CHAPTER 2

Literature review

The decapeptide hormone gonadotrophins releasing hormone (GnRH) or luteinizing hormone releasing hormone (LHRH) is synthesized by neurosecretory cells and stored in the medial basal hypothalamus (Bearden and Fuquay, 1997). In response to neural signals, GnRH is released by hypothalamic nerve endings into the portal capillaries, and is delivered to the anterior pituitary gland by the hypophyseal portal veins ,where it stimulates release of FSH and LH (Bearden and Fuquay, 1997). The GnRH neuronal network is extremely complex and is influenced by several different areas of the brain. Both the frequency and the amplitude of GnRH release are important features of its function in stimulating the release of FSH and LH. With a neuroendocrine mechanism, GnRH controls gonadotrophin synthesis by acting toward at pituitary receptors in a pulsatile manner to exert its physiological effect of activating and maintaining the reproductive status from gonadotrophin synthesis ,and releasing to gametogenesis and gonadal steroidogenesis (Miller, 1993). It is well recognized that two pituitary gonadotrophins control gonadal function in mammals, LH and FSH. Triple-point control of gonadotrophin synthesis and secretion are accompanied with control point 1 which is involved GnRH output from the hypothalamus in a pulsatile manner, control point 2 which is involved GnRH receptor number and/or its sensitivity to GnRH at the pituitary gonadotrope level and control point 3 which is involved regulation of gene expression within the pituitary gland (Miller, 1993).

The number of GnRH receptors on the pituitary gonadotropes can be changeable. Changes in the number of GnRH receptors in the pituitary glands have been characterized during many physiological conditions, particularly in the female. The number of GnRH receptors increases (up-regulated) during estrous cycle and get maximal level at pre-ovulatory surge of LH (Conn, 1994). However, many treatments *in vitro* can alter the number of receptors for GnRH. Continuous presence of GnRH results in a down-regulation of its receptors on the pituitary gonadotropes and a shutdown of

in a down-regulation of its receptors on the pituitary gonadotropes and a shutdown of gonadotrophin release which subsequently suppress reproductive function in male and female dogs (Trigg et al., 2001). Down-regulation of receptors is believed to involve agonist-occupied receptors (Barden et al., 1989). In addition to pituitary receptor down-regulation, GnRH can interact directly with homologous, high affinity and specificity receptors on the Leydig cell (Clayton et al., 1980 cited by Vickery, 1985a). Very potent agonistic analogues of GnRH are now available and undergoing clinical evaluation in many species. Synthetic agonists frequently share the presence of a D-amino acid which enhances its affinity to bind receptors (Conn, 1994). Their increased potency over GnRH itself results from increased receptor binding affinity and/or resistance to metabolism (Vickery, 1985a).

After daily GnRH agonist administration to prepubertal dogs, LH-secreting cells in the pituitary gland of male and female dogs have high levels of glycogen particles in their cytoplasm and tend to be either of normal appearance with dilated rough endoplasmic reticulum (RER) or strongly atrophied with a dark-stained cytoplasm, a contraction of RER, and a decrease in the number of secretory granules (Lacoste et al., 1989). Also the GnRH-stimulated high levels of LH can down regulate its homologous receptors at the gonad, leading to inhibitory effects on testicular function (Chasalow et al., 1979).

The marked inhibitory effect of chronic treatment with GnRH agonists on testicular testosterone formation was first observed in the rat (Belanger et al., 1980). A potent inhibitory effect of such treatment on plasma testosterone levels has also been observed in man (Labrie et al., 1980), ram (Fraser and Lincoln, 1980) and dog (Vickery et al., 1981). Daily administration of GnRH agonists to mature rats was early shown to have suppressive effects on both steroidogenesis and spermatogenesis (Labrie et al., 1978). These observations were forecast as leading to an eventual contraceptive use for men (Labrie et al., 1980).

More detailed studies have revealed however that, whether judged by histology or by mating experiments, complete suppression of fertility cannot be achieved in rats by use of the agonists (Vickery et al., 1983). GnRH agonist administration caused an impairment of spermatogenesis which was also not completely reversible in the rat (Pelletier et al., 1978), whereas normal spermatogenesis and fertility were obtained after cessation of GnRH agonist treatment in the dog (Tremblay et al., 1984) and man (Linde et al., 1981 cited by Dude et al., 1987). This is in spite of the findings of multiple mechanisms of action of GnRH agonists in the rat but not in other species.

The male dog is particularly sensitive to the suppressive effects of GnRH agonists on testicular function, which however appear to be mediated solely through pituitary down regulation (Vickery et al., 1984). Inhibition of spermatogenesis is rapid at higher dose (2 or 10 μg/kg/day) of nafarelin acetate, a potent GnRH agonist, noticeable histologically by 10 days of treatment but slower at 0.5 μg/kg/day, suggesting probably a reflection of the time to down regulation at the pituitary (Vickery et al., 1985b). Eventually however at all of dose levels, total inhibition of spermatogenesis to presence of spermatogonia and Sertoli cells is achieved (Vickery et al., 1985b). The initial influence on serum testosterone after GnRH agonist treatment in dog was demonstrated by Lacoste et al., 1988. Daily subcutaneous administration of GnRH agonist in adult dogs caused a transient increase in the serum testosterone concentration on days 2 to 4 of treatment and subsequent progressively decreases. After cessation of long term treatment of GnRH agonist, recovery of spermatogenesis become and spermatozoa begin to appear in the ejaculate ,however the recovery period depends on the dosage used.

In mammals, the importance of gonadotrophins in spermatogenesis is generally recognized. Mammalian testes contain two primary elements, the gamete producing seminiferous tubules and the sex steroids producing interstitial tissue. The quantitative changes occurring in the seminiferous tubules and testicular atrophy after GnRH agonist treatment have been described by Vickery et al. (1982), Dube et al. (1987) and Paramo et al. (1993).

The prostate is the only accessory sex gland in the male dog. It is an encapsulated, bilobed, and bilaterally symmetrical ovoid gland, located caudal to the bladder, encircling the proximal urethra (Garraway et al., 1991). Recently, dogs have been living longer, and canine benign prostatic hypertrophy (BPH) has become an important age-related disease (Kawakami et al., 1998). More than 80% of sexually intact male dogs, the prostate hypertrophy were reported to develop by > 5 years of age with gross or microscopic evidence of BPH (Sirinarumitr et al., 2001). To maintain prostatic size, dihydrotestosterone (DHT) is well accepted as a key hormone in men and dogs by enhancing growth in the stromal and glandular compartment (Lee, 1996). Testosterone is metabolized by the enzyme 5α-reductase into DHT. DHT has a receptor binding affinity about twice that of testosterone, and the rate of dissociation of DHT from its receptor is a fifth of the rate of dissociation of testosterone (Grino et al., 1990). Castration is the recommended treatment for most dogs with BPH. However, an effective medical treatment is needed for BPH dogs and for dogs for which anesthesia or surgery poses an unacceptable risk. The use of chemicals including GnRH agonists to induce prostatic regression in dogs has been investigated extensively and has revealed great potential (Lacoste et al., 1989; Iguer-Ouada and Verstegen, 1997; Kawakami et al., 1998; Tsutsui et al., 2001; Murakoshi et al., 2000; Sirinarumitr et al., 2001).