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Revised

Effects of preimplantation factor on interleukin-6 and prostaglandin $F_{2\alpha}$ and E_2 in the bovine endometrium.

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ABSTRACT

Preimplantation factor (PIF) is a pregnancy specific peptide with immune modulatory properties exerted on the human endometrium. Viable bovine embryos secrete PIF, but its effect on the bovine endometrial immune response is unknown, both in native and inflammatory stimulated endometrial tissue. An *ex vivo* bovine endometrial tissue culture model was used with lipopolysaccharide (LPS) as an inflammatory stimulant. The effect of synthetic PIF (sPIF) was assessed, in three separate experiments, on the secretion or mRNA expression of essential prostaglandins and cytokines. Radioimmunoassays were used to assess prostaglandin secretion and ELISA for IL-6 secretion from endometrial explants. mRNA expression of *IL6* and *IL8* was analysed from endometrial explants with real-time PCR. Synthetic PIF reduced native IL-6 secretion from explants when pre-treated for 24 hours. There was no effect of sPIF on IL-6 secretion from LPS challenged explants; however, sPIF increased *IL6* mRNA expression when challenged with 500 ng/mL LPS. There was no effect of sPIF on prostaglandin secretion or mRNA expression of *IL8*. Therefore, sPIF is able to modulate the native IL-6 production pathway in the bovine endometrium, yet demonstrates no effect on prostaglandin secretion or *IL8* expression. Unlike in human studies, effects of sPIF were minimal, thus sPIF is not an effective modulator of the immune targets investigated in the bovine endometrium.

Key words: endometrium; bovine; preimplantation factor; interleukin-6, prostaglandin $F_{2\alpha}$; prostaglandin E_2

1. Introduction

Preimplantation factor (PIF) is a 15 amino acid peptide that is produced by viable embryos as early as the two-cell stage [1]. Bovine embryos produce PIF both pre- and post-implantation [1, 2]. Preimplantation factor works through an immune tolerance pathway in human pregnancy to facilitate the acceptance of the embryo by the mother [3, 4]. It is the action of this pathway that is of interest to studies of disease, as sPIF may have potential as an immune modulator. Applied to healthy human endometrium, sPIF is able to upregulate secretion of several interleukins, including IL-8 and IL-6 of decidualized stromal cells [5]. Interestingly, within a murine multiple sclerosis model, sPIF decreased the secretion of IL-6 from splenocytes in culture showing a tissue specific role of the peptide [6]. In a preliminary study, sPIF was investigated in an equine model of *E. coli* post-mating induced endometritis. It was shown that sPIF was able to reduce prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretion from LPS induced explants 24 hours after challenge [7] but only in mares of the follicular stage of the oestrous cycle. More recently, in CD14+ cells, it has been demonstrated that sPIF does not directly interact with TLR-4, but specific downstream targets within the TLR-4 pathway [8]. As sPIF has been demonstrated to interact with the human endometrium in an immune modulatory manner, it was proposed that the peptide may act in a similar manner in the bovine endometrium. Furthermore, as uterine inflammation is a common cause of infertility in cattle through a dysregulation of endocrine function [9], it is of interest to investigate the role of sPIF as an immune modulator in an endometrial inflammatory environment.

Previous studies have utilised an *ex vivo* bovine model of normal and inflammatory endometrium, which shows responses similar to the whole cow [10, 11] and so this model provides a basis for this initial investigation. Both prostaglandins and interleukins are secreted by

cyclic, pregnant and inflammatory endometrial tissue and so have previously been used as targets to measure in *ex vivo* studies [10-12]. Prostaglandins are eicosanoid hormones produced by the endometrium and have essential functional roles in the bovine oestrous cycle and pregnancy [13, 14]. Furthermore, both $PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2) are involved in the endometrial inflammatory response. Following a challenge with *Escherichia coli*-derived lipopolysaccharide (LPS), there is an increase in secretion of $PGF_{2\alpha}$ and PGE_2 from endometrial tissue explants [12, 15]. Interleukins, such as IL-6 and IL-8 are demonstrated to be expressed during the oestrous cycle [16]. Furthermore, in *ex* vivo studies, IL-6 and IL-8 have been shown to have key roles within the endometrial innate immune response [11, 17].

The aim was to investigate the use of sPIF as a potential immune modulator within the bovine endometrium by assessing the role of the peptide using a previously developed endometrial tissue model, using LPS treatment to model an *E. coli* challenge [11, 12, 15]. It has already been demonstrated that sPIF does not bind to LPS [8]. It was hypothesised that sPIF would reduce key immune (IL-6 and IL-8) and endocrine ($PGF_{2\alpha}$ and PGE_2) factors in the bovine endometrium, at both a native and inflammatory level.

2. Materials and methods

2.1 Sample collection and endometrial explant culture

As these experiments used post-slaughter material, licencing through the Animals (Scientific Procedures) Act 1986 and ethical review were not necessary. Bovine uteri and corresponding blood samples were collected from cows presented for slaughter at a local abattoir. A total of 46 animals were used in the study. Uteri with stage I and IV ovaries were investigated to allow the study of sPIF on endometrial tissues that were not under the immune suppressive effects of

progesterone [18, 19]. Samples were staged by assessing ovarian morphology as previously described [20, 21]. Briefly, stage I was defined as having a newly ruptured corpus luteum with a diameter of 0.5 – 1.5 cm and stage IV as having a regressing corpus luteum with a diameter of < 1 cm [20].

Uteri and blood samples were stored on ice during the one-hour transportation to the laboratory. Tissues were used for explant culture and blood serum for analysis of progesterone concentration via ELISA (DRG Diagnostics, Marburg, Germany). To support ovarian morphology staging, the blood sera were used for progesterone analysis. For stage I and IV uteri, samples were deemed to have high progesterone if serum concentrations were above 1 ng/mL [10]. Progesterone inter- and intra- assay CVs were 8.66 % and 2.18 %, respectively.

Tissue culture was established using the method previously described [11]. Briefly, tissue was sampled from the uterine horn ipsilateral to the staged ovary using an 8 mm biopsy punch. Samples were weighed and placed in 6 well plates (Corning, Amsterdam, The Netherlands) with 3 mL of RPMI 1640 media (Gibco, Life Technologies, Paisley, UK) supplemented with 50 IU/mL penicillin, 50 µg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and 2.5 µg/mL amphotericin B (Sigma-Aldrich). All treatments were run in duplicate or triplicate and described for each experiment. Explants were incubated in a sterile incubator at 37^{o}C and 5 % CO_2 for up to 72 hours. Ultra-pure LPS from *E. coli* 0111:B4 strain was used (InvivoGen, Toulouse, France). Synthetic PIF (MVRIKPGSANKPSDD) was synthesised with > 95 % purity by Bioincept (New Jersey, USA). The amino acid structure of the human 15 amino acid PIF has previously been analysed and the 3D structure predicted [22]. The sPIF used in the present study is utilised in all research investigating sPIF.

97 *2.2 Experiment 1*

98 The aim of experiment 1 was to investigate the effect of sPIF on prostaglandin and IL-6 secretion
99 from bovine endometrial explants with and without an LPS challenge. Bovine stage IV uteri
100 (n=14) were utilised. Preliminary studies from our laboratory, utilising unidentified cattle breeds,
101 showed varied results in terms of prostaglandin secretion following sPIF treatment. Therefore,
102 cattle were separated into two groups at the abattoir through identification of being either: beef
103 heifers (n=7), unlikely to have been pregnant or; dairy type cows (n=7) and having had one or
104 more pregnancies. Tissues were sampled from the endometrium and challenged with the
105 following treatments in triplicate: control (media alone); LPS (1 µg/mL) alone; sPIF at three
106 concentrations (50, 100 or 500 nM); or LPS (1 µg/mL) combined with each of the three sPIF
107 concentrations. The LPS concentration was chosen based on previous studies utilising the same
108 endometrial tissue model [11, 12]. Synthetic PIF concentrations were based on the previously
109 described physiological range within the circulation during human pregnancy (50 and 100 nM)
110 and one supra-physiological concentration (500 nM) [23]. Media supernatants were sampled 24,
111 48 and 72 hours after challenge, from the same well at each time point. Time points of 24 and 48
112 hours were chosen based on previous studies [11, 12] and 72 hours based on an equine
113 endometrial explant study as a persistent infection time point [24]. Supernatant samples were
114 stored at -20^{o}C until analysed for $PGF_{2\alpha}$, PGE_2 and IL-6.

115 *2.3 Experiment 2*

116 The aim of experiment 2 was to investigate the effect of a pre-treatment of sPIF on prostaglandin
117 and IL-6 secretion from bovine endometrial explants with and without an LPS challenge and to
118 ensure that there was no underlying inflammation in tracts that may cause variability in the
119 results. Bovine stage I (n=12) and stage IV (n=12) uteri were utilised, as for experiment 1, split

into heifer (n=6 for each stage) or cow (n=6 for each stage) groups. To ensure there was no presence of sub-clinical inflammation, endometrial swabs were collected post-mortem using a modified cytobrush technique [25] and stained and fixed with Kwik-diff (Shandon, Thermo Scientific, Loughborough, UK) to test for inflammation. Samples were assessed for percentage of polymorphonuclear cells (PMN) by counting a minimum of 100 cells at X 400 magnification on Zeiss Axiovert 200M (Zeiss, Jena, Germany). Animals with a PMN percentage greater than 5% were excluded based on the guidelines for the detection of subclinical endometritis [26, 27].

Based on the results of experiment 1, the treatment protocol was modified so that explants were pre-treated with sPIF (50, 100 or 500 nM) or media alone (if explants were not to receive sPIF for the main treatment protocol) for 24 hours before challenging with LPS. At the end of the pre-treatment, the media supernatants were aspirated and replaced with fresh media alone or containing sPIF (50, 100 or 500 nM) or LPS (1 µg/mL) treatments as in experiment 1. Supernatant was sampled at 24 and 48 hours from different explant wells. No samples were collected 96 hours after the beginning of the pre-treatment (72 hours after LPS challenge) because integrity of tissue is likely to be compromised in serum free culture beyond 72 hours. Supernatant samples were stored at -20^{o}C until analysed for $PGF_{2\alpha}$, PGE_2 and IL-6.

2.4 Experiment 3

The aim of experiment 3 was to investigate the effect of a pre-treatment of sPIF on prostaglandin and IL-6 secretion and *IL6 and IL8* mRNA expression in bovine endometrial explants, with and without three low dose LPS challenges to induce a less severe inflammatory response. Bovine stage I (n=4) and stage IV (n=4) uteri were utilised. Only tracts from cows (at least 1 previous pregnancy) were used. As in experiment 2, all tracts were swabbed using the cytobrush technique

and cytology examined for each tract to establish if there was any underlying inflammation. Any animals with greater than 5% PMNs present were discarded.

A 24 hour pre-treatment of sPIF or media alone was used, as in experiment 2. Based on the results of experiment 1 and 2 the treatment choices were modified to use lower concentrations of LPS (5, 50, 500 ng/mL). Although 1 µg/mL of LPS is a more commonly used dose in previous endometrial explant studies, these concentrations of LPS have also previously been shown to significantly induce $PGF_{2\alpha}$, PGE_2 and IL-6 secretion from bovine endometrial explants or cells [11, 12]. One concentration of sPIF (100 nM) was used based on results from experiment 2. Explants were harvested 6 hours after treatment for determination of mRNA expression of *IL6* and *IL8*. Supernatant was collected 24 hours after treatment from separate wells to determine IL-6 secretion.

2.5 Prostaglandin radioimmunoassay and IL-6 ELISA

Supernatant samples were analysed for $PGF_{2\alpha}$ and PGE_2 by radioimmunoassay (RIA) as described previously [28]. Antisera were a kind gift from Professor N. Poyser, University of Edinburgh and Professor Claire Wathes, The Royal Veterinary College. The limits of detection were 0.02 ng/mL for both assays. $PGF_{2\alpha}$ inter- and intra- assay CVs were 12.8 % and 2.19 %, respectively. PGE_2 inter- and intra- assay CVs were 12.69 % and 6.71 %, respectively.

Supernatant samples were analysed for IL-6 concentration as described by the manufacturer (Bovine IL-6 ELISA; Thermo Fisher Scientific). The limit of detection was 75 pg/mL and inter- and intra- assay CVs were 8.19 % and 5.99 %, respectively.

2.6 Real time PCR

In experiment 3 where explants were analysed for gene expression, tissue pieces from each treatment were stored in RNAlater (Invitrogen, Life Technologies, Paisley, UK) and RNA extracted at a later date using the Total RNA purification kit (Norgen Biotek Corp, Ontario, Canada). From each sample, 1 µg RNA was treated for gDNA contamination with DNase I (Thermo Fisher Scientific, Loughborough, UK) and 250 ng subsequently reverse-transcribed to cDNA with the RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Gene expression of *IL6* and *IL8* were analysed using SYBR Green RT-PCR, using Maxima SYBR Green/Rox qPCR master mix (Thermo Fisher Scientific) on a CFX connect (Bio-Rad, Hemel Hempstead, UK). Gene expression was normalised to two housekeeping genes, *ACTB* and *18s* (of which expression was not altered by the experimental treatments) and then the control sample. All primers are outlined in Table 1.

2.7 Statistical analysis

Data were analysed using GenStat statistical software (14th edition, VSN International, Hemel Hempstead, UK) and reported as mean ± standard error of the mean (SEM). RIA and ELISA data were expressed as prostaglandin (ng) or interleukin (pg) secretion per mg of tissue, respectively and grouped into high or low progesterone groups where appropriate. Skewed data were transformed using log+1 or square root transformations in order to make the data normally distributed. Ratios of $PGF_{2\alpha}$ to PGE_2 secretion were calculated from the raw data within each experiment where prostaglandins were measured. Statistical significance was set at $P<0.05$ for all analyses and least significant difference (LSD) used as a *post hoc* significance test. A statistical tendency was defined as $0.05 < P < 0.1$. The following analyses were completed for each experiment.

For experiment 1, a repeated measures ANOVA was performed to assess the effects of treatment, cattle type, progesterone and time. Cows were fitted as a random effect and time point as the repeated measure. Treatment and progesterone were initially used as the main treatments. Once it was established that there was no effect of progesterone, progesterone was removed as a main treatment. Treatment and cattle type were used as the main treatments for the main statistical analysis.

A general ANOVA was performed to assess the effects of treatment, cattle type and progesterone for experiment 2 and treatment and progesterone for experiment 3. Stage I and stage IV explants were analysed separately. Cows were used as blocks, and each time point analysed separately. In experiment 2 treatment and progesterone were used as the main treatments, then progesterone removed and treatment and cattle type used as the main treatments for the main statistical analysis.

3. Results

3.1 Experiment 1

3.1.1 Serum progesterone concentrations

Samples were grouped in a high progesterone (HP) group if progesterone was greater than 1 ng/mL (n=9), with the remaining samples in a low progesterone (LP) group (n=5). Mean progesterone concentrations for LP and HP were 0.27 ± 0.1 ng/mL and 13.86 ± 6.74 ng/mL, respectively. There was no significant effect of progesterone ($P>0.05$) on $PGF_{2\alpha}$, PGE_2 or IL-6 secretion; therefore, the factor of progesterone was removed from the analysis and results presented together.

3.1.2 Prostaglandin $F_{2\alpha}$, E_2 and IL-6 secretion from endometrial tissue

There was no interaction between sPIF treatment and cattle type ($P>0.05$) for prostaglandin or IL-6 secretion. LPS challenge increased ($P<0.001$) $PGF_{2\alpha}$, PGE_2 and IL-6 secretion from explants at all time points (Fig 1). Synthetic PIF did not affect ($P>0.05$) $PGF_{2\alpha}$, PGE_2 or IL-6 secretion from explants with or without a LPS challenge (Fig 1). Synthetic PIF treatment had no effect on the ratio between $PGF_{2\alpha}$ and PGE_2 ($P>0.05$) at all time points, although LPS treatment alone increased the ratio, meaning that PGE_2 secretion was favoured over $PGF_{2\alpha}$ ($P<0.05$; data not shown). In explants not stimulated with LPS, IL-6 secretion was lower ($P<0.05$) with treatment of 100 nM sPIF compared with the 50 nM and 500 nM treatments (Fig 1c).

3.2 Experiment 2

Cytobrush smears showed that no cattle had subclinical inflammation as all samples had less than 5 % PMN.

3.2.1 Serum progesterone concentrations

Stage I samples were assigned to the luteal high progesterone (LHP) group if progesterone concentrations were greater than 1 ng/mL (n=6), with the remaining samples allocated to a luteal low progesterone (LLP) group (n=6). Mean progesterone concentrations for LLP and LHP were 0.66 ± 0.19 ng/mL and 8.2 ± 4.84 ng/mL, respectively.

Stage IV samples were allocated to a follicular high progesterone (FHP) group if progesterone concentrations were greater than 1 ng/mL (FHP; n=7) with the remaining samples assigned to a follicular low progesterone group (FLP; n=5). Mean progesterone concentrations for FLP and FHP were 0.56 ± 0.13 ng/mL and 9.61 ± 3.18 ng/mL, respectively.

There was no effect of progesterone on $PGF_{2\alpha}$, PGE_2 or IL-6 secretion within either stage I or IV explants (P>0.05). Therefore, the factor of progesterone was removed and results displayed together. However, there was an interaction between progesterone group and treatments for $PGF_{2\alpha}$ secretion in Stage I tissue at the 24 hour time point, with $PGF_{2\alpha}$ secretion being significantly lower from LPS and PIF treated explants in the LLP group, compared to the equivalent treatments in the HLP group (P<0.05; data not shown). This particular interaction did not manifest itself in any of the other experiments.

3.2.2 Prostaglandin $F_{2\alpha}$, E_2 and IL-6 secretion from endometrial tissue

There was no interaction (P>0.05) between sPIF treatment and cattle type in stage I cattle. LPS induced secretion (P<0.001) of $PGF_{2\alpha}$, PGE_2 and IL-6 from explants at both 24 and 48 hours (Fig 2). There was no effect (P<0.05) of sPIF on $PGF_{2\alpha}$ or PGE_2 secretion, with or without the presence of LPS (Fig 2). Synthetic PIF treatment of 50 and 100 nM reduced (P<0.05) the $PGF_{2\alpha}$ to PGE_2 secretion ratio compared with the control at 24 hours (Table 2), but not after 48 hours (P>0.05). The 500 nM sPIF treatment had no effect (P>0.05) on the $PGF_{2\alpha}$ to PGE_2 secretion ratio at either time point (Table 2). At 24 hours LPS treatment alone did not increase the $PGF_{2\alpha}$ to PGE_2 secretion ratio compared with the control, but LPS with 100 nM sPIF treatments did increase (P<0.05) the ratio from the control (Table 2). At 48 hours, LPS alone and in combination with all sPIF treatments increased (P<0.05) the $PGF_{2\alpha}$ to PGE_2 secretion ratio compared with the control (Table 2). Synthetic PIF at 100 and 500 nM reduced (P<0.05) native IL-6 secretion from unchallenged explants compared with the control at 24 hours (Fig 2c), but not at 48 hours (P>0.05; Fig 2f). There was no effect (P>0.05) of sPIF on IL-6 secretion from LPS stimulated explants (Fig 2).

In Stage IV cattle, there was no interaction ($P>0.05$) between sPIF treatment and cattle type. As with Stage I cattle, LPS induced ($P<0.001$) $PGF_{2\alpha}$, PGE_2 and IL-6 secretion from explants at both 24 and 48 hours (Fig 3). There was no effect ($P>0.05$) of sPIF on $PGF_{2\alpha}$ or PGE_2 secretion with or without the presence of LPS (Fig 3). Synthetic PIF alone had no effect ($P>0.05$) on the $PGF_{2\alpha}$ to PGE_2 secretion ratio (Table 3). At both time points, LPS alone and with all sPIF treatments increased ($P<0.05$) the $PGF_{2\alpha}$ to PGE_2 secretion ratio compared with the control (Table 3). However, as with Stage I cattle, sPIF at 100 nM reduced ($P<0.05$) IL-6 secretion compared with the control at 24 hours from explants that were not challenged with LPS (Fig 3c), but not at 48 hours ($P>0.05$; Fig 3f). There was no effect of sPIF on LPS stimulated explants ($P>0.05$; Fig 3).

3.3 Experiment 3

Cytobrush smears showed no cattle to have subclinical inflammation as all samples had less than 5% PMN.

3.3.1 Serum progesterone concentrations

Stage I samples were assigned to the luteal high progesterone (LHP) group if progesterone concentrations were greater than 1 ng/mL (n=1), with the remaining samples allocated to a luteal low progesterone (LLP) group (n=3). Mean progesterone concentrations for LLP and LHP were 0.59 ± 0.24 ng/mL and 1.88 ng/mL, respectively.

Stage IV samples were allocated to a follicular high progesterone (FHP) group if progesterone concentrations were greater than 1 ng/mL (FHP; n=2) with the remaining samples assigned to a follicular low progesterone group (FLP; n=2). Mean progesterone concentrations for FLP and FHP were 0.13 ± 0.06 ng/mL and 3.88 ± 1.91 ng/mL, respectively.

There was no effect of progesterone on $PGF_{2\alpha}$, PGE_2 or IL-6 secretion within either stage of cycle (P>0.05). Therefore, the factor of progesterone was removed and results displayed together.

3.3.2 Expression of *IL6* and *IL8* in endometrial tissue

IL6 and *IL8* expression were increased (P<0.05) in both stage I and IV tissue following 50 and 500 ng/mL LPS treatments (Fig 4). The treatment of 5 ng/mL LPS increased (P<0.01) *IL6* gene expression in stage I tissue (Fig 4a). There was no effect (P>0.05) of 5 ng/mL LPS treatment on *IL6* expression in stage IV tissue or *IL8* expression in any tissue (Fig 4b, c, d).

In stage IV tissue, when sPIF was added with 500 ng/mL LPS, *IL6* gene expression was increased (P<0.05) compared with the expression induced by 500 ng/mL LPS treatment alone (Fig 4c). However, there was no effect (P>0.05) of sPIF treatment in combination with any other concentration of LPS or in the control samples in stage I or stage IV tissue (Fig 4a, c). Furthermore, there was no effect (P>0.05) of sPIF on *IL8* expression in control or LPS treated samples (Fig 4b, d).

3.3.3 IL-6 secretion from endometrial tissue

IL-6 secretion from tissue not stimulated with LPS in stage I tissue was undetectable. All three concentrations of LPS induced (P<0.05) IL-6 secretion from stage IV explants at 24 hours (Fig 5b). Only 50 and 500 ng/mL LPS treatments induced (P<0.001) IL-6 secretion from stage I explants (Fig 5a). There was no effect (P>0.05) of sPIF on IL-6 secretion from explants with or without LPS treatment (Fig 5). However, there was a statistical tendency (P<0.1) for sPIF to decrease IL-6 secretion from the 500 ng/mL LPS treatment in stage IV tissue only.

4. Discussion

This is the first study to investigate the effects of sPIF within the bovine endometrium. Although by a small amount, sPIF significantly reduced IL-6 secretion from unstimulated stage I and stage IV endometrial explants when using a pre-treatment of sPIF, but not from LPS challenged explants. Conversely, sPIF increased *IL6* mRNA expression from explants challenged with LPS, demonstrating possible differential effects of sPIF on mRNA expression and protein secretion, which needs further elucidation. There was no effect of sPIF on prostaglandin secretion from explants with or without LPS challenge. Heifers were separated from parous cows and cytobrush analysis on all tracts exhibited < 5% PMN, therefore any lack of effect was not attributed to whether the animal had previously been pregnant or had active inflammation in the uterus, respectively.

The response of endometrial tissue to LPS in the present study is comparable to previous *ex vivo* studies [11, 12, 15]. LPS significantly induced $PGF_{2\alpha}$, PGE_2, IL-6 secretion and expression and *IL8* expression from bovine endometrial explants, as previously demonstrated in bovine endometrial tissue and cell culture [11, 12, 15, 17, 29]. Prostaglandin and IL-6 secretions were above the assay limits of detection unless otherwise stated in figures. There was little change in IL-6 secretion between 24 and 48 hours. Whether IL-6 was simply not secreted after this time point, or whether it was metabolised from the explant, was not clear and further evidence for explant metabolism relative to IL-6 is currently not available.

Synthetic PIF reduced native IL-6 secretion 24 hours post treatment from both stage I and IV explants at 100 nM and at 500 nM in stage I tissue. Yet there was no effect of sPIF on individual prostaglandin secretion. Synthetic PIF is demonstrated to function in a TLR-4

dependent manner in neuronal tissue [30, 31] and IL-6 is controlled directly through the TLR-4 pathway, amongst other pathways. Within a multiple sclerosis model, murine splenocytes secreted less IL-6 and IL-17 into culture media following sPIF treatment compared with the disease control [32]. Furthermore, RAW 264.7 macrophages secrete less IL-6 and TNF-α, when cultured with sPIF, compared to both an unstimulated and irradiated control [33]. However, in decidualized human endometrial stromal cells, an increase in IL-6 protein expression was identified following sPIF treatment, converse to a decrease in IL-6 secretion in the present study [5]. IL-6 is known to be an important cytokine in the implantation period in humans, with maximum expression occurring at this point of the menstrual cycle [34]. In the pregnant bovine endometrium, *IL6* mRNA expression, amongst other immune factors, has been demonstrated to be lower in the endometrium of heifers which carried a viable early stage embryo compared to those carrying a non-viable embryo, although expression was not compared to a reference native endometrium [35]. Yet, during pregnancy, there is a milieu of hormones and immune factors, such as interferon-τ, which circulate and modulate gene expression in the endometrium [36]. The present study has investigated the effects of sPIF on cyclic tissue and so, effects of pregnancy related hormones on the actions of sPIF need to be further elucidated.

A 24 hour pre-treatment period was required to obtain an effect of sPIF on IL-6 secretion compared to the control. Previous human based studies have not used pre-treatment, but treated with sPIF for up to 24 hours [3, 5]. Yet, work on the human endometrium has only been completed on decidualized cells, which constitute the native environment for PIF within pregnancy [3, 5]. Decidua may be more responsive to sPIF as they are pregnancy specific. It is postulated that cyclic endometrial cells, not recently exposed to pregnancy signals, need a period of sPIF priming so that the peptide is able to modulate the uterine immune response.

When examining the effect of sPIF on LPS stimulated prostaglandin or IL-6 secretion in the present study, sPIF had no effect. Synthetic PIF has previously been shown to reduce nitric oxide production by macrophages, following LPS stimulation [31, 37], but this is the first study to investigate the effect of sPIF on the cytokine or prostaglandin secretion from the endometrium following LPS stimulation. As sPIF reduced native IL-6 secretion from tissue, it was considered whether the concentration of LPS was too high; a concentration of 1 µg/mL LPS may induce an acute immune response too extreme for sPIF to have an effect. Therefore, experiment 3 utilised three lower LPS concentrations based on a previous bovine endometrial explant study (5, 50 or 500 ng/mL) [11]. There was no effect of sPIF on IL-6 secretion when explants were stimulated with these lower LPS concentrations, yet stage IV *IL6* mRNA expression was up-regulated following treatment with sPIF and 500 ng/mL LPS. Moreover, in the same tissue, there was a statistical tendency for sPIF to decrease IL-6 secretion. Samples were collected at different time points, 6 hours for mRNA and 24 hours for protein secretion, to detect gene expression changes ahead of the down-stream expression of the corresponding protein secretion. Expression of *IL6* is maximally increased at 6 hours post LPS challenge. Furthermore, expression of other cytokines, such as *TNF* and *IL1B*, are severely reduced by 24 hours post LPS treatment [17, 38]. Secreted IL-6 was sampled at 24 hours to show the accumulation of IL-6 throughout the innate immune response to the LPS challenge and is the general time point used in similar work [10, 11].

The increase in *IL6* mRNA expression following sPIF treatment in LPS stimulated tissue is supported by data from human endometrial stromal cells, where sPIF increased IL-6 secretion, although decidualized cells were used and so, are not directly comparable to the present study [5]. Yet there was a tendency for IL-6 secretion to decrease in tissue where mRNA expression increases following sPIF treatment. Synthetic PIF has been shown to alter the expression of

360 microRNA (miRNA) let-7, which represses translation of proteins and destabilises mRNA, in a
361 TLR-4 dependent manner through the phosphatidylinositol-3 kinase/Akt pathway and
362 subsequent destabilisation of the KH-type splicing regulatory protein, which also regulates
363 several other miRNAs [31]. Such mechanisms of sPIF should be further studied in the bovine
364 endometrium to assess the differential effects of sPIF on *IL6* mRNA expression and subsequent
365 protein secretion.

366 There was no effect of sPIF on *IL8* mRNA expression in experiment 3, with or without
367 LPS treatment. Within decidualized human endometrial stromal cells, sPIF up-regulated IL-8
368 protein secretion to a greater extent than that of IL-6 in the same study [5]. The disagreement
369 with the present study may be due to one of several factors. Firstly, the cellular response to sPIF
370 may be different, the present study used cyclic bovine endometrial tissue, whereas previous
371 studies used decidualized human cells [5]. Additionally, the present study used whole tissue
372 comprised of epithelial and stromal cells, compared with a culture of isolated stromal cells as
373 used in human studies [5].

374 In the context of sPIF as an immune modulator within the bovine endometrium, the
375 evidence provided here shows that sPIF has limited effects. Nonetheless, it would be of interest
376 to determine the response of bovine endometrial stromal cells alone to sPIF, because postpartum
377 stromal cells are intermittently exposed through the syndesmochorial placenta in bovine
378 pregnancy.

379 In conclusion, although to a small extent, sPIF significantly reduced native IL-6 secretion
380 from healthy stage I and IV bovine endometrial explants following a 24 hour pre-treatment with
381 sPIF. Furthermore, when stage IV explants were stimulated with 500 ng/mL LPS, sPIF increased

382 *IL6* mRNA expression at 6 hours post treatment, following an initial 24 hour pre-treatment.
383 There was no effect of sPIF on prostaglandin secretion from endometrial explants. Therefore, the
384 present study demonstrates limited effects of the ability of sPIF to modulate key immune factors
385 of bovine endometrial tissue.

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394 **Conflict of interest**

395 All authors declare no conflict of interest.

396

References

[1] Stamatkin CW, Roussev RG, Stout M, Absalon-Medina V, Ramu S, Goodman C, et al. PreImplantation Factor (PIF) correlates with early mammalian embryo development-bovine and murine models. Reproductive Biol and Endocrinol. 2011;9:63.

[2] Ramu S, Stamatkin C, Timms L, Ruble M, Roussev RG, Barnea ER. PreImplantation factor (PIF) detection in maternal circulation in early pregnancy correlates with live birth (bovine model). Reproductive Biology and Endocrinology. 2013;11:105.

[3] Paidas MJ, Krikun G, Huang SJ, Jones R, Romano M, Annunziato J, et al. A genomic and proteomic investigation of the impact of preimplantation factor on human decidual cells. American Journal of Obstetrics and Gynecology. 2010;202:459 e1-8.

[4] Moindjie H, Santos ED, Gouesse RJ, Swierkowski-Blanchard N, Serazin V, Barnea ER, et al. Preimplantation factor is an anti-apoptotic effector in human trophoblasts involving p53 signaling pathway. Cell Death Dis. 2016;7:e2504.

[5] Barnea ER, Kirk D, Paidas MJ. PreImplantation Factor (PIF) promoting role in embryo implantation: increases endometrial Integrin-α2β3, amphiregulin and epiregulin while reducing betacellulin expression via MAPK in decidua. Reproductive Biology and Endocrinology. 2012;10:50.

[6] Weiss L, Or R, Jones RC, Amunugama R, JeBailey L, Ramu S, et al. Preimplantation factor (PIF*) reverses neuroinflammation while promoting neural repair in EAE model. Journal of Neurological Science. 2012a;312:146-57.

[7] Paddison J, Barnea ER, Nash D. PreImplantation Factor (PIF) displays developmental-related evolving immune anti-inflammatory response of equine endometrial explants *in vitro*. Equine Vet J Suppl. 2012;44 4.

[8] Barnea ER, Hayrabedyan S, Todorova K, Almogi-Hazan O, Or R, Guingab J, et al. PreImplantation factor (PIF*) regulates systemic immunity and targets protective regulatory and cytoskeleton proteins. Immunobiology. 2016;221:778-93.

[9] Sheldon IM, Price SB, Cronin J, Gilbert RO, Gadsby JE. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. Reproduction in Domestic Animals. 2009;44 Suppl 3:1-9.

[10] Saut JP, Healey GD, Borges AM, Sheldon IM. Ovarian steroids do not affect bovine endometrial cytokine or chemokine responses to Escherichia coli or LPS in vitro. Reproduction. 2014;148:593-606.

[11] Borges AM, Healey GD, Sheldon IM. Explants of intact endometrium to model bovine innate immunity and inflammation *ex vivo*. Am J of Reprod Immunol. 2012;67:526-39.

[12] Herath S, Lilly ST, Fischer DP, Williams EJ, Dobson H, Bryant CE, et al. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F_{2alpha} to prostaglandin E_2 in bovine endometrium. Endocrinology. 2009;150:1912-20.

[13] Poyser NL. The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukot Essent Fatty Acids. 1995;53:147-95.

[14] Weems CW, Weems YS, Randel RD. Prostaglandins and reproduction in female farm animals. Vet J. 2006;171:206-28.

[15] Herath S, Fischer DP, Werling D, Williams EJ, Lilly ST, Dobson H, et al. Expression and function of toll-like receptor 4 in the endometrial cells of the uterus. Endocrinology. 2006;147:562-70.

441 [16] Oliveira LJ, Mansouri-Attia N, Fahey AG, Browne J, Forde N, Roche JF, et al.
442 Characterization of the Th profile of the bovine endometrium during the oestrous cycle and early
443 pregnancy. PLoS ONE. 2013;8:e75571.
444 [17] Cronin JG, Turner ML, Goetze L, Bryant CE, Sheldon IM. Toll-like receptor 4 and
445 MYD88-dependent signaling mechanisms of the innate immune system are essential for the
446 response to lipopolysaccharide by epithelial and stromal cells of the bovine endometrium. Biol
447 Reprod. 2012;86:51.
448 [18] Lewis GS. Steroidal regulation of uterine immune defenses. Animal Reproduction Science.
449 2004;82-83:281-94.
450 [19] Lewis GS. Role of ovarian progesterone and potential role of prostaglandin $F_{2\alpha}$ and
451 prostaglandin E_2 in modulating the uterine response to infectious bacteria in postpartum ewes.
452 Journal of Animal Science. 2003;81:285-93.
453 [20] Ireland JJ, Murphee RL, Coulson PB. Accuracy of predicting stages of bovine estrous cycle
454 by gross anatomy of the corpus luteum. Journal of Dairy Science. 1980;63:155-60.
455 [21] Arosh JA, Parent JC, Chapdelaine P, Sirois J, Fortier MA. Expression of cyclooxygenases 1
456 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle.
457 Biology of Reproduction. 2002;67:161-9.
458 [22] Barnea ER, Kirk D, Ramu S, Rivnay B, Roussev R, Paidas MJ. PreImplantation Factor
459 (PIF) orchestrates systemic antiinflammatory response by immune cells: effect on peripheral
460 blood mononuclear cells. Am J Obstet Gynecol. 2012;207:313 e1-11.
461 [23] Duzyj CM, Barnea ER, Li M, Huang SJ, Krikun G, Paidas MJ. Preimplantation factor
462 promotes first trimester trophoblast invasion. Am J Obstet Gynecol. 2010;203:402 e1-4.

463 [24] Nash D, Lane E, Herath S, Sheldon IM. Endometrial explant culture for characterizing
464 equine endometritis. American Journal of Reprodtive Immunology. 2008;59:105-17.
465 [25] Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, et al. A
466 comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in
467 clinically normal postpartum dairy cows. Canadian Veterinary Journal. 2005;46:255-9.
468 [26] Madoz LV, Giuliodori MJ, Jaureguiberry M, Plontzke J, Drillich M, de la Sota RL. The
469 relationship between endometrial cytology during estrous cycle and cutoff points for the
470 diagnosis of subclinical endometritis in grazing dairy cows. Journal of Dairy Science.
471 2013;96:4333-9.
472 [27] Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. Prevalence of endometritis and its
473 effects on reproductive performance of dairy cows. Theriogenology. 2005;64:1879-88.
474 [28] Cheng Z, Robinson RS, Pushpakumara PGA, Mansbridge RJ, Wathes DC. Effect of dietary
475 polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. Journal of
476 Endocrinology. 2001;171:463-73.
477 [29] MacKintosh SB, Schuberth HJ, Healy LL, Sheldon IM. Polarised bovine endometrial
478 epithelial cells vectorially secrete prostaglandins and chemotactic factors under physiological
479 and pathological conditions. Reproduction. 2013;145:57-72.
480 [30] Mueller M, Schoeberlein A, Zhou J, Joerger-Messerli M, Oppliger B, Reinhart U, et al.
481 PreImplantation Factor bolsters neuroprotection via modulating Protein Kinase A and Protein
482 Kinase C signaling. Cell Death and Differentiation. 2015.
483 [31] Mueller M, Zhou J, Yang L, Gao Y, Wu F, Schoeberlein A, et al. PreImplantation factor
484 promotes neuroprotection by targeting microRNA let-7. Proceedings of the National Academy of
485 Sciences U S A. 2014;111:13882-7.

[32] Weiss L, Or R, Jones RC, Amunugama R, JeBailey L, Ramu S, et al. Preimplantation factor (PIF*) reverses neuroinflammation while promoting neural repair in EAE model. J Neurol Sci. 2012;312:146-57.

[33] Shainer R, Almogi-Hazan O, Berger A, Hinden L, Mueller M, Brodie C, et al. PreImplantation factor (PIF) therapy provides comprehensive protection against radiation induced pathologies. Oncotarget. 2016;7:58975-94.

[34] Tabibzadeh S, Kong OF, Babaknia A, May LT. Progressive rise in the expression of interleukin-6 in human endometrium during menstrual-cycle is initiated during the implantation window. Hum Reprod. 1995;10:2793-9.

[35] Beltman ME, Forde N, Lonergan P, Crowe MA. Altered endometrial immune gene expression in beef heifers with retarded embryos. Reprod Fertil Dev. 2013;25:966-70.

[36] Mamo S, Rizos D, Lonergan P. Transcriptomic changes in the bovine conceptus between the blastocyst stage and initiation of implantation. Ani Reprod Sci. 2012;134:56-63.

[37] Azar Y, Shainer R, Almogi-Hazan O, Bringer R, Compton SR, Paidas MJ, et al. PreImplantation Factor Reduces Graft-versus-Host Disease by Regulating Immune Response and Lowering Oxidative Stress (Murine Model). Biology of Blood and Marrow Transplantation. 2013;19:519-28.

[38] Swangchan-Uthai T, Lavender CR, Cheng Z, Fouladi-Nashta AA, Wathes DC. Time course of defense mechanisms in bovine endometrium in response to lipopolysaccharide. Biology of Reproduction. 2012;87:135.

[39] Wathes DC, Cheng Z, Chowdhury W, Fenwick MA, Fitzpatrick R, Morris DG, et al. Negative energy balance alters global gene expression and immune responses in the uterus of postpartum dairy cows. Physiological Genetics. 2009;39:1-13.

510 **Table 1.** Primer sequences, amplicon length and accession numbers for *ACTB*, *18s*, *IL6* and *IL8.* All primers synthesised by Sigma-
511 Aldrich.

512

Gene symbol	Gene name	Primer sequence	Amplicon length (bp)	Genbank accession number	Reference
ACTB	Actin beta	F - CAGAAGGACTCGTACGTGGG R - TTGGCCTTGGGGTTCAGGG	199	NM_173979	Cronin, Turner [17]
18SrRNA	18S ribosomal RNA	F – CGGCGACGACCCATTCGAAC R - GAATCGAACCCTGATTCCCCGTC	99	DQ222453.1	Wathes, Cheng [39]
IL6	Interleukin-6	F – CTTCTGCTTTCCCTACCCCG R - CTCCAGAAGACCAGCAGTGG	296	EU276071	Designed using PrimerBLAST (Pub Med)
IL8	Interleukin-8	F – GCAGGTATTTGTGAAGAGAGCTG R - CACAGAACATGAGGCACTGAA	148	NM_173925	Cronin, Turner [17]

513 **Table 2.** Ratio of $PGF_{2\alpha}$ to PGE_2 secretion from all Stage I endometrial explants in experiment 2
514 challenged with LPS and sPIF at 24 and 48 hours.

Treatment[1]	Time point	
	24 hours	48 hours
Control	1.30 ± 0.38^{abc}	0.85 ± 0.25^{a}
LPS	1.70 ± 0.49^{ade}	1.58 ± 0.46^{b}
sPIF 50	0.87 ± 0.25^{f}	1.10 ± 0.32^{a}
sPIF 100	0.74 ± 0.21^{f}	0.82 ± 0.24^{a}
sPIF 500	1.12 ± 0.32^{bcf}	0.94 ± 0.27^{a}
LPS & sPIF 50	1.83 ± 0.53^{bd}	1.90 ± 0.55^{b}
LPS & sPIF 100	2.01 ± 0.58^{d}	1.73 ± 0.5^{b}
LPS & sPIF 500	1.53 ± 0.44^{abe}	1.99 ± 0.57^{b}

515 [a-e] Means ± SEM within a time point, data not sharing a letter are significantly different ($P<0.05$).
516 [1] Treatments: LPS = 1 µg/mL; sPIF 50 = 50 nM; sPIF 100 = 100 nM; sPIF 500 = 500 nM.

517 **Table 3.** Ratio of $PGF_{2\alpha}$ to PGE_2 secretion from all Stage IV endometrial explants in experiment 2
518 challenged with LPS and sPIF at 24 and 48 hours.

Treatment[1]	Time point	
	24 hours	48 hours
Control	0.57 ± 0.13[a]	0.93 ± 0.29[ab]
LPS	1.14 ± 0.2[b]	1.38 ± 0.26[b]
sPIF 50	0.51 ± 0.1[a]	0.47 ± 0.08[a]
sPIF 100	0.40 ± 0.07[a]	0.67 ± 0.18[a]
sPIF 500	0.69 ± 0.17[a]	0.97 ± 0.26[ab]
LPS & sPIF 50	1.29 ± 0.27[b]	1.45 ± 0.28[b]
LPS & sPIF 100	1.44 ± 0.23[b]	1.49 ± 0.27[b]
LPS & sPIF 500	1.56 ± 0.3[b]	1.42 ± 0.24[b]

519 [a-b] Means ± SEM within a time point, data not sharing a letter are significantly different (P<0.05).
520 [1] Treatments: LPS = 1 µg/mL; sPIF 50 = 50 nM; sPIF 100 = 100 nM; sPIF 500 = 500 nM.

Fig 1. Secretion of a) $PGF_{2\alpha}$, b) PGE_2 and c) IL-6 from endometrial tissue explants in experiment 1 challenged with LPS and sPIF at the same time, displayed as average across all time points (24, 48 and 72 hours). Data are expressed as mean ± SEM. *** indicate significant differences from control (P<0.001). Bars not sharing a letter are significantly different (P<0.05).

Fig 2. Secretion of a,d) $PGF_{2\alpha}$; b,e) PGE_2 and c,f) IL-6 from all Stage I endometrial tissue explants in experiment 2 following a pre-treatment of sPIF for 24 hours and then challenge with LPS and sPIF for 24 (a,b,c) and 48 hours (d,e,f). Data are expressed as mean ± SEM. * (P<0.05); *** (P<0.001) indicates significant differences from the control.

Fig 3. Secretion of a,d) $PGF_{2\alpha}$; b,e) PGE_2 and c,f) IL-6 from all Stage IV endometrial tissue explants in experiment 2 following a pre-treatment of sPIF for 24 hours and then challenge with LPS and sPIF for 24 (a,b,c) and 48 hours (d,e,f). Data are expressed as mean ± SEM. * (P<0.05), *** (P<0.001) indicates significant differences from the control.

Fig 4. Expression of a,c) *IL6* and b,d) *IL8* in stage I (ab) and stage IV (c,d) endometrial tissue explants in experiment 3 following a pre-treatment of sPIF for 24 hours and then a challenge with three LPS concentrations alone (closed bars: 5, 50 and 500 ng/mL) or in combination with sPIF (open bars: 100 nM) for 6 hours. Expression was normalised against two housekeeping genes – *ACTB* and *18s* for each sample (ΔCq). Data are expressed as mean ± SEM. * (P<0.05), ** (P<0.01). *** (P<0.001) indicates significant differences from the control; within a graph, bars not sharing a letter are significantly different (P<0.05).

Fig 5. Secretion of IL-6 from a) stage I, and b) stage IV endometrial tissue explants in experiment 3 following a pre-treatment of sPIF for 24 hours and then a challenge with three LPS concentrations (closed bars: 5, 50 and 500 ng/mL) and sPIF (open bars:100 nM) for 24 hours.

543 Data are expressed as mean ± SEM. * ($P<0.05$); ** ($P<0.01$) indicates significant differences
544 from the control; bars not sharing a letter are significantly different ($P<0.05$). ND signifies no
545 detection of IL-6 in the samples.

546

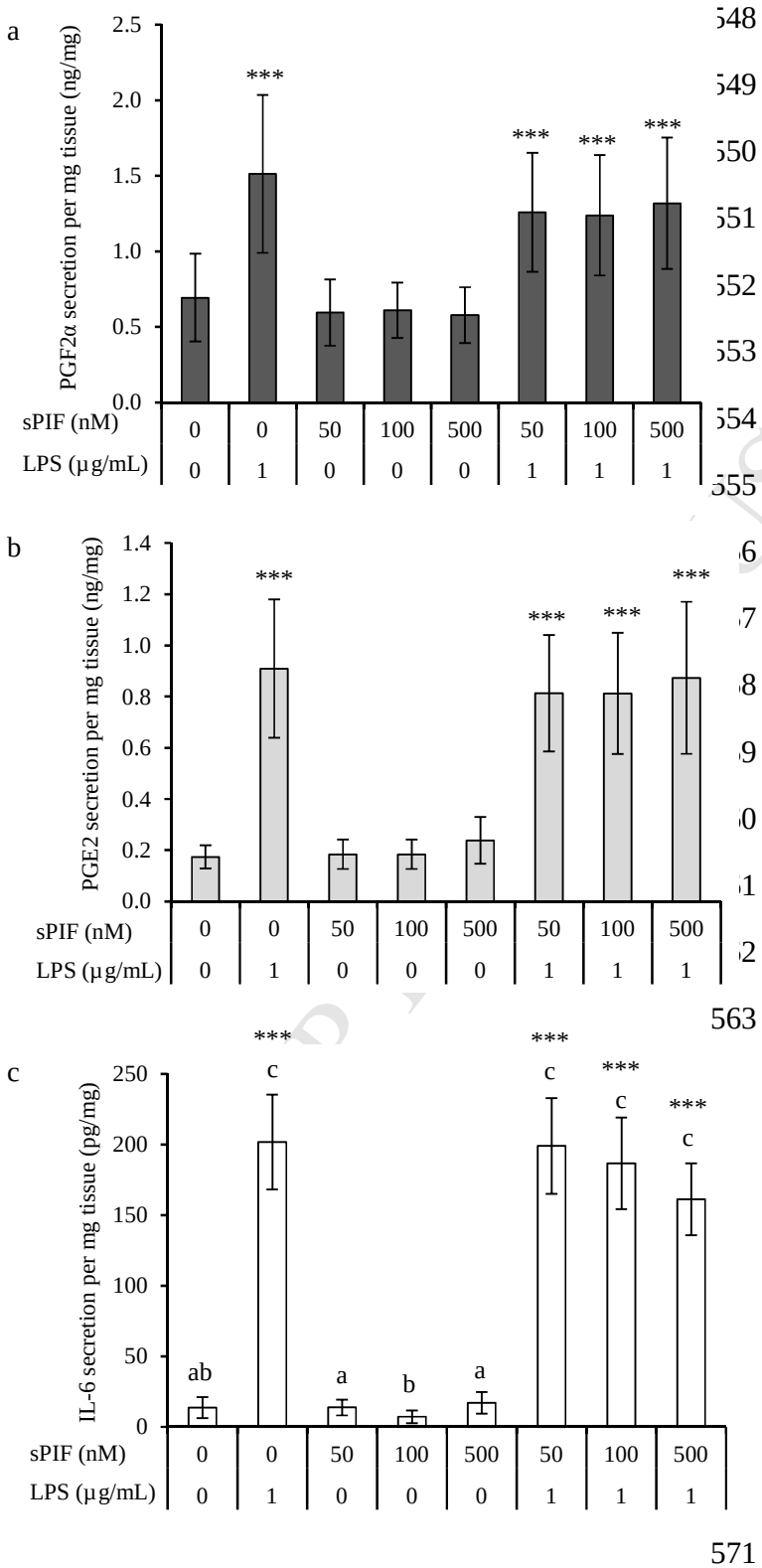
a
PGF2α secretion per mg tissue (ng/mg)
2.5
2.0
1.5
1.0
0.5
0.0
***
***
***
***
sPIF (nM) 0 0 50 100 500 50 100 500
LPS (µg/mL) 0 1 0 0 0 1 1 1
b
PGE2 secretion per mg tissue (ng/mg)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
***
sPIF (nM) 0 0 50 100 500 50 100 500
LPS (µg/mL) 0 1 0 0 0 1 1 1
c
IL-6 secretion per mg tissue (pg/mg)
250
200
150
100
50
0
***
***
***
***
ab
c
a
b
a
c
c
c
sPIF (nM) 0 0 50 100 500 50 100 500
LPS (µg/mL) 0 1 0 0 0 1 1 1

Figure 1

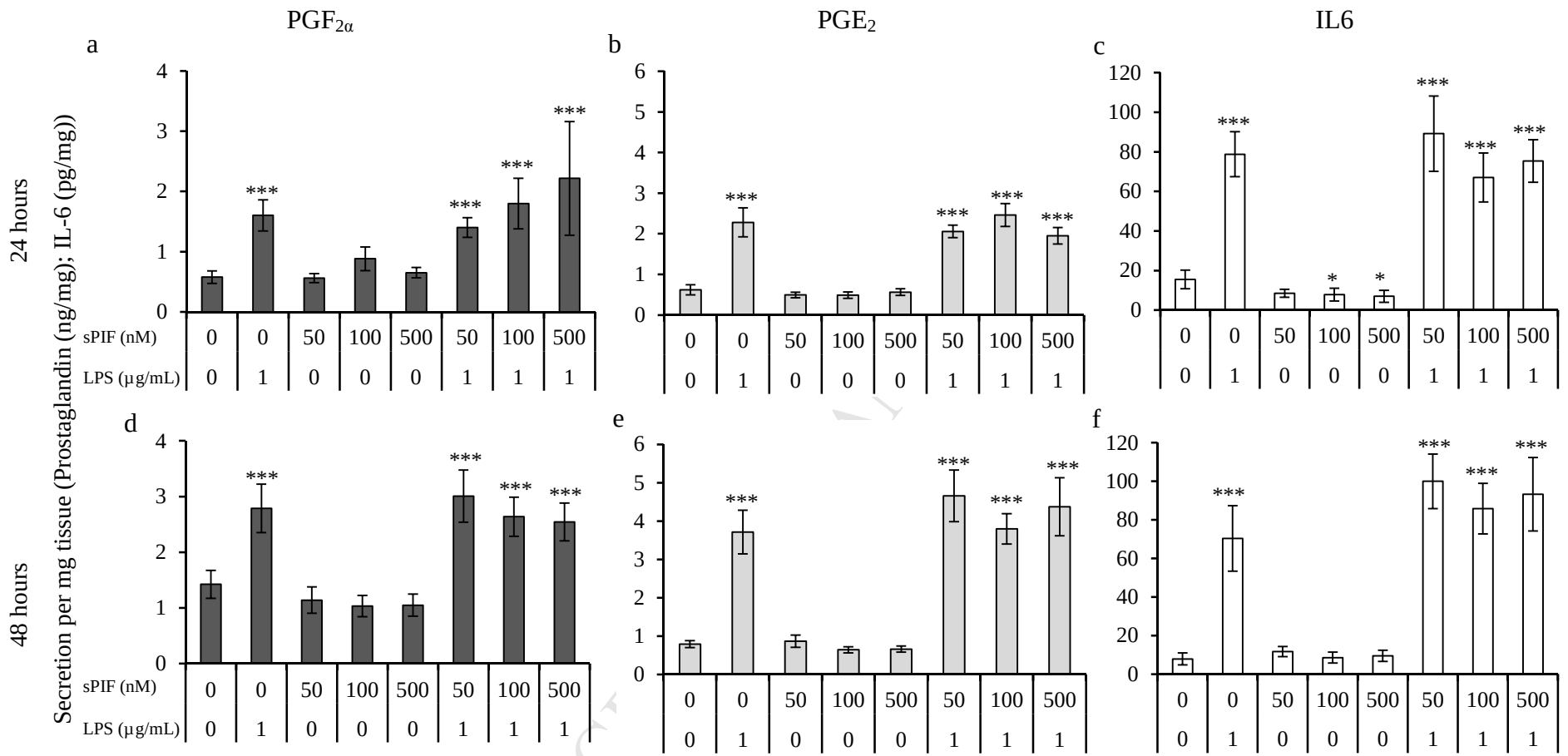
PGF2α
PGE2
IL6
a
b
c
d
e
f
24 hours
48 hours
Secretion per mg tissue (Prostaglandin (ng/mg); IL-6 (pg/mg))
sPIF (nM)
LPS (µg/mL)
0 0 50 100 500 50 100 500
0 1 0 0 0 1 1 1
***
*

Figure 2

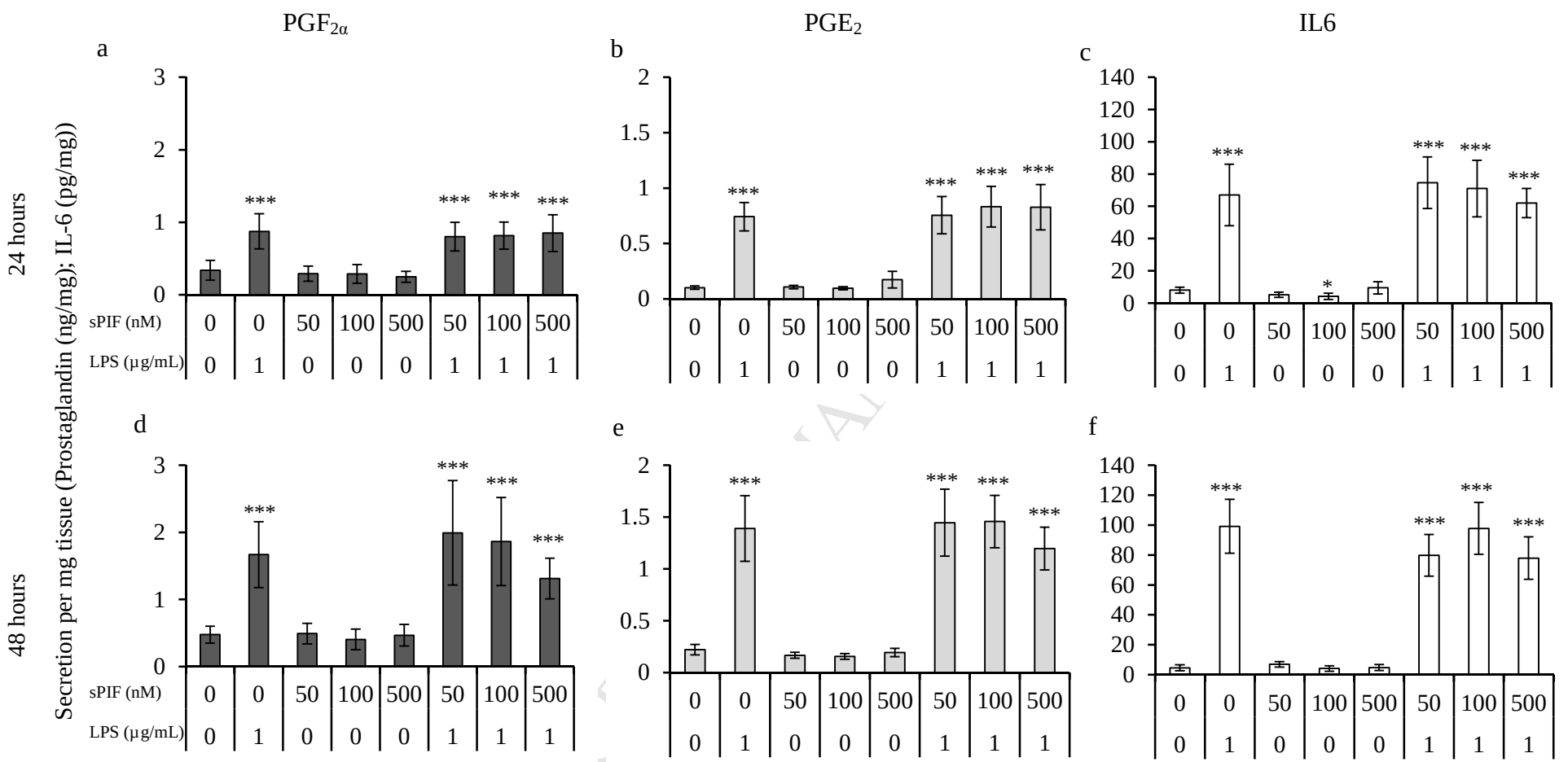
PGF2α
PGE2
IL6
a
b
c
d
e
f
24 hours
48 hours
Secretion per mg tissue (Prostaglandin (ng/mg); IL-6 (pg/mg))
sPIF (nM)
LPS (µg/mL)
0 0 50 100 500 50 100 500
0 1 0 0 0 1 1 1
***
*

Figure 3

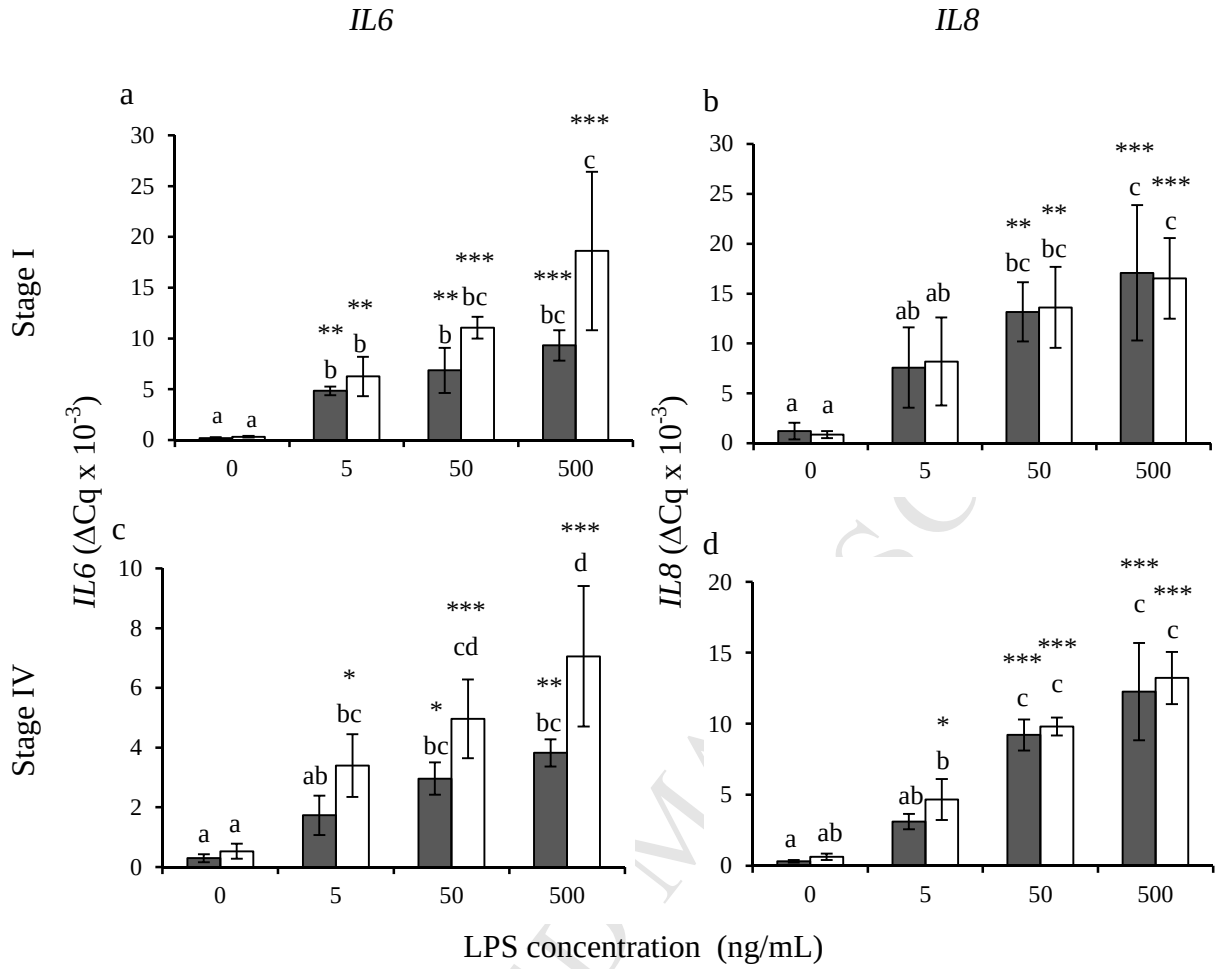

Figure 4

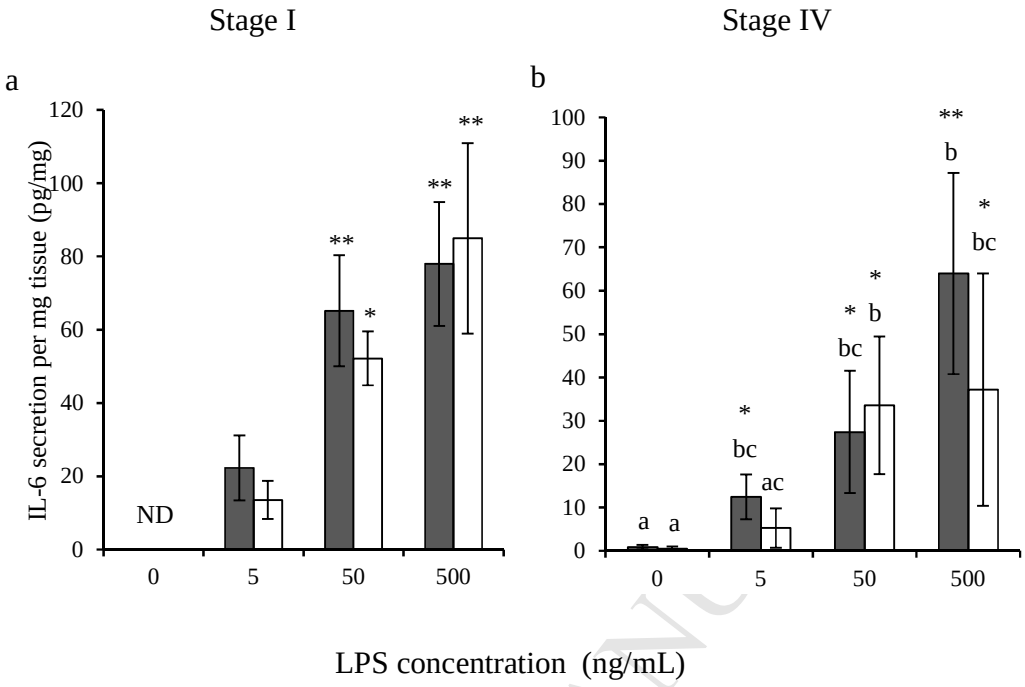

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Figure 5

Revised

Highlights

- The effect of sPIF on bovine endometrial interleukins and prostaglandins was assessed.
- A 24 hour pre-treatment with sPIF was needed to exert effects on the endometrium.
- sPIF reduced native IL-6 secretion from the endometrium.
- sPIF increased *IL6* gene expression in LPS stimulated endometrial tissue.
- There was no effect of sPIF on prostaglandin secretion or *IL8* expression.