CHAPTER 5

Discussion

The results of the present study clearly demonstrates that GnRH agonist deslorelin suppressed reproductive function of male dogs and indicates that such influence can be completely reversible after long-term suppression.

Dogs treated with deslorelin-containing or placebo implants did not show any inflammatory reactions at the site of implantation during the initial two-week intensive observation period or at any time of study. The body condition, whether judged by body weight or by general appearance of dogs in both groups were normal throughout the study, suggesting that the implant has no untoward effects on animal.

GnRH agonists induce a shutdown of male reproductive function by acting directly on the pituitary gonadotropes resulting in a down-regulation of GnRH receptors and subsequently suppressing testosterone produced by Leydig cells. Leydig cells do not store testosterone but rapidly secrete all that is manufactured (Galil and Setchell, 1987). The secreted testosterone first enters interstitial fluid and may then diffuse or be transported onto the seminiferous tubules or into the blood circulation. The amount of testosterone leaving the testis in blood is determined by blood flow (Galil and Setchell, 1987). Since this hormone is crucial for fertility, testosterone deprivation leads to loss of male reproductive functional capacity.

Basal testosterone is usually between 0.5-1.5 ng/ml, rising to a peak of 3.5-6.0 ng/ml (Hewitt, 1998). Pretreatment serum testosterone concentrations of both groups were in normal range. After implantation, mean serum testosterone concentrations of the treatment-group dogs declined rapidly to reach an under basal level within 8 weeks, and exhibited significant decrease from week 8 to week 24. Testosterone levels in the majority of treated dogs fell more rapidly than indicated by the mean which included Dog 3, a slow responder. The reasons for this slower response are speculated on later

Dog 3, a slow responder. The reasons for this slower response are speculated on later in the thesis. Testosterone deprivation shown in this study suggested that the drug was absorbed and directly inhibited endocrine function of the testis in dogs, agreeing with Vickery et al (1984). This has been demonstrated also in rats (Belanger et al., 1980), rams (Fraser and Lincoln, 1980) and men (Labrie et al., 1980). The decrease of mean serum testosterone concentrations following treatment was similar to the testosterone level observed in castrated dogs, which range between 0-0.25 ng/ml (Vickery et al., 1985b), thus suggesting that administration of deslorelin in the dog induces similar changes in serum testosterone concentrations to castration. Blood sample collection for testosterone assay was initially taken at the second week after implantation, so the increasing in testosterone level was not observed. The reason of this event may due to the up-regulation mechanism which responses within a few days after GnRH agonists administration (Lacoste et al., 1998). In the control-group dogs, although mean serum testosterone concentrations significantly increased at several times during this study, those levels still remained in normal range.

The dogs treated with deslorelin allowed the procedure of semen collection throughout the observation period even ejaculates were unable to be obtained which was different from the result of a previous study (Paramo et al., 1993). Those authors reported that dogs treated with the nafarelin derivative allowed collection of semen only during the initial treatment period. There is no evidence that impotence in the dog is caused by low circulating testosterone concentrations (England, 1990), the condition is more likely to be physiological and psychological (Keenan, 1998). All treated dogs showed normal ejaculatory signs with dry ejaculates at semen collection, indicating the lack of sperm production. A marked change was observed in the semen of dogs treated with deslorelin; volume of the sperm-rich fraction considerably lowered within 5.2 weeks and total number of spermatozoa per ejaculation were decreased within 6 weeks of starting treatment. In the dogs, most of the seminal plasma is produced by the prostate and testosterone controls this region of the male reproductive tract (Sirinarumitr et al., 2001). The changes in volume of sperm-rich fraction were considerably reduced one week

before the prostate became significantly smaller. Ejaculates ceased completely at week 7.5 on average in the treatment-group dogs. Surprisingly, one dog (Dog3) prolonged complete cessation of the ejaculate until week 16; this may be due to biological variation or it could be related to a fault with the implant. Excluding this dog, ejaculates ceased more rapidly, the average being at week 6.3.

Testosterone is essential for the maintenance of normal spermatogenesis and fertility in all male mammals (Sharpe, 1994). The formation of spermatogonia from the germinal epithelium in the seminiferous tubules also appears to be stimulated by testosterone (Niswender et al., 1974). The present study shows a remarkable lowering of total sperm count in the ejaculates. Other studies have demonstrated ejaculate volume declined to zero after 4 weeks of treatment with 2 μg/kg of nafarelin acetate (Vickery et al., 1985b) and after 3 weeks of treatment with 50 μg/kg of nafarelin acetate (Paramo et al., 1993). The result of this study was similar to the previous studies on the basis of that time course was less than can be accounted for by the about 8-week duration of the spermatogenic cycle in dogs (Foote et al., 1972). Testosterone is required for devision of the primary spermatocyte to secondary spermatocyte and also to spermatids, whereas the maturation of spermatids into fully developed sperm cells required the presence of FSH (Niswender et al., 1974). This suggested that the effects of GnRH agonists can be exerted distal to the spermatogonia, and the exfoliation of cell stages of spermatogenesis could account for such rapid effects (Vickery et al., 1985b).

The testicular size in the dogs treated with deslorelin was slightly reduced and became obviously smaller than pre-treatment testicular size at week 6. Testicular size whether judged by scrotal circumference measurements or total testicular volume using ultrasonographic measurements was significantly decreased, indicating that deslorelin affects the testicles. Dry ejaculates were found at week 7.5 on average, which was about 1.5 week after significant regression of testicular size, suggesting that the status of spermatogenesis was gradually suppressed by deslorelin treatment prior to significant regression of the testicles. In the testis, seminiferous tubules occupy more

than two-thirds of the testicular volume; measurement of the testicular volume may indicate the status of spermatogenesis (Sherins and Howards, 1978) ,but do not correlate to semen quality in dogs (England, 1991), boars (Cartee et al., 1986) and men (Miskin et al., 1977). However change in testicular volume may correlate well with semen quality in dogs. Apart from lowering serum testosterone, another measurement which provides a good indicator of the effects on sperm production is scrotal circumference (Trigg, 2002). Morphological study of the effects of an GnRH agonist on the canine testis after four months of treatment clearly revealed that marked atrophy taking place in the seminiferous tubules which related to decreased testicular weight (Dube et al., 1987). GnRH agonists disrupted normal spermatogenesis by decreasing the number of germinal cells resulted in disappearance of mature spermatozoa in seminiferous tubules (Paramo et al., 1993). The fully reversible effect in testicular volume was observed in this study, thus indicating that restoration of spermatogenesis within seminiferous tubules could also be reversible. The results of a study showed that deslorelin treated dogs used to mate bitches after recovery were successful in achieving pregnancies (Trigg et al., 2001)

Prior to implantation, mean prostatic volume in both the treatment-group dogs and the control-group dogs was in normal range relative to body weight (Atalan et al., 1999). Dog prostate has been shown to contain androgen receptors (Moore et al., 1979). To maintain prostatic size, DHT, a metabolite of testosterone, is well accepted as a key hormone in men and dogs by enhancing growth in the stromal and glandular compartment (Lee, 1996). Regarding the volume of the prostate prior to treatment as 100%, a change in prostatic volume in the deslorelin-treated group was significantly decreased (50.2%) from week 6 to week 44 of the treatment period. After the maximal regression was attained from week 8 to week 32, prostatic volume gradually increased, and the prostate had recovered to almost the mean original volume by week 48 at the end of study. After castration, the mean prostatic regression rate was 60.1% (Tsutsui et al., 2001). In our study, mean prostatic volume reduced 50.2% which was comparable to the rate seen in castrated dogs (Tsutsui et al., 2001). Whereas, when maximal

prostatic regression rate (57.6%) was compared to the rate of castrated dogs, the two values were much closer (Tsutsui et al., 2001). The mean period prostate became significantly smaller until the prostate returned to the same volume as that prior to treatment was 9.5 months. These findings indicate that it is possible to use a deslorelin implant to induce prostatic regression for a prolonged period in dogs. From the clinical point of view, the further investigation in the effects of deslorelin implantation on BPH dogs is needed to be explored.

Studies of dose-response relationship on male reproductive function in dogs treated with GnRH agonist have been clearly demonstrated (Vickery et al., 1985b; Trigg et al., 2001). Larger doses of GnRH agonists exhibit a more rapid, acute and prolonged suppressive effect on endocrine parameter, semen parameter and anatomic parameter than smaller doses. In this study, using deslorelin at doses between 0.5-1 mg/kg suppressed canine male reproductive function for a long period. However, the results indicated that reproductive functions became fully restored within a 48-week observation period.

In conclusion, the long-acting, reversible effect of deslorelin on reproductive system in male dogs has been clearly demonstrated. The effect of deslorelin on male reproductive suppression was similar to those in castrated dogs over the period which testosterone is suppressed. The results from this present study indicate the use of deslorelin implantation as a non-surgical contraception in male dogs. At doses between 0.5-1 mg/kg, contraceptive efficacy occurred within two months after implantation for all dogs, most dogs being more rapid than that. In this study, the duration of seven months is suggested for contraceptive purpose. Because this method is safe, efficacious, easily performed and long- acting, it is a practical proposition to control large stray dog population. However, individual biological variation among animals requires further investigation. A study on the effective dose and re-implantation time may provide more information on this issue.