REPRODUCTION

Pro-labour myometrial gene expression: are preterm labour and term labour the same?

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Abstract

Preterm labour (PTL) is the most important cause of neonatal morbidity and mortality. While some causes have been identified, the mechanisms involved remain elusive. This study investigates whether term labour (TL) is an appropriate model for PTL by examining pro-labour gene expression, using quantitative rtPCR, and protein synthesis, using Western analysis, in preterm and term myometrial samples obtained from the upper and lower uterine segments before and after the onset of labour. In the lower segment, the levels of prostaglandin H synthase type-2 (*PGHS-2*), interleukin-1β (*IL-1β*), *IL-6* and *IL-8* mRNA expression were significantly higher in TL compared with PTL samples. Compared with non-labour controls, the expression of *IL-1β* and *IL-8* mRNA was increased in both PTL and TL samples and the expression of *PGHS-2* and *IL-6* mRNA was increased in TL samples only. In the upper segment, there were no differences between PTL and TL samples and the mRNA expression of *PGHS-2* and *IL-1β* was increased in TL compared with term no labour samples. No effect of PTL or TL was seen on either oxytocin receptor or connexin-43 mRNA expression or protein levels. The multiple regression analysis and studies in primary cultures of uterine myocytes suggest that the inflammatory cytokines, IL-1β and tumour necrosis factor-α, are the most important regulators of *PGHS-2* and *IL-8*. Our data show that preterm and term labouring myometrium are significantly different and that the most marked labour-induced changes in gene expression are in the lower segment. These changes may occur in response to the release of inflammatory cytokines by the labour-associated inflammatory infiltration.

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Introduction

Preterm labour (PTL), particularly before 32 weeks, is associated with a high fetal morbidity and mortality and consequently is the subject of intense research with the aim of devising preventative or therapeutic strategies. One approach has been to define what triggers term labour (TL) and key roles have been ascribed to the oxytocin receptor (OXTR) and connexin-43 (CX-43). However, more recently, the importance of prostaglandins (PG) and inflammatory cytokines in the process has been realised.

Early studies reported that myometrial OXTR levels are low in early pregnancy, increase with advancing gestation, peak in early labour and decline in advanced labour (Fuchs *et al*. 1984). Other studies suggested that OXTR expression increases with gestational age rather than with the onset of labour (Kimura *et al*. 1996, Wathes

et al. 1999), but more recently *OXTR* mRNA expression was reported to increase with the onset of TL (Mesiano *et al*. 2002, Terzidou *et al*. 2005). CX-43 is also thought to play a critical role in the onset of labour by increasing myometrial cell coupling. In the rat, a small increase in *Cx-43* mRNA and protein levels occurs prior to the onset of labour and a marked increase during labour itself (Risek *et al*. 1990, Lye *et al*. 1993, Ou *et al*. 1997). In the human, Sparey *et al*. (1999) found that CX-43 protein was significantly increased in labouring samples and that this was most marked in the upper segment (US) of the uterus.

There is a massive influx of macrophages, neutrophils and T-lymphocytes into the myometrium and gestational tissues with labour (Thomson *et al*. 1999, Keski-Nisula *et al*. 2000). The activated leucocytes express pro-inflammatory cytokines increasing myometrial levels of interleukin (IL)-1β and IL-8 (Osmers *et al*. 1995, Winkler *et al*. 1999, Osman *et al*. 2003). The latter promotes

further neutrophil infiltration and activation, and the former stimulates PG production. PG production is increased in fetal membranes and myometrium with labour (Turnbull *et al*. 1977, Skinner & Challis 1985) resulting in increased circulating and amniotic fluid PG levels (Sellers *et al*. 1981, Brennecke *et al*. 1985, Romero *et al*. 1994). Consistent with these observations, labour is associated with increased myometrial levels of PGH synthase type-2 (*PGHS-2*) mRNA and protein (Mesiano *et al*. 2002, Sooranna *et al*. 2004). Increased PG levels act on the myometrium to increase uterine contractility and so promote the onset and progression of labour.

In this study, we have tested the hypothesis that there is no difference in myometrial pro-labour gene expression in PTL and TL samples. To achieve this, we have examined the mRNA expression of a group of pro-labour genes, *OXTR*, *CX-43*, *IL-8* and *PGHS-2* and related the pro-labour gene expression to those of the pro-inflammatory cytokines IL-1β, IL-6 and tumour necrosis factor-α (TNF-α) in preterm and term myometrial samples obtained from the upper and lower uterine segments before and after the onset of labour.

Results

Gene expression

PTL versus TL

In the lower segment, the expression of *IL-8*, *PGHS-2*, *IL-1β* and *IL-6* mRNA was greater in TL samples than in PTL samples (P<0.01 for *IL-8* and *PGHS-2*, and P<0.05 for *IL-1β* and *IL-6*; Fig. 1A, B, E and F). There were no significant differences in the US samples for *OXTR*, *IL-8*, *PGHS-2*, *CX-43*, *IL-1β*, *IL-6* and *TNF-α* mRNA expression (Fig. 1A–G).

Non-labour versus labour

In the lower segment PTL samples, *IL-8* and *IL-1β* mRNA expression was increased compared with preterm no labour (PTNL) lower segment samples (both P<0.05; Fig. 1A and E). There were no significant differences in the US samples. In TL lower segment samples, the expression of *IL-8*, *PGHS-2*, *IL-1β* and *IL-6* mRNA was increased compared with term no labour (TNL) samples (P<0.01 for *IL-8*, *IL-6* and *PGHS-2* and P<0.05 for
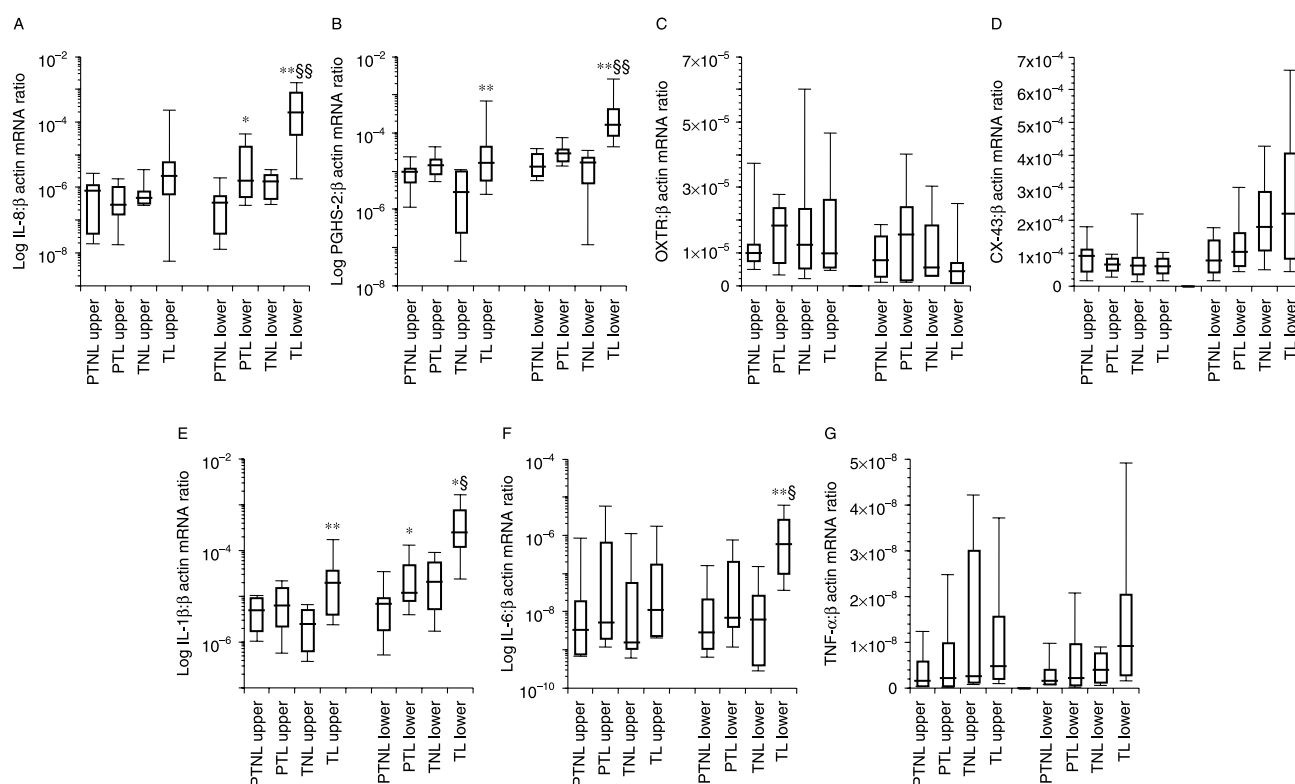

Figure 1 (A) Interleukin-8 (*IL-8*), (B) prostaglandin H synthase-2 (*PGHS-2*), (C) oxytocin receptor (*OXTR*), (D) connexin-43 (*CX-43*), (E) *IL-1β*, (F) *IL-6* and (G) tumour necrosis factor-α (*TNF-α*): β-actin mRNA ratio (median, interquartile range and range) in paired upper and lower human myometrial samples obtained from the following four groups of women: at the time of caesarean section (LSCS) under the conditions of preterm with labour (PTL; 33.9±1.5 weeks, n=9), preterm no labour (PTNL; 30.4±1.5 weeks, n=9), term with labour (TL; 39.2±0.5 weeks, n=10) and term no labour (TNL; 38.4±0.4 weeks, n=8). *A difference of P<0.05 and **a difference of P<0.01; §a difference of P<0.05 and §§a difference of P<0.01 in term labour compared with preterm labour samples and #a difference of P<0.05 and ##a difference of P<0.01 in the upper segment compared with the lower segment samples.

IL-1β; Fig. 1A, B, E and F); and in the US samples, the expression of *PGHS-2* and *IL-1β* mRNA was increased in TL compared with TNL samples (both *P*<0.01; Fig. 1B and E).

Upper versus lower segment

In paired US and lower segment samples (preterm and term, non-labour and labour), the expression of *IL-8*, *PGHS-2*, *IL-1β* and *CX-43* mRNA was greater in the lower segment samples than in the US samples (*P*<0.0001 for *PGHS-2* and *IL-1β* and *P*<0.01 for *IL-8* and *CX-43*; Fig. 1A, B, D and E). OXTR mRNA expression was greater in the US than in the lower segment samples (*P*=0.025, Fig. 1C).

Western blotting for protein concentration

Western analysis for PGHS-2, OXTR (both antibodies) and CX-43 confirmed the mRNA data (Fig. 2A–D). The similarity in the phospho-CX-43 suggests that there is no change in activity (Fig. 2E). The expression of PGHS-2 protein was greater in TL lower segment samples compared with TNL lower segment samples (*P*<0.05, Fig. 2A). The levels of CX-43 tended to be greater in the lower segment and OXTR (detected with the C-terminus antibody) tended to be greater in the US. No differences in PGHS-2 were apparent.

Gestational age

In order to identify changes in gene expression that may occur with advancing gestation and prior to the onset of labour and which may therefore have a role in the onset of labour, we performed a Spearman test for non-parametric data looking for associations between gestational age and mRNA expression in the non-labour samples (*n*=17). In the lower segment, *CX-43* mRNA expression was associated with gestational age (*r*=0.58, *P*=0.014) and *IL-8* mRNA expression was also weakly associated (*r*=0.47, *P*=0.055). In the US, *PGHS-2* mRNA expression was negatively associated with gestational age (*r*=−0.55, *P*=0.023). There was no association between gestational age and *OXTR* mRNA expression in either lower or US samples.

Multiple regression analysis

Multiple regression analysis for *OXTR*, *CX-43*, *PGHS-2* and *IL-8* was performed including *OXTR*, *IL-8*, *CX-43*, *PGHS-2*, *IL-1β*, *TNF-α* and *IL-6* mRNA levels, gestational age, sample site and labour status. For *PGHS-2*, the resulting equation could explain 60% (r^2, *P*<0.0001) of the variance in its levels, for *IL-8*, 77% (r^2, *P*<0.0001), for *OXTR*, 19% (r^2, *P*=0.13) and for *CX-43*, 70% (r^2, *P*<0.0001, Table 1).
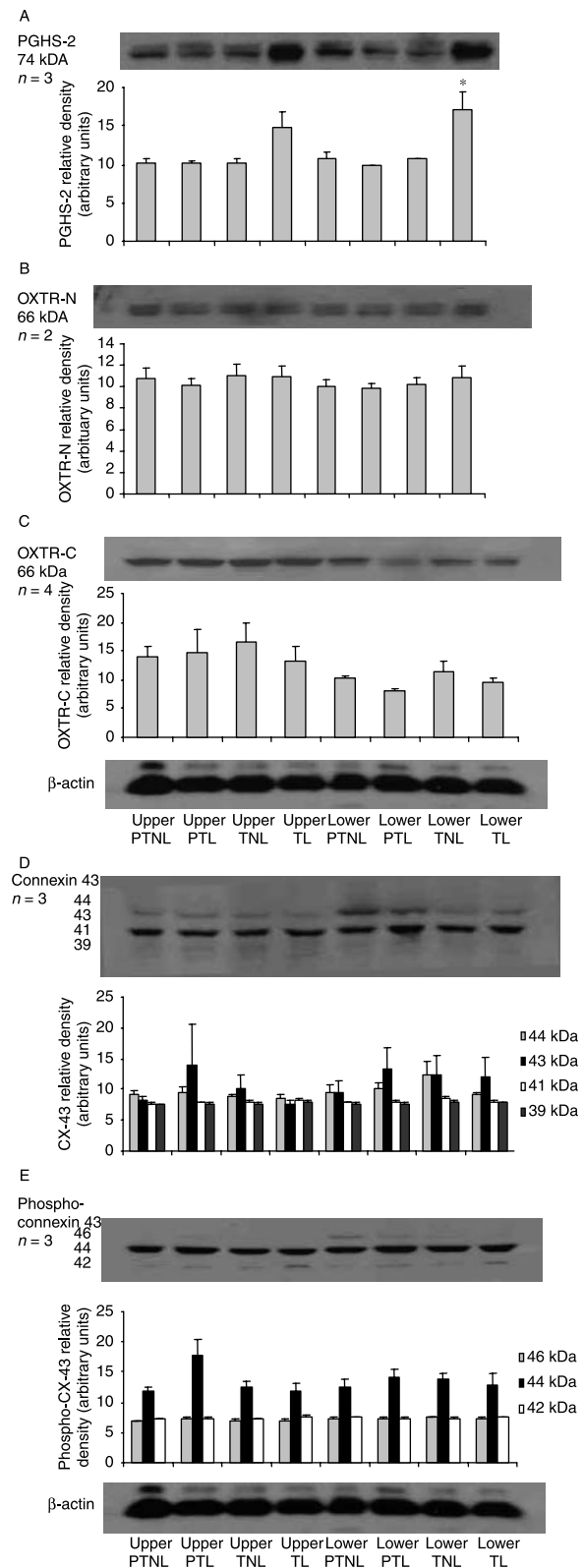

Figure 2 (A) Western analysis of prostaglandin H synthase-2 (PGHS-2), (B) oxytocin receptor (OXTR, antibody directed to the N-terminus), (C) OXTR (antibody directed to the C-terminus), (D) connexin-43 (CX-43) and (E) phospho-CX-43. *A difference of *P*<0.05 in labour compared with non-labour samples.

Table 1 The multiple regression analysis for oxytocin receptor (*OXTR*), connexin-43 (*CX-43*), prostaglandin H synthase type-2 (*PGHS-2*) and interleukin-8 (*IL-8*). The analysis was performed including *OXTR, IL-8, CX-43, PGHS-2 IL-1β*, tumour necrosis factor-α (*TNF-α*) and *IL-6* mRNA levels, gestational age, sample site and labour status.

Pro-labour gene (r^2 and P value)	Related factor	t value (P value)
PGHS-2 (60%, $P<0.0001$)	IL-8	3.54 ($P=0.0008$)
	IL-6	4.26 ($P<0.0001$)
	CX-43	2.63 ($P=0.01$)
	IL-1β	2.09 ($P=0.04$)
OXTR (19%, $P=0.13$)	Upper or lower segment	2.24 ($P=0.03$)
	IL-6	2.18 ($P=0.03$)
CX-43 (70%, $P<0.0001$)	PGHS-2	2.68 ($P=0.01$)
	IL-1β	3.13 ($P=0.003$)
	TNF-α	2.59 ($P=0.01$)
	Upper or lower segment	3.71 ($P=0.0004$)
IL-8 (77%, $P<0.0001$)	PGHS-2	3.54 ($P=0.0008$)
	IL-1β	7.68 ($P<0.0001$)
	IL-6	2.03 ($P=0.047$)

Cell culture

We studied the time course of the effects of TNF-α, IL-1β, IL-6 and IL-8 on *IL-8, PGHS-2, OXTR* and *CX-43* mRNA expression (Fig. 3A–D). The mRNA expression of *IL-8* was increased by IL-1β (at 1, 6 and 24 h, $P<0.05$), TNF-α (at 1, 6 and 24 h, $P<0.05$) and IL-8 (at 24 h, $P<0.05$); of *PGHS-2* was increased by IL-1β (at 1, 6 and 24 h, $P<0.05$, TNF-α (at 1, 6 and 24 h, $P<0.05$), IL-6 (at 24 h, $P<0.05$) and IL-8 (at 24 h, $P<0.05$); of *OXTR* was reduced by *IL-1β* (at 6 and 24 h, $P<0.05$) and of *CX-43* was increased by TNF-α (at 6 and 24 h, $P<0.05$; Fig. 3A–D).

We then studied the time course of the effects of the pro-labour factors PGE$_2$ (10 nM), PGF$_{2\alpha}$ (10 nM) and OXT (100 nM) on *IL-8, PGHS-2, OXTR* and *CX-43* mRNA expression (Fig. 4A–D). The mRNA expression of *IL-8* was increased by PGE$_2$ at 1 h ($P<0.05$) and OXT at 1 h ($P<0.05$) and then reduced by OXT at 24 h ($P<0.05$); of *PGHS-2* was increased by PGE$_2$ (at 1 h, $P<0.05$) and OXT (at 1 and 6 h, $P<0.05$); of *OXTR* was reduced by PGE$_2$ (at 6 and 24 h, $P<0.05$) and OXT (at 6 and 24 h, $P<0.05$) and of *CX-43* unaffected (Fig. 4A–D).
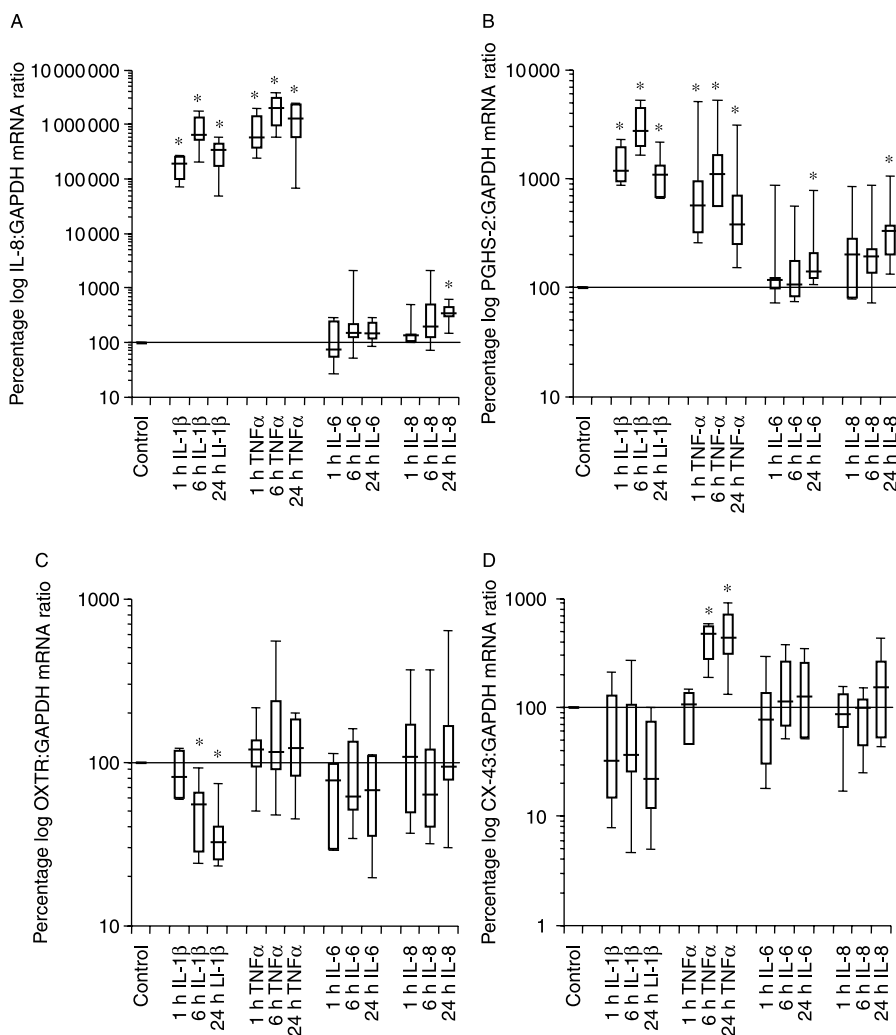

Figure 3 The response of (A) interleukin-8 (*IL-8*), (B) prostaglandin H synthase-2 (*PGHS-2*), (C) oxytocin receptor (*OXTR*), (D) connexin-43 (*CX-43*):GAPDH mRNA ratio (median, interquartile range and range) in primary cultures of human uterine smooth muscle cells to incubation for 0, 1, 6 and 24 h with IL-1β 1 ng/ml), TNF-α 1 ng/ml), IL-6 (1 ng/ml), IL-8 (1 ng/ml) as described in Materials and Methods ($n=6$–12). *A difference of $P<0.05$.
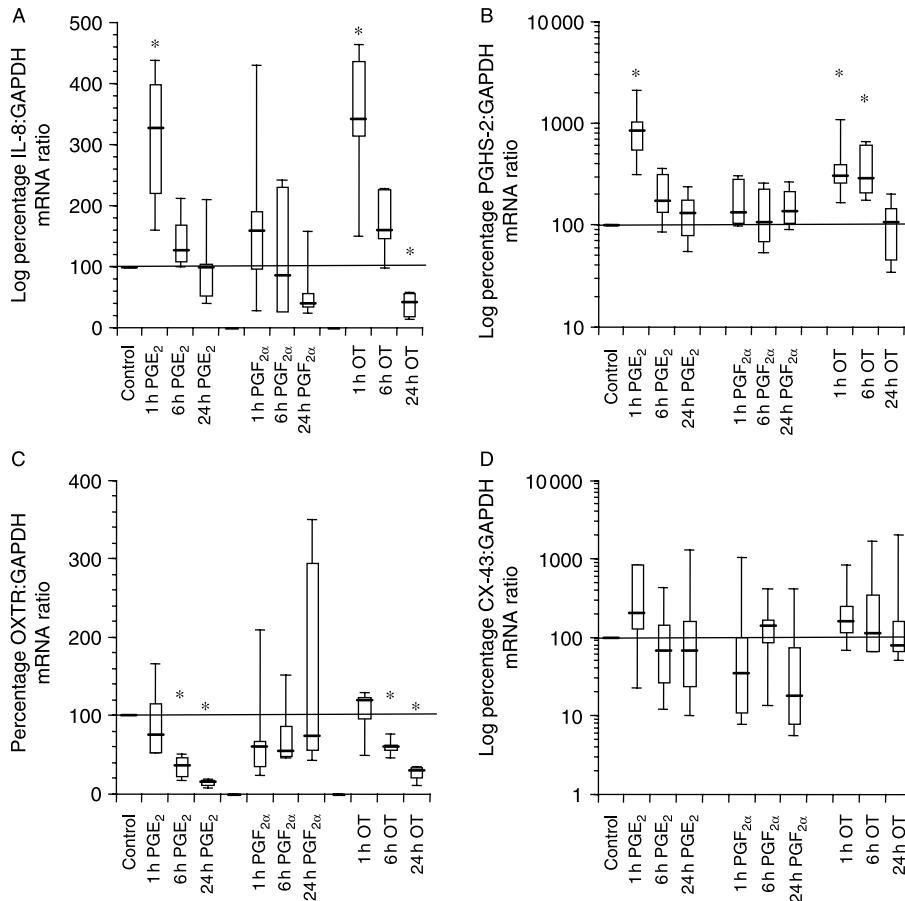
Figure 4 The response of (A) interleukin-8 (*IL-8*), (B) prostaglandin H synthase-2 (*PGHS-2*), (C) oxytocin receptor (*OXTR*), (D) connexin-43 (*CX-43*):GAPDH mRNA ratio (median, interquartile range and range) in primary cultures of human uterine smooth muscle cells to incubation for 0, 1, 6 and 24 h with PGE_2 (10 nM), $PGF_{2\alpha}$ (10 nM) or oxytocin (100 nM) as described in Materials and Methods ($n = 6$–12). *A difference of $P < 0.05$.

Discussion

This study shows that pro-labour gene expression is different in term and preterm myometrial samples and that of the pro-labour genes studied only *PGHS-2* and *IL-8* were significantly elevated with the onset of labour and that these changes were most marked in the lower segment. The multiple regression analysis suggested that the pro-labour gene mRNA expression was associated with that of the cytokines and of PGHS-2, while the cell culture data suggested that the most important modulators of pro-labour gene expression were the inflammatory cytokines IL-1β and TNF-α. Physiologically, these data imply that the myometrial inflammatory infiltration associated with both preterm and TL is important in the onset and progression of labour.

OXTR mRNA expression is a consistent marker of labour in animal models of parturition (Soloff *et al.* 1979), has been reported to be increased in human labour by several groups including our own (Mesiano *et al.* 2002, Terzidou *et al.* 2005) and used by others to differentiate labouring samples (Condon *et al.* 2003). However, it is also reported that *OXTR* mRNA expression does not increase with the onset of labour but with advancing gestation, prior to the onset of labour (Kimura *et al.* 1996, Wathes *et al.* 1999). Our data show no increase in *OXTR* mRNA either with the

onset of labour (whether at or before term) or with advancing gestation. Furthermore, our mRNA data are supported by Western analysis of *OXTR* levels that also show no change. Overall, the evidence suggests that *OXTR* mRNA expression is increased in some but not all women with the onset of labour, and that perhaps the well-recognised increase in OXTR sensitivity to OXT (Fuchs *et al.* 1983) is more important than an increase in *OXTR* mRNA expression. Our data are consistent with several recent gene array studies that failed to find an increase in OXTR with the onset of labour (Aguan *et al.* 2000, Charpigny *et al.* 2003, Esplin *et al.* 2005, Havelock *et al.* 2005, Bukowski *et al.* 2006). Interestingly, the factors we included in the multiple regression analysis accounted for relatively little of the variation in *OXTR* mRNA expression, suggesting that other factors regulate *OXTR* mRNA expression during pregnancy. Our *in vitro* studies found that *OXTR* mRNA expression was either unchanged or reduced (as reported previously for IL-1β (Schmid *et al.* 2001, Helmer *et al.* 2002, Soloff *et al.* 2006) and OXT (Phaneuf *et al.* 2000)) in response to cytokines and pro-labour factors supporting our myometrial data.

CX-43 is held to have an important role in TL. Animal data consistently show that both mRNA and protein levels of *CX-43* increase with the onset of labour (Lye

et al. 1993, McNutt *et al.* 1994, Ou *et al.* 1997, Cook *et al.* 2000) and that ablation of CX-43 delays the onset of parturition (Doring *et al.* 2006). Human data are less robust, with some studies showing increases in protein alone (Sparey *et al.* 1999), some in mRNA but not protein (Chow & Lye 1994, Pierce *et al.* 2002) and some no change in mRNA (Rezapour *et al.* 1997). The spatial expression of CX-43 protein was assessed in one study and found to be greater in the US in both non-labour and labour samples (Sparey *et al.* 1999). Two studies suggested that failure to progress in labour was associated with lower levels of *CX-43* mRNA expression (Pierce *et al.* 2002, Cluff *et al.* 2006). We found that *CX-43* mRNA expression, protein levels and phosphoryl-ation status were unchanged with either term or PTL. Furthermore, several studies have been performed using differential display or gene arrays and none have mentioned *CX-43*, either because it was unchanged or because it was not included in the array (Aguan *et al.* 2000, Chan *et al.* 2002, Charpigny *et al.* 2003, Esplin *et al.* 2005, Havelock *et al.* 2005, Bukowski *et al.* 2006). Our multiple regression studies found associations between the mRNA levels of *CX-43* and those of *IL-1β*, *TNF-α* and *PGHS-2*, but the *in vitro* data found that only TNF-α affected *CX-43* mRNA expression. Overall, these data suggest that in the human at least *CX-43* is not consistently up-regulated with the onset of labour.

The expression of *PGHS-2* mRNA was increased in both the upper and lower uterine segments with TL, but not PTL, and there was no increase with gestational age in either segment. The term data are consistent with most recent reports (Mesiano *et al.* 2002, Sooranna *et al.* 2004), but contrast with our previous observations and the trend reported by Sooranna *et al.* (2006) and Astle *et al.* (2007). The mechanism involved in the up-regulation of *PGHS-2* activity with the onset of labour is uncertain, but several stimuli including IL-1β are known to increase *PGHS-2* expression via the transcription factor nuclear factor κ B (NFκB) (Allport *et al.* 2001). Indeed, most data suggest that inflammatory cytokines increase myometrial con-tractility via increased PG synthesis (Khatun *et al.* 1999). The multiple regression data revealed associations between the levels of *PGHS-2* mRNA and those of *IL-8*, *IL-6* and *IL-1β* and our *in vitro* studies confirmed the important role played by IL-1β; in contrast both IL-6 and IL-8 had a small effect. Interestingly, TNF-α had a marked stimulatory effect on *PGHS-2* mRNA expression, but we found no association between their levels in myometrial samples. OXT is known to activate ERK1/2 and to increase PGHS-2 in an ERK-dependent manner (Strakova *et al.* 1998). OXT-induced ERK1/2 activation may also explain the increase in *IL-8* mRNA expression observed in response to OXT. These inconsistencies may reflect the difference between the behaviour of uterine myocytes in primary culture as opposed to the *in vivo* situation where their function is modulated by other cellular and connective tissue components of the myometrium.

We found that IL-8 is increased in both PTL and TL consistent with the published data (Winkler *et al.* 1999, Elliott *et al.* 2000, Chan *et al.* 2002, Osman *et al.* 2003). The multiple regression analysis found associations between *IL-8* mRNA levels and those of *PGHS-2*, *IL-1β* and *IL-6*, but *in vitro*, uterine myocyte expression of *IL-8* was increased by IL-1β and TNF-α, but not IL-6. Interestingly, neither PGE_2 nor $PGF_{2α}$ affected *IL-8* mRNA expression significantly, suggesting that the association between *IL-8* and *PGHS-2* mRNA levels seen in the multiple regression analysis may reflect a common regulation of both genes by inflammatory cytokines. We also found that IL-8 increased with advancing gestation in non-labour samples albeit weakly, and previously we found that *IL-8* mRNA expression and synthesis were increased by mechanical stretch of uterine myocytes *in vitro* (Loudon *et al.* 2004). Together, these data suggest that myometrial *IL-8* mRNA expression may be physiologically up-regulated by increasing uterine wall tension secondary to the growth of the pregnancy. The increased IL-8 synthesis could then provoke an inflammatory infiltration of the myometrium resulting in the release of IL-1β which stimulates further myometrial synthesis of IL-8, so establishing a positive feedback loop culminating in the onset of labour.

It is possible that the differences we found between PTL and TL are the result of confounding factors, but the groups were similar in most respects and particularly in terms of cervical dilatation, gravidity and parity. Some discrepancy is inevitable between groups, for example, the term group it is more likely have an LSCS for failure to progress and less likely to have an LSCS for malpresenta-tion than the PTL group. The causes of PTL were uncertain for the majority, two having multiple pregnancies and one having an abruption with a non-reassuring fetal heart pattern. There were differences in the racial distribution between groups, the TL group contained relatively more African Americans than the PTNL group, but gene expression in these groups was not directly compared. Certainly, in general terms, labour of an infective aetiology would be expected to be over-represented in the PTL group, but such a cause would also be expected to be associated with an overexpression of inflammatory genes. However, in our study, it appeared the converse was true and inflammatory gene expression was lower in PTL. PTL in multiple pregnancies might be expected to overexpress *PGHS-2* and *IL-8* as these are the most consistent stretch-responsive genes (Sooranna *et al.* 2005); again, this was not the picture we observed. The relatively greater variation in mRNA levels of the genes analysed may have masked significant changes between groups, only a larger study of clinically well-defined cases will allow us to clarify the molecular mechanisms responsible for the onset of PTL of different causes.

The inflammatory cytokines, IL-1β, IL-6 and TNF-α are thought to be released by activated neutrophils and to promote the onset of labour by increasing PGHS-2

synthesis. We found that the pattern of expression of *IL-1β* mRNA in myometrial samples was very similar to that of *IL-8*, but *IL-6* mRNA expression was only increased in TL lower segment samples and *TNF-α* mRNA expression was not significantly different in any group. Others have reported that lower segment *IL-1β* and *IL-6*, but not *TNF-α*, mRNA expression is increased with labour (Maul *et al.* 2002, Osman *et al.* 2003). Thus, our observation of a lack of change in *TNF-α* mRNA expression with the onset of labour is consistent with the literature. However, in the mouse model, TNF-α seems to have an important role, since knocking out either IL-1 or TNF receptors alone failed to prevent infection-induced PTL, but removing both IL-1 and TNF-α receptors significantly reduces infection-induced PTL (Hirsch *et al.* 2006). These data imply that the combination of IL-1 and TNF-α plays a critical role in the process of infection-induced PTL in the mouse. In the primate, intra-amniotic infusions of IL-1β stimulated the most intense contraction pattern, while TNF-α infusions induced labour in two cases and moderate contractions in three others (Sadowsky *et al.* 2006). The exact roles and relative importance of the inflammatory cytokines in human labour are not certain, but the evidence suggests that they are very likely to be involved in the onset of labour.

In this study, the expression of *IL-8*, *PGHS-2*, *IL-1β* and *CX-43* mRNA was greater in the lower segment than in their paired US samples; only *OXTR* mRNA expression was greater in the US than in the lower segment samples. These data are consistent with the literature with regard to *OXTR* (Blanks *et al.* 2003), but not for *CX-43*, the expression of which was previously reported to be greater in the US (Sparey *et al.* 1999). We have previously reported that *cPLA₂*, *sPLA₂*, *PGHS-2*, *PGES-1* and *PGES-2* mRNA expression were greater in the lower than in the US samples (Sooranna *et al.* 2006) in agreement with Sparey *et al.* (1999) in the case of *PGHS-2*. Our observation that the mRNA expression of the inflammatory cytokine IL-1β is greater in the lower than in the US is consistent with the more marked inflammatory infiltration found in the lower segment (Thomson *et al.* 1999). The lower segment starts to form at the end of the second trimester reflecting increased tension in this portion of the uterus. As discussed above, it is possible that the increase tension induces IL-8 production initiating the process that results in the onset of labour. Certainly, the increase in gene expression associated with labour appears to greatest in the lower segment, suggesting that changes in the lower segment drive the process, producing uterotonins that promote the contractile activity of the US.

These data suggest that myometrial gene expression is different in PTL and TL. Clearly, PTL occurs for many reasons, such as infection, ruptured membranes and cervical insufficiency, and our approach to compare all cases of PTL to TL does not allow us to discriminate. Furthermore, we have only analysed genes directly related to labour or inflammation and other sets of genes may

behave differently. Only by studying accurately pheno-typed samples obtained from women in PTL will we be able to define whether some subsets of PTL are in fact accelerated TL while other types of PTL are distinct from TL.

Materials and Methods

Tissue collection and preparation

All procedures involving human myometrial tissues were conducted in compliance with the Institution Review Board of the University of Cincinnati (Cincinnati, OH, USA). Informed consent was obtained from all women prior to any tissue collection. Paired upper and lower segment human myometrial samples were obtained from the following four groups of women (mean gestational age ± s.D. in each case): at PTNL (31.5 ± 3.5 weeks; mean ± s.D.; $n = 9$), PTL (32.3 ± 4.1 weeks; $n = 9$), TNL (38.0 ± 1.2 weeks; $n = 8$) and TL (39.4 ± 0.5 weeks; $n = 10$; Table 2). Labour was defined as the presence of regular uterine contractions (every 3–4 min) resulting in cervical effacement and dilation. Myometrial samples were removed from the upper margin of the incision made in the lower uterine segment and for the US, an Allis clamp was used to group a small segment (1.0 × 0.5 cm) of myometrium below the fundus and tissue excised using Mayo scissors (this included the serosal surface but not the endometrium). Homeostasis was obtained using a single figure of size 8 suture. Tissues were flash frozen in liquid nitrogen prior to storage at −80 °C. The indications for caesarean section included: failure to progress ($n = 4$), fetal distress ($n = 8$), previous uterine surgery ($n = 12$), malpresentation ($n = 5$), severe pre-eclampsia ($n = 5$), placenta praevia ($n = 1$) and gestational diabetes ($n = 1$).

Quantitative RT-PCR

Total RNA was extracted and purified from the upper and lower segment myometrial samples using the Tri-reagent method (Trizol, Sigma–Aldrich Co. Ltd). After quantification, 2.0 μg RNA was pretreated with DNase I (Amp Grade, Invitrogen Ltd) and then reverse transcribed with Oligo dT random primers using SuperScript II reverse transcriptase (Invitrogen Ltd). Total RNA was extracted and purified from myometrial cells grown on 6-well plates using the RNAeasy mini kit from Qiagen Ltd. After quantification, 1.0 μg was reverse transcribed with oligo dT random primers using MuLV reverse transcriptase (Applied Biosystems Ltd, Warrington, UK).

Paired oligonucleotide primers for amplification of pro-labour genes were designed using Primer Designer (Scientific and Educational Software, Durham, NC, USA) against the sequence downloaded from GenBank. The primer sets used (Table 3) produced amplicons of the expected size and flanked intron/exon junctions. Assays were validated for all primer sets by confirming that single amplicons of appropriate size and sequence were generated. Quantitative PCR was performed in the presence of SYBR Green (Qiagen Ltd), and amplicon yield was monitored during cycling in a RotorGene Sequence Detector (Corbett Research Ltd, Mortlake, Sydney, Australia). Pre-PCR cycle was 10 min at 95 °C followed by up to 45 cycles at 95 °C for 20 s, 58–60 °C for 20 s and 72 °C for 20 s followed

Table 2 The demographic data of the study subjects.

	PTNL (*n*=9)	**PTL** (*n*=9)	**TNL** (*n*=8)	**TL** (*n*=10)	
Age	28 (19–36)	26 (18–32)	24 (16–43)	21 (15–44)	NS
Gravidity	3 (1–4)	4 (1–7)	2 (1–7)	1 (1–5)	NS
Parity	1 (0–2)	1 (0–5)	1 (0–2)	0 (0–3)	NS
Race					PTNL versus TL*
African American	2	5	4	9	
White European	7	4	4	1	
Gestational age	32.3 (26–35.3)	33.3 (26.2–36.3)	38.4 (37–39.5)	39.3 (37.3–40.6)	TL versus PTL[†]
					TNL versus PTNL[†]
Dilatation	0 (0–3.5)	5 (1–10)	0 (0–3)	6 (0.5–10)	TL versus PTL NS
					TNL versus PTNL NS
Reason for LSCS					
FTP	–	–	–	4	
NRFHT	3	2	–	3	
PUS	–	3	6	3	
Malpresentation	–	4	1	–	
PET	4	–	1	–	
Placenta praevia	1	–	–	–	
GDM	1	–	–	–	

PTNL, preterm no labour; PTL, preterm labour; TNL, term no labour; TL, term labour; FTP, failure to progress; NRFHT, non-reassuring fetal heart trace; PET, pre-eclampsia; GDM, gestational diabetes. *$P<0.05$ and [†]$P<0.001$.

by an extension at 72 °C for 15 s. A melt over the temperature range of 72–99 °C rising by 1° steps with a wait for 15 s on the first step followed by a wait of 5 s for each subsequent step completes the procedure. The cycle at which the fluorescence reached a preset threshold (cycle threshold) was used for quantitative analyses. The cycle threshold in each assay was set at a level where the exponential increase in amplicon abundance was approximately parallel between all the samples. The r^2 values and efficiencies for the primer pairs are given in Table 3. The mRNA data were expressed relative to the amount of the constitutively expressed housekeeping genes β-actin and *GAPDH* for myometrial tissue samples and primary myometrial cell cultures respectively. Different housekeeping genes were used as we have found that in primary uterine myocytes cell culture *GAPDH* showed less variation and that in myometrial samples β-actin showed less variation (N Engineer, SR Sooranna, S Chalkley, L Myatt & MR Johnson, unpublished observations).

Western analysis

Myometrial tissues were homogenised in five volumes of a buffer containing 20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 2 mM dithiotreitol, 1 mM Na_3VO_4, 1 mM phenylmethylsulphonyl fluoride and 1 µg/ml leupeptin. After centrifugation for 1 min at 12 000 **g**, homogenates were aliquoted and frozen at −80 °C. Protein concentrations were determined by Protein assay (Bio-Rad Laboratories) and BSA reference standards. Electrophoresis was carried out using 15 µg aliquots of protein samples in 2× loading buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue and 0.125 mol/l Tris–HCl, pH 6.8). Samples were boiled for 5 min, quenched on ice and subsequently run on precast 10% SDS-polyacrylamide gels (Bio-Rad Laboratories).

Western blotting was carried out following electrophoretic transfer in 25 mmol/l Tris (pH 8.3), 192 mmol/l glycine and 20% v/v methanol on to Hybond ECL nitrocellulose membrane (Amersham Life Science). Membranes were blocked in 5% milk protein in 0.1% Tween–PBS for 1 h at room temperature. Mouse MAB to β-actin was purchased from Sigma–Aldrich Co. Ltd and goat polyclonal antibodies to OXTR (two antibodies, one directed to the C-terminus and the other to the N-terminus) CX-43 (both phospho and non-phospho forms) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Polyclonal antibody against human PGHS-2 was purchased from Cayman Chemical (Ann Arbor, MI, USA). Specific rabbit polyclonal antibodies directed against the phospho- or total CX-43 (New England Biolabs Ltd, Hitchin, UK). The antibodies were used at a dilution of 1:1000 and incubated overnight at 4 °C. Membranes were washed with 0.1% Tween–PBS and then incubated with anti-goat immunoglobulin G-horseradish peroxidase secondary antibody at a dilution of 1:2000 for 1 h at room temperature. ECL Western blotting detection was carried out using standard protocols (Amersham Life Science). Protein band size was determined using Rainbow coloured protein molecular weight markers (Amersham Life Science). Antibody specificity was confirmed using positive controls. Exposure of cells to u.v. B irradiation (wavelength 356 nm) for 30 min was used as a positive control. Western autoradiographs were quantified by digital densitometry using the Image Master VDS gel documentation system and Image Master VDS Software (Amersham Life Science). Protein bands were digitised, ensuring that the range of pixel densities did not extend to either the minimum or maximum values. Mean pixel density for each band was assessed using a sample gate of the same size. To allow comparisons between blots prepared on different occasions, a single control sample was included on each blot. Final pixel density was adjusted to ensure that this control sample carried the same value for each blot.

Cell culture

Biopsies (0.5 cm³) of term human myometrium were collected at the time of caesarean section (LSCS) from women not in

Table 3 The primer pairs, GenBank accession numbers and nucleotide sequence numbers for the primer pairs used.

	Forward primer sequence	Reverse primer sequence	GenBank/ EMBL accession no.	Nucleotide no.	r^2	Efficiency
GAPDH	5′-TGATGACATCAAGAAGGTGGTGAAG-3′	5′-TCCTTCGAGGCCATGTAGGCCAT-3′	BC014085	1644–1883	0.99999	1.12
β-Actin	5′-GGGAAATCGTCGCGTGACATTAA-3′	5′-TGTGTTGGCGTACAGGTCTTTG-3′	BT019932	617–891	0.99999	0.64
OXTR	5′-AGAAGCACTCGCGCCTCTT-3′	5′-AGGTGATGTCCCACAGCAACT-3′	NM000916	828–929	0.99987	0.96
PGHS-2	5′-TGTGCAACACTTGAGTGG CT-3′	5′-ACTTTCTGTACTGCGCGGTGG CT-3′	AY151286	1381–1677	0.99967	0.67
Connexin-43	5′-TGGATTCAGCTTGAGTGCTG-3′	5′-GGTCGCTCTTTCCCTTAACC-3′	BC026329	619–824	0.99884	1.08
IL-8	5′-GCCTTCCTGATTTCTGCAGC-3′	5′-CGCAGTGTGTGTCCACTCTCA-3′	NM000584	136–285	0.99936	0.93
IL-1β	5′-TACCTGAGCTCGCCAGTGAA-3′	5′-TCGGAGATTCGTAGCTGGATG-3′	NM000576	98–248	0.99971	0.86
IL-6	5′-CCTTCCAAAGATGGCTGAAA-3′	5′-AGCTCTGGCTTGTTCCTCAC-3′	NM000600	335–488	0.99989	0.64
TNF-α	5′-GAGAAGGGTGACCGACTCAG-3′	5′-GGTTGAGGGTGTCTGAAGGA-3′	NM000594	776–951	0.99978	0.84

The r^2 values obtained and the efficiencies during quantitative real-time PCR using the primers pairs are also listed.

labour and stored in DMEM containing 100 mU/ml penicillin and 100 μg/ml streptomycin. Samples were stored at 4 °C for no more than 3 h prior to cell preparation for culture. The indications for LSCS in this group of women were previous LSCS, breech presentation and maternal request. All specimens were obtained after patient consent, and the Riverside Research Ethics Committee approved the study.

Primary human uterine myocytes were isolated using a mixture of collagenases and cultured in DMEM 7.5% fetal calf serum (FCS), 100 munits/ml penicillin and 100 100 μg/ml streptomycin in T75 in an atmosphere of 5% CO_2: 95% air at 37 °C (Sooranna *et al.* 2005). Myometrial cells grown in this manner have previously been characterised (Sooranna *et al.* 2005). Cells from passages 1 to 4 were trypsinised in 0.25% trypsin containing 0.02% EDTA in PBS and cultured in 6-well plates. When cells were 80–90% confluent (days 3–4), old medium was removed and replaced with 1.5 ml fresh medium supplemented with 7.5 mM HEPES with 1% FCS overnight. After 16–18 h, cells were incubated for 1, 6 and 24 h with 1 ng/ml IL-1β, 1 ng/ml TNF-α, 1 ng/ml IL-6, 1 ng/ml IL-8, 10 nM PGE$_2$, 10 nM PGF$_{2\alpha}$ or 100 nM OXT after which media was removed and cells frozen in liquid nitrogen and stored at −80 °C until extraction of RNA.

Statistical analysis

Demographic data were analysed using the Mann–Whitney *U* test for continuously distributed data and a Fisher's exact test for categorical data as appropriate. The myometrial data were not normally distributed and were expressed as median and range. The data were analyzed with a Kruskall–Wallis test and post-testing with Mann–Whitney *U* test for independent samples. The effect of gestational age on gene expression was assessed using a Spearman test for non-parametric data. Multiple regression analysis was used to assess whether there was any relationship between individual gene expression, gestational age, labour status and site of tissue biopsy (upper or lower segment). In cell culture studies, the data were not normally distributed and were expressed as median and range. The data were analyzed with a Friedman test (non-parametric repeated measures ANOVA) and post-testing with Mann–Whitney *U* test.

Acknowledgements

References

Aguan K, Carvajal JA, Thompson LP & Weiner CP 2000 Application of a functional genomics approach to identify differentially expressed genes in human myometrium during pregnancy and labour. *Molecular Human Reproduction* **6** 1141–1145.

Allport VC, Pieber D, Slater DM, Newton R, White JO & Bennett PR 2001 Human labour is associated with nuclear factor-κB activity which mediates cyclo-oxygenase-2 expression and is involved with the 'functional progesterone withdrawal'. *Molecular Human Reproduction* **7** 581–586.

Astle S, Newton R, Thornton S, Vatish M & Slater DM 2007 Expression and regulation of prostaglandin E synthase isoforms in human myometrium with labour. *Molecular Human Reproduction* **13** 69–75.

Blanks AM, Vatish M, Allen MJ, Ladds G, de Wit NC, Slater DM & Thornton S 2003 Paracrine oxytocin and estradiol demonstrate a spatial increase in human intrauterine tissues with labor. *Journal of Clinical Endocrinology and Metabolism* **88** 3392–3400.

Brennecke SP, Castle BM, Demers LM & Turnbull AC 1985 Maternal plasma prostaglandin E2 metabolite levels during human pregnancy and parturition. *British Journal of Obstetrics and Gynaecology* **92** 345–349.

Bukowski R, Hankins GD, Saade GR, Anderson GD & Thornton S 2006 Labor-associated gene expression in the human uterine fundus, lower segment, and cervix. *PLoS Medicine* **3** e169.

Chan EC, Fraser S, Yin S, Yeo G, Kwek K, Fairclough RJ & Smith R 2002 Human myometrial genes are differentially expressed in labor: a suppression subtractive hybridization study. *Journal of Clinical Endocrinology and Metabolism* **87** 2435–2441.

Charpigny G, Leroy MJ, Breuiller-Fouche M, Tanfin Z, Mhaouty-Kodja S, Robin P, Leiber D, Cohen-Tannoudji J, Cabrol D, Barberis C et al. 2003 A functional genomic study to identify differential gene expression in the preterm and term human myometrium. *Biology of Reproduction* **68** 2289–2296.

Chow L & Lye SJ 1994 Expression of the gap junction protein connexin-43 is increased in the human myometrium toward term and with the onset of labor. *American Journal of Obstetrics and Gynecology* **170** 788–795.

Cluff AH, Bystrom B, Klimaviciute A, Dahlqvist C, Cebers G, Malmstrom A & Ekman-Ordeberg G 2006 Prolonged labour associated with lower expression of syndecan 3 and connexin 43 in human uterine tissue. *Reproductive Biology and Endocrinology* **4** 24.

Condon JC, Jeyasuria P, Faust JM, Wilson JW & Mendelson CR 2003 A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. *PNAS* **100** 9518–9523.

Cook JL, Zaragoza DB, Sung DH & Olson DM 2000 Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. *Endocrinology* **141** 1718–1728.

Doring B, Shynlova O, Tsui P, Eckardt D, Janssen-Bienhold U, Hofmann F, Feil S, Feil R, Lye SJ & Willecke K 2006 Ablation of connexin43 in uterine smooth muscle cells of the mouse causes delayed parturition. *Journal of Cell Science* **119** 1715–1722.

Elliott CL, Slater DM, Dennes W, Poston L & Bennett PR 2000 Interleukin 8 expression in human myometrium: changes in relation to labor onset and with gestational age. *American Journal of Reproductive Immunology* **43** 272–277.

Esplin MS, Fausett MB, Peltier MR, Hamblin S, Silver RM, Branch DW, Adashi EY & Whiting D 2005 The use of cDNA microarray to identify differentially expressed labor-associated genes within the human myometrium during labor. *American Journal of Obstetrics and Gynecology* **193** 404–413.

Fuchs AR, Periyasamy S, Alexandrova M & Soloff MS 1983 Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids. *Endocrinology* **113** 742–749.

Fuchs AR, Fuchs F, Husslein P & Soloff MS 1984 Oxytocin receptors in the human uterus during pregnancy and parturition. *American Journal of Obstetrics and Gynecology* **150** 734–741.

Havelock JC, Keller P, Muleba N, Mayhew BA, Casey BM, Rainey WE & Word RA 2005 Human myometrial gene expression before and during parturition. *Biology of Reproduction* **72** 707–719.

Helmer H, Tretzmuller U, Brunbauer M, Kaider A, Husslein P & Knofler M 2002 Production of oxytocin receptor and cytokines in primary uterine smooth muscle cells cultivated under inflammatory conditions. *Journal of the Society of Gynecological Investigation* **9** 15–21.

Hirsch E, Filipovich Y & Mahendroo M 2006 Signaling via the type I IL-1 and TNF receptors is necessary for bacterially induced preterm labor in a murine model. *American Journal of Obstetrics and Gynecology* **194** 1334–1340.

Keski-Nisula L, Aalto ML, Katila ML & Kirkinen P 2000 Intrauterine inflammation at term: a histopathologic study. *Human Pathology* **31** 841–846.

Khatun S, Kanayama N, Md Belayet H, Yonezawa M, Kobayashi T & Terao T 1999 Interleukin-8 potentiates the effect of interleukin-1-induced uterine contractions. *Human Reproduction* **14** 560–565.

Kimura T, Takemura M, Nomura S, Nobunaga T, Kubota Y, Inoue T, Hashimoto K, Kumazawa I, Ito Y, Ohashi K et al. 1996 Expression of oxytocin receptor in human pregnant myometrium. *Endocrinology* **137** 780–785.

Loudon JA, Sooranna SR, Bennett PR & Johnson MR 2004 Mechanical stretch of human uterine smooth muscle cells increases IL-8 mRNA expression and peptide synthesis. *Molecular Human Reproduction* **10** 895–899.

Lye SJ, Nicholson BJ, Mascarenhas M, MacKenzie L & Petrocelli T 1993 Increased expression of connexin-43 in the rat myometrium during labor is associated with an increase in the plasma estrogen:progesterone ratio. *Endocrinology* **132** 2380–2386.

Maul H, Nagel S, Welsch G, Schafer A, Winkler M & Rath W 2002 Messenger ribonucleic acid levels of interleukin-1 beta, interleukin-6 and interleukin-8 in the lower uterine segment increased significantly at final cervical dilatation during term parturition, while those of tumor necrosis factor alpha remained unchanged. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **102** 143–147.

McNutt CM, Nicholson BJ & Lye SJ 1994 ACTH-induced preterm labour in the ewe is associated with increased mRNA and protein levels of myometrial gap junction protein, connexin-43. *Journal of Endocrinology* **141** 195–202.

Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G & Smith R 2002 Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *Journal of Clinical Endocrinology and Metabolism* **87** 2924–2930.

Osman I, Young A, Ledingham MA, Thomson AJ, Jordan F, Greer IA & Norman JE 2003 Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. *Molecular Human Reproduction* **9** 41–45.

Osmers RG, Adelmann-Grill BC, Rath W, Stuhlsatz HW, Tschesche H & Kuhn W 1995 Biochemical events in cervical ripening dilatation during pregnancy and parturition. *Journal of Obstetrics and Gynaecology* **21** 185–194.

Ou CW, Orsino A & Lye SJ 1997 Expression of connexin-43 and connexin-26 in the rat myometrium during pregnancy and labor is differentially regulated by mechanical and hormonal signals. *Endocrinology* **138** 5398–5407.

Phaneuf S, Rodriguez Linares B, TambyRaja RL, MacKenzie IZ & Lopez Bernal A 2000 Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour. *Journal of Reproduction and Fertility* **120** 91–97.

Pierce BT, Calhoun BC, Adolphson KR, Lau AF & Pierce LM 2002 Connexin 43 expression in normal versus dysfunctional labor. *American Journal of Obstetrics and Gynecology* **186** 504–511.

Rezapour M, Kilarski WM, Severs NJ, Gourdie RG, Rothery S, Backstrom T, Roomans GM & Ulmsten U 1997 Quantitative immunoconfocal analysis of human myometrial gap junction connexin43 in relation to steroid hormone concentrations at term labour. *Human Reproduction* **12** 159–166.

Risek B, Guthrie S, Kumar N & Gilula NB 1990 Modulation of gap junction transcript and protein expression during pregnancy in the rat. *Journal of Cell Biology* **110** 269–282.

Romero R, Baumann P, Gonzalez R, Gomez R, Rittenhouse L, Behnke E & Mitchell MD 1994 Amniotic fluid prostanoid concentrations increase early during the course of spontaneous labor at term. *American Journal of Obstetrics and Gynecology* **171** 1613–1620.

Sadowsky DW, Adams KM, Gravett MG, Witkin SS & Novy MJ 2006 Preterm labor is induced by intraamniotic infusions of interleukin-1β and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. *American Journal of Obstetrics and Gynecology* **195** 1578–1589.

Schmid B, Wong S & Mitchell BF 2001 Transcriptional regulation of oxytocin receptor by interleukin-1β and interleukin-6. *Endocrinology* **142** 1380–1385.

Sellers SM, Mitchell MD, Bibby JG, Anderson AB & Turnbull AC 1981 A comparison of plasma prostaglandin levels in term and preterm labour. *British Journal of Obstetrics and Gynaecology* **88** 362–366.

Skinner KA & Challis JR 1985 Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor. *American Journal of Obstetrics and Gynecology* **151** 519–523.

Soloff MS, Alexandrova M & Fernstrom MJ 1979 Oxytocin receptors: triggers for parturition and lactation? *Science* **204** 1313–1315.

Soloff MS, Izban MG, Cook DL Jr, Jeng YJ & Mifflin RC 2006 Interleukin-1-induced NF-κB recruitment to the oxytocin receptor gene inhibits RNA polymerase II-promoter interactions in cultured human myometrial cells. *Molecular Human Reproduction* **12** 619–624.

Sooranna SR, Lee Y, Kim LU, Mohan AR, Bennett PR & Johnson MR 2004 Mechanical stretch activates type 2 cyclooxygenase via activator protein-1 transcription factor in human myometrial cells. *Molecular Human Reproduction* **10** 109–113.

Sooranna SR, Engineer N, Loudon JA, Terzidou V, Bennett PR & Johnson MR 2005 The mitogen-activated protein kinase dependent expression of prostaglandin H synthase-2 and interleukin-8 messenger ribonucleic acid by myometrial cells: the differential effect of stretch and interleukin-1β. *Journal of Clinical Endocrinology and Metabolism* **90** 3517–3527.

Sooranna SR, Grigsby PL, Engineer N, Liang Z, Sun K, Myatt L & Johnson MR 2006 Myometrial prostaglandin E2 synthetic enzyme mRNA expression: spatial and temporal variations with pregnancy and labour. *Molecular Human Reproduction* **12** 625–631.

Sparey C, Robson SC, Bailey J, Lyall F & Europe-Finner GN 1999 The differential expression of myometrial connexin-43, cyclooxygenase-1 and -2, and Gs alpha proteins in the upper and lower segments of the human uterus during pregnancy and labor. *Journal of Clinical Endocrinology and Metabolism* **84** 1705–1710.

Strakova Z, Copland JA, Lolait SJ & Soloff MS 1998 ERK2 mediates oxytocin-stimulated PGE2 synthesis. *American Journal of Physiology* **274** E634–E641.

Terzidou V, Sooranna SR, Kim LU, Thornton S, Bennett PR & Johnson MR 2005 Mechanical stretch up-regulates the human oxytocin receptor in primary human uterine myocytes. *Journal of Clinical Endocrinology and Metabolism* **90** 237–246.

Thomson AJ, Telfer JF, Young A, Campbell S, Stewart CJ, Cameron IT, Greer IA & Norman JE 1999 Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. *Human Reproduction* **14** 229–236.

Turnbull AC, Anderson AB, Flint AP, Jeremy JY, Keirse MJ & Mitchell MD 1977 Human parturition. *Ciba Foundation Symposia* **47** 427–459.

Wathes DC, Borwick SC, Timmons PM, Leung ST & Thornton S 1999 Oxytocin receptor expression in human term and preterm gestational tissues prior to and following the onset of labour. *Journal of Endocrinology* **161** 143–151.

Winkler M, Fischer DC, Ruck P, Marx T, Kaiserling E, Oberpichler A, Tschesche H & Rath W 1999 Parturition at term: parallel increases in interleukin-8 and proteinase concentrations and neutrophil count in the lower uterine segment. *Human Reproduction* **14** 1096–1100.