Potential significance of physiological and pharmacological glucocorticoids in early pregnancy

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BACKGROUND: Despite extensive studies of the developmental consequences of increased glucocorticoid exposure in mid- to late pregnancy, relatively little is known regarding the significance of glucocorticoids in early pregnancy. The objective of this review was to consider potential roles for this family of corticosteroids that might relate to early pregnancy.

METHODS: Although this is a narrative review, 249 source articles addressing potential effects of glucocorticoids on aspects of early pregnancy and development (published between 1997 and 2007) were identified using a systematic literature search. Additional articles (115) were identified if cited by the primary reference articles identified in the systematic phase of the review.

RESULTS: Much of the evidence to implicate glucocorticoids in early pregnancy comes from studies of steroid receptors and the 11β-hydroxysteroid dehydrogenase enzymes, which modulate cortisol action in the endometrium/decidua, trophoblast, placenta and embryo/fetus. The evidence reviewed suggests that in early pregnancy the actions of glucocorticoids are balanced between positive effects that would promote pregnancy (e.g. stimulation of hCG secretion, suppression of uterine natural killer cells, and promotion of trophoblast growth/invasion) versus adverse effects that would be expected to compromise the pregnancy (e.g. inhibition of cytokine-prostaglandin signalling, restriction of trophoblast invasion following up-regulation of plasminogen activation inhibitor-1, induction of apoptosis, and inhibition of embryonic and placental growth).

CONCLUSIONS: Glucocorticoids exert many actions that could impact both negatively and positively on key aspects of early pregnancy. These steroids may also be implicated in obstetric complications, including intra-uterine growth restriction, pre-term labour, pre-eclampsia and chorio-aminionitis.

Keywords: cortisol; glucocorticoid; 11β-hydroxysteroid dehydrogenase; placenta; trophoblast

Introduction

Corticosteroid hormones regulate many of the processes required for successful embryo implantation, as well as for the subsequent growth and development of the fetus and placenta. In utero, the endometrium, placenta and embryo/fetus are each exposed to the major physiological glucocorticoid, cortisol, arising from either the maternal or fetal adrenal glands. There are essentially three mechanisms by which the fetus and placenta can be subjected to increased concentrations of active glucocorticoids in utero, these being:

(i) administration of synthetic glucocorticoids to the mother (as is common practice in pregnancies at risk of pre-term labour)
(ii) elevation of maternal cortisol concentrations (as occurs during maternal stress)
(iii) impaired cortisol metabolism within the decidua, placenta and/or fetus.

In recent years there has been interest in the administration of glucocorticoids to improve (i) pregnancy rates in women undergoing assisted conception by in vitro fertilization-embryo transfer (IVF-ET) (Boomsma et al., 2007), and (ii) pregnancy outcomes in women with a history of recurrent miscarriage (Ogasawara and Aoki, 2000; Quenby et al., 2003, 2005). It is known that uterine receptivity during embryo implantation is influenced, at least in part, by growth factors, cytokines and uterine natural killer (NK) cells. Imbalances between these factors have been implicated in implantation failure and recurrent miscarriage. It has been shown that glucocorticoids may have a role in improving the intra-uterine environment (Quenby et al., 2005), and this may be of particular relevance to women with antiphospholipid syndrome.
Whether this translates to an improvement in clinical outcomes of pregnancy is the aim of ongoing studies. For recurrent miscarriage, this has not been shown and in fact there is a suggestion of increased risks of pregnancy complications (Laskin et al., 1997; Empson et al., 2005). Common medical complications of pregnancy frequently treated by the administration of synthetic glucocorticoids include maternal asthma and rhinitis (Demoly et al., 2003; Namazy and Schatz, 2004; Osur, 2005), although the systemic absorption of inhaled steroids is low and there are a number of studies suggesting no adverse pregnancy effects. A growing body of evidence indicates that increased exposure of the fetus to glucocorticoids in mid- to late pregnancy may result in adverse outcomes, which include:

- intra-uterine growth restriction (IUGR) (Reinisch et al., 1998; Benediktsson et al., 1993; Levitt et al., 1996; Lindsay et al., 1996a; Ikekami et al., 1997; Nyirenda et al., 1998; French et al., 1999; Newnham et al., 1999; Huang et al., 1999; Bloom et al., 2001; Langdown and Sugden, 2001; Lesage et al., 2001; Sloboda et al., 2000; Sugden et al., 2001; Welberg et al., 2001; Jensen et al., 2002; Kerzner et al., 2002; Martins et al., 2003; Field et al., 2005, 2006; Kranendonk et al., 2006a; Enggard et al., 2007);
- increased risk of pre-term labour (Shams et al., 1998; Langdown and Sugden, 2001; Field et al., 2006);
- programming of post-natal hypertension (Tangalakis et al., 1992; Benediktsson et al., 1993; Edwards et al., 1993; Seckl et al., 1995; Levitt et al., 1996; Lindsay et al., 1996a; Dodic et al., 1999; 2002a, b; Doyle et al., 2000; Seckl, 2001; Sugden et al., 2001; Jensen et al., 2002; Trainer, 2002; Banjanin et al., 2004; Jansson and Powell, 2007);
- programming of increased post-natal activity in the hypothalamo–pituitary–adrenal axis (Uno et al., 1994; Levitt et al., 1996; Muneoka et al., 1997; Clark, 1998; Lesage et al., 2001; Liu et al., 2001; Bertram and Hanson, 2002; Matthews, 2002; Sloboda et al., 2002; Banjanin et al., 2004; Matthews et al., 2004; Shoener et al., 2006; de Vries et al., 2007);
- effects on fetal brain development, associated with alterations in pre-natal and post-natal behaviour (Uno et al., 1994; Muneoka et al., 1997; Rodriguez et al., 1998; Huang et al., 1999; Matthews, 2000; Huang, 2001; Welberg et al., 2001; Antonov-Schlorke et al., 2003; Canlon et al., 2003; de Weerth et al., 2003; Matthews et al., 2004; Field et al., 2005; Kranendonk et al., 2006b).

These adverse consequences of glucocorticoids in late pregnancy have been the subject of several reviews (Uno et al., 1994; Seckl et al., 1995; Langley-Evans, 1997a; Seckl, 1997, 2001, 2004, 2007; Nyirenda and Seckl, 1998; Clark, 1998; Newnham, 2001; Newnham and Moss, 2001; O’Regan et al., 2001; Fowden and Forhead, 2004; Welberg and Seckl, 2001; Bertram and Hanson, 2002; Matthews, 2002; Trainer, 2002; Matthews et al., 2004; Seckl and Meaney, 2004; Drake et al., 2007; Meaney et al., 2007). In contrast, relatively little is known regarding the significance of glucocorticoids in early pregnancy and most of the available evidence comes from women undergoing assisted reproduction. While a recent Cochrane review concluded that administration of synthetic glucocorticoids prior to or immediately after embryo transfer had no significant effect on the probability of conceiving, when studies involving intracytoplasmic sperm injection (ICSI) were excluded, the six remaining reports of standard in vitro fertilization (IVF) found pregnancy rates to be significantly increased \( P = 0.02 \) (Boomsma et al., 2007), indicating that glucocorticoids may facilitate conception. This review considers the evidence that glucocorticoids can influence several key aspects of early pregnancy, including effects on the maternal immune response, embryo attachment/implantation, trophoblast outgrowth and invasion, as well as the development of the fetus and associated placenta. Inevitably, much of the experimental evidence comes from studies conducted in animal models, but relevant data from human pregnancies are included wherever available. Evidence is also presented that dysregulation of placental glucocorticoid metabolism might expose the fetus to increased levels of active glucocorticoids in complications of pregnancy, including IUGR, pre-term labour, chorioamnionitis and pre-eclampsia.

**Methodology**

Although this is a narrative (rather than systematic) review of published evidence, source articles were identified using a systematic literature search. An electronic search strategy was developed for medical literature databases [The Cochrane Library 2006: 2, PubMed (1997–2007), Medline (1997–2007)], and searches were updated in November 2007 (Table I). After an initial screen based on article titles, the review of abstracts was performed independently by the two authors in order to identify all peer-reviewed publications relating to the role of glucocorticoids in early pregnancy and development. The full text of each article was reviewed if either author opted to include the paper following the abstract review phase. Additional articles were identified for inclusion if cited by the primary reference articles identified in the systematic phase of the review.

**Cellular actions of glucocorticoids**

Much of the available evidence to implicate glucocorticoids in early pregnancy arises from studies of steroid receptors and/or glucocorticoid metabolism. Hence, this review begins with a brief outline of the cellular mechanisms by which glucocorticoids act and the roles for 11β-hydroxysteroid dehydrogenase (11BHSD) enzymes in modulating glucocorticoid actions.

The chronic actions of glucocorticoid are typically mediated via intracellular glucocorticoid receptors (GR) (Funder, 1997; Kino and Chrousos, 2004; Lu et al., 2006). Having bound steroid, activated GR translocate from the cytoplasm into the nucleus where they act as ligand-dependent transcription factors. This involves formation of phosphorylated GR dimers which recruit co-activator or co-repressor proteins, respectively increasing or decreasing the expression of target genes by controlling histone acetylation (Li et al., 2003; Hayashi et al., 2004; Schoneveld et al., 2004). This nuclear mode of action effects lasting changes in cellular function, but takes several hours to elicit a response. One of the proteins up-regulated by glucocorticoids is the serum and glucocorticoid-induced kinase (sgk)-1, a serine-threonine protein kinase which mediates acute regulation of electrolyte and fluid...
balance (Lang et al., 2006). Sgk-1 is expressed in the human endometrium, where it is up-regulated by progesterone in the secretory phase of the cycle and during decidualization (Feroze-Zaidi et al., 2007). In addition, sgk-1 is also expressed in term human cytotrophoblasts where it can be up-regulated by both glucocorticoids and by aldosterone (Driver et al., 2003). GR are expressed at high levels in decidua, chorion, amnion, stromal fibroblasts, vascular smooth muscle cells and endothelial cells from term placental villi, with moderate expression in term cytotrophoblasts and negligible expression in the term syncytiotrophoblast (Kossmann et al. in term cytotrophoblasts and negligible expression in the term syncytiotrophoblast (Kossmann et al., 1984; Sun et al., 1996; Weisbart and Huntley, 1997; Driver et al., 2001; Chan et al., 2003; Sun and Myatt, 2003; Lee et al., 2005; Chan et al., 2007; Yang et al., 2007). By differentiating between expression of the vacant, non-phosphorylated form of the GR and the activated, phosphorylated form, (Lee et al., 2005) established that placental GR must be activated by physiological glucocorticoids in utero.

While synthetic glucocorticoids can only activate GR, the physiological glucocorticoids, cortisol and corticosterone, can also activate ‘mineralocorticoid receptors’ (MR). Despite their name, MR exhibit little intrinsic specificity, binding aldosterone, cortisol and corticosterone with comparable affinities in vitro (Arriza et al., 1987; Sheppard and Funder, 2001). Since cortisol typically circulates at concentrations 1000-fold greater than aldosterone (nmol/l versus pmol/l, respectively), the MR should be constantly swamped by cortisol, as occurs in the clinical syndrome of apparent mineralocorticoid excess (Ulick et al., 1979; Stewart et al., 1988; Benediktsson and Edwards, 1994; Shimojo and Stewart, 1995; Funder, 1995; Edwards et al., 1996; Mantero et al., 1996; White et al., 1997; Wilson et al., 2001). In the non-pathological state, the 11βHSD enzymes effectively exclude cortisol from the MR, leaving these receptors free to respond appropriately to the renin–angiotensin–aldosterone system (Edwards et al., 1996; White et al., 1997; Krozowski, 1999; Draper and Stewart, 2005).

In potential target cells, including cells of the reproductive system, 11βHSD enzymes catalyse the reversible inactivation of cortisol and corticosterone (Edwards et al., 1996; White et al., 1997; Krozowski, 1999; Michael et al., 2003; Draper and Stewart, 2005) (Fig. 1). To date, two 11βHSD isoenzymes have been cloned and characterized. Type 1 11βHSD, which is a relatively low affinity, bidirectional enzyme that usually regenerates cortisol from biologically inert cortisone (Seckl and Walker, 2001), is expressed in the endometrial epithelium (Thompson et al., 2002; McDonald et al., 2006), decidua (Baggia et al., 2005), is expressed in the endometrial epithelium (Thompson et al., 2002; McDonald et al., 2006), decidua (Baggia et al., 2005), and embryo research (Sun et al., 2001). Type 2 11βHSD, which is expressed in the placenta, has a higher affinity for cortisone compared to cortisol and preferentially inactivates cortisone (Bednarek et al., 2002). Type 2 11βHSD is expressed in the placenta (Muneyyirci-Delale et al., 1996; Pepe et al., 1996a, b; Sun et al., 1997a; Yang, 1997; Alfaidy et al., 2001; Thompson et al., 2002; Klemcke et al., 2003). In contrast, type 2 11βHSD, a high affinity enzyme that inactivates cortisol (Agrawal et al., 1994; Albiston et al., 1994; Zhou et al., 1995), has been localized in the endometrium and placenta (Brown et al., 1993; Burton and Waddell, 1994; Krozowski et al., 1995; Stewart et al., 1995; Li et al., 1996; Pepe et al., 1996a; Petrelli et al., 1997; Smith et al., 1997; Sun et al., 1997a; Yang, 1997; Alfaidy et al., 1998; Hirase et al., 2000; Alfaidy et al., 2001, 2002; Driver et al., 2001; Clarke et al., 2002; Hardy and Yang, 2002; Thompson et al., 2002; Klemcke et al., 2003; Van Beek et al., 2004; Homan et al., 2006). Within the human placenta, there is also substantial metabolism of cortisol (and cortisone) by 5β-reductase, 3α/3βHSD and 20βHSD enzymes to form inactive tetrahydro- and hexahydro-steroid metabolites (Pasqualini, 2005).

**First clues from IVF**

The first evidence to implicate cortisol in early pregnancy was provided by studies of couples undergoing assisted conception by IVF-ET. Eight published studies have reported on the association between the establishment of clinical pregnancies and levels of cortisol–cortisone metabolism within the ovary prior to oocyte retrieval for IVF. These studies have either made direct measurements of ovarian 11βHSD activities in the human granulosa-lutein cells recovered during oocyte collection, or have assessed the

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### Table I.

Search strategy for medical literature databases.

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<th>Search Term</th>
<th>Number of titles identified by search (all titles/abstracts were reviewed)</th>
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<td>4315</td>
<td>249</td>
<td>115</td>
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</tbody>
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**Figure 1:** Cortisol-cortisone inter-conversion by the cloned hydroxysteroid dehydrogenase (11βHSD) enzymes. The conversion of inert cortisone to active cortisol (which can bind and activate glucocorticoid receptors) is catalysed by type 1 11βHSD (11βHSD1), whereas the metabolism of cortisol to cortisone (which cannot activate glucocorticoid receptors) is mediated via both type 1 and type 2 11βHSD (11βHSD2). The pyridine nucleotide cofactors for each isoenzyme are shown in italics.
cortisol:cortisone ratio in follicular fluid as an in vitro index of ovarian cortisol metabolism. Of the eight studies, six reported lower ovarian 11βHSD activities and/or increased cortisol:cortisone ratios in follicular fluid to be associated with an improved probability of achieving pregnancy through IVF-ET (Michael et al., 1993, 1995, 1999; Keay et al., 2002; Lewicka et al., 2003; Thurston et al., 2003). Of the remaining two studies, one was not powered to investigate the link to conception rates (Knaggs et al., 1998) and while the other reported no significant association between levels of cortisol metabolism and pregnancy, the probability of conception was 3-fold higher in those women whose ovarian cells exhibited low rates of cortisol–cortisone metabolism relative to patients with high levels of cortisol oxidation (Thomas et al., 1998).

It is important to note that in the studies linking decreased ovarian cortisol metabolism to conception, differences in pregnancy rates were not accompanied by parallel changes in the proportions of oocytes successfully fertilized in vitro nor with numbers of embryos generated for subsequent embryo transfer (Michael et al., 1993, 1995, 1999; Keay et al., 2002; Lewicka et al., 2003; Thurston et al., 2003). Hence, ovarian 11βHSD activities appeared to correlate with the probability of an oocyte, once fertilized, successfully developing and implanting in vivo (Michael, 2003). One mechanism proposed to explain these findings was that decreased glucocorticoid metabolism in the oocyte and/or subsequent embryo would enable cortisol to retain its immunosuppressive actions within the uterus so as to prevent immune rejection of the blastocyst. It was reasoned that if glucocorticoids promote immune tolerance to the embryonic allograft, then this should be most evident for embryos in which the zona pellucida has been compromised, as with ICSI. This prediction was not borne out by the recent Cochrane review. In their meta-analysis of 13 randomized control trials (RCTs) featuring a total of 1759 couples undergoing IVF-ET or ICSI-ET, Boomsma et al. (2007) found no significant effect of synthetic glucocorticoids administered immediately around the time of embryo transfer on conception. However, when ICSI was excluded and a subgroup analysis performed for six RCTs featuring 650 couples undergoing IVF-ET or ICSI-ET, Boomsma et al. (2003) indicated that decreased glucocorticoid metabolism in the oocyte was not borne out by the recent Cochrane review. In their meta-analysis of 13 randomized control trials (RCTs) featuring a total of 1759 couples undergoing IVF-ET or ICSI-ET, Boomsma et al. (2007) found no significant effect of synthetic glucocorticoids administered immediately around the time of embryo transfer on conception. However, when ICSI was excluded and a subgroup analysis performed for six RCTs featuring 650 couples treated by IVF-ET alone, the clinical pregnancy rate was significantly increased (odds ratio = 1.50, P = 0.02) by glucocorticoid administration (Boomsma et al., 2007). This clinical finding indicates that glucocorticoids may play positive roles in the establishment of early pregnancy over and above their anticipated effects on immune tolerance of the implanting embryo.

**Secretion of hCG**

The peri-implantation secretion of hCG from the trophoblast of the early human embryo is pivotal in maintaining progesterone secretion from the corpus luteum until the luteo-placental shift in progesterone synthesis at around 8 weeks of gestation (Hanson et al., 1971; Stevens, 1979). This gonadotrophin may also play local roles in promoting embryo implantation and differentiation (Islami et al., 2001; Licht et al., 2001; Srisuparp et al., 2001; d’Hauterive et al., 2007; Handschu et al., 2007). The secretion of hCG from human term trophoblast can be stimulated in vitro by up to 10-fold on treatment for 24 to 72 h with synthetic glucocorticoids (dexamethasone and triamcinolone acetate) (Ringler et al., 1989; Guller et al., 1994; Hahn et al., 1999) or by using carbenoxolone to inhibit metabolism of physiological glucocorticoids via the 11βHSD enzymes (Nacharaju et al., 2004). Dexamethasone also stimulated hCG secretion from first trimester cytotrophoblasts (Guller et al., 1994), and when (Mandl et al., 2006) used choriocarcinoma cell lines as in vitro models for first trimester human trophoblast, they found that triamcinolone acetate could double hCG output from GR positive choriocarcinoma cell lines (BeWo and JEG3 cells) without effecting hCG production from the GR negative JAR choriocarcinoma cells. Moreover, triamcinolone had no effect on hCG output when experiments were repeated in the absence of serum (Mandl et al., 2006), suggesting that sgk-1 may also be required for the stimulation of hCG production by glucocorticoids.

**Blastocyst attachment**

Embryo implantation requires a molecular dialogue initiated during blastocyst attachment by cell surface signalling molecules on the trophoblast and endometrium, such as the integrins and fibronectin (Burrows et al., 1996). At physiological concentrations (100 nmol/l), glucocorticoids can suppress the expression of trophoblast integrins (Ryu et al., 1999), hence modulating these initial trophoblast-decidua interactions. The effects of glucocorticoids on fibronectin expression are tissue-specific; while dexamethasone suppresses fibronectin expression in term human cytotrophoblasts and amnion (Guller et al., 1995a, b; Lee et al., 2004), this glucocorticoid acts in synergy with transforming growth factor-β to up-regulate fibronectin in matched samples of chorion and placental mesenchymal cells (Guller et al., 1995a; Lee et al., 2004).

**The inflammatory cascade and embryo implantation**

Following attachment, successful implantation requires a coordinated sequence of inflammatory events with key roles for pro-inflammatory cytokines, such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α, and for prostaglandins (Chard, 1995; Sharkey, 1998; Kelly et al., 2001; Staun-Ram and Shalev, 2005; Achache and Revel, 2006; Makrigiannakis et al., 2006). Glucocorticoids are known to exert several anti-inflammatory actions which could impair the cytokine-prostaglandin signalling cascade required for implantation.

**Glucocorticoid interactions with cytokines**

In first trimester human cytotrophoblast cells, cortisol can suppress the synthesis of the pro-inflammatory IL-1β (Librach et al., 1994). Likewise, in term human placental cytotrophoblasts and decidual surface villous explants, physiological concentrations of cortisol and several synthetic glucocorticoids can each inhibit both basal and bacterial lipopolysaccharide (LPS)-stimulated output of the pro-inflammatory cytokines, TNF-α, IL-6 and IL-8 by over 70% (Rosen et al., 1998; Ma et al., 2004, 2006; Xu et al., 2005) without affecting expression of the anti-inflammatory cytokine IL-10 (Xu et al., 2005). The impact on the ratio of pro-inflammatory to anti-inflammatory cytokines was greater in decidua surface villous explants from placentas delivered at term in pre-eclamptic versus normotensive pregnancies (Xu et al., 2005), indicating that pre-eclampsia may involve altered
sensitivity of cytokine output to modulation by glucocorticoids. In terms of modulating cytokine actions, glucocorticoids can inhibit the activator protein (AP)-1 and nuclear factor (NF)-κB signalling pathways that typically mediate the cellular responses to pro-inflammatory cytokines (van der Burg and van der Saag, 1996; Barnes, 1998, 2006; Adcock and Caramori, 2001; Hayashi et al., 2004).

While glucocorticoids regulate the local synthesis and actions of pro-inflammatory cytokines, these inflammatory mediators can in turn modulate glucocorticoid concentrations within the utero-placental unit. For example, IL-1β and TNF-α both increase the expression and activity of type 1 11βHSD while suppressing the expression of mRNA transcripts encoding type 2 11βHSD in term human choricronic trophoblasts (Chisaka et al., 2005; Li et al., 2006). This would be expected to increase net regeneration of active cortisol from cortisone, creating a negative feedback loop within the placenta between physiological glucocorticoids and cytokines (Fig. 2). When JEG-3 choriocarcinoma cells were treated with IL-1β and TNF-α, both cytokines up-regulated expression of type 1 11βHSD protein, but exerted inconsistent effects on the expression of type 2 11βHSD in two independent studies (Chisaka et al., 2005; Johnstone et al., 2005), indicating possible differences in the regulation of glucocorticoid metabolism between the term placenta and choriocarcinoma cell lines, commonly used as a model for the early human trophoblast.

Glucocorticoid interactions with prostaglandins

In most tissues studied to date, glucocorticoids exert anti-inflammatory effects to inhibit the synthesis of prostaglandins and thromboxanes by decreasing the expression and/or activity of phospholipase A2 (PLA2), so limiting liberation of arachidonic acid for the prostaglandin H synthase (PGHS), cyclo-oxygenase enzyme (Bailey, 1991; Flower and Rothwell, 1994; Barnes, 1998, 2006). In contrast, in the placenta and fetal membranes, glucocorticoids can paradoxically increase prostaglandin synthesis (Sun et al., 2003; Mirazi et al., 2004; Zhang et al., 2006). This uncharacteristic pro-inflammatory action is achieved, in part, by up-regulating the expression and activities of the PLA2 and PGHS-2 enzymes in human amnion fibroblasts and chorionic trophoblasts (Gibb and Breton, 1993; Zakar et al., 1995; Economopoulos et al., 1996; Whittle et al., 2000, 2001; Sun et al., 2003; Li et al., 2006) and of placental prostaglandin synthase enzymes in the placenta (Zhang et al., 2006). Moreover, glucocorticoids can decrease the expression of prostaglandin dehydrogenase (PGDH), so increasing the functional half-life of prostaglandins in the human chorianc and placental trophoblast (Patel et al., 1999a, b, 2003; Whittle et al., 2001; Patel and Challis, 2002).

In the same way that cytokines can modulate the local activity of physiological glucocorticoids, the inter-conversion of cortisol–cortisone within the placenta is also regulated by prostaglandins which up-regulate the expression and reductase activity of type 1 11βHSD in term human placental trophoblasts (Alfaidy et al., 2001) while decreasing the oxidative activity of type 2 11βHSD (Hardy et al., 1999; Hardy et al., 2001) (Fig. 2). In contrast to the negative feedback loop between pro-inflammatory cytokines and glucocorticoids, the reciprocal effects of glucocorticoids on prostaglandin synthesis and of prostaglandins on glucocorticoid metabolism in the placenta establish a positive feed-forward loop which has been implicated in the timing of parturition (Challis et al., 2000; Alfaidy et al., 2001; Michael et al., 2003).

While glucocorticoids stimulate prostaglandin synthesis in term placenta and fetal membranes, dexamethasone exerts a conventional anti-inflammatory action to suppress PGHS-2 expression and inhibit prostaglandin synthesis in the first trimester human trophoblast (Imseis et al., 1997). Hence, further studies are required to establish whether the functional interactions between the glucocorticoid, cytokine and prostaglandin systems in the utero-placental unit differ between early versus late pregnancy.

Immunosuppression

The immunosuppressant actions of glucocorticoids have long been recognized, and indeed exploited, in clinical medicine. For example, in healthy volunteers, daily oral administration of prednisolone halved the total population of lymphocytes within 7 h of the first glucocorticoid treatment, and reduced the proportion of peripheral NK cells (from 16.5 to 9.5% of lymphocytes) over 3 days (Pountain et al., 1993). In light of their immunosuppressant actions, it has been suggested that glucocorticoids might assist in preventing immune rejection of the implanting embryo (Boomsma et al., 2007).

Recent studies have implicated uterine NK cells in recurrent miscarriage. Uterine NK cells constitute a higher proportion of endometrial cells in women with a history of recurrent miscarriage than in endometrial biopsies from women with no history of early pregnancy loss (Quenby et al., 2005; Quenby and Farquharson, 2006). Initial evidence to suggest that synthetic glucocorticoids might influence uterine NK cells was provided by case reports of two individual women who had suffered 10 and 19 recurrent miscarriages, both of whom had successful pregnancies and delivered live infants following oral treatment with methylprednisolone (Ogasawara and Aoki, 2000; Quenby et al., 2003). Subsequent studies established that uterine NK cells are responsive to glucocorticoids in so far as they express GR (Henderson et al., 2003) and the type 1 11βHSD enzyme that modulates access of physiological glucocorticoids to this nuclear receptor (McDonald et al., 2006). In a study of 29 women with a history of recurrent miscarriage and elevated levels of uterine NK cells, oral prednisolone...
administration suppressed uterine NK cells from 14 to 9% of their endometrial biopsies \( (P < 0.001) \) (Quenby et al., 2005), and it was argued that this could decrease the likelihood of uterine NK cells mediating immune rejection of the implanting embryo (Quenby et al., 2005; Quenby and Farquharson, 2006).

A recent study of 22 early pregnancies found that the mean rate of maternal urinary cortisol excretion in the first 3 weeks following planned conception was increased in 13 women undergoing spontaneous early miscarriage (between Days 13 and 47 after ovulation) as compared to 9 women who successfully carried to term (Nepomnaschy et al., 2006). Elevated urinary cortisol excretion in the 3 weeks post-conception was associated with a 2.7-fold increase in the risk of early miscarriage relative to women where urinary cortisol excretion post-conception was the same as the baseline cortisol excretion level pre-conception. Furthermore, in a prospective case control study, antenatal administration of glucocorticoids to 262 women in the first trimester significantly increased the incidence of miscarriages relative to 728 control patients (from 7.0 to 11.5% of pregnancies) \( (P = 0.01) \) (Gur et al., 2004). These findings suggest that physiological glucocorticoids may impede (rather than improve) the early function and implantation of the embryo.

A novel factor emerging as relevant to embryo implantation is corticotrophin releasing hormone (CRH) expressed by the embryonic trophoblast and decidua cells. In addition to limiting trophoblast invasion (via down-regulation of carcinoembryonic antigen-related adhesion molecule-1), trophoblastic CRH also exerts an immunosuppressive effect, acting via type 1 CRH receptor to induce apoptosis of activated endometrial T-lymphocytes (Makrigiannakis et al., 2004; Kalantaridou et al., 2007). Although glucocorticoids suppress CRH expression in the hypotalamus and in pituitary corticoderms may impede (rather than improve) the early function and implantation of the embryo.

Degradation of the extracellular matrix during trophoblast invasion also involves urokinase-type plasminogen activator (uPA) which, in common with the tissue-type enzyme (tPA), catalyses the conversion of inactive plasminogen to plasmin. While uPA promotes the plasmin-associated degradation of the extracellular matrix, tPA is required for the plasmin-dependent breakdown of fibrin, essential for efficient vascular exchange in the early placenta (Vassalli et al., 1991; Loskutoff et al., 1993). The activities of both uPA and tPA can be suppressed by plasminogen activator inhibitor (PAI)-1, a 52 kDa serine protease inhibitor secreted by trophoblast and decidual cells (Feinberg et al., 1989; Vassalli et al., 1991; Hofmann et al., 1994). An in vitro study which featured human cytotrophoblasts isolated from term human placental villi and the HTR-8/SV neo cell line derived from first trimester human extravillous trophoblast (EVT) found that both cortisol and dexamethasone could increase expression of PAI-1 (Ma et al., 2002). It has been suggested that over-expression of PAI-1 in the trophoblast prevents tPA from inducing placen
dependent fibrinolysis which could impede placental nutrient transfer in pre-eclampsia and IUGR (Estelles et al., 1994; Grancha et al., 1996). Hence, glucocorticoids may act in the villous trophoblast to increase expression of PAI-1, limiting trophoblast invasion and plasmin-mediated fibrinolysis by inhibiting uPA and tPA, respectively.

In addition to affecting the invasive properties of the trophoblast, glucocorticoids have been implicated in the fusion of cyt
trophoblast cells to form the syncytiotrophoblast (Morrish et al., 1998, Malassine and Cronier, 2002) and have also been reported to modulate the rate of trophoblast apoptosis. For example, Mandl et al. (2006) found that in the presence of serum, triamcinolone acetate could induce apoptosis in the BeWo choriocarcinoma cell line. Likewise, Crocker et al. (2001) had observed that dexamethasone could induce both apoptosis and necrosis in primary cultures of term human placental trophoblast and in the SGH-PL4 cell line derived from human EVT.

**Trophoblast growth and invasion**

As noted above, choriocarcinoma cell lines have been studied as in vitro models for first trimester human trophoblast. In the presence of serum, triamcinolone acetate increased proliferation of the GR positive BeWo cell line and up-regulated cyclin B1 in a concentration-dependent manner, suggesting that glucocorticoids could stimulate growth of the early trophoblast (Mandl et al., 2006). At the highest tested concentration of 50 \( \mu \text{mol/l} \), triamcinolone acetate also increased the invasion of a Matrigel matrix by the BeWo cells, accompanied by a concentration-dependent up-regulation in the expression of pro-matrix metalloproteinase (proMMP)-2 (Mandl et al., 2006). This finding was in accord with the increased expression of placent MMP2 observed at term following a single course of antenatal betamethasone (Gharraee et al., 2006). However, these findings contrast with an earlier study of first trimester human cytotrophoblasts which found that dexamethasone (100 \( \mu \text{mol/l} \)) suppressed expression of MMP-9 and inhibited the ability of the early cytotrophoblast to invade a Matrigel matrix in vitro (Librach et al., 1994).

**Placental structure and function**

As pregnancy progresses, there are major changes in placental structure which are best exemplified in the pregnant sheep which shows progressive haemophagous eversion of the placental cotyledons from predominantly ‘inverted’ into ‘everted’ placen
tomes. Infusion of glucocorticoids into ewes in either early or late gestation restricts the eversion of the placen
tomes towards term and decreases the total placental weight (Wintour et al., 1994; Jensen et al., 2002; Ward et al., 2006). Likewise, manipulations of cortisol concentrations in the fetal lamb in late pregnancy can alter the proportion of binucleate cells in the ovine tropho
toderm (Ward et al., 2002). Although there are fundamental differences in placental structures between women, sheep and other mammals, these findings establish that glucocorticoids can act in both early and late pregnancy to affect the subsequent growth and development of the placenta.

Since the growth potential for the fetus is influenced by the size of the placenta, the impairment of placental growth has been advanced as a potential mechanism to account for IUGR associ
dated with decreased placental metabolism of glucocorticoids and/or antenatal glucocorticoid administration (Hofmann et al., 2001; Seckl and Meaney, 2004). Infusion of betamethasone to
ewes in late pregnancy induced IUGR associated with decreased placental size (Newnham et al., 1999), and in pregnant rats maternal administration of dexamethasone in the second half of gestation produced a 23% decrease in fetal weight that was accompanied by a 51% decrease in placental weight (P < 0.01) (Ain et al., 2005). The impairment of placental growth was characterized by decreased expression of both prolactin-like protein-B and insulin-like growth factor (IGF)-II, particularly in the junctional zone of the rat placenta. The decreased levels of placental Akt phosphorylation observed following in utero exposure to dexamethasone confirmed that signalling through the mitogenic phosphatidylinositol-3 kinase pathway was decreased in the rat placenta by this synthetic glucocorticoid (Ain et al., 2005). Moreover, maternal administration of dexamethasone decreased the phosphorylation of the pro-apoptotic protein, BAD, and increased the cleavage of poly(ADP-ribose) polymerase (Ain et al., 2005): actions which could account for the pro-apoptotic action of dexamethasone in the junctional zone of the rat placenta (Waddell et al., 2000).

Two of the key molecular pathways involved in placentaation are the WNT pathway, implicated in chorioallantoic attachment and branching morphogenesis (Cross et al., 2006), and the peroxisome proliferator-activated receptors (PPARs) which regulate placentation growth and vascularity (see Schaff et al., 2006). In the placenta, WNT ligands activate a signalling pathway that culminates in the nuclear translocation of β-catenin. This then recruits the transcription factors required for trophoblast proliferation and subsequent placentation growth (Eberhart and Argani, 2001; Cross et al., 2006). In a study of pregnant rats, Hewitt et al. (2006a) found that administration of dexamethasone in the second half of gestation increased the expression of secreted frizzled-related protein-4 and decreased the nuclear levels of β-catenin, indicating that glucocorticoids can inhibit the WNT signalling pathway crucial for early placentaation (Hewitt et al., 2006a). Maternal administration of dexamethasone also suppressed expression of mRNA encoding PPARγ in the labyrinthine zone of the rat placenta by 37% (P < 0.05) (Hewitt et al., 2006b). As an orphan nuclear receptor/transcription factor, PPARγ appears to be particularly important in the growth/development of the labyrinthine zone of the rat placenta (the site of materno–fetal exchange) during the phase of maximal placentation growth (Hewitt et al., 2006b).

In order to fulfil its role as the exchange interface between the maternal and fetal circulations, the placenta must be highly vascularized with good uterine and umbilical blood flow accompanied by local angiogenesis. Although glucocorticoids can induce vasoconstriction in sheep uterine arteries (Xiao et al., 2002, 2003; Jellyman et al., 2004), an in vitro study found cortisol, betamethasone and dexamethasone to each dilate human umbilical arteries (Potter et al., 2002). Moreover, Doppler flow studies have found antenatal administration of synthetic glucocorticoids to have no significant effect on the pulsatility index of the uterine artery in women at risk of pre-term labour (Muller et al., 2003; Urban et al., 2005). In terms of potential effects on angiogenesis, glucocorticoids have been reported to inhibit angiogenesis and destabilize microvessels in three different animal tissue models (rabbit cornea, chick chorioallantoic membrane and rat aorta explants in tissue culture) (McNatt et al., 1992; Phillips et al., 1992; Jaggers et al., 1996), as well as in term human placental vein discs (Jung et al., 2001). However, in each of the cited studies, the angiogenic effects of glucocorticoids were assessed at high pharmacological concentrations (between 10 μmol/l and 7.6 mmol/l) far in excess of the normal physiological concentration range for cortisol (100–600 nmol/l). Nearly 20 years ago, Graf et al. (1989) reported that in vivo administration of dexamethasone and triamcinolone acetate to pregnant rats between Days 16 and 20 of gestation induced major changes in the vascularization of the rat placenta, particularly in the labyrinthine zone (the major site of materno–fetal exchange in rodent placentas). Further studies are required to determine whether physiological concentrations of glucocorticoids can affect human placental vascular supply or angiogenesis in early pregnancy. In a recent review, Jansson and Powell (2007) noted that decreased inactivation of cortisol by the placentatal type 2 11βHSD enzyme at term was associated with increased placental vascular resistance which the authors contended could programme the increased risk of developing post-natal hypertension in the fetus.

Notwithstanding potential effects on the placental vasculature, glucocorticoids can affect the specific placental transport of individual nutrients (Fowden et al., 2006). For example, in pregnant ewes, glucocorticoid administration during either early or late gestation impedes the placental delivery of glucose and lactate to the fetal circulation (Barbera et al., 1997; Moss et al., 2003; Ward et al., 2004, 2006). Results of in vitro studies seem to depend on the steroid concentration and/or the duration of glucocorticoid exposure; Hahn et al. (1999) found that treatment of term human placental trophoblasts with triamcinolone acetate (5 and 50 μmol/l) for 24 h inhibited expression of GLUT-1 and GLUT-3 glucose transporters in a concentration-dependent manner, whereas Ericsson et al. (2005) found no effect of cortisol (tested at 1 μmol/l) over 1 h on glucose transport in villous explants from either first trimester or term human placentas. In contrast, the expression of GLUT-1 and GLUT-3 glucose transporters in the rat placenta can be induced (rather than suppressed) by in vivo administration of glucocorticoids (Langdown and Sudgen, 2001). Hence, further studies are required to clarify the molecular effects of glucocorticoids on placental glucose (and lactate) transport at physiological steroid concentrations.

Glucocorticoids have also been implicated in the transplacental transport of specific amino acids (Fowden et al., 2006). These effects have been attributed to changes in the local concentration gradients between the maternal, placental and fetal circulations for individual amino acids and/or changes in the expression of placental aminotransferase enzymes (Graf et al., 1989; Timmerman et al., 2001; Fowden et al., 2006). Although an initial investigation of first trimester and term human placental villi found no acute effect of cortisol on glucose or amino acid transport within 1 h (Ericsson et al., 2005), a subsequent study found chronic exposure to cortisol (for 24 h) to stimulate amino acid transport in a concentration-dependent manner by up-regulation of the SNAT2 system A (amino acid) transporters in the BeWo choriocarcinoma cell line (Jones et al., 2006). The precise molecular mechanisms via which glucocorticoids control placental transport of glucose/lactate and amino acids have yet to be determined, but are thought to include changes in expression of IGF-II which is a key regulator of placentatal growth (Fowden, 2003; Fowden et al., 2006). The expression of the Igf2 gene is certainly down-regulated following glucocorticoid administration to pregnant rats (Fowden,
11βHSD isoenzymes modulate glucocorticoid actions in the decidua and placenta

Studies of enzyme expression in the human endometrium have established that type 1 11βHSD is expressed at its highest levels in the decidua (Lopez-Bernal and Craft, 1981; Giannopoulos et al., 1982; Stewart et al., 1995; Arcuri et al., 1996, 1997; Burton et al., 1996; Petrelli et al., 1997; Sun et al., 1997a; Ricketts et al., 1998; Driver et al., 2001; McDonald et al., 2006) and menstruating endometrium, with no detectable expression in the proliferative or secretory phases of the non-pregnant menstrual cycle (McDonald et al., 2006). In contrast, type 2 11βHSD is highly expressed in both the proliferative and secretory phase endometrium, with expression localized predominantly to the glandular epithelium (Thompson et al., 2002; McDonald et al., 2006). Although type 1 11βHSD is the predominant enzyme expressed in the decidua, there is still marked up-regulation of type 2 11βHSD expression in the decidua relative to the non-pregnant endometrial stroma (McDonald et al., 2006). Hence, within the first trimester decidua, the balance of cortisol–cortisone metabolism favours local synthesis of active cortisol, as opposed to the net inactivation of cortisol in the non-pregnant endometrium. In the decidua of early pregnancy, expression of type 2 11βHSD is accompanied by the expression of both GR and MR (McDonald et al., 2006) such that the type 2 11βHSD enzyme could operate to limit activation of MR (as well as GR) by cortisol. The functional roles for corticosteroid receptors in the decidua have yet to be defined. The expression of both type 1 11βHSD and GR in the human decidua increase dramatically from the first to the third trimester of pregnancy with parallel increases in the rate of decidual cell apoptosis (Chan et al., 2007). This appears to be a causal relationship since cortisol, cortisone and dexamethasone could each induce the expression of both PGHS-2 and the apoptotic enzyme caspase-3 in human decidua (Chan et al., 2007).

For the past decade it has been accepted that the two cloned 11βHSD enzymes co-operate in the placenta to modulate transfer of active physiological glucocorticoids from the maternal to the fetal circulations (see Lakshmi et al., 1993; Yang, 1995; Benediktsson et al., 1997; Sun et al., 1998a; Murphy et al., 2006). Several studies have assessed the ability of placental 11βHSD enzymes to metabolize synthetic glucocorticoids either in vitro or in perfused placental lobules. While term human placentas could not metabolize budesonide and fluticasone propionate, they were able to metabolize four synthetic corticosteroids: beclomethasone dipropionate, betamethasone, dexamethasone and prednisolone (Beitins et al., 1972; Anderson et al., 1977; Blanford and Murphy, 1977; Levitz et al., 1978; Smith et al., 1988; Addison et al., 1991, 1993; van Runnard-Heimel et al., 2005; Murphy et al., 2007). Their recent findings prompted (Murphy et al., 2007) to conclude that inhaled steroids used to treat maternal asthma should effectively be excluded from the fetal circulation, and so should not pose a threat to fetal growth.

Type 1 11βHSD is expressed specifically in the placental villous endothelial cells, amnion, chorionic and EVT where the enzyme acts predominantly as a reductase to regenerate active cortisol from cortisone (active corticosterone from 11-dehydrocorticosterone in the placentas of rats and mice) (Tanswell et al., 1977; Baggia et al., 1990; Pepe et al., 1996a, b; Sun et al., 1997a, 1998a, 2002; Yang, 1997; Arcuri et al., 1998; Patel et al., 1999b; Driver et al., 2001; Hardy et al., 2001; Alfáidy et al., 2001, 2003; Hardy and Yang, 2002; Thompson et al., 2002; Klemcke et al., 2003; Sun and Myatt, 2003; Van Beek et al., 2004; Li et al., 2006). The expression of type 1 11βHSD increases throughout pregnancy, apparently in response to progesterone (Muneyyirci-Delale et al., 1996; Schoof et al., 2001a; Alfáidy et al., 2003), and can also be up-regulated in term human chorionic trophoblasts by cortisol acting via the nuclear GR (Li et al., 2006) (Table II). As the placenta differentiates, there is progressive up-regulation in expression of the type 2 11βHSD enzyme (Hardy and Yang, 2002) which becomes the major placental isoenzyme, restricting the passage of active glucocorticoids across the placenta into the fetal circulation (Brown et al., 1993; Burton and Waddell, 1994, 1999; Krozsowski et al., 1995; Stewart et al., 1995; Pepe et al., 1996a, 1999; Smith et al., 1997; Sun et al., 1997a; Yang, 1997; Sampath-Kumar et al., 1998; Waddell et al., 1998; Arcuri et al., 1999; Alfáidy et al., 2002; Clarke et al., 2002; Thompson et al., 2002; Klemcke et al., 2003; Staud et al., 2006). Hence, if the expression and/or activity of type 2 11βHSD in the placenta is compromised, increased transfer of cortisol across the placenta would induce the differentiation of fetal tissues at the expense of tissue growth. Indeed, this has been advanced as a mechanism to explain the clinical association between decreased activity of type 2 11βHSD in the human placenta and IUGR (Benediktsson et al., 1993; Edwards et al., 1993; Shams et al., 1998; McTernan et al., 2001; Kajantie et al., 2006). However, Newnham et al. (1999) found that while maternal administration caused IUGR, administration of glucocorticoids directly into the fetal circulation did not restrict fetal growth, suggesting that the growth limiting effects are mediated via actions in the uteroplacental unit rather than effects on fetal tissues.

Type 2 11βHSD is expressed and functional within the first trimester trophoblast where it has been implicated in successful embryonic attachment and implantation (Arcuri et al., 1998). In mid- to late pregnancy, type 2 11βHSD has been co-localized with MR in the fused syncytiotrophoblast (Krozowski et al., 1995; Hirasawa et al., 2000; Driver et al., 2001) and in the invasive EVT (Driver et al., 2001) with no expression in the chorion or amnion (Sun et al., 1997a). In human term trophoblast, expression of type 2 11βHSD is sensitive to the oxygen tension (Alfáidy et al., 2002; Hardy and Yang, 2002; Homan et al., 2006), and is also regulated by nitric oxide, cytokines and steroid hormones (Table II). In a recent study of term placentas from extremely low birth weight infants born weighing <1 kg at 27 ± 2 weeks of gestation, Kajantie et al. (2006) found that antenatal administration of betamethasone was associated with a significant increase in placental type 2 11βHSD activity. Likewise, inhalation of budesonide also increased placental metabolism of cortisol by type 2 11βHSD (Clifton et al., 2006). In contrast, antenatal administration of dexamethasone has been shown to suppress (rather than up-regulate) the expression of type 2 11βHSD in the ovine placenta (Kerzner et al., 2002) and expression of type 2 11βHSD in the ovine placenta decreases at term due to increased fetal output of cortisol (Clarke et al., 2002). These observations emphasize the need to exercise...
Table II. The regulation of cloned 11βHSD enzymes in placenta and decidua.

<table>
<thead>
<tr>
<th>Up-regulate enzyme and/or increase enzyme activity</th>
<th>Down-regulate enzyme and/or decrease enzyme activity</th>
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<tr>
<td><strong>Type 1 11βHSD</strong></td>
<td><strong>Type 2 11βHSD</strong></td>
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<tr>
<td>Dexamethasone</td>
<td>Dexamethasone</td>
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<tr>
<td>PGF2α</td>
<td>Alfaidy et al. (2001)</td>
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<td><strong>Type 1 11βHSD</strong></td>
<td><strong>Type 2 11βHSD</strong></td>
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<tr>
<td>Dexamethasone</td>
<td>Betamethasone</td>
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<tr>
<td>Cyclic AMP</td>
<td>Sun et al. (1998b)</td>
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<tr>
<td>Dexamethasone</td>
<td>Darnel et al. (1999)</td>
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<td>Estradiol</td>
<td>Van Beek et al. (2004)</td>
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<td>IL-1β</td>
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<tr>
<td>Noradrenaline</td>
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<tr>
<td>Progesterone</td>
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<td>Retinoic acid</td>
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<tr>
<td>TNF-α</td>
<td>Chisaka et al. (2005)</td>
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<tr>
<td>ATP</td>
<td>Dexamethasone</td>
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<td>Cadmium</td>
<td>Estradiol</td>
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<td>Calcium</td>
<td>Hypoxia</td>
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<td>HSD enzymes in placenta and decidua.</td>
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HSD, hydroxysteroid dehydrogenase.

cautions in extrapolating findings from animal studies to placental glucocorticoid handling in human pregnancies.

The expression and function of type 2 11βHSD in the placenta is also responsive to maternal stress, though it is not yet clear whether placental glucocorticoid metabolism changes simply in response to the elevation in maternal cortisol as opposed to other endocrine regulators, such as prolactin or β-endorphin. A common experimental model to induce maternal stress in pregnant sheep is to restrict maternal nutrient intake. Using this model, Whorwood et al. (2001) found that restriction of nutrient intake in early pregnancy (Days 28–77 of gestation, where term is 145 days) decreased expression of type 2 11βHSD mRNA. Although restricting nutrient intake by the ewe to 70% of her maintenance diet from Day 27 of gestation to either Day 90 or Day 135 of gestation was associated with a 50% suppression of placental type 2 11βHSD activity (P < 0.001), this was not accompanied by significant changes in the fetal plasma cortisol concentrations (McMullen et al., 2004). As part of their study, McMullen et al. (2004) exposed one group of ewes to the modest nutrient restriction for 30 days prior to mating, but then transferred those ewes to a 100% maintenance diet subsequent to mating for the duration of pregnancy. This pre-conception restriction of nutrient intake had no lasting effect on placental type 2 11βHSD activity but halved the ratio of cortisol:cortisone in the fetal plasma on Day 135 of gestation, more than 4 months after the maternal stress (McMullen et al., 2004). In a subsequent study, ewes were completely fasted for two days 60 days prior to mating, and then transferred to a diet calculated for each ewe to achieve 15% weight loss from 60 days before through to 30 days after mating. After the initial doubling of maternal cortisol concentrations during the 2 day fast, plasma cortisol levels returned to a lower baseline level in the nutrient restricted ewes (relative to the control ewes). This peri-conception maternal stress decreased ovine placental cortisol metabolism by type 2 11βHSD on Day 50 of gestation (P = 0.01), suggesting that maternal stress in early pregnancy can programme placental cortisol metabolism, modulating subsequent exposure of the fetus and placenta to active glucocorticoids (Jaquiey et al., 2006).

**Embryo/fetal growth and development**

The strongest evidence to implicate glucocorticoids in the regulation of fetal growth comes from the clinical association
between IUGR and decreased placental inactivation of cortisol by type 2 11βHSD (Benediktsdsson et al., 1993; Edwards et al., 1993; Stewart et al., 1995; Shams et al., 1998; Hofmann et al., 2001; McTernan et al., 2001; Murphy et al., 2002, 2006; Kajantie et al., 2003; Seckl and Meaney, 2004). While Rogerson et al. (1997) found no relationship between placental levels of this enzyme and either placental or birth weight in 111 normal births, Struwe et al. (2007) reported decreased levels of both cloned 11βHSD enzymes in placenta delivered with small-for-gestational-age infants. Moreover, a study of extremely small birth weight infants (born weighing under 1 kg) also found a correlation between placental type 2 11βHSD activities and infant birth weight (Kajantie et al., 2006), while Field et al. (2006) reported an association between elevated cortisol concentrations in maternal urine and decreased birth weight, consistent with decreased placental cortisol metabolism.

The relevance of placental glucocorticoid metabolism for fetal growth has been tested in vivo using animal models. In pregnant rats, inhibition of the placental 11βHSD enzymes resulted in a significant reduction in birthweight of the pups (Langley-Evans, 1997b). This has been associated with adult hypertension, hyper-insulinaemia and altered post-natal behaviour in the offspring (Lindsay et al., 1996a; b; Nyirenda et al., 1998; Welberg et al., 2000), suggesting that impairment of placental glucocorticoid metabolism could affect multiple aspects of fetal development, not just growth. In recent studies, glucocorticoid metabolism by type 2 11βHSD in the mouse placenta and fetus has been suppressed using a transgenic strategy, and this also resulted in a significant decrease in mean birth weight (Holmes et al., 2006). In studies of pregnant sheep, McMullen et al. (2004) restricted the nutritional intake of ewes in early, mid and late gestation (Days 28–45, 46–90 and 91–135 of gestation, respectively). The moderate nutrient restriction at each stage of pregnancy did not affect cortisol metabolism in the ovine placental cotyledons, but decreased the ratio of cortisol:cortisone in the fetal plasma at term (McMullen et al., 2004). This implies that the exposure of fetal tissues to cortisol may be determined locally by 11βHSD iso-enzymes within the fetus, rather than simply by glucocorticoid metabolism at the maternal–fetal interface (Murphy et al., 1981; Stewart et al., 1994, 1995; Langlois et al., 1995; Brown et al., 1996b; Condon et al., 1998; Hirasaawa et al., 1999; Hundertmark et al., 2001; Speirs et al., 2004; Thompson et al., 2004; McNeil et al., 2007).

In terms of direct evidence for effects of glucocorticoids on human fetal growth, Gur et al. (2004) reported decreased birth weight of infants born to mothers treated with synthetic glucocorticoids in the first trimester of pregnancy. In contrast, a meta-analysis of 5 trials in which 2028 pregnant women were treated with corticosteroids in late pregnancy (22 to 33 weeks of gestation) found no significant effect on birth weight (Crowther and Harding, 2007), indicating that human fetal growth may only be glucocorticoid sensitive in early pregnancy. In experimental studies of pregnant sheep, adrenalectomy accelerated fetal growth and increased birth weight, whereas maternal infusion of betamethasone in late pregnancy decreased birth weight and the size of specific fetal organs (Fowden et al., 1996; Newnham et al., 1999). In a recent study of pregnant pigs, Klemcke et al. (2006) found that suppressing the maternal cortisol concentration on Days 14–19 of pregnancy (where term is 114 days) resulted in small decreases (8–12%) in both the average embryonic weight and the total embryonic weight which could be prevented by co-administration of cortisol. Significant biphasic correlations were reported between maternal cortisol concentrations and both the total embryo weight and the total allantoic weight with peak weights observed at a cortisol concentration of 10 ng/ml (equivalent to around 28 nmol/l) (Klemcke et al., 2006). The correlations fitted second-order regressions, suggesting the requirement for an optimal cortisol concentration for maximum embryo growth and allantoic size. In keeping with this model of an optimal cortisol concentration for fetal growth and development, McNeil et al. (2007) reported that small fetal piglets had decreased (rather than increased) plasma cortisol concentrations on Day 45 of gestation.

In addition to general effects on fetal growth, there is evidence that glucocorticoids can affect the development of specific fetal organs, of which the best studied are the cardiovascular and central nervous systems (CNSs). Much of the attention has been focused on the development of the cardiovascular system following reports that acute exposure of fetal lambs to dexamethasone for just 48 h in early pregnancy (between Days 22 and 29 of gestation) can programme post-natal hypertension in those lambs which persists for up to 7 years (Dodic et al., 1998, 2001). The actions of dexamethasone in early pregnancy included a 12% increase in fetal cardiac output (Dodic et al., 1999) with steroid-induced left ventricular hypertrophy (Dodic et al., 2001) and proliferation of cardiomyocytes (Giraud et al., 2006). In follow up studies, these authors determined that the developmental consequences of in utero exposure to the pharmacological glucocorticoid dexamethasone were not the same as exposure to cortisol (see Moritz et al., 2005). For example, in the fetal kidney, cortisol selectively increased the expression of the type 1 angiotensin II receptor, whereas dexamethasone also increased the renal expression of the type 2 angiotensin II receptor and angiotensinogen (Moritz et al., 2002). Exposure to dexamethasone in early pregnancy also impaired nephrogenesis in the fetal lamb kidney which persisted as a decreased number of nephrons through to post-natal year 7 (Wintour et al., 2003), and programmed the responses of the peripheral vasculature to known vasoconstrictors and vasodilators in the lambs born to steroid-treated ewes (Roghair et al., 2005; Segar et al., 2006). Hence, a transient increase in glucocorticoids in early pregnancy can produce lasting effects on the structure and function of the cardiovascular system which precede the onset of adult hypertension. The findings of similar studies in pregnant rats indicate important species differences and the need for caution in extrapolating findings from animal studies to human pregnancies. For example, Woods and Weeks (2005) found that while antenatal exposure to dexamethasone in late pregnancy (Days 15–20 of 22) increased the mean arterial blood pressure in adult rats born to dexamethasone-treated mothers, there was no hypertensive response following in utero exposure to dexamethasone in early pregnancy (Days 1–10 of gestation) (Woods and Weeks, 2005). In rats, the hypertensive effect of increased glucocorticoid exposure in late pregnancy appears to reflect sclerotic actions of glucocorticoids in the fetal kidney, rather than effects on the cardiac or vascular systems (Ortiz et al., 2001, 2003; Martins et al., 2003; Woods and Weeks, 2005).

Actions on the developing CNS may underlie the effects of glucocorticoids on the in utero programming of post-natal behaviour.
Antenatal treatment of pregnant rats with dexamethasone impaired post-natal learning and cognitive function in their offspring which exhibited increased susceptibility of their cholinergic neurones to neurotoxins (Engard et al., 2007). Likewise, pharmacological elevation of the physiological glucocorticoid concentration following inhibition of 11\betaHSD activities in pregnant rats altered the post-natal behaviour, and the hypothalamic expression of GR, in the pups born to carbenoxolone-treated mothers (Welberg et al., 2000). In addition, Holmes et al. (2006) have reported that transgenic mice lacking the type 2 11\betaHSD enzyme exhibit increased anxiety, consistent with developmental effects of glucocorticoids on the mouse CNS in utero. In marmoset monkeys, prenatal exposure to dexamethasone for 1 week in either early or late pregnancy (Days 42–48 and 90–96 of gestation, respectively, where term is 148 days) impaired proliferation of dentate gyrus cells without affecting cell differentiation (Tauber et al., 2006).

In terms of assessing CNS function in human neonates, Brazleton scores provide indexes of an infant’s strength and adaptive behavioural responses between 37 weeks of gestation and 2 months of age. Using this scoring system, Field et al. (2006) found that elevated cortisol levels in pregnant women were associated with lower Brazleton habituation scores and higher Brazleton reflex scores in their babies, while de Weerth et al. (2003) found elevated maternal cortisol concentrations in late pregnancy (weeks 37 and 38) to be associated with increased crying, fussing and negative facial expressions in their babies in the first 20 weeks following delivery. In their review, Kapoor et al. (2006) emphasized that the effects of glucocorticoid exposure on brain development and post-natal behaviour are highly sensitive to the gestational age at which the CNS is exposed to glucocorticoids; the maximum impact on brain development/behaviour occurs if the fetus is exposed to elevated glucocorticoid concentrations at the time of maximal brain growth. These authors also commented on the fact that passage of cortisol across the placenta is greater for female fetuses than male fetuses (Kapoor et al., 2006). Since the expression of each of the cloned 11\betaHSD enzymes in the placenta and decidua is sensitive to sex steroids (Table II), steroid output from the developing fetus could affect the balance between type 1 versus type 2 11\betaHSD, and hence determine the net inactivation of cortisol within the placenta.

While effects of glucocorticoids on the cardiovascular and CNS are the best characterized, the development of other embryonic/fetal organ systems may also be sensitive to glucocorticoids. For example, glucocorticoids have been implicated in regulating the development of the endocrine pancreas (Blondeau et al., 2001; Gesina et al., 2004, 2006; Breant et al., 2006; de Vries et al., 2007) and may programme post-natal insulin secretion and glucose homeostasis (de Blasio et al., 2007; de Vries et al., 2007).

Placental glucocorticoid metabolism in complications of pregnancy

Pre-term labour
As alluded to above, the timing of parturition, both at term and pre-term, has previously been linked to changes in the local metabolism of glucocorticoids within the placenta and associated tissues in the latter stages of pregnancy (Challis et al., 2000; Schoof et al., 2001a; Alfaidy et al., 2003; Ma et al., 2003; Murphy and Clifton, 2003). In terms of direct evidence to support a link between pre-term labour and increased glucocorticoid exposure in early pregnancy, Field et al. (2006) reported a significant association between increased cortisol clearance in maternal urine and pre-term labour. Moreover, the administration of synthetic glucocorticoids in the first trimester of pregnancy doubled the incidence of pre-term delivery (which rose from 10.8 to 22.7%) in the prospective case–control study reported by Gur et al. (2004). While a subsequent meta-analysis, conducted by Rahimi et al. (2006), reported no link between administration of inhaled corticosteroids and incidence of pre-term delivery, the surveyed studies did not assess the impact of glucocorticoids administered in early pregnancy, nor did they take account of variation in the placental metabolism of the different synthetic steroids.

Placental CRH has also been implicated in the timing of parturition with plasma CRH concentrations rising at an earlier stage of gestation and to higher levels in pre-term labour (McLean et al., 1995; Wadhwa et al., 1998; Hobel et al., 1999; Erickson et al., 2001; Leung et al., 2001; Beshay et al., 2007; Smith and Nicholson, 2007). In a study of 203 pregnant women for whom plasma CRH and cortisol levels were assessed at 15, 19, 25 and 31 weeks of gestation, the magnitude and timing of the increase in CRH levels was highly predicted by elevated concentrations of cortisol in the maternal plasma in early pregnancy, specifically at 15 weeks of gestation (Sandman et al., 2006). Hence elevated plasma cortisol concentrations in early pregnancy may predict subsequent risk of pre-term labour with an apparent mediatory role for increased placental CRH output.

Chorioamnionitis
Increased placental expression of type 1 11\betaHSD accompanied by decreased placental expression and activity of type 2 11\betaHSD has been documented in chorioamnionitis (Johnstone et al., 2005). Since glucocorticoids paradoxically increase the net synthesis of active prostaglandins in the human placenta (Whittle et al., 2001; Li et al., 2006) with pro-inflammatory cytokines regulating the expression of both placental 11\betaHSD isoenzymes (Chisaka et al., 2005; Johnstone et al., 2005; Li et al., 2006) (Fig. 2), it is not yet possible to determine whether changes in placental glucocorticoid metabolism in chorioamnionitis are the cause or consequence of the associated intra-uterine inflammation. A recent meta-analysis of 21 RCTs found that the incidence of chorioamnionitis is not increased by a single maternal course of betamethasone administered across a range of gestational ages (Roberts and Dalziel, 2006), which would seem to imply that changes in glucocorticoid metabolism are more likely to be a consequence of chorioamnionitis than a cause. In pregnancies complicated by chorioamnionitis, decreased expression of sgk-1 in the fetal lungs has been implicated in the dysregulation of pulmonary fluid balance (Wirbelauer et al., 2007).

Pre-eclampsia
The pathogenesis of pre-eclampsia is increasingly thought to arise from abnormalities in placental implantation or function in the first
trimester of pregnancy with evidence of increased impedance to uterine artery blood flow, altered maternal serum markers and reduced placental volume as assessed by 3D ultrasound evident long before the onset of the clinical signs (Papageorghiou and Campbell, 2006). Given the importance of glucocorticoids in implantation, trophoblast invasion and placental growth, is there any evidence to implicate glucocorticoids in the aetiology of pre-eclampsia? The majority of published investigations have reported that the expression and/or activity of type 2 11βHSD in human placentas is decreased (typically by 50–70%) in established pre-eclampsia relative to placentas from normotensive pregnancies (McCalla et al., 1998; Schoof et al., 2001b; Alfaidy et al., 2002; Causevic and Mohaupt, 2007). Whether such changes pre-date the onset of pre-eclampsia is not known, but in vitro studies conducted using both first trimester placental villous explants (isolated between weeks 5 and 8 of pregnancy) and term human placental trophoblasts have shown that the expression of type 2 11βHSD mRNA and protein are both suppressed by ~60% in tissue incubated at low oxygen tensions (either 3 or 1% by volume) than compared with 20% (v/v) oxygen (Alfaidy et al., 2002; Hardy and Yang, 2002). Moreover, the decreased expression of type 2 11βHSD was accompanied by suppression of the net cortisol oxidation by up to 50%, dependent on the gestational age of the trophoblast and the degree of hypoxia (Alfaidy et al., 2002; Hardy and Yang, 2002). Hence the decreased expression and activity of type 2 11βHSD in pre-eclamptic placentas, and the accompanying increase in exposure of the placenta and embryo/fetus to active glucocorticoids, could be a consequence of impaired placental perfusion and hypoxia.

Crocker et al. (2003) noted that pre-eclampsia and IUGR were both characterized by increased susceptibility of the placental trophoblast to apoptosis. In light of their earlier finding that glucocorticoids could induce apoptosis in cytотrophoblast and syncytiotrophoblast (Crocker et al., 2001), increased exposure to glucocorticoids following down-regulation of type 2 11βHSD could contribute to the increased rate of trophoblast apoptosis in pre-eclampsia. Pre-eclampsia is also characterized by increased production of placental CRH (Laatikainen et al., 1991; Goland et al., 1995; Florio et al., 2004) which may also be a reflection of increased local concentrations of active glucocorticoids in pre-eclampsia.

Published data relating to dysregulation of glucocorticoid metabolism in pregnancy-induced hypertension (PIH) are equivocal. While Walker et al. (1995) found no difference in urinary ratios of cortisol/cortisone in 12 patients with PIH relative to 16 women with normotensive pregnancies, Heilmann et al. (2001) reported significant increases in the urinary cortisol/cortisone ratios for 59 PIH patients relative to 67 normotensive controls.

**Hydatidiform moles**

As noted above, pre-eclampsia may have its origins in defective implantation and placentation in early pregnancy (Papageorghiou and Campbell, 2006). However, it has yet to be determined whether the fetus and placenta are exposed to elevated concentrations of active glucocorticoid in early pregnancy, or just in the later stages of gestation. In contrast, hydatidiform moles represent complications of early pregnancy and trophoblast invasion. Hence, it is relevant to this review that a recent study reported that cortisol oxidation by both type 1 and type 2 11βHSD enzymes was also decreased by >80% in hydatidiform moles relative to normal placentas (Muneyyirci-Delale et al., 2006). Since the abilities of both enzymes to oxidize cortisol were compromised in hydatidiform moles, local exposure of the invasive trophoblast to active cortisol would be increased in molar pregnancies, as in chorioamnionitis and pre-eclampsia.

**Conclusions**

While glucocorticoids exert predominantly adverse effects on the fetus and placenta in late pregnancy, the effects of these adrenal corticosteroids in early pregnancy are far less well defined. Glucocorticoids can exert a range of positive effects which would be expected to promote establishment of early pregnancy, such as suppression of uterine NK cells and stimulation of hCG secretion, as well as promotion of trophoblast proliferation and invasion (Fig. 3). However, glucocorticoids can also exert a range of adverse effects that would be expected to impede pregnancy, including up-regulation of PAI-1, induction of placental and/or decidual apoptosis and impairment of placental nutrient transport (Fig. 3). Since physiological glucocorticoids have the potential to activate both MR and GR (whereas synthetic glucocorticoids act via the GR only), the divergent actions of cortisol (and corticosterone) in pregnancy may be mediated via different intracellular receptors and signalling pathways.

Given the opposing beneficial and adverse actions of glucocorticoids, the apparent lack of effect of glucocorticoid administration on conception rates in the studies included in the meta-analysis reported by Boomsma et al. (2007) could reflect opposing positive versus negative consequences of increased glucocorticoid exposure in early pregnancy, rather than a complete lack of action of these corticosteroids. The one certainty to emerge from this review is that further research is required to elucidate the roles for glucocorticoids in early pregnancy. With such research, the possible relevance of glucocorticoids in the first trimester of pregnancy to Figure 3: Overview of the possible beneficial and adverse impacts of the physiological glucocorticoid, cortisol, in early pregnancy. Positive effects on enzyme activity and/or protein expression are indicated by solid green arrows, while negative effects are indicated by broken red lines. hCG, human chorionic gonadotrophin; IGF-II, insulin-like growth factor II; MMP-9, matrix metalloproteinase-9; NK, natural killer; PAI-1, plasminogen activator inhibitor-1; PPAR, peroxisome proliferator-activated receptor; proMMP-2, pro-matrix metalloproteinase-2; tPA, tissue plasminogen activator; troph, trophoblast; uPA, urokinase plasminogen activator; CRH, corticotrophin releasing hormone.
normal embryo implantation and feto–placental development, as well as to the pathogenesis of obstetric complications (e.g. IUGR, pre-term labour and pre-eclampsia) should emerge.

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