



## Clinical Applications of Neuroendocrinology in Female Farm Animals

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### Abstract

The control of female mammalian reproduction is governed by the hypothalamo-pituitary-ovarian endocrine axis. Pulsatile pattern of gonadotrophin-releasing hormone (GnRH) secreted by hypothalamus, stimulates the secretion of luteinizing hormone (LH) and Follicle stimulating hormone (FSH) from anterior pituitary gland. These two gonadotrophins, in turn, are responsible for ovarian follicular growth, steroidogenesis and ovulation. Any factor i.e. environmental, physiological, managemental or physical altering the normal pattern of any of these reproductive hormones compromise fertility.

**Keywords:** Neuroendocrinology; Neurohumoral; Female mammalian reproduction

### Introduction

Earlier, hypothalamic GnRH used to be considered as key determinant of reproductive function, however, now it has been well established that different neurotransmitters and neuropeptides secreted in higher brain centres control the activity of hypothalamic pulse generator [1]. In this context, Geoffrey Harris' 1955 monograph 'Neural Control of the Pituitary Gland' provided the first evidence for neurohumoral control of the anterior pituitary hormones by hypophysial portal vessel blood of chemical substances from the hypothalamus to the anterior pituitary gland. This revolutionized the understanding of endocrine disorders such as pubertal abnormalities and infertility, and their therapeutic management using pituitary and ovarian hormones. However, in farm animals, clinical applications of neuroendocrinology are still far from complete understanding. For profitable farm animal rearing, anestrus and repeat breeding are the major constraints due to huge economic losses encountered with 20%-39% incidence of repeat breeding in cattle and buffalo, and 80% of non-pregnant buffalo failing to exhibit estrus during summer season [2,3]. The failure to detect estrus in a shy breeder like buffalo leads to temporary infertility. For the successful establishment of pregnancy, the prerequisites are accurate detection of estrus onset for predicting the correct time of artificial insemination (AI), pre-ovulatory follicular development and AI in stress-free environment, optimal duration of estrus, AI-ovulation synchrony, post-breeding luteal sufficiency and suppression of luteolytic signal. Various hormonal interventions acting at the level of pituitary or gonads have been tried for the fertility enhancement of dairy cattle and buffalo namely induction of synchronized ovulation in anestrus buffalo, development of an estrus synchronization protocol for buffalo reared in hot and humid conditions, increasing pre-ovulatory follicle diameter, improvement in post-breeding luteal sufficiency and antiluteolytic strategies to establish pregnancy and endocrine strategies to normalize duration of estrus and establish pregnancy in repeat breeder crossbred cattle with history of prolonged estrus. Besides all these efforts, the success rate in problematic animals following application of these protocols is up to 50%-60% [2]. Thus, interventions targeting central level may act in a more usual way to improve the function of pituitary and gonads. To further augment the reproductive efficiency of farm animals, the scientists are aiming at the hypothalamus, the black box. This warrants strategies in farm animals aiming at neuroendocrine control of GnRH and hence pituitary and gonadal axis.

### Gonadotrophin-releasing-hormone (GnRH)

In response to environmental factors, external stimuli or physiological demands of the body, messages are transmitted from higher brain centers *via* different neurotransmitters/neuropeptides to regulate the GnRH secretion from hypothalamus. This GnRH is responsible for gonadotrophin release (LH and FSH) from anterior pituitary which in turn causes the secretion of ovarian steroids (progesterone and oestradiol). These latter hormones through their positive and negative feedback on higher brain centers or hypothalamus or at pituitary controls the secretion of GnRH/gonadotrophins and this way normal endocrine system of reproduction is maintained. The positive and negative feedback of ovarian steroids controlling GnRH secretion depends upon stage of reproductive cycle [4] and mediated by different neurotransmitters/neuropeptides at brain level where opioids, GABA, noradrenaline, kisspeptin etc are the possible candidates mediating this process during luteal and follicular phase [1,5]. Thus, GnRH being key determinant of gonadotrophins and ovarian steroids is being used clinically in farm animals in regulation of reproduction and treatment of infertility such as ovarian follicular cysts, delayed ovulation or anovulation, acyclicity, to improve pregnancy rates and estrus synchronization.

### Oxytocin

Oxytocin is synthesized in the supraoptic and para-ventricular nucleus of the hypothalamus and transported in the small vesicles enclosed by membrane down the hypothalamic-hypophyseal nerve axons and stored in the posterior pituitary as neurosecretory granules until released in circulation. Stretching of cervix during parturition or tactile stimuli during suckling or milking causes reflex release of oxytocin in circulation. Its main action is contraction of smooth muscles of the uterus and cells of the mammary glands. Thus used

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as induction of parturition in horses and bitches, to precipitate labor, accelerate normal parturition, evacuation of uterine debris and in the treatment of retention of placenta, post-operative uterine hemorrhage, metritis, uterine involution, post-partum prolapse and letdown of milk. However, full dilatation of cervix should be ensured for its use in clinical cases.

## Kisspeptin

The role and interactions of kisspeptin in the hypothalamus suggests various potential targets for the treatment of subfertility. In fact, kisspeptin also participates in the translation of signals of nutritional state and stress into reproductive capacity *via* GnRH signaling.

Kisspeptin, a protein product of the *KISS1* gene, and acting *via* the kisspeptin receptor, depolarizes hypothalamic gonadotrophin releasing hormone (GnRH) neurons to control GnRH release. Kisspeptin neurons exist in close apposition with GnRH neurons in the hypothalamus and GnRH neurons express the kisspeptin receptor [6]. In addition, Kisspeptin also has a potentially direct gonadal impact [6]. Ongoing research regarding the neuroendocrine control of GnRH neurons have revealed that Kisspeptin, expressed in hypothalamic arcuate and preoptic area in sheep, is one of the candidates involves in regulation of GnRH during the estrous cycle [7,8]. Kisspeptin plays a critical role in the onset of puberty in humans [6]. Preliminary studies have been carried out for the induction of LH by kisspeptin administration in pre-pubertal calves and ovariectomized cows [9]. Also, Kisspeptin expression increases in the ovary at the time of ovulation in rats [10]. Administration of a single intravenous dose of kisspeptin induced short-lived stimulation of LH release, whereas, a repeated administration induced a sustained FSH and LH release in ewe. The systemic delivery of kisspeptin induced LH surges by activating estradiol-positive feedback on gonadotropin secretion in acyclic ewes [11]. However, treatment protocols are yet to be fully standardized with regard to kisspeptin in farm animals. In goats, continuous subcutaneous infusion of kisspeptin *via* osmotic mini pumps leads to receptor desensitization and marked suppression of LH by day 3 post-treatment [12].

Kisspeptin can stimulate the quiescent hypothalamo-hypophyseal ovarian axis of ewes in anestrus. Kisspeptin resulted in ovulation in >80% anoestrus ewes [13]. The response to kisspeptin treatment was most effective in the late follicular phase [14]. Prolactin hormone was suggested to be elevated in summer season in buffalo. In this regard, Kisspeptin can be useful as hyperprolactinemia-induced ovarian acyclicity was reversed by kisspeptin administration in humans [15]. Thus, summer season acyclicity can be treated by kisspeptin infusion in buffalo during summer season.

Kisspeptin is also a regulator of seasonal reproduction. Increased arcuate kisspeptin expression was observed in ewes during short day conditions, but there was no change in kisspeptin expression levels in preoptic area. Conversely, during long day periods kisspeptin expression in the arcuate of ewes is reduced. Furthermore, kisspeptin administration in seasonally acyclic ewes induces ovulation. Also, GnRH responses to kisspeptin are greater in anestrus ewes compared with luteal phase ewes. In addition, kisspeptin receptor expression on GnRH neurons was greater during the non-breeding season compared with the breeding season [6].

## Melatonin

Neuroendocrine studies in ewes strongly suggest a hypothalamic and pituitary target of melatonin to modulate the reproductive axis. Melatonin, a pineal gland hormone and a chemical expression of

darkness, is synthesized and secreted mainly during the hours of darkness. Declining day length is associated with a longer pattern of melatonin secretion, which is stimulatory to reproductive axis in short-day breeder like ewe [16]. In this species, the variations in GnRH/LH pulse frequency between long-day (one pulse/6 h) and short-day (10 pulses/6 h) photoperiod is determined by the duration of melatonin release [17]. The Mediterranean buffalo showing seasonal reproductive trend had highest nighttime plasma melatonin in winter and lowest in summer [18]. In another study carried out on buffalo heifers and buffalo cows, the melatonin trend showed remarkable differences between seasons. In peak summer because of the shortest night, the lowest plasma melatonin with less persistence of melatonin peak were found, while the highest concentrations were noted in early winter corresponding to the start of hypothalamus-pituitary-ovarian axis activity [19]. Moreover, low plasma melatonin was associated with a low seasonal ovulatory activity in buffaloes [18]. This decrease in ovulatory activity during long days happens despite the presence of ovulatory size (12–14 mm) follicles on the ovaries of buffaloes [20]. In European countries, during summer anoestrous, subcutaneous insertion of melatonin implants in ewes is widely practiced for the activation of hypothalamo-pituitary GnRH/LH axis [21]. Treatment with slow-release melatonin implant mimics the effect of short days by lengthening the daily presence of melatonin. However, this activation of gonadotropic system in anoestrous ewes leading to the onset of oestrous requires several weeks of exposure to melatonin, during which a change in the feedback action of oestradiol on GnRH/LH plays a pivotal role [17]. Intriguingly, daily administration of melatonin in form of injection induced oestrous in summer anoestrous buffaloes [22]. A preliminary study in buffalo revealed induction of ovulation of ovulatory size non-ovulatory follicles and initiation of ovarian cyclicity in summer anoestrous buffalo heifers using melatonin implants [23]. The treatment induced rise in circulating melatonin, through its action both on hypothalamus and pituitary is, in turn, responsible for at least 10-fold increase in plasma concentrations of GnRH and gonadotrophins, thus leading to follicular growth and ovulation in ewe [17]. However, studies in buffalo are warranted to identify the differences in individual buffalo with regard to endogenous melatonin rhythms, and alterations in endogenous melatonin and LH following melatonin treatment of summer anoestrous buffalo heifers. Nevertheless, in recent studies conducted at Central Institute for Research in Buffalo, Hisar (India), melatonin treatment of buffalo heifers during out-of-breeding season under tropical conditions increased serum melatonin, increased the diameter of the largest follicle and the number of large follicles between days 0 and 35 of melatonin treatment, as well as increased the conception rate, however, without any significant impact on the LH pulse frequency during the treatment period [24,25].

## Noradrenaline and GABA

It has been suggested that brainstem noradrenergic systems have a stimulatory role during oestradiol-induced surge induction process but a suppressive effect has been reported on tonic LH release [26]. Associated with the preovulatory surge, an increase in noradrenaline release occurs in the POA and ARC in ewe [27]. Conversely, GABA has an inhibitory effect on GnRH/LH secretion. GABA neurons, as well as a dense plexus of GABA fibers are located within the ewe POA. In this area, GABA may directly control GnRH perikarya as GABA terminals synapse directly on GnRH cell bodies in the ewe and GABAA receptor mRNA co-localizes with GnRH neurons [28,29]. In ewe, about 44% neurons synthesizing GABA co-localize ER $\alpha$  in the POA and these account for 30% of all ER $\alpha$ -positive cells in this area [30].

Estrogen-receptive GABA neurons could mediate negative feedback of estrogen on GnRH neurons as GABA concentrations are increased in the POA following estrogen signal in ewes. Moreover, there is marked decrease in GABA tone before the generation of an estrogen-induced preovulatory LH surge in ewe [31]. The start of a slow decline in GABA concentrations well in advance (by 10 h) of first rise in the LH surge indicates the permissive nature of GABA neurons [31]. Thus, use of noradrenergic and GABA receptor agonist and antagonist in control of fertility has promising future.

## Opioids

The various opioid effects on GnRH neurons are mediated through three well-known opioid receptor (OR) subtypes designated as mu ( $\mu$ -), delta ( $\delta$ -) or kappa ( $\kappa$ -) and are encoded from the genes; *Mor-1*, *Dor-1* and *Kor-1* [26]. The ORs exist in two potential sites of opioid action in the ewe hypothalamus viz., POA and MBH. Opioids in the hypothalamus form important inhibitory pathways of neural circuitry that are involved in regulation of gonadotrophin secretion. Although opioids act in both the POA and MBH to inhibit GnRH/LH secretion, different populations of opioid neurons may be activated during different phases of the estrous cycle with a negative effect on LH secretion particularly during luteal phase [4,28].

Either direct or a local inter-neuronal network in the POA between the MBH neurons and GnRH perikarya transmits opioid effects from the MBH to the POA. Further, in ewe a delayed and transient effect of an OR agonist on plasma LH concentrations suggests that opioids influence GnRH cell bodies through inter-neurons. Noradrenaline, GABA, glutamate and/or nitric oxide-positive neurons may be potential mediators as opioids restrain stimulatory catecholamine, glutamate and nitric oxide input and accelerate inhibitory GABA input to GnRH neurons [28]. Opioid agonist met-enkephalin abolished pulsatile LH secretion during follicular phase and resulted anovulation and this effect was reversed by naloxone administration [32]. Similarly, in anestrus ewes during non-breeding season, naloxone treatment resulted early and prolonged LH surge in induced oestrus [33]. Thus, experimentally, opioid antagonist naloxone/naltrexone has been demonstrated and well accepted to initiate abrupt release of LH and also to reverse stress-induced inhibition of LH surge [34,35]. However, its role in clinical cases is yet to be established.

## Stress hormone antagonists

Ovulatory defects are known to impair dairy cow fertility. Although the underlying mechanism is not entirely clear, stress-induced alteration in adrenal hormone secretion can cause these anovulation and ovarian pathologies [4]. Stressors and administration of ACTH throughout preovulatory follicle development alters follicular steroidogenesis in association with impaired angiogenesis, thus suggesting the mechanism underlying ovulation failure and the formation of persistent or cystic follicles under stress [36-38]. Hypothalamic corticotropin-releasing hormone (CRH) plays a prominent role in mediating the effect of stressors on the hypothalamic-pituitary-adrenocortical axis, and in coordinating the endocrine, autonomic, behavioral and immune responses to stress [39]. Promising results regarding reducing stress measures can be obtained using the new class of peptide and non-peptide antagonists of CRH receptors. The neuroendocrine information for these drugs is still incomplete and studies are warranted in female farm animals regarding the drugs that could inhibit the neuroendocrine response to stressors. In addition, Opioid antagonist naloxone reversed stress-induced inhibition of LH surge [34] thus its role in clinical cases of stress-induced infertility is advocated.

Stress inhibits reproductive function by suppressing GnRH release through main role of hypothalamic corticotrophin releasing factor (CRF). The expression of kisspeptin and its receptor is reduced in the arcuate and medial preoptic area of mice in response to central injection of CRF. Reduced kisspeptin expression was also observed in response to stressors like restraint, insulin-induced hypoglycaemia and lipopoly-saccharide, thus suggesting that altered kisspeptin may contribute to stress-induced suppression of reproductive function [6] and its application in reversal of stress-induced infertility is expected.

## Conclusions

Existing standard endocrine interventions aiming at the pituitary and gonadal level have side-effects associated with premature or delayed occurrence of events such as LH surge, thus appropriate level of fertility is not being attained in subfertile farm animals. On the other hand, neuroendocrine approaches may represent a novel target in the treatment of fertility disorders. These approaches tend to be more natural as they target the central system in the hypothalamus, which ultimately controls reproductive axis downstream. Following clinical application of neuroendocrine approaches, the ovarian changes during estrous cycle may be more sequential and may lead restore adequate level of fertility in subfertile animals. However, the neuroendocrine applications in farm animals for fertility improvement are expensive and are at preliminary stage of scientific research. Nevertheless, these approaches may be successfully used to develop new or improve existing fertility treatments and thus have the potential to revolutionize the therapeutic strategies for fertility disorders.

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