CHAPTER 1

Introduction

Statement of the Problem

1.1 The need for oestrus control

Canine overpopulation is a considerable problem in many countries all over the world including Thailand. Packs of roaming dogs present serious public health problems in a variety of ways : by biting humans and domestic animals, by spreading diseases such as rabies, by creating pollution by urinating and defecating in streets and on sidewalks and parkways, consequently providing a breeding ground for flies, food for rats and mice, and source of contamination of water and food supplies by a variety of zoonotic pathogens such as plague, salmonellosis (Carter, 1990), by causing property damage and road accidents (Lloyd, 1988). Despite the fact that general health of many dogs is poor and many succumb to traffic, overpopulation prevails. The dog population can be separated to 3 groups:

A. Those which have owner responsibility,

B. Those supplied food by someone but having no owner responsible for them and

C. Those without owners (stray dogs).

Dogs start their reproductive lives at a very young age, and if uncontrolled, can deliver many litters of unwanted offspring throughout their lifetime. Many of these will be abandoned as stray dogs. So the ultimate aim is to increase owner responsibility and compliance to neuter their pet.
Summing up, it may be stated that canine overpopulation contributes a problem, increasingly recognized by the authorities, but that for majority of dog owners other considerations for seeking oestrus control in their bitches are more important. Yet, the main aim of oestrus control has not become the limitation of the canine overpopulation by birth control, but has remained the avoidance of the annoyances associated with heat in dog owners and health reasons of the bitches.

1.2 Methods of contraception

Contraceptive and oestrous control methods have been available for many years to use in the control of unwanted breeding in dogs' and assist pet's owners in reducing annoyance with sexual behavior. There are some procedures to control the fertility in the dog (table 1) and the bitch (table 2) and those have different of advantages or limits.

For dog population control in Thailand, beside the efficacy of fertility control agents or surgical technique, the attitude of the dog owner on contraception should be considered. Swangchanuthai and Siriviayapong (2002) collected data from 446 dog owners at Chulalongkorn University Veterinary Teaching Hospital during August and September 2001 using questionnaire and direct interview. Attitude to contraception

was 61.7 %for and 38.3 %against. From those in favour of contraception (276), 13.8 % preferred to perform in male and 39.9 % in female whilst 46.4 % agreed to do in both sexes. The attitudes on the appropriate age to start contraception, the percentages (44.5 %) which was 1 to 5 years. With male contraception, castration after one year old was found to be the most preferable (57.9 %), vasectomy (19.9 %), prepubertal castration (14.6 %) and GnRH agonists implantation (7.61 %), respectively. With female contraception, ovariohysterectomy (OVH) after first pubertal was the most favorable (39.7 %), a prepubertal OVH (23.6 %), progesterone administration (16.9 %), annual GnRH agonists implantation (9.3 %), physical separation during oestrus (6.7 %), pregnancy termination (2.5 %) and orally hormonal administration (1.3 %), respectively..

Table1. Procedures for fertility control, efficacy and safety in male dogs (WHO and WSPA, 1990)

Procedure	Efficacy and safety, concern or duration of effect
Bilateral Orchidectomy or Castration	-100 % and permanent -inexpensive, more time consuming than non-surgical method -complication of anesthesia, incision dehiscence and postoperative infections
Vasectomy	-100 % and permanent -no affect with sexual behavior
Phamacological agents	
- Anti-androgenic steroids	-at least 90 % -temporary effect
- Antimetabolites	-at least 90 % -no available in some countries
- GnRH agonists or GnRH antogonists	-at least 95 % -effectiveness for 7-12 months
Active immunization against LH or GnRH	-at least 90 % -temporary effect

Table 2. Procedures for fertility control, efficacy and safety in female dogs, modified from Concannon and Meyers-Wallen (1991) and WHO and WSPA (1990)

Procedure	Efficacy and safety concern or duration of effect
Ovariohysterectomy	-100 % and permanent -major operation, expensive, more time consuming than non-surgical method -complication of anesthesia, incision dehiscence and postoperative infections
Phamacological agents	
- Megestrol acetate	-at least 90 % -oestrous cycle can return, fertility control for 1-2 cycles
- Mibolerone	-at least 90 % -oestrous cycle can return, can use for 1-2 years
- Medroxy-Progesterone acetate	-at least 90 % -oestrous cycle can return -fertility control for 6-18 months
- Proligestone	-at least 90 % -oestrous cycle can return -fertility control for 6-18 months
- GnRH agonists or antagonists	-at least 90 % -oestrous cycle can return -can use for 1-2 years
Antimetabolites	
- Busulfan	-at least 90 % -effectiveness for 12 months
Pregnancy termination agents	
- Oestrogens	-alter zygote transport time and impair implantation
- Prostaglandins	-development of CEH and pyometra
- Prolactin inhibitors e.g. Bromocriptine	-efficacy but increasing in side-effects -at least 90 % -vomit is the most common adverse effect
- Non-hormonal Compounds ; RU486	-efficacy but increasing in toxicity
- Corticostroids	-efficacy but increasing in side-effects -use in some countries
Immunization	
- Active immunization against LH or GnRH	-variable ; at least 90 % -oestrous cycle can return
- Zona Pellucida vaccination	-at least 75 % -oestrous cycle can return

1.2.1 Surgical neutering of male and female dogs

Surgical neutering of male dogs, Orchidectomy is the main method of control dog population, because it is simple, inexpensive and highly efficient. An alternative surgical neutering procedure in male dogs that can leave the testis *in situ* but infertile is vasectomy using a modified method involving no incisions. The leader stud dogs remain dominant in their bitch group. However, this method requires well-trained veterinarians and specific surgical instruments (Lohachit and Chatdarong, 1997). Therefore, surgical neutering of male dogs fails to contribute significantly to birth control because for an increase in population only a relatively small number of entire males is required. Thus the only feasible and practical method of birth control in dogs is to prevent heat in bitches. Originally, surgical neutering in female (OVH) was the available method to achieve this end (Faulkner, 1971). The clear advantage in the surgical option is that irreversible infertility is attained immediately. The disadvantages are that surgery is expensive and labor-intensive and is not performed without risks such as complications associated with anesthesia, incision dehiscence, postoperative infections. Other important factors affecting the decision-making for neutering pets is their attitudes and their beliefs (Santiwattanatam, 1998) including the belief that surgically sterilized animals become lethargic and prone to obesity (Lloyd, 1988; Carter, 1990). OVH not only prevents unwanted pups from being born, but also protects the bitch from future ovarian and uterine disease (Olson and Johnston, 1993).

1.2.2 Chemical control of oestrus

Whilst surgical neutering is obviously effective, there also appears to be a place for reversible methods if these nonsurgical methods are affordable, reliable, safe and convenient. For the bitch, particularly, progestagens were at one time expected to fill this role (Sokolowski et al., 1968). The synthetic hormones include medroxyprogesterone acetate (MPA) and proligestone. However, unacceptable side effects, specifically endometritis, pyometra and mammary nodules of MPA have made these compounds useful only in short term applications such as the postponement of oestrus. Injection of proligestone is available in suspension as an injectible canine contraceptive in the United Kingdom and Europe, but is not currently marketed in the United States. However, only 2 products are commercially available and approved for use as canine contraceptives in the United States (Concannon and Meyers-Wallen, 1991). They are the progestin, megestrol acetate, and the androgen derivative, mibolerone. Diabetes mellitus is a contraindication to the use of megestrol acetate, as are mammary tumors and uterine or liver disease. Mibolerone has the inconvenience of daily oral administration, high interbreed differences in dosage requirements and the masculinizing effects of this androgenic steroid have combined to impede wide scale usage of the drug (Weissinger and McRae, 1991).

Luteinizing hormone releasing hormone (LHRH) or Gonadotrophin releasing hormone (GnRH) (Matsuo et al., 1971) and its more potent

agonistic analogues can exert anti-fertility effects. Although the high dose requirements, delivery problems and cost for long-term use of the LHRH antagonists are the disadvantages of these products in the past (Vickery et al., 1989). A product recently approved in Australia and New Zealand that meets these criteria. Suprelorin, contains the GnRH agonist deslorelin, in a slow release implant, can be used to suppress oestrous for up to and in some cases over 12 months following one implantation using a novel implant formulation for delivery (Trigg et al., 2001). However, oestrous induction during 5-28 days after the first usage of GnRH agonists in some bitches is the major problem for this contraceptive application. Wright et al. (2001) demonstrated that the oestrus induced in by deslorelin treatment may be suppressed by progestin treatment but the requirement for daily dosing make compliance and additional costs an issue. On the other hand, Trigg et al. (2001) found that, provided bitches had a minimum plasma progesterone concentration at the time of implantation of > 5 ng/ml, no oestrus was induced. Measurement of plasma progesterone prior to implantation will provide an means of overcoming a problem with this method.

As the same contraceptive effect, GnRH antagonist analogues had been produced. By contrast, the antagonists preferentially bind to the GnRH receptor, but fail to activate the second messenger cascade. Thus, both agonist and antagonist analogues can be used to suppress the reproductive functions of the pituitary gland (Lyncoln, 1992). Unfortunately, the high dose required to prevent oestrous cycles of the antagonists make the cost per unit dose considerably higher than agonists. (Olson and Johnston, 1993; Vickery, 1985; Vickery et al., 1989).

1.2.3 Future developments

Another potential method to avoid deslorelin induced oestrus might be to implant animals whilst they are still in their prepubertal state. One study reported that prepubertal dogs (4 months old) did not show oestrous sign and the oestrous interval was postponed when using the GnRH agonist (McRae et al., 1985). On the other hand, Vickery et al.(1989) reported that prepubertal dogs (10-11 months old) could be induced oestrus and ovulation following GnRH agonist treatment. If incidence of induced oestrus is affected by physiological development then it is probable several other factors including breed and nutrition will be implicated. This study is designed to provide more data on the effect of age on contraception by the GnRH agonist, deslorelin in prepubertal dogs.

Objectives

1. To further clarify the age of prepubertal female dog that show no sign of oestrous induction after using GnRH agonist implantation.
2. To study the effect of oestrous suppression after implanting of GnRH agonist in the dogs at various prepubertal ages in tropical climate country (Thailand).
3. To investigate the effect of GnRH agonist implantation on vulvar appearance and changing in histology of the ovary and uterus.

Definitions

1. Oestrous cycle

The oestrous cycle consists of four stages; proestrus, oestrus and dioestrus and anoestrus. The stages of the oestrous cycle are defined by the bitch's behavior, type of vaginal discharge present, changes in vaginal cytology and concentrations of progesterone. Because an assay for canine serum LH is not widely available, and detecting of LH surge requires daily blood sampling, measurement of serum progesterone concentration has been used as an indirect indicator of the LH peak and of subsequent ovulation. Serum progesterone level begins to increase coincidentally with the LH peak, preceding ovulation by several days (Johnson and Root, 1999). During anoestrus and proestrus, serum progesterone levels are basal (<0.16 ng/ml). At the time of the LH peak, the progesterone levels have usually risen to 2 and 3 ng/ml (0.9-3.0) and ovulation occurs 1-2 days later at a progesterone level of approximately 4-8 ng/ml (Concannon et al., 1975 ; Linde-Forsberg, 1995).

1.1 Proestrus

Proestrus is the first stage of heat. Various clinical signs can be taken as evidence of proestrus. The physiological changes are a response to increasing concentration of oestrogens. As the oestrogen levels rise, the vulva begins to swell, enlarge and redden, the appearance of a serosanguineous vaginal discharge (Figure 1) which is usually taken as marking the first day of proestrus. Although the bitch begins to show interest in the male, she is not yet ready to stand for mating.

1.2 Oestrus

At the end of proestrus and at the beginning of oestrus, the circulatory estrogen levels decrease and the progesterone concentration rises. These changes result in decrease in swelling of the vulva and trigger the onset of the receptive period for mating. (Jeffcoate, 1998). The first day of oestrus determined by vaginal cytology; superficial cell percentage was > 90 %. This stage lasts an average of nine days with a range from 0-17 days (Feldman and Nelson, 1996). Oestrus is derived from the Latin for gadfly, and literally refers to frenzied behavior (Jeffcoate, 1998). Oestrus is characterized by the bitch standing firmly and presenting its perineal region when exposed to male. The onset of oestrus usually occurs within a day or two of the preovulatory Luteinizing hormone (LH) surge and is a

response to the declining oestrogen to progesterone ratio outlined above (Sirivaidyapong, 2000). This stage lasts an average of nine days with a range from 3-21 days (Feldman and Nelson, 1996).

1.3 Dioestrus

The bitch enters dioestrus when she will no longer stand to be bred. The first day of dioestrus was the day in which the percentage of superficial cell had decreased by at least 20 %. Progesterone dominates during the dioestrus, this phase begins approximately 4-7 days after ovulation and lasts until circulatory progesterone concentrations fall below 1 ng/ml. (Johnston, 1992). Dioestrus lasts an average of 60 days (Feldman and Nelson, 1996).

1.4 Anoestrus

Anoestrus is the period of ovarian quiescence that separates oestrus cycles. The bitch shows no physical and behavioral changes due to the static hormonal level. The first day of anoestrus was the day of the progesterone level return to basal levels (< 1 ng/ml) (Feldman and Nelson, 1996). It is variable in length but typically lasts between 4 and 4.5 months (Concannon, 1991; Johnston, 1992).

a. b.

Figure 1. External genital (vulvar) appearance. a: anoestrous stage b: Proestrous stage (vulvar swelling and serosanguineous vaginal discharge was observed).

The wild predecessors of the dog, like most feral canines found today, exhibited oestrus only once a year. Among domestic dogs only the Basenji has retained this trait (Jochle and Andersen, 1977; McDonald, 1980). Most reports agree that oestrus can occur at any time throughout the year (Engle, 1946; Sokolowski, 1973). Such differences between wild predecessors and the breeds kept as pet may point to an influence of domestication on the cycle length. A complex of internal and external factors may play an additional role, such as genetic (breed) influences and geographic differences in latitude, photoperiodicity, nutrition and climate. In view of the apparent large variation in the length of the bitch's cycle, the lack of uniformity in the literature on this subject is not surprising. The mean length of interoestrous interval varies between breeds, ranging from 5 to 8 months (Christie and Bell, 1971; Sokolowski et al., 1971; Prole, 1973). In the previously mentioned study with 500 individually kept bitches the interoestrous interval was 5-7 months in 68 %, usually longer than 7 months in 27 % and usually shorter than 5 months in 3 % and 2 % of the animals showed no signs of oestrus at all (Frost, 1963). Although there are large individual variations bitches usually stop cycling at 10-12 years of age. Yet, oestrus has been observed even in 20 years old bitches an in many bitches there seems to be no menopause. For some period before a bitch stops cycling the interoestrous intervals tend to become longer and more irregular (Feldman and Nelson, 1996).

2. *Prepuberty*

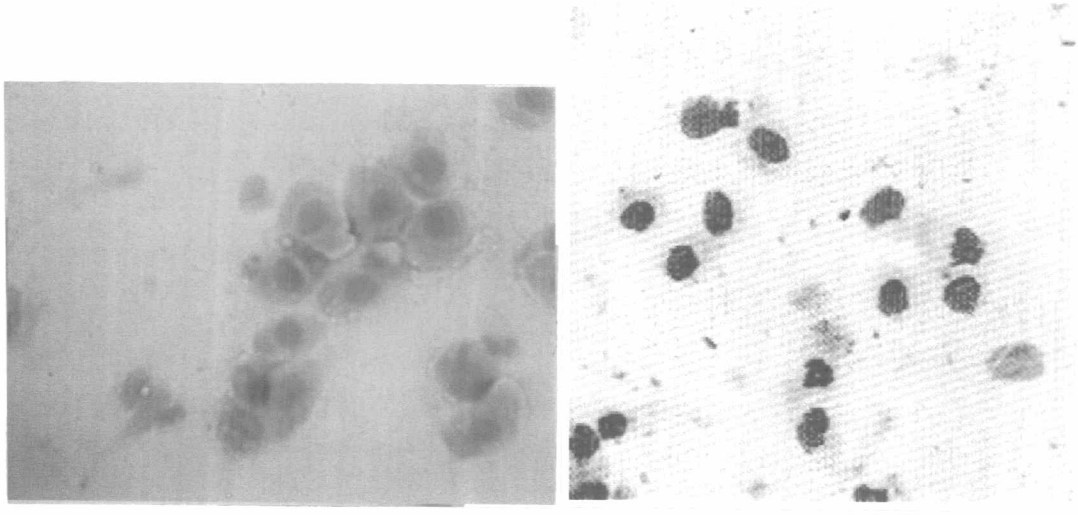
Prepuberty is the period before the bitch reaches its first reproductive cycle (Cunningham, 1997). In recent data, the term "puberty" in the bitch is used to define the onset of reproductive life (as indicated by the onset of the first cycle or sexual activity). The most precise definition is the time of first ovulation, which on average, occurs at 9 month of age (range : 6-12 months), but according to various reports between 4 to 22 months have been noted (Cunningham, 1997). Large breeds generally reach puberty at later age than smaller breeds (Hancock and Rowlands, 1949), although a large breed bitch may also begin her first proestrus before one year of age, some normal large-breed bitches may not begin to cycle until 18 to 24 month of age. Kenneled dogs reach puberty at later age than individually kept bitches (McDonald, 1980) and free roaming dogs. Breed has a significant effect on the timing of a 1st oestrus. Generally, bitches exhibit their first cycle several months after they achieve adult height and body weight. There is, however, considerable variation within a breed as well as between different breeds (Feldman and Nelson, 1996).

The physiological mechanisms involving control of puberty in domestic animals are best known in sheep (Cunningham, 1997). One of the fundamental concepts of the onset of puberty involves and increases in the synthesis and release of GnRH from hypothalamus, which drives pulsatile gonadotrophin secretion and follicular growth. Prior to puberty, GnRH and gonadotrophin secretion are kept in check, because the hypothalamus is highly sensitive to negative feedback inhibition by estrogens. One of the keys to puberty in lambs is a maturation of the hypothalamus, which results in reduced sensitivity to negative feedback by estrogen. Puberty onset is not held back because of lack

of responsiveness of the prepubertal gonads, because ovarian follicle development can be elicited by gonadotrophin administration (Cunningham, 1997). However, the molecular mechanism of GnRH and gonadotrophin regulation at the onset of puberty is unclear. Several studies have reported that Excitatory amino acid (EAA) neurotransmission is an essential component of the neuroendocrine transmission line that regulates LH and FSH secretion. Also, EAAs and ovarian steroids appear to play a role in puberty. Moreover, sensitivity of the receptor at hypothalamus and brain that response to EAAs and ovarian steroids appear different at the prepubertal and peripubertal age in mammals (Brann and Mahesh, 1994)

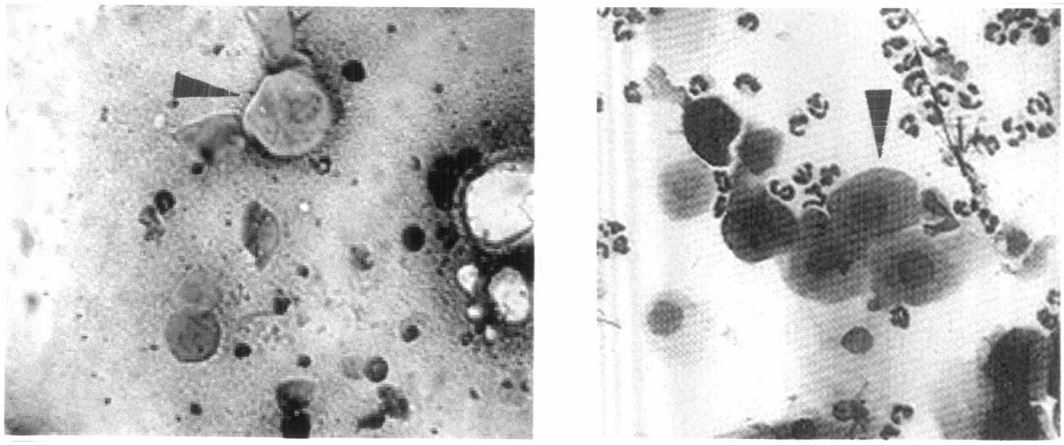
3. *Vaginal cytology*

Vaginal exfoliative cytology consists of the observation of types and quantities of vaginal cells is placed to examine under a microscope and arriving at a determination of a bitch's reproductive or physiological status at any given time. It is thus possible to identify a specific stage of the cycle. The parabasal cells (Figure 2) are the least mature epithelial cells. They are small ovoid cells with a large nuclear-to-cytoplasmic ratio. The intermediate cells (Figure 3) included a wide range of sizes and types because they represent all stages of maturation between parabasal and fully mature superficial cells. The less mature intermediate cells are small and round in shape, with a relatively large nucleus. They become more angular, enlarged and flattened as they mature. The relative size of the nucleus decreases as the cell enlarge. Superficial intermediate or large intermediate cells (Figure 4) have nonpyknotic nuclei and the mature superficial configuration of the cytoplasm. Superficial or cornified cells (Figure 5) were fully mature squamous epithelial cells. These cells have keratin incorporated in cytoplasm. The nucleus may be pyknotic, barely visible as a shadow or absent. Superficial cells were dead cells, incapable of further change.



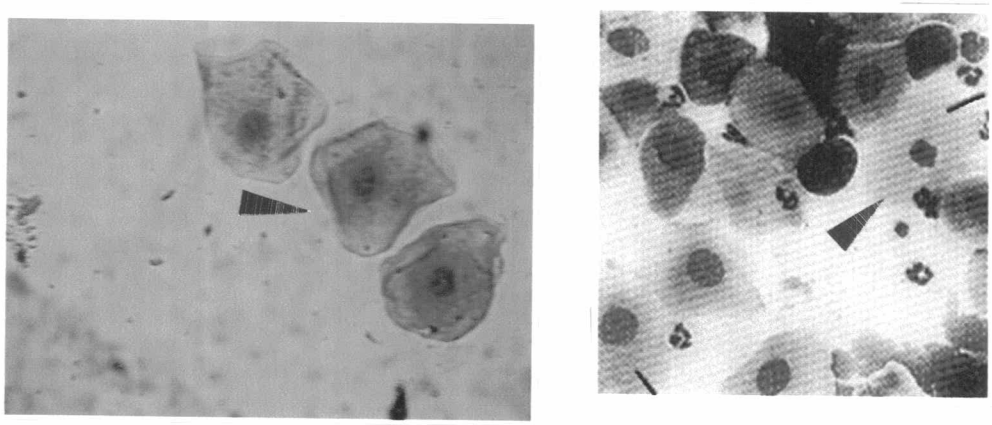
a. b.

Figure 2. Parabasal cells on vaginal cytology (Diff-quik, x 400)
a. present study b. Bacha and Wood, 1990.



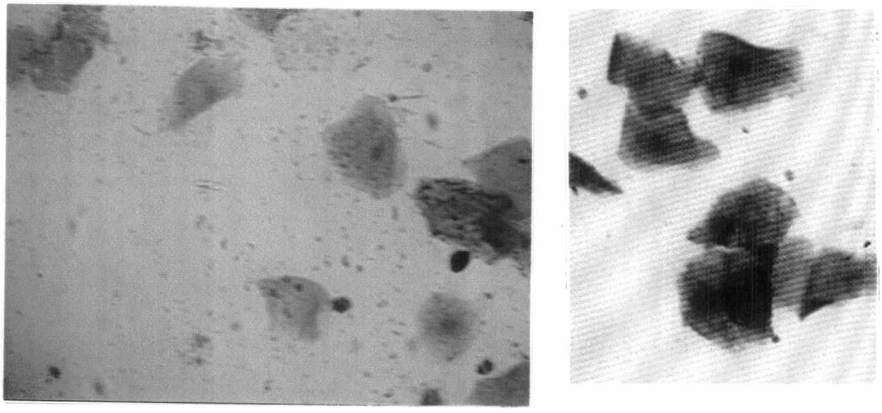
a. b.

Figure 3. Intermediate cell (straight arrow) on vaginal cytology (Diff-quik, x 400)
a. present study b. Bacha and Wood, 1990.



a. b.

Figure 4. Superficial intermediate or large intermediate cells (straight arrow) on vaginal cytology (Diff-quik, x 400) a. present study
b. Bacha and Wood, 1990.



a. b.

Figure 5. Superficial or cornified cells on vaginal cytology (Diff-quik, x 400)
a. present study b. Bacha and Wood, 1990.