Altered expression of interleukin-6, interleukin-8 and their receptors in decidua of women with sporadic miscarriage

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STUDY QUESTION: Are alterations in decidual expression of interleukin (IL)-6 and IL-8 associated with sporadic miscarriage?

SUMMARY ANSWER: IL-6 and IL-8 secretion from decidual uterine natural killer (uNK) cells and macrophages isolated from women with spontaneous miscarriage was reduced compared with normal controls.

WHAT IS KNOWN ALREADY: Miscarriage is a common gynaecological problem with huge financial and personal implications. Eleven to twenty per cent of all clinically recognized pregnancies are lost before the 20th week of gestation, with miscarriages often being divided into early (≤12 completed weeks from last menstrual period) and late (≥13 weeks). Spiral artery remodelling is a key feature of early pregnancy; failure of this process has been implicated in sporadic miscarriage. The molecular triggers that initiate spiral artery remodelling are not clear, although cytokines such as IL-6 and IL-8 may play a role.

STUDY DESIGN, SIZE, DURATION: This was a laboratory-based study using decidual and placental bed biopsy samples from women with sporadic miscarriage (n = 30) and termination of pregnancy controls (n = 30).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Total adherent decidual cells, CD10+ stromal cells, CD14+ macrophages and CD56+ uNK cells were isolated from decidua from apparently normal pregnancies that were terminated at either 8–10 or 12–14 weeks’ gestation. In addition, CD14+ macrophages and CD56+ uNK cells were isolated from decidua from sporadic miscarriage at 8–10 weeks’ gestation. Secreted IL-8 was measured in all isolated cell populations, while IL-6 was measured in CD14+ macrophages and CD56+ uNK cells from both sporadic miscarriage and normal controls. Placental bed biopsies were taken from women after sporadic miscarriage or termination of pregnancy at ≤12 completed weeks’ or >13 weeks’ gestational age, formalin-fixed, paraffin-embedded and immunostained for IL-6, IL-6Rα, GP130, IL-8, CXCR1, CXCR2 and CD13 (aminopeptidase N). Staining intensity for each factor was assessed in extravillous trophoblast cell populations, myometrial and decidual stroma, myometrial and decidual spiral arteries and decidual glandular epithelium. A CPA model was used to assess the potential role of IL-6 and IL-8 in spiral artery remodelling.

MAIN RESULTS AND THE ROLE OF CHANCE: IL-8 was secreted by total adherent decidual cells, CD10+ stromal cells and CD14+ macrophages at both 8–10 and 12–14 weeks’ gestation, with CD14+ cells secreting the highest levels. Both CD14+ and CD56+ cells isolated from decidua of early sporadic miscarriage produced lower IL-6 (P = 0.04, P = 0.01, respectively) and IL-8 levels (P = 0.0007, P = 0.002, respectively) compared with normal cases. In addition, altered expression of IL-6, IL-8 and their receptors was observed in various cell types in placental bed (myometrial stroma, glandular epithelium, interstitial extravillous trophoblast cells, vascular smooth muscle cells and endothelial cells) in sporadic miscarriage, particularly from later gestational ages. IL-6 and IL-8 disrupted vascular smooth muscle morphology and organization in an in vitro model of spiral artery remodelling.

LIMITATIONS, REASONS FOR CAUTION: By the nature of sampling at the time of miscarriage, it was not possible to ascertain the cause or effect in the observed alterations of levels of IL-6 and IL-8 in sporadic miscarriage.

† J.N.B. and G.E.L. contributed equally to this manuscript.

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WIDER IMPLICATIONS OF THE FINDINGS: Alterations in the expression of IL-6, IL-8 and their receptors may be associated with the aetiology of sporadic miscarriage, especially given the potential role of these cytokines in the regulation of trophoblast invasion and spiral artery remodelling.

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TRIAL REGISTRATION NUMBER: Not applicable.

Key words: interleukin-6 / interleukin-8 / sporadic miscarriage / spiral artery remodelling / decidua

Introduction

Miscarriage is a common gynaecological problem with huge financial and personal implications (Everett, 1997). It has been estimated that 11–20% of all clinically recognized pregnancies are lost before the 20th week of gestation (Everett, 1997), with miscarriages often being divided into early (≤12 completed weeks from the last menstrual period) and late (≥13 weeks) (Regan and Rai, 2000). Early miscarriages account for the majority, with approximately 50% being associated with aneuploidy. Miscarriage after 12 weeks’ gestational age (GA) affects only 1–2% of pregnancies (Regan and Rai, 2000) and is less likely to be associated with aneuploidy. The cause of many sporadic miscarriages is not known, although it has been proposed to be linked to incomplete placentation and spiral artery remodelling and therefore may be associated with altered levels of the cytokines and growth factors that regulate these processes.

Extravillous trophoblast (EVT) cells invade uterine decidua and myometrium (interstitial EVT) throughout the first half of pregnancy, accumulating around uterine spiral arteries and facilitating their remodelling (Pijnenborg et al., 1981, 2006). Failure of EVT invasion and spiral artery remodelling has been implicated in several pregnancy complications, including early and late miscarriages (Hustin et al., 1990; Jauniaux et al., 2000; Ball et al., 2006a,b).

Despite its importance, the mechanisms that control EVT invasion and spiral artery remodelling are poorly understood; decidual factors are likely to play an important role, and two such factors are interleukin (IL)-6 and IL-8. IL-6 is a pleiotropic cytokine that signals via the receptors IL-6Rα and gp130 (a promiscuous cytokine receptor) and plays important roles in host defence mechanisms and growth of cancer cells (Heinrich et al., 2003; Lin et al., 2007). Recent studies suggest that IL-6 is a potent pro-angiogenic cytokine stimulating the growth and proliferation of vascular smooth muscle cells (VSMC) and endothelial cells in vitro, including the formation of endothelial cell tubules (Nilsson et al., 2005). IL-8 is a multifunctional chemokine, best characterized as a chemo-attractant for neutrophils at sites of inflammation (Matsushima et al., 1988), although it is also chemotactic for other normal and neoplastic cells, and has angiogenic properties (Martin et al., 2009). IL-8 signals via two membrane-bound receptors, CXCR1 and CXCR2, which mediate different functional responses (Rosenkilde and Schwartz, 2004). IL-8 can also bind a non-signalling (‘decoy’) receptor, DARC, and can be inactivated by the cell-surface metalloproteinase, aminopeptidase N (CD13). The role of IL-6 in regulating EVT invasion is unclear, with one report suggesting stimulation of invasion (Jovanovic and Vićovac, 2009) and another no effect (Champion et al., 2012). IL-8 has been shown to stimulate EVT invasion and contributes to uterine natural killer (uNK) cell-mediated stimulation of trophoblast invasion (Hanna et al., 2006; De Oliveira et al., 2010). We have recently reported that IL-6 is secreted by a number of decidual cell populations including CD10+ decidual stromal cells, CD8+ T cells, CD14+ macrophages and CD56+ uNK cells, with levels secreted by CD10+ decidual stromal cells being increased at 12–14 weeks’ compared with 8–10 weeks’ GA and CD14+ macrophages secreting the highest levels of any of the individual cell types that were tested (Champion et al., 2012). In addition, we have previously demonstrated that IL-8 is secreted by total decidual cell isolates, CD8+ T cells and CD56+ uNK cells, with levels secreted by uNK cells being increased at 12–14 weeks’ compared with 8–10 weeks’ GA (De Oliveira et al., 2010).

There have been few studies of IL-8 and IL-6 in miscarriage and most have focused on circulating levels with varying results. There is only one report of uteroplacental IL-8 in miscarriage; levels were higher in products of conception from women with two or more miscarriages compared with normal pregnancy or first miscarriage (Madhappan et al., 2003). There are no reports of uteroplacental IL-6 in miscarriage in humans. However, in a mouse model, increased levels of IL-6 at the fetal maternal interface were associated with fetal loss (Zenclussen et al., 2003). The IL-8 and IL-6 receptor status has not been previously investigated in miscarriage.

We hypothesized that levels of IL-6 and/or IL-8 secreted by decidual cell populations isolated from women with sporadic miscarriage would be reduced; and that IL-6 and/or IL-8 would play roles in the initial stages of spiral artery remodelling.

Materials and Methods

Samples

All samples were collected at The Royal Victoria Infirmary, Newcastle upon Tyne, and all participants gave written informed consent. The study was approved by the local ethics committee (Ref: 10/H0908/52; County Durham and Tees Valley Research Ethics Committee). Placental bed biopsies (8–18 weeks’ GA) were taken under ultrasound guidance from women undergoing evacuation of products of conception after miscarriage (n = 5 early ≤12 weeks’ GA euploid, n = 5 late ≥13 weeks’ GA euploid, n = 5 early ≤12 weeks’ GA aneuploid and n = 5 ≥13 weeks’ GA aneuploid) or at surgical termination of an apparently normal pregnancy (n = 10 early ≤12 weeks’ GA, n = 10 ≥13 weeks’ GA) as previously described (Robson et al., 2002; Ball et al., 2006a,b). Miscarriage samples underwent karyotyping as previously reported (Ball et al., 2006a). Placental bed biopsies were immediately frozen in liquid nitrogen-cooled isopentane (Sigma Chemical Co., Poole, Dorset, UK) and stored at −80 °C until required for sectioning. Additional samples of decidua parietalis were obtained from both miscarriages (n = 10, early ≤11 weeks’ GA, karyotype not determined) and pregnancy terminations (n = 10, 8–11 weeks’ GA; n = 10, 12–14
weeks’ GA). Decidua was identified macroscopically, washed in phosphate-buffered saline, pH 7.4, to remove excess blood and used for the isolation of different cell populations, as described below. Chorionic plate arteries were dissected from term placenta obtained after elective Caesarean section.

**Isolation and culture of decidual cell populations**

Total adherent decidual, CD56+ uNK cell, CD10+ stromal cell and CD14+ macrophage cultures were prepared as previously described (Champion et al., 2012). Positive selection with CD14 will isolate both macrophages and immature dendritic cells; phenotypic analysis after isolation suggested that only 1–2% of isolated cells are immature dendritic cells (data not shown), and therefore for simplicity this cell population will be termed CD14+ macrophages. Briefly, decidual tissue was minced, subjected to DNase/collagenase enzymatic digestion and incubated overnight at 37°C in complete RPMI1640 medium (containing 10% fetal bovine serum, 1000 U/ml penicillin, 1 mg/ml streptomycin and 2 mM L-glutamine, all from Sigma Chemical Co.) to allow cells to adhere. The non-adherent fraction was used for the isolation of CD56+ uNK cells and CD10+ stromal cells, while the adherent fraction was used for the isolation of total adhered cells and CD14+ macrophages. The cells were either used as a total unfraccionated decidual cell suspension or different cell populations were isolated by positive selection with anti-CD56 (Coulter, High Wycombe, UK) or anti-CD10 (Leica, Newcastle upon Tyne, UK) and immunomagnetic beads (MidMACS; Miltenyi Biotec., Surrey, UK) as previously described (Champion et al., 2012). For the isolation of CD14+ cells, directly conjugated anti-CD14 immunomagnetic beads (Miltenyi Biotec) were used according to the manufacturer’s instructions. Total decidual cell suspensions, CD56+ uNK cells, or CD10+ stromal cells, were plated into a 96-well plate seeded at 1 × 10^5 cells per well in 100 μl of complete medium. CD14+ macrophages were plated into a 48-well plate seeded at 2 × 10^5 cells per well in 200 μl of complete medium. Conditioned medium was harvested after 24 h and stored at −20°C until required for analysis. Purity was assessed by routine immunostaining of isolated cell smears for the appropriate CD protein: using this methodology, the cell-enriched isolates were consistently >95% pure.

**IL-6 and IL-8 enzyme-linked immunosorbent assay**

Levels of IL-6 and IL-8 secreted by different decidual cell populations were determined using either a human IL-6 ELISA Development Kit (Peprotech EC Ltd, London, UK) or IL-8 ELISA Duoset (R&D Systems, Abingdon, UK) according to the manufacturer’s instructions as previously described (De Oliveira et al., 2010; Champion et al., 2012). Samples were diluted 1:100 or 1:2 (as appropriate) in the specified reagent diluent. There was no detectable IL-6 or IL-8 in non-conditioned medium (data not shown).

**Immunohistochemistry**

Formalin-fixed paraffin-embedded sections (3 μm) were dewaxed in xylene and rehydrated prior to incubation in 1% H2O2 in methanol for 10 min to block endogenous peroxidase activity. Cryosections (3 μm) were directly incubated in 1% H2O2 in methanol for 10 min to block endogenous peroxidase activity. All washes were performed in 0.15 M Tris-buffered 0.05 M saline, pH 7.6. For any given antibody, all tissue sections were immunostained in the same staining run to avoid day-to-day variation. The immunostaining procedure has been described in detail previously (Pongcharoen et al., 2004). Details for all primary antibodies used are given in Table I. Detection was with the mouse Vectastain Elite ABC kit with the exception of IL-6, which was detected using the polymer-based Impression Kit (Vector Laboratories, Peterborough, UK). The reaction was developed with 3,3′-diaminobenzidine.

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**Table I** Details of primary antibodies used in the study of IL-6, IL-8 and their receptors in decidua of women with sporadic miscarriage.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Method</th>
<th>Species</th>
<th>Dilution</th>
<th>Incubation time</th>
<th>Pretreatment</th>
<th>Clone or catalogue #</th>
<th>Positive control tissue</th>
</tr>
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<tr>
<td>LP34a</td>
<td>Elitef,g</td>
<td>Mouse</td>
<td>1:80</td>
<td>RT, 30 min</td>
<td>Nil (frozen)</td>
<td>NCL-LP34</td>
<td>Placenta</td>
</tr>
<tr>
<td>H-Caldesmonb</td>
<td>Elitef,g</td>
<td>Mouse</td>
<td>1:100</td>
<td>RT, 30 min</td>
<td>Nil (frozen)</td>
<td>h-CD</td>
<td>Myometrium</td>
</tr>
<tr>
<td>IL-6c</td>
<td>Immpressf,h</td>
<td>Rabbit</td>
<td>1:1500</td>
<td>RT, 30 min</td>
<td>Nil (frozen)</td>
<td>Ab6672</td>
<td>Tonsil</td>
</tr>
<tr>
<td>IL-8d</td>
<td>Immpressf,h</td>
<td>Mouse</td>
<td>1:20</td>
<td>RT, 60 min</td>
<td>Nil (frozen)</td>
<td>Ab10769</td>
<td>Tonsil</td>
</tr>
<tr>
<td>gp130g</td>
<td>Elitef,g</td>
<td>Mouse</td>
<td>1:50</td>
<td>RT, 60 min</td>
<td>Nil (frozen)</td>
<td>gp130 (AN-112)</td>
<td>Tonsil</td>
</tr>
<tr>
<td>IL-6Ra</td>
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<td>Rabbit</td>
<td>1:50</td>
<td>RT, 60 min</td>
<td>Nil (frozen)</td>
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<tr>
<td>CXCR1h</td>
<td>Immpressf,h</td>
<td>Rabbit</td>
<td>1:80</td>
<td>4°C, O/N</td>
<td>Nil (frozen)</td>
<td>42705-111</td>
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<tr>
<td>CXCR2h</td>
<td>Immpressf,h</td>
<td>Rabbit</td>
<td>1:40</td>
<td>4°C, O/N</td>
<td>Nil (frozen)</td>
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<tr>
<td>CD13e</td>
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<td>Mouse</td>
<td>1:200</td>
<td>RT, 30 min</td>
<td>Nil (frozen)</td>
<td>CD13-22A5</td>
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<tr>
<td>Myosin heavy chaina</td>
<td>Elitef,g</td>
<td>Mouse</td>
<td>1:600</td>
<td>RT, 60 min</td>
<td>PC in citrate buffer pH 6.0, 1 min (paraffin)</td>
<td>hSM-V</td>
<td>Stomach</td>
</tr>
<tr>
<td>CD31a</td>
<td>Elitef,g</td>
<td>Mouse</td>
<td>1:20</td>
<td>RT, 60 min</td>
<td>PC in citrate buffer pH 6.0, 1 min (paraffin)</td>
<td>IA10</td>
<td>Lymph node</td>
</tr>
</tbody>
</table>

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*a* Leica Biosystems, Newcastle upon Tyne, UK.

*b* Dako Cytomation, Denmark.

*c* Abcam, Cambridge, UK.

*d* Santa Cruz Biochemicals, California, USA.

*e* R&D Systems, Abingdon, UK.

*f* Vector Laboratories, Peterborough, UK.

*g* Vector ABC ELITE Kit, avidin–biotin peroxidase method (mouse or rabbit as appropriate).

*h* Vector Immpress Kit (polymer based, mouse or rabbit as appropriate).

RT, room temperature; O/N, overnight; PC, pressure cook.
Assessment of immunohistochemical staining

Each immunostained section was analysed semi-quantitatively using a modified ‘quickscore’ method (Schiessl et al., 2009) to take into account both the intensity of staining (0 = negative, 1 = weak, 2 = moderate, 3 = strong) and the percentage of positive cells for each staining intensity (1 = 0–25%, 2 = 25–50%, 3 = 50–75%, 4 = 75–100%). The whole of the section was assessed by one operator (HP) who was blinded to the origin of the sample. The intensity and percentage scores were then multiplied and scored summed to give a possible score range of 0–12. For example, negative staining in 20% of a given cell type (0 × 1 = 0), weak staining in 40% (1 × 2 = 2) and moderate staining in 40% (2 × 2 = 4) would give a total score of 0 + 2 + 4 = 6. The glandular epithelium, decidual stromal cells, myometrial stromal cells, decidual spiral artery endothelial cells, myometrial spiral artery endothelial cells, decidual spiral artery VSMC, myometrial spiral artery VSMC, interstitial EVT cells, endovascular EVT and trophoblast giant cells were all scored separately.

Chorionic plate artery model

Intact chorionic plate artery (CPA) segments (5 mm) were dissected from normal-term placenta and incubated in a 48-well plate at 37°C in 5% CO2 as previously described (Robson et al., 2012): (i) control RPMI complete medium (RPMI 1640, 5% charcoal-stripped fetal calf serum, 1% penicillin/streptomycin and 1% l-glutamine); (ii) 1 ng/ml recombinant IL-6 (Peprotech); (iii) 10 ng/ml recombinant IL-6; (iv) 5 ng/ml recombinant sIL-6Rα; (v) 10 ng/ml recombinant IL-6 + 5 ng/ml recombinant sIL-6Rα; (vi) 1 ng/ml recombinant IL-8; (vii) 10 ng/ml recombinant IL-8. All cytokines were made to the desired concentration in RPMI complete medium, which was changed every 48 h. CPAs were harvested after 120 h, fixed in 10% neutral buffered formalin and embedded in paraffin wax for immunohistochemistry. Three micrometre serial sections were immunostained using an avidin biotin peroxidase technique for CD31 to confirm endothelial integrity after culture; CD31-negative vessels were excluded from this study.

To investigate the effect of the cytokines on CPA VSMC morphology, sections were immunostained for myosin heavy chain. The slides were assessed for changes in VSMC rounding, disorganization of VSMC layers and the misalignment of VSMC layers, using a four-point scale (1 < 10%, 2 = 10–25%, 3 = 25–75%, 4 ≥ 75%) as previously described (Robson et al., 2012).

Secretion of IL-8 by decidual cell populations

High levels of IL-8 were found to be secreted by all cell types investigated (n = 10 in each group; Fig. 1). Secretion of IL-8 by the total adhered fraction was higher at 12–14 weeks’ than at 8–10 weeks’ gestation (P = 0.04; Fig. 1).

Secretion of IL-6 and IL-8 by macrophages and uNK cells in sporadic miscarriage

Based on our published data regarding IL-6 and IL-8 secretion by decidual cell populations in normal pregnancy, we investigated secretion of IL-6 and IL-8 in CD14+ macrophages and CD56+ uNK cells isolated from sporadic miscarriage and normal decidua at 7–11 weeks’ GA (n = 10 in each group). In sporadic miscarriage samples, lower levels of both IL-6 and IL-8 were secreted by both cell types compared with normal pregnancy controls (IL-6: CD14+ macrophages P = 0.04, CD56+ uNK cells P = 0.01; IL-8: CD14+ macrophages P = 0.0007, CD56+ uNK cells P = 0.002; Fig. 2A and B).

Immunolocalization of IL-6, IL-8 and their receptors in the placental bed of women with sporadic miscarriage

IL-6 and its receptors IL-6Rα and gp130, and IL-8 and its receptors CXCR1 and CXCR2 as well as the IL-8 modulating protein CD13 (aminopeptidase N) were immunolocalized in the placental bed of women with sporadic miscarriage (n = 10 in each GA group) or termination of apparently normal pregnancy (n = 5 in each GA group). Two GA periods were investigated: early (≤ 12 weeks’ gestation) and late (≥ 13 weeks’ gestation). Initial analysis showed no difference between euploid or aneuploid miscarriage and therefore these groups were combined for comparison with normal pregnancy groups.

Immunostaining of all the proteins investigated was widespread throughout the placental bed, with the majority of cell types studied showing some degree of immunostaining. Representative immunostaining of IL-6, IL-8 and their receptors in early sporadic miscarriage placental bed biopsies is shown in Fig. 3. In the early GA groups (≤ 12 weeks’ gestation), immunostaining for CD13 was weaker on glandular epithelial cells in decidua from sporadic miscarriage compared with normal
pregnancy ($P = 0.04$; Table II; Fig. 3K and L). There were no differences observed for any of the other factors investigated in the early GA groups. In the late GA group ($\geq 12^{+6}$ weeks’ gestation), CD14 expression was stronger on interstitial EVT ($P = 0.04$; Table III; Fig. 3M and N) and on myometrial stroma ($P = 0.03$; Table III; Fig. 3Q and P) in sporadic miscarriage compared with normal pregnancy. In contrast, CD13 immunostaining was weaker on myometrial spiral artery VSMCs in late sporadic miscarriage compared with normal pregnancy ($P = 0.01$; Table III; Fig. 3U and V). In addition to changes in receptor immunostaining, a reduction in IL-6 immunostaining was observed on myometrial spiral artery VSMCs in late sporadic miscarriage compared with normal pregnancy ($P = 0.05$; Table III; Fig. 3S and T), but this difference was not observed on decidual artery VSMCs or any other cell types. Staining for gp130 receptor was intense on the glandular epithelium and interstitial EVT, but there were no differences in immunostaining patterns between normal pregnancy and miscarriage. No other alterations in expression patterns of the IL-6 family members studied were observed.

### Role of IL-6 and IL-8 in spiral artery remodelling

Given the alterations in IL-6 and IL-8 receptor expression on spiral arteries in sporadic miscarriage and the decrease in IL-6 and IL-8 secretion by decidual cell types in sporadic miscarriage, we sought to investigate the potential role of these two cytokines in spiral artery remodelling using a CPA model (Robson et al., 2012).

Treatment with IL-6 had no effect on CPA VSMC rounding, but did increase separation of VSMC layers and misalignment of VSMCs within the vessel (Fig. 4A and B). Treatment of CPA with 10 ng/ml IL-6 or 10 ng/ml IL-6 + 5 ng/ml sIL-6Rx increased separation of VSMC layers compared with the 5-day media control ($P = 0.04$; $P = 0.009$, respectively). The combination of 10 ng/ml IL-6 + 5 ng/ml sIL-6Rx also increased misalignment of VSMC compared with 5-day media control ($P = 0.04$).

Treatment of CPA with IL-8 had no effect on rounding or separation of VSMC but did increase VSMC misalignment compared with the 5-day media control ($P = 0.02$) (Fig. 4C and D).

### Discussion

In the current study, we have demonstrated IL-8 production by a range of decidual cell types at two different GAs, with secretion by total adhered and CD56$^+$ uNK cells being increased at 12–14 compared with 8–10 weeks’ gestation. In addition, secretion of IL-6 and IL-8 by CD14$^+$ macrophages and CD56$^+$ uNK cells was reduced in early miscarriage compared with normal pregnancy of similar GA. IL-6, IL-8 and their receptors were immunolocalized in placental bed biopsies from both early and late normal and miscarriage patients. Immunostaining intensity of CD13 (aminopeptidase N), IL-6, IL-6R and CD56$^+$ decidual cell types at two different GAs, with secretion by total adhered uNK cells being increased at 12–14 compared with 8–10 weeks’ gestation. In addition, secretion of IL-6 and IL-8 by CD14$^+$ macrophages and CD56$^+$ uNK cells was reduced in early miscarriage compared with normal pregnancy of similar GA. IL-6, IL-8 and their receptors were immunolocalized in placental bed biopsies from both early and late normal and miscarriage patients. Immunostaining intensity of CD13 (aminopeptidase N), IL-6, IL-6R and CD56$^+$ decidual cell types at two different GAs, with secretion by total adhered uNK cells being increased at 12–14 compared with 8–10 weeks’ gestation. In addition, secretion of IL-6 and IL-8 by CD14$^+$ macrophages and CD56$^+$ uNK cells was reduced in early miscarriage compared with normal pregnancy of similar GA. IL-6, IL-8 and their receptors were immunolocalized in placental bed biopsies from both early and late normal and miscarriage patients. Immunostaining intensity of CD13 (aminopeptidase N), IL-6, IL-6R and CD56$^+$ decidual cell types at two different GAs, with secretion by total adhered uNK cells being increased at 12–14 compared with 8–10 weeks’ gestation. In addition, secretion of IL-6 and IL-8 by CD14$^+$ macrophages and CD56$^+$ uNK cells was reduced in early miscarriage compared with normal pregnancy of similar GA. IL-6, IL-8 and their receptors were immunolocalized in placental bed biopsies from both early and late normal and miscarriage patients. Immunostaining intensity of CD13 (aminopeptidase N), IL-6, IL-6R and CD56$^+$ decidual cell types at two different GAs, with secretion by total adhered uNK cells being increased at 12–14 compared with 8–10 weeks’ gestation. In addition, secretion of IL-6 and IL-8 by CD14$^+$ macrophages and CD56$^+$ uNK cells was reduced in early miscarriage compared with normal pregnancy of similar GA. IL-6, IL-8 and their receptors were immunolocalized in placental bed biopsies from both early and late normal and miscarriage patients.

We have previously reported secretion of IL-8 by total non-adherent decidual cells, CD56$^+$ uNK cells and CD8$^+$ T cells, with levels of IL-8 secreted by uNK cells increasing with GA (De Oliveira et al., 2010). In the current study, these data were extended to also demonstrate secretion of IL-8 by CD14$^+$ macrophages and CD10$^+$ decidual stromal cells, with no alteration in secretion profile with GA. It should be noted that the CD14$^+$ cell population would also contain a small proportion of immature dendritic cells and therefore not all IL-6 and IL-8 secretion can be attributed to the macrophages. In contrast, in common with the uNK cell population, total adherent decidual cells did show increased secretion with GA. It is not clear which cell type contributes to this increase in IL-8 secretion in the total adherent decidual cells, although it may...

![Figure 2](image-url)
Figure 3 Representative photomicrographs of immunolocalization of IL-6, IL-8 and their receptors in sporadic miscarriage placental bed biopsies. (A–C) Early normal placental bed biopsy immunostained for (A) LP34 antibody/periodic acid-Schiff (PAS) showing interstitial EVT (iEVT), intramural EVT (imEVT) and endovascular EVT (eEVT), (B) H-cal/PAS showing a decidual spiral artery and (C) H-cal/PAS showing a myometrial spiral artery. (D–J) Early miscarriage placental bed biopsy immunostained for (D) IL-6Ra showing a myometrial spiral artery, (E) GP130 showing interstitial EVT and the glandular epithelium, (F) IL-6 showing a decidual spiral artery, (G) IL-8 showing the glandular epithelium and a decidual spiral artery, (H) CD13 showing the glandular epithelium and a decidual spiral artery, (I) CXCR2 showing giant EVT cells and (J) CXCR1 showing a decidual spiral artery. (K and L) CD13 immunostaining of the glandular epithelium in early normal (K) and miscarriage (L) placental bed biopsies. (M and N) CD13 immunostaining of interstitial EVT in late normal (M) and miscarriage (N) placental bed biopsies. (O and P) CD13 immunostaining of the myometrium in late normal (O) and miscarriage (P) placental bed biopsies. (Q and R) CD13 immunostaining of myometrial VSMCs in late normal (Q) and miscarriage (R) placental bed biopsies. (S and T) IL-6 immunostaining of myometrial VSMCs in late normal (S) and miscarriage (T) placental bed biopsies. (U and V) IL-6Ra immunostaining of the glandular epithelium in late normal (U) and miscarriage (V) placental bed biopsies. (W and X) CXCR2 immunostaining of decidual endothelial cells in late normal (W) and miscarriage (X) placental bed biopsies. (Y and Z) No primary antibody negative controls of late normal (Y) and miscarriage (Z) placental bed biopsies.
Table II Immunoalignment and semi-quantitative ‘quickscore’ results in early (≤12 weeks’ gestation) placental bed samples from sporadic miscarriage (SM, n = 10) and normal termination of pregnancies (TOP, n = 10).

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<tr>
<th></th>
<th>gp130</th>
<th>IL-6Rx</th>
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<td>SM</td>
<td>TOP</td>
</tr>
<tr>
<td>Decidual stroma</td>
<td>3 ± 1</td>
<td>4.5 ± 0.9</td>
<td>1 ± 0</td>
<td>0.9 ± 0.3</td>
<td>7.5 ± 4.5</td>
<td>9.2 ± 0.8</td>
<td>5.5 ± 3.5</td>
</tr>
<tr>
<td>Myometrium</td>
<td>3.6 ± 0.8</td>
<td>2.5 ± 0.5</td>
<td>1.2 ± 0.2</td>
<td>0.2 ± 0.6</td>
<td>7.4 ± 4.1</td>
<td>6.3 ± 1</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Epithelium</td>
<td>12 ± 0</td>
<td>10 ± 1.1</td>
<td>0.6 ± 0.3</td>
<td>3.1 ± 1</td>
<td>1 ± 0</td>
<td>8 ± 1.2</td>
<td>10.5 ± 1.5</td>
</tr>
<tr>
<td>iEVT</td>
<td>12 ± 0</td>
<td>10.8 ± 0.9</td>
<td>0 ± 0</td>
<td>1.9 ± 1</td>
<td>8 ± 0</td>
<td>9.2 ± 0.8</td>
<td>12 ± 0</td>
</tr>
<tr>
<td>eEVT</td>
<td>8 ± 0</td>
<td>5.5 ± 2.5</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>imEVT</td>
<td>6 ± 0</td>
<td>12 ± 0</td>
<td>0 ± 0</td>
<td>2.6 ± 2.4</td>
<td>4 ± 0</td>
<td>10.5 ± 0.9</td>
<td>12 ± 0</td>
</tr>
<tr>
<td>EVT giant cells</td>
<td>6 ± 0</td>
<td>4 ± 1</td>
<td>0 ± 0</td>
<td>3.5 ± 2.8</td>
<td>8 ± 0</td>
<td>5.6 ± 1.2</td>
<td>6 ± 0</td>
</tr>
<tr>
<td>SpA VSMC (d)</td>
<td>2.5 ± 1.5</td>
<td>1.14 ± 0.5</td>
<td>6 ± 0</td>
<td>7.3 ± 1.4</td>
<td>12 ± 0</td>
<td>11.6 ± 0.2</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>SpA VSMC (m)</td>
<td>1.4 ± 0.3</td>
<td>1.7 ± 0.5</td>
<td>9.6 ± 1.8</td>
<td>11 ± 0.7</td>
<td>6.6 ± 2.6</td>
<td>9.2 ± 0.7</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>SpA EC (d)</td>
<td>6 ± 2</td>
<td>6 ± 1.1</td>
<td>1 ± 1</td>
<td>1.5 ± 1</td>
<td>1.5 ± 1.5</td>
<td>2.6 ± 1.8</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>SpA EC (m)</td>
<td>3.4 ± 0.3</td>
<td>5.2 ± 1.1</td>
<td>0.4 ± 0.4</td>
<td>0 ± 0</td>
<td>3.8 ± 1.7</td>
<td>3.3 ± 1.3</td>
<td>8 ± 4</td>
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</tbody>
</table>

Data are shown as mean ± SEM. Significant differences are highlighted in bold.

iEVT, interstitial extravillous trophoblast (EVT); eEVT, endovascular EVT; imEVT, intramural EVT; SpA, spiral artery; VSMC, vascular smooth muscle cell; d, decidua; m, myometrium; EC, endothelial cell.

\*P = 0.04
**Table III**  Immunolocalization and semi-quantitative ‘quickscore’ results in late (≥ 13 weeks' gestation) placental bed samples from SM (n = 10) and normal TOPs (n = 10).

<table>
<thead>
<tr>
<th></th>
<th>gp130</th>
<th>IL-6RA</th>
<th>CXCR1</th>
<th>CXCR2</th>
<th>CD13</th>
<th>IL-4</th>
<th>IL-8</th>
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<tr>
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<td>TOP</td>
<td>SM</td>
<td>TOP</td>
<td>SM</td>
<td>TOP</td>
</tr>
<tr>
<td>Decidual stroma</td>
<td>4.5 ± 1.9</td>
<td>5.7 ± 0.7</td>
<td>0.3 ± 0.3</td>
<td>1 ± 0.2</td>
<td>7.5 ± 0.9</td>
<td>8.5 ± 0.8</td>
<td>5.8 ± 1.4</td>
</tr>
<tr>
<td>Myometrium</td>
<td>2.2 ± 1</td>
<td>2.4 ± 0.3</td>
<td>3.8 ± 1</td>
<td>3.4 ± 0.8</td>
<td>7.8 ± 1.2</td>
<td>9.5 ± 0.8</td>
<td>4 ± 0.6</td>
</tr>
<tr>
<td>Epithelium</td>
<td>12 ± 0</td>
<td>11.6 ± 0.4</td>
<td>1 ± 0a</td>
<td>3.8 ± 0.8a</td>
<td>3.3 ± 1.6</td>
<td>6.1 ± 1</td>
<td>5.3 ± 1.6</td>
</tr>
<tr>
<td>iEVT</td>
<td>12 ± 0</td>
<td>11.6 ± 0.4</td>
<td>0.8 ± 0.5</td>
<td>1.4 ± 0.4</td>
<td>8.4 ± 1.6</td>
<td>10.1 ± 0.8</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td>eEVT</td>
<td>10 ± 2</td>
<td>10.7 ± 1.3</td>
<td>0 ± 0</td>
<td>0.7 ± 0.7</td>
<td>8 ± 0</td>
<td>8 ± 0</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>iEVT giant cells</td>
<td>8.8 ± 1.5</td>
<td>10 ± 1</td>
<td>1.3 ± 1.3</td>
<td>0.2 ± 0.2</td>
<td>9.8 ± 0.9</td>
<td>9.1 ± 1.1</td>
<td>8 ± 0</td>
</tr>
<tr>
<td>EVT giant cells</td>
<td>12 ± 0</td>
<td>12 ± 0</td>
<td>0 ± 0</td>
<td>1.6 ± 1.2</td>
<td>5.2 ± 2</td>
<td>8.2 ± 0.8</td>
<td>9.4 ± 1.2</td>
</tr>
<tr>
<td>SpA-VSMC (d)</td>
<td>1 ± 0</td>
<td>3 ± 1.2</td>
<td>5 ± 2.1</td>
<td>6 ± 1.8</td>
<td>10 ± 2</td>
<td>11.5 ± 0.5</td>
<td>2 ± 0.4</td>
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<tr>
<td>SpA-VSMC (m)</td>
<td>1.2 ± 0.4</td>
<td>1.6 ± 0.3</td>
<td>1.3 ± 0.9</td>
<td>11.2 ± 0.8</td>
<td>9.6 ± 1.1</td>
<td>11.3 ± 0.5</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>SpA-EC (d)</td>
<td>4.8 ± 0.8</td>
<td>5.6 ± 1.3</td>
<td>0 ± 0</td>
<td>0.3 ± 0.3</td>
<td>6.3 ± 2.4</td>
<td>5.9 ± 2</td>
<td>1.8 ± 1.4c</td>
</tr>
<tr>
<td>SpA-EC (m)</td>
<td>5.8 ± 1.4</td>
<td>5.3 ± 0.9</td>
<td>0 ± 0</td>
<td>0.2 ± 0.2</td>
<td>5.6 ± 2.1</td>
<td>4.1 ± 1.4</td>
<td>5.4 ± 0.2</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SEM. Significant differences are highlighted in bold.
tiEVT, interstitial extravillous trophoblast (EVT); eEVT, endovascular EVT; imEVT, intramural EVT; SpA, spiral artery; VSMC, vascular smooth muscle cell; d, decidua; m, myometrium; EC, endothelial cell.

*P = 0.01.
*P = 0.009.
*P = 0.03.
*P = 0.04.
*P = 0.01.
*P = 0.05.
Figure 4 Effect of IL-6 and IL-8 on VSMC morphology and organization in a chorionic plate artery (CPA) model. (A) Graphical representation of morphological scores for separation, misalignment and rounding of VSMCs in the CPA model treated with IL-6 and/or sIL-6Rα ($n = 10$ in each group). (B) Representative photomicrographs of chorionic plate arteries immunostained with myosin heavy chain showing differences in separation (three left panels) and misalignment (two right panels). The photomicrograph shows an overview of the whole-vessel cross section that was analysed, while the inset shows a much higher magnification to demonstrate separation and misalignment. Original magnification $\times 200$. (C) Graphical representation of morphological scores for separation, misalignment and rounding of VSMCs in the CPA model treated with IL-8. (D) Representative photomicrographs of chorionic plate arteries immunostained with myosin heavy chain showing differences in misalignment. The photomicrograph shows an overview of the whole-vessel cross section that was analysed, while the inset shows a much higher magnification to demonstrate misalignment. Original magnification $\times 200$. 
arise from co-ordination of the different cell types, which is not seen when they are separated. Alternatively, as shown by immunohistochemistry, the glandular epithelium is highly reactive for IL-8 and this may be a potent cellular source of this cytokine in the early pregnancy decidua. IL-8 secretion has been previously reported for all of these cell types (Engert et al., 2007; Vaccia et al., 2008; Li et al., 2009), although we are the first group to investigate differences in cytokine secretion at different GAs. It is interesting to note that CD56+ uNK cells secrete the lowest levels of IL-8 of any of the cell types investigated to date. We have previously reported IL-6 secretion by the same cell types, with CD10+ decidual stromal cell secretion of IL-6 increasing with GA (Champion et al., 2012).

There are few reports of IL-6 and IL-8 in miscarriage, with results varying significantly. In the current study, we demonstrate reduced secretion of both IL-6 and IL-8 by CD56+ and CD14+ cells isolated from decidua from miscarriage compared with normal pregnant samples of comparable GA. In our studies of IL-6 and IL-8 secretion by different decidual cell types, we have found that CD14+ cells are one of the major sources of these cytokines within the decidua, while CD56+ cells have shown some GA differences. In addition, these two cell types are the major leucocyte populations within the decidua in early pregnancy and have known non-immune type functions in early pregnancy. Previous studies in our laboratory have demonstrated no change in uNK cell numbers, but an increase in macrophage numbers in sporadic miscarriage (Scaife et al., 2004). It is not clear, however, whether the increase in macrophage numbers would be able to compensate for the reduction in uNK cell and macrophage secretion of IL-6 and IL-8. In contrast, Madhappan et al. (2003) reported higher levels of uteroplacental IL-8 in products of conception from women suffering recurrent miscarriage compared with normal pregnancy controls. However, specific cell types were not investigated and differences may arise between sporadic and recurrent miscarriage. There are also conflicting reports of IL-6 in miscarriage. Jasper et al. (2007) reported a significant reduction in non-pregnant endometrial IL-6 mRNA expression in women suffering from recurrent miscarriage, although different cell types were not investigated. In contrast, in murine pregnancy, Zenclussen et al. (2003) reported increased IL-6 at the fetal—maternal interface in fetal loss with increased immunohistochemical expression by trophoblast cells, increased production by decidual monocyte cells and up-regulated IL-6 mRNA expression in decidual and placental tissue at day 18 of gestation. Plasma levels of both IL-8 and IL-6 have also been reported to be increased in women with a history of second trimester abortion (Galazios et al., 2011) and euploid sporadic miscarriage (Calleja-Agius et al., 2012). The discrepancies between studies may be due to the difference in species, gestational time points and tissues investigated. In addition, in the current study, the karyotype of the miscarriage samples was not determined, which may introduce further variation in results. However, recent studies have provided evidence that there is no difference in decidual environment in euploid or aneuploid pregnancies (Salker et al., 2010; Teklenburg et al., 2010).

We have previously reported immunohistochemical localization of IL-6, IL-8 and their receptors, IL-6Ra, gp130, CXCR1 and CXCR2, in the placental bed during early pregnancy (De Oliveira et al., 2010; Champion et al., 2012). Immunolocalization of IL-6 and IL-8 was widespread, and the receptors for these cytokines were immunolocalized to EVT cells as well as to spiral arteries, both potential targets for IL-6 and IL-8 biological activity during early pregnancy. CD13 (aminopeptidase N) has been reported to be expressed by decidual stromal cells, with its expression being regulated by estrogen (Seli et al., 2001). In the current study, CD13 was immunolocalized to decidual stromal cells, myometrium, glandular epithelium, EVT cells and spiral arteries. Both IL-6 and IL-8 have been proposed to play roles in regulating trophoblast invasion and spiral artery remodelling. We, and others, have previously demonstrated that IL-8 can stimulate trophoblast invasion and likely contributes to uNK cell stimulation of EVT invasion (Hanna et al., 2006; De Oliveira et al., 2010; Jovanovic et al., 2010). The role of IL-6 in regulating trophoblast invasion is less clear, with one study suggesting that it enhances trophoblast invasion (Jovanovic and Vico, 2009), although we did not find any effect of IL-6 on trophoblast invasion (Champion et al., 2012). The reduced uNK cell secretion of IL-8 observed in miscarriage may help contribute to the aetiology of this condition by attenuating the ability of uNK cells to stimulate EVT invasion.

In the current study, we examined the potential role of IL-6 and IL-8 in spiral artery remodelling, using a previously published CPA model (Robson et al., 2012). Both IL-6 (alone and in combination with sIL-6Ra) and IL-8 were able to induce morphological changes in VSMCs in the CPA model. The effects were modest and we have previously demonstrated that angiopoietin 2 contributes to uNK cell induction of similar morphological changes in this model (Robson et al., 2012). Therefore, it is likely that a combination of factors are able to induce these observed morphological changes that mimic the initial stages of spiral artery remodelling. Leucocytes, in particular uNK cells and macrophages, are observed surrounding spiral arteries in the early stages of remodelling (Pijnborg et al., 1983; Smith et al., 2009) and our in vitro studies suggest a role for leucocyte-derived cytokines and angiogenic growth factors in the remodelling process. Therefore, it is possible that the reduced secretion of IL-6 and IL-8 by both uNK cells and macrophages contributes to the aetiology of miscarriage and the reduced spiral artery remodelling observed in these cases.

In the current study, immunostaining intensity of these factors was examined in a number of cell types in the placental bed in women with early or late miscarriage and appropriate controls. Several reports suggest that IL-6 and IL-8 have an involvement in miscarriage, and receptor expression of these cytokines has not yet been investigated in miscarriage samples compared with normal pregnancy. We hypothesized that IL-8 and IL-6 levels within the decidua would alter in miscarriage, also resulting in altered receptor expression on target cells. Euploid and aneuploid samples from early miscarriage (≤12 + 6 weeks’ GA) (Ball et al., 2006b) and euploid and aneuploid samples from late miscarriage (≥13 weeks’ GA) (Ball et al., 2006a) were assessed semi-quantitatively and there were no differences in immunostaining between euploid and aneuploid samples within the GA groups studied; therefore, euploid and aneuploid samples were combined for analysis. In the current study, there was also no difference in immunostaining intensity for any of the factors investigated or in any of the cell types investigated; therefore, to increase sample numbers and the power of the study, these groups were combined for each GA group. In addition, recent evidence suggests that the decidual environment does not differ depending on whether a euploid or aneuploid embryo is present (Salker et al., 2010; Teklenburg et al., 2010).

CD13 immunostaining was more intense on interstitial EVT and myometrial stroma in miscarriage ≥13 weeks’ GA compared with interstitial EVT from normal pregnancy of comparable GA. CD13 immunostaining was reduced, however, in the glandular epithelium in early miscarriage
and in myometrial spiral artery VSMCs in late miscarriage compared with appropriate controls. Conflicting reports exist on the role of CD13 and its association with IL-8. Initial reports suggested that CD13 inactivated IL-8 by degradation (Kanayama et al., 1995; Seli et al., 2001), but a later study reported that expression of CD13 by human embryonic kidney cells down-regulated CXCR4 expression and inhibited CXCL12-stimulated migration but did not affect the activity of IL-8 (Wulfaenger et al., 2008). Until we fully understand the role of CD13 in regulating IL-8 activity, we can only speculate on the role the observed alterations in CD13 expression patterns may play in the aetiology of early or late miscarriage. CXCR2 immunostaining was also increased on the endothelial cells of decidual spiral arteries in late miscarriage. IL-8 appears to act directly on VSMCs to facilitate spiral artery remodelling, and therefore it is not clear how increased CXCR2 on endothelial cells may contribute to miscarriage. Expression of IL-6Rα was increased in the glandular epithelium of late miscarriage compared with normal pregnancy, while IL-6 immunostaining was decreased in myometrial spiral arteries in late miscarriage. The biological role of IL-6 on the glandular epithelium is not clear but it may regulate secretion of various cytokines and growth factors, as is the case in EVT cells (Champion et al., 2012), and therefore may further alter the cytokine milieu of the decidual during miscarriage. The decreased IL-6 observed in myometrial spiral arteries may contribute to their decreased remodelling. It was interesting to note that, with the exception of CD13 expression by the glandular epithelium, all other significant differences were observed in late miscarriage. We have previously demonstrated decreased spiral artery remodelling in late, but not in early, miscarriage and these alterations in IL-6- and IL-8-related molecules may play a physiological role in the aetiology of this condition. However, it cannot be ruled out that the changes observed in the current study with miscarriage are a consequence of this condition and not causative for it.

In conclusion, levels of IL-6 and IL-8 secreted by CD56+ uNK cells and CD14+ macrophages were decreased in women with early miscarriage, although later miscarriage samples were not investigated in this study. Immunohistochemical assessment of early and late miscarriage placental bed biopsies predominantly showed alterations in IL-6 and IL-8 regulators in late miscarriage. In addition to our previous studies on the regulation of trophoblast invasion, we now show that IL-6 and IL-8 may play a role in the initial stages of spiral artery remodelling that requires induction of VSMC separation and morphological changes. The reduced levels of IL-6 and IL-8 observed in miscarriage may contribute to reduced trophoblast invasion as well as reduced spiral artery remodelling, both features of late miscarriage. We therefore conclude that IL-6 and IL-8 play vital roles in the establishment and maintenance of pregnancy, and that alterations in their levels or biological activity may contribute to the aetiology of miscarriage, particularly in the second trimester.

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Authors’ roles

H.P.: performed experiments, wrote manuscript. B.A.I.: performed experiments. S.C.R.: sample collection. J.N.B.: study design, wrote manuscript. G.E.L.: study design, wrote manuscript. All authors approved the submitted version of the manuscript.

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Conflict of interest

None declared.

References


Jovanović M, Stefanoska I, Radojčić L, Vićovac L. Interleukin-8 (CXCL8) stimulates trophoblast cell migration and invasion by increasing levels of matrix metalloproteinase (MMP)2 and MMP9 and integrins α5β1 and β3. *Reproduction* 2010;139:789–798.