

## Peripheral corticotropin-releasing hormone is produced in the immune and reproductive systems: actions, potential roles and clinical implications

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### 1. ABSTRACT

Corticotropin-releasing hormone (CRH), the principal regulator of the hypothalamic-pituitary-adrenal axis, has been identified in various organ systems, including the immune and the female and male reproductive systems. CRH-like immunoreactivity has been reported in peripheral inflammatory sites and in a number of reproductive organs, including the ovaries, endometrial glands, decidualized endometrial stroma, placenta, decidua, and the testes. Therefore, “immune” and “reproductive” CRH are forms of “tissue” CRH; i.e., CRH found in peripheral tissues. Immune CRH plays a direct immunomodulatory role as an autocrine/paracrine mediator of inflammation. Immune CRH participates in several experimental inflammations and, in humans, in inflamed tissues from patients with autoimmune and inflammatory diseases. One of the early effects of immune CRH is the degranulation of mast cells and the release of histamine and several inflammatory cytokines. Reproductive CRH is regulating reproductive functions with an inflammatory component, such as ovulation, luteolysis, decidualization, implantation, and early maternal tolerance. Placental CRH participates in the physiology of pregnancy and the onset of labor. Circulating placental CRH is responsible for the physiologic hypercortisolism of the latter half of pregnancy. Postpartum, this hypercortisolism is followed by a transient adrenal suppression, which may explain the blues/depression and increased autoimmune phenomena observed during this period.

### 2. INTRODUCTION

Corticotropin-releasing hormone (CRH) is a 41-amino acid neuropeptide secreted by the paraventricular nucleus of the hypothalamus (1). CRH and its receptors are also found in many extra-hypothalamic sites of the central nervous system (CNS). There, CRH plays a major coordinative role for the stress response, including activation of the arousal and sympathetic systems, central suppression of the immune system and elicitation of stress-related behaviors (2). CRH is part of a family of mammalian peptides that includes the urocortins (UCN I, UCN II, and UCN III), as well as fish urotensin I and frog sauvagine (3-5). Signals from CRH and CRH-related peptides are transduced across cell membranes via activation of two types of G-coupled CRH receptors, R1 and R2, encoded by different genes. The CRH-R1 gene expresses four known subtypes, CRH-R1 alpha, R1 beta, R1c, and R1d, whereas the CRH-R2 gene expresses three known subtypes, CRH-R2 alpha, R1 beta, and R1 gamma (6). CRH-R1 binds CRH, UCN I, urotensin I and sauvagine with approximately equal affinity, whereas CRH-R2 binds UCN I, UCN II, UCN III, sauvagine and urotensin I with significantly higher affinity than it does CRH, indicating that these may be the natural or preferred ligands (6).

CRH and its receptors have been identified, not only throughout the CNS, but also in various organ systems, such as the immune and the female and male reproductive systems. Indeed, CRH-like immunoreactivity has been described in peripheral inflammatory sites (7-11)

**Table 1.** Sites of production, proposed functions and potential pathogenic effects of peripheral corticotropin-releasing hormone in the immune and reproductive systems

Peripheral CRH	Sites of Production	Proposed functions	Potential Pathogenic Effects
<b>Immune CRH</b>			
	Inflammatory sites	Stimulation of local inflammation	Autoimmune inflammatory disorders Stress-induced allergic or vasokinetic conditions, such as asthma, eczema and migraine headaches
<b>Reproductive CRH</b>			
Ovarian CRH	Theca; Stroma; Ovum	Inhibition of female sex steroid production; Ovulation; Luteolysis; Follicular maturation	Premature ovarian failure; Anovulation; Corpus luteum dysfunction; Ovarian dysfunction
Uterine CRH	Endometrial glands; Decidualized stroma	Decidualization; Blastocyst implantation; Early maternal tolerance	Infertility; Recurrent spontaneous abortion
Blastocyst	Invasive trophoblast	Blastocyst implantation; Early maternal tolerance	Recurrent spontaneous abortion
Placental CRH	Cytotrophoblast ; Syncytiotrophoblast ; Amnion; Chorion	Regulation of fetoplacental circulation; Fetal adrenal steroidogenesis; Induction of labor; Maternal hypercortisolism	Premature labor; Delayed labor; Preeclampsia and eclampsia
Testicular CRH	Leydig cells; Germ cells; Spermatozoa	In rat: inhibition of male sex steroid production; In mouse: stimulation of male sex steroid production	In rat: hypogonadism

and in a number of reproductive organs, including the ovaries, endometrial glands, decidualized endometrial stroma, placental trophoblast, syncytiotrophoblast, decidua, and the testes (12-22). Thus, “immune” and “reproductive” CRH are forms of “tissue” CRH, i.e., CRH found in peripheral tissues. “Immune” CRH is secreted at inflammatory sites and possesses potent proinflammatory properties influencing innate and acquired immune processes (Table 1). “Reproductive” CRH is regulating reproductive functions with an inflammatory component, such as ovulation, luteolysis, decidualization, implantation, and early maternal tolerance (Table 1). In addition, UCN I is widely distributed in immune and reproductive systems, suggesting that it has important physiological roles in these tissues (4,5).

The biological effects of CRH and CRH-related peptides are modulated not only by CRH receptors, but also by the CRH-binding protein (CRH-BP) (23). The CRH-BP has been shown to exist in numerous species; expressed in a highly tissue-specific pattern. In primates, CRH-BP gene is expressed in liver, placenta, and brain, whereas in rats, it has only been detected in the brain. The source of human peripheral CRH-BP is the liver and the placenta (23). The role of CRH-BP is still unclear; it has been proposed to act as a modulator of the HPA axis activation and CRH endocrine, autocrine or paracrine actions.

## 3. CRH IN THE IMMUNE SYSTEM

“Immune CRH” is secreted peripherally from the spleen, thymus and inflamed tissues and plays a direct immunomodulatory role, as an autocrine or paracrine mediator of inflammation (7,24). Whereas central, CNS CRH, via glucocorticoids and catecholamines, inhibits the inflammatory reaction; directly secreted immune CRH stimulates local inflammation. UCN I expression has also been shown in spleen, thymus, macrophages, and lymphocytes (24,25), whereas the existence of additional CRH-related peptides secreted by immune cells cannot ruled out. UCN I expression appears to be increased in various inflammatory diseases (5).

Endothelial cells, macrophages, and tissue fibroblasts have CRH in their cytoplasm (26). Inflammatory sites, examined by immunohistochemistry and extraction-chromatography, contain large amounts of immune CRH, which is identical to hypothalamic CRH (7). However, under normal conditions, in which the immune system is not activated, levels of CRH and its mRNA are very low. CRH is produced in peripheral inflammatory sites, acutely from peripheral nerves (postganglionic sympathetic neurons and primary somatosensory fibers) and chronically from immune cells (27).

Our laboratory localized immunoreactive CRH in local immune accessory cells in various experimental models of inflammation, including carrageenin-induced aseptic inflammation in Sprague-Dawley rats, acute and chronic streptococcal cell wall- and adjuvant-induced arthritis in Lewis rats (8) and peptide R-16 (epitope of the interphotoreceptor retinoid-binding protein)-induced uveitis in Lewis rats and B10.A mice (11). Immune neutralization and CRH antagonist experiments have demonstrated marked inhibition of inflammation (7,11,26,28). Immunoreactive CRH has also been found in human tissues undergoing inflammatory processes, including joints of patients with rheumatoid arthritis, thyroid glands of patients with Hashimoto thyroiditis, and colonic mucosa of patients with ulcerative colitis (9,10,28). Inflamed tissues contain large amounts of CRH, reaching levels similar to those observed in the hypophyseal portal system. The concentrations of CRH-BP are significantly elevated in active “immune” states, such as rheumatoid arthritis and septacemia, probably due to increased secretion by the liver (29).

The exact mechanisms by which immune CRH/UCN I exert their proinflammatory actions are not known, but one major mechanism is the degranulation of mast cells (30,31). CRH induces a marked increase in vascular permeability and mast cell degranulation in a dose-dependent and CRH receptor 1-depedent fashion (31). Thus, CRH causes vasodilation, increases vascular permeability, and allows extravasation of plasma throughout the capillary vessel walls (31). CRH also

stimulates secretion of IL-1 from monocytes, IL-2 from lymphocytes, and IL-6 from mononuclear cells (32-34). In addition, CRH promotes lymphocyte proliferation, and IL-2 receptor expression and chemotaxis by mononuclear leukocytes, and enhances production of oxygen radicals by macrophages (35-38). Immune CRH has been also shown to play a role in local, opioid-mediated analgesia (39). Secretion of immune CRH is suppressed by glucocorticoids and somatostatin (40).

In early stages of inflammation, there is a major discrepancy between the abundance of the peptide and the paucity of its mRNA, which is undetectable or present in minute quantities. The demonstration of CRH-like immunoreactivity in the dorsal horn of the spinal cord and dorsal root ganglia and in sympathetic nerve cell bodies and sympathetic ganglia, including post-ganglionic neurons and nerve fibers in spleen after IL-2 administration, support the hypothesis that the majority of immune CRH in early inflammation is of peripheral nerve rather than immune cell origin (41-44).

The production of CRH from the postganglionic sympathetic neurons may be responsible for the stress-induced activation of allergic/autoimmune diseases, such as asthma and eczema, via mast cell degranulation (30). Therefore, a CRH-R1 antagonist could be used for the treatment of stress-induced allergic or vasokinetic conditions (30,31).

## 4. CRH IN THE REPRODUCTIVE SYSTEM

### 4.1. Ovarian CRH

Ovarian CRH is primarily localized in thecal cells surrounding the ovarian follicles, in luteinized cells of the stroma and also in the cytoplasm of the ovum (12,13). CRH-R1 and CRH-R2 alpha receptors are also detected in the ovary, especially in the regressing corpus luteum (45). *In vitro* experiments have shown that CRH exerts an inhibitory effect on ovarian steroidogenesis in a dose-dependent, interleukin (IL)-1-mediated manner (46,47). This finding suggests that ovarian CRH has anti-reproductive actions that might be related to premature ovarian failure observed in women exposed to high psychosocial stress (48).

There is no detectable CRH in oocytes of primordial follicles in human ovaries, whereas there is abundant expression of the CRH and CRH-R1 genes in mature follicles, suggesting that CRH may play auto/paracrine roles in follicular maturation (12,13,45). However, polycystic ovaries present diminished amounts of CRH immunoreactivity, suggesting that decreased ovarian CRH might be related to the anovulation of polycystic ovarian syndrome (13). Finally, the concentration of CRH is higher in the premenopausal than the postmenopausal ovaries, indicating that ovarian CRH may be related to normal ovarian function during the reproductive life span (49).

UCN I is also expressed in the human ovary, mainly in steroidogenic luteal cells, especially during the

regression of the corpus luteum (50). Like CRH, it appears that UCN I may suppress ovarian steroidogenesis (50).

### 4.2. Uterine CRH

Human and rat uterus express the CRH gene, suggesting a local effect of endometrial CRH (14,15). Epithelial cells are the main source of endometrial CRH, while stroma does not express it, unless it differentiates to decidua (14,15,51,52). Similarly, epithelial cells are the main source of endometrial UCN I (4). In addition, CRH-R1 alpha, CRH-R1 beta and CRH-2 alpha are present in the human endometrium (6,53).

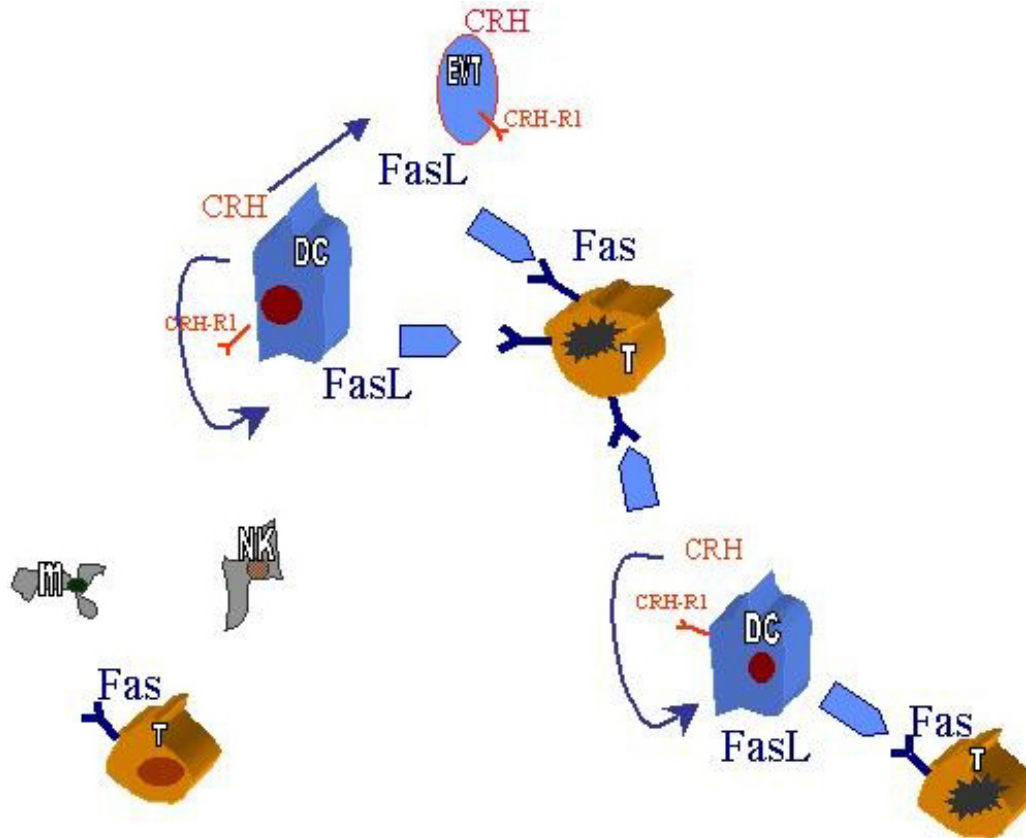
Inducers of CRH, such as 8-bromo-cAMP, forskolin and epidermal growth factor, stimulate the activity of the CRH promoter (54). Estrogens and glucocorticoids inhibit and prostaglandin E<sub>2</sub> stimulates the promoter of human CRH gene in transfected human endometrial cells, suggesting that the endometrial CRH gene is under the control of these agents (55). The inhibitory effect of glucocorticoids on endometrial CRH is in agreement with that found in the hypothalamus and opposite to that observed in the human placenta, indicating that the regulation of the transcription of the CRH gene is tissue-specific. The cytokines interleukin-1 (IL-1) and IL-6 stimulate the activity of the CRH promoter, an effect possibly mediated by prostaglandins, as has been described in both the hypothalamus and the placenta (55).

The endometrial glands are full of CRH during both the proliferative and the secretory phases of the cycle (14,15). However, the concentration of CRH is significantly higher in the secretory phase, associating endometrial CRH with intrauterine phenomena of the secretory phase of the menstrual cycle, such as decidualization and implantation (51). Indeed, in the human endometrium, a phenomenon with characteristics of an "aseptic" inflammatory reaction takes place during the differentiation of endometrial stroma to decidua. It has been shown that CRH induces the decidualization of endometrial stroma and that it potentiates the decidualizing effect of progesterone (52,56). Of note, progestins stimulate the expression of endometrial CRH in a cAMP-dependent manner (57).

### 4.3. CRH and blastocyst implantation

Intrauterine CRH participates in local immune phenomena associated with embryo implantation. We have reported that CRH may play a crucial role in the implantation and the anti-rejection process that protects the fetus from the maternal immune system, primarily by killing activated T cells through the Fas-Fas ligand interaction (58). In addition, UCN I may also play a role in implantation; it was recently shown that UCN I levels are significantly increased in the endometrium of women with spontaneous abortions (59).

During blastocyst implantation, the maternal endometrial response to the invading embryo has characteristics of an acute, aseptic inflammatory response; yet, once implanted, the embryo suppresses this response and prevents rejection (51). Indeed, during blastocyst



**Figure 1.** Involvement of CRH/Fas ligand system in the early phase of human implantation. CRH is produced by both extravillous trophoblast and decidual cells; acting in an autocrine/paracrine manner to stimulate FasL expression and to induce apoptosis of activated maternal T lymphocytes carrying Fas receptor.

implantation, several immune mediators of the inflammatory response, such as interleukin-1 (IL-1) and IL-6, are produced in the endometrium, whereas IL-1 and tumor necrosis-alpha receptors are expressed in endometrial cells (60,61). The implanting blastocyst secretes inflammatory mediators, including CRH, IL-1, IL-6, leukemia inhibiting factor (LIF) and PGE<sub>2</sub> (62,63). It has been suggested that blastocyst-derived IL-1 plays an essential role in implantation, since in mice blockage of its action by the antagonist IL-1ra inhibits it (64). The effects of LIF are equally important (63).

Early in pregnancy, the implantation sites in rat endometrium contain 3.5-fold higher concentrations of CRH compared to the interimplantation regions (51). Furthermore, human trophoblast and decidualized endometrial cells express Fas ligand (FasL), a pro-apoptotic molecule. These findings suggest that intrauterine CRH may participate in blastocyst implantation, while FasL may assist with maternal immune tolerance to the semi-allograft embryo. We have shown that CRH induces the expression of apoptotic FasL on invasive extravillous trophoblast and maternal decidual cells at the fetal-maternal interface (58). Furthermore, CRH increases the apoptosis of activated T lymphocytes through FasL induction, participating in the processes of both implantation and early pregnancy

tolerance (Figure 1). This effect of CRH is specifically mediated through CRH-R1.

Preliminary data suggested that implantation could be blocked in mice by the administration of a polyclonal rabbit antiserum generated against rat or human CRH (65). This observation is further supported by experiments in rats using antalarmin, a CRH-R1 specific antagonist (58). Indeed, invasive trophoblast promoted apoptosis of activated Fas-expressing human T-lymphocytes, an effect potentiated by CRH and inhibited by CRH antagonist (58). In support of these findings, female rats treated with the CRH antagonist in the first 6 days of gestation had a dose-dependent decrease of endometrial implantation sites and markedly diminished endometrial FasL expression (58). Our data are in agreement with previously published reports suggesting that expression of FasL by fetal extravillous trophoblast cells can induce apoptosis of activated T lymphocytes expressing the Fas receptor (66,67). It should be noted here that mice with missense or inactivating mutations of FasL gene (*gld*) can reproduce, suggesting that trophoblast FasL expression is not obligatory for maternal immunotolerance. Thus, in the absence of a functional Fas-FasL system, other mechanisms supporting maternal immunotolerance are sufficient to prevent total pregnancy failure.

If CRH-R1 blockade by CRH-R1 antagonists prevents implantation, by reducing the inflammatory-like reaction of the endometrium to the invading blastocyst, they might represent a new class of non-steroidal inhibitors of pregnancy at its very early stages. Although the systemic toxicity of this class of compounds has not yet been fully determined, preliminary studies have indicated that they are relatively safe with no apparent toxicity or adverse effects in rats and nonhuman primates (21). Given the promising future of CRH antagonists in the therapy of depression and anxiety disorders (68), their ability to cause hypofertility or early miscarriages should be seriously considered. Nevertheless, in rats, administration of antalarmin after gestation day 5, and until the end of pregnancy, did not affect the embryos, suggesting that other than CRH-mediated FasL expression mechanisms occur in mid- and late-gestation.

#### 4.4. Placental CRH

The human placenta also contains CRH, UCN I, and UCN II. The production of placental CRH occurs in humans and higher primates, but not in other mammals, suggesting that there are different mechanisms regulating human pregnancy (69,70).

Placental CRH is produced in syncytiotrophoblast cells, in placental decidua and fetal membranes (20,71). In pregnant women, plasma concentrations of CRH are low during the first trimester, rise exponentially from mid-gestation to term to reach concentrations as much as 1000-fold greater during the last 6 to 8 weeks of pregnancy compared to concentrations found in the plasma of non-pregnant women (18). The biologic activity of CRH in maternal plasma is attenuated by the presence of a circulating CRH binding protein (CRH-BP), produced by the liver and placenta, which prevents inappropriate stimulation of the stress axis by placental CRH (72,73). Nevertheless, CRH-BP concentrations decrease during the last 6 weeks of pregnancy, leading to elevations of free CRH (72,73). Thus, placental CRH is responsible for the hypercortisolism observed during the latter half of pregnancy. This hypercortisolism is followed by a transient suppression of hypothalamic CRH secretion in the postpartum period, which may explain the blues/depression and autoimmune phenomena seen during this period (68,74,75).

There is no change in UCN I expression throughout gestation (76). During pregnancy, UCN I and II may modulate myometrial contractility (6).

CRH-R1 alpha, CRH-R1 beta and CRH-2 beta are present in human non-pregnant myometrium (77). As pregnancy progresses, the myometrium starts to express the CRH-R2 alpha and CRH-R2 beta, while at term it expresses the CRH-R1c and CRH-R1d receptor subtypes, indicating that these receptor subtypes play a role at the end of pregnancy (78). Therefore, it appears that the biological actions of CRH/UCN I in myometrium during the different trimesters of pregnancy are mediated via different CRH receptor variants (78).

The human placenta expresses the CRH-R1 alpha and CRH-R1c and the fetal membranes express the CRH-R1 alpha, CRH-R1c and CRH-R1d (6). Placental CRH induces dilation of uterine and fetal placental vessels through nitric oxide synthetase activation, and stimulation of smooth muscle contractions through prostaglandin F<sub>2alpha</sub> and E<sub>2</sub> production by fetal membranes and placental decidua (79,80). Placental CRH may also stimulate the production of cortisol and dehydroepiandrosterone from the fetal adrenal gland at the end of the pregnancy (81). Because the placenta cannot directly synthesize estradiol, the fetal adrenal gland is the predominant source of its precursor, dehydroepiandrosterone. This positive loop may also mediate other components of labor, such as expression of oxytocin receptor, gap junctions and prostaglandins (80).

Placental CRH secretion is stimulated by glucocorticoids, inflammatory cytokines, and anoxic conditions, including the stress of preeclampsia or eclampsia (68,82,83), whereas it is suppressed by estrogens (84).

McLean and colleagues suggested that there is a "CRH placental clock" which is active from the early stages of human pregnancy and determines the length of gestation and the timing of parturition and delivery (85). In some women with idiopathic preterm labor, concentrations of CRH increase up to 10 weeks before the development of any symptoms (86). High concentrations of UCN I are also found in women with preterm or term labor (87). Of note, experimental data have shown that CRH receptor type 1 antagonism in the sheep fetus, using antalarmin, can delay the onset of parturition (88). It is possible that in cases of premature labor, a CRH-R1 antagonist might delay or prevent labor (68,89).

#### 4.5 Testicular CRH

CRH is present in the testis of several animal species (90,91) and is localized in Leydig and germ cells and in spermatozoa (22). A major stimulus for Leydig-cell derived CRH is LH/hCG, which results in CRH secretion in a dose-dependent fashion (22). In the rat testis, CRH acts as an anti-reproductive hormone, exerting autocrine inhibitory actions on Leydig cell steroidogenesis (22,92). To the contrary, in mouse Leydig cells, CRH exerts stimulatory effects on steroidogenesis (93).

To our knowledge, there are no studies investigating if CRH is expressed in the human testis and if it exerts inhibitory or stimulatory effects. However, if CRH is expressed in the human testis and has stimulatory actions on testicular function, then blockade of CRH by CRH-non-steroidal inhibitors may represent a new class of male contraceptives.

## 5. SUMMARY AND PERSPECTIVE

CRH, the principal regulator of the hypothalamic-pituitary-adrenal axis, CRH-related peptides and their receptors, have been identified in various organ systems, including the immune and the female and male

reproductive systems. “Immune” and “reproductive” CRH are forms of “tissue” CRH, i.e., CRH found in peripheral tissues.

“Immune” CRH is secreted at inflammatory sites and possesses potent proinflammatory properties, influencing innate and acquired immune processes (4-11). Immune CRH is found in experimentally-induced aseptic inflammations and, in humans, in inflamed tissues from patients with a host of autoimmune and inflammatory diseases (4-11). In addition, immune CRH activates mast cells via a CRH-R1 dependent mechanism, leading to release of histamine and, hence, vasodilation and increased vascular permeability, suggesting potential involvement of the neuropeptide in allergic and vasokinetic processes (30,31). CRH-R1 antagonism, using antalarmin, prevents several proinflammatory effects of CRH, thus revealing its therapeutic potential in some forms of inflammation (4,11,26,28).

Reproductive CRH participates in various reproductive functions with an inflammatory component (12-22). These include ovarian and endometrial CRH, which may participate in the regulation of steroidogenesis and the inflammatory processes of the ovary (ovulation and luteolysis) and the endometrium (decidualization and blastocyst implantation); and placental CRH, which is secreted mostly during the latter half of pregnancy and is responsible for the onset of labor and the physiologic hypercortisolism seen during this period (68,74,85). It has been suggested that there is a “CRH placental clock” which determines the length of gestation and the timing of parturition and delivery. Indeed, all types of premature labor have been associated with elevations in maternal plasma CRH (85). It is possible that, in cases of premature labor, a CRH-R1 antagonist might delay or prevent labor (68).

## 6. REFERENCES

- Vale W., J. Spiess, C. Rivier, & B. Rivier: Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213, 1394-1397 (1981)
- Chrousos G.P. & P.W. Gold: The concepts of stress and stress system disorders. *JAMA* 267,1244-1252 (1992)
- Vaughan J., C. Donaldson, J. Bittencourt, M.H. Perrin, K. Lewis, S. Sutton, R. Chan, A.V. Turnbull, D. Lovejoy, C. Rivier, J. Rivier, P.E. Sawchenko & W. Vale: Urocortin, a mammalian neuropeptide related to urotensin I and to corticotropin-releasing factor. *Nature* 378, 287-292 (1995)
- Florio P., W. Vale & F. Petraglia: Urocortins in human reproduction. *Peptides* 25, 1751-1757 (2004)
- Oki Y. & H. Sasano: Localization and physiological roles of urocortin. *Peptides* 25, 1745-1749 (2004)
- Karteris E., D.K. Grammatopoulos, H.S. Randeva & E.W. Hillhouse: The role of corticotropin-releasing hormone receptors in placenta and fetal membranes during human pregnancy. *Mol Genet Metab* 2001;72:287-296
- Karalis K., H. Sano, J. Redwine, S. Listwak, R.L. Wilder & G.P. Chrousos: Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone *in vivo*. *Science* 254, 421-423 (1991)
- Crofford L. J., H. Sano, K. Karalis, E. L. Webster, E. A. Goldmuntz, G. P. Chrousos & R. L. Wilder: Local secretion of corticotropin-releasing hormone in the joints of Lewis rats with inflammatory arthritis. *J Clin Invest* 90, 2555-2564 (1992)
- Crofford L.J., H. Sano, K. Karalis, T.C. Friedman, H. R. Epps, E. F. Remmers, P. Mathern, G. P. Chrousos & R. L. Wilder: Corticotropin-releasing hormone in synovial fluids and tissues of patients with rheumatoid arthritis and osteoarthritis. *J Immunol* 151, 1587-1596 (1993)
- Kawahito Y., H. Sano, S. Mukai, K. Asai, K. Kimura, Y. Yamamura, H. Kato, G. P. Chrousos, R. L. Wilder & M. Kondo: Corticotropin releasing hormone in colonic mucosa in patients with ulcerative colitis. *Gut* 37, 544-551(1995)
- Mastorakos G., E.A. Bouzas, P.B. Silver, G. Sartani, T.C. Friedman, C.C. Chan, R.R. Caspi & G.P. Chrousos: Immune corticotropin-releasing hormone is present in the eyes of and promotes experimental autoimmune uveoretinitis in rodents. *Endocrinology* 136, 4650-4658 (1995)
- Mastorakos G., E.L. Webster, T.C. Friedman & G.P. Chrousos: Immunoreactive corticotropin-releasing hormone and its binding sites in the rat ovary. *J Clin Invest* 92, 961-968 (1993)
- Mastorakos G., C.D. Scopa, A. Vryonidou, T.C. Friedman, D. Kattis, C. Phenekos, M.J. Merino & G.P. Chrousos: Presence of immunoreactive corticotropin-releasing hormone in normal and polycystic ovaries. *J Clin Endocrinol Metab* 79, 934-939 (1994)
- Mastorakos G., C.D. Scopa, L.C. Kao, A. Vryonidou, T.C. Friedman, D. Kattis, C. Phenekos, D. Rabin & G.P. Chrousos: Presence of immunoreactive corticotropin releasing hormone in human endometrium. *J Clin Endocrinol Metab* 81, 1046-1050 (1996)
- Makriganakis A., E. Zoumakis, A.N. Margioris, P. Theodoropoulos, C. Stournaras & A. Gravanis: The corticotropin-releasing hormone in normal and tumoral epithelial cells of human endometrium. *J Clin Endocrinol Metab* 80, 185-193 (1995)
- Grino M., G.P. Chrousos & A.N. Margioris: The corticotropin releasing hormone gene is expressed in human placenta. *Biochem Biophys Res Commun* 148, 1208-1214 (1987)
- Clifton V.L., J.F. Telfer, A.J. Thompson, I.T. Cameron, T.G. Teoh, S.J. Lye & J.R. Challis: Corticotropin-releasing hormone and proopiomelanocortin-derived peptides are present in human myometrium. *J Clin Endocrinol Metab* 83, 3716-3721 (1998)
- Frim D.M., R.L. Emanuel, B.G. Robinson, C.M. Smas, G.K. Adler & J.A. Majzoub: Characterization and gestational regulation of corticotropin-releasing hormone messenger RNA in human placenta. *J Clin Invest* 82, 287-292 (1988)
- Petraglia F., S. Tabanelli, M.C. Galassi, G.C. Garuti, A.C. Mancini, A.R. Genazzani & E. Gurpide: Human decidua and *in vitro* decidualized endometrial stromal cells at term contain immunoreactive corticotropin-releasing factor (CRF) and CRF-messenger ribonucleic acid. *J Clin Endocrinol Metab* 74, 1427-1431 (1992)

20. Jones S.A., A.N. Brooks & J.R. Challis: Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. *J Clin Endocrinol Metab* 68, 825-830 (1989)
21. Grammatopoulos D. & G.P. Chrousos: Structural and signaling diversity of corticotropin-releasing hormone (CRH) and related peptides and their receptors: potential clinical applications of CRH receptor antagonists. *Trends Endocrinol Metab* 13, 436-444 (2002)
22. Fabri A., J.C. Tinajero & M.L. Dufau: Corticotropin-releasing factor is produced by rat Leydig cells and has a major local antireproductive role in the testis. *Endocrinology* 127, 1541-1543 (1990)
23. Kemp C.F., R.J. Woods & P.J. Lowry: The corticotropin-releasing factor-binding protein: an act of several parts. *Peptides* 19, 1119-1128 (1998)
24. Baigent S.M.: Peripheral corticotropin-releasing hormone and urocortin in the control of the immune response. *Peptides* 22, 809-820 (2001)
25. Bamberger C.M., M. Wald, A.M. Bamberger, S. Ergun, F.U. Beil & H.M. Schulte: Human lymphocytes produce urocortin, but not corticotropin-releasing hormone. *J Clin Endocrinol Metab* 83, 708-711 (1998)
26. Webster E.L., D.B. Lewis, D.J. Torpy, K.E. Zacman, K.C. Rice & G.P. Chrousos: *In vivo* and *in vitro* characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* 137:5747-5750 (1996)
27. Webster E.L., D.J. Torpy, I.J. Elenkov & G.P. Chrousos: Corticotropin-releasing hormone and inflammation. *Ann NY Acad Sci* 840, 21-32 (1998)
28. Scopa C.D., G. Mastorakos, T.C. Friedman, M. Melachrinou, M.J. Merino & G.P. Chrousos: Presence of immunoreactive corticotropin-releasing factor in thyroid lesion. *Am J Pathol* 195, 1159-1167 (1994)
29. Woods R., J. David, S. Baigent, J. Gibbins & P. Lowry: Elevated levels of corticotrophin releasing factor binding protein in the blood of patients suffering from arthritis and septicaemia and the presence of novel ligands in synovial fluid. *Br J Rheumatol* 35, 120-124 (1996)
30. Theoharides T.C., C. Spanos, X. Pang, L. Alferes, K. Ligris, R. Letourneau, J.J. Rozniecki, E. Webster & G.P. Chrousos: Stress-induced intracranial mast cell degranulation. A corticotropin-releasing hormone-mediated effect. *Endocrinology* 136, 5745-5750 (1995)
31. Theoharides T.C., L.K. Singh, W. Boucher, X. Pang, R. Letourneau, E. Webster, & G. Chrousos: Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects. *Endocrinology* 139, 403-413 (1998)
32. Kavelaars A., R.E. Ballieux & C.J. Heijnen: The role of IL-1 in the corticotropin-releasing factor and arginine-vasopressin-induced secretion of immunoreactive  $\beta$ -endorphin by human peripheral blood mononuclear cells. *J Immunol* 142, 2338-2342 (1989)
33. Singh V.K. & C.S.J. Leu: Enhancing effect of corticotropin-releasing neurohormone on the production of interleukin-1 and interleukin-2. *Neuroscience* 120, 151-154 (1990)
34. Genedani S., M. Bernardi, M.G. Baldini & A. Bertolini: Influence of CRH and alpha-MSH on the migration of human monocytes *in vitro*. *Neuropeptides* 23, 99-102 (1992)
35. Angioni S., F. Petraglia, A. Gallinelli, A. Cossarizza, C. Franceschi, M. Muscettola, A.D. Genazzani, N. Surico & A.R. Genazzani: Corticotropin-releasing hormone modulates cytokines release in cultured human peripheral blood mononuclear cells. *Life Sci* 53, 1735-1742 (1993)
36. Singh V.K.: Stimulatory effect of corticotropin-releasing neurohormone on human lymphocyte proliferation and interleukin-2 receptor expression. *J Neuroimmunol* 23, 256-262 (1989)
37. McGillis J.P., A. Park, P. Rubin-Fletter, C. Turck, M.F. Dallman & D.G. Payan: Stimulation of rat B-lymphocyte proliferation by corticotropin-releasing factor. *J Neurosci Res* 23, 346-352 (1989)
38. Koshida H. & Y. Kotake: Corticotropin-releasing hormone enhances the superoxide anion production of rabbit peritoneal macrophages stimulated with N-formyl-methionyl-leucyl-phenylalanine. *Life Sci* 54, 539-543 (1994)
39. Schafer M., S.A. Mousa, Q. Zhang, L. Carter & C. Stein: Expression of corticotropin-releasing factor in inflamed tissue is required for intrinsic peripheral opioid analgesia. *Proc Natl Acad Sci USA* 93, 6096-6100 (1996)
40. Elenkov I.J. & G.P. Chrousos: Stress, hormone, proinflammatory and anti-inflammatory cytokines and autoimmunity. *Ann NY Acad Sci* 966, 290-303 (2002)
41. Merchenthaler I., M.A. Hynes, S. Vingh, A.V. Schally & P. Petrusz: Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat spinal cord. *Brain Res* 275, 373-377 (1983)
42. Skofitsch G., N. Zamir, C. Helke, J.M. Savitt & D.M. Jacobowitz: Corticotropin releasing factor-like immunoreactivity in sensory ganglia and capsaicin sensitive neurons of the rat central nervous system: Colocalization with other neuropeptides. *Peptides* 6, 307-318 (1985)
43. Suda T., N. Tomori, F. Tozawa, T. Mouri, H. Demura & K. Shizume: Distribution and characterization of immunoreactive corticotropin-releasing factor in human tissues. *J Clin Endocrinol Metab* 59, 861-866 (1984)
44. Udelman R., J.P. Harwood, M.A. Millan, G.P. Chrousos, D.S. Goldstein, R. Zimlichman, K.J. Catt & G. Aguilera: Functional corticotropin-releasing factor receptors in the primate peripheral nervous system. *Nature* 319, 146-150 (1986)
45. Asakura H., L.H. Zwain & S.S.C. Yen: Expression of genes encoding corticotropin-releasing factor (CRF), type 1 CRF receptor, and CRF-binding protein and localization of the gene products in the human ovary. *J Clin Endocrinol Metab* 82, 2720-2725 (1997)
46. Calogero A.E., N. Burrello, P. Negri-Cesi, L. Papale, M.A. Palumbo, A. Cianci, S. Sanfilippo & R. D'Agata: Effects of corticotropin-releasing hormone on ovarian estrogen production *in vitro*. *Endocrinology* 137, 4161-4166 (1996)
47. Ghizzoni L., G. Mastorakos, A. Vottero, A. Barreca, M. Furlini, A. Cesarone, B. Ferrari, G.P. Chrousos & S. Bernasconi: Corticotropin-releasing hormone (CRH) inhibits steroid biosynthesis by cultured human granulosa-lutein cells in a CRH and interleukin-1 receptor mediated fashion. *Endocrinology* 138, 4806-4811 (1997)

48. Bromberger J.T., K.A. Matthews, L.H. Kuller, R.R. Wing, E.N. Meilahn & P. Plantinga: Prospective study of the determinants of age at menopause. *Am J Epidemiol* 145, 124-133 (1997)
49. Zoumakis E., E. Chatzaki, I. Charalampopoulos, A.N. Margioris, E. Angelakis, E. Koumantakis & A. Gravanis: Cycle and age-related changes in corticotropin-releasing hormone levels in human endometrium and ovaries. *Gynecol Endocrinol* 15, 98-102 (2001)
50. Muramatsu Y., N. Sugino, T. Suzuki, K. Totsune, K. Takahashi, A. Tashiro A, M. Hongo, Y. Oki & H. Sasano: Urocortin and corticotropin-releasing factor receptor expression in normal cycling human ovaries. *J Clin Endocrinol Metab* 86, 1362-1369 (2001)
51. Makrigiannakis A., A.N. Margioris, C. Le Goascogne, E. Zoumakis, G. Nikas, C. Stournaras, A. Psychoyos & A. Gravanis: Corticotropin-releasing hormone (CRH) is expressed at the implantation sites of early pregnant rat uterus. *Life Sci* 57, 1869-1875 (1995)
52. Ferrari, A., F. Petraglia & E. Gurbide: Corticotropin-releasing factor decidualizes human endometrial stromal cells in vitro. Interaction with progesterin. *J Steroid Biochem Mol Biol* 54, 251-255 (1995)
53. Di Blasio A.M., F.P. Giraldo, P. Vigano, F. Petraglia, M. Vignali & F. Cavagnini: Expression of corticotropin-releasing hormone and its R1 receptor in human endometrial stromal cells. *J Clin Endocrinol Metab* 82, 1594-1597 (1997)
54. Makrigiannakis A., E. Zoumakis, A.N. Margioris, C. Stournaras, G.P. Chrousos & A. Gravanis: Regulation of the promoter of the human corticotropin-releasing hormone gene in transfected human endometrial cells. *Neuroendocrinology* 64, 85-93 (1996)
55. Makrigiannakis A., A.N. Margioris, E. Zoumakis, C. Stournaras & A. Gravanis: The transcription of corticotropin-releasing hormone in human endometrial cells is regulated by cytokines. *Neuroendocrinology* 70, 451-459 (1999)
56. Zoumakis E., A. Margioris, C. Stournaras, E. Dermizaki, E. Angelakis, A. Makrigiannakis, E. Koumantakis & A. Gravanis: Corticotropin-releasing hormone (CRH) interacts with inflammatory prostaglandins and interleukins and affects decidualization of human endometrial stroma. *Mol Hum Reprod* 6, 344-351 (2000)
57. Makrigiannakis A., A. Margioris, E. Chatzaki, E. Zoumakis, G.P. Chrousos & A. Gravanis: The decidualizing effect of progesterone may involve direct transcriptional activation of corticotropin-releasing hormone (CRH) from human endometrial stroma cells. *Mol Hum Reprod* 5, 788-796 (1999)
58. Makrigiannakis A., E. Zoumakis, S. Kalantaridou, C. Coutifaris, A.N. Margioris, G. Coukos, K.C. Rice, A. Gravanis & G.P. Chrousos: Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance. *Nat Immunol* 2, 1018-1024 (2001)
59. Madhappan B., D. Kempuraj, S. Christodoulou, S. Tsapikidis, W. Boucher, V. Karagiannis, A. Athanassiou & T.C. Theoharides: High levels of intrauterine corticotropin-releasing hormone, urocortin, tryptase, and interleukin-8 in spontaneous abortions. *Endocrinology* 144, 2285-2290 (2003)
60. Tabibzadeh S.: Cytokine regulation of human endometrial function. *Ann NY Acad Sci* 622, 89-98 (1991)
61. Innis E., M. Gatanaga, F. Cappuccini, C. Dett, R. Yamamoto, G. Granger & T. Gatanaga: Growth of the endometrial adenocarcinoma cell line AN3CA is modulated by tumor necrosis factor and its receptor is up-regulated by estrogen *in vitro*. *Endocrinology* 130, 1852-1856 (1992)
62. Sheth K.V., G.L. Roca, S.T. Al-Sedairy, R.S. Parhar, C.J. Hamilton, F. Al-Abdul Jabbar: Prediction of successful embryo implantation by measuring interleukin-1-alpha and immunosuppressive factor(s) in preimplantation. *Fertil Steril* 55:952-958 (1991)
63. Lass A., W. Weiser, A. Munafo & E. Loumaye: Leukemia inhibitory factor in human reproduction. *Fertil Steril* 76, 1091-1096 (2001)
64. Simon C., A. Frances, G. Piquette, I. Danasouri, G. Zurawski, W. Dang & M. Polan: Embryonic implantation in mice is blocked by interleukin-1 receptor antagonist. *Endocrinology* 134, 521-529 (1992)
65. Athanassakis I., V. Farmakiotis, I. Aifantis, A. Gravanis & S. Vassiliadis: Expression of corticotropin-releasing hormone in the mouse uterus: participation in embryo implantation. *J Endocrinol* 163, 221-227 (1999)
66. Itoh N., S. Yonehara, A. Ishii, M. Yonehara, S. Mizushima, M. Sameshima, A. Hase, Y. Seto & S. Nagata: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66, 233-243 (1991)
67. Yamashita H., Y. Otsuki, K. Matsumoto, K. Ueki & M. Ueki: Fas ligand, Fas antigen and Bcl-2 expression in human endometrium during the menstrual cycle. *Mol Hum Reprod* 5, 358-364 (1999)
68. Chrousos G.P., D.J. Torpy & P.W. Gold: Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. *Ann Intern Med* 129, 229-240 (1998)
69. Robinson B.G., J.L. Arbiser, R.L. Emanuel & J.A. Majzoub: Species-specific placental corticotropin releasing hormone messenger RNA, and peptide expression. *Mol Cell Endocrinol* 62, 337-341 (1989)
70. Smith R., E.J. Wickings, M.E. Bowman, A. Belleoud, G. Dubreuil, J.J. Davies & G. Madsen: Corticotropin-releasing hormone in chimpanzee and gorilla pregnancies. *J Clin Endocrinol Metab* 84, 2820-2825 (1999)
71. Riley S.C., J.C. Walton, J.M. Herlick & J.R. Challis: The localization and distribution of corticotropin-releasing hormone in the human placenta and fetal membranes throughout gestation. *J Clin Endocrinol Metab* 72, 1001-1007 (1991)
72. Challis J.R., S.G. Matthews, C. Van Meir & M.M. Ramirez: Current topic: the placental corticotropin-releasing hormone-adrenocorticotrophin axis. *Placenta* 16, 481-502 (1995)
73. Linton E.A., A.V. Perkins, R.J. Woods, F. Eben, C.D. Wolfe, D.P. Behan, E. Potter, W.W. Vale & P.J. Lowry: Corticotropin releasing hormone-binding protein (CRH-BP): plasma levels during the third trimester of normal human pregnancy. *J Clin Endocrinol Metab* 76, 260-262 (1993)
74. Magiakou M.A., G. Mastorakos, D. Rabin, B. Dubbert, P.W. Gold & G.P. Chrousos: Hypothalamic corticotropin releasing hormone suppression during the postpartum period: implications for the increase of psychiatric manifestations during this time. *J Clin Endocrinol Metab* 81, 1912-1917 (1996)



75. Elenkov I.J., R.L. Wilder, V.K. Bakalov, A.A. Link, M.A. Dimitrov, S. Fisher, M. Crane, K.S. Kanik & G.P. Chrousos: Interleukin 12, tumor necrosis factor-alpha and hormonal changes during late pregnancy and early postpartum: Implications for autoimmune disease activity during these times. *J Clin Endocrinol Metab* 86, 4933-4938 (2001)
76. Clifton V.L., G. Qing, V.E. Murphy, J. Schwartz, G. Madsen & R. Smith: Localization and characterization of urocortin during human pregnancy. *Placenta* 21, 782-788 (2000)
77. Hillhouse E.W., D. Grammatopoulos, N.G. Milton & H.W. Quintero: The identification of a human myometrial corticotropin-releasing hormone receptor that increases in affinity during pregnancy. *J Clin Endocrinol Metab* 76, 736-741 (1993)
78. Karteris E., N. Papadopoulou, D.K. Grammatopoulos & E.W. Hillhouse: Expression and signaling characteristics of the corticotrophin-releasing hormone receptors during the implantation phase in the human endometrium. *J Mol Endocrinol* 32:21-32 (2004)
79. Chrousos G.P.: Reproductive placental corticotropin-releasing hormone and its clinical implications. *Am J Obstet Gynecol* 180(Suppl), 249-250 (1999)
80. Grammatopoulos D.K. & E.W. Hillhouse: Role of corticotropin-releasing hormone in onset of labour. *Lancet* 354, 1546-1549 (1999)
81. Karalis K., G. Goodwin & J.A. Majzoub: Cortisol blockade of progesterone: a possible molecular mechanism involved in the initiation of human labor. *Nat Med* 2, 556-560 (1996)
82. Robinson B.G., R.L. Emanuel, D.M. Frim & J.A. Majzoub: Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proc Natl Acad Sci (USA)* 85, 5244-5248 (1988)
83. Goland R.S., I.M. Conwell & S. Jozak: The effect of preeclampsia on human placental corticotropin-releasing hormone content and processing. *Placenta* 16, 375-382 (1995)
84. Ni X., R.C. Nicholson, B.R. King, E.C. Chan, M.A. Read & R. Smith: 2002. Estrogen represses whereas the estrogen-antagonist ICI 182780 stimulates placental CRH gene expression. *J Clin Endocrinol Metab* 87, 3774-3778 (2002)
85. McLean M., A. Bisits, J. Davies, R. Woods, P. Lowry & R. Smith: A placental clock controlling the length of human pregnancy. *Nat Med* 1, 460-463 (1995)
86. Wolfe C.D.A., S.P. Patel, E.A. Linton, E.A. Campbell, J. Anderson, A. Dornhorst, P.J. Lowry & M.T. Jones: Plasma corticotropin-releasing factor (CRF) in abnormal pregnancy. *Br J Obstet Gynaecol* 95, 1003-1006 (1988)
87. Florio P., M. Torricelli, L. Galleri, G. De Falco, E. Leucci, G. Calonaci, E. Picciolini, G. Ambrosini, E.A. Linton, F. Petraglia: High fetal urocortin levels at term and preterm labor. *J Clin Endocrinol Metab* 90, 5361-5365 (2005)
88. Cheng-Chan E., J. Falconer, G. Madsen, K.C. Rice, E.L. Webster, G.P. Chrousos & R. Smith: A corticotropin-releasing hormone type 1 receptor antagonist delays parturition in sheep. *Endocrinology* 139, 3357-3360 (1998)
89. Erickson K., P. Thorsen, G. Chrousos, D.E. Grigoriadis, O. Khongsaly, J. Mc Gregor & J. Schulkin: Preterm birth: associated neuroendocrine, medical, and behavioral risk factors. *J Clin Endocrinol Metab* 86, 2544-2552 (2001)
90. Yoon D.J., C. Sklar & R. David: Presence of immunoreactive corticotropin-releasing factor in rat testis. *Endocrinology* 122, 559-561 (1988)
91. Audhya T., C.S. Hollander, D.H. Schlesinger & B. Hutchinson: Structural characterization and localization of corticotropin-releasing factor in testis. *Biochim Biophys Acta* 996, 10-16 (1989)
92. Dufau M.L., J.C. Tinajero & A. Fabbri: Corticotropin-releasing factor: an antireproductive hormone of the testis. *FASEB J* 7, 299-307 (1993)
93. Huang B.M., D.M. Stocco, J.C. Hutson & R.L. Norman: Corticotropin-releasing hormone stimulates steroidogenesis in mouse Leydig cells. *Biol Reprod* 53, 620-626 (1995)

**Abbreviations:** CRH: corticotropin-releasing hormone; CRH-R1: CRH receptor type 1; DC: decidual cell; EVT: extravillous trophoblast cell; Fas: Fas receptor; FasL: Fas ligand; T: T lymphocyte; NK: natural killer cell; m: macrophage

**Key Words:** Peripheral CRH, Immune CRH, Inflammation, Reproductive CRH, Ovarian CRH, Uterine CRH, Implantation, Placental CRH, Testicular CRH

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