Reproduction (2003) **126**, 425–441

Review

Glucocorticoid metabolism and reproduction: a tale of two enzymes

Anthony E. Michael[1,2], Lisa M. Thurston[1,2] and Michael T. Rae[3]

[1]*Department of Biochemistry and Molecular Biology, University College London, Rowland Hill Street, London NW3 2PF, UK;* [2]*Reproduction and Development Group, Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU, UK; and* [3]*Section of Obstetrics and Gynaecology, Division of Reproductive and Developmental Sciences, Centre for Reproductive Biology, University of Edinburgh, Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK*

Within potential target cells, the actions of physiological glucocorticoids (cortisol and corticosterone) are modulated by isoforms of the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD). To date, two isoforms of 11βHSD have been cloned: 11βHSD1 acts predominantly as an NADP(H)-dependent reductase to generate active cortisol or corticosterone, and 11βHSD2 is a high affinity NAD^+-dependent enzyme that catalyses the enzymatic inactivation of glucocorticoids. Whereas the regeneration of active glucocorticoids by 11βHSD1 has been implicated in the cellular mechanisms of pituitary function, ovulation and parturition, the enzymatic inactivation of cortisol and corticosterone by 11βHSD enzymes appears to be central to the protection of gonadal steroidogenesis, prevention of intra-uterine growth retardation, and lactation. Recent evidence indicates that follicular fluid contains endogenous modulators of cortisol metabolism by 11βHSD1, the concentrations of which are associated with the clinical outome of assisted conception cycles and are altered in cystic ovarian disease. In conclusion, the two cloned isoforms of 11βHSD fulfil diverse roles in a wide range of reproductive processes from conception to lactation.

The definitive role of glucocorticoids, synthesized in the zona fasciculata of the adrenal cortex in response to adrenocorticotrophic hormone (ACTH), is to increase plasma glucose concentrations. However, these steroids exert diverse actions throughout the body, many of which have important implications for fertility. Before excretion in the urine or faeces, glucocorticoids must be rendered water soluble by the sequential actions of hepatic 5α- or 5β-reductase and 3α- or 3β-hydroxysteroid dehydrogenase (3αHSD or 3βHSD) which reduce C=C double bonds and ketones, respectively, to generate hydrophilic dihydro- and tetrahydro-steroid metabolites. It was recognized in the late 1950s that within potential target cells, the actions of glucocorticoids are modulated by 11β-hydroxysteroid dehydrogenases (11βHSD) (EC 1.1.1.146) which catalyse the reversible inactivation of cortisol and corticosterone to their inert 11-ketosteroid metabolites, cortisone and 11-dehydrocorticosterone, respectively (Bush *et al*., 1968) (Fig. 1). Although the biochemistry of 11βHSD is well established, it is only over the past decade that studies have started to define the physiological significance of glucocorticoid metabolism by these enzymes. This review focuses on the emerging importance of 11βHSD isoenzymes in the germ cells, testis, ovary, reproductive tracts, placenta and mammary glands. As this field of reproductive biology is comparatively new, this review summarizes the current understanding of the physiological roles played by 11βHSD in diverse processes including reproductive suppression, ovulation, luteinization, cystic ovarian disease, the developmental potential of oocytes, intra-uterine growth retardation (IUGR), parturition and lactation. Where sufficient robust data have been published, this review summarizes the defined roles for the 11βHSD enzymes. However, this review also includes working hypotheses which fit all of the data currently available, but which will be subject to change as further experimental evidence is reported.

Physiology and molecular biology of 11βHSD

Before discussing specific roles for 11βHSD in reproductive tissues, it is necessary to outline major milestones in the history of this enzyme. To date, two biochemically distinct isoforms of 11βHSD, designated type 1 11βHSD

Email: t.michael@rfc.ucl.ac.uk

1470-1626/2003

Table 1. Biochemical properties of cloned isoforms of 11β-hydroxysteroid dehydrogenase (11βHSD)

	11βHSD1	11βHSD2
Gene	*HSD11B1*	*HSD11B2*
Enzyme cofactor	NADPH > $NADP^+$ (>>> NADH > NAD^+)	NAD^+ only
K_m – cortisol	17–27 μmol l^{-1}	14–60 nmol l^{-1}
(K_m – corticosterone)	(2 μmol l^{-1})	(1–10 nmol l^{-1})
K_m – cortisone	300 nmol l^{-1}	∞
Direction of reaction	Bi-directional: 11-ketosteroid reductase > 11β- dehydrogenase	Uni-directional: 11β-dehydrogenase only

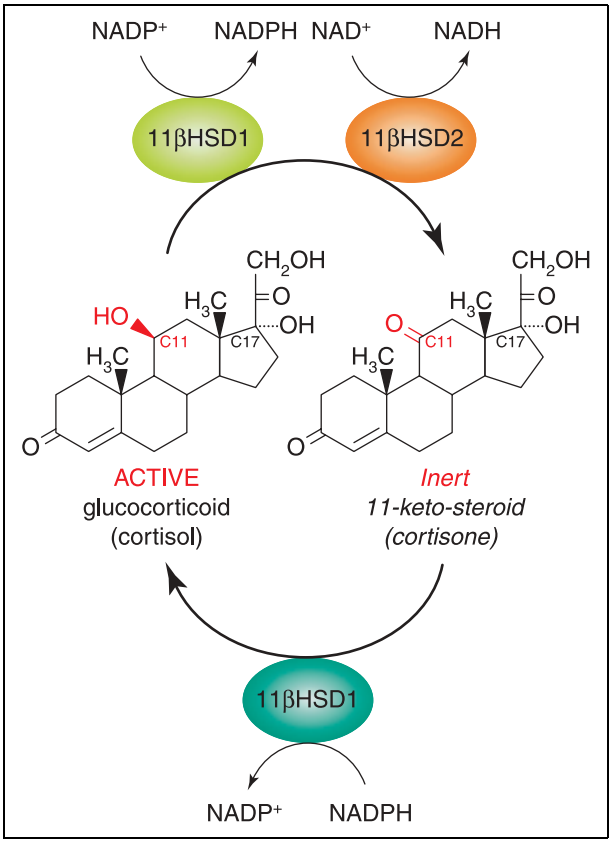

Fig. 1. Interconversion of cortisol and cortisone by enzymatic oxidation or reduction at carbon position 11 (C11), catalysed by the two cloned isoforms of 11β-hydroxysteroid dehydrogenase (11βHSD). By convention, bonds below the plane of the molecule (α bonds) are represented by dotted lines; bonds which have no definitive orientation are represented by solid lines; and bonds above the plane of the molecule (β bonds) are represented by solid triangles. Corticosterone and 11-dehydrocorticosterone have similar structures to cortisol and cortisone, respectively, but lack the α-hydroxyl groups at position C17.

(11βHSD1) and type 2 11βHSD (11βHSD2), have been cloned. (By convention, terms in standard upper case text refer to the enzyme proteins, 11βHSD1 and 11βHSD2, whereas the corresponding genes are referred to in italicized text, *HSD11B1* and *HSD11B2*, respectively.) Both cloned isoforms of 11βHSD are members of the short-chain alcohol dehydrogenase superfamily of enzymes, which includes not only other mammalian and bacterial HSD enzymes, but also 15-hydroxyprostaglandin dehydrogenase (PGDH), retinol dehydrogenase, and the hepatic alcohol dehydrogenase (Penning, 1997). Whereas the 11βHSD enzymes are closely related to other hydroxysteroid dehydrogenases, such as the 3βHSD, 17βHSD and 20αHSD enzymes, 11βHSD1 and 11βHSD2 are distinct gene products (encoded by the *HSD11B1* and *HSD11B2* genes, respectively) which share only 14% homology in their primary sequence (Albiston *et al.*, 1994). Although both enzymes have similar active sites, with a conserved catalytic triad comprising tyrosine, serine and lysine (Penning, 1997), 11βHSD1 acts as a dimer or tetramer whereas 11βHSD2 appears only to be active in its monomeric form. The biochemical properties of the two cloned 11βHSD enzymes are summarized (Table 1). 11βHSD2 has an absolute requirement for the oxidized form of nicotinamide adenine dinucleotide (NAD^+) as an enzyme cofactor, whereas 11βHSD1 preferentially utilizes nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH).

11βHSD1 was originally isolated from liver (Lakshmi and Monder, 1988), where it acts as an NADPH-dependent reductase to generate active glucocorticoids from inert 11-ketosteroids (Fig. 1). After cloning of 11βHSD1 (Agarwal *et al.*, 1989; Tannin *et al.*, 1991), this reductive enzyme isoform was shown to be widely expressed, co-localizing with glucocorticoid receptors (Whorwood *et al.*, 1992; Seckl and Walker, 2001). On the basis of this pattern of expression, it is generally accepted that the principal role of 11βHSD1 is to regenerate cortisol or corticosterone to maximize activation of the glucocorticoid receptors (Seckl and Walker, 2001). However, whereas 11βHSD1 acts predominantly as a ketosteroid reductase in intact cells, this enzyme is inherently bi-directional; in cell homogenates provided with $NADP^+$, 11βHSD1 can inactivate glucocorticoids, albeit with a low affinity (K_m for cortisol = 27 μmol l^{-1}) (Lakshmi and Monder, 1988; Agarwal *et al.*, 1989) (Table 1). Hence, the predominant direction of reaction catalysed by 11βHSD1 depends on the redox state of

NADP(H) in a particular cell. In liver cells, with an abundant supply of glucose (glycogen) and an active pentose phosphate pathway, most NADP will be in the reduced form, favouring the NADPH-dependent ketosteroid reductase activity of 11βHSD1. However, in the steroidogenic cells of the testis, ovary and placenta, the activity of NADPH-dependent cytochrome P450 enzymes, required for the biosynthesis of steroids, favours the oxidation of NADPH to $NADP^+$, thus promoting the oxidative activity of 11βHSD1.

Failure of 11βHSD1 to act as an 11-ketosteroid reductase in the liver manifests as 'apparent cortisone reductase deficiency' (ACRD) (Phillipou and Higgins, 1985; Jamieson *et al.*, 1999). As ACRD has been reported to date in only five women, each diagnosed with anovulatory infertility, hyperandrogenism and polycystic ovaries, the molecular basis and reproductive consequences of this condition will be discussed later. A second clinical syndrome of defective glucocorticoid metabolism is that of 'apparent mineralocorticoid excess' (AME) (Ulick *et al.*, 1979; Stewart *et al.*, 1988). In this syndrome, glucocorticoids are not inactivated, due to a defect in the oxidative activity of 11βHSD, and so gain access to mineralocorticoid receptors which display little inherent specificity for their normal ligand, aldosterone (Krozowski and Funder, 1983; Arriza *et al.*, 1987). As glucocorticoids circulate at concentrations 1000-fold higher than aldosterone, the mineralocorticoid receptors are hyperstimulated, resulting in excessive sodium transport and significant changes in sodium-dependent fluid fluxes (Funder *et al.*, 1988). Increased resorption of water in the distal nephron and colon increases blood volume and pressure, suppressing plasma renin activity and the concentration of aldosterone (Ulick *et al.*, 1979; Stewart *et al.*, 1988).

After the cloning of the human *HSD11B1* gene, patients with the inherited form of AME were screened for mutations. The lack of any mutations in these patients (Nikkila *et al.*, 1993), and the low affinity of 11βHSD1, implicated the existence of a second isoform of 11βHSD to protect the non-specific mineralocorticoid receptors. In 1994, 11βHSD2 was cloned (Agarwal *et al.*, 1994; Albiston *et al.*, 1994) and identified as the enzymatic 'gatekeeper' that excludes glucocorticoids from mineralocorticoid receptors (Fig. 2). Unlike 11βHSD1, 11βHSD2 has been localized to mineralocorticoid target cells in the kidney, colon and parotid glands, as well as to the pancreas and placenta (Mercer and Krozowski, 1992; Brown *et al.*, 1993; Albiston *et al.*, 1994). With physiological substrates, 11βHSD2 acts exclusively as an NAD^+-dependent, high affinity 11β-dehydrogenase (K_m for cortisol = 60 nmol l^{-1}) (Mercer and Krozowski, 1992; Brown *et al.*, 1993; Albiston *et al.*, 1994) (Table 1). Although this enzyme can catalyse limited reduction of the synthetic steroid, 11-dehydrodexamethasone, it has no reductive activity against cortisone or 11-dehydrocorticosterone, even in the presence of excess NADH.

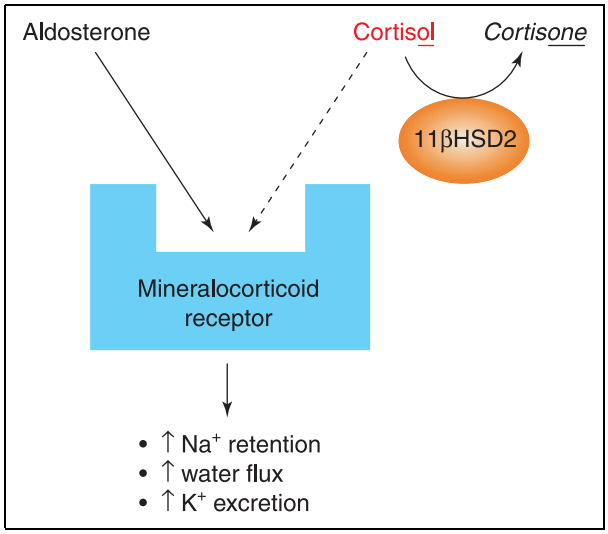

Fig. 2. Modulation of glucocorticoid access to the 'mineralocorticoid receptor' by 11β-hydroxysteroid dehydrogenase 2 (11βHSD2), a molecular 'gatekeeper'. Deficiency of 11βHSD2 activity allows cortisol to gain access to the promiscuous mineralocorticoid receptor.

Although patients with the inherited form of AME have been shown to inherit recessive mutations in the *HSD11B2* gene that encodes 11βHSD2 (Mune *et al.*, 1995; Ferrari *et al.*, 1996), such congenital defects are rare. By comparison, failure of 11βHSD2 due to enzyme inhibition is relatively common. Important inhibitors of 11βHSD2 include glycyrrhizic and glycyrrhetinic acids (metabolites of liquorice) (Stewart *et al.*, 1987; Monder *et al.*, 1989), and, particularly relevant for the current review, progesterone (Lopez-Bernal *et al.*, 1980; Sun *et al.*, 1998; Burton and Waddell, 2002).

11βHSD in the hypothalamus and anterior pituitary gland

In reproductive biology, any review should logically begin at the level of the hypothalamo–pituitary complex, where both cloned isoforms of 11βHSD are known to be expressed and functional. In 11βHSD1 knockout mice, which are unable to reduce 11-ketosteroids to active glucocorticoids, there is increased hypothalamic secretion of corticotrophin-releasing hormone (CRH) and increased ACTH drive to the adrenal gland, indicating that 11βHSD1 is required in the hypothalamus or anterior pituitary corticotrophs for glucocorticoids to exert full negative feedback in the hypothalamo–pituitary–adrenal (HPA) axis (Harris *et al.*, 2001). A recent study confirmed that regeneration of glucocorticoids by 11βHSD1 limits the sensitivity of corticotrophs to CRH by decreasing the expression of CRH receptors (Hanafusa *et al.*, 2002). Moreover, pilot studies in the present authors' laboratory

have found that one or more of the 11βHSD enzymes is active in αT3-1 and LβT2 gonadotroph cells, and that 11βHSD activities respond acutely to GnRH in these pituitary gonadotroph cell lines (Thurston *et al*., 2003a).

Testicular 11βHSD: fertility and reproductive suppression

Over the past three decades, it has emerged that glucocorticoids exert a number of deleterious effects on the interstitial Leydig cells of the testis, including direct inhibition of testosterone biosynthesis, suppression of LH receptor expression, and induction of Leydig cell apoptosis (Bambino and Hsueh, 1981; Monder *et al*., 1994; Gao *et al*., 2002). Shortly after the cloning of rat 11βHSD1, Phillips *et al*. (1989) confirmed expression of this enzyme in rat Leydig cells. In subsequent studies, 11βHSD1 was shown to alleviate inhibition of testosterone biosynthesis by corticosterone (Monder *et al*., 1994). This protective role presumes that in rat Leydig cells, 11βHSD1 acts predominantly as an 11β-dehydrogenase. However, this assumption was challenged by Leckie *et al*. (1998) who found that 11βHSD1 acts as an 11-ketosteroid reductase in rat Leydig cells maintained in primary culture. Detailed *in vitro* studies have since revealed that the predominant direction of testicular 11βHSD1 activity depends on the age of the rat from which Leydig cells have been harvested, the duration of incubation with enzyme substrates, and the precise composition of the cell culture medium in which enzyme activities are assessed. Expression of 11βHSD1 is highest, and the reductase activity of this enzyme predominates, in immature Leydig cells (Ge *et al*., 1997a), on prolonged incubation with steroid substrates (Ge and Hardy, 2000), and in culture medium containing high concentrations of D-glucose or pyruvate (Ferguson *et al*., 1999; Ge and Hardy, 2000). However, in adult Leydig cells, on acute incubation with corticosterone (< 1 h) or in Dulbecco's modified Eagle's medium without added glucose or pyruvate, expression of 11βHSD1 mRNA is decreased and the oxidative activity of 11βHSD predominates (Ge *et al*., 1997a; Ferguson *et al*., 1999; Ge and Hardy, 2000).

In attempting to resolve disputes over the preferred direction of action of 11βHSD1 in Leydig cells in rats, evidence has accumulated to indicate the existence of an enzyme activity that is biochemically distinct from either of the cloned isoforms of 11βHSD. In addition to the low affinity, NADPH-dependent activity characteristic of 11βHSD1, Leydig cells also exhibit an $NADP^+$-dependent enzyme activity which acts preferentially as a high affinity dehydrogenase (K_m for corticosterone = 42 nmol l^{-1}) (Ge *et al*., 1997b). Available evidence indicates that this represents a novel isoform of 11βHSD, rather than a post-translationally modified form of 11βHSD1.

Investigation of the hormonal regulation of 11βHSD1 in testis Leydig cells (Gao *et al*., 1997) established that over 3 days in primary culture, both LH and epidermal growth factor (EGF) upregulated expression of 11βHSD1 mRNA. In the case of EGF, this increase in 11βHSD1 expression was accompanied by a significant increase in 11-ketosteroid reductase activity, whereas both LH and EGF decreased the net 11β-dehydrogenase activity in cultured Leydig cells (Gao *et al*., 1997). Recent studies have revealed that increase of intracellular calcium favours testicular 11-ketosteroid reductase activity, whereas activation of protein kinase C (PKC) inhibits the net reductase activity and instead stimulates the net oxidation of glucocorticoids in rat Leydig cells (Ge and Hardy, 2002). Analysis of the primary amino acid sequences of the 11βHSD1 and 11βHSD2 proteins reveals multiple phosphorylation sequences for calmodulin kinase, casein kinase II and various tyrosine kinases. However, whereas the catalytic domain of 11βHSD1 contains a consensus phosphorylation site for PKC, there is no such sequence in 11βHSD2, and neither 11βHSD1 nor 11βHSD2 contains a consensus phosphorylation sequence for protein kinase A (PKA). Given that the enzymes contain binding sites for both calcium and for adenosine triphosphate (ATP), it seems probable that in Leydig cells direct binding of calcium to 11βHSD1 stimulates the reductase activity of this enzyme, whereas phosphorylation of the active site via PKC favours the oxidative activity of this enzyme.

With respect to the physiological relevance of 11βHSD in Leydig cells, the fact that 11βHSD1 protein cannot be detected before day 31 after birth (Schafers *et al*., 2001), and the developmental switch from predominant reductase activity in mesenchymal-like Leydig cell progenitors to an oxidase in adult Leydig cells (Ge *et al*., 1997a), indicates a role for testicular 11βHSD enzymes in increased testosterone synthesis at puberty. Moreover, latest reports indicate that failure of testicular 11βHSD to protect Leydig cells from the adverse effects of corticosterone contributes to decreased testosterone output in socially subordinate male rats whereas increased testicular 11βHSD activities are observed in dominant males (Hardy *et al*., 2002). As these trends mirror changes in serum LH concentrations within the first week of establishing the social hierarchy (Hardy *et al*., 2002), the authors propose that LH upregulates the protective 11βHSD system in sexually active dominant male rats (allowing their Leydig cells to maintain testosterone production in the face of increased plasma corticosterone), whereas suppression of LH in social subordinates contributes to the decrease in testicular 11βHSD activities.

11βHSD in spermatozoa and the male reproductive tract

Within testis Leydig cells, expression of 11βHSD1 protein coincides with the maturation of elongate spermatids in neighbouring seminiferous tubules (Neumann *et al*.,

1993). 11βHSD activity has also been reported in spermatozoa and other cellular components of human semen (Nacharaju *et al*., 1997). Moreover, the net oxidative activities of 11βHSD were 10-fold higher in semen from men with low sperm counts or a high percentage of morphologically abnormal spermatozoa compared with ejaculates with high sperm counts or good sperm morphology (Nacharaju *et al*., 1997). Although the physiological role for 11βHSD in spermatozoa has yet to be defined, it is relevant to note that human seminal plasma contains relatively high concentrations of cortisol (Brotherton, 1990) and that in men with increased serum cortisol concentrations (due to Cushing's disease), pathological changes occur in the seminiferous tubules (McKenna *et al*., 1979).

Enzyme activity was localized histochemically to the entire length of the mouse epididymis in initial studies of testicular 11βHSD (Rastogi *et al*., 1976). In the epithelium of the vas deferens in pigs, glucocorticoids have been associated with increased sodium absorption (Phillips and Schultz, 2002), which would be expected to reduce seminal fluid volume, leading to a high viscosity luminal environment for the mature spermatozoa. In addition, corticosteroid-dependent sodium absorption will limit the activity of sodium–proton co-transporters in the epithelia of the male reproductive tract which participate in the control of seminal pH (Pushkin *et al*., 2000). Hence, alterations in 11βHSD-mediated glucocorticoid metabolism within the male reproductive tract modify the luminal environment for maturing spermatozoa.

11βHSD in the oocyte

In a wide range of teleost species, cortisol, 11-deoxycortisol and related hydroxylated metabolites of progesterone stimulate oocyte maturation and developmental potential (for example see Kime *et al*., 1992; Petrino *et al*., 1993; Pinter and Thomas, 1999). To date, investigations of the direct effects of glucocorticoids on mammalian oocytes, confined to studies of pig oocytes, have found that glucocorticoids inhibit meiotic development (Yang *et al*., 1999). In contrast, in follicular aspirates of women undergoing assisted conception, follicular glucocorticoid concentrations, which increase following the preovulatory LH surge (Andersen and Hornnes, 1994; Harlow *et al*., 1997), have been positively correlated with oocyte maturity (Fateh *et al*., 1989; Jimena *et al*., 1992).

Metabolism of glucocorticoids by 11βHSD in mammalian oocytes further implicates these steroids in mammalian oogenesis. Although 11βHSD expression has been reported in human oocytes (Ricketts *et al*., 1998; Smith *et al*., 2000), the most convincing data have been obtained in the ovary in rats, wherein expression of 11βHSD1 mRNA and protein were both very high in the oocyte (Benediktsson *et al*., 1992). In interpreting this observation, it is important to note that in oocytes, transcription of new mRNA is blocked between the preovulatory resumption of meiosis and the second mitotic division of the two-cell embryo. Hence, high expression of 11βHSD1 in the preovulatory oocyte could reflect roles for this reductive isoform of 11βHSD in the ovulatory oocyte, zygote or early embryo.

11βHSD across the ovarian cycle: roles in ovulation and luteinization

As in Leydig cells of the testis, glucocorticoids act directly on ovarian cells to inhibit both gonadotrophin action and steroid biosynthesis (Hsueh and Erickson, 1978; Michael *et al*., 1993). Moreover, before the cloning of 11βHSD isoforms, it was established that in granulosa lutein cells in humans, 11βHSD inactivates glucocorticoids, so increasing the concentration of cortisol required to inhibit LH-stimulated steroidogenesis by an order of magnitude (Michael *et al*., 1993).

Within ovarian follicles in humans, 11βHSD2 protein has been localized to theca cells with low 11βHSD2 activity measured in theca cells isolated from immature follicles (Ricketts *et al*., 1998; Yong *et al*., 2000). As regards enzyme expression in granulosa cells, this is highly dependent on the functional phenotype and differentiation of the cells. In parallel studies of ovaries in rats and humans, Tetsuka *et al*. (1997, 1999a) demonstrated that granulosa cells that have not experienced an LH surge co-express mRNA encoding 11βHSD2 and mineralocorticoid receptors. Thurston *et al*. (2003b) observed expression of 11βHSD2 protein in bovine follicles at all stages of development. Given the pivotal role for 11βHSD2 in regulating stimulation of sodium-dependent fluid fluxes via mineralocorticoid receptors, this implicates corticosteroids in the control of follicular fluid accumulation during the expansion of the antral follicle.

In human and rat granulosa cells which have luteinized following exposure to an ovulatory dose of LH *in vivo*, there is no detectable expression of 11βHSD2 mRNA or protein (Michael *et al*., 1997; Tetsuka *et al*., 1997, 1999a; Thurston *et al*., 2003c). Instead, the predominant enzyme isoform in such cells is 11βHSD1 (Michael *et al*., 1997; Tetsuka *et al*., 1997, 1999a), expression of which increases progressively as the cells undergo functional luteinization (Thurston *et al*., 2003c). Moreover, the only cloned isoform of 11βHSD expressed in the corpus luteum of pregnant rats (until such time as the gland undergoes functional regression) is 11βHSD1 (Waddell *et al*., 1996). This transition from expression of 11βHSD2 in follicular granulosa cells to 11βHSD1 in luteinized granulosa cells is accompanied by a switch in expression from mineralocorticoid receptors in the follicle to glucocorticoid receptors in luteinized cells (Tetsuka *et al*., 1997, 1999a) (Fig. 3).

Hillier and colleagues have determined the molecular basis for the transition from 11βHSD2 to 11βHSD1

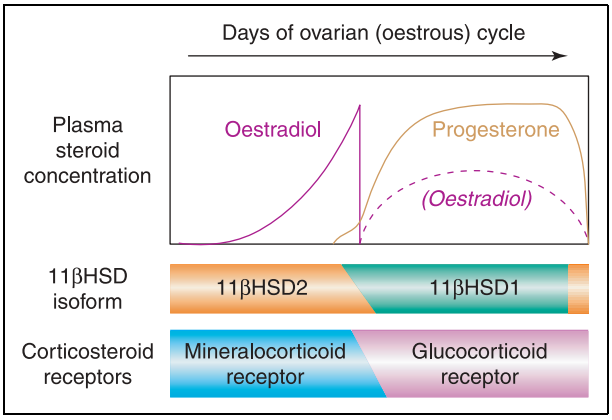

Fig. 3. Temporal pattern of expression of 11β-hydroxysteroid dehydrogenase (11βHSD) isoforms and corticosteroid receptors across ovarian cycles in rats and humans. The upper panel depicts changes in oestradiol (purple line) and progesterone (brown line) to illustrate the phase of the ovarian cycle. (The broken purple line indicates that oestradiol is not secreted in the luteal phase for non-primate species). The horizontal coloured bars indicate the stages of the ovarian cycles in rats and humans at which follicular granulosa cells or luteinized granulosa or luteal cells express a particular isoform of 11βHSD or a particular corticosteroid receptor. Note the transient expression of 11βHSD2 in luteal cells coincident with functional regression of the corpus luteum.

during luteinization, concluding that 11βHSD1 upregulation at ovulation is induced by gonadotrophins. As ovulation is an inflammatory event characterized by increased synthesis of interleukins and prostaglandins (Espey, 1980; Terranova and Rice, 1997; Ando *et al*., 1998), increased generation of anti-inflammatory glucocorticoids by the reductase activity of 11βHSD1 at ovulation may present a physiological mechanism to limit the ovarian inflammatory process (Hillier and Tetsuka, 1998; Andersen, 2002). Certainly, glucocorticoids inhibit the synthesis of both prostaglandins and pro-inflammatory cytokines in the ovary (Goppelt-Struebe, 1997; Telleria *et al*., 1998). As expression of 11βHSD1 is upregulated in granulosa cells by LH and by pro-inflammatory cytokines such as interleukin 1β (IL-1β) (Evagelatou *et al*., 1997; Tetsuka *et al*., 1999b), the synthesis of glucocorticoids via 11βHSD1 may be increased by gonadotrophins or cytokines as an integral aspect of the inflammatory cascade of ovulation. Consistent with this hypothesis, the total and free concentrations of cortisol in human follicular fluid increase in response to the preovulatory LH surge (Fateh *et al*., 1989; Andersen and Hornnes, 1994; Harlow *et al*., 1997; Yong *et al*., 2000). The increase in free cortisol also involves competitive displacement of cortisol from cortisol-binding globulin by the high concentrations of progesterone within the ovulatory follicle (Andersen and Hornnes, 1994; Harlow *et al*., 1997; Andersen, 2002).

Whilst accepting the roles for LH and interleukins in the transition from expression of 11βHSD2 to expression of 11βHSD1 at ovulation, this periovulatory switch in enzyme isoforms at luteinization could also involve steroidal regulation of enzyme expression. Specifically, 11βHSD2 expression in follicular granulosa cells may be dependent on local synthesis of oestrogens, whereas progesterone may suppress expression of 11βHSD2 or induce expression of 11βHSD1 in luteinizing cells. Evidence to support a role for progesterone was obtained in a study of the pregnant rat corpus luteum. In this transient endocrine gland, expression of 11βHSD1 is high until the last day of pregnancy when the corpus luteum undergoes functional regression. At this time, 11βHSD1 expression decreases markedly with a resurgence in expression of 11βHSD2 (Waddell *et al*., 1996) (Fig. 3). The simplest interpretation of this observation is that upregulation of 11βHSD1 and suppression of 11βHSD2 at luteinization is dependent on progesterone, such that the decline in progesterone output with luteal regression causes a parallel loss of 11βHSD1 expression accompanied by an increase in 11βHSD2 expression. In a recent study of human granulosa lutein cells undergoing functional luteinization *in vitro*, this possibility has been examined. Whereas suppression of progesterone synthesis increased both the oxidative and reductive activities of 11βHSD (reflecting decreased competitive inhibition by progesterone), expression of 11βHSD1 protein was unaffected by progesterone suppression and increased progressively as cells luteinized (Thurston *et al*., 2003c). Hence, upregulation of 11βHSD1 expression in luteinizing cells does not rely on local synthesis of progesterone.

Tetsuka *et al*. (2003) reported data for the bovine ovary which differ fundamentally from the observations of ovarian cells in humans and rats. Bovine granulosa cells in large antral follicles expressed only mRNA encoding 11βHSD1 whereas 11βHSD2 mRNA was not detected by RT–PCR. In the corpus luteum in cows, both 11βHSD1 and 11βHSD2 were co-expressed, with 11βHSD1 mRNA predominating in the active corpus luteum and 11βHSD2 predominating in the regressing corpus albicans (Tetsuka *et al*., 2003). Hence, although enzyme expression does change with the functional activity of follicles and corpus luteum, the cow does not show the absolute switch from expression of 11βHSD2 to 11βHSD1 at ovulation. The species specificity of this phenomenon further proves that the transition between 11βHSD isoforms as granulosa cells in rats and humans luteinize cannot be ascribed solely to changes in follicular and luteal steroid production. In large antral follicles in cows, the synthesis of oestradiol is not accompanied by expression of 11βHSD2, and in the corpus luteum in cows, 11βHSD2 is expressed in the absence of oestradiol and in the presence of progesterone.

With respect to luteinizing granulosa cells in humans, the issue of whether 11βHSD1 acts in these cells as either a reductase or an oxidase has been disputed by

independent studies (Michael *et al*., 1997; Thomas *et al*., 1998). It seems likely that in granulosa lutein cells, as in rat Leydig cells, the predominant direction of action is influenced by subtle factors such as cell differentiation or age and differences between culture media. In studying the detailed kinetics of 11βHSD in granulosa lutein cell homogenates in humans, an $NADP^+$-dependent enzyme activity that appeared to act predominantly as an 11β-dehydrogenase with a high affinity for cortisol (K_m = 400 nmol l^{-1}) was identified (Michael *et al*., 1997). The biochemical properties of this ovarian enzyme activity are very similar to the high affinity 11βHSD activity described in Leydig cells in rats (Ge *et al*., 1997b).

11βHSD in the ovarian surface epithelium

Historically, studies of ovarian function have overlooked the surface epithelium in favour of more dynamic ovarian events and structures. However, emerging evidence indicates that this single-layered epithelium participates in repair of the ovarian surface after the 'wounding' of ovulation. Recent studies have shown that human ovarian surface epithelial (OSE) cells express 11βHSD1, which is upregulated by pro-inflammatory cytokines, such as IL-1α (Yong *et al*., 2002). Regeneration of cortisol by the reductive activity of 11βHSD1 may create an anti-inflammatory environment as part of a co-ordinated response to the preovulatory LH surge (Yong *et al*., 2002). In addition, ovulation also involves collagenolysis and tissue remodelling at the ovarian surface with apoptosis of OSE cells from the follicular apex (Murdoch *et al*., 1999). Therefore, the remaining 'bystander' OSE cells surrounding the ovulatory follicle and, after ovulation, the ovulatory rupture site, may play functional roles both in the tissue breakdown that precedes ovulation and in repair of the damaged ovarian surface. These 'bystander' OSE cells are spared from apoptotic damage (Murdoch *et al*., 1999), indicating that a highly localized inflammatory environment is present at ovulation. In the OSE cells, 11βHSD1 will participate in the local generation of cortisol at and around the site of ovulation to facilitate increased anti-inflammatory and surface repair mechanisms (Yong *et al*., 2002). In human OSE cells *in vitro*, cortisol inhibits the induction of metalloproteinase activity by pro-inflammatory cytokines (Rae *et al*., 2003).

Ovarian 11βHSD and assisted conception

Michael *et al*. (1995) reported an inverse correlation between levels of oxidation of cortisol by 11βHSD in granulosa lutein cells in humans and the clinical outcome of gonadotrophin-stimulated assisted conception cycles. Whereas none of the *in vitro* fertilization–embryo transfer (IVF–ET) cycles characterized by detectable net conversion of cortisol to cortisone resulted in conception, the probability of establishing a clinical pregnancy in IVF–ET cycles characterized by undetectably low ovarian 11βHSD activities was 64% (Michael *et al*., 1995). As ovarian 11βHSD activities were only weakly associated with the probability of oocyte fertilization *in vitro* (Michael *et al*., 1995), it was concluded that ovarian 11βHSD activities in the granulosa lutein cells reflected the development potential of ova after fertilization. In patients studied over consecutive treatment cycles, ovarian 11βHSD activities appeared to be a feature of the ovarian response to treatment in a particular IVF–ET cycle for a given patient (Michael *et al*., 1995).

In an independent study, Thomas *et al*. (1998) found no significant difference between mean ovarian 11βHSD activities in conception versus non-conception IVF cycles, although the probability of conception was threefold higher in patients with low ovarian 11βHSD activities than in patients whose granulosa lutein cells exhibited high rates of cortisol–cortisone interconversion. Subsequently, the ratio of cortisol:cortisone in follicular fluid was assessed as a reflection of 11βHSD activities within follicles *in vivo*. In gonadotrophin-stimulated IVF–ET cycles, high follicular cortisol:cortisone ratios have been found to be associated with a greater than 50% probability of conception, whereas low follicular cortisol:cortisone ratios are typically associated with a probability of establishing a pregnancy through IVF–ET of less than 10% (Michael *et al*., 1999; Thurston *et al*., 2003d). Although a study by Andersen *et al*. (1999) found no significant correlation between IVF outcome and follicular cortisol:cortisone ratios, Keay *et al*. (2002) reported a significant association between increased follicular cortisol:cortisone ratios and the probability of conception in women undergoing natural cycle IVF. High cortisol:cortisone ratios in follicular fluid aspirated from women who conceive by IVF–ET would reflect either a low net oxidation of cortisol to cortisone in the mature follicle (in agreement with the present authors' initial observations) or an increase in the opposing reduction of cortisone to cortisol.

Ovarian modulators of 11βHSD: IVF outcome, ovarian cysts and apparent cortisone reductase deficiency

In initial studies, granulosa lutein cells were stored for up to 3 days in follicular fluid before being isolated and cultured. Thurston *et al*. (2002, 2003e) have showed that follicular fluid from women, cows and pigs contains at least two distinct classes of compound capable of selectively modulating the NADP(H)-dependent activities of the 11βHSD1 isoform. Specifically, follicular fluid contains a hydrophilic compound(s) that can stimulate 11βHSD1 activity by up to threefold within 1 h, and a hydrophobic component(s) that acutely inhibits 11βHSD1 activity by up to 84%.

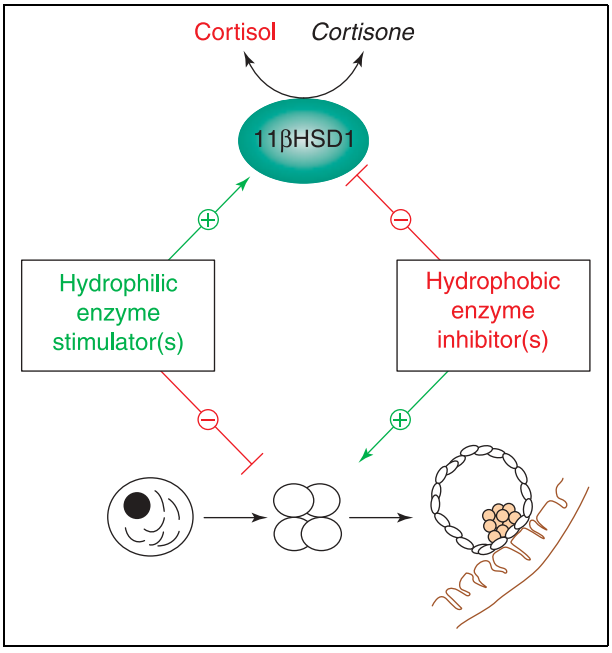

Fig. 4. Independent effects of ovarian modulators of 11β-hydroxysteroid dehydrogenase (11βHSD) on 11βHSD1 activity and the developmental potential of the oocyte. Green lines with a positive symbol indicate a stimulatory effect on enzyme activity or on the developmental potential of the oocyte or embryo. Red lines with a negative symbol indicate an inhibitory effect on enzyme activity or on the developmental potential of the oocyte or embryo.

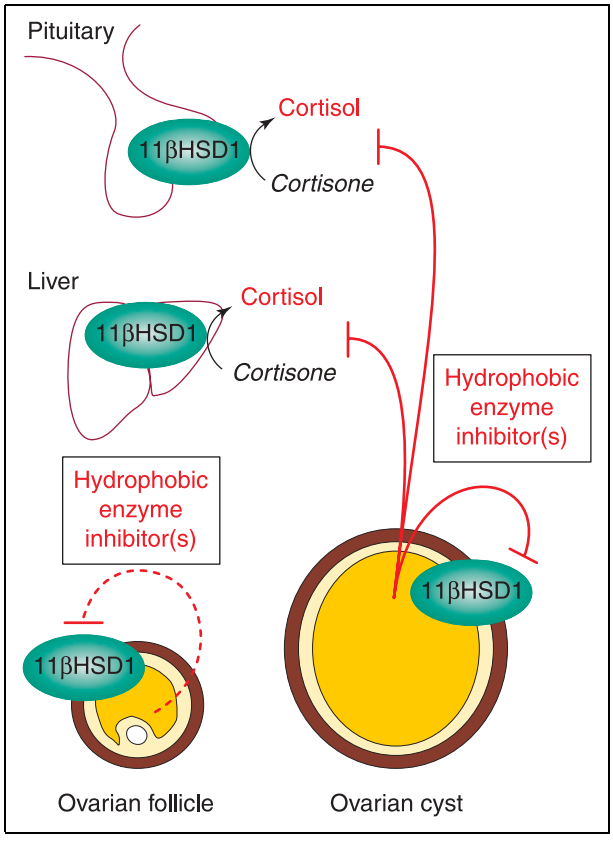

Fig. 5. Contrasting paracrine and endocrine actions of hydrophobic ovarian inhibitors of 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) produced by a maturing ovarian follicle versus an ovarian cyst. Red lines indicate a paracrine or endocrine inhibition of cortisol–cortisone inter-conversion by 11βHSD1 in the ovary, liver or pituitary gland. Broken lines indicate relatively weak inhibition; solid lines indicate relatively strong inhibition.

Within human follicular fluid, high activity of the hydrophilic stimulator(s) of 11βHSD1 correlates with a low intrafollicular cortisol:cortisone ratio and predicts a low probability of conception, whereas high activity of the hydrophobic inhibitor(s) of 11βHSD1 are associated with a high cortisol:cortisone ratio and an increased likelihood of establishing a clinical pregnancy (Thurston *et al.*, 2003d). Hence, in the present authors' initial studies, low 11βHSD activities may have reflected the activity of enzyme modulators present in the follicular fluid samples in which cells were stored before culture. The working model is that the association between the follicular content of enzyme stimulator(s), enzyme inhibitor(s) and IVF outcome reflects independent effects of these as yet unidentified compounds on ovarian glucocorticoid metabolism and the developmental competence of oocytes in maturing follicles (Fig. 4).

In ovaries in cows and pigs, aspirates from spontaneous ovarian cysts contain much lower contents of the hydrophilic stimulator(s) of NADP(H)-dependent glucocorticoid metabolism and higher contents of the hydrophobic inhibitor(s) of 11βHSD1 than does follicular fluid aspirated from large antral follicles (Thurston *et al.*, 2003e). If this picture within follicular and ovarian cyst fluid is reflective of circulating modulators of 11βHSD1 activities, this could explain, at least in part, the association between polycystic ovaries and ACRD in hyperandrogenic, anovulatory women (Fig. 5). Certainly, in patients with ACRD, no mutations have yet been identified in the *HSD11B1* gene (Nikkila *et al.*, 1993; Jamieson *et al.*, 1999), indicating a post-transcriptional defect in the reductase activity of hepatic 11βHSD1. The present authors, based on latest findings, would attribute ACRD to a post-translational inhibition of hepatic 11βHSD1 activity in cystic ovarian disease, possibly due to increased secretion of a hydrophobic inhibitor of 11βHSD1 from the ovarian cysts. Given that 11βHSD1 is required in the pituitary gland for glucocorticoids to suppress ACTH synthesis and secretion (Harris *et al.*, 2001), the present authors propose that in cystic ovarian disease, increased circulating concentrations of the hydrophobic inhibitor(s) of 11βHSD1 not only alters hepatic glucocorticoid metabolism, but also impedes negative feedback within the HPA axis. The consequent increase in pituitary ACTH secretion would then, in turn, contribute to the stimulation of adrenal hyperandrogenism typical of cystic ovarian disease (Fig. 5). Although it is yet to be proved definitively that the

inhibitor(s) of 11βHSD1 present in follicular or cyst fluid arise locally within the ovaries, the fact that first degree male relatives of women with ACRD show normal hepatic cortisone reductase activity (Jamieson *et al*., 1999) supports the view that the major inhibitor of 11βHSD1 is produced in a tissue or gland found only in females.

11βHSD in the uterus

In the uterus, both cloned isoforms of 11βHSD co-operate to control access of glucocorticoids to the glucocorticoid receptors (rather than the mineralocorticoid receptors). Initial studies of rat uteri found that 11βHSD1 mRNA expression varied across the oestrous cycle from a peak at dioestrus to a nadir at oestrus, consistent with suppression of 11βHSD1 by oestrogens (Albiston *et al*., 1995). Subsequent studies confirmed that uterine expression of both 11βHSD1 and 11βHSD2 is indeed oestrogen sensitive (Burton *et al*., 1998). However, studies of 11βHSD1 expression in liver and hippocampus established that in these tissues, suppression of 11βHSD1 mRNA by oestradiol is mediated indirectly via increased plasma growth hormone (Low *et al*., 1994). The suppression of 11βHSD1 expression by growth hormone is in turn mediated by insulin-like growth factor 1 (IGF-I) (Moore *et al*., 1999). As the IGF system is active in the uterus, expression of 11βHSD1 here is likely to be controlled by both direct effects of oestradiol and indirect effects mediated via IGF-I. As in the ovary, expression of 11βHSD1 mRNA in the uterus in rats can also be increased by gonadotrophins (Ho *et al*., 1999), although indirect effects secondary to stimulation of ovarian steroidogenesis cannot be excluded.

Within the myometrium, glucocorticoids exert important effects on such parameters as myometrial contractility (Challis *et al*., 2000) and expression of oestrogen receptors (Wu *et al*., 1996). In the uteri in rats, 11βHSD1 and 11βHSD2 have both been co-localized with glucocorticoid receptors in the myometrial smooth muscle cells (Burton *et al*., 1996a; Waddell and Burton, 2000; Burton and Waddell, 2002). Myometrial expression of 11βHSD2 mRNA and protein both increase from day 10 of pregnancy, peak at day 16 of gestation, and are significantly suppressed within 24 h after birth (Burton and Waddell, 2002). However, in pregnancy, NAD^+-dependent oxidation of corticosterone is suppressed (rather than increased) relative to the non-pregnant rat myometrium, and remains constantly low throughout pregnancy, returning to pre-conception values within 24 h of parturition. This apparent paradox reflects competitive inhibition of myometrial 11βHSD2 by progesterone throughout pregnancy (Burton and Waddell, 2002). During pregnancy, the expression of 11βHSD1 mRNA and protein in the rat myometrium also increases markedly to a maximum on day 22 of gestation (that is 1 day before birth), decreasing to non-pregnant values by day 1 after birth (Burton *et al*., 1996a; Waddell and Burton, 2000; Burton and Waddell, 2002). Unlike 11βHSD2, the 30-fold increase in expression of 11βHSD1 at term is accompanied by a 10-fold increase in the 11-ketosteroid reductase activity which peaks on the day before parturition (Burton *et al*., 1996a; Waddell and Burton, 2000). As the oxidative activity of myometrial 11βHSD2 remains constant throughout pregnancy, the pre-partum induction of 11βHSD1 favours net regeneration of active glucocorticoids within the myometrium (Burton and Waddell, 2002). As discussed below, this change from inactivation to regeneration of glucocorticoids within the myometrium assists in parturition. However, this role for myometrial 11βHSD is species specific as there is little detectable expression of 11βHSD1 mRNA in the myometrium in sheep from day 60 of gestation to 2 days before term (Yang *et al*., 1996).

Although the data reviewed above indicate up-regulation of myometrial 11βHSD1 by progesterone as pregnancy progresses, Waddell and Burton (2000) observed that in unilaterally pregnant rats there was only limited endocrine upregulation of 11βHSD1 mRNA in the contralateral, non-gravid uterine horn, concluding that full induction of myometrial 11βHSD1 expression in the gravid horn was reliant on paracrine factors. This comprehensive study established that paracrine upregulation of 11βHSD1 expression and activity in the pregnant uterine horn was attributable to the placenta rather than to uterine distension or to the presence of a fetus. Waddell and Burton (2000) concluded that placental hormones (for example placental cytokines) are required for local upregulation of myometrial 11βHSD1 in pregnancy.

In the uterine endometrium, in myomorph rodents and sheep, 11βHSD1 is absent from the endometrial stroma and glandular epithelium, but has been localized to the luminal epithelium where it is co-expressed with glucocorticoid receptors (Yang *et al*., 1996; Burton *et al*., 1998; Thompson *et al*., 2002). As in the myometrium, endometrial expression of 11βHSD1 mRNA is increased in the luteal phase of the oestrous cycle and in pregnancy, reflecting upregulation by progesterone (Yang *et al*., 1996). In cultured human endometrial stroma cells, the expression and activity of 11βHSD1 was increased by treatment of cells with progesterone for 3 days. Although treatment with oestradiol alone had no effect, co-treatment of stromal cells with oestradiol potentiated the induction of 11βHSD1 by progesterone. This observation led Arcuri *et al*. (1996) to conclude that upregulation of 11βHSD1 is a feature of endometrial decidualization.

In the endometrium of pregnant rats and mice, 11βHSD2 is expressed only in the sub-epithelial stroma, and there is no detectable expression in the uterine epithelia (Burton and Waddell, 2002; Thompson *et al*.,

2002). In contrast, in the endometrium of non-pregnant women, there is limited expression of 11βHSD2 in the stroma, but this oxidative enzyme isoform is highly expressed in both the glandular and luminal epithelia (Smith *et al*., 1997). Although endometrial 11βHSD2 activity increases in the secretory phase of the menstrual cycle, this almost certainly reflects expansion of the glandular epithelium under the action of progesterone (Smith *et al*., 1997). Although roles for endometrial 11βHSD have been proposed in pregnancy (particularly for the decidua), the physiological roles for glucocorticoid metabolism in the non-pregnant endometrium have yet to be defined.

11βHSD in pregnancy

Early studies of glucocorticoid metabolism focused on human placenta as a source of enzyme activity (Osinski, 1960; Murphy *et al*., 1974). As the complex role for 11βHSD in this organ has been discussed in several excellent reviews (for example Yang, 1997; Burton and Waddell, 1999; Bertram and Hanson, 2002), coverage in the current review will focus on major points of note and recent developments.

In the placenta of all species studied to date, there are two biochemically distinct 11βHSD activities which have been attributed to the co-expression of both 11βHSD1 and 11βHSD2 (Murphy, 1981; Lakshmi *et al*., 1993; Yang, 1995; Burton *et al*., 1996b; Pepe *et al*., 1996; Sun *et al*., 1997a; Thompson *et al*., 2002). 11βHSD1 is predominantly expressed in the decidua, chorion, amnion and vascular endothelial cells, whereas 11βHSD2 is expressed in both the decidua and in the placenta (Murphy, 1981; Sun *et al*., 1997a; Burton and Waddell, 1999; Driver *et al*., 2001; Thompson *et al*., 2002). In the baboon placenta, 11βHSD1 and 11βHSD2 are co-expressed in the syncitiotrophoblast (Pepe *et al*., 1996), and in the mouse placenta, both cloned isoforms of 11βHSD are co-expressed with glucocorticoid receptors in the labyrinthine zone in the later stages of gestation (Thompson *et al*., 2002). In all species, this localization of 11βHSD2 at the materno–fetal interface reflects the role for 11βHSD2 as a mechanism to limit transfer of glucocorticoids between the maternal and fetal circulations.

At different stages of gestation, the balance between the reductase activity of 11βHSD1 and the oxidative activity of 11βHSD2 in the placenta changes in a species-specific manner. In ewes and guinea-pigs, placental 11β-dehydrogenase activity decreases as pregnancy progresses (Yang, 1997; Sampath-Kumar *et al*., 1998), whereas in human, baboon, pig and rat placentas, 11β-dehydrogenase activity increases in the later stages of gestation (Lopez-Bernal *et al*., 1980; Pepe *et al*., 1988; Klemcke and Christenson, 1996; Burton and Waddell, 1999). Although the net oxidative activity of 11βHSD increases at term in the rat placenta, this reflects increasing expression of 11βHSD2 in the basal zone of the placenta (the major site of placental hormone synthesis), which masks a decrease in the expression and activity of 11βHSD2 in the labyrinthine zone (the site of feto–maternal exchange) (Burton *et al*., 1996b).

As in other reproductive tissues, the expression and activities of placental 11βHSD isoforms is hormonally regulated. For example, in the placenta in sheep, suppression of 11βHSD2 activity at term is mediated by cortisol (Clarke *et al*., 2002). In placental and chorionic trophoblasts in humans, both progesterone and oestradiol selectively inhibit the oxidative activity of 11βHSD2 without affecting 11βHSD1 reductase activity (Sun *et al*., 1998). In addition, progesterone represses the expression of 11βHSD2 mRNA (Sun *et al*., 1998). Expression or activity of this high affinity 11β-dehydrogenase is also inhibited in human term placental trophoblasts by noradrenaline (Sarkar *et al*., 2001), nitric oxide (mediated, at least in part, via cyclic GMP) (Sun *et al*., 1997b), prostaglandins and leukotriene B_4 (which inhibit enzyme activity at the post-transcriptional level via increased intracellular calcium) (Hardy *et al*., 1999, 2001). In contrast to these suppressive actions, placental 11βHSD2 is selectively upregulated by cyclic AMP (Sun *et al*., 1998). As 11βHSD2 does not contain a consensus phosphorylation sequence for the cyclic AMP-dependent kinase, PKA, the selective upregulation of placental 11βHSD2 must be attributable either to a direct effect of cyclic AMP on the enzyme (via interaction with the ATP-binding site) or to an effect on enzyme translation or stability. (The endocrine regulation of 11βHSD1 expression and activity in the placenta is of primary importance at parturition, and so will be discussed below.)

Recent studies have focused on the regulation of placental 11βHSD2 by hypoxia. Although two studies have shown that hypoxia represses expression of 11βHSD2 (without affecting 11βHSD1) in term human trophoblasts and villous explants (Alfaidy *et al*., 2002; Hardy and Yang, 2002), Driver *et al*. (2002) found that hypoxia upregulates 11βHSD2 and have identified a hypoxia-inducible factor 1 response element in the 11βHSD2 promoter. Moreover, decreased oxygen tension indirectly influences the activities of placental 11βHSD by altering the redox balances of pyridine nucleotide cofactors in favour of NADPH (so favouring the reductive activity of 11βHSD1) and NADH (thereby limiting the NAD^+-dependent oxidative activity of 11βHSD2). In either event, the placental hypoxia associated with pre-eclampsia alters the trans-placental passage of glucocorticoids from mother to fetus and the local balance of glucocorticoid regeneration from 11-ketosteroids (Alfaidy *et al*., 2002).

Endogenous inhibitors of glucocorticoid metabolism have been described in human urine (Morris *et al*., 1992). Pending identification, these compounds were named 'glycyrrhetinic acid-like factors' (GALFs), reflecting their ability to inhibit both 11βHSD and 5β-reductase:

characteristic actions of the liquorice derivative, glycyrrhetinic acid. The urinary GALF content, which is higher in women than men, increases progressively during pregnancy (Morris *et al*., 1992), consistent with production of GALF by the ovary or placenta. Although GALF and identified inhibitor(s) of 11βHSD1 from follicular fluid share similar biophysical properties (Thurston *et al*., 2002), it can only be speculated at present that the urinary GALFs and ovarian enzyme inhibitor(s) may be related (if not the same) compounds.

11βHSD, fetal development and intra-uterine growth retardation

The widely accepted view is that in the placenta, 11βHSD2 serves as a barrier to limit transfer of active glucocorticoids from mother to fetus. Hence, if placental 11βHSD2 activity is compromised, increased transport of glucocorticoids (before activation of the fetal HPA axis) stimulates premature differentiation of fetal tissues, limiting subsequent tissue growth and culminating in growth retardation. This model was first advanced by Benediktsson *et al*. (1993) after observing a direct correlation between placental 11βHSD activities and fetal weights in rats at term. That placental 11βHSD activities are decreased in pregnancies complicated by IUGR was subsequently corroborated in studies of pregnancies in humans (Shams *et al*., 1998; McTernan *et al*., 2001). However, it is important to note that within the fetus, both cloned isoforms of 11βHSD are widely expressed in a tissue-specific manner (Murphy, 1981; Stewart *et al*., 1994). Hence, any decrease in placental inactivation of glucocorticoids may not necessarily influence the concentrations to which individual fetal tissues are exposed. It seems far more likely that the exposure of fetal tissues to glucocorticoids is determined at the intracrine level by the activities of 11βHSD1 and 11βHSD2, rather than at the relatively distant level of the placenta.

Recent studies have demonstrated that maternal nutrient restriction at defined stages of pregnancy results in both fetal growth retardation and in significant decreases in the expression and activity of placental 11βHSD2 (Bertram *et al*., 2001; Lesage *et al*., 2001). Although this would permit increased passage of active glucocorticoid into the fetal circulation, it is not possible to comment on the physiological significance of the decrease in placental glucocorticoid metabolism versus changes in the expression or activities of 11βHSD within affected fetuses.

Although the relative impact of placental versus fetal 11βHSD in modulating fetal exposure to glucocorticoids remains contentious, 11βHSD enzymes in tissues such as the decidua are able to influence fetal development *in utero*. Specifically, 11βHSD1 acts in the decidua to increase the concentration of active glucocorticoid in amniotic fluid. As the amniotic fluid bathes the fetal lungs, a high cortisol:cortisone (or corticosterone:11-dehydrocorticosterone) ratio in amniotic fluid promotes maturation of the fetal lungs. To guard against premature lung maturation, the fetal lung expresses the oxidative 11βHSD2 enzyme throughout the first and second trimester (Murphy, 1981; Stewart *et al*., 1994), but in late gestation, this oxidative enzyme is replaced by the reductive 11βHSD1 enzyme, so allowing glucocorticoid derived from the amniotic fluid to stimulate synthesis of lung surfactant.

11βHSD and parturition

Over the past 5 years, studies conducted primarily by Challis and colleagues have implicated the reductive activity of 11βHSD1 in the mechanism of parturition (Fig. 6). Specifically, it has been proposed that glucocorticoids generated by 11βHSD1 in the myometrium and decidua increase (rather than suppress) uterine concentrations of prostaglandins which stimulate contractions of the myometrium to expel the fetus (reviewed by Challis *et al*., 2000). This unusual pro-inflammatory (rather than anti-inflammatory) action of glucocorticoids at term involves upregulation of prostaglandin H synthase 2 accompanied by suppression of PGDH in the chorion (Patel *et al*., 1999; Whittle *et al*., 2001) (Fig. 6). PGDH, which normally oxidizes prostaglandins to inactive metabolites, belongs to the same enzyme superfamily as the 11βHSD enzymes, and shares over 90% primary sequence homology at the active site with 11βHSD1 (Penning, 1997).

Within the placenta, prostaglandins increase both the expression and activity of 11βHSD1 (Alfaidy *et al*., 2001). The consequent increase in the concentration of active glucocorticoids is further enhanced by the abilities of prostaglandins E_2 and $F_{2\alpha}$ to inhibit the oxidative 11βHSD2 isoform in the placenta (Hardy *et al*., 1999). Recent studies have shown that glucocorticoids can also upregulate placental 11βHSD1 expression (Sun *et al*., 2002) and suppress the inactivation of glucocorticoids by placental 11βHSD2 (Clarke *et al*., 2002), thereby accelerating the paracrine glucocorticoid–prostaglandin spiral (Fig. 6).

At term, this positive feed-forward loop may be activated by glucocorticoids generated as the fetal HPA axis matures (Whittle *et al*., 2001). However, this mechanism could also participate in the initiation of pre-term labour following an increase in either maternal or fetal glucocorticoids (and catecholamines) under conditions of maternal or fetal stress. In addition, this paracrine positive feed-forward loop provides a mechanism whereby increase of uterine prostaglandin concentrations (for example in response to an intra-uterine infection) would prematurely trigger parturition. As placental 11βHSD2 appears to be sensitive to oxygen tension, changes in glucocorticoid metabolism have also been implicated in the mechanism of pre-term labour

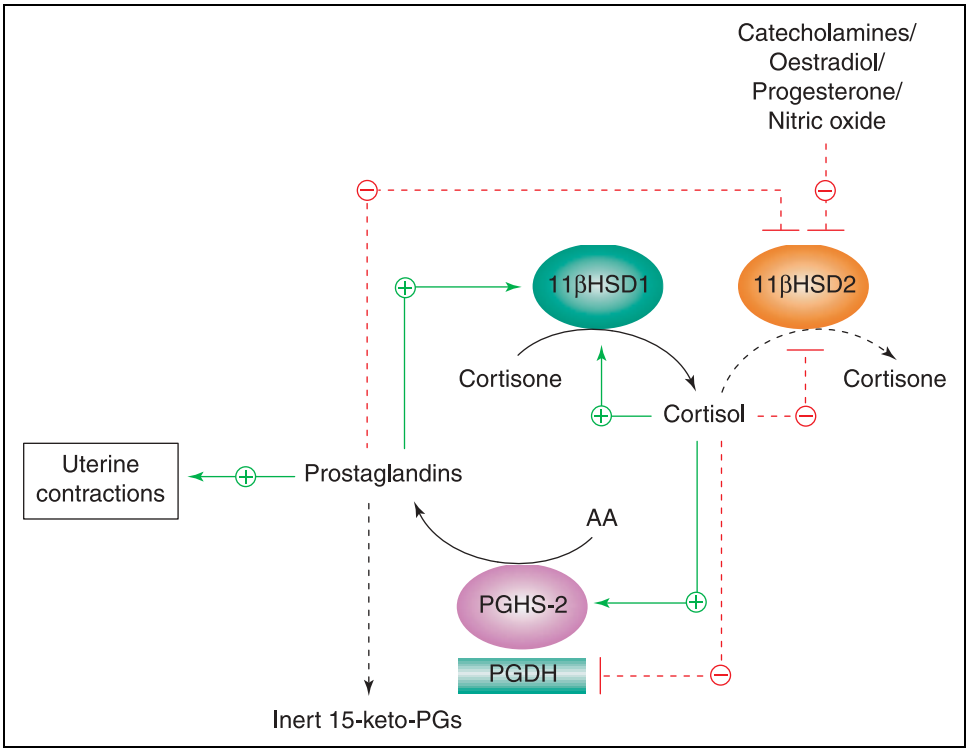

Fig. 6. A potential glucocorticoid–prostaglandin paracrine feed-forward loop contributing to the mechanism of parturition. Black lines indicate an enzyme-catalysed reaction; solid black lines are used for major metabolic pathways; and broken lines indicate minor pathways. Solid green lines with a positive symbol indicate a stimulatory effect on the expression or activity of the appropriate enzyme, or on myometrial contraction. Broken red lines with a negative symbol indicate an inhibitory effect on the expression or activity of the appropriate enzyme. AA: arachidonic acid; 11βHSD1: 11β-hydroxysteroid dehydrogenase 1; PGDH: 15-hydroxyprostaglandin dehydrogenase; PGHS-2: prostaglandin H synthase 2.

in hypoxic states such as pre-eclampsia (Alfaidy *et al*., 2002).

11βHSD and the mammary gland

Within the mammary gland, oxidative 11βHSD activity is 20-fold higher in adipose cells than in mammary epithelial cells (Quirk *et al*., 1990). Moreover, whereas 11βHSD activities are comparably high in mammary cells from virgin and pregnant rats, the ability of 11βHSD to inactivate glucocorticoids decreases by over 75% during lactation (Quirk *et al*., 1990). As glucocorticoids induce the expression of milk proteins such as casein and lactalbumin (Ono and Oka, 1980), the postpartum decrease in glucocorticoid oxidation is a prerequisite for the endocrine induction of lactogenesis (Quirk *et al*., 1990), whereas inactivation of glucocorticoids within the mammary gland prevents futile antagonism between the positive effects of glucocorticoids and the suppressive actions of progesterone during pregnancy. As to the mechanism for the decrease in mammary 11βHSD activities during lactation, this is unlikely to reflect changes in gonadal or placental steroids as enzyme activities were the same in virgin versus pregnant rats. Instead, the decrease in mammary 11βHSD activities probably reflects unique changes to the endocrine milieu of the mammary gland post partum (for example increased prolactin levels).

Hundertmark *et al*. (1997) reported inactivation of physiological and synthetic glucocorticoids by 11βHSD in the MCF-7 and ZR-75-1 human breast cancer cell lines. As the cells exhibited only limited 11-ketosteroid reductase activity, and because the oxidation of glucocorticoids could be inhibited by glycyrrhetinic acid, these authors concluded that 11βHSD2 is the predominant enzyme isoform in the human breast. Moreover, inhibition of 11βHSD activity with glycyrrhetinic acid potentiated the anti-proliferative action of prednisolone (Hundertmark *et al*., 1997), confirming that oxidation modulates the actions of glucocorticoids in mammary cells.

Concluding remarks

The evidence outlined in this review indicates that the metabolism of glucocorticoids by isoforms of 11βHSD is important in a wide range of reproductive tissues and

Table 2. Overview of the confirmed and speculative physiological roles for 11β-hydroxysteroid dehydrogenases (11βHSD) in reproductive tissues

Tissue or organ	Cell	11βHSD isoform	Proposed physiological role
Pituitary	Corticotrophs	1	Maximizes negative feedback on ACTH
	Gonadotrophs	1 + 2	Unknown
Testis	Leydig cells	1 (+ ?)	Protects testosterone biosynthesis from inhibition by glucocorticoids
	Spermatozoa	Unknown	Unknown
Epididymis/vas deferens	Not specified	1	Modulates control of sodium content/viscosity of seminal plasma
Ovary	Theca cells	2	Unknown
	Granulosa cells	2 (+ ?)	Protects oestradiol biosynthesis from inhibition by glucocorticoids
	Oocyte	1	Modulates effects of glucocorticoids on oocyte maturation
	Luteal cells	1 (+ ?)	Protects progesterone biosynthesis from inhibition by glucocorticoids
	Surface epithelial cells	1	Facilitates anti-inflammatory effects of glucocorticoids at ovulation
Myometrium	Smooth muscle cells	1 + 2	Modulates actions of glucocorticoids on contractility and oestrogen receptors
Endometrium	Epithelial cells + sub-epithelial stroma	1 + 2	Unknown
Placenta	Decidua + chorion + amnion + vascular endothelial cells	1	Facilitates effects of glucocorticoids on prostaglandin synthesis or metabolism to induce parturition (implicated in pre-term labour)
	Decidua + syncitiotrophoblast	2	Limits transfer of cortisol from the maternal to the fetal circulation (defective in IUGR)
Mammary gland	Adipose cells	2	Modulates actions of glucocorticoids on lactogenesis

1: 11βHSD1; 2: 11βHSD2; (+ ?): the possible existence of an as yet uncloned isoform of 11βHSD; ACTH: adrenocorticotrophic hormone; IUGR: intra-uterine growth retardation.

a number of key reproductive processes (summarized in Table 2). The apparent significance of glucocorticoid inactivation or reactivation by 11βHSD in reproductive suppression, ovulation, luteinization, cystic ovarian disease, the developmental potential of oocytes, IUGR, parturition and lactation indicates significant roles for glucocorticoids in each of these fundamental processes.

The authors would like to thank S. G. Hillier and K. C. Jonas for their constructive feedback on early drafts of this review. The original research contributions of the authors to the work reviewed herein has been supported by grants from the BBSRC (48/S15850), Freemedic plc, MRC (project grants G9309070 and G9519816; programme grant 0000066), Stanley Thomas Johnson Foundation, Wellbeing and the Wellcome Trust (052970/Z/97).

References

Key references are indicated by asterisks.

Agarwal AK, Monder C, Eckstein B and White PC (1989) Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase *Journal of Biological Chemistry* **264** 18 939–18 943

Agarwal AK, Mune T, Monder C and White PC (1994) NAD^+-dependent isoform of 11β-hydroxysteroid dehydrogenase. Cloning and characterization cDNA from sheep kidney *Journal of Biological Chemistry* **269** 25 959–25 962

Albiston AL, Obeyesekere VR, Smith RE and Krozowski ZS (1994) Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme *Molecular and Cellular Endocrinology* **105** R11–R17

Albiston AL, Smith RE and Krozowski ZS (1995) Changes in the levels of 11β-hydroxysteroid dehydrogenase mRNA over the oestrous cycle in the rat *Journal of Steroid Biochemistry and Molecular Biology* **52** 45–48

***Alfaidy N, Xiong ZG, Myatt L, Lye SG, MacDonald JF and Challis JRG** (2001) Prostaglandin $F_{2\alpha}$ potentiates cortisol production by stimulating 11β-hydroxysteroid dehydrogenase 1: a novel feedback loop that may contribute to human labor *Journal of Clinical Endocrinology and Metabolism* **86** 5585–5592

Alfaidy N, Gupta S, DeMarco C, Caniggia I and Challis JRG (2002) Oxygen regulation of placental 11β-hydroxysteroid dehydrogenase 2: physiological and pathological implications *Journal of Clinical Endocrinology and Metabolism* **87** 4797–4805

***Andersen CY** (2002) Possible new mechanism of cortisol action in female reproductive organs: physiological implications of the free hormone hypothesis *Journal of Endocrinology* **173** 211–217

Andersen CY and Hornnes P (1994) Intrafollicular concentrations of free cortisol close to follicular rupture *Human Reproduction* **9** 1944–1949

Andersen CY, Morineau G, Fukuda M, Westergaard LG, Ingerslev HJ, Fiet J and Byskov AG (1999) Assessment of the follicular cortisol:cortisone ratio *Human Reproduction* **14** 1563–1568

Ando M, Kol S, Kokia E, Ruutiainen-Altman K, Sirois J, Rohan RM, Payne DW and Adashi EY (1998) Rat ovarian prostaglandin endoperoxide

synthase-1 and -2: periovulatory expression of granulosa cell-based interleukin-1-dependent enzymes *Endocrinology* **139** 2501–2508

Arcuri F, Monder C, Lockwood CJ and Schatz F (1996) Expression of 11β-hydroxysteroid dehydrogenase during decidualization of human endometrial stromal cells *Endocrinology* **137** 595–600

Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE and Evans RM (1987) Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor *Science* **237** 268–275

Bambino TH and Hsueh AJ (1981) Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis *in vivo* and *in vitro*. *Endocrinology* **108** 2142–2148

***Benediktsson R, Yau JLW, Low SC, Brett LP, Cooke BE, Edwards CRW and Seckl JR** (1992) 11β-hydroxysteroid dehydrogenase in the rat ovary: high expression in the oocyte *Journal of Endocrinology* **135** 53–58

Benediktsson R, Lindsay RS, Noble J, Seckl JR and Edwards CRW (1993) Glucocorticoid exposure *in utero*: new model for adult hypertension *Lancet* **341** 339–341

***Bertram CE and Hanson MA** (2002) Prenatal programming of postnatal endocrine responses by glucocorticoids *Reproduction* **124** 459–467

Bertram CE, Trowern AR, Copin N, Jackson AA and Whorwood CB (2001) The maternal diet during pregnancy programmes altered expression of the GR and 11βHSD2: potential molecular mechanisms underlying the programming of hypertension *in utero*. *Endocrinology* **142** 2841–2853

Brotherton J (1990) Cortisol and transcortin in human seminal plasma and amniotic fluid as estimated by modern specific assays *Andrologia* **22** 197–204

Brown RW, Chapman KE, Edwards CRW and Seckl JR (1993) Human placental 11β-hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent iosform *Endocrinology* **132** 2614–2621

Burton PJ and Waddell BJ (1999) Dual function of 11β-hydroxysteroid dehydrogenase in placenta: modulating glucocorticoid passage and local steroid action *Biology of Reproduction* **60** 234–240

Burton PJ and Waddell BJ (2002) Myometrial expression of 11β-hydroxysteroid dehydrogenase type 2 in rat pregnancy *Journal of Endocrinology* **172** 519–526

Burton PJ, Dhamarajan AM, Hisheh S and Waddell BJ (1996a) Induction of myometrial 11β-hydroxysteroid dehydrogenase type 1 messenger ribonucleic acid and protein expression in late pregnancy *Endocrinology* **137** 5700–5706

Burton PJ, Smith RE, Krozowski ZS and Waddell BJ (1996b) Zonal distribution of 11β-hydroxysteroid dehydrogenase types 1 and 2 messenger ribonucleic acid expression in the rat placenta and decidua during late pregnancy *Biology of Reproduction* **55** 1023–1028

Burton PJ, Krozowski ZS and Waddell BJ (1998) Immunolocalization of 11β-hydroxysteroid dehydrogenase types 1 and 2 in rat uterus: variation across the estrous cycle and regulation by estrogen and progesterone *Endocrinology* **139** 376–382

Bush IE, Hunter SA and Meigs RA (1968) Metabolism of 11-oxygenated steroids *Biochemical Journal* **107** 239–258

***Challis JRG, Matthews SG, Gibb W and Lye SJ** (2000) Endocrine and paracrine regulation of birth at term and preterm *Endocrine Reviews* **21** 514–550

Clarke KA, Ward JW, Forhead AJ, Giussani DA and Fowden AL (2002) Regulation of 11β-hydroxysteroid dehydrogenase type 2 activity in ovine placenta by fetal cortisol *Journal of Endocrinology* **172** 527–534

Driver PM, Kilby MD, Bujalska I, Walker EA, Hewison M and Stewart PM (2001) Expression of 11β-hydroxysteroid dehydrogenase isozymes and corticosteroid hormone receptors in primary cultures of human trophoblast and placental bed biopsies *Molecular Human Reproduction* **7** 357–363

Driver PM, Hewison M, Kilby MD and Stewart PM (2002) Oxygen tension regulates placental 11β-hydroxysteroid dehydrogenase type 2 *Endocrine Abstracts* **3** P221 (Abstract)

***Espey LL** (1980) Ovulation as an inflammatory reaction – a hypothesis *Biology of Reproduction* **22** 73–106

Evagelatou M, Peterson SL and Cooke BA (1997) Leukocytes modulate 11β-hydroxysteroid dehydrogenase (11β-HSD) activity in human granulosa lutein cell cultures *Molecular and Cellular Endocrinology* **133** 81–88

Fateh M, Ben-Rafael Z, Benadiva CA, Mastroianni L, Jr and Flickinger GL (1989) Cortisol levels in human follicular fluid *Fertility and Sterility* **51** 538–541

***Ferguson SE, Pallikaros Z, Michael AE and Cooke BA** (1999) The effects of different culture media, glucose, pyridine nucleotides and adenosine on the activity of 11β-hydroxysteroid dehydrogenase in rat Leydig cells *Molecular and Cellular Endocrinology* **158** 37–44

Ferrari P, Obeyesekere VR, Li K, Wilson RC, New MI, Funder JW and Krozowski ZS (1996) Point mutations abolish 11β-hydroxysteroid dehydrogenase type II activity in three families with the congenital syndrome of apparent mineralocorticoid excess *Molecular and Cellular Endocrinology* **119** 21–24

Funder JW, Pearce PT, Smith R and Smith AI (1988) Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated *Science* **242** 583–585

Gao H-B, Ge R-S, Lakshmi V, Marandici A and Hardy MP (1997) Hormonal regulation of oxidative and reductive activities of 11β-hydroxysteroid dehydrogenase in rat Leydig cells *Endocrinology* **138** 156–161

Gao H-B, Tong M-H, Hu Y-Q, Guo Q-S, Ge R-S and Hardy MP (2002) Glucocorticoid induces apoptosis in rat leydig cells *Endocrinology* **143** 130–138

Ge R-S and Hardy MP (2000) Initial predominance of the oxidative activity of type 1 11β-hydroxysteroid dehydrogenase in primary rat Leydig cells and transfected cell lines *Journal of Andrology* **21** 303–310

Ge R-S and Hardy MP (2002) Protein kinase C increases 11β-hydroxysteroid dehydrogenase oxidation and inhibits reduction in rat Leydig cells *Journal of Andrology* **23** 135–143

Ge R-S, Hardy DO, Catterall JF and Hardy MP (1997a) Developmental changes in glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase oxidative and reductive activities in rat Leydig cells *Endocrinology* **138** 5089–5095

***Ge R-S, Gao H-B, Nacharaju VL, Gunsalus GL and Hardy MP** (1997b) Identification of a kinetically distinct activity of 11β-hydroxysteroid dehydrogenase in rat Leydig *cells Endocrinology* **138** 2435–2442

Goppelt-Struebe M (1997) Molecular mechanisms involved in the regulation of prostaglandin biosynthesis by glucocorticoids *Biochemical Pharmacology* **53** 1389–1395

Hanafusa J, Mune T, Tanahashi T, Isomura Y, Suwa T, Isaji M, Daido H, Morita H, Murayama M and Yasuda K (2002) Altered corticosteroid metabolism differentially affects pituitary corticotropin response *American Journal of Physiology – Endocrinology and Metabolism* **282** E466–E473

Hardy DB and Yang K (2002) The expression of 11β-hydroxysteroid dehydrogenase type 2 is induced during trophoblast differentiation: effects of hypoxia *Journal of Clinical Endocrinology and Metabolism* **87** 3696–3701

Hardy DB, Pereira LE and Yang K (1999) Prostaglandins and leukotriene B4 are potent inhibitors of 11β-hydroxysteroid dehydrogenase type 2 activity in human choricarcinoma JEG-3 cells *Biology of Reproduction* **61** 40–45

Hardy DB, Dixon SJ, Narayan N and Yang K (2001) Calcium inhibits human placental 11β-hydroxysteroid dehydrogenase type 2 activity *Biochemical and Biophysical Research Communications* **283** 756–761

***Hardy MP, Sottas CM, Ge R et al.** (2002) Trends of reproductive hormones in male rats during psychosocial stress: role of glucocorticoid metabolism in behavioural dominance *Biology of Reproduction* **67** 1750–1755

Harlow CR, Jenkins JM and Winston RML (1997) Increased follicular fluid total and free cortisol during the luteinizing hormone surge *Fertility and Sterility* **68** 48–53

Harris HJ, Kotelevtsev Y, Mullins JJ, Seckl JR and Holmes MC (2001) Intracellular regeneration of glucocorticoids by 11β-hydroxysteroid dehydrogenase (11β-HSD)-1 plays a key role in regulation of the hypothalamo–pituitary–adrenal axis: analysis of 11β-HSD-1-deficient mice *Endocrinology* **142** 114–120

***Hillier SG and Tetsuka M** (1998) An anti-inflammatory role for glucocorticoids in the ovaries? *Journal of Reproductive Immunology* **39** 21–27

Ho CK, Tetsuka M and Hillier SG (1999) Regulation of 11β-hydroxysteroid dehydrogenase isoforms and glucocorticoid receptor gene expression in the rat uterus *Journal of Endocrinology* **163** 425–431

Hsueh AJ and Erickson GF (1978) Glucocorticoid inhibition of FSH-induced estrogen production in cultured rat granulosa cells *Steroids* **32** 639–648

Hundertmark S, Buhler H, Rudolf M, Weitzel HK and Ragosch V (1997) Inhibition of 11β-hydroxysteroid dehydrogenase activity enhances the antiproliferative effect of glucocorticosteroids on MCF-7 and ZR-75-1 breast cancer cells *Journal of Endocrinology* **155** 171–180

Jamieson A, Wallace AM, Andrew R, Nunez BS, Walker BR, Fraser R, White PC and Connell JMC (1999) Apparent cortisone reductase deficiency: a functional defect in 11β-hydroxysteroid dehydrogenase type 1 *Journal of Clinical Endocrinology and Metabolism* **84** 3570–3574

Jimena P, Castilla JA, Peran F, Ramirez JP, Vergara F, Jr, Molina R, Vergara F and Herruzo A (1992) Adrenal hormones in follicular fluid *Acta Endocrinologica (Copenhagen)* **127** 403–406

Keay SD, Harlow CR, Wood PJ, Jenkins JM and Cahill DJ (2002) Higher cortisol:cortisone ratios in the preovulatory follicle of completely unstimulated IVF cycles indicate oocytes with increased pregnancy potential *Human Reproduction* **17** 2410–2414

Kime DE, Scott AP and Canario AV (1992) *In vitro* biosynthesis of steroids, including 11-deoxycortisol and 5α-pregnane-3β,7α,17,20β-tetrol, by ovaries of the goldfish *Carassius auratus* during the stage of oocyte final maturation *General and Comparative Endocrinology* **87** 375–384

Klemcke HG and Christenson RK (1996) Porcine placental 11β-hydroxysteroid dehydrogenase activity *Biology of Reproduction* **55** 217–223

Krozowski ZS and Funder JW (1983) Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity *Proceedings National Academy of Sciences USA* **80** 6056–6060

Lakshmi V and Monder C (1988) Purification and characterization of the corticosteroid 11β-dehydrogenase component of the rat liver 11β-hydroxysteroid dehydrogenase complex *Endocrinology* **123** 2390–2398

Lakshmi V, Nath N and Muneyyirci-Delale O (1993) Characterization of 11β-hydroxysteroid dehydrogenase of human placenta: evidence for the existence of two species of 11β-hydroxysteroid dehydrogenase *Journal of Steroid Biochemistry and Molecular Biology* **45** 391–397

Leckie CM, Welberg LAM and Seckl JR (1998) 11β-hydroxysteroid dehydrogenase is a predominant reductase in intact Leydig cells *Journal of Endocrinology* **159** 233–238

Lesage J, Blondeau B, Grino M, Breant B and Dupouy JP (2001) Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo–pituitary adrenal axis in the newborn rat *Endocrinology* **142** 1692–1702

Lopez-Bernal A, Flint APF, Anderson ABM and Turnbull AC (1980) 11β-hydroxysteroid dehydrogenase activity in human placenta and deciduas *Journal of Steroid Biochemistry* **13** 1081–1087

Low SC, Chapman KE, Edwards CRW, Wells T, Robinson ICAF and Seckl JR (1994) Sexual dimorphism of hepatic 11β-hydroxysteroid dehydrogenase in the rat: the role of growth hormone patterns *Journal of Endocrinology* **143** 541–548

McKenna TJ, Lorber D, Lacroix A and Rabin D (1979) Testicular activity in Cushing's disease *Acta Endocrinologica (Copenhagen)* **91** 501–510

McTernan CL, Draper N, Nicholson H, Chalder SM, Driver P, Hewison M, Kilby MD and Stewart PM (2001) Reduced placental 11βHSD2 mRNA levels in human pregnancies complicated by IUGR: analysis of possible mechanisms *Journal of Clinical Endocrinology and Metabolism* **89** 4979–4983

Mercer WR and Krozowski ZS (1992) Localization of an 11β-hydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney *Endocrinology* **130** 540–543

Michael AE, Pester LA, Curtis P, Shaw RW, Edwards CRW and Cooke BA (1993) Direct inhibition of ovarian steroidogenesis by cortisol and the modulatory role of 11β-hydroxysteroid dehydrogenase *Clinical Endocrinology* **38** 641–644

***Michael AE, Gregory L, Piercy EC, Walker SM, Shaw RW and Cooke BA** (1995) Ovarian 11β-hydroxysteroid dehydrogenase activity is inversely related to the outcome of *in vitro* fertilization–embryo transfer treatment cycles *Fertility and Sterility* **64** 590–598

***Michael AE, Evagelatou M, Norgate DP *et al*.** (1997) Isoforms of 11β-hydroxysteroid dehydrogenase in human granulosa–lutein cells *Molecular and Cellular Endocrinology* **132** 43–52

Michael AE, Collins TD, Norgate DP, Gregory L, Wood PJ and Cooke BA (1999) Relationship between ovarian cortisol:cortisone ratios and the clinical outcome of *in vitro* fertilization and embryo transfer (IVF–ET) *Clinical Endocrinology* **51** 535–540

Monder C, Stewart PM, Lakshmi V, Valentino R, Burt D and Edwards CRW (1989) Liquorice inhibits corticosteroid 11β-dehydrogenase of rat kidney and liver: *in vivo* and *in vitro* studies *Endocrinology* **125** 1046–1053

***Monder C, Miroff Y, Marandici A and Hardy MP** (1994) 11β-hydroxysteroid dehydrogenase alleviates glucocorticoid-mediated inhibition of steroidogenesis in rat Leydig cells *Endocrinology* **134** 1199–1204

Moore JS, Monson JP, Kaltsas G, Putignano P, Wood PJ, Sheppard MC, Besser GM, Taylor NF and Stewart PM (1999) Modulation of 11β-hydroxysteroid dehydrogenase isozymes by growth hormone and insulin-like growth factor: *in vivo* and *in vitro* studies *Journal of Clinical Endocrinology and Metabolism* **84** 4172–4177

Morris DJ, Semafuko WEB, Latif SA, Vogel B, Grimes CA and Sheff MF (1992) Detection of glycyrrhetinic acid-like factors (GALFs) in human urine *Hypertension* **20** 356–360

Mune T, Rogerson FM, Nikkila H, Agarwal AK and White PC (1995) Human hypertension caused by mutations in the kidney isozyme of 11β-hydroxysteroid dehydrogenase *Nature Genetics* **10** 394–399

Murdoch WJ, Wilken C and Young DA (1999) Sequence of apoptosis and inflammatory necrosis within the formative ovulatory site of sheep follicles *Journal of Reproduction and Fertility* **17** 325–329

***Murphy BEP** (1981) Ontogeny of cortisol–cortisone interconversion in human tissues: a role for cortisone in human fetal development *Journal of Steroid Biochemistry* **14** 811–817

Murphy BEP, Clark SD, Donald IR, Pinsky M and Vedady DL (1974) Conversion of maternal cortisol to cortisone during placental transfer to the human fetus *American Journal of Obstetrics and Gynaecology* **118** 538–541

***Nacharaju VL, Muneyyirci-Delale O and Khan N** (1997) Presence of 11β-hydroxysteroid dehydrogenase in human semen: evidence of correlation with semen characteristics *Steroids* **62** 311–314

Neumann A, Haider SG and Hilscher B (1993) Temporal coincidence of the appearance of elongated spermatids and of histochemical reaction of 11β-hydroxysteroid dehydrogenase in rat Leydig cells *Andrologia* **25** 263–269

Nikkila H, Tannin GM, New MI, Taylor NF, Kalaitzoglou G, Monder C and White PC (1993) Defects in the HSD11 gene encoding 11β-hydroxysteroid dehydrogenase are not found in patients with apparent mineralocorticoid excess or 11-oxoreductase deficiency *Journal of Clinical Endocrinology and Metabolism* **77** 687–691

Ono M and Oka T (1980) The differential actions of cortisol on the accumulation of α-lactalbumin and casein in midpregnant mouse mammary gland in culture *Cell* **19** 473–480

Osinski PA (1960) Steroid 11β-ol dehydrogenase in human placenta *Nature* **187** 777

***Patel FA, Sun K and Challis JRG** (1999) Local modulation by 11β-hydroxysteroid dehydrogenase of glucocorticoid effects on the activity of 15-hydroxyprostaglandin dehydrogenase in human chorion and placental trophoblast cells *Journal of Clinical Endocrinology and Metabolism* **84** 395–400

Penning TM (1997) Molecular endocrinology of hydroxysteroid dehydrogenases *Endocrine Reviews* **18** 281–305

Pepe GJ, Waddell BJ, Stahl SJ and Albrecht ED (1988) The regulation of transplacental cortisol–cortisone metabolism by oestrogen in pregnant baboons *Endocrinology* **122** 78–83

Pepe GJ, Babischkin JS, Burch MMG, Leavitt MG and Albrecht ED (1996) Developmental increase in expression of the messenger ribonucleic acid and protein levels of 11β-hydroxysteroid dehydrogenase types 1 and 2 in the baboon placenta *Endocrinology* **137** 5678–5684

Petrino TR, Lin YW, Netherton JC, Powell DH and Wallace RA (1993) Steroidogenesis in *Fundulus heteroclitus* V: purification, characterization, and metabolism of 17α,20β,-dihydroxy-4-pregnen-3-one by intact follicles and its role in oocyte maturation *General and Comparative Endocrinology* **92** 1–15

Phillipou G and Higgins BA (1985) A new defect in the peripheral conversion of cortisone to cortisol *Journal of Steroid Biochemistry* **22** 435–436

Phillips DM, Lakshmi V and Monder C (1989) Corticosteroid 11β-dehydrogenase in rat testis *Endocrinology* **125** 209–216

Phillips ML and Schultz BD (2002) Steroids modulate transepithelial resistance and Na^+ absorption across cultured porcine vas deferens epithelia *Biology of Reproduction* **66** 1016–1023

Pinter J and Thomas P (1999) Induction of ovulation of mature oocytes by the maturation-inducing steroid 17,20β,21-trihydroxy-4-pregnen-3-one in the spotted seatrout *General and Comparative Endocrinology* **115** 200–209

Pushkin A, Clark I, Kwon TH, Nielsen S and Kurtz I (2000) Immunolocalization of NBC3 and NHE3 in the rat epididymis: colocalization of NBC3 and the vacuolar H^+-ATPase *Journal of Andrology* **21** 708–720

Quirk SJ, Slattery JA and Funder JW (1990) Epithelial and adipose cells isolated from mammary glands of pregnant and lactating rats differ in 11β-hydroxysteroid dehydrogenase activity *Journal of Steroid Biochemistry and Molecular Biology* **37** 529–534

Rae MT, Niven D, Critchley HOD and Hillier SG (2003) Anti-inflammatory actions of cortisol on human ovarian surface epithelia (HOSE) cells *Society for Gynecologic Investigation 50th Annual Scientific Meeting* Abstract 674

Rastogi RK, Saxena PK and Iela L (1976) Histochemical localization of some hydroxysteroid dehydrogenases in the mouse epididymis *Hormone Research* **7** 43–48

Ricketts ML, Verhaeg JM, Bujalska I, Howie AJ, Rainey WE and Stewart PM (1998) Immunohistochemical localisation of type 1 11β-hydroxysteroid dehydrogenase in human tissues *Journal of Clinical Endocrinology and Metabolism* **83** 1325–1335

Sampath-Kumar R, Matthews SG and Yang K (1998) 11β-hydroxysteroid dehydrogenase type 2 is the predominant isozyme in the guinea pig placenta: decreases in messenger ribonucleic acid and activity at term *Biology of Reproduction* **59** 1378–1384

Sarkar S, Tsai S-W, Nguyen TT, Plevyak M, Padbury JF and Rubin LP (2001) Inhibition of placental 11β-hydroxysteroid dehydrogenase type 2 by catecholamines via α-adrenergic signaling *American Journal of Physiology (Regulatory and Integrative Comparative Physiology)* **281** R1966–R1974

Schafers BA, Schlutius BG and Haider SG (2001) Ontogenesis of oxidative reaction of 17β-hydroxysteroid dehydrogenase and 11β-hydroxysteroid dehydrogenase in rat Leydig cells, a histochemical study *Histochemical Journal* **33** 585–595

Seckl JR and Walker BR (2001) 11β-hydroxysteroid dehydrogenase type 1 – a tissue-specific amplifier of glucocorticoid action *Endocrinology* **142** 1371–1376

Shams M, Kilby MD, Somerset DA, Howie AJ, Gupta A, Wood PJ, Afnan M and Stewart PM (1998) 11β-hydroxysteroid dehydrogenase type 2 in human pregnancy and reduced expression in intrauterine growth restriction *Human Reproduction* **13** 799–804

Smith MP, Mathur RS, Keay SD, Hall L, Hull MGR and Jenkins JM (2000) Periovulatory human oocytes, cumulus cells, and ovarian leukocytes express type 1 but not type 2 11β-hydroxysteroid dehydrogenase RNA *Fertility and Sterility* **73** 825–830

Smith RE, Salamonsen LA, Komesaroff PA, Li KXZ, Myles KM, Lawrence M and Krozowski Z (1997) 11β-hydroxysteroid dehydrogenase type II in the human endometrium: localization and activity during the menstrual cycle *Journal of Clinical Endocrinology and Metabolism* **82** 4252–4257

Stewart PM, Wallace AM, Valentino R, Burt D, Shackleton CH and Edwards CRW (1987) Mineralocorticoid activity of liquorice: 11β-hydroxysteroid dehydrogenase deficiency comes of age *Lancet* **2** 821–824

Stewart PM, Corrie JET, Shackleton CHL and Edwards CRW (1988) Syndrome of apparent mineralocorticoid excess. A defect in the cortisol-cortisone shuttle *Journal of Clinical Investigation* **82** 340–349

Stewart PM, Murry BA and Mason JI (1994) Type 2 11β-hydroxysteroid dehydrogenase in human fetal tissues *Journal of Clinical Endocrinology and Metabolism* **78** 1529–1532

Sun K, Yang K and Challis JRG (1997a) Differential expression of 11β-hydroxysteroid dehydrogenase types 1 and 2 in human placenta and fetal membranes *Journal of Clinical Endocrinology and Metabolism* **82** 300–305

Sun K, Yang K and Challis JRG (1997b) Differential regulation of 11β-hydroxysteroid dehydrogenase type 1 and 2 by nitric oxide in cultured human placental trophoblast and chorionic cell preparation *Endocrinology* **138** 4912–4920

***Sun K, Yang K and Challis JRG** (1998) Regulation of 11β-hydroxysteroid dehydrogenase type 2 by progesterone, estrogen, and the cyclic adenosine 5′-monophosphate pathway in cultured human placental and chorionic trophoblasts *Biology of Reproduction* **58** 1379–1384

Sun K, He P and Yang K (2002) Intracrine induction of 11β-hydroxysteroid dehydrogenase type 1 expression by glucocorticoid potentiates prostaglandin production in the human chorionic trophoblast *Biology of Reproduction* **67** 1450–1455

Tannin GM, Agarwal AK, Monder C, New MI and White PC (1991) The human gene for 11β-hydroxysteroid dehydrogenase. Structure, tissue distribution and chromosomal localization *Journal of Biological Chemistry* **266** 16 653–16 658

Telleria CM, Ou J, Sugino N, Ferguson S and Gibori G (1998) The expression of interleukin-6 in the pregnant rat corpus luteum and its regulation by progesterone and glucocorticoid *Endocrinology* **139** 3597–3605

Terranova PF and Rice VM (1997) Review: cytokine involvement in ovarian processes *American Journal of Reproductive Immunology* **37** 50–63

Tetsuka M, Thomas FJ, Thomas MJ, Anderson RA, Mason JI and Hillier SG (1997) Differential expression of messenger ribonucleic acids encoding 11β-hydroxysteroid dehydrogenase types 1 and 2 in human granulosa cells *Journal of Clinical Endocrinology and Metabolism* **82** 2006–2009

***Tetsuka M, Milne M, Simpson GE and Hillier SG** (1999a) Expression of 11β-hydroxysteroid dehydrogenase, glucocorticoid receptor, and mineralocorticoid receptor genes in rat ovary *Biology of Reproduction* **60** 330–335

Tetsuka M, Haines LC, Milne M, Simpson GE and Hillier SG (1999b) Regulation of 11β-hydroxysteroid dehydrogenase type 1 gene expression by LH and interleukin-1β in cultured rat granulosa cells *Journal of Endocrinology* **163** 417–423

Tetsuka M, Yamamoto S, Hayashida N, Hayashi KG, Hayashi M, Scosta TJ and Miyamoto A (2003) Expression of 11β-hydroxysteroid dehydrogenase in bovine follicle and corpus luteum *Journal of Endocrinology* **177** 445–452

Thomas FJ, Thomas MJ, Tetsuka M, Mason JI and Hillier SG (1998) Corticosteroid metabolism in human granulosa–lutein cells *Clinical Endocrinology* **48** 509–513

Thompson A, Han VKM and Yang K (2002) Spatial and temporal patterns of expression of 11β-hydroxysteroid dehydrogenase types 1 and 2 messenger RNA and glucocorticoid receptor protein in the murine placenta and uterus during late pregnancy *Biology of Reproduction* **67** 1708–1718

***Thurston LM, Norgate DP, Jonas KC, Chandras C, Kloosterboer HJ, Cooke BA and Michael AE** (2002) Ovarian modulators of 11β-hydroxysteroid dehydrogenase (11βHSD) in follicular fluid from gonadotrophin-stimulated assisted conception cycles *Reproduction* **124** 801–812

Thurston LM, Fowkes RC, Burrin JM and Michael AE (2003a) 11β-hydroxysteroid dehydrogenase (11βHSD) activities in αT3-1 and LβT2 gonadotroph cell lines: acute effects of GnRH and cAMP *Reproduction Abstract Series* **30** Abstract O8

Thurston LM, Michael AE, Jonas KC and Abayasekara DRE (2003b) 11β-hydroxysteroid dehydrogenase (11βHSD) expression and activities in the developing ovarian follicle at defined phases of the bovine oestrus cycle *Reproduction Abstract Series* **30** Abstract O7

Thurston LM, Chin E, Jonas KC, Abayasekara DRE and Michael AE (2003c) Expression of 11β-hydroxysteroid dehydrogenase (11βHSD) proteins in

luteinizing human granulosa–lutein cells *Journal of Endocrinology* **178** 127–135

***Thurston LM, Norgate DP, Jonas KC, Gregory L, Wood PJ, Cooke BA and Michael AE** (2003d) Ovarian modulators of type 1 11β-hydroxysteroid dehydrogenase (11βHSD) activity and intra-follicular cortisol:cortisone ratios correlate with the clinical outcome of *in vitro* fertilization–embryo transfer (IVF–ET) *Human Reproduction* **18** 1603–1612

Thurston LM, Jonas KC, Abayasekara DRE and Michael AE (2003e) Ovarian modulators of 11β-hydroxysteroid dehydrogenase (11βHSD) activity in follicular fluid from bovine and porcine large antral follicles and spontaneous ovarian cysts *Biology of Reproduction* **68** 2517–2163

Ulick S, Levine LS, Gunczler P, Zanconato G, Ramirez LC, Rauh W, Rosler A, Bradlow HL and New MI (1979) A syndrome of apparent mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol *Journal of Clinical Endocrinology and Metabolism* **49** 757–764

Waddell BJ and Burton PJ (2000) Full induction of rat myometrial 11β-hydroxysteroid dehydrogenase type 1 in late pregnancy is dependent on intrauterine occupancy *Biology of Reproduction* **62** 1005–1009

Waddell BJ, Benediktsson R and Seckl JR (1996) 11β-hydroxysteroid dehydrogenase type 2 in the rat corpus luteum: induction of messenger ribonucleic acid expression and bioactivity coincident with luteal regression *Endocrinology* **137** 5386–5391

***Whittle WL, Patel FA, Alfaidy N, Holloway AC, Fraser M, Gyomorey S, Lye SJ, Gibb W and Challis JRG** (2001) Glucocorticoid regulation of human and ovine parturition: the relationship between fetal hypothalamic–pituitary–adrenal axis activation and intrauterine prostaglandin production *Biology of Reproduction* **64** 1019–1034

Whorwood CB, Franklyn JA, Sheppard MC and Stewart PM (1992) Tissue localization of 11β-hydroxysteroid dehydrogenase and its relationship to the glucocorticoid receptor *Journal of Steroid Biochemistry and Molecular Biology* **41** 21–28

Wu W-X, Derks JB and Nathanielsz PW (1996) Effects of glucocorticoids on estrogen receptor messenger ribonucleic acid in the pregnant ovine myometrium *in vivo* and *in vitro*. *Biology of Reproduction* **54** 230–241

Yang K (1995) Co-expression of two distinct isoforms of 11β-hydroxysteroid dehydrogenase in the ovine placenta *Journal of Steroid Biochemistry and Molecular Biology* **52** 337–343

Yang K (1997) Placental 11β-hydroxysteroid dehydrogenase: barrier to maternal glucocorticoid *Reviews of Reproduction* **2** 129–132

Yang J-G, Chen W-Y and Li PS (1999) Effects of glucocorticoids on maturation of pig oocytes and their subsequent fertilizing capacity *in vitro*. *Biology of Reproduction* **60** 929–936

Yang K, Fraser M, Yu M, Krkosek M, Challis JRG, Lamming GE, Campbell LE and Darnel A (1996) Pattern of 11β-hydroxysteroid dehydrogenase type 1 messenger ribonucleic acid expression in the ovine uterus during the estrous cycle and pregnancy *Biology of Reproduction* **55** 1231–1236

***Yong PYK, Thing KJ, Andrew R, Walker BR and Hillier SG** (2000) Development-related increase in cortisol biosynthesis by human granulosa cells *Journal of Clinical Endocrinology and Metabolism* **85** 4728–4733

Yong PYK, Harlow C, Thong KJ and Hillier SG (2002) Regulation of 11β-hydroxysteroid dehydrogenase type 1 gene expression in human ovarian surface epithelial cells by interleukin-1 *Human Reproduction* **17** 2300–2306