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

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## 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  $\mu$ M OA and challenged with control medium, oxytocin (OT, 250 nM), lipopolysaccharide (LPS, 0.1  $\mu$ g/ml) or dexamethasone (DEX, 5  $\mu$ M). Spent medium was harvested at 2 and 24 h after challenge for quantifying PGs.

*Results:* In ME cells OA increased PGE<sub>2</sub> production moderately but attenuated PGF<sub>2 $\alpha$</sub>  production leading to a doubling of the PGE<sub>2</sub>:PGF<sub>2 $\alpha$</sub>  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<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  production by up to 70% (P<0.01) whereas in LPS treated cells OA increased the E:F ratio. In FC cells PGE<sub>2</sub> production at 2 h was stimulated by 100  $\mu$ M OA (P<0.05). In these cells LPS caused a 3-fold increase in PGE<sub>2</sub> (P<0.01), an effect which was completely inhibited by DEX.

*Discussion:* OA supplementation favours basal PGE<sub>2</sub> 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,  $\Delta 6$ ,  $\Delta 5$  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 NF $\kappa$ B 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<sub>2</sub> 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<sub>2</sub> causes cervical dilatation whereas PGF<sub>2 $\alpha$</sub>  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 dexamethasone (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)A<sub>2</sub> and *PTGS2*, and inhibits *PGDH* [25, 26]. LPS induces NF $\kappa$ B 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  $\mu$ m 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  $\mu$ M was made in the dilution medium. LPS from E. Coli 026.B6 (Sigma) was dissolved in distilled water at 500  $\mu$ g/ml then diluted to 50  $\mu$ g/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  $\mu$ m 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 transferrin and 0.5  $\mu$ g/ml selenium; Sigma) for 3 h. The cells were then cultured in 2 ml test medium containing 0 (CONT), 20 or 100  $\mu$ M 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  $\mu$ g/ml LPS, (iv) 5  $\mu$ M DEX or (v) 0.1  $\mu$ g/ml LPS + 5  $\mu$ M 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<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  in the spent medium were quantified using charcoal-dextran coated RIA methods as described previously [29]. The samples were diluted ( $\times$ 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<sub>2 $\alpha$</sub>  antiserum, 34% with PGF<sub>1 $\alpha$</sub>  and 25% with PGF<sub>3 $\alpha$</sub> ; PGE<sub>2</sub> antiserum, 23% with PGE<sub>1</sub> and 15% with PGE<sub>3</sub>. The limit of detection was 2 pg/tube for PGE<sub>2</sub> and 1 pg/tube for PGF<sub>2 $\alpha$</sub> . The limit of detection was

2 pg/tube for PGE<sub>2</sub> and 1 pg/tube for PGF<sub>2α</sub>. The intra-assay and inter-assay coefficients of variation were 3.5% and 6.3% for PGE<sub>2</sub> (n = 6), and 4.1% and 9.6% for PGF<sub>2α</sub>, 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 PGE<sub>2</sub> and PGF<sub>2α</sub> generation at 2 h and 24 h. LPS increased PGE<sub>2</sub> and PGF<sub>2α</sub> production at 24 h only, but to a lesser extent than OT and statistical significance was only achieved for PGF<sub>2α</sub> compared with the cells without challenge. DEX did not alter PG production in most of the treatment groups except that it decreased the PGF<sub>2α</sub> concentration at 24 h. OT and LPS did not change the ratios of PGE<sub>2</sub> to PGF<sub>2α</sub> (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 PGE<sub>2</sub> 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<sub>2α</sub> production significantly whereas supplementation with 20 and 100 μM OA for 24 h significantly decreased PGF<sub>2α</sub> 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<sub>2</sub> and PGF<sub>2α</sub> 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  $\mu\text{M}$  OA stimulated  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  production at 2 h and 100  $\mu\text{M}$  OA increased  $\text{PGE}_2$  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  $\text{PGE}_2$  or  $\text{PGF}_{2\alpha}$  or the E:F ratios. The only significant change was in the 100  $\mu\text{M}$  OA supplementation group for  $\text{PGF}_{2\alpha}$  production at 2 h, where  $\text{PGF}_{2\alpha}$  concentrations were higher than the CONT group.

#### *Effect of OA supplementation on $\text{PGE}_2$ production by FC cells*

Only  $\text{PGE}_2$  production was measured in FC cells as we have shown previously that production of  $\text{PGF}_{2\alpha}$  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  $\text{PGE}_2$  concentrations after 2 h. In contrast, LPS stimulated high production of  $\text{PGE}_2$  after 24 h whilst DEX not only suppressed  $\text{PGE}_2$  production at this time point but also completely prevented the stimulatory effect of LPS (Fig 6). OA alone caused a slight increase in  $\text{PGE}_2$  concentrations in non-challenged FC cells, although this was only significant with 100  $\mu\text{M}$  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  $\text{PGE}_2$  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  $\text{PGE}_2$  to  $\text{PGF}_{2\alpha}$  produced.

Arachidonic acid (AA) released from the cell phospholipid membrane store is metabolized to  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  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  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  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<sub>2</sub> and PGF<sub>2α</sub> 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<sub>2</sub> production whereas it significantly decreased PGF<sub>2α</sub> production, thus altering the E:F ratios. OA also increased PGE<sub>2</sub> 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<sub>2α</sub> to PGE<sub>2</sub> 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<sub>2α</sub> is principally produced by epithelial cells and PGE<sub>2</sub> 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<sub>2</sub> and PGF<sub>2α</sub> outputs reduced by about 45 and 70%, respectively. OA did not, however, prevent the rise in PGE<sub>2</sub> 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<sub>2</sub> 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<sup>2+</sup>, 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 NFκB 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<sub>2</sub> 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<sub>2α</sub> 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<sub>2</sub> production in both ME and FC cells. OA supplementation favours basal PGE<sub>2</sub> production to increase the ratios of PGE<sub>2</sub> to PGF<sub>2α</sub> in both non-challenged and LPS treated cells, whereas it significantly reduced the stimulatory effect of OT on uterine PGE<sub>2</sub> and PGF<sub>2α</sub> production. These results suggest that raised circulating OA or chronic OA supplementation may affect both the initiation and progression of parturition.

### **Acknowledgements**

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## 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  $\mu\text{M}$  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  $\mu\text{M}$  oleic acid, OA100 = 100  $\mu\text{M}$  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  $\mu\text{g/ml}$  lipopolysaccharide, DEX = 5  $\mu\text{M}$  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  $\mu\text{M}$  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  $\mu\text{M}$  oleic acid, OA100 = 100  $\mu\text{M}$  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  $\mu\text{M}$  OA. All cells were challenged with 0.1  $\mu\text{M}$  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  $\mu\text{M}$  oleic acid, OA100 = 100  $\mu\text{M}$  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  $\mu\text{M}$  OA. Then, all cells were challenged with 5  $\mu\text{M}$  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  $\mu\text{M}$  oleic acid, OA100 = 100  $\mu\text{M}$  oleic acid. \*  $P < 0.05$  compared with CONT.

**Fig. 6.** The effect of oleic acid supplementation on prostaglandin  $\text{E}_2$  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  $\mu\text{M}$  OA and then challenged with control medium (a, CM), 0.1  $\mu\text{g/ml}$  lipopolysaccharide (b, LPS), 5 $\mu\text{M}$  dexamethasone (c, DEX) or 0.1  $\mu\text{g/LPS}$  + 5  $\mu\text{M}$  DEX (d, LPS+DEX). CONT = OA free control medium, OA20 = 20  $\mu\text{M}$  oleic acid, OA100 = 100  $\mu\text{M}$  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.

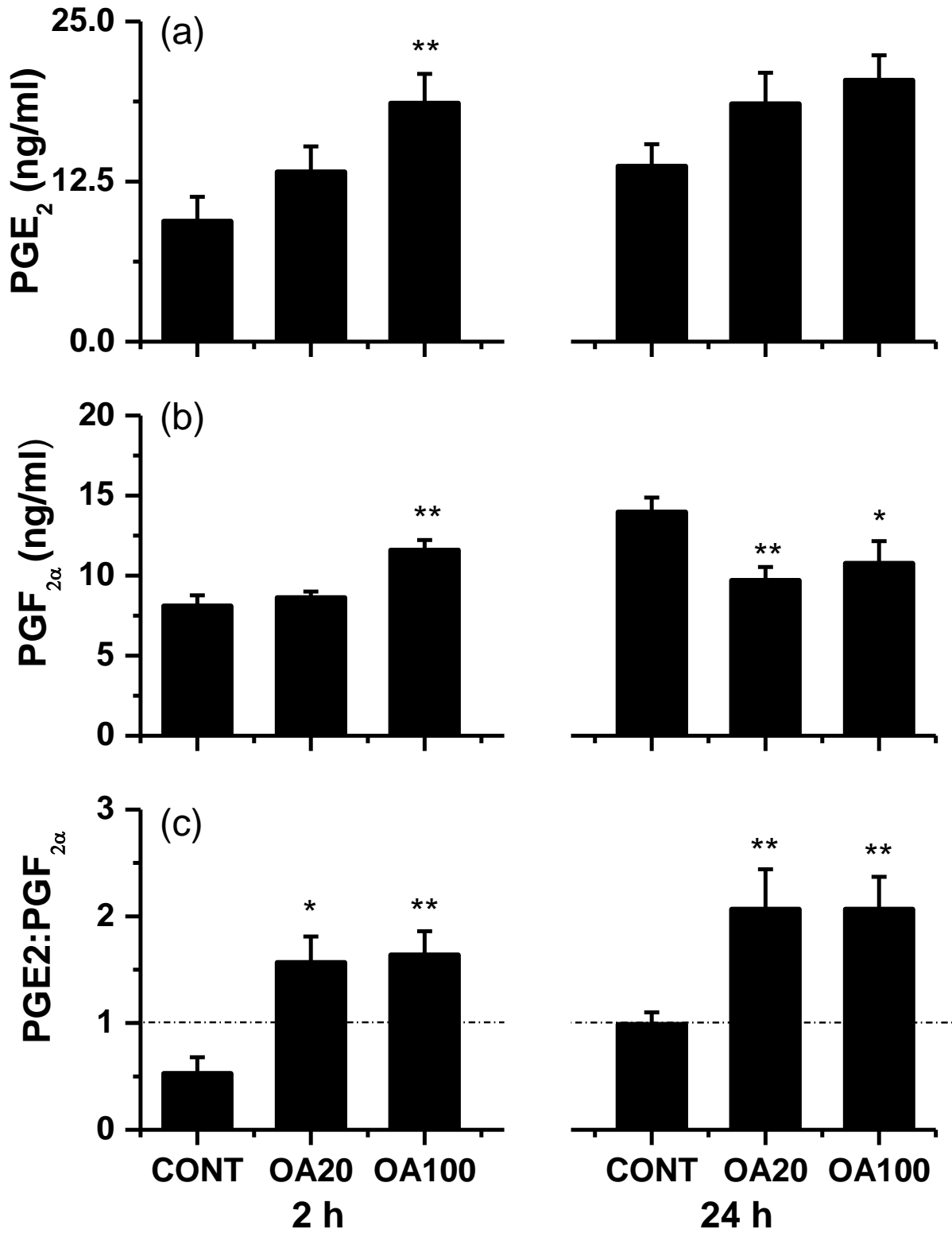


Fig 2

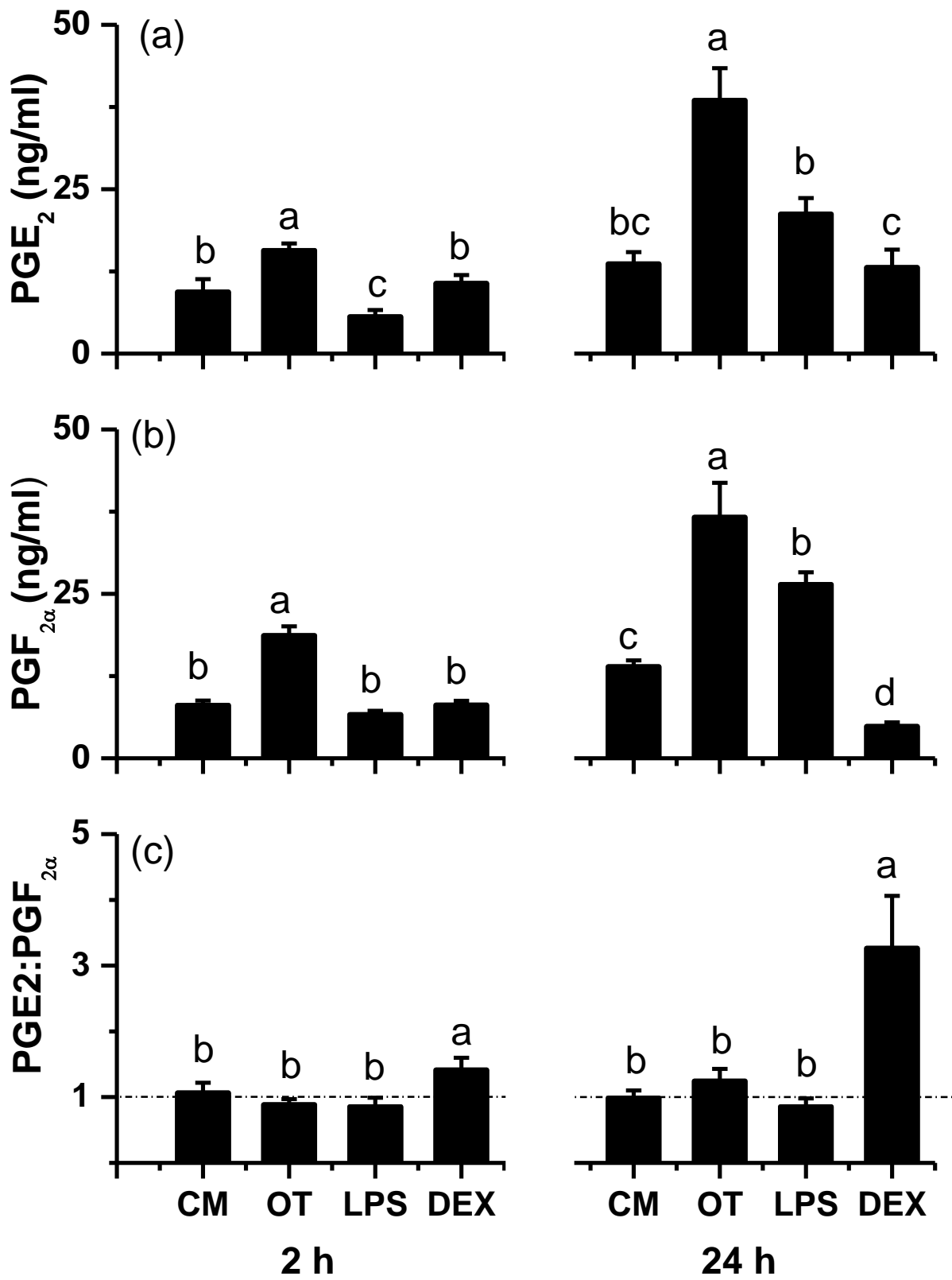


Fig 3.

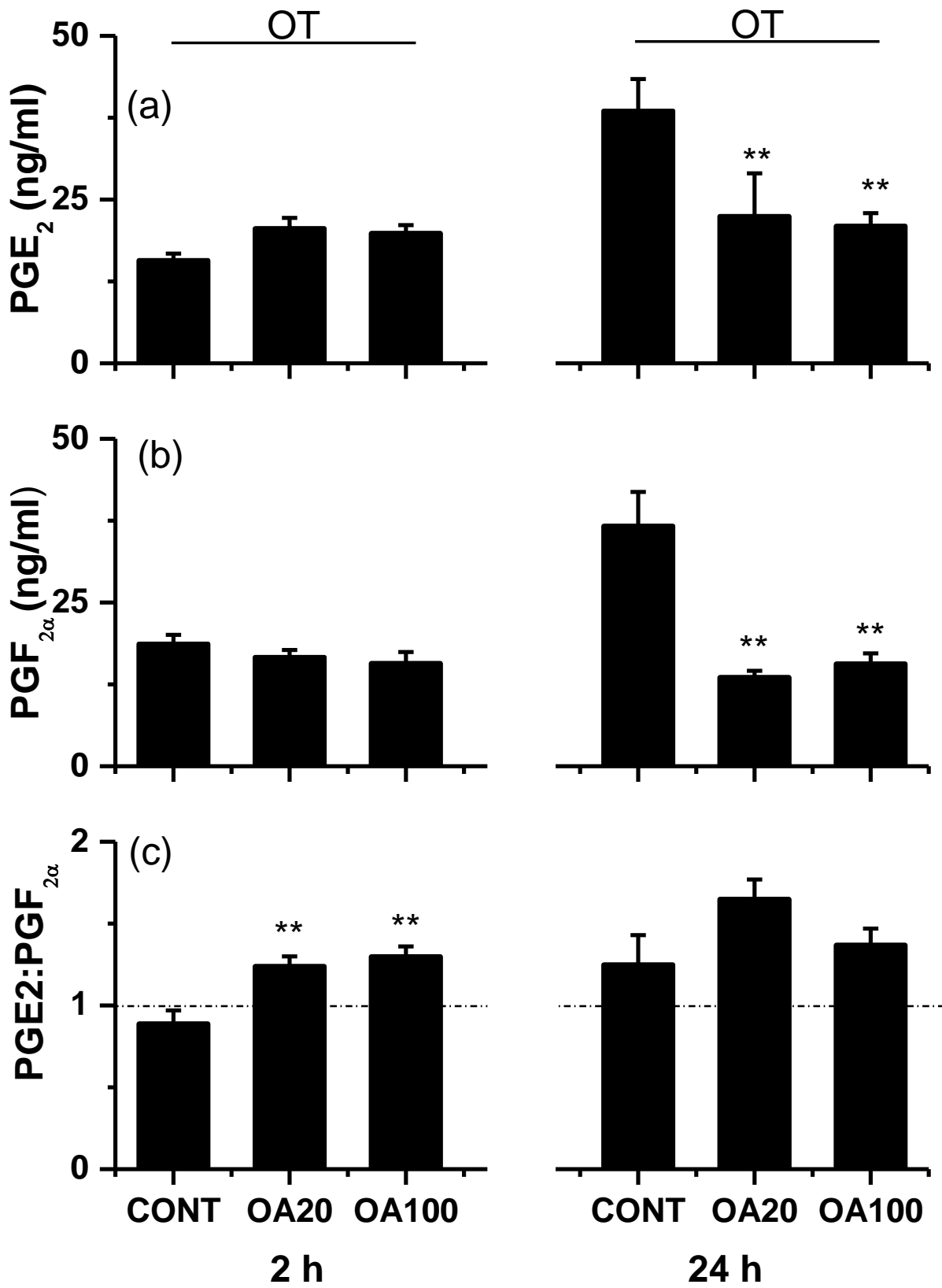


Fig 4

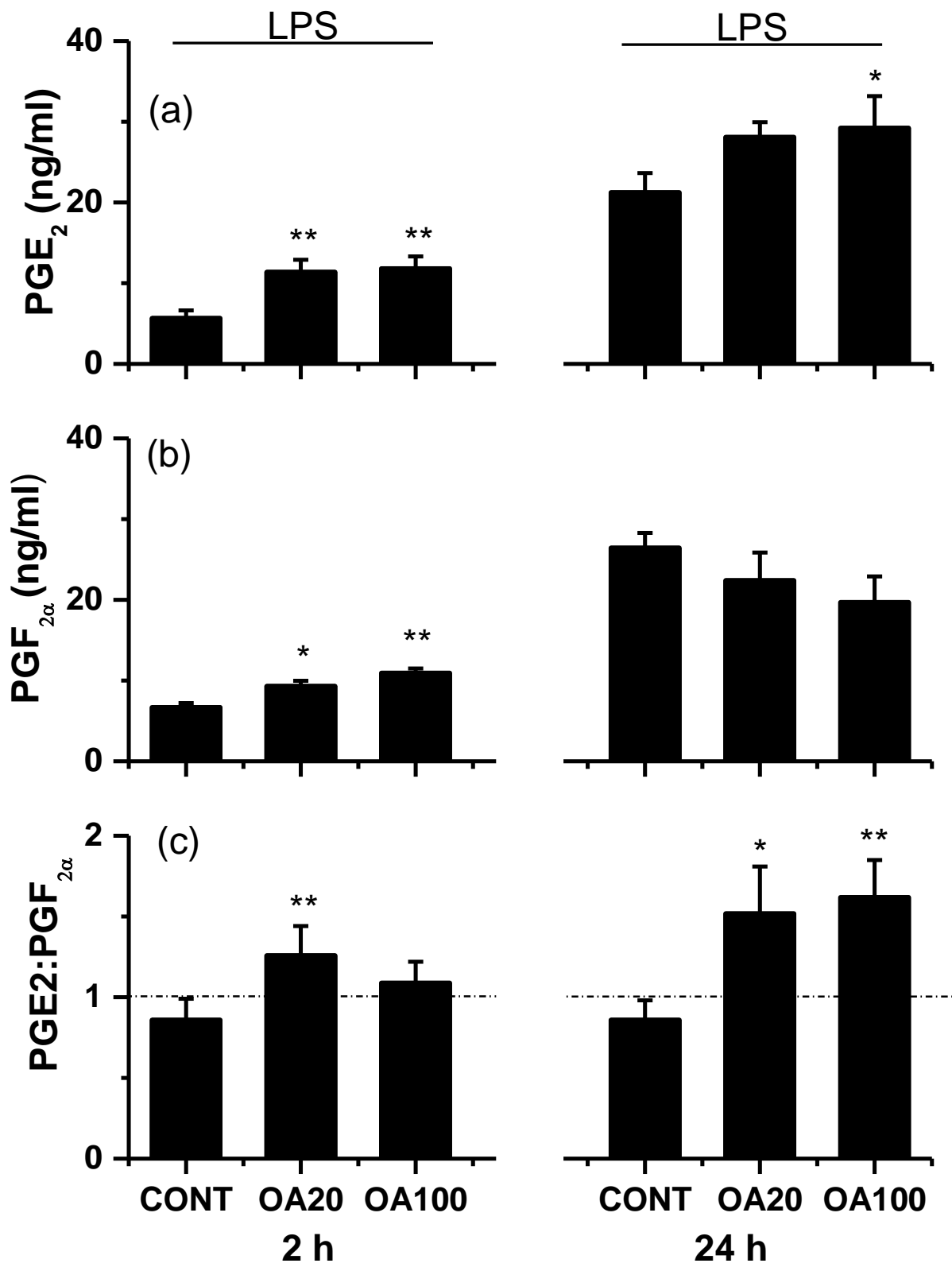


Fig 5

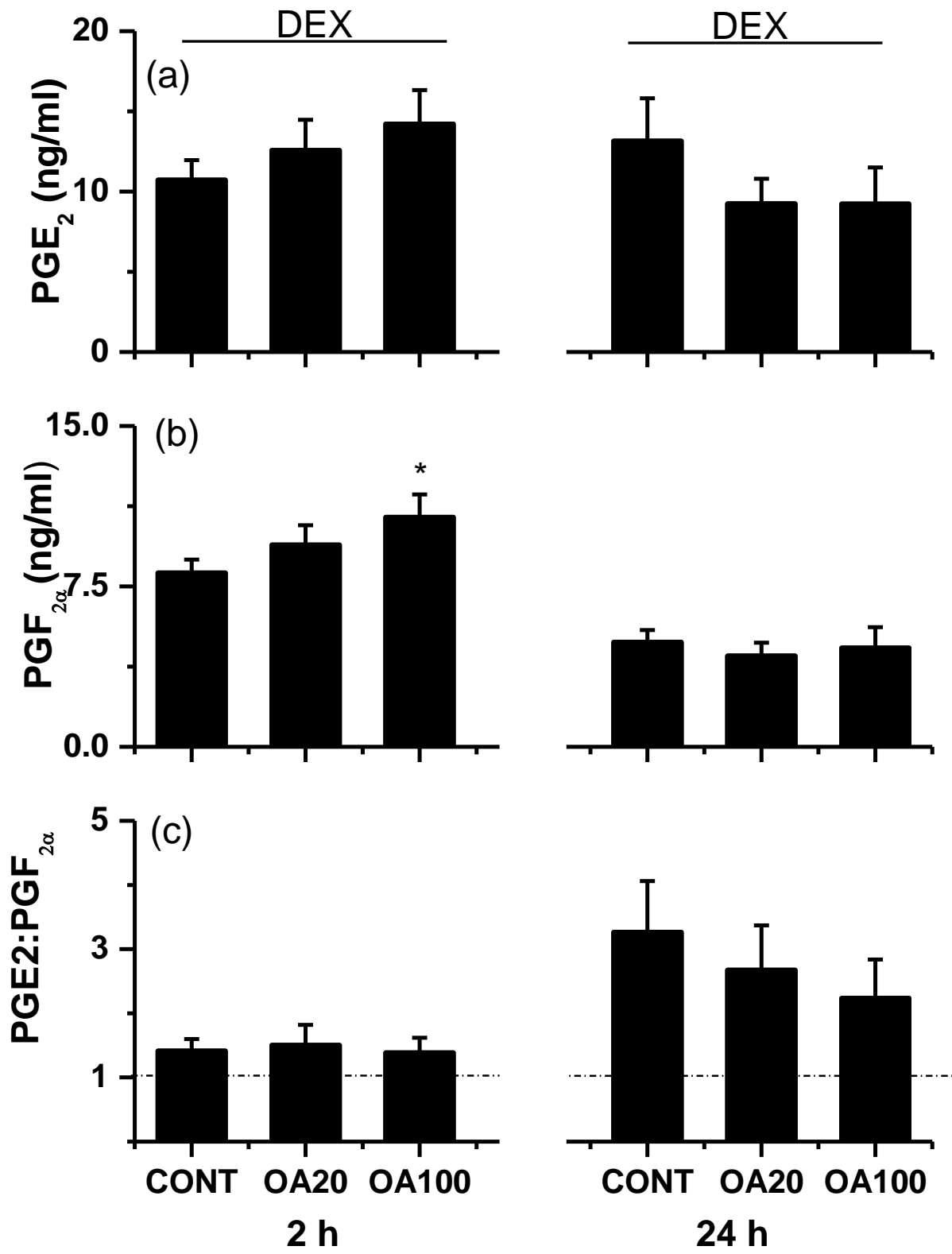
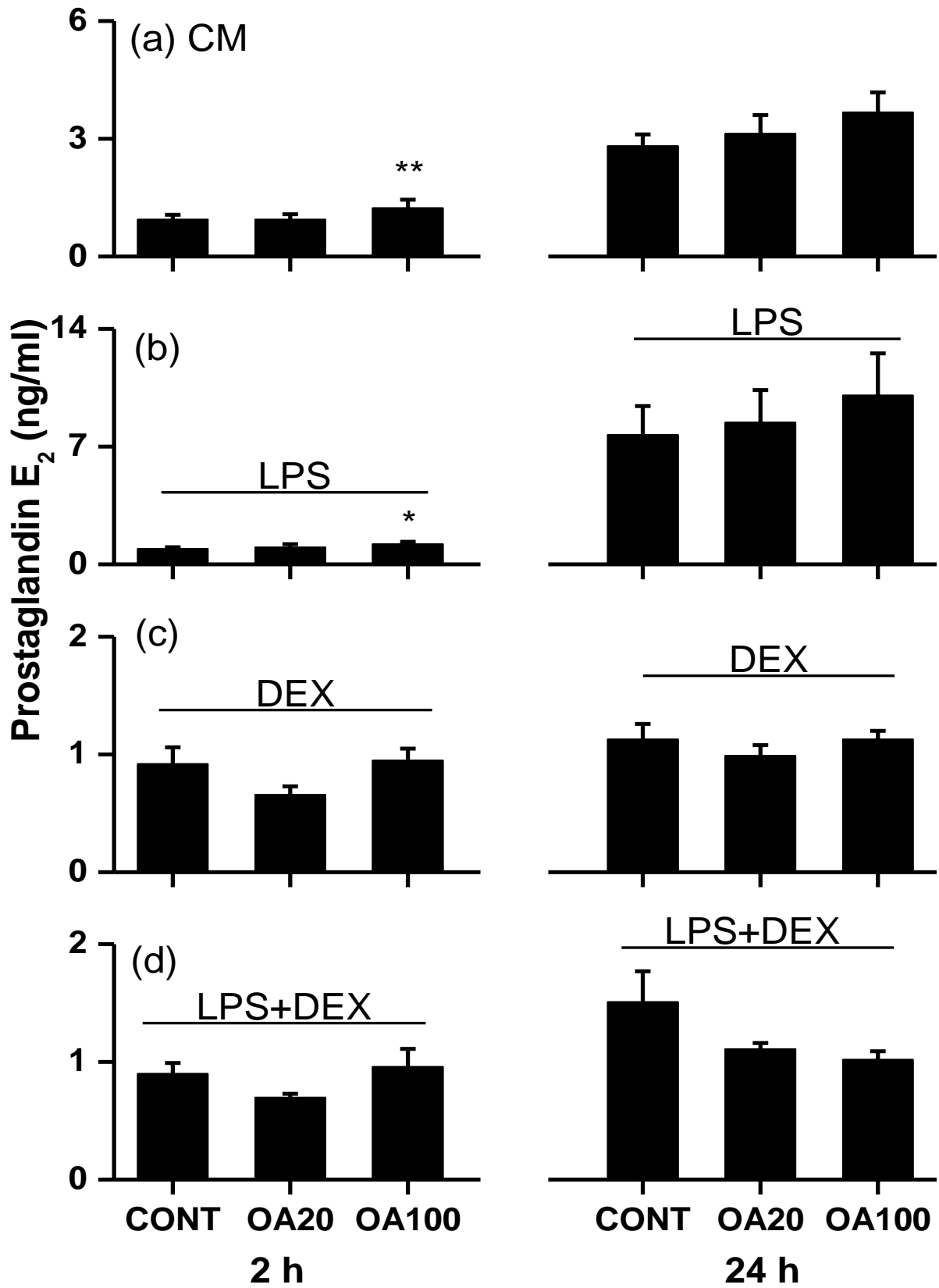


Fig 6





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(Max 45 Refs)

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