CHAPTER 5

Discussion

The results of present study demonstrate the relationship of age on the response of the young female dog implanted with the GnRH agonist, deslorelin.

The major effects of GnRH agonist in this study were to suppress or postpone oestrus in most animals and to induce the 1st oestrus in the more mature prepubertal female dogs.

GnRH agonist analogues have been studied in female reproduction as *contraceptive agents* by many authors (Nester, 1984 cited by Vickery et al., 1989; McRae et al., 1985; Vickery et al., 1987 cited by Vickery et al., 1989; Vickery et al. 1989; Trigg et al. 2001; Wright et al., 2001); as *induction of oestrous agents* (Vanderlip et al., 1987; Cain et al., 1988; Cain, 1989; Concannon, 1989; Vickery et al., 1989; Cain et al., 1990; Cinone et al., 1996; Concannon et al., 1997; Concannon, 1998; Inaba et al., 1998 ; Kutzler et al., 2002) and for other uses such as *treatment of mammary gland tumors* (Lombardi et al., 1999). Up to now, the development of delivery system that economically provided slow release of GnRH has not been forthcoming, until the system described by Trigg et al (2001) became available.

In this study, dogs treated with deslorelin-containing or placebo implants did not show allergic or inflammatory reaction at the site of implantation at 10-day intensive observation period or at any time of study, suggesting that the deslorelin and its delivery system is safe for subcutaneous implantation into animal (Trigg et al., 2001; Ponglowhapan et al., 2002). In addition these data show also that it is safe for young animals of 4 months.

The present study clearly demonstrates that GnRH agonist deslorelin postponed the 1st oestrus and caused long-term suppressed reproductive function of all (6/6) prepubertal female dogs when implanted at 4 month of age. In the 4 month of age-GnRH implantation group GnRH successfully delayed of pubertal development for the duration of the trial, as indicated by all variables monitored. Pubertal development in mammals is triggered by an increase in hypothalamic GnRH secretion into the hypophysial blood by hypothalamic GnRH-secreting neurons (Pralong et al., 2000). The result of continuous availability of exogenous-releasing factors that saturate the receptor sites of neurohormone in the anterior pituitary gland and, is the desensitization and hypo-responsiveness to the increasing of hypothalamic GnRH stimulation. This has been demonstrated in the prepubertal rat by (Debeljuk et al., 1972). The application of high doses of exogenous GnRH may inhibit endogenous GnRH synthesis in the hypothalamus as reported in ovarian cancer cell of mammal (Kang et al., 2000). In addition prepubertal dogs do not have the ability to respond to gonadal steroids.

Exogenous GnRH treatment induced the onset of puberty by shortening non-oestrous period before 1st oestrus in 7 month of age treatment group ($P < 0.003$). This indicates

that the systems of pituitary-gonadal axis were more developed in 7-month-old dog compared with the 4 month old litter mates.

In mammals, GnRH receptors have been shown to undergo biphasic (down- and up-regulation) homologous regulation (the desensitization of the receptor is initiated by the activation of the same receptor by the agonist; Sallese et al., 2000) by physiological concentrations of GnRH, pulsatile GnRH stimulation up-regulated the expression of its own receptor mRNA (Yasin et al., 1995; Haisenleder et al., 1997), where as high amplitude pulsatile or continuous treatment with GnRH generally down-regulates the levels of GnRH receptor (Adam et al., 1996; Vizcarra et al., 1997). Deslorelin induced oestrus in all 6 of the 7 months old bitches, two of which failed to ovulate described by the lack of elevation in serum progesterone concentration. The incomplete oestrus induced in *2 dogs* in this study was similar to those that sometimes occur naturally, such as the "false oestrus" which due to a failure for LH surge and thus a failure for ovulation following the end of proestrus. One dog from the 7 month of age-treatment group showed evidence of the increasing of superficial cell percentage from vaginal cytology and the swelling of vulva but not in progesterone concentration. This may be due to follicular phases and resultant failure of a spontaneous ovulatory LH surge. The changes in vulva, vagina and behavior primarily reflect the normal rise and fall only in oestrogen (England and Concannon, 2002). Vulvar swelling with serosanguineous discharge in this case may due to the slightly rising in oestrogen but inadequate to cause the changing of vaginal cytology. Whether incomplete oestrus that occur naturally or those seen in the present study reflect inadequate or atypical oestrogen secretion as Concannon et al. (1997) observed, or a hypothalamic or pituitary inadequacy is not clear. The normal bitch cycles in which follicles fail to ovulate (and enter the progesterone secreting luteal phase) are therefore likely to be characterized by a period of proestrus behaviour which wanes and then followed by a return to proestrus later (Jeffcoate, 1998). Due to the continuous release of deslorelin in this dog a long suppression of oestrus (> 23 weeks) occurred. Another dog that showed incomplete oestrus with no evidence of either increasing of percentage of superficial cell or progesterone concentration. Vulvar swelling with serosanguineous discharge in this case may due to the slightly rising in oestrogen but inadequate to cause the changing of vaginal cytology (Dr S Siriviayapong, personal communication). The abnormal oestradiol hormone pattern in this dog indicated that inadequate oestrogen may be due to low level of follicular growth caused by lack of FSH. FSH plays an important role in the maturation of the follicle and in equipping its cells for conversion to corpora lutea after ovulation. This process is of key importance in the bitch's cycle because there is a rise in progesterone secretion by the pre-ovulatory follicles, which appears to play a central role in triggering ovulation and standing estrus. However, the amount of progesterone circulating in pre-ovulatory bitches is still relatively low. The pre-ovulatory LH surge is often depicted as the central event of the cycle because of its role in stimulating ovulation and transition to the progesterone dominated luteal phase, metoestrus (Jeffcoate, 1998).

The groups implanted with GnRH showed decreased plasma progesterone concentration within one month (dioestrous duration), corresponding with the result of Wright et al. (2001). They reported that signs of pro-oestrus-oestrus were observed in all the bitches that received the deslorelin implant (9/9), four bitches that received deslorelin were mated, two of these bitches were found to be pregnant by

pregnancies failed at about day 40 of gestation. Possibly the luteotrophic mechanism had not been down-regulated in early pregnancy resulting in the maintenance of foetal viability at first 40 days. However, extended release of deslorelin from the implant appeared to have resulted in premature luteal failure of the synchronized bitches in Wright et al's study (2001). The associated decrease in progesterone concentration may be caused by regression of the corpus luteum due to low plasma concentrations of LH, reflecting continuous deslorelin-induced down regulation or desensitization of pituitary gland GnRH receptors (Wright et al, 2001). LH and prolactin are necessary for the maintenance of the corpus luteum (Olson et al., 1989; Onclin et al., 2000). In this study, the dogs showing induced oestrus were not allowed to mate. In recently study by Concannon et al. (2001) pregnancy specific increases in luteal function were found. These were described as the luteotrophic effect of increasing serum concentration of prolactin after days 30 of pregnancy.

An alternative method to induce fertile oestrus using deslorelin (Ovuplant®, Fort Dodge) was demonstrated by Kutzler et al. (2002). They suggested that implanting beneath the vestibular mucosa then removing the implant at the time of the surge appears "to offer a pragmatic method for oestrus synchronization of anoestrous bitches" which is followed by normal fertility (pregnancy and birth).

Agonists often induced oestrus as a result of an initial stimulatory effect on gonadotrophin release and gonadal steroid output before the suppression of the pituitary-gonadal axis (Vickery et al., 1989). Many of the current therapeutic agents act as agonists to the receptor. They cause an initial rise in hormone levels before the desensitization of the receptor. This phenomenon, known as the 'flare effect'. Many studies have attempted to solve this problem. In one study, progestin treatment suppressed GnRH agonist (deslorelin) induced estrus. The mechanism through which progestin suppresses the pro-estrous-estrous response is not clear (Wright et al., 2001). The effect may involve inhibition of the responsiveness of the pituitary gland to deslorelin, a direct effect in the ovaries that inhibits follicular responses to gonadotropin and effects on the action of estrogens at the target organs (vagina, uterus).

From the measurement of vulva at the end of study, GnRH agonist implantation into both groups had no effects on vulvar width and height compared with control, possibly that all of them were not in follicular phase at the end of experiment. No interaction between treatment and vulvar growth were observed.

Histological results in this study demonstrate non-inflammatory response in ovary and uterus as shown by no inflammatory agents found either in the control dogs or in GnRH agonists-deslorelin implanted dog. There was no evidence of cystic endometrial hyperplasia, one of the most life-threatening endocrine disorders often found in the bitch treated with other hormones for contraception such as progesterone (Concannon and Meyers-Wallen, 1991; Buergelt, 1997),

Follicular atrophy was observed in some dogs in both treatment groups, suggesting that the GnRH agonist effects follicular development in prepubertal female dogs by suppression the releasing of gonadotropins, FSH and LH. The histological changes in 2 control dogs showed multiple islands of CL's in the ovary, and endometrial hyperplasia with subendometrial haemorrhage in uterus. These changes occur normally in the dioestrus stage which was the time both dogs were

ovariohystectomized (Batha and Wood, 1990). The cause of the subendometrial congestion, seen in 2 deslorelin implanted dogs, the etiology is not known. It may have been a result of physiological changing or deslorelin but no major lesion which alter the reproductive health in these dogs.

In conclusion, GnRH agonist-deslorelin given as a subcutaneous implant and containing 10 mg active can be used as anti-fertility agent. It results in safe, long acting contraception. In this study it was more practicable to implant at 4 months of age as no dogs treated at this time showed signs of oestrus. The site of implantation, and reproductive organs such as the ovaries and uterus showed no chronic or acute inflammation.

In this study, deslorelin suppressed oestrus more than 9 months in some animals. The full duration of the effect could not be determined due to the time limitations of the study protocol. Others (Trigg et al. 2001) have reported that with certain formulations of deslorelin it was possible to suppress oestrus for longer periods of up to 27 months.

Control of dog numbers in both in-house and strays can be made with this technology development. The effects of repeated implantation of deslorelin, given before the effects of the existing implant expire, require further investigation.