Is interleukin-1α a luteotrophic or luteolytic agent in cattle?

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Abstract

Cytokines are thought to regulate prostaglandin (PG) secretion in the bovine endometrium. However, there is no consensus about the role of interleukin-1α (IL1A) on PG secretion. The objective of this study was to examine the influence of IL1A on basal and interferon-τ (IFNT)-regulated PG in vitro secretion, as well its effects on PG secretion, progesterone (P₄) output, and corpus luteum (CL) in vivo lifespan. Explants of bovine endometrium (days 16–17 of the estrous cycle or early pregnancy) were stimulated with IL1A (10 ng/ml), IFNT (30 ng/ml), or IL1A combined with IFN. IL1A alone strongly stimulated luteotropic PGF₂α secretion by endometrial tissues of both pregnant and nonpregnant cows. IL1A also stimulated luteotropic PGF₂α output in the late luteal phase. IFNT augmented the stimulatory effect of IL1A on PGE₂ secretion. In an in vivo experiment, saline or IL1A at different doses (0.001–10 μg/per animal) was applied to the uterine lumen on day 16 of the cycle. Only the highest dose of IL1A caused a temporal increase in PGF₂α secretion, while it had no effect on P₄ secretion or CL lifespan. Application of 0.1 and 1 μg IL1A stimulated P₄ and PGE₂ output and prolonged the CL lifespan. Although IL1A may stimulate in vitro luteotropic PGF₂α secretion during the estrous cycle, it only acts as a luteotropic factor in vivo. IL1A increased luteotropic PGE₂ and P₄ output, inhibiting spontaneous luteolysis. These luteotropic effects may result in appropriate luteal development and function in cows during the estrous cycle and early pregnancy.

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Introduction

The cyclic nature of the bovine estrous cycle depends on endometrial prostaglandin (PG) production and its action on the corpus luteum (CL; McCracken et al. 1999, Skarzynski et al. 2008). PGs are synthesized from arachidonic acid in several organs including the female reproductive tract, i.e. in the uterus and ovary during the estrous cycle and pregnancy (Okuda et al. 2002, Arosh et al. 2004a, 2004b, Weems et al. 2006). Uterine and ovarian PGs are considered important factors for the regulation of reproductive events such as ovulation, luteolysis, embryo implantation, and maintenance of pregnancy (Weems et al. 2006). Generally, PGE₂ exerts luteotropic and luteoprotective actions to lengthen the lifespan of CL and sustain progesterone (P₄) production (Weems et al. 1997, Kotwica et al. 2003). PGF₂α is the main luteolytic agent in ruminants (McCracken et al. 1999, Skarzynski et al. 2008).

The relative proportion of both PGE₂ and PGF₂α seems to be more important than their absolute values for establishment and maintenance of pregnancy (Weems et al. 1997, Arosh et al. 2004a, 2004b). Several immunological factors, including cytokines such as tumor necrosis factor-α (TNF), are thought to participate in luteolysis and to stimulate luteolytic PGF₂α production and output from the bovine uterus and CL (Miyamoto et al. 2000, Skarzynski et al. 2000, 2007, Pate & Keyes 2001, Korzekwa et al. 2008). In ruminants, another cytokine, interferon-τ (IFNT), is considered to be responsible for the blockade of the luteolytic signal and maintenance of CL function in early pregnancy (Thatcher et al. 1986, Spencer et al. 2007a, 2007b). IFNT modulates PG secretion by inhibiting the expression of oxytocin (OXT) receptor. IFNT also increases the expression of PGH synthase-2 (PTGS2; COX2), which leads to a shift in the PG production profile from PGF₂α toward PGE₂ (Spencer et al. 1996, Bazer et al. 1997). Cytokines including TNF and interleukin (IL) have been proposed to act as mediators/modulators of IFNT actions during maternal recognition of pregnancy (Leung et al. 2000, Parent et al. 2002, 2003, Okuda et al. 2004, Lea & Sandra 2007).

IL1A belongs to the IL1 superfamily and is known as a pro-inflammatory factor that is synthesized mainly by immune cells such as macrophages, monocytes, and dendritic cells. This pleiotropic cytokine regulates the inflammatory process, immune response, and hematopoiesis, and also regulates PG production in several
organs and tissues (Takes et al. 1998). Besides acting as an immune factor, IL1A is one of the main cytokines that participates in the local regulation of many reproductive events. IL1A also has a role in regulating ovulation (Rae et al. 2004). The mRNAs for IL1A and IL1B (IL1β) and receptor IL1R are present in the bovine CL and endometrium throughout the estrous cycle (Nishimura et al. 2004, Tanikawa et al. 2005) and during early pregnancy (Leung et al. 2000). In vitro studies suggest that ILs act as local modulators of both luteolytic PGF2α and luteotrophic PGE2 production in the bovine CL and endometrium (Betts & Hansen 1992, Leung et al. 2001, Nishimura et al. 2004, Tanikawa et al. 2005).

A number of in vitro studies have also shown that IL1 (α and β) as well as other cytokines can regulate endometrial PG secretion in a cell type-restricted manner (Betts & Hansen 1992, Davidson et al. 1995, Tanikawa et al. 2005, 2008). Previous results suggest that stromal cells are the target of IL1A and IL1B for stimulating the production of both PGs (Tanikawa et al. 2008). The action of IL1 and IL2 on both OXT receptor (OXTR) mRNA expression and endometrial PG production may alter luteolysis (Leung et al. 2001). Recent in vitro studies from our laboratory suggest that IL1A plays some role in regulating the PGF2α to PGE2 ratio during the estrous cycle, and that this effect is mediated via regulation of PGE synthase (Tanikawa et al. 2005, 2008). However, based on in vitro studies, there is no consensus whether IL1A has luteotropic or luteolytic properties (Betts & Hansen 1992, Leung et al. 2001, Tanikawa et al. 2005). Therefore, to determine the physiological role of IL1A during the estrous cycle and early pregnancy in cows, we examined the influence of IL1A on PG secretion by cultured bovine endometrial tissues obtained from cyclic or early pregnant cows (days 16–17). We also examined whether IL1A modulates the effects of IFNT on the bovine endometrium in vitro, and whether it affects PG and P4 secretions and the lifespan of the CL in vivo.

Results

Experiment 1: effect of IL1A on basic and IFNT-regulated PG secretion by the bovine endometrium: in vitro

In the present study, TNF, used as a positive control, increased in vitro secretion of both PGE2 and PGF2α by bovine endometrium at the luteal phase (P<0.01; Figs 1A and 2A), indicating that the tissues were reactive in the present experimental condition. Nevertheless, this cytokine did not affect any PG secretions on days 16–17 of the pregnancy (P>0.05; Figs 1B and 2B).

On days 16–17 of the estrous cycle, IL1A increased PGE2 secretion by bovine endometrium (Fig. 1A, P<0.05). Nevertheless, IFNT at a dose of 30 ng/ml did not influence basal PGE2 secretion by cow endometrium collected on the same days (P>0.05). However, this cytokine significantly augmented IL1A-stimulated PGE2 production on days 16–17 of the estrous cycle (P>0.05).

On days 16–17 of pregnancy, IL1A strongly increased PGE2 secretion by the bovine endometrium (Fig. 1B, P<0.05). IFNT that was administered alone did not influence PGE2 secretion and did not modulate IL1A influence on PGE2 secretion on days 16–17 of pregnancy (P>0.05).

Figure 1 PGE2 production by bovine endometrial tissue from cows on days 16–17 of estrous cycle (A) and pregnancy (B) in response to TNF (0.6 nM), IL1A (10 ng/ml), IFNT (30 ng/ml), and both IL1A and IFNT. Superscript letters: a, b, and c indicate statistical differences between control and treated groups (P<0.05), as determined by one-way ANOVA, followed by the Newman–Keuls test.

Figure 2 PGF2α production by bovine endometrial tissue from cows on days 16–17 of estrous cycle (A) and pregnancy (B) in response to TNF (0.6 nM), IL1A (10 ng/ml), IFNT (30 ng/ml), and both IL1A and IFNT. Superscript letters: a, b, and c indicate statistical differences between control and treated groups (P<0.05), as determined by one-way ANOVA, followed by the Newman–Keuls test.
IL1A increased PGF$_{2\alpha}$ production by the bovine endometrium on days 16–17 of the estrous cycle (Fig. 2A, $P<0.05$). IFNT did not influence the stimulatory effect of IL1A on PGF$_{2\alpha}$ concentration on days 16–17 of the estrous cycle (Fig. 2A, $P<0.05$).

On days 16–17 of pregnancy, IL1A and IFNT did not influence PGF$_{2\alpha}$ production ($P>0.05$; Fig. 2B). Also, IFNT did not modulate IL1A influence on PGF$_{2\alpha}$ secretion on days 16–17 of pregnancy ($P>0.05$).

**Experiment 2: effects of IL1A on PGs, P$_4$ output, and the CL lifespan: in vivo**

IL1A at doses of 0.1 and 1 $\mu$g stimulated P$_4$ output in peripheral blood during 24 h in comparison to the control cows ($P<0.01$). Moreover, IL1A at doses of 0.1 and 1 $\mu$g elevated the total amount of secreted P$_4$ in the blood plasma in comparison to the control cows (Table 1; $P<0.01$). Administration of other selected doses of IL1A did not influence P$_4$ concentration and total amount of secreted P$_4$ in the blood plasma in comparison to the control cows (Fig. 3A, Table 1; $P>0.05$). Figure 3A shows P$_4$ concentrations in the blood plasma of control and experimental cows.

IL1A at doses of 0.1 and 1 $\mu$g stimulated PGE$_2$ output in peripheral blood during 24 h in comparison to the control cows ($P<0.01$). Moreover, IL1A at doses of 0.1 and 1 $\mu$g elevated the total amount of secreted PGE$_2$ in the blood plasma in comparison to the control cows (Table 1; $P<0.01$). Administration of other selected doses of IL1A did not influence PGE$_2$ concentration and total amount of secreted PGE$_2$ in the blood plasma in comparison to the control cows (Fig. 3B, Table 1; $P>0.05$). Figure 3B shows PGE$_2$ concentrations in the blood plasma of control and experimental cows.

IL1A at a dose of 10 $\mu$g stimulated PGFM (13,14-dihydro, 15-keto-prostaglandin F$_{2\alpha}$) output in peripheral blood during 12 h after application in comparison to the control cows ($P<0.01$). Moreover, IL1A at doses of 0.1 and 1 $\mu$g was capable of prolonging the luteal phase of the estrous cycle – the lifespan of CL (Fig. 4, $P<0.05$). The length of the estrous cycle was prolonged to more than 30 days by infusion of IL1A at a dose of 0.1 and 1 $\mu$g as compared to that in control heifers (22.1 ± 0.5 days; $P<0.05$).

**Discussion**

Both PGE$_2$ and PGF$_{2\alpha}$ are secreted by the bovine endometrium throughout the estrous cycle (Miyamoto et al. 2000, Skarzynski et al. 2003) and may play opposite roles, i.e. PGE$_2$ is luteotrophic and luteoprotective (Pratt et al. 1977, Magness et al. 1981, Kotwica et al. 2003, 2010).

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![Image](https://via.placeholder.com/150)

**Figure 3** Concentrations of progesterone (A), PGE$_2$ (B), and PGFM (C) in peripheral blood plasma of cows infused with saline (gray bars) and various doses of IL1A (lines). All reagents were infused into the uterus. Different subscript letters indicate significant differences ($P<0.05$) between treated groups, as assessed by repeated measures ANOVA test followed by Newman–Keuls test.

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**Table 1** Effects of different doses of interleukin-1z (IL1A) administered into the uterus on the total amounts of released progesterone (P$_4$), prostaglandin E$_2$ (PGE$_2$) and PGFM in cows on days 16–17 of the estrous cycle. Values indicate the area under the curve (relative units, means ± S.E.M.).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P$_4$ (ng/ml, 24 h)</th>
<th>PGE$_2$ (ng/ml, 24 h)</th>
<th>PGFM (ng/ml, 24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>12.50 ± 0.94$^a$</td>
<td>8.29 ± 1.21$^a$</td>
<td>525 ± 198$^a$</td>
</tr>
<tr>
<td>IL1A 0.001 (μg)</td>
<td>9.61 ± 0.82$^a$</td>
<td>14.60 ± 2.69$^a$</td>
<td>1494 ± 297$^{ab}$</td>
</tr>
<tr>
<td>IL1A 0.01 (μg)</td>
<td>34.70 ± 4.77$^a$</td>
<td>8.45 ± 3.18$^a$</td>
<td>1393 ± 404$^{ab}$</td>
</tr>
<tr>
<td>IL1A 0.1 (μg)</td>
<td>57.37 ± 18.12$^a$</td>
<td>42.24 ± 27.16$^a$</td>
<td>1053 ± 433$^{ab}$</td>
</tr>
<tr>
<td>IL1A 1 (μg)</td>
<td>75.42 ± 26.52$^{ab}$</td>
<td>41.05 ± 7.73$^{ab}$</td>
<td>630 ± 147$^{a,b}$</td>
</tr>
<tr>
<td>IL1A 10 (μg)</td>
<td>51.31 ± 15.54$^a$</td>
<td>7.65 ± 0.48$^a$</td>
<td>941 ± 277$^{b}$</td>
</tr>
</tbody>
</table>

The baseline was determined by means of hormone concentrations in blood collected 2 h before IL1A administration. Differences between hormone concentrations were analyzed using one-way ANOVA, followed by the Newman–Keuls test. $^{ab}$Different subscript letters within a column indicate significant differences ($P<0.05$) between treated groups.
roles in reproductive functions by regulating local PGE2

Weems et al. 2006), whereas PGF2α is luteolytic (McCacken et al. 1999, Weems et al. 2006). Moreover, it has been shown that the relative proportion of PGE2 and PGF2α secretion is thought to be more important than the absolute amounts of each PG to exert physiological effects on female reproductive functions (Murakami et al. 2001, Weems et al. 2006). One of the goals of our study was to examine whether IL1A has an effect on the secretion of PGs in the bovine endometrium during the estrus cycle and early pregnancy.

In the in vitro experiment, as expected, IL1A stimulated both luteolytic PGF2α and luteotrophic PGF2α, by the bovine endometrium during the late luteal phase of the estrous cycle. Another in vitro study has suggested that IL1A is produced in the bovine endometrium during the estrus cycle, and that it plays crucial effects on female reproductive functions (Murakami et al. 2001, Weems et al. 2006). This cytokine, together with IL1B, also affects bovine CL in vitro by modulating the secretions of both PGE2 and PGF2α, depending on the stage of the estrous cycle (Davidson et al. 1995, Nishimura et al. 2004). However, IL1A is thought to stimulate PGE2 production more strongly than IL1B in stromal cells of the bovine endometrium (Tanikawa et al. 2005). In bovine stromal cells, IL1A induced PGE2 production by increasing the expressions of PTGS2 and PGE2 synthase type 1 mRNA and their proteins without any effects on PGF2α synthase (PGFS) expression (Tanikawa et al. 2008).

In the present study, IL1A increased both PGE2 and PGF2α secretion in tissue explants from cows in the luteal phase of the estrous cycle. However, during early pregnancy, IL1A only stimulated the secretion of luteolytic PGE2 without having any influence on the luteolytic PGF2α secretion. Furthermore, IL1A administered into the uteri of cows in the luteal phase at doses of 0.1 and 1 µg significantly stimulated PGE2 and P4 output. Only at a dose of 10 µg did IL1A increase temporally PGFM plasma concentration without affecting P4 secretion and CL lifespan. These findings agree with data of Leung et al. (2001) showing that pro-inflammatory ILs suppress OXTR expression in the late luteal phase. The cytokines IL1 and IL2 are therefore unlikely to initiate/modulate luteolysis. However, ILs can cause increased production of PGF2α during uterine infection (Leung et al. 2001). Therefore, we suggest that during the luteal phase and early pregnancy, IL1A action is directed toward stimulating PGF2α synthesis only and increasing the PGE2:PGF2α ratio. However, at the time of luteolysis and during inflammatory processes, this cytokine may stimulate PGF2α production/output as a supporting/modulating factor rather than as a mandatory factor (Leung et al. 2001, Tanikawa et al. 2005, 2008).

Based on in vitro data (Leung et al. 2001, Tanikawa et al. 2005, 2008), it appears that some local modulators may exist during early pregnancy that switch IL1A action from stimulation of luteolytic PGF2α to the stimulation of luteotrophic PGE2 production. One of the candidate factors is IFNT. In our study, IFNT had no significant effect on IL1A-stimulated PGE2 secretion in tissues from the pregnant endometrium, but augmented IL1A-stimulated PGE2 secretion. Moreover, in early pregnancy, IL1A strongly stimulated PGE2 production by the endometrium without having any effect on luteolytic PGF2α output. These results suggest that during early pregnancy, IFNT modulates IL1A action and changes it toward the stimulation of luteotrophic PGE2 synthesis. In ruminants, IFNT produced by the trophoblast tissue at the time of recognition of pregnancy acts on maintaining the CL function (Helmer et al. 1989, Bazer et al. 1997). Data from in vivo experiments showed that IFNT inhibits PGF2α secretion and promotes the maintenance of CL (Meyer et al. 1995, Spencer & Bazer 2004). IFNT inhibits PGF2α secretion also in vitro (Meyer et al. 1996, Spencer et al. 2007a, 2007b). The effect of IFNT on the decrease of PGF2α secretion in bovine endometrial cells may be due to its inhibitory actions on PGFS in the endometrium and myometrium (Arosh et al. 2004a, 2004b). Depending upon the dose and biological activity, IFNT may increase PGE2 production and stimulate PTGS2, the inducible form of cyclooxygenase in bovine endometrial cell cultures (Asselin et al. 1997, Spencer et al. 2007a, 2007b). However, there are some conflicting reports in literature about the ability of IFNT to stimulate or inhibit PG production (Xiao et al. 1999, Arosh et al. 2004a, 2004b, Okuda et al. 2004, Spencer & Bazer 2004).

IFNT in the present study had no significant influence on the basal PG secretion from endometrial tissue explants on days 16–17 of the estrous cycle and pregnancy. The purpose of the present work was not to determine whether IFNT regulates basal PG production but only to use the lowest effective IFNT dose that can modulate IL1A-stimulated PG production exclusively. Therefore, according to Takahashi et al. (2003) and Okuda et al. (2004), we used IFNT at a relatively small dose of 30 ng/ml that shows bioactivity and was able to

Figure 4 Concentrations of progesterone in peripheral blood plasma of cows infused with saline (gray bars) and various doses of IL1A (lines). All reagents were infused into the uterus. Different subscript letters indicate significant differences (P<0.05) between treated groups, as assessed by repeated measures ANOVA test followed by Newman–Keuls test.

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influence TNF-regulated PGF<sub>2α</sub> production. Moreover, some differences in IFNT action on basal PG production depend on the type and biological activity of IFNT used in the studies as well as on diverse doses (Asselin et al. 1997, Parent et al. 2002, Takahashi et al. 2003, Spencer et al. 2007a, 2007b).

The results of the in vitro experiment were supported by the in vivo experiment, which showed that an infusion of IL1A at doses of 0.1 and 1 µg increased plasma concentrations of P<sub>4</sub> and PGE<sub>2</sub>. Besides, we found that infusion of IL1A at a dose of 10 µg increased PGFM plasma concentration that may reflect the stimulation of luteolytic PGF<sub>2α</sub> secretion by the uterus. Thus, IL1A as well other cytokines (e.g. IFNs, TNF) has a diverse spectrum of biological activities, including stimulation of different metabolites of arachidonic acid (luteotrophic versus luteolytic), cell proliferation and differentiation, and induction of apoptosis (Beutler & Huffel 1994, Dinarello 1994, 1996, Skarzynski et al. 2003, 2007, Korzekwa et al. 2008). The ability of cytokines to exert a wide variety of effects is likely due to actions exerted via multiple signaling pathways involving two or more distinct receptors and numerous intracellular mediators/pathways (Arend 1991, Beutler & Van Huffel 1994, Colotta et al. 1994, Dinarello 1994, Tartaglia & Goeddel 1994, Korzekwa et al. 2008). The highest dose of IL1A may act not only locally on the endometrial cells, but also remotely on other structures of the reproductive tract (i.e. on epithelium of blood vessels, immune cells, myometrium, etc.), inducing uterine PGF<sub>2α</sub> production. In our study, 10 µg of IL1A induced temporal rise in PGF<sub>2α</sub> secretion, but didn’t have any effect on P<sub>4</sub> production or the CL lifespan. Thus, for such luteolytic action of IL1A, both sites of action are needed (uterus and CL), and direct amplification of luteolytic signals in the CL is mandatory (Nishimura et al. 2004, Skarzynski et al. 2008). It is controversial to suppose that IL1A can participate in luteolysis as one of mandatory auto-paracrine factors inducing PGF<sub>2α</sub> production and output in the endometrium (Tanikawa et al. 2005). Our in vivo experiment showed that IL1A administered directly into the uterus at the late luteal stage of the estrous cycle significantly stimulated P<sub>4</sub> and PGE<sub>2</sub> concentrations and prolonged CL lifespan. These findings suggest that in vivo IL1A plays mainly luteoprotective roles. The present results show that IL1A possibly has a direct positive influence on the CL function via the stimulation of P<sub>4</sub> secretion. However, the IL1A indirect role seems also probable because of its influence on PGE<sub>2</sub> secretion. It should also be noted that the relatively small effect of IL1A treatments on PG levels was shown in in vitro experiments comparing with in vivo effects. The endometrium is composed of epithelial and stromal cells and also blood vessels and leukocytes. This mixture of cell types is the evident source of the basal levels of PGs as well as the source of PGs that were secreted after stimulation during the in vitro experiments. However, in in vivo procedures, many other tissues including the main bloodstream can respond to IL1A. In response to IL1A, these tissues may release some PGs, the amounts of which can be estimated by jugular vein sampling.

In summary, IL1A may play some autocrine or paracrine roles in regulating PG production in the endometrium during the estrous cycle and early pregnancy in cattle. Although IL1A may stimulate in vitro luteolytic PGF<sub>2α</sub> secretion during the estrous cycle, in vivo it acts only as a luteotrophic factor. IL1A increased luteotropic PGE<sub>2</sub> and P<sub>4</sub> output, inhibiting spontaneous luteolysis and prolonging the lifespan of the bovine CL. These luteotropic effects may result in appropriate development and function of the bovine CL during the estrous cycle and early pregnancy. Moreover, IFNT produced during the early pregnancy can modulate IL1A action and change it toward the stimulation of luteotropic PGE<sub>2</sub> synthesis. These luteotropic effects of IL1A during early pregnancy may support early embryo development and implantation, and may play an additional role in the maternal recognition of pregnancy in cattle.

**Materials and Methods**

**Animals**

All procedures were approved by the Local Animal Care and Use Committee in Olsztyn, Poland (agreements no. 73/2002/N and 06/2007/N).

For all experiments, normally-cycling Holstein/Polish Black and White (75/25%; respectively) cows (4–6 lactations; n = 39) were chosen. The animals were culled by the owner from two dairy cow herds (years 2005–2008) because of their low milk production. In order to carry out these experiments, estrus of the cows was synchronized using two analogs of PGF<sub>2α</sub> (dinoprost, Dinolytic; Upjohn – Pharmacia N.V.S.A.) injections with an 11–14-day interval, as recommended and described recently (Skarzynski et al. 2009). The onset of estrus was determined by observing its signs (i.e. vaginal mucus, standing behavior) and was confirmed by a veterinarian by transrectal internal genitalia ultrasonography (USG) examination using Draminski Animal profi Scanner (Draminski Electronics in Agriculture, Olsztyn, Poland). Only the cows with behavioral signs of estrus and USG confirmation were chosen for the study. Estrus was considered as day 0 of the estrous cycle.

**Experimental procedure**

**Experiment 1: the effect of IL1A on basal and IFNT-regulated PG secretion by the bovine endometrium: in vitro**

Bovine uteri were obtained at a local slaughterhouse (Zaklady Miesne ‘Warmia’ in Biskupiec) within 20 min of exsanguination and were transported on ice to the laboratory within 40 min. Before slaughter, the cows were assigned into two groups, i.e. pregnant and cyclic animals. The animals were randomly chosen for the pregnant group and underwent
artificial insemination with semen from the same bull. Pregnancy was confirmed by flushing the uterus for the collection of the viable embryo as described recently (Woclawek-Potocka et al. 2009). Endometria were obtained from cows on days 16–17 of the cycle \( (n=7) \) and on days 16–17 of pregnancy \( (n=6) \). Moreover, the flushed uterine fluid was collected and the antiviral activity of IFNT was measured, as described recently (Gierek et al. 2006). In addition, the sensitivity of cultured endometrial tissue \( (\text{days 16–17}) \) to OXT treatment was tested. Only nonpregnant bovine endometrium, in the late luteal and follicular phases of the cycle, is sensitive to OXT treatment and PGF\(_{2\alpha}\) release (Miyamoto et al. 2000). Therefore, only the uterus which responded properly to those tests were chosen for the respective pregnancy groups as described previously (Woclawek-Potocka et al. 2009).

The endometrial tissues were incubated in a shaking water bath at 37 \(^\circ\)C as described previously (Miyamoto et al. 2000, Tanikawa et al. 2005). Shortly, endometrial strips were washed thrice in sterile saline containing 100 IU/ml penicillin and 100 \( \mu \)g/ml streptomycin. The tissues were then cut into small pieces \( (~20–30 \text{ mg}) \). The individual pieces of endometrial tissues were placed in culture glass tubes \( (12\times75 \text{ mm}) \) containing 2 ml culture medium \( (\text{DMEM and Hams F-12 medium} 1:1(\text{v/v}); \Sigma \text{Chemical Co., #8900}) \) supplemented with 0.1% BSA \( (\Sigma \text{, #A9056}) \), 5 ng/ml sodium selenite \( (\Sigma \text{, #S1382}) \), 0.5 mM ascorbic acid \( (\Sigma \text{, #A1417}) \), and 20 \( \mu \)g gentamycin \( (\Sigma \text{, #G1397}) \). Media were continuously gassed with 5% CO\(_2\) during incubation.

After 1-h preincubation, endometrial slices were exposed to IL1A \( (10 \text{ ng/ml}) \), IFNT \( (30 \text{ ng/ml}) \), or TNF \( (0.6 \text{ nM}) \) as a positive control. IL1A \( (\text{HL-18}) \) was obtained from Dainippon Pharmaceutical Co., Osaka, Japan. Recombinant bovine IFNT, classified into the rb-1a group, was produced by an Autographa californica nuclear polyhedrosis virus expression system, and was a gift from Dr Hitomi Takahashi (National Institute of Livestock and Grassland Science, Tsukuba, Japan). Doses of IL1A, TNF, and IFNT were chosen based on our previous studies (Miyamoto et al. 2000, Takahashi et al. 2003, Okuda et al. 2004, Tanikawa et al. 2005).

After 18 h of stimulation, the conditioned media were collected in tubes with 10 \( \mu \)l EDTA, 1% aspirin \( (\Sigma \text{, #A1093}) \), solution \( (\text{pH }7.3) \), and frozen at \(-20 \text{ \degree C}\) until PGE\(_2\), PGFM, and \( P_4 \) measurement. The tissues were blotted with filter paper and weighed to obtain the concentration per gram of tissue.

**Experiment 2: effects of IL1A on PGs, \( P_4 \) output, and the CL lifespan: in vivo studies**

On day 16 of the cycle, a polyvinyl catheter was inserted into the jugular vein for frequent collection of blood samples (Skarzynski et al. 2003). To test the hypothesis that IL1A acts locally on the bovine uterus to regulate PG secretion and the lifespan of the bovine CL, we administered saline \( (5 \text{ ml}; n=5) \) or 0.001 \( (n=3) \), 0.01 \( (n=5) \), 0.1 \( (n=5) \), 1 \( (n=5) \), and 10 \( \mu \)g of IL1A \( (\text{dissolved in 5 ml of saline}) \) directly into the uterine lumen as a single intrauterine infusion described previously (Skarzynski et al. 2007). Blood was collected 2 h before and every 2–4 h after IL1A administration during 24 h, via cannula placed in the jugular vein. Furthermore, blood was collected every 1, 3, or 6 days during the estrous cycle. Plasma was obtained by centrifugation \( (3000 \text{ g}, 10 \text{ min}, 4 \text{ \degree C}) \) and stored at \(-20 \text{ \degree C}\) until PGE\(_2\), PGFM, and \( P_4 \) assessment.

**Determination of hormone concentrations**

The concentrations of PGE\(_2\) in plasma samples and culture medium were determined with the direct enzyme immunoassay (EIA) test, as described previously (Skarzynski et al. 2000, 2003). The PGE\(_2\) standard curve ranged from 0.07 to 20 ng/ml. The intra- and interassay coefficients of variation (CV) were on average 6.9 and 9.7% respectively.

PGF\(_{2\alpha}\) concentration in the culture medium was assayed using direct EIA as described previously (Skarzynski et al. 2000). The PGF\(_{2\alpha}\) standard curve was produced for PGF\(_{2\alpha}\) concentrations ranging from 15.6 to 4000 pg/ml. The intra- and interassay CV were on average 7.9 and 10.4% respectively.

The concentration of PGFM in the culture medium was determined with the direct EIA test, as described previously (Skarzynski et al. 2003). The anti-PGFM serum (WS44685) was donated by Dr W J Silvia, University of Kentucky, Lexington, KY, USA. The PGFM standard curve ranged from 15.6 to 4000 pg/ml. The intra- and interassay CV were on average 6.1 and 8.7% respectively.

\( P_4 \) concentrations in plasma were assayed using direct EIA as described previously (Skarzynski et al. 2003). The \( P_4 \) standard curve ranged from 0.07 to 20 ng/ml. The intra- and interassay CV were on average 6.9 and 9.7% respectively.

**Statistical analysis**

Least-squares means and s.e.m. were determined. Differences in the length of the estrous cycle and in vitro PG production were analyzed using one-way ANOVA followed by Newman–Keuls multiple comparison test (ANOVA; GraphPAD PRISM Version 4.00, San Diego, CA, USA). Moreover, the differences in in vitro PG production between control and experimental groups were analyzed using paired Student’s \( t \)-test (GraphPAD PRISM). \( P_4 \) and arachidonic acid metabolites (PGE\(_2\), PGFM) in the jugular plasma samples were analyzed using a repeated measure design approach (Skarzynski et al. 2003, 2007). All analyses were performed using repeated measures ANOVA tests followed by Newman–Keuls multiple comparison test (GraphPAD PRISM; \( P<0.05 \) was considered significant). Moreover, in the in vivo study, the total amounts of released \( P_4 \), PGE\(_2\), and PGFM were shown by the areas under the curve (relative units; Table 1) and analyzed using one-way ANOVA followed by Newman–Keuls multiple comparison test (ANOVA; GraphPAD PRISM).

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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