyogenes* and *E. coli* infusions [32]. Bacteria were infused into the uterus two days before commencement of progesterone injections to determine whether the uterus would eliminate the bacteria soon after they were introduced. The bacteria were apparently able to survive in the uterus until exogenous progesterone prevented the uterus from suppressing their proliferation. Results from these studies with postpartum and seasonally anestrous ewes support the hypothesis that, when progesterone and estradiol are basal, the uterus is resistant to infectious bacteria, although the uterus does not seem to eliminate bacteria as soon as they arrive. A direct effect of estradiol on the resistance or susceptibility of the uterus in cattle, sheep, and pigs to infections has not been established, but authors usually acknowledge the temporal associations between reductions in progesterone, increases in estradiol, and resistance to uterine infections [18,20]. Thus, because the role of estradiol has not been established and because progesterone unequivocally down-regulates uterine immune functions, progesterone seems to be the ovarian steroid that primarily determines whether the uterus can prevent bacteria from proliferating into an infection.

Progesterone and Eicosanoids

Eicosanoids, including prostaglandins and leukotrienes, belong to a family of compounds that are synthesized from arachidonic acid through the cyclooxygenase and lipoxygenase pathways [33,34]. The family is large; so only selected eicosanoids will be discussed.

Peripheral progesterone and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) concentrations were quantified in postpartum dairy cows to characterize the relationships among progesterone, PGFM, and onset of uterine infections [15,35,36]. 13,14-Dihydro-15-keto-$PGF_{2\alpha}$ is a metabolite of $PGF_{2\alpha}$ with a half-life of approximately 15 min, compared with approximately 1 min for $PGF_{2\alpha}$ [37,38]. During the postpartum period, jugular concentrations of PGFM are a close reflection of uterine secretion of $PGF_{2\alpha}$; however, that does not seem to be the case during the

estrous cycle, when the uterus produces considerably less $PGF_{2\alpha}$ [31,37,39]. The results of studies with postpartum dairy cows indicated that PGFM concentrations were less in cows that later developed uterine infections than they were in cows that did not develop uterine infections [15,36]. Only small increases in progesterone, presumably luteal, were necessary to initiate the onset of uterine infections [15], and PGFM concentrations increased at the onset of uterine infections [35]. The increase in PGFM most likely reflected a uterine inflammatory response to the proliferation of bacteria and release of endotoxin [40,41].

One interpretation of the results of the studies with dairy cows and sheep is that uterine $PGF_{2\alpha}$ production is related to the ability of the uterus to prevent or resolve infections. Moreover, one may speculate that progesterone-$PGF_{2\alpha}$ interactions modulate the ability of the uterus to prevent infections. Stated more directly, progesterone can down-regulate immune functions and prevent the uterus from resisting infections, whereas $PGF_{2\alpha}$, and probably other eicosanoids such as leukotriene B_4 (LTB_4), can up-regulate immune functions and override the effects of progesterone. In fact, in vitro experiments indicate that $PGF_{2\alpha}$, LTB_4, 5-hydroxyeicosatetraenoic acid, 15-hydroxyeicosatetraenoic acid, and lipoxin B_4 are chemoattractant to neutrophils [42]. Neutrophils are thought to be the initial defense against pathogens that enter the uterus, and suppressed neutrophil functions are thought to predispose the uterus to infections [22,23,25]. Prostaglandin E_2 can suppress a number of immune functions, including neutrophil functions, and predispose cows to uterine infections [24]. Progesterone-eicosanoid interactions are clearly broader than just $PGF_{2\alpha}$ interactions. However, because of the large number of compounds that can interact with progesterone and affect immune functions, our research has focused on progesterone-$PGF_{2\alpha}$ interactions.

Even though exogenous $PGF_{2\alpha}$ will induce luteolysis and allow the uterus to clear infections, authors have mentioned that exogenous $PGF_{2\alpha}$ helped resolve uterine infections in cows that did not have any evidence of luteal function [6]. Clinical veterinarians often speculate that exogenous $PGF_{2\alpha}$ stimulates uterine contractions that expel the bacteria (personal communications). But this ignores the issue that bacteria are likely to remain in the uterus and will be present when luteal function is reinitiated, and it ignores the fact that literature is not available to substantiate the idea that uterine contractions cleanse the uterus. Indeed, a study with mares indicates that uterine contractions may reduce the fluid associated with uterine infections, but uterine contractions do no seem to eliminate the bacteria [43]. However, one can reason, based on a good deal of literature, that exogenous $PGF_{2\alpha}$ has direct effects on uterine immune functions.

A key point, from a mechanism-of-action perspective, is that the direct effects of exogenous $PGF_{2\alpha}$ on uterine immune functions and the effects of $PGF_{2\alpha}$ on luteal function and progesterone concentrations are completely confounded. In other words, is exogenous $PGF_{2\alpha}$ effective because it stimulates immune functions, or is exogenous $PGF_{2\alpha}$ effective because it removes progesterone? We have conducted studies with sheep and pigs to address these questions.

Prostaglandin $F_{2\alpha}$ is not luteolytic in pigs until after approximately day 12 of the estrous cycle [44]. We used this information to determine whether $PGF_{2\alpha}$ had direct effects on uterine immune functions. Sows (i.e., parous pigs) were assigned to a 2 × 2 factorial array of treatments (n = 6 sows/group); intrauterine infusion of *A. pyogenes* and *E. coli* (i.e., bacteria vs. phosphate-buffered saline [PBS]) and $PGF_{2\alpha}$ (i.e., 10 mg of $PGF_{2\alpha}$ vs. saline) were the two main effects. Bacteria or PBS was infused on day 7 of the estrous cycle, and $PGF_{2\alpha}$ or saline was injected i.m. once on day 9 of the estrous cycle. Uteri were collected on day 11 of the same estrous cycle. Vena caval blood was collected throughout the experiment. Vena caval progesterone concentrations did not differ among groups and averaged 64 ng/mL during the study, which indicates that $PGF_{2\alpha}$ did not affect luteal function. Vena caval estradiol-17β concentrations did not differ among groups and averaged 1 ng/mL, which is a typical luteal phase concentration. The $PGF_{2\alpha}$ injection increased vena caval $PGF_{2\alpha}$ concentrations, and this is consistent with the results of studies with sheep in which exogenous $PGF_{2\alpha}$ stimulated uterine secretion of $PGF_{2\alpha}$ [37]. Exogenous $PGF_{2\alpha}$ also enhanced unstimulated and LPS-stimulated lymphocyte proliferation, and it increased vena caval PGE_2 concentrations. None of the PBS-treated sows developed uterine infections, but all of the bacteria-treated sows developed uterine infections. Based on the volume of sediment, which is composed of leucocytes and bacteria, in the uterine flushings collected on day 11 and the ability to culture of *A. pyogenes* and *E. coli* from the flushings, the $PGF_{2\alpha}$-treated sows were resolving the uterine infections (sediment was 70% of total flushing volume for bacteria-saline, 30% for bacteria-$PGF_{2\alpha}$, and <5% for PBS-saline and PBS-$PGF_{2\alpha}$ sows). Based on these results, $PGF_{2\alpha}$ treatment allowed the uterus to begin resolving the infections, even though progesterone remained at luteal phase concentrations and estradiol concentrations were basal.

Because $PGF_{2\alpha}$ is luteolytic in sheep after approximately day 4 of the estrous cycle, we used ovariectomized, progesterone-treated ewes to test the hypothesis that $PGF_{2\alpha}$ has effects on uterine immunity that are independent of progesterone concentrations. Ewes were assigned to a 2 × 2 × 2 factorial array of treatments (n = 8 ewes/group); ovariectomy (i.e., ovariectomy vs. sham procedure), proges-

terone (5 mg of progesterone at 12-hour intervals vs. sesame oil diluent at the same times), and $PGF_{2\alpha}$ (15 mg of $PGF_{2\alpha}$ vs. saline) were main effects. On the day of estrus (i.e., day 0), ewes were either ovariectomized or a sham procedure was performed. Either progesterone or sesame oil was injected i.m. from day 0 through day 11. Catheters were positioned in the vena cava on day 5, and vena caval blood was collected from then until the end of the experiment. On day 6, all ewes received intrauterine infusions of *A. pyogenes* and *E. coli*. Prostaglandin $F_{2\alpha}$ or saline was injected i.m. on day 9, and uteri were collected on day 12. Vena caval progesterone concentrations in all eight groups of ewes behaved as anticipated. For example, progesterone concentrations in sham ovariectomy-sesame oil-saline ewes were indicative of a "normal" luteal phase. Prostaglandin $F_{2\alpha}$ induced luteolysis and reduced progesterone concentrations to basal values in ewes that did not receive exogenous progesterone. Ovariectomy reduced progesterone to basal concentrations, and exogenous progesterone maintained or increased (i.e., in sham ovariectomized, progesterone-treated ewes) progesterone concentrations. As expected, exogenous $PGF_{2\alpha}$ increased vena caval $PGF_{2\alpha}$ concentrations. All of the sham-ovariectomy ewes developed uterine infections. However, based on sediment volume and ability to culture *A. pyogenes* and *E. coli* from the uterine flushings, the sham-oil-saline ewes and the sham-oil-$PGF_{2\alpha}$ ewes were resolving (sediment volume approximately 8%) the infections by day 12. The sham-progesterone-saline ewes had severe uterine infections on day 12 (sediment volume of 28%, which is considerably greater than usually seen for sheep with uterine infections), but the sham-progesterone-$PGF_{2\alpha}$ ewes were resolving the infections on day 12 (sediment volume of approximately 15% vs. the 28%). The ovariectomy-oil-saline ewes did not have uterine infections on day 12 (sediment volume of 5%), but the ovariectomy-progesterone-saline ewes had typical infections (sediment volume of approximately 16%) on day 12. The ovariectomy-oil-$PGF_{2\alpha}$ ewes did not have uterine infections on day 12 (sediment volume of approximately 2%), and the ovariectomy-progesterone-$PGF_{2\alpha}$ ewes had nearly resolved the infections (sediment volume of approximately 4%) by day 12. Progesterone reduced unstimulated lymphocyte proliferation, whereas exogenous $PGF_{2\alpha}$ increased unstimulated, Con A-stimulated, and LPS-stimulated lymphocyte proliferation. The results of this experiment indicate that exogenous $PGF_{2\alpha}$ enhances the ability of the uterus to resolve infections, regardless of progesterone concentrations. The ewes in this experiment and the sows in the other experiment discussed received one injection of $PGF_{2\alpha}$, and this was enough to begin resolving or to nearly resolve the uterine infections. We have speculated that a second injection of $PGF_{2\alpha}$ 12 to 24 hours after the first would resolve all of the induced infections, but that possibility has not yet been evaluated experimentally.

Based on current literature, the ability of the uterus to resist or to resolve uterine infections seems to be related to the ability of the uterus to secrete $PGF_{2\alpha}$. Exogenous $PGF_{2\alpha}$ enables the uterus to resolve uterine infections, even when circulating progesterone concentrations are maintained at luteal phase concentrations or greater. Thus, $PGF_{2\alpha}$ can up-regulate immune functions and override the down-regulatory effects of progesterone.

Mechanisms

Progesterone and eicosanoids have a variety of independent effects on immune cell functions. The negative effects of progesterone on immune functions have been reviewed extensively, especially the effects of progesterone on the synthesis of immunosuppressants and blocking factors, and will not be reviewed in this article [26-28]. Instead, this section will focus on relationships between progesterone and eicosanoids, because manipulating this relationship at the cellular or molecular level seems to have great potential for preventing or resolving uterine infections in livestock-production settings.

During estrus, when progesterone concentrations are decreased and estradiol concentrations are increased, uterine production of $PGF_{2\alpha}$ is increased, endometrial LTB_4 production is increased, and the uterus is normally able to prevent infections from developing [45-48]. Uterine $PGF_{2\alpha}$ and LTB_4 production decrease to basal within a few days after estrus, when progesterone concentrations begin to increase and the uterus again becomes susceptible to infections [46,47,49]. Prostaglandin $F_{2\alpha}$ enhanced neutrophil chemotaxis and the ability of neutrophils to ingest bacteria, and LTB_4 enhanced chemotaxis, random migration, and antibody-independent cell-mediated cytotoxicity [42]. Prostaglandin $F_{2\alpha}$, which is considered a proinflammatory molecule, may stimulate the production of proinflammatory cytokines that enhance phagocytosis and lymphocyte functions [19,50]. Leukotriene B_4 may promote uterine involution and reduce the risk of uterine infections in cows [49]. Exogenous $PGF_{2\alpha}$ increases uterine secretion of $PGF_{2\alpha}$ and luteal production of LTB_4 [37,51]. Because definitive data are not available, one can only speculate that $PGF_{2\alpha}$ enhances uterine LTB_4 production. However, nordihydroguaiaretic acid, which inhibits lipoxygenase activity and LTB_4 production, prolonged the luteal phase in cattle and sheep, and the uterus seems to have mediated the effect [52,53]. The increase in uterine $PGF_{2\alpha}$ production after exogenous $PGF_{2\alpha}$ probably increases phospholipase A_2 (PLA_2) and cyclooxygenase 2 activities [54-56]. Increased PLA_2 would increase the amount of free arachidonic acid that could be used to produce cyclooxygenase (e.g., $PGF_{2\alpha}$ and PGE_2) and lipoxygenase (e.g., LTB_4) products. In addition, tumor-necrosis factor α (TNFα), which mediates inflammatory and cytotoxic responses [40], stimulates endometrial $PGF_{2\alpha}$ pro-

duction, and PLA_2 seems to mediate this effect [57,58]. Therefore, enhancing uterine secretion of $PGF_{2\alpha}$ and LTB_4 should up-regulate immune functions and enable the uterus to prevent or resolve infections. Exogenous fenprostalene (i.e., a long-acting $PGF_{2\alpha}$ analogue), which should increase uterine $PGF_{2\alpha}$ secretion, injected subcutaneously once sometime between days 7 and 10 postpartum reduced the incidence of endometritis in cows with dystocia and/or retained fetal membranes [36]. A single subcutaneous fenprostalene injection on the day endometritis was detected (i.e., sometime between days 14 and 28 postpartum) reduced the interval from parturition to conception in dairy cows [36]. However, studies to define the mechanisms of action of $PGF_{2\alpha}$ in livestock have not been reported. This seems to be a fruitful area of study with considerable benefit to the livestock industries.

If the assertion that enhancing uterine secretion of $PGF_{2\alpha}$ and LTB_4 will enable the uterus to resolve infections is correct, it means that $PGF_{2\alpha}$ and LTB_4 must override the inhibitory effects of progesterone. In addition to its ability to stimulate the production of immunosuppressants and blocking factors, progesterone can stimulate prostaglandin E synthase activity and decrease the activity of a number of proinflammatory molecules [26-28,59,60]. For example, progesterone suppresses the production of IL-8, which promotes chemotaxis, superoxide release, and granule release from phagocytic cells, in reproductive tissues [61-65]. It may also suppress the production of IL-6, which promotes B-cell differentiation and production of acute-phase proteins [66]. In addition, progesterone inhibits the production of IL-12, which induces interferon-γ production and enhances natural killer-cell cytotoxicity, and PLA_2, presumably via increased free arachidonic acid, seems to mediate the effects of IL-12 [29]. Even though progesterone has a variety of inhibitory effects, they are not always consistent among reports, much of the research has only been conducted with in vitro systems, and very little of the research has been focused on the relationship between production of various proinflammatory molecules and the ability of the uterus in livestock to resistant or resolve infections.

Conclusions

Progesterone seems to be the primary ovarian steroid that governs the ability of the uterus to resist infections. In livestock, progesterone typically down-regulates immune functions and makes the uterus susceptible to infections. Exogenous $PGF_{2\alpha}$ increases uterine secretion of $PGF_{2\alpha}$, and probably LTB_4, and these two eicosanoids are associated with enhanced uterine immune responses and resistance to infections. Even though progesterone and eicosanoids affect a variety of proinflammatory molecules that can alter uterine immune responses, the role of these molecules in determining whether the uterus in livestock is resistant or susceptible to infections has not been elucidated. Indeed, determining how uterine $PGF_{2\alpha}$ is able to stimulate the uterus to resolve infections, even when progesterone concentrations are increased, should be important to scientists and clinicians working to understand the underlying causes of uterine infections. Information from this line of research should yield important new prevention and treatment strategies for uterine infections that do not rely on antibiotic and antimicrobial compounds.

List of abbreviations

Concanavalin A, Con A; Interleukin, IL; Intramuscular, i.m.; Leukotriene B_4, LTB_4; Lipopolysaccharides, LPS; Phosphate-buffered saline, PBS; Phospholipase A_2, PLA_2; Prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$; 13,14-dihydro-15-keto-$PGF_{2\alpha}$, PGFM; Tumor-necrosis factor α, TNFα;

Acknowledgements

The author is grateful to the contributions from his trainees and collaborators, particularly RP Del Vecchio, I Matamoros, AA Ramadan, RC Seals, S. Wang, and MC Wulster-Radcliffe.

References

1. Arthur GH, Noakes DE, Pearson H: *Veterinary Reproduction and Obstetrics* 6th edition. *Philadelphia: Baillière Tindall*; 1989.
2. Lewis GS: **Uterine health and disorders.** *J Dairy Sci* 1997, **80:**984-994.
3. Dhaliwal GS, Murray RD, Woldehiwet Z: **Some aspects of immunology of the bovine uterus related to treatments for endometritis.** *Anim Reprod Sci* 2001, **67:**135-152.
4. Griffin JFT, Hartigan PJ, Nunn WR: **Non-specific uterine infection and bovine fertility. I. Infection patterns and endometritis during the first seven weeks post-partum.** *Theriogenology* 1974, **1:**91-106.
5. Griffin JFT, Hartigan PJ, Nunn WR: **Non-specific uterine infection and bovine fertility. II. Infection patterns and endometritis before and after service.** *Theriogenology* 1974, **1:**107-114.
6. Del Vecchio RP, Matsas DJ, Fortín S, Sponenberg DP, Lewis GS: **Spontaneous uterine infections are associated with elevated prostaglandin $F_{2\alpha}$ metabolite concentrations in postpartum dairy cows.** *Theriogenology* 1994, **41:**413-421.
7. Usmani RH, Ahmad N, Shafiq P, Mirza MA: **Effect of subclinical uterine infection on cervical and uterine involution, estrous activity and fertility in postpartum buffaloes.** *Theriogenology* 2001, **55:**563-571.
8. Tzora A, Leontides LS, Amiridis GS, Manos G, Fthenakis GC: **Bacteriological and epidemiological findings during examination of the uterine content of ewes with retention of fetal membranes.** *Theriogenology* 2002, **57:**1809-17.
9. Chenault JR, McAllister JF, Chester ST, Dame KJ, Kausche FM: **Efficacy of ceftiofur hydrochloride administered parenterally for five consecutive days for treatment of acute postpartum metritis in dairy cows.** *34th Annual Convention Proceedings, Am Assoc Bovine Practitioners* 2001, **34:**137-138.
10. Black WG, Ulberg LC, Kidder HE, Simon J, McNutt SH, Casida LE: **Inflammatory response of the bovine endometrium.** *Am J Vet Res* 1953, **14:**179-183.
11. Rowson LEA, Lamming GE, Fry RM: **The relationship between ovarian hormones and uterine infection.** *Vet Rec* 1953, **65:**335.
12. Hawk HW, Turner GD, Sykes JF: **Variation in the inflammatory response and bactericidal activity of the sheep uterus during the estrous cycle.** *Am J Vet Res* 1961, **22:**689-692.
13. Hawk HW, Brinsfield TH, Turner GD, Whitmore GW, Norcross MA: **Effect of ovarian status on induced acute inflammatory responses in cattle uteri.** *Am J Vet Res* 1964, **25:**362-366.
14. Lander Chacin MF, Hansen PJ, Drost M: **Effects of stage of the estrous cycle and steroid treatment on uterine immu-**

noglobulin content and polymorphonuclear leukocytes in cattle. *Theriogenology* 1990, **34:**1169-1184.
15. Seals RC, Matamoros I, Lewis GS: **Relationship between postpartum changes in 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ concentrations in Holstein cows and their susceptibility to endometritis.** *J Anim Sci* 2002, **80:**1068-1073.
16. Seals RC, Wulster-Radcliffe MC, Lewis GS: **Modulation of the uterine response to infectious bacteria in postpartum ewes.** *Am J Reprod Immunol* 2002, **47:**57-63.
17. Lewis GS: **Role of ovarian progesterone and potential role of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 in modulating the uterine response to infectious bacteria in postpartum ewes.** *J Anim Sci* 2003, **81:**285-293.
18. Ramadan AA, Johnson GL III, Lewis GS: **Regulation of uterine immune function during the estrous cycle and in response to infectious bacteria in sheep.** *J Anim Sci* 1997, **75:**1621-1632.
19. Seals RC, Wulster-Radcliffe MC, Lewis GS: **Uterine response to infectious bacteria in estrous cyclic ewes.** *Am J Reprod Immunol* 2003, **49:**269-278.
20. Wulster-Radcliffe MC, Seals RC, Lewis GS: **Progesterone increases susceptibility of gilts to uterine infections after intrauterine inoculation with infectious bacteria.** *J Anim Sci* 2003, **81:**1242-1252.
21. Saad AM, Concha C, Åström G: **Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition.** *J Vet Med Ser* 1989, **36:**337-345.
22. Hussain AM: **Bovine uterine defense mechanisms: a review.** *J Vet Med Ser* 1989, **36:**641-651.
23. Hussain AM, Daniel RCW: **Bovine endometritis: current and future alternative therapy.** *J Vet Med Ser* 1991, **38:**641-651.
24. Slama H, Vaillancourt D, Goff AK: **Pathophysiology of the puerperal period: relationship between prostaglandin E_2 (PGE_2) and uterine involution in the cow.** *Theriogenology* 1991, **36:**1071-1090.
25. Cai TQ, Weston PG, Lund LA, Brodie B, McKenna DJ, Wagner WC: **Association between neutrophil functions and periparturient disorders in cows.** *Am J Vet Res* 1994, **55:**934-943.
26. Segerson EC, Gunsett FC: **Interference with the cytolytic activity of interleukin-2-treated lymphocytes by bovine uterine luminal protein.** *Biol Reprod* 1993, **48:**1036-1041.
27. Hansen PJ: **Regulation of uterine immune function by progesterone – lessons from the sheep.** *J Reprod Immunol* 1998, **40:**63-79.
28. Szekeres-Bartho J, Barakonyi A, Par G, Polgar B, Palkovics T, Szereday L: **Progesterone as an immunomodulatory molecule.** *Int Immunopharmacol* 2001, **1:**1037-1048.
29. Par G, Geli J, Kozma N, Varga P, Szekeres-Bartho J: **Progesterone regulates IL12 expression in pregnancy lymphocytes by inhibiting phospholipase A_2.** *Am J Reprod Immunol* 2003, **49:**1-5.
30. Benoit AM, Dailey RA: **Catheterization of the caudal vena cava via lateral saphenous vein in the ewe, cow, and gilt: an alternative to uteroovarian and medial coccygeal vein catheters.** *J Anim Sci* 1991, **69:**2971-2979.
31. Fortín S, Sayre BL, Lewis GS: **Does exogenous progestogen alter the relationships among $PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, progesterone, and estrogens in ovarian-intact ewes around the time of luteolysis?** *Prostaglandins* 1994, **47:**171-187.
32. Mink LE, Wulster-Radcliffe MC, Lewis GS: **Ability of the uterus in anestrous ewes to resist infection.** In *Proceedings of the Western Section American Society of Animal Science* 2002, **53:**3-6.
33. Pace-Asciak C, Granström E: *Prostaglandins and Related Substances New York: Elsevier*; 1983.
34. Müller-Peddinghaus R, Kast R: **Leukotriene synthesis (flap) inhibition: biochemistry and pharmacology of bay × 105.** In *Eicosanoids From Biotechnology to Therapuetic Applications* Edited by: *Folco GC, Samuelsson B, Maclouf J, Velo GP. New York: Plenum Press*; 1996:18-204.
35. Del Vecchio RP, Matsas DJ, Inzana TJ, Sponenberg DP, Lewis GS: **Effect of intrauterine bacterial infusions and subsequent endometritis on prostaglandin $F_{2\alpha}$ metabolite concentrations in postpartum beef cows.** *J Anim Sci* 1992, **70:**3158-3162.
36. Nakao T, Gamal A, Osawa T, Nakada K, Moriyoshi M, Kawata K: **Postpartum plasma PGF metabolite profile in cows with dystocia and/or retained placenta, and effect of fenprostalene on uterine involution and reproductive performance.** *J Vet Med Sci* 1997, **59:**791-794.
37. Wade DE, Lewis GS: **Exogenous prostaglandin $F_{2\alpha}$ stimulates uteroovarian release of prostaglandin $F_{2\alpha}$ in sheep: A possible component of the luteolytic mechanism of action of prostaglandin $F_{2\alpha}$.** *Domestic Anim Endocrinol* 1996, **13:**383-395.
38. Williams WF, Lewis GS, Thatcher WW, Underwood CS: **Plasma 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) in pregnant and nonpregnant heifers prior to and during surgery and following intrauterine injection of $PGF_{2\alpha}$.** *Prostaglandins* 1983, **25:**891-899.
39. Guilbault LA, Thatcher WW, Drost M, Hopkins SM: **Source of F series prostaglandins during the early postpartum period in cattle.** *Biol Reprod* 1984, **31:**879-887.
40. Roitt I, Brostoff J, Male D: *Immunology* 5th edition. *London: Mosby*; 1998.
41. Leung ST, Cheng Z, Sheldrick EL, Derecka K, Derecka K, Flint AP, Wathes DC: **The effects of lipopolysaccharide and interleukins-1alpha, -2 and -6 on oxytocin receptor expression and prostaglandin production in bovine endometrium.** *J Endocrinol* 2001, **168:**497-508.
42. Hoedemaker M, Lund LA, Wagner WC: **Influence of arachidonic acid metabolites and steroids on function of bovine polymorphonuclear neutrophils.** *Am J Vet Res* 1992, **53:**1534-1539.
43. Nikolakopoulos E, Watson ED: **Uterine contractility is necessary for the clearance of intrauterine fluid but not bacteria after bacterial infusion in the mare.** *Theriogenology* 1999, **52:**413-423.
44. Guthrie HD, Polge C: **Luteal function and oestrus in gilts treated with a synthetic analogue of prostaglandin $F_{2\alpha}$ (ICI 79,939) at various times during the oestrous cycle.** *J Reprod Fertil* 1976, **48:**423-425.
45. Ottobre JS, Lewis GS, Thayne WV, Inskeep EK: **Mechanism by which progesterone shortens the estrous cycle of the ewe.** *Biol Reprod* 1980, **23:**1046-1053.
46. Kindahl H, Basu S, Fredriksson G, Goff A, Kunavongkrit A, Edqvist LE: **Levels of prostaglandins $F_{2\alpha}$ metabolites in blood and urine during early pregnancy.** *Anim Reprod Sci* 1984, **7:**133-148.
47. Zarco L, Stabenfeldt GH, Quirke JF, Kindahl H, Bradford GE: **Release of prostaglandin $F_{2\alpha}$ and the timing of events associated with luteolysis in ewes with oestrous cycles of different lengths.** *J Reprod Fertil* 1988, **83:**517-526.
48. Vagnoni KE, Abbruzzese SB, Christiansen ND, Holyoak GR: **The influence of the phase of the estrous cycle on sheep endometrial tissue response to lipopolysaccharide.** *J Anim Sci* 2001, **79:**463-469.
49. Slama H, Vaillancourt D, Goff AK: **Leukotriene B_4 in cows with normal calving, and in cows with retained fetal membranes and/or uterine subinvolution.** *Can J Vet Res* 1993, **57:**293-299.
50. Kelly RW, King AE, Critchley HO: **Cytokine control in human endometrium.** *Reproduction* 2001, **121:**3-19.
51. Steadman LE, Murdoch WJ: **Production of leukotriene B_4 by luteal tissues of sheep treated with prostaglandin $F_{2\alpha}$.** *Prostaglandins* 1988, **36:**741-745.
52. Milvae RA, Alila HW, Hansel W: **Involvement of lipoxygenase products of arachidonic acid metabolism in bovine luteal function.** *Biol Reprod* 1986, **35:**1210-1215.
53. Cooke RG, Ahmad N: **Delayed luteolysis after intra-uterine infusions of nordihydroguaiaretic acid in the ewe.** *Anim Reprod Sci* 1998, **52:**113-121.
54. Binelli M, Guzeloglu A, Badinga L, Arnold DR, Sirois J, Hansen TR, Thatcher WW: **Interferon-τ modulates phorbol ester-induced production of prostaglandin and expression of cyclooxygenase-2 and phospholipase-A(2) from bovine endometrial cells.** *Biol Reprod* 2000, **63:**417-424.
55. Diaz FJ, Anderson LE, Wu YL, Rabot A, Tsai SJ, Wiltbank MC: **Regulation of progesterone and prostaglandin $F_{2\alpha}$ production in the CL.** *Mol Cell Endocrinol* 2002, **191:**65-80.
56. Narayansingh RM, Senchyna M, Carlson JC: **Treatment with prostaglandin $F_{2\alpha}$ increases expression of prostaglandin synthase-2 in the rat corpus luteum.** *Prostaglandins Other Lipid Mediat* 2002, **70:**145-160.
57. Miyamoto Y, Skarzynski DJ, Okuda K: **Is tumor necrosis factor alpha a trigger for the initiation of endometrial prostaglandin $F_{2\alpha}$ release at luteolysis in cattle?** *Biol Reprod* 2000, **62:**1109-1115.
58. Skarzynski DJ, Miyamoto Y, Okuda K: **Production of prostaglandin $F_{2\alpha}$ by cultured bovine endometrial cells in response to tumor necrosis factor α: cell type specificity and intracellular mechanisms.** *Biol Reprod* 2000, **62:**1116-1120.

59. Hunt JS, Miller L, Roby KF, Huang J, Platt JS, DeBrot BL: **Female steroid hormones regulate production of pro-inflammatory molecules in uterine leukocytes.** *J Reprod Immunol* 1997, **35:**87-99.
60. Arosh JA, Parent J, Chapdelaine P, Sirois J, Fortier MA: **Expression of cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle.** *Biol Reprod* 2002, **67:**161-169.
61. Ito A, Imada K, Sato T, Kubo T, Matsushima K, Mori Y: **Suppression of interleukin 8 production by progesterone in rabbit uterine cervix.** *Biochem J* 1994, **301:**183-186.
62. Loudon JA, Elliott CL, Hills F, Bennett PR: **Progesterone represses interleukin-8 and cyclo-oxygenase-2 in human lower segment fibroblast cells and amnion epithelial cells.** *Biol Reprod* 2003, **69:**331-337.
63. Kelly RW, Illingworth P, Baldie G, Leask R, Brouwer S, Calder AA: **Progesterone control of interleukin-8 production in endometrium and chorio-decidual cells underlines the role of the neutrophil in menstruation and parturition.** *Hum Reprod* 1994, **9:**253-258.
64. Mitchell SE, Robinson JJ, King ME, McKelvey WA, Williams LM: **Interleukin 8 in the cervix of non-pregnant ewes.** *Reproduction* 2002, **124:**409-416.
65. Mitchell GB, Albright BN, Caswell JL: **Effect of interleukin-8 and granulocyte colony-stimulating factor on priming and activation of bovine neutrophils.** *Infect Immun* 2003, **71:**1643-1649.
66. Montes MJ, Tortosa CG, Borja C, Abadia AC, Gonzalez-Gomez F, Ruiz C, Olivares EG: **Constitutive secretion of interleukin-6 by human decidual stromal cells in culture. Regulatory effect of progesterone.** *Am J Reprod Immunol* 1995, **34:**188-194.