Is interleukin-1 α 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-1 α (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 luteotrophic PGE₂ secretion by endometrial tissues of both pregnant and nonpregnant cows. IL1A also stimulated luteolytic PGF_{2 α} 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 μ g/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 PGF_{2 α} secretion, while it had no effect on P₄ secretion or CL lifespan. Application of 0.1 and 1 μ g IL1A stimulated P₄ and PGE₂ output and prolonged the CL lifespan. Although IL1A may stimulate *in vitro* luteolytic PGF_{2 α} secretion during the estrous cycle, it only acts as a luteotrophic factor *in vivo*. IL1A increased luteotrophic PGE₂ and P₄ output, inhibiting spontaneous luteolysis. These luteotrophic 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 luteotrophic and luteoprotective actions to lengthen the lifespan of CL and sustain progesterone (P₄) production (Weems *et al.* 1997, Kotwica *et al.* 2003). PGF_{2 α} is the main luteolytic agent in ruminants (McCracken *et al.* 1999, Skarzynski *et al.* 2008).

The relative proportion of both PGE₂ and PGF_{2 α} 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 PGF_{2 α} 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 PGF_{2 α} 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 (IL1 β) 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 PGF_{2 α} 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 OXTR 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 PGF_{2 α} 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 luteotrophic 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 PGF_{2 α} 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

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.

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 2 PGF_{2 α} 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 α} 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 α} 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 α} production ($P > 0.05$; Fig. 2B). Also, IFNT did not modulate IL1A influence on PGF_{2 α} secretion on days 16–17 of pregnancy ($P > 0.05$).

Experiment 2: effects of IL1A on PGs, P₄ output, and the CL lifespan: in vivo

IL1A at doses of 0.1 and 1 μ g stimulated P₄ 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 μ g elevated the total amount of secreted P₄ 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 P₄ concentration and total amount of secreted P₄ in the blood plasma in comparison to the control cows (Fig. 3A, Table 1; $P > 0.05$). Figure 3A shows P₄ concentrations in the blood plasma of control and experimental cows.

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IL1A at a dose of 10 μ g stimulated PGFM (13,14-dihydro, 15-keto-prostaglandin F_{2 α}) output in peripheral blood during 12 h after application in comparison to the control cows ($P < 0.01$). Moreover, IL1A at doses of

Figure 3 Concentrations of progesterone (A), PGE₂ (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.

0.001 and 10 μ g elevated the total amount of secreted PGFM in the blood plasma in comparison to the control cows (Table 1; $P < 0.01$). Administration of other doses of IL1A did not influence PGFM concentration and total amount of secreted PGFM in the blood plasma in comparison to the control cows (Fig. 3C, Table 1; $P > 0.05$). Figure 3C shows PGFM concentrations in the blood plasma of control and experimental cows.

IL1A at doses of 0.1 and 1 μ 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 μ g as compared to that in control heifers (22.1 ± 0.5 days; $P < 0.05$).

Discussion

Both PGE₂ and PGF_{2 α} 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. PGE₂ is luteotrophic and luteoprotective (Pratt *et al.* 1977, Magness *et al.* 1981, Kotwica *et al.* 2003,

Table 1 Effects of different doses of interleukin-1 α (IL1A) administered into the uterus on the total amounts of released progesterone (P₄), prostaglandin E₂ (PGE₂) and PGFM in cows on days 16–17 of the estrous cycle. Values indicate the area under the curve (relative units, means $\pm$ S.E.M).

Treatment	P ₄	PGE ₂	PGFM
Saline (control)	12.50 $\pm$ 0.94 ^a	8.29 $\pm$ 1.21 ^a	525 $\pm$ 198 ^a
IL1A 0.001 (μ g)	9.61 $\pm$ 0.82 ^a	14.60 $\pm$ 2.69 ^a	1494 $\pm$ 297 ^b
IL1A 0.01 (μ g)	34.70 $\pm$ 4.77 ^a	8.45 $\pm$ 3.18 ^a	1393 $\pm$ 404 ^{a,b}
IL1A 0.1 (μ g)	57.37 $\pm$ 18.12 ^b	42.24 $\pm$ 27.16 ^b	1053 $\pm$ 437 ^a
IL1A 1 (μ g)	75.42 $\pm$ 26.52 ^b	41.05 $\pm$ 7.73 ^b	630 $\pm$ 147 ^{a,b}
IL1A 10 (μ g)	51.31 $\pm$ 15.54 ^a	7.65 $\pm$ 0.48 ^a	941 $\pm$ 277 ^b

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. ^{a,b}Different subscript letters within a column indicate significant differences ($P < 0.05$) between treated groups.

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.

Weems *et al.* 2006), whereas $\text{PGF}_{2\alpha}$ is luteolytic (McCracken *et al.* 1999, Weems *et al.* 2006). Moreover, it has been shown that the relative proportion of PGE_2 and $\text{PGF}_{2\alpha}$ 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 estrous cycle and early pregnancy.

In the *in vitro* experiment, as expected, IL1A stimulated both luteolytic $\text{PGF}_{2\alpha}$ and luteotrophic PGE_2 , 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 estrous cycle, and that it plays crucial roles in reproductive functions by regulating local PGE_2 and $\text{PGF}_{2\alpha}$ production in the bovine endometrium (Tanikawa *et al.* 2005). This cytokine, together with IL1B, also affects bovine CL *in vitro* by modulating the secretions of both PGE_2 and $\text{PGF}_{2\alpha}$, depending on the stage of the estrous cycle (Davidson *et al.* 1995, Nishimura *et al.* 2004). However, IL1A is thought to stimulate PGE_2 production more strongly than IL1B in stromal cells of the bovine endometrium (Tanikawa *et al.* 2008). In bovine stromal cells, IL1A induced PGE_2 production by increasing the expressions of *PTGS2* and PGE_2 synthase type 1 mRNA and their proteins without any effects on $\text{PGF}_{2\alpha}$ synthase (PGFS) expression (Tanikawa *et al.* 2008).

In the present study, IL1A increased both PGE_2 and $\text{PGF}_{2\alpha}$ secretion in tissue explants from cows in the luteal phase of the estrous cycle. However, during early pregnancy, IL1A only stimulated the secretion of luteotrophic PGE_2 without having any influence on the luteolytic $\text{PGF}_{2\alpha}$ secretion. Furthermore, IL1A administered into the uteri of cows in the luteal phase at doses of 0.1 and 1 µg significantly stimulated PGE_2 and P_4 output. Only at a dose of 10 µg did IL1A increase temporally PGFM plasma concentration without effecting P_4

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 $\text{PGF}_{2\alpha}$ during uterine infection (Leung *et al.* 2001). Therefore, we suggest that during the luteal phase and early pregnancy, IL1A action is directed toward stimulating PGE_2 synthesis only and increasing the $\text{PGE}_2:\text{PGF}_{2\alpha}$ ratio. However, at the time of luteolysis and during inflammatory processes, this cytokine may stimulate $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ to the stimulation of luteotrophic PGE_2 production. One of the candidate factors is IFNT. In our study, IFNT had no significant effect on IL1A-stimulated PGE_2 secretion in tissues from the pregnant endometrium, but augmented IL1A-stimulated PGE_2 secretion. Moreover, in early pregnancy, IL1A strongly stimulated PGE_2 production by the endometrium without having any effect on luteolytic $\text{PGF}_{2\alpha}$ output. These results suggest that during early pregnancy, IFNT modulates IL1A action and changes it toward the stimulation of luteotrophic PGE_2 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 $\text{PGF}_{2\alpha}$ secretion and promotes the maintenance of CL (Meyer *et al.* 1995, Spencer & Bazer 2004). IFNT inhibits $\text{PGF}_{2\alpha}$ secretion also *in vitro* (Meyer *et al.* 1996, Spencer *et al.* 2007a, 2007b). The effect of IFNT on the decrease of $\text{PGF}_{2\alpha}$ 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 PGE_2 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

influence TNF-regulated PGF_{2 α} 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 μ g increased plasma concentrations of P₄ and PGE₂. Besides, we found that infusion of IL1A at a dose of 10 μ g increased PGFM plasma concentration that may reflect the stimulation of luteolytic PGF_{2 α} 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_{2 α} production. In our study, 10 μ g of IL1A induced temporal rise in PGF_{2 α} secretion, but didn't have any effect on P₄ 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_{2 α} 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 P₄ and PGE₂ 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 P₄ secretion. However, the IL1A indirect role seems also probable because of its influence on PGE₂ 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_{2 α} secretion during the estrous cycle, *in vivo* it acts only as a luteotrophic factor. IL1A increased luteotrophic PGE₂ and P₄ output, inhibiting spontaneous luteolysis and prolonging the lifespan of the bovine CL. These luteotrophic 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 luteotrophic PGE₂ synthesis. These luteotrophic 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_{2 α} (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 (Zakłady 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 (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 α} release (Miyamoto *et al.* 2000). Therefore, only the uteri 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 °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 µg/ml streptomycin. The tissues were then cut into small pieces (~20–30 mg). The individual pieces of endometrial tissues were placed in culture glass tubes (12×75 mm) containing 2 ml culture medium (DMEM and Hams F-12 medium 1:1(v/v); Sigma Chemical Co. #8900) supplemented with 0.1% BSA (Sigma, #A9056), 5 ng/ml sodium selenite (Sigma, #S1382), 0.5 mM ascorbic acid (Sigma, #A1417), and 20 µg gentamycin (Sigma, #G1397). Media were continuously gassed with 5% CO₂ during incubation.

After 1-h preincubation, endometrial slices were exposed to IL1A (10 ng/ml), IFNT (30 ng/ml), both IL1A and IFNT, or TNF (0.6 nM) as a positive control. IL1A (HL-18) was obtained from Daiinippon 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 µl EDTA, 1% aspirin (Sigma, #A1093), solution (pH 7.3), and frozen at –20 °C until PGF_{2 α} and PGE₂ measurements. The tissues were blotted with filter paper and weighed to obtain the concentration per gram of tissue.

Experiment 2: effects of IL1A on PGs, P₄ 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 ml; $n=5$) or 0.001 ($n=3$), 0.01 ($n=5$), 0.1 ($n=5$), 1 ($n=5$), and 10 µg of IL1A (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 g, 10 min, 4 °C) and stored at –20 °C until PGE₂, PGFM, and P₄ assessment.

Determination of hormone concentrations

The concentrations of PGE₂ in plasma samples and culture medium were determined with the direct enzyme immunoassay (EIA) test, as described previously (Skarzynski *et al.* 2000, 2003). The PGE₂ 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 α} concentration in the culture medium was assayed using direct EIA as described previously (Skarzynski *et al.* 2000). The PGF_{2 α} standard curve was produced for PGF_{2 α} 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 (WS4468-5) 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.

P₄ concentrations in plasma were assayed using direct EIA as described previously (Skarzynski *et al.* 2003). The P₄ 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) P₄ and arachidonic acid metabolites (PGE₂, 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 P₄, PGE₂, 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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