Trends in Endocrinology & Metabolism

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Feature Review

Glucocorticoids and Reproduction: Traffic Control on the Road to Reproduction

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Glucocorticoids are steroid hormones that regulate diverse cellular functions and are essential to facilitate normal physiology. However, stress-induced levels of glucocorticoids result in several pathologies including profound reproductive dysfunction. Compelling new evidence indicates that glucocorticoids are crucial to the establishment and maintenance of reproductive function. The fertility-promoting or -inhibiting activity of glucocorticoids depends on timing, dose, and glucocorticoid responsiveness within a given tissue, which is mediated by the glucocorticoid receptor (GR). The GR gene and protein are subject to cellular processing, contributing to signaling diversity and providing a mechanism by which both physiological and stress-induced levels of glucocorticoids function in a cell-specific manner. Understanding how glucocorticoids regulate fertility and infertility may lead to novel approaches to the regulation of reproductive function.

Trends

Glucocorticoids exhibit both central and peripheral regulation of the reproductive axis.

Within the reproductive organs, basal glucocorticoids contribute to reproductive function while stress-induced levels induce suppression of reproductive function.

Transgenic animal models have aided in the discovery of the tissue-specific functions of glucocorticoids. However, studies in reproductive tissues are needed.

Immune cells play an active role in male and female fertility, and administration of glucocorticoids or stress may disrupt the immune system resulting in reproductive dysfunction.

Introduction

Glucocorticoids are essential for stress adaptation through the regulation of metabolic activity, behavior, and reproduction, in which trade-offs are made between immediate survival and future offspring. Stress-induced levels of glucocorticoids mediate this process through direct and indirect actions on the hypothalamic–pituitary–gonadal (HPG) axis. Interestingly, the presenting complaint of the first **Cushing's syndrome** (see Glossary) patient in 1912 was secondary amenorrhea. However, the molecular mechanisms by which high levels of glucocorticoids suppress fertility are complex and remain to be fully discovered. Moreover, basal glucocorticoid levels are critically important for the establishment and maintenance of fertility, as evidenced in patients with **Addison's disease** who present with premature ovarian failure and oligospermia [1,2]. Our current understanding of glucocorticoid signaling in the context of reproductive physiology is limited. Notably, a balance between low and high levels of glucocorticoids differentiates between fertility and infertility and elucidating the molecular mechanisms governing this shift is a potential new direction for future research.

Circulating levels of glucocorticoids are a function of the activation status of the HPA axis (Box 1). The biological effects of glucocorticoids are determined by serum concentrations in concert with relative expression of the **11β-hydroxysteroid dehydrogenase (11β-HSD) isoenzymes** and the GR (gene: *NR3C1*), a ligand-dependent transcription factor (Box 2). The transcriptional activity of the GR demonstrates profound diversity, stimulating or suppressing the expression of 10–20% of all genes in the human genome (Box 3) [3]. Identifying which genes are targets of GR regulation in reproductive tissues will provide a basis for understanding the mechanisms by which glucocorticoids regulate reproduction and a framework for the complex regulatory networks. Establishing which signaling networks are associated with stress-induced

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Box 1. Glucocorticoid Production and Bioavailability

Glucocorticoids are steroid hormones synthesized and released by the adrenal cortex under the regulation of the HPA axis. The production of glucocorticoids by the HPA axis is pulsatile, displaying both circadian and ultradian rhythms. Corticotrophin-releasing hormone (CRH) and arginine vasopressin are secreted from the parvicellular neurons of the hypothalamus into the pituitary portal circulation, which stimulates adrenocorticotropic hormone (ACTH) release from the anterior pituitary gland. ACTH acts on the adrenal gland to induce steroidogenesis and the production of glucocorticoids. Endocrine feedback loops exist where glucocorticoids inhibit CRH expression and secretion and ACTH output [119–121]. The rhythmic secretion of glucocorticoids is critical to the maintenance of physiological homeostasis, but acute exposures to stress transiently induce HPA activity and glucocorticoid release.

Approximately 95% of secreted glucocorticoids circulate bound to cortisol-binding globulin (CBG) or albumin and, therefore, expression of these binding proteins determines the level of biologically free glucocorticoids [122,123]. The circulating half-life of glucocorticoids is variable (66–120 min) but glucocorticoids bound to CBG have a longer half-life than unbound glucocorticoids [124,125]. Unbound glucocorticoids are highly lipophilic and able to cross the plasma membrane through simple diffusion. In cells that express the multidrug-resistance p-glycoprotein, entry of glucocorticoids into the intracellular compartment can be restricted by active transport out of the cell [126]. Within the cell interconversion of glucocorticoids to the active (cortisol and corticosterone) or inactive (cortisone and 11-dehydrocorticosterone) forms by 11β-HSD I and II controls ligand availability to the GR [127]. Collectively, the biological activity of glucocorticoids is a balance between synthesis and secretion, diffusion and transport, and metabolism and clearance.

infertility will ultimately provide new avenues for therapy. Here we review recent findings on glucocorticoid action in the reproductive system, summarizing historical data and highlighting new insights where research is ongoing.

Glucocorticoid Actions in the Hypothalamus and Pituitary: Recent Developments

The hypothalamus and pituitary preside over reproductive physiology by acting as a central terminal for the input and output of endocrine signals [4]. Gonadotropin-releasing hormone (GnRH) is synthesized and secreted by the hypothalamus and then crosses through the hypophyseal portal system into the anterior pituitary. In the pituitary GnRH induces the synthesis and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the gonadotropes, which trigger testosterone release from the testis and estradiol and progesterone release from the ovaries. A feedback loop exists by which testosterone inhibits the secretion of GnRH and therefore FSH and LH production. In females, ovarian estradiol also exerts negative feedback on the hypothalamus and pituitary. However, during the late follicular phase of the menstrual cycle rising levels of estradiol from the follicle trigger a switch from negative to positive feedback on the hypothalamus resulting in increased GnRH secretion. During the luteal phase of the menstrual cycle, rising levels of progesterone negatively feed back to suppress activity in the hypothalamus and pituitary. At the late luteal phase, the levels of estradiol and progesterone decline, allowing gonadotrophins to again rise and triggering the beginning of a new cycle.

Increased glucocorticoid exposure, either by stress or by exogenous treatment, leads to profound reproductive dysfunction through well-described effects in the hypothalamus and pituitary [5–7]. Glucocorticoids modulate the HPG axis by directly inhibiting the release of GnRH from the hypothalamus and the synthesis and release of gonadotropins from the pituitary. However, the signaling pathways by which this occurs remain under investigation and are further complicated by the recent discovery of new neuropeptides such as kisspeptin (KISS1) and gonadotropin-inhibitory hormone (GnIH). These neuropeptides have opposing effects on GnRH release from the hypothalamus and are responsive to high levels of glucocorticoids. KISS1 exerts stimulatory effects on GnRH secretion through its cognate receptor KISS-1R [also known as G protein-coupled receptor 54 (GPR54)], which is coexpressed in GnRH neurons. KISS1 neurons in the anteroventral periventricular nucleus and periventricular nucleus continuum of the preoptic area of the hypothalamus express GR, suggesting that glucocorticoids can

Glossary

11β-Hydroxysteroid dehydrogenase (11β-HSD) isoenzymes: enzymes that catalyze the interconversion of active glucocorticoids (11-oxoreductase activity) and their inactive metabolites (11β-dehydrogenase activity). The type I isoenzyme demonstrates both 11β-dehydrogenase and 11-oxoreductase activities while the type II isoenzyme has only 11β-dehydrogenase activity.

Addison's disease: severe or total deficiency of the hormones made in the adrenal cortex; also known as primary adrenal insufficiency or hypocortisolism.

Adrenalectomy: surgical removal of the adrenal glands (bi- or unilateral).

Bishop score: a prelabor cervix score used to predict the response to labor induction or chance of spontaneous labor. A low Bishop score is associated with a greater probability that induction of labor will fail.

Blood–testis barrier (BTB): a physical barrier within the seminiferous tubules formed by tight junctions, adherens junctions, and desmosome junctions between adjacent Sertoli cells; forms an immunological barrier that segregates the basal and adluminal compartments.

Chromatin immunoprecipitation sequencing (ChIP-seq): a method to identify genome-wide DNA-binding sites for proteins. Proteins crosslinked to chromatin are isolated by antibody immunoprecipitation. Enriched DNA-binding sites are sequenced and mapped to a reference genome. Sequencing identifies the location and quantity of sites bound by a protein.

Cre–lox technology: system for the creation of tissue-specific or inducible knockout mouse models by catalyzing the recombination of two loxP recognition (floxed) sites introduced into the gene of interest with tissue-specific/inducible expression of the Cre enzyme.

Cushing's syndrome: a disorder resulting from prolonged exposure to elevated levels of the endogenous glucocorticoid cortisol; often results from exogenous glucocorticoid use, but can also be caused by pituitary adenomas and adrenal tumors.

Developmental origins of health and disease (DOHaD) hypothesis:

Box 2. The GR: Structure and Regulation

The molecular response to glucocorticoids is mediated by intracellular GR. The GR is a member of the nuclear receptor superfamily of ligand-dependent transcription factors [128]. All members of this superfamily share a similar domain structure: an N-terminal transactivation (AF1) domain, a central DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD). In addition to comprising the ligand-binding pocket, the LBD also contains sequences important for receptor dimerization and nuclear localization and a second transactivation domain (AF2) that mediates ligand-dependent interactions with coregulators. The DBD and the LBD are separated by a small, flexible hinge region, which plays a role in ligand binding-mediated conformational changes.

The human GR is the product of one gene (*NR3C1*) comprising nine exons. Exon 1 forms the 5′ untranslated region of the human GR and exon 2 encodes the NTD, which is poorly conserved and therefore the most variable domain among nuclear receptors. The DBD, the most conserved region in the nuclear receptor family, comprises exons 3 and 4 and exons 5–9 encode the hinge region and LBD. Alternative splicing of the *NR3C1* gene primarily from the C-terminal end produces the human GRα, GRβ, GRγ, GR-A, and GR-P transcriptional isoforms [129–132]. Research has mainly focused on glucocorticoid signaling through GRα and GRβ, which are identical proteins through amino acid 727 that diverge in C-terminal exon 9. GRβ lacks sequences that encode helices 11 and 12 of the LBD and is therefore unable to bind glucocorticoids [133]. GRβ was thought to function primarily as a dominant-negative regulator of GRα, but genome-wide transcriptional analysis of overexpressed human GRβ indicates that it maintains inherent transcriptional activity [130,134,135]. The sequence of GRγ differs from that of GRα by the inclusion of an arginine residue between exons 3 and 4 of the DBD from the use of an alternative intronic splice donor site [132]. This single amino acid insertion demonstrates the functional importance of this region, as the transcriptional activity of GRγ is significantly reduced [136,137]. Interestingly, differences in the DBD of GRγ alter the DNA-binding sequence specificity and result in a unique transcriptome [138,139]. The GR-A and GR-P isoforms contain incomplete LBDs and relatively little is known about their functions *in vivo* [131,140]. Each GR transcriptional splice variant is capable of generating additional isoforms through alternative translation initiation mechanisms [141]. In exon 2 eight highly conserved AUG start codons generate the GR-A, -B, -C1, -C2, -C3, -D1, -D2, and -D3 isoforms. These translational isoforms have distinct tissue distribution and subcellular localization, regulate unique genes, and differentially regulate the cellular response to glucocorticoids [142–144].

act directly on these neurons [8]. In mice corticosterone treatment diminished hypothalamic expression of KISS1 during the estradiol-induced LH surge and decreased the activation of KISS1 neurons [9]. Impairment of KISS1 neurons represents a newly discovered mechanism by which glucocorticoids are able to suppress the HPG axis. GnIH [mammalian ortholog: RFamide-related peptide-3 (RFRP3)] neurons inhibit the activity of GnRH neurons and KISS1 neurons [10]. Acute (audiovisual imposition of a predator, hypoglycemia, or lipopolysaccharide challenge) and chronic (synthetically induced plasma cortisol levels) stress in sheep increase the function of GnIH neurons and increased contacts with GnRH neurons [11]. Immobilization stress-induced upregulation of GnIH led to inhibition of LH release from the pituitary [12]. **Adrenalectomy** blocked the induction of GnIH, suggesting that glucocorticoids released in response to stress mediated this effect. *NR3C1* is coexpressed in GnIH neurons and corticosterone treatment in quail and a rat hypothalamic neuronal cell line increased *GnIH* expression through **glucocorticoid response elements (GREs)** in the *GnIH* promoter [13]. Regulation of GnRH expression and release by KISS1 and GnIH represents two novel mechanisms by which glucocorticoids indirectly modulate the reproductive functions of the hypothalamus and pituitary.

Glucocorticoids Regulate the Function of Organs in the Reproductive Tract

Ovary

In addition to the effects on ovarian cyclicity mediated through the hypothalamus and pituitary, glucocorticoids impact ovarian physiology through regulating the functions of granulosa cells, oocytes, cumulus cells, and luteal cells [9]. Glucocorticoids differentially induce and repress steroidogenesis in the ovary. In rat preovulatory granulosa cells, dexamethasone increased production of progesterone by increasing the expression of caspase-3 and the steroidogenic proteins steroidogenic acute regulatory protein (StAR) and cytochrome P450 cholesterol side chain-cleavage enzyme [14]. Glucocorticoids (cortisol and dexamethasone) have also been shown to decrease LH-induced StAR protein levels and progesterone production in rat and

a theory, previously known as the fetal origins hypothesis, based on David Barker's analysis of epidemiological studies in the early 1900s that states that environmental factors encountered by a developmentally plastic organism early in life influence health and disease outcomes throughout adulthood.

Glucocorticoid response element (GRE): a short palindromic sequence of DNA comprising two 6-bp sequences separated by a three-nucleotide spacer that the GR recognizes and binds to directly regulate transcription [5′ RGRACAnnnTGTYCY3′; R = purine (A or G), Y = pyrimidine (C or T), n = any nucleotide].

Inflammasome: a multiprotein intracellular complex that activates caspase enzymes leading to the processing and secretion of proinflammatory cytokines in response to pathogenic microorganisms and sterile stressors.

Negative glucocorticoid response element (nGRE): related DNA sequences bound by the GR distinct from the transrepression activities of glucocorticoids. The nGRE motif tolerates single-base-pair mutations and contains a 0–2-bp spacer between inverted repeated motifs.

Post-translational modification: chemical modifications to a protein, including phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation, and proteolysis, that increase the functional diversity of the proteome. Post-translational modifications can occur at many stages in the life of a protein and can also be reversible.

Single-nucleotide polymorphism (SNP): a DNA sequence variation at a single nucleotide that is appreciable within a population. The variation may be a substitution, deletion, or insertion. SNPs are found within genes, in noncoding regions of DNA, and in intergenic regions. When occurring within a gene, the SNP may lead to an amino acid substitution. SNPs can be associated with traits or diseases or have no known function.

Box 3. GR Signaling

Once in the cytoplasm, glucocorticoids bind the GR resulting in a conformational change, unmasking of nuclear localization signals, and subsequent nuclear translocation of the glucocorticoid–GR complex. Classically, glucocorticoids were thought to act exclusively through the genomic actions of the activated GR. Recent evidence indicates that glucocorticoids can also act through signaling cascades secondary to the conformational change of the glucocorticoid–GR complex or through extranuclear GRs, although the identity of the membrane-associated GR remains unknown [145–147]. In the nucleus the ligand-bound GR mediates the genomic actions of the GR by regulating gene transcription in direct and indirect mechanisms. Direct transcriptional regulation requires binding of GR homodimer complexes to well-defined GREs. However, motif analysis of GR ChIP-seq data sets evaluating GR-bound regions indicates that <42% of binding peaks contain a high-stringency motif match [148]. This suggests that GRE binding is not a requirement for DNA association. Alternatively, the glucocorticoid–GR complex can directly bind distinct **negative GREs (nGREs)** to inhibit gene transcription [149]. GR association with DNA can also occur through mechanisms of indirect gene regulation by the GR that involve interactions with other DNA-bound transcription factors (protein–protein tethering) or through cooperative binding with other transcription factors at neighboring sites (composite regulation). The DNA- or complex-bound GR recruits cofactors that facilitate conversion of the signal into transcriptional activity via RNA polymerases or by regulating paused elongation complexes [150].

The GR is constitutively expressed in almost all cells, but the actions of glucocorticoids demonstrate tissue- and cell type-specific diversity. The glucocorticoid response is determined by many factors including the availability of glucocorticoids and the sensitivity to glucocorticoids in a target tissue. The relative level of the GR and the relative expression of the isoforms can determine the response to glucocorticoids in a given tissue or cell [151]. The GR is subject to various post-translational modifications, which can mediate subcellular trafficking, promoter specificity, cofactor interaction, receptor stability, and turnover [152]. At specific gene loci, the response to glucocorticoids is regulated by the recruitment of cofactors, which can act in various mechanisms including remodeling of chromatin, facilitating the assembly of transcriptional machinery, or modifying histones or other components of the transcription factor complex [153–156]. These coregulators of GR function maintain tissue-specific expression [157,158]. The unique transcriptional profiles of the GR also rely on distal enhancers that loop to the promoter of target genes and open regions of chromatin that are highly cell-type specific [159,160]. Individual glucocorticoid sensitivity has been associated with SNPs in *NR3C1* that alter protein stability, ligand binding affinity, transactivating capacity, and interactions with coactivators or corepressors [161]. Polymorphisms in the genes that encode the 11β-HSD isoenzymes have been identified and may also contribute to glucocorticoid sensitivity [162–164].

human granulosa cells [15,16]. These differences may reflect differences in dose or stage of follicular development. Cortisol treatment or elevated glucocorticoid levels due to restraint stress in mice impairs oocyte development potential when evaluated *ex vivo* [17]. Isolated mural granulosa cells displayed increased apoptosis in response to elevated cortisol, which was associated with higher levels of Fas ligand, and cumulus cells were sensitized to undergo apoptosis following serum-starved culture conditions. Glucocorticoid exposure is also associated with decreased mRNA expression of *NR3C1* and the ovarian growth factors insulin-like growth factor-1 (*IGF-1*) and brain-derived neurotrophic factor (*BDNF*) in mural granulosa cells. Interestingly, culture of *in vitro*-fertilized bovine oocytes increased blastocyst development rates, indicating that the response of oocytes to glucocorticoids may be context dependent [18]. Moreover, what is known regarding the function of endogenous glucocorticoids in ovarian physiology stems largely from exposure studies and may not represent direct functions that occur at physiological levels in the ovary.

Uterus, Placenta, and Labor

Glucocorticoid regulation of uterine biology has historically been described in terms of the antagonistic effects of glucocorticoids on estrogen action and has only recently been explored as an independent regulator of uterine function. Dexamethasone blocks estrogen-induced uterine growth and proliferation in mice, reducing the number of implantation sites when administered before estradiol-induced implantation [19,20]. Gene ontology analysis of dexamethasone- and estradiol-regulated genes in the mouse uterus indicates that the cell cycle and embryonic development are among the top regulated networks [21]. Dexamethasone administration in mice also uniquely regulates the expression of genes whose functions are related to cellular development, growth and proliferation, and signaling. In the neonatal mouse uterus, dexamethasone inhibits epithelial cell proliferation through both GR-dependent and

-independent mechanisms [22]. Decidualized human endometrial cells also display a unique GR-dependent transcriptome enriched for Krüppel-associated box domain-containing zinc-finger proteins, a family of transcriptional repressors [23]. Interestingly, GR signaling is responsible for repressing global expression of trimethylated H3K9 levels during stromal cell differentiation, suggesting that the GR can both directly regulate gene expression and globally alter transcription through regulation of histone modifications in human uterine cells.

The placenta is developmentally plastic and therefore responsive to multiple stimuli including altered glucocorticoid levels. Dexamethasone administered during early placentation inhibits the proliferation, migration, and invasion of trophoblasts, induces placental oxidative damage, and manifests as reduced placental and fetal size [24]. Maternal administration of glucocorticoids reduces placental weight and is associated with reduced expression of proliferative markers and increased expression of apoptotic factors [25,26]. In animal models of maternal prenatal stress, increased glucocorticoid levels are correlated with intrauterine growth restriction. Fetal growth restriction mediated through excess endogenous corticosterone or synthetic dexamethasone exposure results in altered placental transporter function and glucose transport and reduced expression of proangiogenic factors [27,28]. Glucocorticoid exposure at the maternal–fetal interface is in part regulated through expression of the 11β-HSD isoenzymes in discrete maternal and fetal compartments [29]. Dysregulated expression of the 11β-HSD isoenzymes by glucocorticoid excess further potentiates the adverse effects of high levels of glucocorticoid on placental function. Interestingly, expression of the *NR3C1* translational isoforms varies between term and preterm human placenta and with prenatal glucocorticoid exposure, suggesting a mechanism by which changes to the GR protein profile may also mediate the response to increased glucocorticoids [30].

Studies conducted on animals and pregnant woman have indicated the importance of glucocorticoid signaling for the initiation of labor [31,32]. In sheep glucocorticoids drive the shift to an estrogen-primed contractile myometrium through induction of the placental enzyme cytochrome P450 17α-hydoxylase (*P450c17*) [adrenal dehydroepiandrosterone sulfate (*DHEAS*) in humans] [31]. Once the myometrium has achieved an estrogen-primed state, prostaglandins critically stimulate myometrial contractions, induce cervical ripening, and trigger fetal membrane rupture. Glucocorticoids contribute to the synthesis of prostaglandins by stimulating the production of cyclooxygenase-2 (*COX-2*) in human amnion cells and ovine placental trophoblast cells [33,34]. During parturition cortisol also contributes to the rupture of fetal membranes by inducing prostaglandins and inducing apoptosis in the amnion epithelial cells [35]. Intravenous dexamethasone improved the **Bishop score** of the cervix and reduced the time between labor induction and active labor in women. However, synthetic glucocorticoids administered preterm for fetal lung maturation do not induce labor, which may denote differential responses dependent on the extent of myometrial priming.

Fetal Development *in Utero*

It is well appreciated that the *in utero* environment directly contributes to physiological disorders that manifest postpartum [the **developmental origins of health and disease (DOHaD) hypothesis**]. Fetal exposure to high levels of glucocorticoids is believed to be a major contributor to early-life programming of adult-onset disease. Data supporting the role of stress and elevated glucocorticoids in fetal programming have been documented in many species and thoroughly reviewed [36–41]. Interestingly, programming of the HPA axis in response to prenatal stressors demonstrates sex-specific differences [42]. A systematic review of human studies concluded that the placenta of female offspring altered permeability to maternal glucocorticoids through regulation of the 11β-HSD enzymes in response to maternal stress [42]. Maternal exposure to dexamethasone in rats results in sexually dimorphic behavior in offspring associated with structural changes to neuronal populations [43,44]. In mice the cardiovascular and renal renin–angiotensin–aldosterone systems also respond to elevated prenatal corticosterone in a sexually dimorphic manner, potentially through alteration of adrenal function [45–47]. Epigenetic factors may also play a key role in the glucocorticoid-driven effects of fetal programming, including altered methylation status of the GR following neonatal dexamethasone exposure [48]. It is important to recognize the timing of exposure when considering the correlation between animal models and human fetuses. Development in the prenatal and early postnatal rodent is more similar to the first two trimesters of human pregnancy and therefore may not accurately model the response in humans during the third trimester. Synthetic analogs of glucocorticoids are routinely administered during the third trimester to women at risk of early- or late-preterm delivery; therefore, elucidating the long-term effects of late-term glucocorticoid exposure is more accurately evaluated in a human population than a rodent model.

Testis

Both physiological and stress-induced levels of glucocorticoids also mediate testicular function. Adrenalectomy in male rats demonstrated that homeostatic levels of adrenal hormones, including glucocorticoids, are required to maintain steroidogenesis, spermatogenesis, and sperm maturation [49]. Testosterone production increased following adrenalectomy in rats and this was prevented by glucocorticoid replacement [50]. Decreased spermatid number following adrenalectomy was rescued by concurrent dexamethasone treatment, indicating that adrenally produced hormones are responsible for maintaining sperm production. Interestingly, glucocorticoid replacement exacerbated the morphological defects evident in the seminiferous tubules of adrenalectomized males, which may reflect differences between the endogenous and synthetic hormones. Alternatively, other endocrine hormones produced by the adrenal gland, such as mineralocorticoids and epinephrine, could be responsible for the maintenance of testicular morphology, or morphology within the seminiferous epithelium could be sensitive to glucocorticoid dose. High levels of circulating glucocorticoids related to stress, Cushing's disease, or exogenous hormone treatment suppress male fertility through repressing the expression of steroidogenic enzymes, inducing testicular oxidative stress, reducing the testicular response to gonadotropins, and prompting apoptosis of Leydig and germ cells [5]. Elevated glucocorticoids may also control Leydig cell number through the induction of cell cycle arrest, as was demonstrated following dexamethasone treatment in R2C rat Leydig tumor cells *in vitro* [51]. Understanding the physiological range of adrenal steroids that supports male fertility is critical in patients who undergo adrenalectomy or have Addison's disease or Cushing's syndrome.

Mouse Models to Uncover GR Actions

Whole-body GR-null mice exhibit perinatal lethality due to defects in lung maturation and subsequent respiratory failure, which precludes the study of reproductive function in these mice [52]. Investigators have made use of tissue transplants from embryonic GR-null mice into syngeneic hosts, but this method is limited by the types of tissues that can be transplanted and the end points that can be measured. The advent of **Cre–lox technology** allowed researchers to investigate the cell-autonomous versus indirect effects of GR in tissues that critically support reproductive function. However, models to investigate the direct actions of glucocorticoids are limited. Currently in the reproductive system, the tissue- or cell type-specific functions of the GR have been evaluated in the uterus, Sertoli cells, mammary epithelial cells, and prostate epithelial cells [53–56].

Conditional ablation of uterine *NR3C1* demonstrated the direct actions of glucocorticoids in early pregnancy, indicating that glucocorticoid signaling is critical for the establishment of uterine receptivity and the subsequent endometrial remodeling that occurs during decidualization. [53]. The immune response is likely to play a key role in establishing endometrial receptivity [57] and genes related to the inflammatory response and immune cell trafficking were substantially dysregulated in the absence of uterine GR [53]. Thus, the GR can locally coordinate the immune response in early pregnancy, thereby contributing to endometrial receptivity. Moreover, the expression of genes known to contribute to proper implantation was altered when *NR3C1* was conditionally ablated from the uterus, suggesting that uterine glucocorticoid signaling may be necessary for the molecular framework that decides uterine receptivity. Glucocorticoid signaling in the uterus may also be required for the cell-fate decision of stromal cells during decidualization, although the direct gene targets of the GR that regulate cell proliferation or apoptosis in the uterus remain to be determined. Deletion of *NR3C1* in Sertoli cells through the use of anti-Müllerian hormone (*AMH*) Cre produced a profound phenotype where the GR was determined to be required for Sertoli cell maintenance [54]. Although male Sertoli cell-specific *NR3C1*-knockout mice are fertile, numbers of Sertoli cells and stage-specific spermatocytes/spermatids were significantly reduced. The Sertoli cell *NR3C1*-knockout model also revealed that glucocorticoid signaling in Sertoli cells mediates testicular steroidogenesis and critically maintains endocrine feedback pathways. The uterine and Sertoli cell transgenic mouse models have proved that glucocorticoids play a homeostatic role in the regulation of fertility and also provide a direct link between the stress response and fertility.

Before the creation of a tissue-specific model of glucocorticoid action in mammary epithelial cells, researchers utilized mice harboring a deletion in the *NR3C1* gene (GR^{dim}), which was reported to prevent GR transcriptional regulation through direct DNA binding, and tissue-transplantation techniques from GR-deficient mice [58,59]. In the viable GR^{dim} mice, mammary gland development was impaired due to reduced ductal epithelial cell proliferation in virgin mice, although mammary gland differentiation and milk production during pregnancy were normal [59]. The GR^{dim} mutant maintains transcriptional regulation through protein–protein interactions and direct transactivation by the GR^{dim} mutant has been described for some genes, indicating that the mechanisms of GR action in the mammary gland remain poorly understood in this model [60]. Embryonic mammary buds from mice with a disrupted second exon of *NR3C1* were transplanted into the cleared mammary fat pad of 3-week-old recipient mice to circumvent perinatal lethality and study the role of the GR in mammary gland development [52,58]. These transplants displayed abnormal ductal morphogenesis in virgin mice but no defects in development during pregnancy, lactation, or involution. However, the exon 2 hypomorph yields a ligand-responsive truncated GR fragment, which suggests that studies with this model were unable to fully appreciate the functions of the GR [61]. Transgenic mice with mammary epithelial cell-specific deletion of *NR3C1* late in pregnancy exhibited defects in lobuloalveolar development due to reduced cell proliferation, although expression of milk proteins and milk secretion were not affected [55]. Interestingly, none of the *in vivo* models of disrupted glucocorticoid signaling in the mammary gland demonstrated defects in milk protein production compared with previous experiments that determined that glucocorticoids are essential for the transcription of milk protein genes and support milk secretion [62]. The differences in the observed phenotypes may reflect the limits of current transgenic models and may be resolved with targeted deletion of *NR3C1* in other mammary cell types.

The prostate expresses the GR and the direct actions of glucocorticoid signaling in prostate physiology are not well understood. A prostate epithelial cell-specific *NR3C1* knockout was created using *Probasin* Cre to determine the cell-specific role of glucocorticoids in the prostate [56]. Deletion of prostate epithelial cell *NR3C1* demonstrated that glucocorticoid signaling in epithelial cells is not required for prostate development or for the prostate's morphological response to the presence or absence of androgens. This model showed that glucocorticoid-mediated prostate epithelial cell hyperplasia results from paracrine actions of the GR originating in the stroma and not direct signaling within the epithelial cells. These studies suggest that the actions of glucocorticoids vary by prostate cell type, which is likely to be important in interpreting results from human prostate cell lines *in vitro* and understanding the role of the GR in prostate cancer.

Changes in the bioavailability of glucocorticoids have been modeled through the creation of whole-body and tissue-specific 11β-HSD I- and II-knockout mice and rats [63–67]. These models have provided insights into the consequences of intrauterine glucocorticoid excess during pregnancy. In the mouse *11β-HSD II* is expressed in the stromal cells of the endometrium and the labyrinthine zone of the placenta, the primary site of maternal–fetal exchange where maternal blood flows over embryo-derived trophoblasts [68]. Therefore, impaired expression of 11β-HSD II exposes both the placenta and the fetus to locally high levels of glucocorticoids. *11β-HSD II*-null mice weigh less at birth, which was correlated with reduced fetal capillary development accompanied by lower placental expression of the angiogenic factors vascular endothelial growth factor A (*Vegfa*) and peroxisome proliferator-activated receptor gamma (*Pparγ*), decreased expression of glucose transporter 3 (*Slc2a3*; GLUT3), reduced placental transport of glucose to the offspring, and a smaller placenta [69]. The free transfer of maternal glucocorticoids through the placenta in the absence of 11β-HSD II indicates that glucocorticoid overexposure is detrimental to placentation and placental function. The role of physiological levels of cortisol has not been determined.

It is important to note that tissue-specific deletion of *NR3C1* has been accomplished through the use of several unique floxed constructs that excise different exons of *NR3C1*. For instance, exon 3-floxed mice were used to create the Sertoli cell, mammary epithelial cell, and prostate epithelial cell knockout mice, and the uterine knockout model utilized mice floxed for exon 3 and 4 [70,71]. Exon 2-floxed mice have been generated by two groups, although this model has not been utilized to develop tissue-specific knockouts of the GR in the reproductive system [72,73]. Zebrafish have also been utilized to study the actions of the GR. Reported models include the use of morpholinos and a transgenic model harboring a point mutation in the DNA-binding region [74,75]. In both zebrafish models, disruption of GR signaling alters embryo development and adult physiology, although an assessment of fertility was not reported. Zebrafish offer a promising alternative model for the study of glucocorticoid signaling in the reproductive tract [76]. New gene editing techniques will expand our understanding of glucocorticoid signaling through the ability to insert, delete, or replace DNA at precise regions of the genome and will enable researchers to use other experimental animal models (Box 4).

Box 4. Insights from Non-classical Models

In most vertebrates studied, the exon length and amino acid sequence of the *NR3C1* gene is well conserved and the HPA response to a physiological stressor is intact [165]. Therefore, studies describing the impact of stress on reproductive fitness may provide insight into the evolutionarily conserved mechanisms linking fertility to glucocorticoid signaling. Fertility in both male and female cheetahs is sensitive to the physiological stress that accompanies captivity. Living arrangements on-exhibit compared with off-exhibit increased glucocorticoid concentrations, reduced total mobile sperm number in males, and decreased ovarian cyclicity in females [166,167]. Heat stress leads to infertility in dairy cows by inhibiting follicular development. Specifically, heat stress dramatically alters gene expression, reduces estrogen synthesis, and induces apoptosis in the follicle-supporting granulosa cells [168]. In male zebra finches, fasting-increased levels of glucocorticoids were associated with decreased testicular parameters, including lower testosterone and expression of steroidogenic enzymes, in the absence of hypothalamic input, indicating that glucocorticoids integrate the cues from stress directly in the testis in this species [169]. As seen in rodent models, a single exposure to dexamethasone decreased serum testosterone and expression of genes involved in cholesterol synthesis and steroidogenesis in the stallion testis [170]. Dexamethasone exposure in breeder roosters decreased testosterone, sperm motility, and sperm viability [171]. Further evidence for a conserved mechanism of glucocorticoid regulation of steroidogenesis was demonstrated in the three-spot wrasse, *Halichoeres trimaculatus* [172]. In this species prolonged exposure to cortisol induced female-to-male sex change and repression of plasma estradiol. Deciphering the mechanisms by which stress impacts fertility in other species is not only important in understanding the origin of infertility in humans but also essential to alleviate the financial consequences of infertility in agricultural species.

Reproductive Immunology: Immunomodulatory Actions of Glucocorticoids

Endometrial receptivity and successful pregnancy require immune adaptations that precede embryo implantation and allow the semiallogenic fetus to be tolerated (Figure 1A). During the menstrual cycle and increasingly in early pregnancy, immune cells are recruited to and activated in the endometrial compartment in a tightly regulated manner [77–79]. Conditional ablation of *NR3C1* in the mouse uterus demonstrated that glucocorticoid signaling in the endometrium is essential for appropriate immune cell recruitment [53]. Studies utilizing exogenous glucocorticoid administration have also demonstrated effects on immune cell numbers and function in the uterus [57]. Importantly, various immune cells are present in the uterus that work to create the dynamic immune response essential for implantation and tolerance. Disturbances of this immune balance are likely to contribute to the pathogenesis of miscarriage, preeclampsia, or preterm labor. Direct actions on immune cells and indirect actions through signaling networks in the endometrium represent two mechanisms by which glucocorticoids can regulate the uterine immune system. Uterine natural killer (uNK) cells, stemming from resident and recruited populations, are the predominant immune cell population in the uterus at implantation, representing approximately 70% of all leucocytes [80,81]. During early pregnancy uNK cells regulate trophoblast invasion and the vascular changes that support placental development. uNK cells express the GR, and uNK cell-mediated cytotoxicity is sensitive to cortisol treatment [82,83]. Prednisolone treatment in women with recurrent miscarriage is associated with reduced numbers of uNK cells, although a link between the glucocorticoid-mediated reduction in uNK cell numbers and pregnancy success was not demonstrated [84]. Moreover, uNK cells differ in function from circulating NK cells and the specific effects of glucocorticoids on each of these populations have not been investigated.

Infiltrating monocytes that develop into macrophages and uterus-resident myeloid cells constitute the second largest population of immune cells in the uterus during early pregnancy [85].

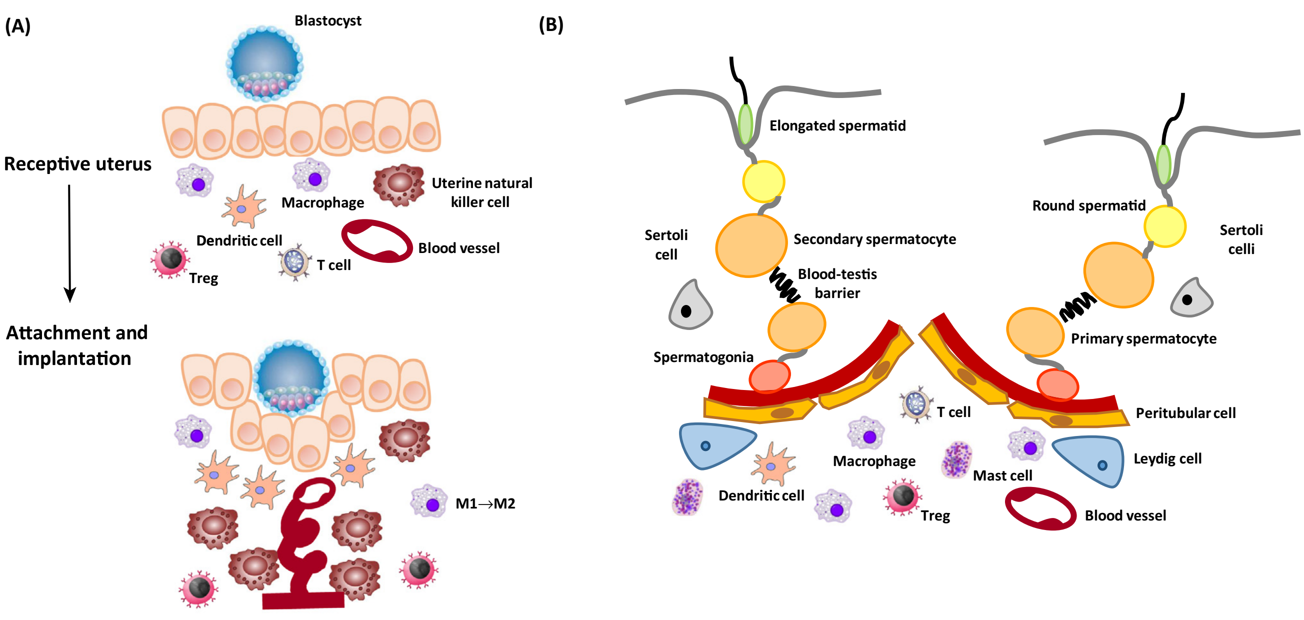


Figure 1. Schematic Illustration of the Mammalian Uterus in Early Pregnancy (A) and the Seminiferous Tubule and Interstitium of the Testis (B). (A) Immune cells are recruited and activated within the endometrium in response to pregnancy. Following implantation immune cells contribute to the endometrial and vascular remodeling that is required to support pregnancy. (B) Under normal physiological conditions, immune cells are present in the interstitial compartment of the testis. Changes in the cell junctions of the blood–testis barrier resulting from inflammation alter the exposure of immunoprivileged cells to cytokines and chemokines, resulting in activation of apoptotic pathways.

Macrophages exist as two distinct subpopulations: an M1 'proinflammatory' phenotype and an M2 phenotype linked to tissue remodeling, immunosuppression, and angiogenesis [86]. Endometrial macrophages are skewed toward the M1 phenotype during the peri-implantation period and polarized toward the M2 phenotype post-implantation [87,88]. Human endometrial macrophages express the GR during the secretory phase, corresponding to the period of uterine receptivity and implantation, the menstrual phase, and at term, although the function of glucocorticoid signaling in macrophages is not well understood [89,90]. Macrophage-specific *NR3C1*-knockout mice have been generated using *LysM* Cre recombinase, although no associated infertility has been reported [91].

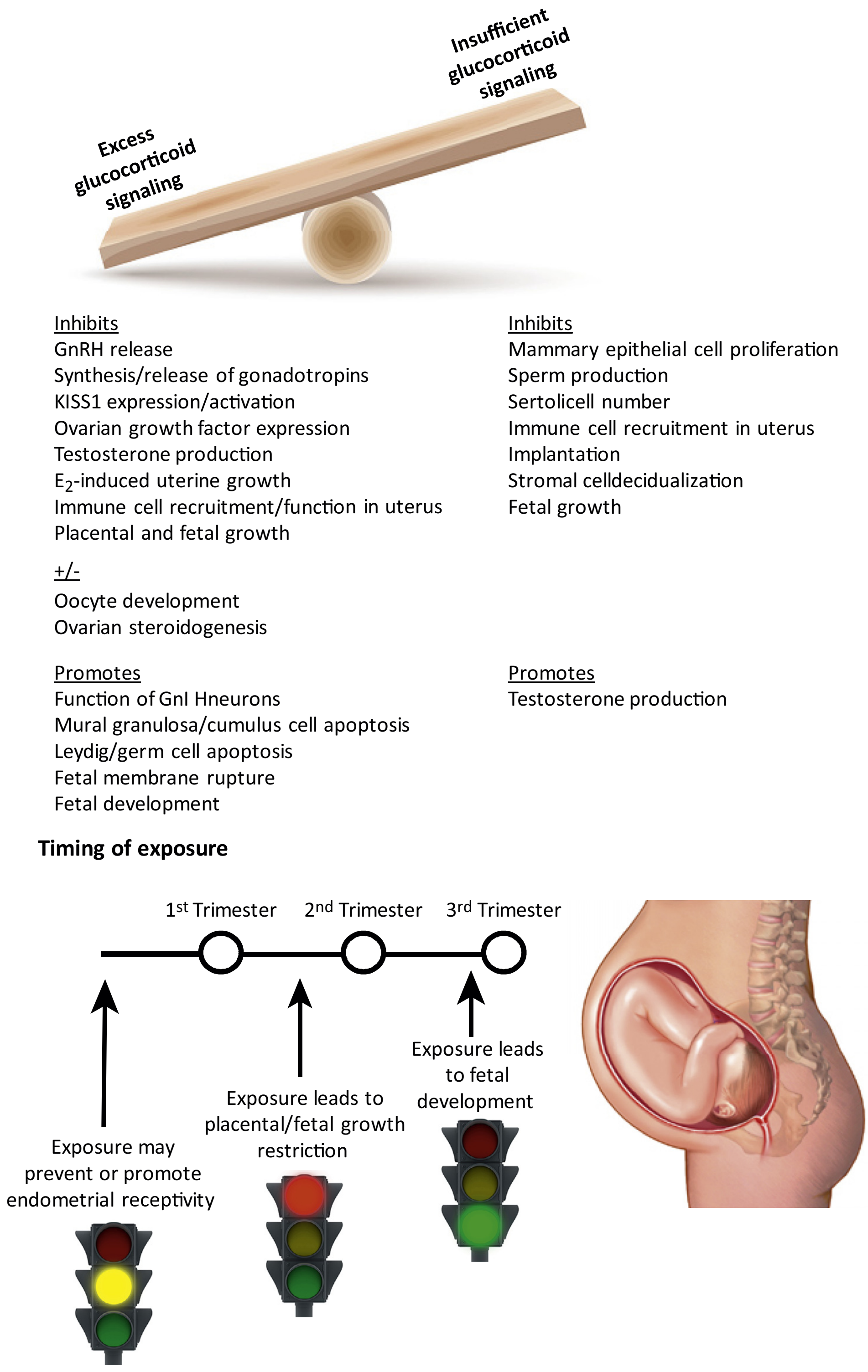
Dendritic cells control the innate adaptive immune response, but mouse studies suggest that they also contribute to uterine receptivity. Selective ablation of $CD11c^{+}$ dendritic cells in the murine uterus caused implantation failure, impaired stromal cell decidualization, and altered angiogenesis, indicating that dendritic cells are important for the initiation of pregnancy [92]. Dendritic cells are antigen-presenting cells and play a key role in determining tolerance to or rejection of the blastocyst. Regulatory T cells are critical for the maintenance of tolerance at the maternal–fetal interface and are generated from $CD4^{+}$ T cells through antigen presentation in the thymus or uterus. Depletion of regulatory T cells in mice leads to pregnancy loss and fetal resorption [93]. The relative abundance of another derivative of the $CD4^{+}$ T cell, Th17 cells, has been associated with miscarriage, although the importance of this immune cell type in normal pregnancy has not been investigated [94]. Moreover, the physiological effects of glucocorticoids on dendritic cells, regulatory T cells, and Th17 cells in the endometrium have not been studied. Dendritic cell-specific *NR3C1*-knockout mice have been utilized in models of sepsis and arthritis but have not been evaluated for functions relating to fertility [95,96]. T cell-specific ablation of *NR3C1* has been reported, although Cre recombinase activity occurs early in T cell development and prevents discrimination of the contribution of glucocorticoid signaling among T cell subtypes [91].

Much like the uterus during pregnancy, immune cells present in the testis mediate the process of spermatogenesis through a delicate balance of inflammation and immunotolerance (Figure 1B) [97]. The testis is an immunoprivileged site by virtue of the **blood–testis barrier (BTB)**, systemic immune tolerance, and local immunosuppression, which protects the immunogenic germ cells against provoking an immune response [98]. However, effective local innate immunity is required to prevent bacterial or viral infections in the testis. Cells of both the innate and the adaptive immune response reside within the interstitial space of the testis and are able to interact with the steroidogenic Leydig cells and the seminiferous tubules where spermatogenesis occurs. Under physiological conditions, subsets of dendritic cells and regulatory T cells mediate tolerance to germ cells. Under inflammatory conditions, however, proinflammatory cytokines produced by macrophages, dendritic cells, and effector T cells disrupt the BTB and induce apoptosis of germ cells. To date there are no studies in humans or mouse models assessing the impact of glucocorticoids on immune cells in the testis. Endocrine regulation of the testicular immune system by androgens suggests that glucocorticoids may also have indirect effects on immune cell functions.

In the peripheral immune system, glucocorticoids display various direct actions on immune cell development and function, which may influence the relative pool of immune cells able to be recruited to the reproductive tract [99]. Glucocorticoids prevent the generation of immature dendritic cells from monocytes and inhibit the differentiation of immature dendritic cells into mature dendritic cells *in vitro* [100,101]. High serum glucocorticoid levels induced by psychoactive drug administration were shown to be associated with rapid accumulation of mature NK cells in the peripheral circulation *in vivo* [102]. Glucocorticoids also induce robust apoptosis of T and B cells, mature dendritic cells, basophils, and eosinophils [103]. Glucocorticoids also

Key Figure

Impact of Glucocorticoids on Reproductive Physiology.



Trends in Endocrinology & Metabolism

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regulate the immune system by impacting the function of immune cells. For example, glucocorticoids inhibit the transcription of proinflammatory cytokines and chemokines in macrophages and drive macrophages toward the M2 phenotype [57]. Dexamethasone can also induce components of the **inflammasome** to sensitize the innate immune response in macrophages [104]. The nature of the response to glucocorticoids in the immune system is dependent on physiological or stress-induced levels of hormone and the duration of the stimulus, suggesting that the immune response governing reproduction is regulated in part through physiological glucocorticoid signaling and is sensitive to hormone fluctuations.

Concluding Remarks

Stress-induced activation of the HPA axis or administration of exogenous glucocorticoids produces adverse effects on male and female fertility and negatively impacts *in utero* development. In models of adrenal insufficiency, the absence of glucocorticoids is also associated with reduced fertility and disturbances of fetal growth. However, basal physiological concentrations of glucocorticoids are supportive of all stages of reproduction, indicating that the dose is critically important in the outcome. The timing of glucocorticoid exposure may also dictate whether glucocorticoid signaling promotes or inhibits fertility. The tipping point between supportive and adverse effects is unclear (Figure 2, Key Figure). For example, exogenous glucocorticoids are utilized clinically during *in vitro* fertilization (IVF) and in patients with recurrent miscarriage with the rationale that the immunomodulatory actions of glucocorticoids improve the intrauterine environment [105,106]. Following conception, women may receive glucocorticoids during pregnancy as treatment for asthma, autoimmune diseases, or adrenal insufficiency or for the management of preterm labor [107–109]. Glucocorticoid use during preterm labor critically improves neonatal outcomes through enhanced fetal development. However, exposure to exogenous or stress-induced levels of glucocorticoids during the window of receptivity or during fetal development may attenuate the maternal immune system required to establish pregnancy and alter fetal development *in utero*. Evidence from animal studies demonstrates that prenatal exposure to synthetic or stress-induced levels of glucocorticoids leads to reduced fetal growth and dysfunction of the cardiovascular, metabolic, endocrine, nervous, and reproductive systems in adults [26]. The effects of overexposure are dependent on the dose and type of glucocorticoid and determined by the gestational age at exposure. In humans prenatal glucocorticoid exposure is associated with higher blood pressure and insulin levels and mild behavioral defects, although it is difficult to separate the effects of glucocorticoid exposure from the impact of premature birth [110]. Given the clear benefits to fetal survival, prenatal glucocorticoids are part of the standard management for women who present with preterm labor. However, the additional benefit of repeated treatment or the long-term consequences of exposure during early pregnancy remain uncertain and call for further investigation (see Outstanding Questions).

Other deficits in our current understanding of glucocorticoid functions in the reproductive system stem from a lack of knowledge regarding the sites of direct GR action and the crosstalk of glucocorticoids with other nuclear receptors. Glucocorticoids and estrogens have exhibited antagonistic actions in the uterus [19,20]. The presence of estradiol with dexamethasone alters GR recruitment to target genes in immortalized uterine cells and is

Outstanding Questions

The ovarian hormones and their receptors regulate reproductive functions before and during pregnancy. Many *in vitro* studies have demonstrated dynamic molecular interplay in GR and ER binding, and glucocorticoids and estrogen regulate many common genes. What role does the GR play in mediating estrogen responses, if any?

Glucocorticoid sensitivity in the reproductive system may also be mediated by the relative expression of the 11β-HSD isoenzymes and the mineralocorticoid receptor (MR). Do glucocorticoids signal through the MR in the normal physiology of reproduction or the pathologies of adverse events? Could 11β-HSD be a therapeutic target in women?

What are the signaling pathways regulated by glucocorticoids during parturition in humans and could our knowledge of these improve pregnancy outcomes?

Antenatal corticosteroid administration for fetal maturation is critical for survival in preterm delivery. Recent updates have shown that antenatal corticosteroids may be beneficial in late-preterm birth (34 to <37 weeks) and should now be considered in treatment. What is the magnitude of the benefits or risk of long-term effects in this population?

How is glucocorticoid signaling in reproduction impacted by environmental exposures? Are there tissue-specific effects or long-term consequences? The stress response to environmental compounds may represent a new adaptive mechanism to incorporate exposures into reproductive fitness.

Figure 2. The impact of glucocorticoids on reproductive physiology is dependent on dose and timing. Both excess and insufficient levels of glucocorticoids result in reproductive dysfunction, although these occur through differing pathophysiologies. The timing of exposure also dictates the relative benefit or harm of glucocorticoid exposure. The example is provided in which early exposure to high levels of glucocorticoids may inhibit endometrial receptivity, although physiological levels of glucocorticoids may be required for this process. Excess glucocorticoid exposure during early placentation is associated with reduced placental weight and fetal size. However, glucocorticoid signaling in the third trimester is required for fetal development. Timing of exposure also plays a role in fetal programming of postnatal development, with more profound influence occurring as a result of elevated glucocorticoids during the first and second trimesters.

dependent on estrogen receptor alpha (ERα) [111]. It is unclear whether this molecular antagonism is a result of direct interference with GR–DNA interactions and the biological significance of GR–ER crosstalk is not well understood. Glucocorticoids bind with low affinity to PR and, similarly, progesterone weakly binds GR [112,113]. The abortifacient drug RU486 acts as both an antiprogestin and an antiglucocorticoid, which prevents discrimination between the GR- and PR-mediated effects in the uterus [114]. Cells in culture have provided a genetically tractable model to dissect the relative contributions of GR and PR and suggest crosstalk between progesterone and the GR in cells of the reproductive tract [115–118]. New techniques utilizing cell-specific knockdown or targeted genome editing *in vivo* will help clarify the specific roles of glucocorticoids in normal reproductive physiology and stress-induced infertility. By identifying GR target genes in tissues of the reproductive tract and framing these data in reference to published **ChIP-seq** databases, we will begin to provide a picture of the regulatory networks that orchestrate fertility. Additionally, future studies should assess the contribution of the *NR3C1* isoforms, **post-translational modifications**, and **SNPs** in the heterogeneity of stress responses among individuals. In the placenta GR isoform expression varied between women who delivered with no complications at term and those who delivered preterm [30]. Context-specific expression provides insight on the molecular mechanisms by which stress impacts individuals uniquely. This knowledge is increasingly important for the identification of new avenues of treatment for infertility and potential new mechanisms to enhance fertility in agricultural species.

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References

1. Erichsen, M.M. *et al.* (2010) Sexuality and fertility in women with Addison's disease. *J. Clin. Endocrinol. Metab.* 95, 4354–4360
2. Kowal, B.F. *et al.* (2006) Addison's disease presenting as male infertility. *Fertil. Steril.* 85, 1059 e1051–e1054
3. Oakley, R.H. and Cidlowski, J.A. (2011) Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. *J. Biol. Chem.* 286, 3177–3184
4. Dagklis, T. *et al.* (2015) Common features and differences of the hypothalamic–pituitary–gonadal axis in male and female. *Gynecol. Endocrinol.* 31, 14–17
5. Whirledge, S. and Cidlowski, J.A. (2013) A role for glucocorticoids in stress-impaired reproduction: beyond the hypothalamus and pituitary. *Endocrinology* 154, 4450–4468
6. Breen, K.M. and Mellon, P.L. (2014) Influence of stress-induced intermediates on gonadotropin gene expression in gonadotrope cells. *Mol. Cell. Endocrinol.* 385, 71–77
7. Geraghty, A.C. and Kaufer, D. (2015) Glucocorticoid regulation of reproduction. *Adv. Exp. Med. Biol.* 872, 253–278
8. Takumi, K. *et al.* (2012) Immunohistochemical analysis of the colocalization of corticotropin-releasing hormone receptor and glucocorticoid receptor in kisspeptin neurons in the hypothalamus of female rats. *Neurosci. Lett.* 531, 40–45
9. Luo, E. *et al.* (2016) Corticosterone blocks ovarian cyclicity and the LH surge via decreased kisspeptin neuron activation in female mice. *Endocrinology* 157, 1187–1199
10. Ubuka, T. *et al.* (2009) Identification of human GnIH homologs, RFRP-1 and RFRP-3, and the cognate receptor, GPR147 in the human hypothalamic pituitary axis. *PLoS One* 4, e8400
11. Clarke, I.J. *et al.* (2016) Stress increases gonadotropin inhibitory hormone cell activity and input to GnRH cells in ewes. *Endocrinology* 157, 4339–4350
12. Kirby, E.D. *et al.* (2009) Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. *Proc. Natl. Acad. Sci. U. S. A.* 106, 11324–11329
13. Son, Y.L. *et al.* (2014) Molecular basis for the activation of gonadotropin-inhibitory hormone gene transcription by corticosterone. *Endocrinology* 155, 1817–1826
14. Yuan, X.H. *et al.* (2014) Dexamethasone altered steroidogenesis and changed redox status of granulosa cells. *Endocrine* 47, 639–647
15. Huang, T.J. and Shirley Li, P. (2001) Dexamethasone inhibits luteinizing hormone-induced synthesis of steroidogenic acute regulatory protein in cultured rat preovulatory follicles. *Biol. Reprod.* 64, 163–170
16. Michael, A.E. *et al.* (1993) Direct inhibition of ovarian steroidogenesis by cortisol and the modulatory role of 11 β-hydroxysteroid dehydrogenase. *Clin. Endocrinol.* 38, 641–644
17. Yuan, H.J. *et al.* (2016) Glucocorticoids impair oocyte developmental potential by triggering apoptosis of ovarian cells via activating the Fas system. *Sci. Rep.* 6, 24036
18. da Costa, N.N. *et al.* (2016) Effect of cortisol on bovine oocyte maturation and embryo development *in vitro*. *Theriogenology* 85, 323–329
19. Rhen, T. *et al.* (2003) Dexamethasone blocks the rapid biological effects of 17β-estradiol in the rat uterus without antagonizing its global genomic actions. *FASEB J.* 17, 1849–1870
20. Johnson, D.C. and Dey, S.K. (1980) Role of histamine in implantation: dexamethasone inhibits estradiol-induced implantation in the rat. *Biol. Reprod.* 22, 1136–1141
21. Whirledge, S. *et al.* (2013) Global gene expression analysis in human uterine epithelial cells defines new targets of glucocorticoid and estradiol antagonism. *Biol. Reprod.* 89, 66
22. Nanjappa, M.K. *et al.* (2015) Maximal dexamethasone inhibition of luminal epithelial proliferation involves progesterone receptor (PR)- and non-PR-mediated mechanisms in neonatal mouse uterus. *Biol. Reprod.* 92, 122
23. Kuroda, K. *et al.* (2013) Induction of 11β-HSD 1 and activation of distinct mineralocorticoid receptor- and glucocorticoid

receptor-dependent gene networks in decidualizing human endometrial stromal cells. *Mol. Endocrinol.* 27, 192–202

24. Zhang, D. *et al.* (2016) Glucocorticoid exposure in early placentation induces preeclampsia in rats via interfering trophoblast development. *Gen. Comp. Endocrinol.* 225, 61–70
25. Braun, T. *et al.* (2015) Early dexamethasone treatment induces placental apoptosis in sheep. *Reprod. Sci.* 22, 47–59
26. Fowden, A.L. and Forhead, A.J. (2015) Glucocorticoids as regulatory signals during intrauterine development. *Exp. Physiol.* 100, 1477–1487
27. Vaughan, O.R. *et al.* (2015) Corticosterone alters materno-fetal glucose partitioning and insulin signalling in pregnant mice. *J. Physiol.* 593, 1307–1321
28. Ozmen, A. *et al.* (2015) Glucocorticoid exposure altered angiogenic factor expression via Akt/mTOR pathway in rat placenta. *Ann. Anat.* 198, 34–40
29. Yang, Q. *et al.* (2016) Compartmentalized localization of 11β-HSD 1 and 2 at the feto-maternal interface in the first trimester of human pregnancy. *Placenta* 46, 63–71
30. Saif, Z. *et al.* (2015) Expression of eight glucocorticoid receptor isoforms in the human preterm placenta vary with fetal sex and birthweight. *Placenta* 36, 723–730
31. Li, X.Q. *et al.* (2014) Roles of glucocorticoids in human parturition: a controversial fact? *Placenta* 35, 291–296
32. Laloha, F. *et al.* (2015) Effect of intravenous dexamethasone on preparing the cervix and labor induction. *Acta Med. Iran.* 53, 568–572
33. Wang, W. *et al.* (2015) Phosphorylation of STAT3 mediates the induction of cyclooxygenase-2 by cortisol in the human amnion at parturition. *Sci. Signal.* 8, ra106
34. Challis, J.R.G. *et al.* (2000) Endocrine and paracrine regulation of birth at term and preterm. *Endocr. Rev.* 21, 514–550
35. Wang, W. *et al.* (2016) Induction of amnion epithelial apoptosis by cortisol via tPA/plasmin system. *Endocrinology* 157, 4487–4498
36. Gopalakrishnan, G.S. (2004) Programming of adult cardiovascular function after early maternal undernutrition in sheep. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R12–R20
37. De Blasio, M.J. *et al.* (2007) Maternal exposure to dexamethasone or cortisol in early pregnancy differentially alters insulin secretion and glucose homeostasis in adult male sheep offspring. *Am J Physiol. Endocrinol. Metab.* 293, E75–E82
38. Slotkin, T.A. *et al.* (1996) Programming of brainstem serotonin transporter development by prenatal glucocorticoids. *Brain Res. Dev. Brain Res.* 93, 155–161
39. Pechnick, R.N. *et al.* (2006) Developmental exposure to corticosterone: behavioral changes and differential effects on leukemia inhibitory factor (LIF) and corticotropin-releasing hormone (CRH) gene expression in the mouse. *Psychopharmacology* 185, 76–83
40. Fowden, A.L. *et al.* (2016) Glucocorticoid programming of intrauterine development. *Domest. Anim. Endocrinol.* 56 (Suppl), S121–S132
41. Holmes, M.C. *et al.* (2015) Fetal programming of adult behaviour by stress and glucocorticoids. *Psychoneuroendocrinology* 61, 9
42. Carpenter, T. *et al.* (2017) Sex differences in early-life programming of the hypothalamic–pituitary–adrenal axis in humans suggest increased vulnerability in females: a systematic review. *J. Dev. Orig. Health Dis.* Published online January 20, 2017. http://dx.doi.org/10.1017/S204017441600074X
43. Gillies, G.E. *et al.* (2016) Enduring, sexually dimorphic impact of *in utero* exposure to elevated levels of glucocorticoids on midbrain dopaminergic populations. *Brain Sci.* Published online December 30, 2016. http://dx.doi.org/10.3390/brainsci7010005
44. Hiroi, R. *et al.* (2016) Sex-dependent programming effects of prenatal glucocorticoid treatment on the developing serotonin system and stress-related behaviors in adulthood. *Neuroscience* 320, 43–56
45. Cuffe, J.S. *et al.* (2016) Maternal corticosterone exposure in the mouse programs sex-specific renal adaptations in the renin–angiotensin–aldosterone system in 6-month offspring. *Physiol. Rep.* 4, e12754
46. O'sullivan, L. *et al.* (2015) Excess prenatal corticosterone exposure results in albuminuria, sex-specific hypotension, and altered heart rate responses to restraint stress in aged adult mice. *Am. J. Physiol. Renal Physiol.* 308, F1065–F1073
47. Cuffe, J.S. *et al.* (2017) Prenatal corticosterone exposure programs sex-specific adrenal adaptations in mouse offspring. *J. Endocrinol.* 232, 37–48
48. Sun, Y. *et al.* (2016) Prenatal dexamethasone exposure increases the susceptibility to autoimmunity in offspring rats by epigenetic programing of glucocorticoid receptor. *Biomed. Res. Int.* 2016, 9409452
49. Silva, E.J. *et al.* (2014) Impact of adrenalectomy and dexamethasone treatment on testicular morphology and sperm parameters in rats: insights into the adrenal control of male reproduction. *Andrology* 2, 835–846
50. Gao, H.B. *et al.* (1996) Suppression of endogenous corticosterone levels *in vivo* increases the steroidogenic capacity of purified rat Leydig cells *in vitro*. *Endocrinology* 137, 1714–1718
51. Panza, S. *et al.* (2016) Glucocorticoid receptor as a potential target to decrease aromatase expression and inhibit Leydig tumor growth. *Am. J. Pathol.* 186, 1328–1339
52. Cole, T.J. *et al.* (1995) Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. *Genes Dev.* 9, 1608–1621
53. Whirledge, S.D. *et al.* (2015) Uterine glucocorticoid receptors are critical for fertility in mice through control of embryo implantation and decidualization. *Proc. Natl. Acad. Sci. U. S. A.* 112, 15166–15171
54. Hazra, R. *et al.* (2014) *In vivo* actions of the Sertoli cell glucocorticoid receptor. *Endocrinology* 155, 1120–1130
55. Wintermantel, T.M. *et al.* (2005) The epithelial glucocorticoid receptor is required for the normal timing of cell proliferation during mammary lobuloalveolar development but is dispensable for milk production. *Mol. Endocrinol.* 19, 340–349
56. Zhao, B. *et al.* (2014) Glucocorticoid receptor in prostate epithelia is not required for corticosteroid-induced epithelial hyperproliferation in the mouse prostate. *Prostate* 74, 1068–1078
57. Robertson, S.A. *et al.* (2016) Corticosteroid therapy in assisted reproduction – immune suppression is a faulty premise. *Hum. Reprod.* 31, 2164–2173
58. Kingsley-Kallesen, M. (2002) The mineralocorticoid receptor may compensate for the loss of the glucocorticoid receptor at specific stages of mammary gland development. *Mol. Endocrinol.* 16, 2008–2018
59. Reichardt, H.M. *et al.* (2001) Mammary gland development and lactation are controlled by different glucocorticoid receptor activities. *Eur. J. Endocrinol.* 145, 519–527
60. Jewell, C.M. *et al.* (2012) Complex human glucocorticoid receptor dim mutations define glucocorticoid induced apoptotic resistance in bone cells. *Mol. Endocrinol.* 26, 244–256
61. Mittelstadt, P.R. and Ashwell, J.D. (2003) Disruption of glucocorticoid receptor exon 2 yields a ligand-responsive C-terminal fragment that regulates gene expression. *Mol. Endocrinol.* 17, 1534–1542
62. Casey, T.M. and Plaut, K. (2007) The role of glucocorticoids in secretory activation and milk secretion, a historical perspective. *J. Mammary Gland Biol. Neoplasia* 12, 293–304
63. Kotelevtsev, Y. *et al.* (1997) 11β-Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc. Natl. Acad. Sci. U. S. A.* 94, 14924–14929
64. Kotelevtsev, Y. *et al.* (1999) Hypertension in mice lacking 11β-hydroxysteroid dehydrogenase type 2. *J. Clin. Invest.* 103, 683–689
65. Lavery, G.G. *et al.* (2012) Lack of significant metabolic abnormalities in mice with liver-specific disruption of 11β-hydroxysteroid dehydrogenase type 1. *Endocrinology* 153, 3236–3248
66. Wyrwoll, C. *et al.* (2015) Fetal brain 11β-hydroxysteroid dehydrogenase type 2 selectively determines programming of adult

depressive-like behaviors and cognitive function, but not anxiety behaviors in male mice. *Psychoneuroendocrinology* 59, 59–70

67. Mullins, L.J. *et al.* (2015) Mineralocorticoid excess or glucocorticoid insufficiency: renal and metabolic phenotypes in a rat Hsd11b2 knockout model. *Hypertension* 66, e20
68. Thompson, A. *et al.* (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. *Biol. Reprod.* 67, 1708–1718
69. Wyrwoll, C.S. *et al.* (2009) Altered placental function of 11β-hydroxysteroid dehydrogenase 2 knockout mice. *Endocrinology* 150, 1287–1293
70. Tronche, F. *et al.* (1999) Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat. Genet.* 23, 99–103
71. Oakley, R.H. *et al.* (2013) Essential role of stress hormone signaling in cardiomyocytes for the prevention of heart disease. *Proc. Natl. Acad. Sci. U. S. A.* 110, 17035–17040
72. Jeanneteau, F.D. *et al.* (2012) BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1305–1310
73. Brewer, J.A. *et al.* (2003) T-cell glucocorticoid receptor is required to suppress COX-2-mediated lethal immune activation. *Nat. Med.* 9, 1318–1322
74. Pikulkaew, S. *et al.* (2011) The knockdown of maternal glucocorticoid receptor mRNA alters embryo development in zebrafish. *Dev. Dyn.* 240, 874–889
75. Griffiths, B.B. *et al.* (2012) A zebrafish model of glucocorticoid resistance shows serotonergic modulation of the stress response. *Front. Behav. Neurosci.* 6, 68
76. Hoo, J.Y. *et al.* (2016) Zebrafish: a versatile animal model for fertility research. *Biomed Res. Int.* 2016, 9732780
77. Zhang, J. *et al.* (2016) To serve and to protect: the role of decidual innate immune cells on human pregnancy. *Cell Tissue Res.* 363, 249–265
78. Figueiredo, A.S. and Schumacher, A. (2016) The T helper type 17/regulatory T cell paradigm in pregnancy. *Immunology* 148, 13–21
79. Vinketova, K. *et al.* (2016) Human decidual stromal cells as a component of the implantation niche and a modulator of maternal immunity. *J. Pregnancy* 2016, 8689436
80. Mori, M. *et al.* (2016) The decidua – the maternal bed embracing the embryo – maintains the pregnancy. *Semin. Immunopathol.* 38, 635–649
81. Bartmann, C. *et al.* (2014) Quantification of the predominant immune cell populations in decidua throughout human pregnancy. *Am. J. Reprod. Immunol.* 71, 109–119
82. Henderson, T.A. *et al.* (2003) Steroid receptor expression in uterine natural killer cells. *J. Clin. Endocrinol. Metab.* 88, 440–449
83. Chen, Y. *et al.* (2012) Mifepristone increases the cytotoxicity of uterine natural killer cells by acting as a glucocorticoid antagonist via ERK activation. *PLoS One* 7, e36413
84. Quenby, S. *et al.* (2005) Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertil. Steril.* 84, 980–984
85. Trundley, A. *et al.* (2006) Methods for isolation of cells from the human fetal–maternal interface. *Methods Mol. Med.* 122, 109–122
86. Porta, C. *et al.* (2015) Molecular and epigenetic basis of macrophage polarized activation. *Semin. Immunol.* 27, 237–248
87. Gustafsson, C. *et al.* (2008) Gene expression profiling of human decidual macrophages: evidence for immunosuppressive phenotype. *PLoS One* 3, e2078
88. Heikkinen, J. *et al.* (2003) Phenotypic characterization of human decidual macrophages. *Clin. Exp. Immunol.* 131, 498–505
89. Thiruchelvam, U. *et al.* (2016) Cortisol regulates the paracrine action of macrophages by inducing vasoactive gene expression in endometrial cells. *J. Leukoc. Biol.* 99, 1165–1171
90. Horton, J.S. *et al.* (2011) Relaxin modulates proinflammatory cytokine secretion from human decidual macrophages. *Biol. Reprod.* 85, 788–797
91. Tuckermann, J.P. *et al.* (2007) Macrophages and neutrophils are the targets for immune suppression by glucocorticoids in contact allergy. *J. Clin. Invest.* 117, 1381–1390
92. Plaks, V. *et al.* (2008) Uterine DCs are crucial for decidua formation during embryo implantation in mice. *J. Clin. Invest.* 118, 3954–3965
93. Aluvihare, V.R. *et al.* (2004) Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* 5, 266–271
94. Wang, W.J. *et al.* (2010) Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients. *J. Reprod. Immunol.* 84, 164–170
95. Li, C.C. *et al.* (2015) Suppression of dendritic cell-derived IL-12 by endogenous glucocorticoids is protective in LPS-induced sepsis. *PLoS Biol.* 13, e1002269
96. Baschant, U. *et al.* (2011) Glucocorticoid therapy of antigen-induced arthritis depends on the dimerized glucocorticoid receptor in T cells. *Proc. Natl. Acad. Sci. U. S. A.* 108, 19317–19322
97. Perez, C.V. *et al.* (2013) Dual role of immune cells in the testis: protective or pathogenic for germ cells? *Spermatogenesis* 3, e23870
98. Zhao, S. *et al.* (2014) Testicular defense systems: immune privilege and innate immunity. *Cell. Mol. Immunol.* 11, 428–437
99. Busillo, J.M. and Cidlowski, J.A. (2013) The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore. *Trends Endocrinol. Metab.* 24, 109–119
100. Woltman, A.M. *et al.* (2000) The effect of calcineurin inhibitors and corticosteroids on the differentiation of human dendritic cells. *Eur. J. Immunol.* 30, 1807–1812
101. Matasic, R. *et al.* (1999) Dexamethasone inhibits dendritic cell maturation by redirecting differentiation of a subset of cells. *J. Leukoc. Biol.* 66, 909–914
102. Bigler, M.B. *et al.* (2015) Stress-induced *in vivo* recruitment of human cytotoxic natural killer cells favors subsets with distinct receptor profiles and associates with increased epinephrine levels. *PLoS One* 10, e0145635
103. Cruz-Topete, D. and Cidlowski, J.A. (2015) One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. *Neuroimmunomodulation* 22, 20–32
104. Busillo, J.M. *et al.* (2011) Glucocorticoids sensitize the innate immune system through regulation of the NLRP3 inflammasome. *J. Biol. Chem.* 286, 38703–38713
105. Polak de Fried, E. *et al.* (1993) Improvement of clinical pregnancy rate and implantation rate of *in-vitro* fertilization–embryo transfer patients by using methylprednisone. *Hum. Reprod.* 8, 393–395
106. Quenby, S. *et al.* (2003) Successful pregnancy outcome following 19 consecutive miscarriages: case report. *Hum. Reprod.* 18, 2562–2564
107. Breton, M.C. *et al.* (2010) Risk of perinatal mortality associated with inhaled corticosteroid use for the treatment of asthma during pregnancy. *J. Allergy Clin. Immunol.* 126, 772–777
108. Ponticelli, C. and Moroni, G. (2015) Immunosuppression in pregnant women with systemic lupus erythematosus. *Expert Rev. Clin. Immunol.* 11, 549–552
109. Msan, A.K. *et al.* (2015) Use of antenatal corticosteroids in the management of preterm delivery. *Am. J. Perinatol.* 32, 417–426
110. Harris, A. and Seckl, J. (2011) Glucocorticoids, prenatal stress and the programming of disease. *Horm. Behav.* 59, 279–289
111. Whirledge, S. and Cidlowski, J.A. (2013) Estradiol antagonism of glucocorticoid-induced GILZ expression in human uterine epithelial cells and murine uterus. *Endocrinology* 154, 499–510
112. Haslam, S.Z. *et al.* (1981) An empirical basis for the competition by dexamethasone to progesterone receptors as estimated with the synthetic progestin R5020. *J. Recept. Res.* 2, 435–451
113. Attardi, B.J. *et al.* (2007) Comparison of progesterone and glucocorticoid receptor binding and stimulation of gene expression by progesterone, 17-α hydroxyprogesterone caproate, and

related progestins. *Am. J. Obstet. Gynecol.* 197, 599 e591–e597

114. Schreiber, J.R. *et al.* (1983) Binding of the anti-progestin RU-486 to rat ovary steroid receptors. *Contraception* 28, 77–85
115. Lei, K. *et al.* (2012) Progesterone acts via the nuclear glucocorticoid receptor to suppress IL-1β-induced COX-2 expression in human term myometrial cells. *PLoS One* 7, e50167
116. Leo, J.C. *et al.* (2004) Glucocorticoid and mineralocorticoid cross-talk with progesterone receptor to induce focal adhesion and growth inhibition in breast cancer cells. *Endocrinology* 145, 1314–1321
117. Louw-du Toit, R. (2014) Medroxyprogesterone acetate differentially regulates interleukin (IL)-12 and IL-10 in a human ectocervical epithelial cell line in a glucocorticoid receptor (GR)-dependent manner. *J. Biol. Chem.* 289, 31136–31149
118. Lei, K. *et al.* (2015) Progesterone and the repression of myometrial inflammation: the roles of MKP-1 and the AP-1 system. *Mol. Endocrinol.* 29, 1454–1467
119. Sawchenko, P.E. (1987) Evidence for a local site of action for glucocorticoids in inhibiting CRF and vasopressin expression in the paraventricular nucleus. *Brain Res.* 403, 213–223
120. Plotsky, P.M. *et al.* (1986) Inhibition of immunoreactive corticotropin-releasing factor secretion into the hypophysial–portal circulation by delayed glucocorticoid feedback. *Endocrinology* 119, 1126–1130
121. Aguilera, G. (1994) Regulation of pituitary ACTH secretion during chronic stress. *Front. Neuroendocrinol.* 15, 321–350
122. Daughaday, W.H. (1956) Binding of corticosteroids by plasma proteins. II. Paper electrophoresis and equilibrium paper electrophoresis. *J. Clin. Invest.* 35, 1434–1438
123. Baker, M.E. (2002) Albumin, steroid hormones and the origin of vertebrates. *J. Endocrinol.* 175, 121–127
124. Weitzman, E.D. *et al.* (1971) Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J. Clin. Endocrinol. Metab.* 33, 14–22
125. Wood, P.J. *et al.* (1997) Evidence for the low dose dexamethasone suppression test to screen for Cushing's syndrome – recommendations for a protocol for biochemistry laboratories. *Ann. Clin. Biochem.* 34, 222–229
126. Pariante, C.M. (2008) The role of multi-drug resistance p-glycoprotein in glucocorticoid function: studies in animals and relevance in humans. *Eur. J. Pharmacol.* 583, 263–271
127. Woods, C. and Tomlinson, J.W. (2015) The dehydrogenase hypothesis. *Adv. Exp. Med. Biol.* 872, 353–380
128. Wira, C. and Munck, A. (1970) Specific glucocorticoid receptors in thymus cells. Localization in the nucleus and extraction of the cortisol–receptor complex. *J. Biol. Chem.* 245, 3436–3438
129. Bamberger, C.M. *et al.* (1995) Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *J. Clin. Invest.* 95, 2435–2441
130. Oakley, R.H. *et al.* (1996) The human glucocorticoid receptor beta isoform Expression, biochemical properties, and putative function. *J. Biol. Chem.* 271, 9550–9559
131. Moalli, P.A. *et al.* (1993) Alternatively spliced glucocorticoid receptor messenger RNAs in glucocorticoid-resistant human multiple myeloma cells. *Cancer Res.* 53, 3877–3879
132. Rivers, C. *et al.* (1999) Insertion of an amino acid in the DNA-binding domain of the glucocorticoid receptor as a result of alternative splicing. *J. Clin. Endocrinol. Metab.* 84, 4283–4286
133. Hollenberg, S.M. (1985) Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* 318, 635–641
134. Kino, T. *et al.* (2009) Glucocorticoid receptor (GR) beta has intrinsic, GRα-independent transcriptional activity. *Biochem. Biophys. Res. Commun.* 381, 671–675
135. He, B. *et al.* (2015) Human glucocorticoid receptor beta regulates gluconeogenesis and inflammation in mouse liver. *Mol. Cell. Biol.* 36, 714–730
136. Ray, D.W. *et al.* (1996) Glucocorticoid receptor structure and function in glucocorticoid-resistant small cell lung carcinoma cells. *Cancer Res.* 56, 3276–3280
137. Meijsing, S.H. *et al.* (2009) DNA binding site sequence directs glucocorticoid receptor structure and activity. *Science* 324, 407–410
138. Thomas-Chollier, M. (2013) A naturally occurring insertion of a single amino acid rewires transcriptional regulation by glucocorticoid receptor isoforms. *Proc. Natl. Acad. Sci. U. S. A.* 110, 17826–17831
139. Morgan, D.J. *et al.* (2016) Glucocorticoid receptor isoforms direct distinct mitochondrial programs to regulate ATP production. *Sci. Rep.* 6, 26419
140. Gaitan, D. *et al.* (1995) Glucocorticoid receptor structure and function in an adrenocorticotropin-secreting small cell lung cancer. *Mol. Endocrinol.* 9, 1193–1201
141. Lu, N.Z. and Cidlowski, J.A. (2005) Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. *Mol. Cell* 18, 331–342
142. Lu, N.Z. *et al.* (2007) Selective regulation of bone cell apoptosis by translational isoforms of the glucocorticoid receptor. *Mol. Cell. Biol.* 27, 7143–7160
143. Nehme, A. *et al.* (2009) Glucocorticoids with different chemical structures but similar glucocorticoid receptor potency regulate subsets of common and unique genes in human trabecular meshwork cells. *BMC Med. Genomics* 2, 58
144. Gross, K.L. *et al.* (2011) Glucocorticoid receptor alpha isoform-selective regulation of antiapoptotic genes in osteosarcoma cells: a new mechanism for glucocorticoid resistance. *Mol. Endocrinol.* 25, 1087–1099
145. Song, I.H. and Buttgereit, F. (2006) Non-genomic glucocorticoid effects to provide the basis for new drug developments. *Mol. Cell. Endocrinol.* 246, 142–146
146. Nahar, J. *et al.* (2015) Rapid nongenomic glucocorticoid actions in male mouse hypothalamic neuroendocrine cells are dependent on the nuclear glucocorticoid receptor. *Endocrinology* 156, 2831–2842
147. Nahar, J. *et al.* (2016) Further evidence for a membrane receptor that binds glucocorticoids in the rodent hypothalamus. *Steroids* 114, 33–40
148. Starick, S.R. *et al.* (2015) ChIP-exo signal associated with DNA-binding motifs provides insight into the genomic binding of the glucocorticoid receptor and cooperating transcription factors. *Genome Res.* 25, 825–835
149. Surjit, M. *et al.* (2011) Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor. *Cell* 145, 224–241
150. Gupte, R. *et al.* (2013) Glucocorticoid receptor represses proinflammatory genes at distinct steps of the transcription cycle. *Proc. Natl. Acad. Sci. U. S. A.* 110, 14616–14621
151. Lu, N.Z. and Cidlowski, J.A. (2006) Glucocorticoid receptor isoforms generate transcription specificity. *Trends Cell Biol.* 16, 301–307
152. Ramamoorthy, S. and Cidlowski, J.A. (2016) Corticosteroids: mechanisms of action in health and disease. *Rheum. Dis. Clin. North Am.* 42, 15–31
153. Barnes, P.J. (2009) Histone deacetylase-2 and airway disease. *Ther. Adv. Respir. Dis.* 3, 235–243
154. Trotter, K.W. *et al.* (2015) Glucocorticoid receptor transcriptional activation via the BRG1-dependent recruitment of TOP2β and Ku70/86. *Mol. Cell. Biol.* 35, 2799–2817
155. Grontved, L. *et al.* (2013) C/EBP maintains chromatin accessibility in liver and facilitates glucocorticoid receptor recruitment to steroid response elements. *EMBO J.* 32, 1568–1583
156. Jones, C.L. *et al.* (2014) Loss of TBL1XR1 disrupts glucocorticoid receptor recruitment to chromatin and results in glucocorticoid resistance in a B-lymphoblastic leukemia model. *J. Biol. Chem.* 289, 20502–20515
157. Goi, C. *et al.* (2013) Cell-type and transcription factor specific enrichment of transcriptional cofactor motifs in ENCODE ChIP-seq data. *BMC Genomics* 14 (Suppl. 5), S2
158. Smith, C.L. and O'Malley, B.W. (2004) Coregulator function: a key to understanding tissue specificity of selective receptor modulators. *Endocr. Rev.* 25, 45–71

159. John, S. *et al.* (2011) Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. *Nat. Genet.* 43, 264–268
160. John, S. *et al.* (2008) Interaction of the glucocorticoid receptor with the chromatin landscape. *Mol. Cell* 29, 611–624
161. Koper, J.W. *et al.* (2014) Glucocorticoid receptor polymorphisms and haplotypes and their expression in health and disease. *Steroids* 92, 62–73
162. Konstantakou, P. *et al.* (2017) Dysregulation of 11β-hydroxysteroid dehydrogenases: implications during pregnancy and beyond. *J. Matern. Fetal Neonatal Med.* 30, 284–293
163. Lutz, S.Z. *et al.* (2016) Genetic variation in the 11β-hydroxysteroid-dehydrogenase 1 gene determines NAFLD and visceral obesity. *J. Clin. Endocrinol. Metab.* 101, 4743–4751
164. Ragnarsson, O. *et al.* (2014) Common genetic variants in the glucocorticoid receptor and the 11β-hydroxysteroid dehydrogenase type 1 genes influence long-term cognitive impairments in patients with Cushing's syndrome in remission. *J. Clin. Endocrinol. Metab.* 99, E1803–E1807
165. Stolte, E.H. *et al.* (2006) Evolution of glucocorticoid receptors with different glucocorticoid sensitivity. *J. Endocrinol.* 190, 17–28
166. Wells, A. *et al.* (2004) The stress response to environmental change in captive cheetahs (*Acinonyx jubatus*). *J. Zoo Wildl. Med.* 35, 8–14
167. Koester, D.C. *et al.* (2015) Motile sperm output by male cheetahs (*Acinonyx jubatus*) managed *ex situ* is influenced by public exposure and number of care-givers. *PLoS One* 10, e0135847
168. Li, L. *et al.* (2016) The effect of heat stress on gene expression, synthesis of steroids, and apoptosis in bovine granulosa cells. *Cell Stress Chaperones* 21, 467–475
169. Lynn, S.E. *et al.* (2015) Food, stress, and circulating testosterone: cue integration by the testes, not the brain, in male zebra finches (*Taeniopygia guttata*). *Gen. Comp. Endocrinol.* 215, 1–9
170. Ing, N.H. *et al.* (2014) Dexamethasone acutely down-regulates genes involved in steroidogenesis in stallion testes. *J. Steroid Biochem. Mol. Biol.* 143, 451–459
171. Min, Y. *et al.* (2016) Vitamin C and vitamin E supplementation alleviates oxidative stress induced by dexamethasone and improves fertility of breeder roosters. *Anim. Reprod. Sci.* 171, 1–6
172. Nozu, R. and Nakamura, M. (2015) Cortisol administration induces sex change from ovary to testis in the protogynous wrasse, *Halichoeres trimaculatus*. *Sex. Dev.* 9, 118–124