

# Hormonal Regulation of Female Reproduction

**Authors** A. Christensen<sup>1,2</sup>, G. E. Bentley<sup>3,4</sup>, R. Cabrera<sup>5</sup>, H. H. Ortega<sup>6,7</sup>, N. Perfito<sup>3,4</sup>, T. J. Wu<sup>8</sup>, P. Micevych<sup>1,2</sup>

**Affiliations** Affiliation addresses are listed at the end of the article

## Key words

- HPG axis
- GnRH
- estradiol
- LH surge

## Abstract

Reproduction is an event that requires the coordination of peripheral organs with the nervous system to ensure that the internal and external environments are optimal for successful procreation of the species. This is accomplished by the hypothalamic-pituitary-gonadal axis that coordinates reproductive behavior with ovulation. The primary signal from the central nervous system is gonadotropin-releasing hormone (GnRH), which modulates the activity of anterior pituitary gonadotropes regulating follicle stimulating hormone (FSH) and luteinizing hormone (LH) release. As ovarian follicles develop they release estradiol, which negatively regulates further

release of GnRH and FSH. As estradiol concentrations peak they trigger the surge release of GnRH, which leads to LH release inducing ovulation. Release of GnRH within the central nervous system helps modulate reproductive behaviors providing a node at which control of reproduction is regulated. To address these issues, this review focuses on several critical questions. How is the HPG axis regulated in species with different reproductive strategies? What internal and external conditions modulate the synthesis and release of GnRH? How does GnRH modulate reproductive behavior within the hypothalamus? How does disease shift the activity of the HPG axis?

## Introduction

The complexity of controlling the hypothalamo-pituitary-gonadal (HPG) axis is apparent on several levels including peripheral hormonal regulation and the extensive network of neuronal and glial cells that mediate this function. While there is a single neuroendocrine output, gonadotropin releasing hormone (GnRH), its regulation depends on the precise coordination of internal and external signals. The relative influence of each environmental signal varies between organisms; some organisms are highly responsive to changes in day length, whereas others are more sensitive to fluctuations in temperature, olfactory input, food availability, or acoustic stimuli. In other vertebrates, this coordination of reproduction is dependent on internal signals such as steroid feedback and protein hormone modulation. In general, though, there is a complex interaction between external environmental cues and a number of internal signaling processes that converge to elicit regulation of the GnRH system, the final common pathway for

transduction of these cues into a reproductive output. This physiological regulation of reproduction to induce ovulation via GnRH needs to be coordinated to regulate behavioral outcome for successful reproduction.

Female mammals exhibit an internal hormonal milieu that is in a daily state of flux and can be perturbed by disturbances in external inputs. Regardless of whether the organism is more internally or externally biased in terms of its reproductive control, estradiol produced by developing follicles in the ovaries has a central role. The ovarian synthesis of estradiol is a carefully regulated system that is coordinated by feedback mechanisms between the hypothalamus, anterior pituitary, and ovaries. It is this estradiol signal that regulates the neural network either inhibiting or facilitating the release of GnRH to act on the pituitary causing the synthesis and release of FSH or LH, ovulation, and reproductive behaviors.

A circuit that includes hypothalamic and limbic nuclei coordinating olfactory, hormonal and environmental information controls female sexual

received 09.12.2011  
accepted 13.02.2012

## Bibliography

**DOI** <http://dx.doi.org/10.1055/s-0032-1306301>  
Published online:  
March 21, 2012  
Horm Metab Res 2012;  
44: 587–591  
© Georg Thieme Verlag KG  
Stuttgart · New York  
ISSN 0018-5043

## Correspondence

### A. Christensen

Department of Neurobiology  
University of California, Los  
Angeles  
650 Charles E Young Drive South  
Los Angeles  
CA 90095  
USA  
Tel.: +1/310/206 0533  
Fax: +1/310/825 2224  
achristensen@mednet.ucla.edu



behavior in rodents, which is precisely timed to coincide with ovulation. Estradiol initially activates the circuit by acting in the arcuate nucleus of the hypothalamus (ARH), sending a projection to the medial preoptic nucleus (MPN) where olfactory and limbic system inputs are integrated and then activates the final common output of the hypothalamus ventromedial nucleus (VMH). Projections of the VMH descend to the periaqueductal gray, the vestibular complex and eventually directly and indirectly innervate motoneurons mediating the reproductive behavior.

There are many reproductive strategies that exist that evolved based on their environmental cues. Whatever the strategy, these animals are submitted to stressors and disease. This review examines the regulation and dysregulation of the LH surge that triggers ovulation, hypothalamic control of reproductive behavior and how these circuits are affected by photoperiod and disease.

### Control of the LH Surge

The surge release of GnRH is triggered by a positive feedback loop involving the rapid increase in plasma estradiol concentration. In rats and humans, both estradiol and progesterone are involved in stimulating the LH surge. In the early stages of the estrus cycle, estradiol levels are low and the release of GnRH is inhibited. As estradiol levels surge at proestrus, GnRH is released and causes the release of LH, which stimulates the production of progesterone in the ovaries. This GnRH release is not directly through the action of estradiol on GnRH cells, but rather through intermediate cells which contain the neuropeptide kisspeptin. Kisspeptin has been shown to be vital for the release of GnRH, and its receptor, GPR54, is located on GnRH cells (reviewed in [1]). According to Micevych and co-workers (recently reviewed in [2]), estradiol has 2 main effects on the neural-glia network mediating the GnRH system in females. As estradiol rises during diestrus, it induces the expression of progesterone receptors (PRs) in kisspeptin neurons, which strongly stimulate GnRH neurons. When estradiol levels peak on proestrus, membrane estrogen receptors (ERs) on astrocytes are activated, which transactivate metabotropic glutamate receptor type 1a (mGluR1a) and initiate the rapid synthesis of neuroprogesterone (neuroP; [2,3]). The local neuroP then stimulates the kisspeptin cells to release kisspeptin onto GnRH cells and causes the release of GnRH onto the pituitary. Cabrera and his colleagues suggest a different hypothesis. They observed that GnRH release may be modified by the neurosteroid allopregnanolone, which is synthesized from progesterone. Allopregnanolone stimulates the release of glutamate activating NMDA receptors on GnRH neurons [4]. The results in this work indicate that allopregnanolone strongly stimulates GnRH release. Neurosteroids have been proposed to interact both directly and indirectly; they bind with their classical receptors and with ionic channels, such as the GABA<sub>A</sub> [5] and the glutamic acid receptors [6]. Such interactions could produce fast modifications of neuronal excitability. Further evidence suggests that gamma-aminobutyric acid (GABA) containing neurons may enhance LH release [7]. The GABAergic system regulates LH release via modulation of catecholaminergic systems that control GnRH secretion. In addition, Cabrera et al. present results suggesting that the neurosteroid allopregnanolone might have an important physiologic function due to its possible action on NMDA receptors [4]. They show an acute

response to allopregnanolone in an in vitro system on GnRH release. These results suggest that allopregnanolone effects on GnRH release could be specifically mediated by NMDA receptors since co-administration of AP-7, an NMDA antagonist, decreased the stimulatory action of the neurosteroid. The reversion observed in the effect of allopregnanolone on glutamate release suggests that this action is also mediated by NMDA receptors. One possible explanation could be the existence of presynaptic NMDA receptors regulating glutamate release in glutamatergic terminals. These competing theories of estrogen positive feedback through neuroP and allopregnanolone are not mutually exclusive because neuroP may be converted to allopregnanolone by 5- $\alpha$  reductase and 3- $\alpha$  hydroxysteroid oxidase reductase, both of which are present in astrocytes [5]. Indeed, both mechanisms are probably involved.

Wu and colleagues focus on the complexity of GnRH function in regulating reproductive function and behavior. They argue that GnRH-(1-5), a metabolite of the decapeptide GnRH, composed of the first 5 amino acids, may be bioactive [8-12]. Previous studies in the laboratory showed that this metabolite stimulates GnRH mRNA expression in the neuronal GT1-7 cell line [12] as well as increasing secreted GnRH pulse amplitude from hypothalamic explants [13]. GnRH treatment, on the other hand, had a negative autoregulatory effect on its own gene expression and peptide secretion, indicating that these effects are not mediated through the GnRH-R. In addition, this pentapeptide is also capable of regulating reproductive behavior [11].

GnRH-(1-5) is produced by the 75-kDa EP24.15 (also known as thimet oligopeptidase). This endopeptidase was identified in the soluble fraction of rat brain homogenates [14] and is widely distributed in a variety of cell and tissue types [15-19]. The enzyme is regulated by protein kinase A [19] and as a thermolysin-like metalloendopeptidase, the enzyme is dependent on zinc [20]. Substrate specificity is dependent upon peptide size (< 17 amino acids), without preference for the amino acid sequence [21]. However, a hydrophobic amino acid residue in the P1 and P2 positions along with a bulky hydrophobic residue in the P3' position increases specificity [22]. The results of studies show that GnRH-(1-5) is biologically active and supports EP24.15's role as an extracellular processing peptidase and converting enzyme. Processing of GnRH by EP 24.15 to GnRH-(1-5) presents itself as an additional layer of regulation of this already complex system.

In seasonal breeders, several nonreproductive hormones affect the release of LH. In birds, it has been proposed that changing day length results in an increase in type 2 iodothyronine deiodinase (*Dio2*), which activates thyroid hormone locally in the mediobasal hypothalamus (MBH). Some potentially exciting findings on the role of thyroid hormones in the avian photoperiodic response have been published in recent years. The first of these findings was that long day lengths induce the gene for *Dio2*, an enzyme which activates thyroid hormone [23]. Long days increase conversion of T<sub>4</sub> into its more bioactive form T<sub>3</sub>. Under long-day conditions, the hypothalamic content of T<sub>3</sub> was about 10-fold higher than under short-day conditions. In addition, the ICV infusion of T<sub>3</sub> induced testicular growth in quail held under non-stimulatory short days. The second finding was that there is high expression of *Dio3* [type 3 deiodinase, an enzyme which inactivates thyroid hormone via conversion of T<sub>4</sub> and T<sub>3</sub> to reverse (r) T<sub>3</sub> and T<sub>2</sub>, respectively] and low expression of *Dio2* under short-day conditions. Conversely, there was low expression of *Dio3* and high expression of *Dio2* under long-day



conditions, indicating increased activation and decreased inactivation of thyroid hormone in response to long days. These findings indicate that thyroid hormone gene switching is one of the earliest events detected in the photoperiodic cascade and that it must occur at or before hour 16 of a long day, as a single 16-h day can induce LH secretion [24,25]. These opposite effects on gene activation are thought to amplify the localized action of thyroid hormones and lead to neuroendocrine changes that cause GnRH secretion a few hours later (although no study on this putative mechanism implying this action on GnRH has measured GnRH synthesis or release – but see below). Exactly how signals from this local regulation of thyroid hormones are processed and transferred to the GnRH system is not yet known, but there is possible involvement of mechanical actions of glia in the median eminence [26]. More recently, a wave of gene expression was identified at hour 14 of a single long day, and included increased thyrotropin (TSH) beta-subunit expression in the pars tuberalis [27]. Central administration of TSH to short-day quail stimulated gonadal growth and expression of *Dio2*. Thus, there appears to be a role for TSH in the regulation of the avian photoperiodic response. However, it is entirely possible that the increase in *Dio2* is not involved in the avian photoperiodic response and is instead a system that co-varies with day length. In support of this, thyroidectomized birds continue to exhibit a long-day gonadal response, despite having no circulating thyroxine for *Dio2* to act on and produce  $T_3$  in the MBH [28]. Furthermore, in a species of wild songbird, exposure to a single long day resulted in an increase in LH without an increase in *Dio2* [29]. This difference was population specific with the more northern population of the same species showing the expected gene switching (i.e., an increase in *Dio2* and decrease of *Dio3* mRNA) while a more southern population did not. Yet both populations show an ultimate increase in FSH mRNA expression in the anterior pituitary and in LH secretion to plasma. These findings suggest that that regulation of *Dio2/Dio3* expression is not required for GnRH and/or gonadotropin stimulation. Current data cannot yet determine whether the differences observed are due to evolutionary or experiential effects. However, they open up new avenues for future work examining ecological and evolutionary variation in the physiological organization of reproductive timing.

### Ovulation and Disease

▼ Dysregulation of ovulation can occur during disease. One variable is an increase in the secretion of LH associated with the development of cystic ovarian disease in cattle (COD). In this disease, variable hormone levels reflect the hypothalamic-pituitary-gonadal axis attempting to maintain homeostatic control of circulating hormones.

COD in cattle is characterized by disruptions of folliculogenesis and the formation of cysts in the ovaries. These changes are correlated with alterations in the expression of ER $\alpha$ , ER $\beta$ , androgen receptor (AR), and PR. High expression of ER $\alpha$  has been described in granulosa and theca cells of cystic follicles, concomitant with a decrease in the expression of ER $\beta$  in cattle [30,31], rats [32], and women [33]. Gonadotrophins and estrogen downregulate granulosa expression of the ER $\beta$  isoforms [34,35] and that both ERs show a tendency to be upregulated together as estrogen levels increase in the follicular fluid. This correlates with the upregulation in the LH and FSH receptors [36]. In mice, ER $\alpha$

overexpression produces downregulation of the ER $\beta$  gene and subfertility [37], which raises the possibility that the decrease in ER $\beta$  observed in cystic follicles may be secondary to ER $\alpha$  upregulation. In this sense, studies with ER $\alpha$ -knockout mice that were anovulatory also indicate that ER $\alpha$  is not required for follicular recruitment or early differentiation but is necessary for subsequent follicular growth [38], an important component of COD pathogenesis in cattle.

PRs are mainly expressed in bovine granulosa cells [39,40]. But, cystic follicles show a higher expression in the thecal cells compared with healthy follicles [31]. Multiple PR isoforms may partly explain the intracellular processes involved in progesterone activation of a target cell. The 116 kDa B-receptor (PRb) and the 94 kDa A-receptor (PRa) can have very different transcriptional functions that are cell specific and promoter specific. Although the PRb and PRa isoforms have similar ligand-binding affinities and similar DNA-binding affinities [41], the different isoforms have different functions and PRb significantly increases in the granulosa of cystic follicles [31]. While selective ablation of PRb does not affect ovarian function, double PR (PRa and PRb) knockout mice fail to ovulate [42].

Animals with COD usually present high levels of circulating estrogen and LH [43], and this could cause alterations in PR expression. One possible scenario is that changes in the ratio of PR isoform expression could regulate the biological activity of progesterone and result in functional hormone withdrawal in the absence of changes in serum concentrations or total progesterone-binding activities of the reproductive tissues [44–47]. Ovaries from animals with COD exhibited an altered steroid receptor expression compared with ovaries from control animals and numerous studies provide evidence that an altered steroid signalling system may be present in the bovine cystic follicles. Therefore, it is reasonable to suggest that in conditions characterized by altered ovulation, such as COD, changes in the expression of ovarian steroid receptors could play a fundamental role in the pathogenesis of this disease.

### Reproductive Behavior

▼ Reproductive behavior in female mammals is timed to coincide with ovulation. Since reproduction in many cases requires some risk to the well-being of the animal, it is only during ovulation, when pregnancy could result, that the risk is acceptable. Estradiol from developing ovarian follicles begins to activate neural circuits that control receptive behavior. In the ARH, estradiol rapidly activates mER $\alpha$  and transactivates the mGluR1 [48]. In both the VMH and the ARH, estradiol increases spine density [49,50]. This increase in dendritic spines is important for the regulation of female sexual behavior [50]. The growth of estradiol-induced spines may play a role in the timing of sexual behavior. A bolus of estradiol given to an ovariectomized rat initially produces immature filopodial spines, which are thought to be insufficient for synaptic transmission. However, at later time points, when the female is sexually receptive, the spines mature to functional mushroom-shaped spines. This change in synaptic morphology underlies critical changes in neuronal connections within the lordosis regulating circuit. When spinogenesis is pharmacologically inhibited in the ARH, females do not display full sexual receptivity.

The interaction between ER $\alpha$  and mGluR1, which has been shown to be critical for reproductive behavior [48], was shown



to be important for the growth of estradiol-mediated spinogenesis as well [50]. The actin depolymerizing factor, cofilin, is rapidly phosphorylated and inactivated during spinogenesis. Estradiol causes the phosphorylation of cofilin. However, when mGluR1 is inhibited, estradiol is unable to increase the phosphorylation of cofilin and the growth of new spines is decreased.

## Conclusions

The regulation of reproduction is multi-faceted. It begins with the regulation of the ovaries. The level of estradiol released by these structures ultimately determines the readiness of the system to advance to the next stage of fertility. The release of LH is then regulated in several ways. Not only must the level of estradiol increase beyond some critical point, but progesterone and allopregnanolone must begin to be synthesized by astrocytes in the MBH. These neurosteroids have several effects, but ultimately lead to the release of GnRH and finally LH. As another level of regulation, GnRH level is controlled by an enzyme that cleaves it. In this way its own concentration of release is increased.

Diseases of the ovary which perturb the secretion of sex steroids disrupt the entire axis. Changes in the secretion of estradiol initiate disruptions in ability of the animal to produce the LH surge required for regulation of the rest of the HPG axis. Indeed, dysregulation of any part of the axis will result in a loss of normal reproductive behavior.

Because ovulation is so closely tied to reproductive behavior, the estradiol that causes the release of LH also has other roles within the reproductive circuit. Importantly, it is able to alter the synaptic connections of the hypothalamus to increase the sexual receptivity of the female. It is only when the entire system is healthy and ready for reproduction that sexual behavior is able to occur successfully.

Seasonal breeders must be able to detect the appropriate time of year for reproduction. In order to do this some part of their internal milieu must change in response to changing day length. It is possible that an increase in the thyroid hormone activating enzyme, *Dio2*, is one method used by some seasonal breeders to prepare for the mating season.

The regulation of the reproductive system is complex and requires the coordination of multiple central and peripheral systems and the input of internal and external environments. There is much known about the neurosteroids and enzymes that are involved centrally in regulating the release of GnRH as well as the roles of steroids and their receptors peripherally. Many questions remain to be answered such as: how are the neuroP and allopregnanolone systems coordinated together to regulate GnRH release? Is the loss of steroid receptors seen in COD the cause or result of the disease? And can this change in receptor levels be observed with other diseases or reproductive senescence? And what is the timing in these cases?

Although neuroendocrinology has unraveled a variety of physiological processes that require the coordination of the brain and body to respond to the external environment to ensure reproductive success, a large number of unresolved questions remain to be answered. Answering these questions will provide the basis for understanding the integration that is regulated by the reproductive neuroendocrine axis.

## Acknowledgements

Supported by NSF IOS#1052288 (TJW) and DoD RO85FN (TJW).

### Affiliations

<sup>1</sup>Department of Neurobiology, University of California, Los Angeles, USA

<sup>2</sup>Laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, USA

<sup>3</sup>Department of Integrative Biology, University of California, Berkeley, USA

<sup>4</sup>Helen Wills Neuroscience Institute, University of California, Berkeley, USA

<sup>5</sup>Instituto de Investigaciones Biomédicas-IMBECU-CONICET, Universidad de Mendoza, Argentina

<sup>6</sup>Morphological Sciences Department, Universidad Nacional de Litoral, Santa Fe, Argentina

<sup>7</sup>Argentine National Research Council, Buenos Aires, Argentina

<sup>8</sup>Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

### References

- 1 Millar RP, Roseweir AK, Tello JA, Anderson RA, George JT, Morgan K, Pawson AJ. Kisspeptin antagonists: unraveling the role of kisspeptin in reproductive physiology. *Brain Res* 2010; 1364: 81–89
- 2 Micevych P, Sinchak K. The neurosteroid progesterone underlies estrogen positive feedback of the LH surge. *Frontiers in Genomic Endocrinology* 2011; 2: 1–11
- 3 Kuo J, Hamid N, Bondar G, Prossnitz ER, Micevych P. Membrane estrogen receptors stimulate intracellular calcium release and progesterone synthesis in hypothalamic astrocytes. *J Neurosci* 2010; 30: 12950–12957
- 4 Giuliani FA, Yunes R, Mohn CE, Laconi M, Rettori V, Cabrera R. Allopregnanolone induces LHRH and glutamate release through NMDA receptor modulation. *Endocrine* 2011; 40: 21–26
- 5 Corpechot C, Young J, Calvel M, Wehrey C, Veltz JN, Touyer G, Mouren M, Prasad VV, Banner C, Sjoval J. Neurosteroids: 3 alpha-hydroxy-5 alpha-pregnan-20-one and its precursors in the brain, plasma, and steroidogenic glands of male and female rats. *Endocrinology* 1993; 133: 1003–1009
- 6 Cabrera RJ, Bregonzio C, Laconi M, Mampel A. Allopregnanolone increase in striatal N-methyl-D-aspartic acid evoked [3H]dopamine release is estrogen and progesterone dependent. *Cell Mol Neurobiol* 2002; 22: 445–454
- 7 Favit A, Wetsel WC, Negro-Vilar A. Differential expression of gamma-aminobutyric acid receptors in immortalized luteinizing hormone-releasing hormone neurons. *Endocrinology* 1993; 133: 1983–1989
- 8 Baldwin EL, Wegorzewska IN, Flora M, Wu TJ. Regulation of type II luteinizing hormone-releasing hormone (LHRH-II) gene expression by the processed peptide of LHRH-I, LHRH-(1-5) in endometrial cells. *Exp Biol Med (Maywood)* 2007; 232: 146–155
- 9 Bourguignon JP, Alvarez Gonzalez ML, Gerard A, Franchimont P. Gonadotropin releasing hormone inhibitory autocrine feedback by subproducts antagonist at N-methyl-D-aspartate receptors: a model of autocrine regulation of peptide secretion. *Endocrinology* 1994; 134: 1589–1592
- 10 Walters K, Chin YP, Wu TJ. A processed metabolite of luteinizing hormone-releasing hormone has proliferative effects in endometrial cells. *Am J Obstet Gynecol* 2007; 196: e31–e35
- 11 Wu TJ, Glucksman MJ, Roberts JL, Mani SK. Facilitation of lordosis in rats by a metabolite of luteinizing hormone releasing hormone. *Endocrinology* 2006; 147: 2544–2549
- 12 Wu TJ, Mani SK, Glucksman MJ, Roberts JL. Stimulation of luteinizing hormone-releasing hormone (LHRH) gene expression in GT1-7 cells by its metabolite, LHRH-(1-5). *Endocrinology* 2005; 146: 280–286
- 13 Roberts JL, Mani SK, Woller MJ, Glucksman MJ, Wu TJ. LHRH-(1-5): a bioactive peptide regulating reproduction. *Trends Endocrinol Metab* 2007; 18: 386–392
- 14 Orłowski M, Michaud C, Chu TG. A soluble metalloendopeptidase from rat brain. Purification of the enzyme and determination of specificity with synthetic and natural peptides. *Eur J Biochem* 1983; 135: 81–88
- 15 Grundker C, Volker P, Emons G. Antiproliferative signaling of luteinizing hormone-releasing hormone in human endometrial and ovarian cancer cells through G protein alpha(1)-mediated activation of phosphotyrosine phosphatase. *Endocrinology* 2001; 142: 2369–2380
- 16 Muller FO, Terblanche J, Schall R, van Zyl Smit R, Tucker T, Marais K, Groenewoud G, Porchet HC, Weiner M, Hawarden D. Pharmacokinetics of triptorelin after intravenous bolus administration in healthy males and in males with renal or hepatic insufficiency. *Br J Clin Pharmacol* 1997; 44: 335–341



- 17 Ramakrishnappa N, Rajamahendran R, Lin YM, Leung PC. GnRH in non-hypothalamic reproductive tissues. *Anim Reprod Sci* 2005; 88: 95–113
- 18 Tiong JD, Pakiam JG, Wray S. Gonadotropin releasing hormone-1 expression in incisors of mice. *Endocrinology* 2004; 145: 3608–3612
- 19 Tullai JW, Cummins PM, Pabon A, Roberts JL, Lopingco MC, Shrimpton CN, Smith AI, Martignetti JA, Ferro ES, Glucksman MJ. The neuropeptide processing enzyme EC 3.4.24.15 is modulated by protein kinase A phosphorylation. *J Biol Chem* 2000; 275: 36514–36522
- 20 Dong KW, Yu KL, Roberts JL. Identification of a major up-stream transcription start site for the human progadotropin-releasing hormone gene used in reproductive tissues and cell lines. *Mol Endocrinol* 1993; 7: 1654–1666
- 21 Ray K, Hines CS, Coll-Rodriguez J, Rodgers DW. Crystal structure of human thimet oligopeptidase provides insight into substrate recognition, regulation, and localization. *J Biol Chem* 2004; 279: 20480–20489
- 22 Swanson TA, Kim SI, Myers M, Pabon A, Philibert KD, Wang M, Glucksman MJ. The role of neuropeptide processing enzymes in endocrine (prostate) cancer: EC 3.4.24.15 (EP24.15). *Protein Pept Lett* 2004; 11: 471–478
- 23 Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S. Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. *Nature* 2003; 426: 178–181
- 24 Meddle SL, Follett BK. Photoperiodically driven changes in Fos expression within the basal tuberal hypothalamus and median eminence of Japanese quail. *J Neurosci* 1997; 17: 8909–8918
- 25 Meddle SL, Follett BK. Photoperiodic activation of fos-like immunoreactive protein in neurons within the tuberal hypothalamus of Japanese quail. *J Comp Physiol A* 1995; 176: 79–89
- 26 Yamamura T, Hirunagi K, Ebihara S, Yoshimura T. Seasonal morphological changes in the neuro-glial interaction between gonadotropin-releasing hormone nerve terminals and glial endfeet in Japanese quail. *Endocrinology* 2004; 145: 4264–4267
- 27 Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, Yasuo S, Katou Y, Kageyama S, Uno Y, Kasukawa T, Iigo M, Sharp PJ, Iwasawa A, Suzuki Y, Sugano S, Niimi T, Mizutani M, Namikawa T, Ebihara S, Ueda HR, Yoshimura T. Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature* 2008; 452: 317–322
- 28 Bentley GE, Goldsmith AR, Dawson A, Glennie LM, Talbot RT, Sharp PJ. Photorefractoriness in European starlings (*Sturnus vulgaris*) is not dependent upon the long-day-induced rise in plasma thyroxine. *Gen Comp Endocrinol* 1997; 107: 428–438
- 29 Perfeto N, Jeong S, Silverin B, Calisi R, Bentley GE, Hau M. Anticipating spring: wild populations differ in expression of key genes for photoperiodic time measurement. *PLoS ONE*, [in revision]
- 30 Salvetti NR, Acosta JC, Gimeno EJ, Muller LA, Mazzini RA, Taboada AF, Ortega HH. Estrogen receptors alpha and beta and progesterone receptors in normal bovine ovarian follicles and cystic ovarian disease. *Vet Pathol* 2007; 44: 373–378
- 31 Alfaro NS, Salvetti NR, Velazquez MM, Stangafarro ML, Rey F, Ortega HH. Steroid receptor mRNA expression in the ovarian follicles of cows with cystic ovarian disease. *Res Vet Sci* 2011 [in press]
- 32 Salvetti NR, Baravalle C, Mira GA, Gimeno EJ, Dallard BE, Rey F, Ortega HH. Heat shock protein 70 and sex steroid receptors in the follicular structures of induced ovarian cysts. *Reprod Domest Anim* 2009; 44: 805–814
- 33 Walters KA, Allan CM, Handelsman DJ. Androgen actions and the ovary. *Biol Reprod* 2008; 78: 380–389
- 34 Byers M, Kuiper GG, Gustafsson JA, Park-Sarge OK. Estrogen receptor-beta mRNA expression in rat ovary: down-regulation by gonadotropins. *Mol Endocrinol* 1997; 11: 172–182
- 35 Sharma SC, Clemens JW, Pisarska MD, Richards JS. Expression and function of estrogen receptor subtypes in granulosa cells: regulation by estradiol and forskolin. *Endocrinology* 1999; 140: 4320–4334
- 36 Berisha B, Pfaffl MW, Schams D. Expression of estrogen and progesterone receptors in the bovine ovary during estrous cycle and pregnancy. *Endocrine* 2002; 17: 207–214
- 37 Tomic D, Frech MS, Babus JK, Symonds D, Furth PA, Koos RD, Flaws JA. Effects of ERalpha overexpression on female reproduction in mice. *Reprod Toxicol* 2007; 23: 317–325
- 38 Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M. Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. *Development* 2000; 127: 4277–4291
- 39 Van den Broeck W, D'Haeseleer M, Coryn M, Simoens P. Cell-specific distribution of progesterone receptors in the bovine ovary. *Reprod Domest Anim* 2002; 37: 314–320
- 40 Jo M, Komar CM, Fortune JE. Gonadotropin surge induces two separate increases in messenger RNA for progesterone receptor in bovine preovulatory follicles. *Biol Reprod* 2002; 67: 1981–1988
- 41 Fang X, Wong S, Mitchell BF. Messenger RNA for progesterone receptor isoforms in the late-gestation rat uterus. *Am J Physiol Endocrinol Metab* 2002; 283: E1167–E1172
- 42 Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM, O'Malley BW. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 1995; 9: 2266–2278
- 43 Vanholder T, Opsomer G, de Kruif A. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. *Reprod Nutr Dev* 2006; 46: 105–119
- 44 Graham JD, Clarke CL. Expression and transcriptional activity of progesterone receptor A and progesterone receptor B in mammalian cells. *Breast Cancer Res* 2002; 4: 187–190
- 45 Schams D, Kohlenberg S, Amselgruber W, Berisha B, Pfaffl MW, Sinowatz F. Expression and localisation of oestrogen and progesterone receptors in the bovine mammary gland during development, function and involution. *J Endocrinol* 2003; 177: 305–317
- 46 Amrozi Kamimura S, Ando T, Hamana K. Distribution of estrogen receptor alpha in the dominant follicles and corpus luteum at the three stages of estrous cycle in Japanese black cows. *J Vet Med Sci* 2004; 66: 1183–1188
- 47 Goldman S, Weiss A, Almalah I, Shalev E. Progesterone receptor expression in human decidua and fetal membranes before and after contractions: possible mechanism for functional progesterone withdrawal. *Mol Hum Reprod* 2005; 11: 269–277
- 48 Dewing P, Boulware MI, Sinchak K, Christensen A, Mermelstein PG, Micevych P. Membrane estrogen receptor-alpha interactions with metabotropic glutamate receptor 1a modulate female sexual receptivity in rats. *J Neurosci* 2007; 27: 9294–9300
- 49 Calizo LH, Flanagan-Cato LM. Estrogen selectively regulates spine density within the dendritic arbor of rat ventromedial hypothalamic neurons. *J Neurosci* 2000; 20: 1589–1596
- 50 Christensen A, Dewing P, Micevych P. Membrane-initiated estradiol signaling induces spinogenesis required for female sexual receptivity. *J Neurosci* 2011; 31: 17583–17589

