



CHALLENGES IN ASSESSMENT OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS

The feedback system that regulates the endocrine control of reproduction is called the hypothalamic-pituitary-gonadal (HPG) axis. Dysfunction of the HPG-axis can result in several clinical problems, for example: primary or secondary anestrus, decreased libido in males or impaired spermatogenesis. Furthermore, in cases of suspected remnant ovarian tissue or a disorder of sexual development, functioning of the HPG-axis may be altered. Knowledge about the regulation of the HPG-axis is essential for clinical research concerning diagnostic and therapeutic possibilities in patients with endocrine-related reproductive problems.

The synthesis and secretion of gonadotropin releasing hormone (GnRH) by the hypothalamus and luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior lobe of the pituitary gland are regulated by many factors, including the gonadal steroids (estrogens, progesterone, and testosterone). One of the fundamental questions regarding the operation of the HPG axis is the means by which sex steroids act to modulate GnRH secretion, since GnRH-producing cells do not express the relevant sex steroid receptors, i.e., the androgen receptor and the estrogen receptor. In 2003, two independent research groups discovered that the kisspeptin receptor (KiSS1R) and by implication, its ligands, kisspeptins (KPs), was essential for normal reproduction (Seminara et al. 2003; de Roux et al. 2003). Kisspeptins, a group of peptides encoded by the *KiSS1* gene, are potent stimulators of GnRH neurons and together with their receptor, KiSS1R, they are required for normal functioning of the HPG axis. Loss-of-function mutations in the *KiSS1R* gene or the *KiSS1* gene, as demonstrated in humans and mice, lead to isolated hypogonadotropic hypogonadism (IHH), caused by deficient GnRH secretion and consequently deficient pituitary secretion of LH and FSH. This results in impairment of both pubertal maturation and reproductive function. Kisspeptin-secreting neurons in the hypothalamus express receptors for gonadal steroids and they project directly to GnRH cell bodies which contain the KiSS1R. Gonadal steroids modulate the expression of KiSS1 mRNA in these hypothalamic areas. These findings strongly suggest that sex steroid feedback regulation of GnRH-producing cells is predominantly exerted via KP cells. Kisspeptin

signaling appeared to be essential for normal reproductive function. Administration of KP results in a rise of plasma LH, FSH and gonadal steroid concentration. The dog appeared to be relatively sensitive to KP, as the LH and FSH response is both quicker and higher compared to most other species ¹.

Due to the pulsatile character of pituitary and gonadal hormones, a single determination of the plasma hormone concentration is often not sufficient to draw a conclusion about functioning of the HPG-axis. Provocative tests with exogenous GnRH can be used to assess the HPG-axis (a so-called GnRH stimulation test). After intravenous injection of a GnRH analogue, a significant rise in plasma estradiol concentration is observed in anestrus bitches and male dogs, but not in ovariectomized bitches and castrated male dogs ². In both male and female dogs, mean basal plasma LH and FSH concentrations increase after gonadectomy, due to the loss of negative feedback of gonadal hormones. Despite the high basal plasma concentration of gonadotropic hormones in gonadectomized dogs, exogenous GnRH administration results in an increase of plasma LH concentrations as soon as 10 minutes after the GnRH administration. For the assessment of the HPG-axis, for example to determine the presence or function of ovarian tissue, both gonadotropic hormones and estradiol should be determined during a GnRH stimulation test. Cycle stage should be taken into account, as it can affect the results.

Another challenge can be the assessment of the functionality of the testes during temporary chemical castration by the use of a slow-release GnRH agonist (deslorelin) implant. The GnRH receptor on the gonadotropes becomes desensitized after long-term stimulation. The duration of downregulation of the HPG axis is dependent on the dose of deslorelin, albeit with large inter-individual variation. The time to complete recovery of the HPG axis has been reported to vary between 360 and 680 days after implantation of 6 mg deslorelin ³. Downregulation of the GnRH receptors on the gonadotropes results in a decrease in the plasma LH and FSH concentrations. To evaluate the function of testicular tissue in a male dog treated with a slow-release GnRH agonist, the size of the testicles and/or the prostate gland can be measured, as both will decrease in size when downregulation of the HPG axis is achieved. However, the size of the testicles and/or the prostate will only be a reliable parameter if they can be compared with

COMPANION ANIMAL

ENDOCRINOLOGY

pretreatment size. If this is unknown, it can be difficult to ascertain that the deslorelin implant was administered correctly and that full downregulation of the HPG axis has been achieved. A GnRH stimulation test can be used to evaluate the HPG axis during deslorelin treatment.

In conclusion, for an assessment of the HPG-axis, a GnRH stimulation test is the most reliable and complete diagnostic procedure. Additionally, a GnRH stimulation test is the only reliable way to prove absence of functional gonadal tissue. However, it is invasive, requires more than one blood sample, and is somewhat time consuming (the maximum plasma estradiol concentration after GnRH injection is only reached after 60-120 minutes)².

References

- 1 Albers-Wolthers KHJ, De Gier J, Kooistra HS, Rutten VPMG, Van Kooten PJS, De Graaf JJ, Leegwater PAJ, Millar RP, Schaefer-Okkens AC. Identification of a novel kisspeptin with high gonadotrophin stimulatory activity in the dog. *Neuroendocrinology* 2014;99:178-89
- 2 de Gier J, Buijtels JJCWM, Albers-Wolthers CHJ, Oei CHY, Kooistra HS, Okkens AC. Effects of gonadotropin-releasing hormone administration on the pituitary-gonadal axis in male and female dogs before and after gonadectomy. *Theriogenology* 2012;77:967-78
- 3 Junaidi A, Williamson PE, Martin GB, Blackberry MA, Cummins JM, Trigg TE. Dose-response studies for pituitary and testicular function in male dogs treated with the GnRH superagonist, deslorelin. *Reprod Domest Anim* 2009;44:725-34.