Effect of oleic acid supplementation on prostaglandin production in maternal endometrial and fetal allantochorion cells isolated from late gestation ewes

Z Cheng[a*], DRE Abayasekara[a], M Elmes[b], S Kirkup[a], DC Wathes[a]

[a] Department of Veterinary Basic Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK.

[b] School of Biosciences, Sutton Bonington Campus, Loughborough, Leicestershire , LE12 5RD, UK.

* Corresponding author. Tel +44 1707 666 663. E-mail address: zcheng@rvc.ac.uk.

Abstract

Introduction: Elevated circulating non-esterified fatty acids including oleic acid (OA) are associated with many pregnancy related complications. Prostaglandins (PGs) play crucial roles during parturition. We investigated the effect of OA supplementation on PG production using an in vitro model of ovine placenta.

Methods: Maternal endometrium (ME) and fetal allantochorion (FC) were collected in late pregnancy (day 135). Confluent cells were cultured in serum-free medium supplemented with 0, 20 or 100 µM OA and challenged with control medium, oxytocin (OT, 250 nM), lipopolysaccharide (LPS, 0.1 µg/ml) or dexamethasone (DEX, 5 µM). Spent medium was harvested at 2 and 24 h after challenge for quantifying PGs.

Results: In ME cells OA increased PGE_2 production moderately but attenuated $PGF_{2\alpha}$ production leading to a doubling of the PGE_2:$PGF_{2\alpha}$ ratio (E:F) ($P<0.01$). Without OA, both OT and LPS stimulated PG production about 3-fold ($P<0.01$) without changing the E:F ratio. In the ME cells challenged with OT, OA decreased both PGE_2 and $PGF_{2\alpha}$ production by up to 70% ($P<0.01$) whereas in LPS treated cells OA increased the E:F ratio. In FC cells PGE_2 production at 2 h was stimulated by 100 µM OA ($P<0.05$). In these cells LPS caused a 3-fold increase in PGE_2 ($P<0.01$), an effect which was completely inhibited by DEX.

Discussion: OA supplementation favours basal PGE_2 production in both ME and FC. In ME OA increased E:F ratios and antagonized the stimulatory effect of OT on PG production. This suggests that raised circulating OA may affect both the initiation and progression of parturition.

Keywords: Oleic acid, Prostaglandins, Maternal endometrial, fetal allantochorion, Ewe.

Introduction

Maternal obesity now affects around 20% of pregnancies in many westernised countries [1]. Obesity increases the risk of many pregnancy related complications including pre-eclampsia [2], pre-term birth [3], prolonged labour [4] and emergency caesarean section [5]. Obesity is associated with aberrant lipid metabolism and higher circulating concentrations of free fatty acids [6, 7]. Women with non-esterified fatty acids (NEFA) levels in the highest tertile were at about twice the risk of spontaneous pre-term birth than those in the lowest tertile [3]. Amongst these, oleic acid (OA, 18:1n-9) accounts for about 30% of the NEFAs circulating during

pregnancy in women and up to 45% in sheep [8]: its concentration increases in women with gestational diabetes mellitus [9] and OA was the NEFA with the greatest increase (67%) associated with pre-eclampsia [6]. OA also constituted around 10% of the fatty acids present in amniotic fluid [10] and 30% of total membrane fatty acids in immortalised pregnant human myometrial cells [11]. Circulating NEFA concentrations including OA also rise during negative energy balance when body lipids are mobilised to supply extra energy [7]. In addition, OA is a principal effective ingredient within foodstuffs such as olive oil and peanuts which are believed to have positive effects on human health, such as protection from cardiovascular disease [12, 13]. Both in vivo and in vitro studies have shown that OA can reduce the negative metabolic effects of elevated dietary saturated fatty acids [14].

In mammals, various eicosanoids including prostaglandins (PGs) and leukotrienes are produced by the metabolism of n-3 and n-6 polyunsaturated fatty acids (PUFAs). The key enzymes in these metabolic pathways, Δ6, Δ5 desaturase and prostaglandin-endoperoxide synthase (PTGS), are rate limiting to a large number of substrates, including 18 and 22 carbon fatty acids [15]. Therefore, OA supplementation may alter metabolism of PUFA and the PG synthetic pathways through enzyme competition. Unsaturated fatty acids (UFAs) are important signalling molecules controlling many cellular processes [16] so OA may compete with PUFAs for incorporation into phospholipid membranes and reduce availability of the PG precursors. Such evidence was found in a study showing that unsaturated fatty acids, including OA, inhibited induced activation of NFkB and *PTGS2* expression mediated through toll-like 4 receptors [16]; [17].

PGs, especially the 2-series, are key signalling molecules in reproductive processes including ovulation, luteolysis, menstruation and parturition [18, 19]. Parturition in the ewe is initiated by activation of the fetal hypothalamic-pituitary-adrenal axis, followed by a concurrent increase in plasma cortisol and oestradiol production, that up-regulates *PTGS2* expression and down-regulates PG dehydrogenase (*PGDH*), resulting in increased PG synthesis [20]. In the late pregnant ewe, PGE_2 is produced primarily by the fetal component of the placentome, and concentrations rise over the last 3 weeks of gestation to aid fetal maturation [21]. At term, PGE_2 causes cervical dilatation whereas $PGF_{2\alpha}$ is released through oxytocin (OT) binding to its receptors in the intercotyledonary endometrium and stimulates uterine contractions and delivery [20, 22, 23]. Synthetic glucocorticoids, such as dexamethesone (DEX) mimic the endogenous cortisol rise and induce parturition and pre-term birth [20]. Intrauterine infection is another major risk factor for preterm labour [24]. The bacterial endotoxin lipopolysaccharide (LPS) increases the activities of phospholipase (PL)A2 and PTGS2, and inhibits PGDH [25, 26]. LPS induces NFkB activation which is a key signalling pathway in infection-induced preterm labour [27].

The aim of the present study was, therefore, to test the hypothesis that supplementing maternal endometrial (ME) and fetal allantochorion (FC) cells isolated from late gestation ewes with different concentrations of OA would: (i) influence PG production and (ii) alter their response to the agonists OT, LPS and DEX.

Materials and methods

All reagents were from Sigma (Sigma-Aldrich Company Ltd, Poole, Dorset, UK) or BDH Merck Ltd (Lutterworth, Leics, UK) unless otherwise stated. Solutions were sterilised by passage through a 0.20 μm filter (Nalge Nunc International, Rochester, NY14602-0365, USA or Millipore Company, Bedford, MA01730, USA). All culture media used included 50,000

units/L penicillin and 50 mg/L streptomycin. All animal experiments were carried out under the Animal (Scientific Procedures, UK) Act 1986.

Animals, cell isolation and culture

Tissues for the study were obtained post mortem from 9 ewes on Day 135 of gestation (term is approximately 145 days). ME and FC cells were separated and cultured following the methods described previously [28]. The FC cells were taken from the intercotyledonary regions of the placenta to avoid possible contamination with maternal tissue.

OA supplementation and cell challenge

OA (cis-9-Octadecenoic acid, Sigma) was dissolved in 100% ethanol to a concentration of 100 mM. It was diluted further to 10 mM in the dilution medium (serum free DMEM/F12 medium containing 0.1125 % BSA as a carrier). OT (Sigma) was dissolved in 0.01 M acetic acid at a concentration of 5 mM. Further dilution to 500 μM was made in the dilution medium. LPS from E. Coli 026.B6 (Sigma) was dissolved in distilled water at 500 μg/ml then diluted to 50 μg/ml in dilution medium. DEX (Sigma) was dissolved in 100 % ethanol to 20 mM, then diluted to 2 mM in the dilution medium. The solutions were sterilised by passing through a 0.20 μm filter (Nalge Nunc International, Rochester, NY14602-0365, USA).

The isolated endometrial cells were cultured in DMEM/F12 medium containing 10% fetal calf serum (FCS) and culture medium was changed every 48 h for 7–8 days to allow the cells to grow to confluence as checked using microscopy. Prior to the OA supplementation, the FCS present in the confluent cells was removed via incubation with 2 ml serum-free test medium (DMEM/F12 medium containing 0.1125 % BSA, 50,000 units/L penicillin and streptomycin, and 1 ml/L ITS (0.5 mg/ml insulin, 0.5 mg/ml transferin and 0.5 μg/ml selenium; Sigma) for 3 h. The cells were then cultured in 2 ml test medium containing 0 (CONT), 20 or 100 μM OA for 45 h. After this, the cells were incubated for a further 2 h or 24 h with either: (i) control medium (CM); (ii) 250 nM OT (endometrial cells only), (iii) 0.1 μg/ml LPS, (iv) 5 μM DEX or (v) 0.1 μg/ml LPS + 5 μM DEX in 1 ml of the above OA supplemented medium. Spent medium was collected and stored at -20°C until analysis. There were four replicate wells per treatment for each ewe and all treatments were repeated in cells isolated from at least 3 separate ewes. The doses of OA and challenge were selected to fall within the effective range following preliminary work using a wider dose response curve. The maximum ethanol concentration in the incubation medium was less than 0.05%.

PG radioimmunoassay (RIA)

PGE_2 and $PGF_{2\alpha}$ in the spent medium were quantified using charcoal-dextran coated RIA methods as described previously [29]. The samples were diluted (×10-200) in the RIA buffer. This allowed the measured PG concentrations to fall within the effective analytical area of the standard curves and minimized the effect of medium on the RIA to a negligible level, making extraction unnecessary. The PG antisera were a kind gift from Dr N L Poyser (University of Edinburgh, Edinburgh, UK). Their cross-reactivities were as follows: $PGF_{2\alpha}$ antiserum, 34% with $PGF_{1\alpha}$ and 25% with $PGF_{3\alpha}$; PGE_2 antiserum, 23% with PGE_1 and 15% with PGE_3. The limit of detection was 2 pg/tube for PGE_2 and 1 pg/tube for $PGF_{2\alpha}$.The limit of detection was

2 pg/tube for PGE_2 and 1 pg/tube for $PGF_{2\alpha}$. The intra-assay and inter-assay coefficients of variation were 3.5% and 6.3% for PGE_2 (n = 6), and 4.1% and 9.6% for $PGF_{2\alpha}$, respectively (n = 6).

Statistical analysis

The data are expressed as mean ± standard error (S.E.) unless otherwise stated. Data analysis was carried out using an SPSS V22 software package (SPSS Inc, Chicago, IL, USA). The data were checked for distribution using a Levene's test of equality of error variance. Where necessary, logarithmic transformation was carried out to achieve homogeneity. Analysis of variance (ANOVA) with repeated measurement was carried out via a linear mixed effect model, which took the differences between treatments, time, challenges and their interactions as the fixed effects and ewe as the random effect. Where a significant difference ($P<0.05$) was achieved, the least square means based on Fisher's LSD for multiple comparisons were calculated to examine the differences between the above fixed effects.

Results

Effect of challenge on PG production by ME cells

The effects of challenge with CONT, OT, LPS or DEX in the ME cells without OA supplementation are shown in Fig. 1. OT challenge stimulated both PGE2 and PGF2α generation at 2 h and 24 h. LPS increased PGE2 and PGF2α production at 24 h only, but to a lesser extent than OT and statistical significance was only achieved for PGF2α compared with the cells without challenge. DEX did not alter PG production in most of the treatment groups except that it decreased the PGF2α concentration at 24 h. OT and LPS did not change the ratios of PGE2 to PGF2α (E:F) while DEX increased those ratios at both 2 h and 24 h.

Effect of OA supplementation on PG production by non-challenged maternal endometrial (ME) cells

The effects of OA supplementation on unchallenged ME cells are shown in Fig. 2. OA dose-dependently increased PGE2 production at both time points compared with the CONT group although statistical significance was achieved in the cells supplemented with 100 µM OA at 2 h. At 2 h the 100 µM OA also increased $PGF_{2\alpha}$ production significantly whereas supplementation with 20 and 100 µM OA for 24 h significantly decreased $PGF_{2\alpha}$ synthesis. The E:F ratios were increased significantly following OA supplementation at both concentrations and time points.

Effect of OA supplementation on PG production by challenged ME cells

The combined effects of challenging endometrial cells with OT, LPS or DEX in the presence of OA are shown in Figs 3, 4 and 5 respectively. OA did not alter the effect of OT on PG production at 2 h whereas at 24 h production of both PGE_2 and $PGF_{2\alpha}$ was significantly inhibited. The E:F ratios were significantly increased by OA supplementation at 2 h compared to the cells without OA supplementation (Fig 3).

In the presence of LPS, supplementation with 20 or 100 μM OA stimulated PGE_2 and $PGF_{2\alpha}$ production at 2 h and 100 μM OA increased PGE_2 generation at 24 h. The E:F ratios were increased by OA supplementation compared with the CONT cells at both time points (Fig 4).

In cells treated with DEX (Fig 5), OA did not generally alter production of either PGE_2 or $PGF_{2\alpha}$ or the E:F ratios. The only significant change was in the 100 μM OA supplementation group for $PGF_{2\alpha}$ production at 2 h, where $PGF_{2\alpha}$ concentrations were higher than the CONT group.

Effect of OA supplementation on PGE_2 production by FC cells

Only PGE2 production was measured in FC cells as we have shown previously that production of PGF2α is negligible/undetectable [30]. FC cells were also not challenged with OT as they are unresponsive (data not shown). Neither LPS, DEX nor DEX + LPS given alone influenced PGE2 concentrations after 2 h. In contrast, LPS stimulated high production of PGE2 after 24 h whilst DEX not only suppressed PGE2 production at this time point but also completely prevented the stimulatory effect of LPS (Fig 6). OA alone caused a slight increase in PGE2 concentrations in non-challenged FC cells, although this was only significant with 100 μM OA after 2 h (Fig 6a). Similar results were seen after LPS challenge (Fig 6b). In FC cells challenged with DEX (Fig 6c) or the combination of DEX + LPS (Fig 6d), OA supplementation did not significantly alter PGE2 production.

Discussion

Prostaglandins generated in the fetal placenta and maternal endometrium play crucial roles in fetal maturation and parturition [19, 20]. The ewe is a well-established model for studying these mechanisms [31, 32]. OA is a major component of NEFAs with evidence to suggest that concentrations of OA are elevated in women with obesity and that this may contribute to complications such as pre-eclampsia and premature delivery [3, 6, 9]. On the other hand, OA supplementation is credited with beneficial actions on neutralising the negative effects of dietary saturated fatty acids [14, 33]. There is, however, very little evidence available to show the effects of OA on PG production in tissues from the female reproductive tract. In the present study we investigated the effect of OA supplementation alone on PG produced by cells isolated from the endometrium and fetal placenta from late gestation ewes. We also determined the effects of OA on a variety of factors known to regulate uterine PG production during pregnancy and parturition. The key findings of the study are that supplementing ME and FC cells with OA altered: 1) basal PG production; 2) the effects of OT, LPS and DEX on PG production and 3) the ratio of PGE_2 to $PGF_{2\alpha}$ produced.

Arachidonic acid (AA) released from the cell phospholipid membrane store is metabolized to PGE_2 and $PGF_{2\alpha}$ by the action of PTGS enzymes PTGS1 and PTGS2 [34]. PTGS1 is considered as a house keeping gene and is responsible for immediate responses whereas PTGS2 is an inducible enzyme and crucial for more-sustained PG production [35]. Previous studies have shown that both OT and LPS stimulate A2 and PTGS2 expression and result in increased PG output [26, 36]. In the present study, OT challenge stimulated both PGE_2 and $PGF_{2\alpha}$ production at 2 h followed by an intensive increase at 24 h while the stimulatory effect of LPS was only observed at 24 h. This is in accord with our previous studies showing the presence of OTR in luminal epithelium of late pregnant ewes [22] so OT may not only induce PTGS2 expression, but also cause an immediate release of PGs from the cells. LPS might act on PTGS2 only since this inducible enzyme needs about 6 h to reach peak expression following induction [37].

In the absence of OA, similar concentrations of PGE_2 and $PGF_{2\alpha}$ were synthesised by the endometrium and challenges with OT and LPS significantly increased PG production without altering the E:F ratios. OA supplementation to unchallenged ME cells caused a moderate increase in PGE_2 production whereas it significantly decreased $PGF_{2\alpha}$ production, thus altering the E:F ratios. OA also increased PGE_2 production in the fetal placenta. Our previous work has similarly demonstrated that supplementation of ME cells isolated from late gestation ewes with LA, conjugated LA, GLA and AA also increased the E:F ratios [28, 38]. In contrast to our data, it was demonstrated that LPS induced an endocrine switch from $PGF_{2\alpha}$ to PGE_2 in bovine uterine endometrial cells [39]. The reasons for this difference are not clear but the previous study was carried out in tissue from non-pregnant cows while the endometrial cells used here were isolated from late gestation ewes. In the non-pregnant ewe, $PGF_{2\alpha}$ is principally produced by epithelial cells and PGE_2 by stromal cells [40]. Different cell types may have different capacities for fatty acid incorporation, metabolism and signalling pathways due to differences in enzyme systems, cellular structures and membrane receptors present, and they may use different AA pools for PG production.

In endometrium both tested concentrations of OA caused a marked inhibition in the response to OT after 24 h, with PGE_2 and $PGF_{2\alpha}$ outputs reduced by about 45 and 70%, respectively. OA did not, however, prevent the rise in PGE_2 after LPS treatment. There are a number of mechanisms through which OA could reduce PG synthesis. These include competition with AA for incorporation into the phospholipid membrane so reducing AA availability for n-6 PUFA metabolism. OA may also compete for PTGS enzymes systems to reduce conversion of AA into PGs due to substrate diversity of the desaturase and PTGS isozymes [15]. This does not, however, explain the increased PGE_2 concentrations following OA treatment in unchallenged ME and FC cells. It is perhaps more likely that OA supplementation disrupted OT signalling. Both cholesterol concentrations and localization within caveolin-1 enriched domains have previously been shown to alter OT signalling [41, 42]. No evidence for this was, however, found in a study of immortalised human myometrial cells in which there were no significant differences between BSA and OA treatments with respect to changes in intracellular Ca^{2+}, phosphatidylinositol turnover or oxytocin binding capacity or binding affinity following an OT challenge [11]. Using a different approach, we have recently correlated circulating NEFA concentrations in postpartum cows treated to induce differing degrees of negative energy balance as described previously [43] with microarray gene expression data in the endometrium. The NEFA level was significantly associated with expression of over 1000 genes and activation of over 100 pathways and biological processes. Of particular relevance here were NFkB signalling and numerous inflammatory/immune pathways and its positive correlation with PTGS2 expression ($r = 0.71$) (Z. Cheng and D.C. Wathes, unpublished observations).

In terms of physiological relevance, an increase in basal PGE_2 production by OA may benefit fetal maturation, membrane rupture and cervical ripening [23]. However the inhibition of OT stimulated PG production by OA may have adverse effects on parturition and delivery as $PGF_{2\alpha}$ synthesised by the ME induces myometrial contractions and delivery. In support of this, a recent epidemiological study has shown that pregnant women consuming higher levels of OA had increased OA concentrations and OA:n-6 PUFA ratios in their maternal and neonatal plasma, and were at increased risk of complications during labour, with 50% of the women having caesarean section [44]. This was 31% higher than those that consumed low levels of OA. In contrast, preterm infants had significant lower OA in their sera, indicating insufficient OA transport in the maternal placenta [45]. Thus specific epidemiological studies are required to confirm these interesting findings.

In conclusion, chronic supplementation of ME and FC cells isolated from late gestation ewes with OA significantly changed the proportion and concentrations of PG produced and altered the responsiveness of these cells to challenges with OT and LPS. OA increased PGE_2 production in both ME and FC cells. OA supplementation favours basal PGE_2 production to increase the ratios of PGE_2 to $PGF_{2\alpha}$ in both non-challenged and LPS treated cells, whereas it significantly reduced the stimulatory effect of OT on uterine PGE_2 and $PGF_{2\alpha}$ production. These results suggest that raised circulating OA or chronic OA supplementation may affect both the initiation and progression of parturition.

Acknowledgements

The study was supported by the BBSRC, UK. The authors wish to thank Dr N.L. Poyser for his generous gift of PG antibodies.

Figure legends

Fig. 1. The effect of oleic acid (OA) supplementation on basal prostaglandin (PG) production by maternal endometrial cells isolated from late gestation ewes (day 135). Cells were grown to confluence, rinsed with fatty acid free medium, cultured for 45 h in medium containing 0, 20 or 100 µM OA, then changed with the same media and cultured for 2 and 24 h. There were four replicates per treatment for each ewe and 3 ewes per treatment. CONT = control medium, OA20 = 20 µM oleic acid, OA100 = 100 µM oleic acid. * $P<0.05$ and ** $P<0.01$ compared with CONT.

Fig. 2. The effect of challenge on prostaglandin (PG) production by maternal endometrial cells isolated from late gestation ewes (day 135). Cells were grown to confluence, rinsed with fatty acid free medium, cultured for 45 h in medium without OA, and then received the challenges. There were four replicates per treatment for each ewe and 3 ewes per treatment. Medium was collected at 2 and 24 h after challenge. CM = challenge free control, OT = 250 nM oxytocin, LPS = 0.1 µg/ml lipopolysaccharide, DEX = 5 µM dexamethasone. a>b>c>d, $P<0.05$-0.01.

Fig. 3. The effect of oleic acid supplementation on prostaglandin (PG) production by maternal endometrial cells isolated from late gestation ewes (day 135) and challenged with oxytocin. Cells were grown to confluence, rinsed with fatty acid free medium and cultured for 45 h in medium containing 0, 20 or 100 µM OA. All cells were challenged with 250 nM oxytocin for 2 and 24 h. There were four replicates per treatment for each ewe and 3 ewes per treatment. CONT = OA free control medium, OA20 = 20 µM oleic acid, OA100 = 100 µM oleic acid. ** $P<0.01$ compared with CONT.

Fig. 4. The effect of oleic acid supplementation on prostaglandin (PG) production by maternal endometrial cells isolated from late gestation ewes (day 135) and challenged with lipopolysaccharide (LPS). Cells were grown to confluence, rinsed with fatty acid free medium and cultured for 45 h in medium containing 0, 20 or 100 µM OA. All cells were challenged with 0.1 µM LPS for 2 and 24 h. There were four replicates per treatment for each ewe and 3 ewes per treatment. CONT = OA free control medium, OA20 = 20 µM oleic acid, OA100 = 100 µM oleic acid. * $P<0.05$ and ** $P<0.01$ compared with CONT.

Fig. 5. The effect of oleic acid supplementation on prostaglandin production by maternal endometrial cells isolated from late gestation ewes (day 135) and challenged with dexamethasone (DEX). Cells were grown to confluence, rinsed with fatty acid free medium and cultured for 45 h in medium containing 0, 20 or 100 µM OA. Then, all cells were challenged with 5 µM DEX for 2 and 24 h. There were four replicates per treatment for each ewe and 3 ewes per treatment. CONT = OA free control medium, OA20 = 20 µM oleic acid, OA100 = 100 µM oleic acid. * $P<0.05$ compared with CONT.

Fig. 6. The effect of oleic acid supplementation on prostaglandin E_2 production by fetal allantochorion cells isolated from late gestation ewes (day 135) at absence or presence of

different challenges. Cells were grown to confluence, rinsed with fatty acid free medium and cultured for 45 h in medium containing 0 (CONT), 20 or 100 µM OA and then challenged with control medium (a, CM), 0.1 µg/ml lipopolysaccharide (b, LPS), 5µM dexamethasone (c, DEX) or 0.1 µg/LPS + 5 µM DEX (d, LPS+DEX). CONT = OA free control medium, OA20 = 20 µM oleic acid, OA100 = 100 µM oleic acid. There were four replicates per treatment for each ewe and 3 ewes per treatment. Medium was collected at 2 and 24 h after challenge. * $P<0.05$ and ** $P<0.01$ compared with CONT.

Fig 1.

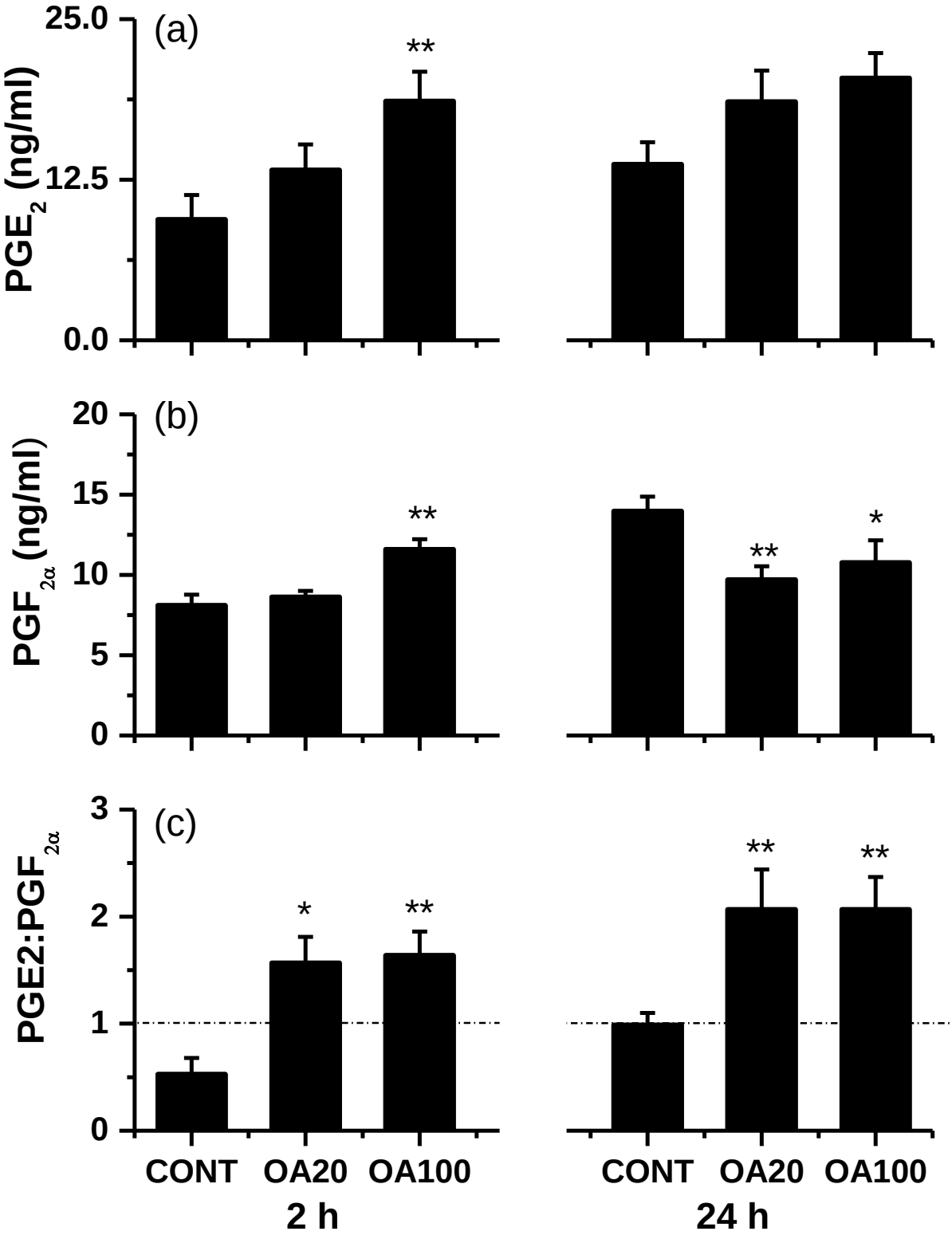

(a)
25.0
12.5
0.0
PGE2 (ng/ml)
**
(b)
20
15
10
5
0
PGF2α (ng/ml)
**
**
*
(c)
3
2
1
0
PGE2:PGF2α
*
**
**
**
CONT
OA20
OA100
2 h
CONT
OA20
OA100
24 h

Fig 2

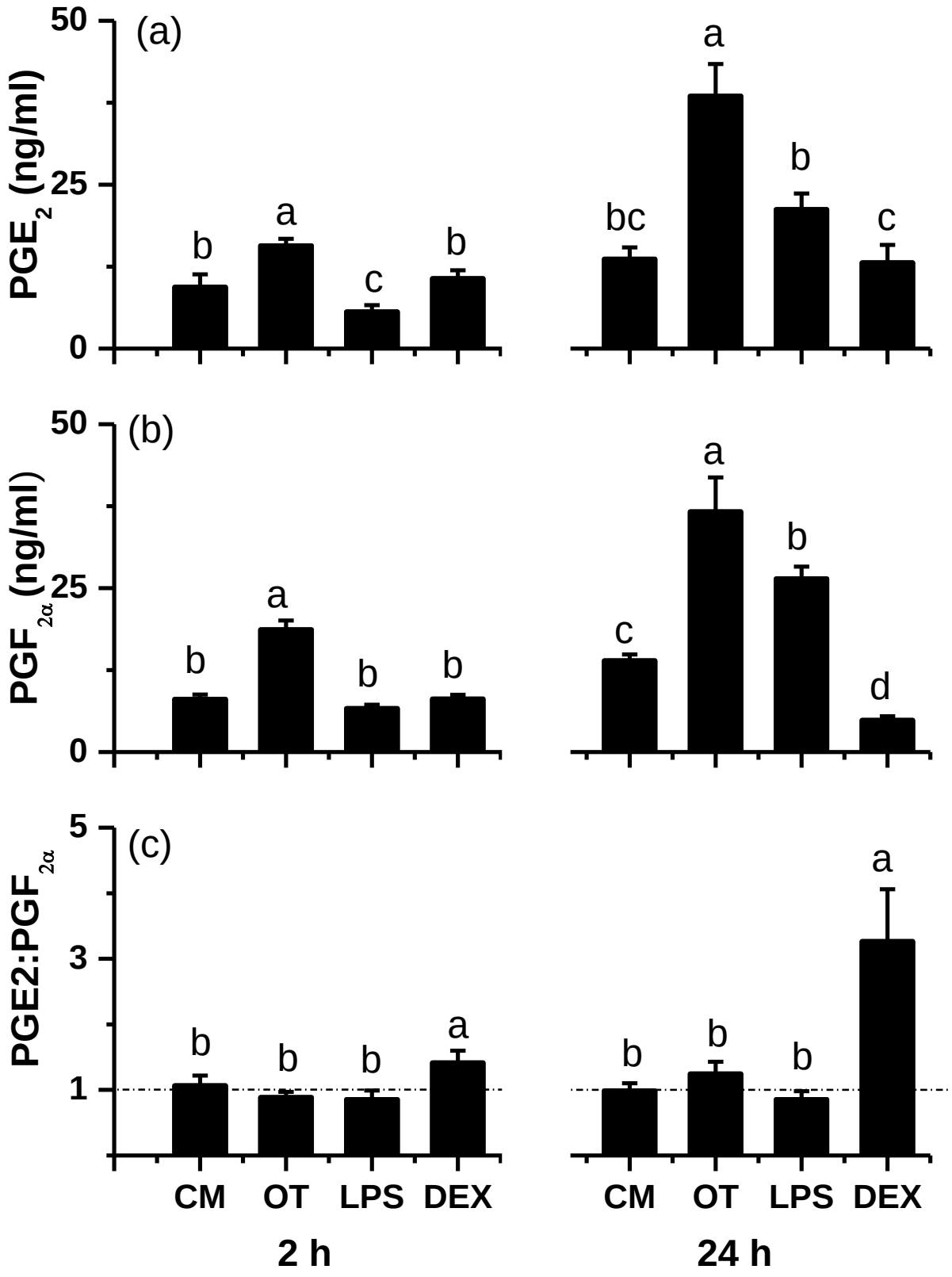

(a)
PGE2 (ng/ml)
50
25
0
b
a
c
b
bc
a
b
c
(b)
PGF2α (ng/ml)
50
25
0
b
a
b
b
c
a
b
d
(c)
PGE2:PGF2α
5
3
1
b
b
b
a
b
b
b
a
CM
OT
LPS
DEX
2 h
CM
OT
LPS
DEX
24 h

Fig 3.

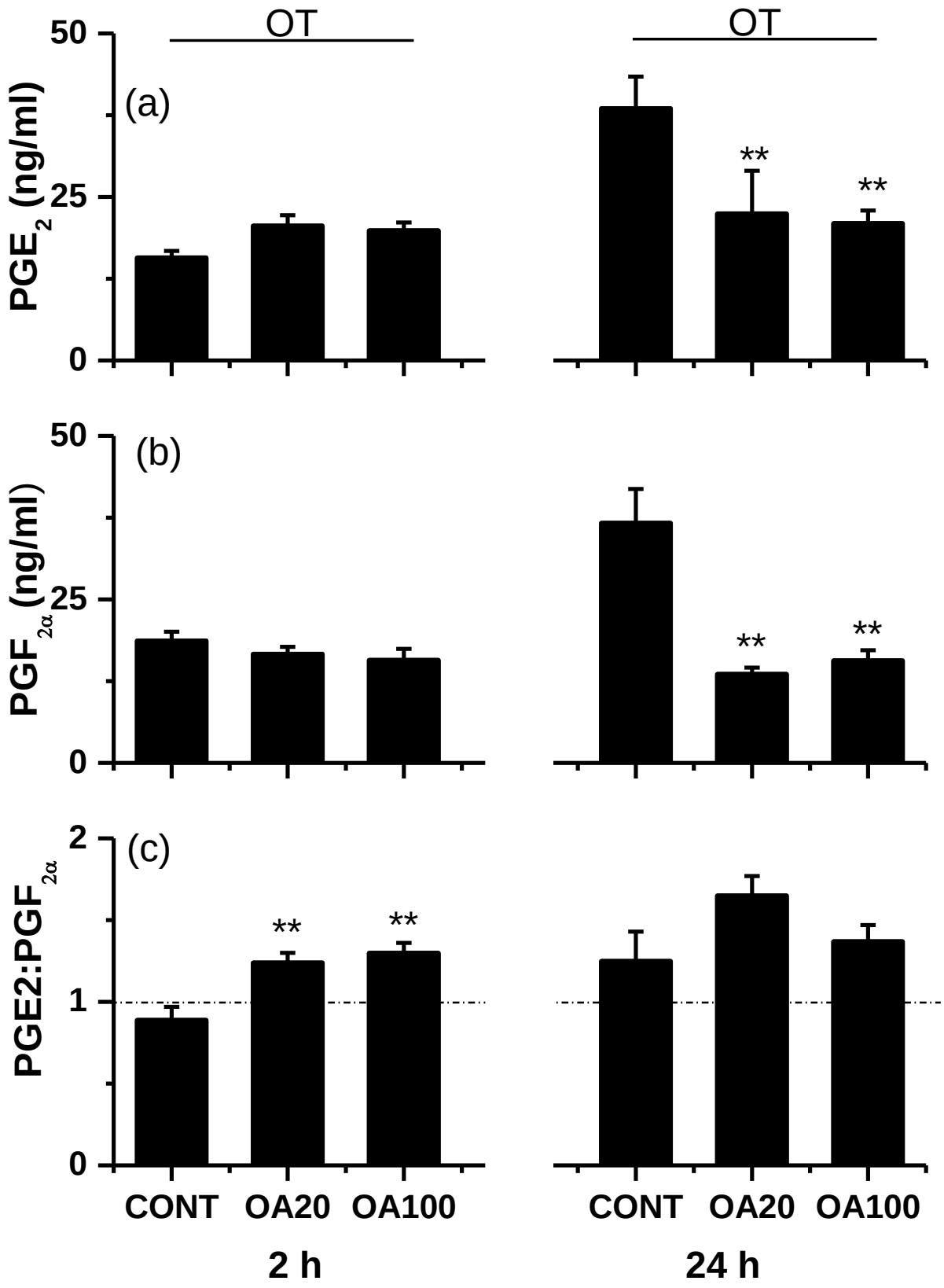

OT
OT
(a)
(b)
(c)
PGE2 (ng/ml)
PGF2α (ng/ml)
PGE2:PGF2α
50
25
0
2
1
**
CONT
OA20
OA100
2 h
24 h

Fig 4

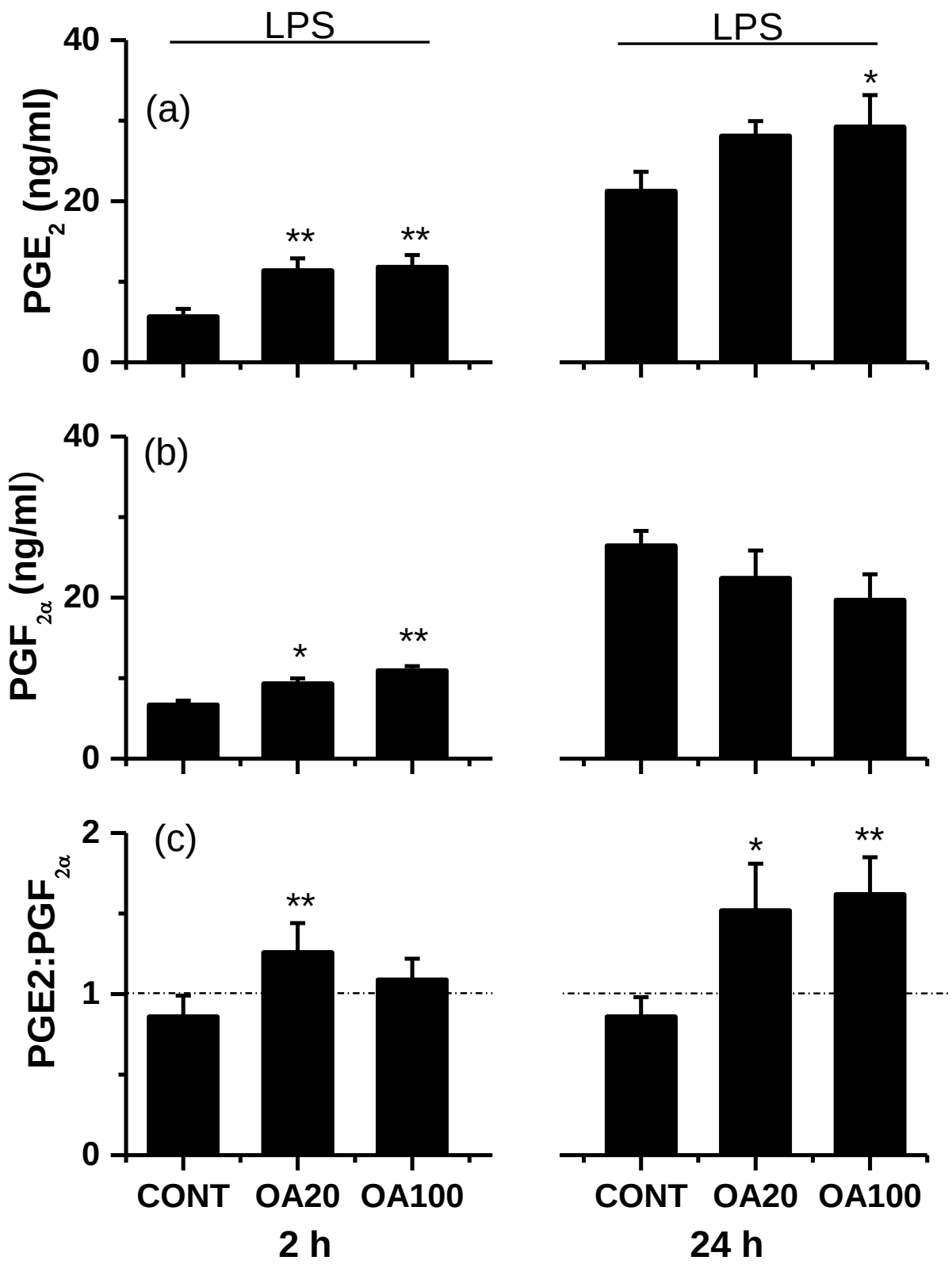

LPS
LPS
(a)
(b)
(c)
PGE2 (ng/ml)
PGF2α (ng/ml)
PGE2:PGF2α
40
20
0
2
1
0
**
**
*
**
*
**
CONT
OA20
OA100
CONT
OA20
OA100
2 h
24 h

Fig 5

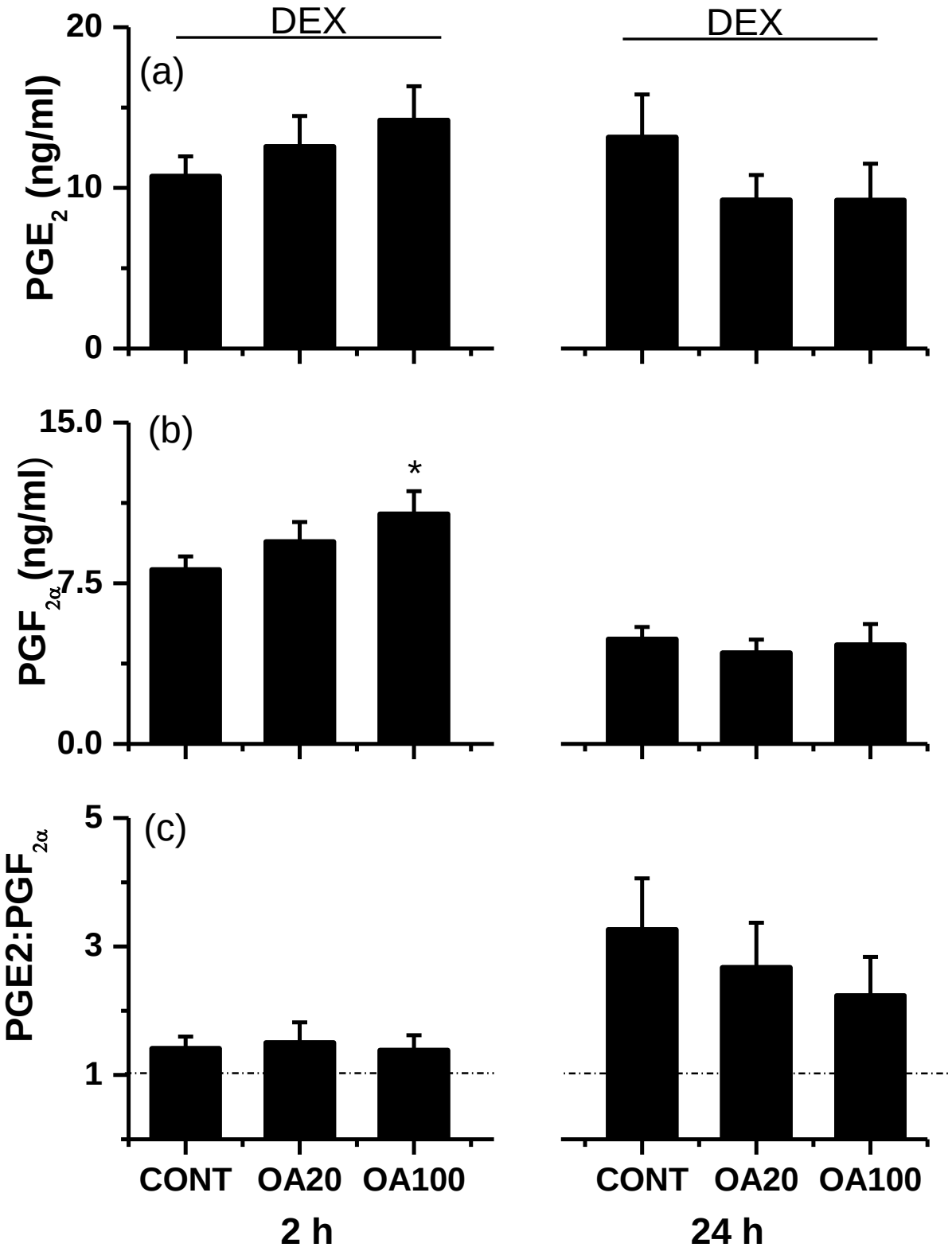
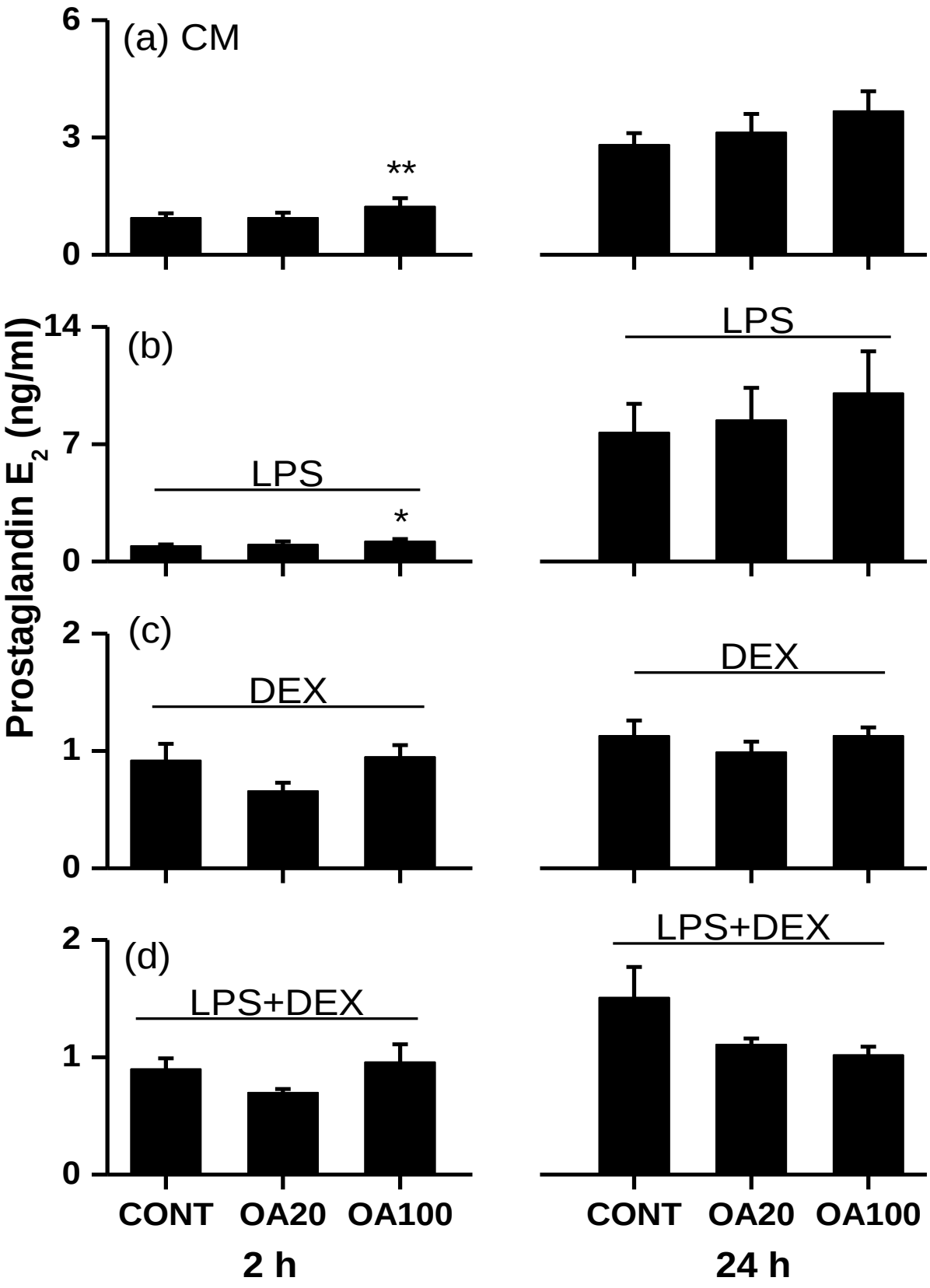

DEX
DEX
(a)
20
10
0
PGE2 (ng/ml)
(b)
15.0
7.5
0.0
PGF2α (ng/ml)
*
(c)
5
3
1
PGE2:PGF2α
CONT
OA20
OA100
2 h
CONT
OA20
OA100
24 h

Fig 6


(a) CM
(b)
(c)
(d)
LPS
DEX
LPS+DEX
**
*
Prostaglandin E2 (ng/ml)
6
3
0
14
7
2
1
CONT
OA20
OA100
2 h
24 h

References

(Max 45 Refs)

[1] Chu SY, Kim SY and Bish CL. Prepregnancy obesity prevalence in the United States, 2004-2005. Matern Child Health J 2009; 13(5):614-20.
[2] Robinson NJ, Minchell LJ, Myers JE, Hubel CA and Crocker IP. A potential role for free fatty acids in the pathogenesis of preeclampsia. J Hypertens 2009; 27(6):1293-302.
[3] Catov JM, Bertolet M, Chen YF, Evans RW and Hubel CA. Nonesterified fatty acids and spontaneous preterm birth: a factor analysis for identification of risk patterns. Am J Epidemiol 2014; 179(10):1208-15.
[4] Zhang J, Bricker L, Wray S and Quenby S. Poor uterine contractility in obese women. BJOG 2007; 114(3):343-8.
[5] Crane SS, Wojtowycz MA, Dye TD, Aubry RH and Artal R. Association between pre-pregnancy obesity and the risk of cesarean delivery. Obstet Gynecol 1997; 89(2):213-6.
[6] Villa PM, Laivuori H, Kajantie E and Kaaja R. Free fatty acid profiles in preeclampsia. Prostaglandins Leukot Essent Fatty Acids 2009; 81(1):17-21.
[7] Nielsen TS, Jessen N, Jorgensen JO, Moller N and Lund S. Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease. J Mol Endocrinol 2014; 52(3):R199-222.
[8] Noble RC, Steele W and Moore JH. The plasma lipids of the ewe during pregnancy and lactation. Res Vet Sci 1971; 12(1):47-53.
[9] Chen X, Scholl TO, Leskiw M, Savaille J and Stein TP. Differences in maternal circulating fatty acid composition and dietary fat intake in women with gestational diabetes mellitus or mild gestational hyperglycemia. Diabetes Care 2010; 33(9):2049-54.
[10] Witkin SS, Skupski D, Herway C, Rudge MV, Saito F and Harris M. Fatty acid composition of mid-trimester amniotic fluid in women of different ethnicities. J Matern Fetal Neonatal Med 2012; 25(6):818-21.
[11] Kim PY, Zhong M, Kim YS, Sanborn BM and Allen KG. Long chain polyunsaturated fatty acids alter oxytocin signaling and receptor density in cultured pregnant human myometrial smooth muscle cells. PLoS One 2012; 7(7):e41708.
[12] Carluccio MA, Massaro M, Bonfrate C, Siculella L, Maffia M, Nicolardi G, Distante A, Storelli C and De Caterina R. Oleic acid inhibits endothelial activation : A direct vascular antiatherogenic mechanism of a nutritional component in the mediterranean diet. Arterioscler Thromb Vasc Biol 1999; 19(2):220-8.
[13] Lunn J and Theobald HE. The health effects of dietary unsaturated fatty acids. Nutrition Bulletin 2006; 31(3):178-224.
[14] Alves RD, Moreira AP, Macedo VS, de Cassia Goncalves Alfenas R, Bressan J, Mattes R and Costa NM. Regular intake of high-oleic peanuts improves fat oxidation and body composition in overweight/obese men pursuing a energy-restricted diet. Obesity (Silver Spring) 2014; 22(6):1422-9.
[15] Guillou H, D'Andrea S, Rioux V, Jan S and Legrand P. The surprising diversity of Delta6-desaturase substrates. Biochem Soc Trans 2004; 32(Pt 1):86-7.
[16] Wendell SG, Baffi C, Holguin F. Fatty acids, inflammation, and asthma
J Allergy Clin Immunol 2014; 133 (5): 1255–1264.
[17] Lee JY, Sohn KH, Rhee SH and Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 2001; 276(20):16683-9.

[18] Poyser NL. The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukot Essent Fatty Acids 1995; 53(3):147-95.
[19] Jabbour HN, Sales KJ, Catalano RD and Norman JE. Inflammatory pathways in female reproductive health and disease. Reproduction 2009; 138(6):903-19.
[20] Challis JR, Sloboda DM, Alfaidy N, Lye SJ, Gibb W, Patel FA, Whittle WL and Newnham JP. Prostaglandins and mechanisms of preterm birth. Reproduction 2002; 124(1):1-17.
[21] Thorburn GD. The placenta and the control of fetal breathing movements. Reprod Fertil Dev 1995; 7(3):577-94.
[22] Leung ST, Wathes DC, Young IR and Jenkin G. Effect of labor induction on the expression of oxytocin receptor, cytochrome P450 aromatase, and estradiol receptor in the reproductive tract of the late-pregnant ewe. Biol Reprod 1999; 60(4):814-20.
[23] Whittle WL, Patel FA, Alfaidy N, Holloway AC, Fraser M, Gyomorey S, Lye SJ, Gibb W and Challis JR. Glucocorticoid regulation of human and ovine parturition: the relationship between fetal hypothalamic-pituitary-adrenal axis activation and intrauterine prostaglandin production. Biol Reprod 2001; 64(4):1019-32.
[24] Goncalves LF, Chaiworapongsa T and Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev 2002; 8(1):3-13.
[25] Lamont RF, Anthony F, Myatt L, Booth L, Furr PM and Taylor-Robinson D. Production of prostaglandin E2 by human amnion in vitro in response to addition of media conditioned by microorganisms associated with chorioamnionitis and preterm labor. Am J Obstet Gynecol 1990; 162(3):819-25.
[26] Flynn JT and Hoff H, 3rd. Lipopolysaccharide induces time-dependent increases in prostaglandin H synthase-2 and cytosolic phospholipase A2 mRNA in cultured human microvessel-derived endothelial cells. Shock 1995; 4(6):433-40.
[27] Sharp GC, Ma H, Saunders PT and Norman JE. A computational model of lipopolysaccharide-induced nuclear factor kappa B activation: a key signalling pathway in infection-induced preterm labour. PLoS One 2013; 8(7):e70180.
[28] Cheng Z, Elmes M, Abayasekara DR and Wathes DC. Effects of conjugated linoleic acid on prostaglandins produced by cells isolated from maternal intercotyledonary endometrium, fetal allantochorion and amnion in late pregnant ewes. Biochim Biophys Acta 2003; 1633(3):170-8.
[29] Cheng Z, Robinson RS, Pushpakumara PG, Mansbridge RJ and Wathes DC. Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. J Endocrinol 2001; 171(3):463-73.
[30] Cheng Z, Elmes M, Kirkup S, Abayasekara DR and Wathes DC. Effects of n-6 polyunsaturated fatty acids on prostaglandin production in ovine fetal chorion cells in vitro in late gestation ewes 2011; 32(10):752-756.
[31] Karsch FJ and Battaglia DF. Mechanisms for endotoxin-induced disruption of ovarian cyclicity: observations in sheep. Reprod Suppl 2002; 59:101-13.
[32] Lewis GS. Role of ovarian progesterone and potential role of prostaglandin F2alpha and prostaglandin E2 in modulating the uterine response to infectious bacteria in postpartum ewes. J Anim Sci 2003; 81(1):285-93.
[33] Aardema H, Vos PL, Lolicato F, Roelen BA, Knijn HM, Vaandrager AB, Helms JB and Gadella BM. Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. Biol Reprod 2011; 85(1):62-9.
[34] Smith WL, Marnett LJ and DeWitt DL. Prostaglandin and thromboxane biosynthesis. Pharmacol Ther 1991; 49(3):153-79.
[35] Smith WL, Garavito RM and DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 1996; 271(52):33157-60.

[36] Asselin E, Drolet P and Fortier MA. Cellular mechanisms involved during oxytocin-induced prostaglandin F2alpha production in endometrial epithelial cells in vitro: role of cyclooxygenase-2. Endocrinology 1997; 138(11):4798-805.
[37] Reddy ST, Tiano HF, Langenbach R, Morham SG and Herschman HR. Genetic evidence for distinct roles of COX-1 and COX-2 in the immediate and delayed phases of prostaglandin synthesis in mast cells. Biochem Biophys Res Commun 1999; 265(1):205-10.
[38] Cheng Z, Elmes M, Kirkup S, Abayasekara DR and Wathes DC. Alteration of prostaglandin production and agonist responsiveness by n-6 polyunsaturated fatty acids in endometrial cells from late-gestation ewes. J Endocrinol (2004); 182(2): 249-256.
[39] Herath S, Lilly ST, Fischer DP, Williams EJ, Dobson H, Bryant CE, and Sheldon IM. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2alpha to prostaglandin E2 in bovine endometrium. Endocrinology 2009; 150(4):1912–1920
[40] Cherny RA and Findlay JK. Separation and culture of ovine endometrial epithelial and stromal cells: evidence of morphological and functional polarity. Biol Reprod 1990; 43(2):241-250.
[41] Klein U, Gimpl G and Fahrenholz F. Alteration of the myometrial plasma membrane cholesterol content with beta-cyclodextrin modulates the binding affinity of the oxytocin receptor. Biochemistry 1995; 34(42):13784-93.
[42] Rimoldi V, Reversi A, Taverna E, Rosa P, Francolini M, Cassoni P, Parenti M and Chini B. Oxytocin receptor elicits different EGFR/MAPK activation patterns depending on its localization in caveolin-1 enriched domains. Oncogene 2003; 22(38):6054-60.
[43] Wathes DC, Cheng Z, Chowdhury W, Fenwick MA, Fitzpatrick R, Morris DG, Patton J, Murphy JJ. Negative energy balance alters global gene expression and immune responses in the uterus of postpartum dairy cows. Physiol Genomics 2009; 39(1):1-13.
[34] Wang Y. Comparative fatty acid status of population groups from inland, lake/river and coastal regions of China. Implications for pregnancy and non-communicable diseases 2010; Ph.D. London Metropolitan University.
[45] Arsic A, Vucic V, Prekajski N, Tepsic J, Ristic-Medic D, Velickovic V and Glibetic M. Different fatty acid composition of serum phospholipids of small and appropriate for gestational age preterm infants and of milk from their mothers. Hippokratia 2012; 16(3):230-5.