“STUDIES ON EFFECT OF ADMINISTRATION OF GnRH AT THE TIME OF MATING ON THE CONCEPTION RATE AND LITTER SIZE IN PUG FEMALE DOGS”

HARSHA, R.V.

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS

VETERINARY COLLEGE, HEBBAL, BANGALORE

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR- 585 401

JUNE, 2013

"STUDIES ON EFFECT OF ADMINISTRATION OF GnRH AT THE TIME OF MATING ON THE CONCEPTION RATE AND LITTER SIZE IN PUG FEMALE DOGS"

Thesis submitted to the

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

In partial fulfillment of the requirements

For the award of the degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY GYNAECOLOGY AND OBSTETRICS

By

HARSHA, R.V.

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS

VETERINARY COLLEGE, HEBBAL, BANGALORE

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR- 585 401

JUNE, 2013

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS

VETERINARY COLLEGE, HEBBAL, BANGALORE

CERTIFICATE

This is to certify that the thesis entitled "***STUDIES ON EFFECT OF ADMINISTRATION OF GnRH AT THE TIME OF MATING ON THE CONCEPTION RATE AND LITTER SIZE IN PUG FEMALE DOGS***" submitted by **Mr. HARSHA, R.V., ID No. MVHK-1133** in partial fulfilment of the requirements for the award of degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY GYNAECOLOGY AND OBSTETRICS** of the Karnataka Veterinary, Animal and Fishers Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision. And the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
June, 2013

Dr. A. KRISHNASWAMY
Major Advisor

Approved by:

Chairman : ______________________________
(Dr. A. KRISHNASWAMY)

Members : 1. ______________________________
(Dr. T. G. HONNAPPA)

2. ______________________________
(Dr. G. SUDHA)

3. ______________________________
(Dr. B. N. NAGARAJA)

This thesis is Dedicated to

my Beloved Parents, Sister

and my Dear Friends for their Love,

Endless Support and Encouragement

ACKNOWLEDGEMENT

Pages will not suffice to describe this pleasant moment of expressing gratitude to all those who have supported and helped me in completing this master degree programme

*Foremost, I would like to express my sincere gratitude to my advisor **Dr. A. Krishnaswamy**, Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bangalore, for his excellent supervision, unflagging support, stead-fast encouragement, incredible suggestions, immense patience, his painstaking help and enthusiasm for the work is a source for my motivation towards this work. I confess that it has been a great fortune and proud privilege for me to be associated with him during my master degree programme. I could not have imagined having a better advisor and mentor for my M.V.Sc study.*

*I feel much honor to express my deep sense of gratitude to my advisory committee member **Dr. T. G. HONNAPPA**, Associate Professor, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bangalore, for his sustained encouragement, technical guidance, and timely suggestions and support at all the stages of my master degree programme as a member of my advisory committee.*

*I cordially extend my thanks to **Dr. G. SUDHA**, Assistant Professor, Department of Gynaecology and Obstetrics, Veterinary College, Bangalore, for her good nature, valuable suggestions, and meticulous corrections in my thesis and support at all the stages of my master degree programme as a member of my advisory committee.*

*I would also take the honor to thank **Dr. B. N. NAGARAJA**, Professor, Department of Veterinary Surgery and Radiology,Veterinary College, Bangalore, as a member of my advisory committee for his valuable suggestions, and also help that enabled me to complete my research work.*

*I consider it would be incomplete without extending my heartfelt special thanks and profound sense of gratitude to **Dr. V. Chandrashekara Murthy**, Associate*

Professor, Department of Gynaecology and Obstetrics, Veterinary College, Bangalore, and ***Dr. Ravindranath, B. M.*** *Assistant Professor, Department of TVCC, Veterinary College, Bangalore, for all their help, guidance and critical advice throughout my study without which this study was not easy to accomplish.*

I wish to express my hearty thanks to my seniors ***Drs. Shwetha K S, Vibha R K, Ashwartha, G, Hakim , Dost and Dr. Narasimha Murthy*** *for their suggestions with whom I have always got help from one way or other and a nice time in the department.*

*I also acknowledge my colleagues****, Prashant, Ashok, Yathish, Sowmya,*** *and* ***Chithra*** *for providing good working environment during my stay in the department and also to my junior colleagues* ***Drs. Abhignya, Sharan, Yugandhar, Malli, Prem sir, Prashanth sir*** *and* ***Suja*** *for their help and support.*

With utmost pleasure and a deep sense of gratitude I acknowledge my indebtedness to ***Dr. Amaregouda S Patil*** *for his valuable suggestions, helping nature at critical situations.*

I wish to express my hearty thanks to PhD Scholars, ***Drs. Jayakumar, Lakshmikanth, Mahesh Doddamani*** *and* ***Kantharaj*** *for their suggestions with whom I have always got help from one way or other and a nice time in the department.*

Words fail to express the magnitude of support, infinite love, inspiration, joyous atmosphere, generous cooperation and inspiring moral support extended during the course of my studies given by my friends ***Drs. Akshatha, Navya, Hema, Kavya, Nagu, Sharath, Rakesh, Chethan****,* ***Vinay*** *and* ***Ms. Thanu****.*

*I wish to express my deep sense of gratitude to the team '***ಗೋಕುಲ** - ಅರಳುವ ಪ್ರತಿಭೆಗಳ ವೇದಿಕೆ*' where I learnt not to give reasons for failure, for where I learnt what dedication is. Thanks to all the members of Gokula for their support and encouragement.*

A big hug to my beloved friends ***Drs. Harish patel, Nithin, Balaji, Vishwa, Swamy, Mahesh, Colonel, Goutham, 110, Mahendra, Madhu, Mutta, Sridev, Lanki, IP, Chari, Balu, Basavarajappa,***

My vocabulary utterly fails in expressing my accolade to my roommates ***Rajesh, Rahul*** *and* ***Dr. Chandrashekar.*** *My seniors,* ***Drs. Sudhakar, Santosh C.N, Mahesh Kadagi*** *, my juniors* ***Drs. Manjunath, Abhilash, Lakshmi,*** *and* ***Sowmya B.*** *I deeply express my sincere thanks to all my friends, whose continuous inspiration, encouragement and affection, boosted up my morale during the period of study.*

My sincere thanks to ***Vijayalakshmi, Puttu, Siddaram, Rajanna, Satish, Manjunath*** *Dept. of Veterinary Gynaecology & Obstetrics and other non- teaching staff who have helped me in completion of my thesis; either directly or indirectly.*

With all my love and affection, I record my indebtedness to my Parents, ***Sri. Vasu & Smt. Lalitha,*** *my Sister,* ***Sowmyashree*** *and my brother in law* ***Mohan*** *who had to sacrifice many things in their life to nurture my ambition and without whose encouragement, inspiration and moral support, it could have not been possible for me to achieve this task and I would not be in this position today.*

"Finally, but immensely I extend thanks to all those who directly or indirectly rendered their help for flagging it off on its journey into the world of life"

Bangalore, ***(HARSHA, R.V)***

June, 2013

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

I. INTRODUCTION

Traditionally, the female dog is considered as monoestrous, ovulating once or twice a year at 5- 12 months interval. Because of limited estrous cycles during the year, every infertile cycle is a worrisome for the dog breeder as it limits the number of litters that can be obtained in the breedable lifespan of bitch and limits the profit. Further, the female dog has a prolonged estrus period with the ovulation occurring at variable times after the onset of behavioral estrus. This phenomenon necessitates multiple matings so that fertile sperms are always present in the female genital tract around the time of ovulation. However, most male dog owners provide only one or two services of their studs and it become important that the limited number of matings provided coincide with the time of ovulation.

Improper timing of mating is considered to be the most important cause of breeding failure in the bitch (Concannon, 1986; Concannon *et al.*1989). Many of the studies have established that the fertile period of the bitch is short, lasting not more than 5 to 7 days (Goodman, 1998), and commencing from a time shortly after Luteinizing Hormone (LH) surge and ending with the degeneration of the ova in the oviduct 5 to 7 days later. Natural mating outside this period invariably result in poor conception rate and therefore, establishing the correct time of mating which can result in pregnancy and above average litter size becomes important. Determination of fertile period in the bitch has been attempted using several techniques. These include vaginal exfoliative cytology (Linde and Karlson, 1984; Olson *et al.,* 1984; Wright and Perry, 1989), vaginoscopy(Lindsay, 1983; Jeffocate and Lindsay, 1989) and plasma / serum progesterone concentration estimation (Eckersall and Harvey, 1987; Hegstead and Johnston 1989; Concannon, 1997).These techniques aim to determine the mating time and does not involve any therapeutic strategies to further optimize the conception rate.

In farm animals, Gonadotropin releasing hormone (GnRH) is routinely used for induction of ovulation and to improve the conception rate (Sterry *et al.*, 2006). Several studies have reported a significant positive effect of GnRH administered at the time of artificial insemination on pregnancy rate in cows (Lee *et al.*, 1983;Phatak *et al.*, 1986; Stevenson *et al.*, 1988). GnRH administered at the time of service has also improved the conception rate in several other species of farm animals such as ewe (Martemucci, 2010), goat (Mohamed, 2002) and mare (Kanitz, 2007).

In contrast to the extensive use of GnRH in farm animals for augmentation of fertility, the clinical application of GnRH in companion animals has received little attention. Most common clinical application of GnRH in companion animals appear to be for induction of estrus in anestrous female dog.(Vanderlip *et al.*,1987; Cain *et al.*, 1988;Concannon, *et al.*, 1977). There are occasional reports of GnRH recommended for induction of early ovulation to cause regression of vaginal hyperplasia, and in male dogs, it has been recommended as a line of treatment to cause descent of testes in cryptorchid pups (Humke, 1977).

There have been several unpublished claims by the field veterinarians that they have observed an improved conception rate as well as higher litter size following administration of GnRH prior to mating in female dogs. A through scanning of available literature did not reveal any information on the effect of GnRH administered at the time of mating on the conception rate in female dogs. Therefore there is a need to scientifically verify the claims of field veterinarians that administration of GnRH at the time of mating in female dogs improves conception rate and also the litter size. The present study was therefore designed with the following specific objectives.

1) To assess the conception rate and litter size of pugs following recommendation of mating time on the basis of vaginal exfoliative cytology.

2) To evaluate the effect of administration of GnRH at the time of mating improves the conception rate and litter size in pugs.

Review of Literature

II. REVIEW OF LITERATURE

2.1. General

Domestic dogs are monoestrous and predominantly nonseasonal breeders. With large individual and breed specific variations there are several months of anestrous between active reproductive phases (Stabenfeldt and Shille 1977).

The classical signs of canine estrous cycle introduced by Heape (1900) were proestrus, estrus, metestrus and anestrus. In recent years, the metestrus has been largely replaced by the term diestrus (Olson and Nett 1986).

Proestrus clinically begins with vulvar swelling and the onset of blood tinged discharge (Goodman 1998). The average duration of proestrus in mature bitches is stated to 9 days ranging from 0-27 days. (Bell and Christie 1971; Olson and Nett 1986; Allen and England 1990).

Feldman and Nelson (1996) stated that proestrous is typically but not always associated with varying quantities of blood tinged discharge. They further stated that, vaginal bleeding begins because of diapedesis of erythrocytes through the endometrium subepithelilly as capillaries rupture within the endometrium. This blood seeps through a slightly relaxed cervix and enters into the vaginal canal. In most cases, the blood tinged discharge fades and become transparent to straw colour as proestrus progress to estrus, but change in colour of discharge may be inconsistent with some bitches showing blood discharge throughout proestrus, estrus and diestrus as well, others may bleed little or only at the proestrus.

The vulva slowly enlarges during proestrus (Feldman and Nelson 1996). As proestrus proceeds into estrus, vulva relaxes and softens dramatically. Behaviorally, the bitch will attract the male but refuse to let them to mount (Olson and Nett 1986; Goodman 1998).

Conconnan (1980) states that both sexes begin to show an increased behavioral interest in each other during this phase and as a part of courtship pattern, episodes of playing may be seen, though most bitches show a high degree of resistance to all males and will either avoid or attack them.

Feldman and Nelson (1996) stated that early proestrus includes; an increase in playtime and teasing activity, but active discouragement of any mounting attempt by male which is exhibited as antisexual growling, moving away, baring the teeth and snapping. The bitch may also keep her tail tight against the perineum, between the legs, and covering the vulva. This initial behavior pattern gradually changes as proestrus progresses. The female become more receptive, as demonstrated by increasingly seeking males, playing and teasing. Male attraction involves pheromone secretion, and methyl p-hydroxybenzoate was identified as a sex attractant in vaginal secretions of estrous bitches (Concannon, 2011). In late proestrus, the bitch may stand or sit passively when mounted with or without intermittent displacement of tail deviation (Feldman and Nelson, 1996).

Johnston *et al.* (2001), stated that bitches in proestrus exhibited three sexual reflexes namely; the upward tipping or winking of vulva in response to touching the skin immediately dorsal to vulva, the ipsilateral curvature of the rear legs in response to the tapping of the skin to the right or left of the vulva, and contralateral or vertical deviation or flagging of tail in response to tapping the skin on either side of the vulva (Beach *et al*., 1982). These sexual reflexes were stated to be absent during anestrus, increased during proestrus and reached their peak during early to mid estrus.

Serum estradiol increases throughout, from 5 to15 pg/ml (~20–55 pmol/l) initially, to reach peaks of 40–120 (mean 70) pg/ml (~150–450, mean 255 pmol/l). Proestrus ends with the onset of receptive behavior typically occurring 0.5–3 days after the peak in estradiol and within a day of the preovulatory LH surge. Endocrinologically, physiological proestrus ends with the preovulatory LH surge (Concannon, 2011).

Serum concentration of progesterone remained at basal level (< 1ng/ml) prior to and during the first few days of proestrus followed by a gradual increase. This increase was related to preovulatory luteinization of the follicles (Phemister *et al.,* 1973; Conconnan *et al.,* 1977).

Proestrus proceeds into estrus, which is the stage of the cycle characterized by the bitches acceptance of the male for mounting. The first day the bitch allows mating (standing estrus) is considered as the start of estrus and this phase ends when she no longer accepts the male. (Feldman and Nelson, 1996). Estrus behavior is characterized by proactive receptivity to mounting by males and increased male seeking behavior (Concannon, 2011). The bitch in estrus may crouch or elevate the perineum towards the male and the tail to be shifted on one side with an obvious tensing of the rear legs to support the weight of a mounting male. Other clinical changes indicative of estrus described were; relaxation or softening of vulva and the presence of straw or pink coloured vaginal discharge (Feldman and Nelson, 1996).

Bell and Christie (1971) reported the average duration of estrus based on behavioural signs of estrus as 9 days, ranging from 4-24 days. Concannon (2011) states that, estrus lasts a variable length of time, wanes slowly or rapidly after 5–10 days (mean 9 days) but can persist to some extent well beyond the day 8 end of “fertile estrus”.

Endocrinologically, estrus begins with the luteinizing hormone (LH) surge and is characterized by rise in progesterone (P_4) and decline in estrogen levels (Goodman, 1998).

The bitch appears to become receptive to the male after the serum concentration of estrogen begins to fall and concentration of progesterone begins to rise (Concannon, 1975; Concannon *et al.*, 1979; Beach *et al.*, 1982). Estradiol continues its decline from peak values of late proestrus to intermediate values of 10 to 20 pg/ml (~40 to 90 pmol/l). Serum progesterone rapidly increases above 1 to 3 ng/ml (3 to 6 nmol/l) during the preovulatory LH surge, and immediately (or after a 1 to 3 days pause) rapidly increases further, reaching 10 to 25 ng/ml (~30 to 80 nmol/l) by day 10, at or shortly after the end of estrus Concannon (2011). Although estrogen alone can induce sexual behavior, progesterone seems to further enhance and synchronize this behavior (Leedy, 1988).

Some investigators have reported that preovulatory LH surge occurred on the first day of standing heat (Nett *et al.*, 1975; Remier *et al.*, 1978), while others have found no such correlation (Mellin *et al.*, 1976; Wildt *et al.*, 1978). In some bitches, the onset of behavioral estrus was found to occur as early as 2 to 3 days before LH surge, while many others it did not occur until 4 to 5 days after LH surge (Johnston *et al.*, 2001). Concannon (1987) stated that in the extreme case an aggressive male might mate a bitch in late proestrus as early as 4 to 5 days before the LH surge, while some bitches may refuse a male until more than 6 days after the surge.

The duration of preovulatory LH surge in the bitch appears to vary, with range of 24 to 96 hours. (Smith and Mcdonald 1971; Nett *et al.,* 1975; Concannon *et al.,* 1977; Reimers *et al.,* 1978; Wildt *et al.,* 1978; Olson *et al.,* 1982). Ovulation occurred approximately 2 to 3 days following preovulatory LH surge (Phemister *et al.,* 1973;Concannon *et al.,* 1977; Wildt *et al.,* 1978; Tsutsui 1989). The average interval from LH surge to ovulation was 2 days in 10 Beagle bitches. (Phemister *et al.,* 1973)

Canine oocytes are ovulated as primary oocytes and ovulated 48 to 60 hours post LH surge undergo maturation at 96 to108 hours in the distal uterine tube (oviduct), and secondary oocytes remain viable for another 24 to 48 hours in most bitches, for 72 to 96 hours in some bitches, and 120 to 144 hours in the extreme Concannon (2011).

Fertility for single matings is maximal from the LH surge (day 0) until day 5 and wanes rapidly over the next 3 days, in part due to a “cervical closure” at day 7 to 8. Conception rates following intrauterine AI at day 9 to 10 after the LH surge average 60 percent (Tsutsui *et al.,* 2009) vs. rates of less than 10 per cent with vaginal inseminations.

Concannon *et al.* (2001a, b) states that, following implantation on day 21, parturition routinely occurs 65±1 days after the LH surge, as early as 55 days and as late as 68 days after mating.

Canine sperm survive up to 7 days in utero, with mating 5 days before ovulation often being fertile, and super fecundation is not uncommon. Artificial insemination with poor quality or frozen thawed semen is most successful when performed at day 5 or 6 post LH surge and after oocyte maturation. Normal litter sizes range from 2 to 16, average about 7, breed averages varying from 5 to 8, roughly positively correlated to breed size (Concannon, 2011). Therefore, it becomes necessary to identify the fertile period in a bitch, so that mating time can be recommended which can result in conception and above average litter size.

2.2 Ovulation

Determining the time of ovulation is often critical in breeding management, a rapid reduction in vulval turgor due to preovulatory declines in the estrogen to progesterone (E:P) ratio typically indicates ovulation is either eminent or has just occurred (Concannon, 2011).

The LH surge to ovulation interval is characterized by a rapid increase in follicle mural cell luteinization, growth of theca and blood vessels, abrupt increases in serum progesterone and 17-hydroxyprogesterone, and typically further decline in estradiol (Concannon *et al.,* 2009).

Bouchard (1991) monitored estrous cycle of 16 mature mongrel female dogs to evaluate the accuracy of vaginal cytology and quantitative ELISA progesterone assay to determine ovulation. Ovulation was considered to take place 48 hours after the preovulatory LH peak. Ovulation was estimated to occur when P_4 concentration was 4.9 ± 1.0 ng/ml (mean ± SD, n = 15), with a range of 3.4 to 6.6 ng/ml. Based on vaginal cytology, ovulation took place 6.9 ± 1.6 d (n = 15) after 80% of the squamous cells were superficial and 6.8 ± 1.4 d (n = 16) before day 1.

Ovulation took place 2.1 ± 3.9 d (n = 11) after the first day of standing estrus and 8.8 ± 1.5 d (n = 10) before the last day of receptivity.

Hormonal assays have been found to accurately determine the time of ovulation. Elevation of P_4 concentrations prior to ovulation is peculiar to bitches. A serum P_4 concentration of 5.44 ± 0.93 ng/ml (mean ± SEM) as measured by RIA was found to correspond to ovulation (Concannon *et al.*, 1977).

2.3 Timing the fertile period by Vaginal exfoliative cytology

The technique of using vaginal smears to determine the stages of the oestrous cycle has been practiced for many years. The vaginal mucosa is a target organ for the ovarian hormones and during estrus the vaginal epithelium changes from 2 to 4 layers into a multi layered epithelium. The changes are observed in the vaginal smear as an increasing percentage of keratinized epithelial cells (Catharina, 1984).

The increasing circulatory levels of estradiol-17β stimulate the growth of vaginal epithelium from bistratified cuboidal epithelium to a stratified squamous epithelium of greater than 30 cell layers with every estrous cycle (Holst, 1986). As the vaginal mucosa develops, number of superficial epithelial cells slough into vaginal fluid. The changes in the percentage of superficial cells in a vaginal smear have been used to study the progression of proestrus into estrus in bitch and recommend the appropriate time of mating.

The increase in keratinization of the vaginal epithelial cells is correlated with the increasing estrogen levels during proestrus (Allen and Doisy, 1923). During later years the hormonal changes during the estrus period in the bitch have been extensively studied (Concannon *et al.*, 1975; Concannon *et al.*, 1977 and Wildt *et al.*, 1978). The vaginal cells have in this study been grouped according to a modification of the method described by Christie *et al.* (1972). The cells were divided into four groups: parabasals, intermediates, superficials and anuclears (Catharina, 1984).

Flower *et al.* (1971) examined the correlation between vaginal smear and morphological features of ovary and uterus in 34 mature bitches. In approximately 25 percent of bitches examined, a poor correlation existed between the type of desquamated vaginal epithelial cells and the histological appearance of ovaries and uterus.

In evaluating vaginal smear obtained from 12 beagle bitches on a daily proestrus, estrus and early diestrus, the day of maximum cornification was variable from 91 to 99 percent and occurring between -1 to +7 days after the LH surge (Christie *et al.*, 1972). The first day, when atleast 90 percent of epithelial cells were of superficial type ranged from -6 to +4 days after LH surge.

Olson *et al*. (1983) opinioned that, breeding must occur while the smear is fully cornified regardless of all other observation including behavior.

Catharina and Inger (1984), studied the correlation between the vaginal smear and the calculated time of ovulation in 22 bitches, during a total of 24 heat periods. In all 24 heat periods maximum keratinization of the vaginal epithelial cells was observed at least 3 days after peak estradiol levels and in 17 of the heat periods (70.8 per cent) maximum keratinization was seen when the peripheral plasma level of progesterone had increased to more than 5.44 ± 0.93 ng/ml, which has been calculated as the ovulatory level.

Olson (1989) and Wright (1990) recommended the routine use of vaginal exfoliative cytology to monitor the estrous cycle in bitch and suggest the optimum time for mating.

Bouchard *et al.* (1991) monitored the estrus cycle of 16 mature mongrel bitches by studying the vaginal cytology twice weekly. The bitch was considered to be in estrus when more than 90 per cent cells were superficial keratinized epithelial cells. Based on vaginal cytology, ovulation was estimated to take place 6.9 ± 1.6 days after 80 per cent of the vaginal epithelial cells were superficially keratinized.

England (1992), determined the mating time in 50 bitches by examining vaginal exfoliative cytology starting from within four days of the onset of proestrus and subsequently daily on alternative days. Mating was recommended when maximum percentage of a nuclear cells and ferning pattern of cervicovaginal fluid. 92 per cent of the 50 bitches were mated on basis of vaginal cytology became pregnant and 348 pups were born, the mean litter size is being 7.5 ± 2.9. The conception rate recorded was significantly higher than the conception rate in control group of bitches, which were mated on day 10 and 12 after the onset of proestrus. Further, it was identified that bitch may demonstrate one or two anuclear cell peaks during their estrous cycle. In 58 percent of bitches, one anuclear cell was identified with a maximum peak value between 43 per cent and 94 per cent of bitches, two anuclear cell peaks were observed. For the bitches in which one peak of anuclear cells were identified, the mean interval between the calculated LH surge and the anuclear peak was 1.9 ± 2.1 days, while it was 0.8 ± 2.6 days in bitches with two anuclear peaks. The author recommended the mating or insemination at the second peak of the anuclear cells or two days after a single peak of anuclear cells.

2.4. Timing the fertile period based on plasma / serum progesterone concentration

Progesterone (P_4) measurement in the peripheral blood is an objective parameter for determination of reproductive functions in the bitch. This study evaluates an Enzyme-Linked Fluorescence Assay (ELFA) (Biomerieux, France) for the determination of progesterone validated for use in human.

Determining the mating time using the progesterone assay focuses on using changes in P_4 level to identify the occurrence of LH surge. Basal progesterone levels have been reported to typically range from 0.0 to 1.0 ng/ml during anestrus and proestrus (Concannon *et al.*, 1977). At the time of LH surge, serum progesterone rise rapidly (0.8 to 3.0ng/ml), continued to rise at ovulation (1.0 to 8.0ng/ml), and was even higher towards the end of fertile period (4.0 to 20.0 ng/ml), by day 21 of post LH surge, progesterone levels ranged between 15 to 50 ng/ml and remained elevated for 2 to 3 months (Concannon, 1986; Jeffocate and Lindsay, 1989).

Kutzler *et al*. (2003) reported that, in clinical practice, various methods have been used to monitor serum P_4 concentrations to determine the optimal time for insemination, since this is more convenient and economical than monitoring serum LH. Previous studies of canine reproductive physiology indicate that P_4 concentrations can vary widely after the LH surge. Reports using quantitative results from P_4 radioimmunoassay (RIA) to estimate ovulation (2 days after LH surge; P4 ¼ 5–6 ng/ml) or the time of insemination

(4 to 7 days after LH surge; P4 ¼ 6 to 12 ng/ml), have predicted gestation lengths ranging from 58 to 70 days.

However, little variation was reported in peripheral P_4 concentrations (0.8 ± 0.1, 1.6 ± 0.2, and 2.6 ± 0.2) near the time of the preovulatory LH surge (day before, day of, and day following the LH surge, respectively) (Concannon, 1975).

Van *et al. (*1989*)* determined the optimum time for mating bitches by measuring plasma P_4 concentrations three times a week after the start of vaginal bleeding. The owners were advised to mate their bitches within 9 hours, if the P_4 concentration exceeded 12 ng/ml, within 9 to 33 hours, if it was between 6 to 12 ng/ml and within 33 to 57 hours, if it was between 5 to 6 ng/ml of 104 bitches with reduced fertility, 81 (78 per cent) became pregnant and out of 112 bitches with normal fertility, 105 (94 per cent) became pregnant. The mean interval between start of vaginal bleeding and optimum time for mating in 88 bitches was 11.8 ± 31 days. The authors concluded that the progesterone is an excellent indicator of timing mating in bitches with reduced fertility.

Wright (1990) opinioned that, the clinical parameters like behavior, vaginal cytology and appearance of the vaginal mucosa show characteristic changes before and after ovulation, and that the subjective nature of their assessment is a significant drawback. By contrast, the progesterone concentration is a functional parameter, which can be measured quantitatively. The estimate of the time of ovulation based on the P_4 measurement is therefore more reliable than the estimate based on vaginal cytology.

Gunzel-Apel *et al.* (1990a,b), Ververidis *et al.* (2002) stated that, P_4 levels rise significantly from the time of the LH peak and ovulation is assumed to occur when a concentration of 5 ng ⁄ ml is reached. They opinioned that along with clinical examinations like vaginal cytology, vaginoscopy, observation of the external genitalia as well as behaviour, the increasing level of P_4 in peripheral blood is a reliable indicator of ovulation.

Dietrich and Mollar, (1993) reported that 11 out of 13 bitches became pregnant when mating occurred within 2 to 3 days following serum progesterone concentration of 5 ng/ml or above.

Kutzler *et al.* (2003) recorded serum progesterone concentrations as serial samples from 63 bitches (19 breeds) were analyzed. P_4 concentrations were measured by radioimmunoassay (RIA) or Chemiluminescent immunoassay (CLIA). The CLIA method was validated for use in determining P_4 concentrations in canine serum and results were comparable to those obtained with RIA. Bitches were grouped by nonpregnant body weight (BW) and litter size (LS). Day 0 (D0), the day of preovulatory rise in serum P_4, was defined as the day that P_4 concentration rose to l.5 ng/ml and was atleast twice the baseline concentration. The predicted parturition date, 65 days following the day of preovulatory rise in serum P_4 (D65), was compared to actual parturition date, the day the first pup was delivered. He determined that mean P_4 concentration at D0 for all BW groups was 2.02 ± 0.18 ng/ml and there was significant variation in P_4 concentrations between BW groups after D1. In addition, he determined that the accuracy of parturition date prediction within a ±1, ±2, and ±3 day interval using prebreeding serum progesterone concentrations was 67, 90, and 100%, respectively, and that the accuracy was not affected by body weight or litter size.

Brugger, (2011) stated that P_4 measurement in the peripheral blood is an objective parameter for determination of reproductive functions in the bitch. This study evaluates an enzyme-linked fluorescence assay (ELFA) (Biomerieux, France) for the determination of P_4 validated for use in human. The ELFA is to be performed on the automated analyzer MiniVidas which provides quantitative results within 45 min. Blood samples from a total of 27 female dogs of different breeds were used to test the correctness of the ELFA 15 blood samples with a range of 0.3 to 40.0 ng⁄ ml were compared to a radioimmunoassay (RIA) validated in the dog. The values obtained with the Mini Vidas showed a high agreement (mean deviation 15%), deviations were in both directions and the correlation coefficient was 0.989. He reported that, there was increase of P4 above 5 to 10 ng/ml indicating ovulation was accompanied by corresponding results of the clinical examinations in all seven bitches. Four out of seven bitches were bred on the second day after the day of ovulation and three of them conceived. This result underlines that the increase of P_4 values which occurs during oestrus in the bitch can be reliably measured quantitatively with the Mini Vidas system.

2.5. Gonadotropin releasing hormone (GnRH)

2.5.1. General

Gonadotropin releasing hormone (GnRH) or Luteinizing Hormone releasing hormone (LHRH) is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) hypothalamic hormone that acts upon GnRH receptors in the pituitary. It is released in a pulsatile manner and has a short half life of 2 to 5 minutes due to rapid cleavage by proteases. GnRH stimulates, in the pituitary to production and secretion of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) which in turn act on the gonads regulating steroid production, spermatogenesis, ovarian follicular development and ovulation (Hull and Kenigsberg, 1987; Jiang *et al.*, 2001).

Oral administration of GnRH analogs is unsuitable (0.1% bioavailability) and they require parenteral administration (Chrisp and Goa, 1990; Padula, 2005).

Efforts were successful in generating potent agonists by substitution of two amino acids (positions 6 and 10) with D-aminoacid at position 6 and or an N-ethylamide at position 10 (Nestor, 1984).

The design of GnRH agonists has been directed toward stabilization of the molecule against enzymatic attack, increasing binding to plasma proteins and membranes, and increasing the affinity of the agonist for the GnRH receptor (Conn and Crowley, 1991)

An initial disadvantage with the use of agonists was the need for frequent subcutaneous (s/c) injections or of minipumps implanted subcutaneously (s/c) over prolonged time periods to mimic the pulsatile, physiological GnRH secretion. A significant advance for their clinical use was obtained by the development of slow release agonists that can be easily administered intramuscularly or implanted subcutaneously, every 3, 6 or 12 months depending on the formulation (Weckerman and Harzmann, 2004). Pulsatile administration of GnRH at doses of 0.2 to 0.4 µg/kg B.W, at 90 min intervals is sufficient to obtain increase in LH similar to the endogenous pulses that normally occur at the end of proestrus. GnRH delivery methods have been much less developed in companion than in farm animals. Two delivery systems have been created and either equine or human products have also been used in some studies (Inaba *et al.*, 1996; Kutzler *et al.*, 2001a, b; Trigg *et al.*, 2001; Herbert and Trigg, 2005; Rubion *et al.*, 2003 and Rubion *et al.*, 2006) Finally, with both classes of analogs, that is agonists and antagonists, no significant changes in blood biochemistry or hematological values have been noted in other species (Tarlatzis and Bili, 2004).

Canine reproductive physiology has unique characteristics that make the simple extrapolation of exogenous control of reproduction techniques used in farm animals unsuccessful in this species.

GnRH agonists used in studies with dogs have varied in potency in relation to natural GnRH, with differences due to the chemistry of the molecules and also among reports;

GnRH agonists	**Potency**	**Reference**
Histrelin and nafarelin	200	Herbert and Trigg (2005)
Lutrelin	150–180	Vickery *et al.* (1989) and Concannon *et al.* (2006)
Deslorelin	150	Concannon (2005)
Azagly nafarelin	50–100	Rubion *et al.* (2003)
Buserelin	15–30	Cinone *et al.* (1996) and Herbert and Trigg (2005)
Leuprolide	15	Herbert and Trigg (2005)

Cinone *et al.* (1996) made first study of a sustained-release formulation of an agonist in dogs. A single buserelin silastic implant was used for estrus induction in 29 anestrous bitches. All the females ovulated and whelped normal litters.

Cristina (2007) opinioned that, there is a promising place for both agonists and antagonists in future canine reproduction. They can be used in the control of estrous cycle, hormone dependent diseases and as well as in contraception.

2.5.2. Pro fertility effects of GnRH in Canine Reproduction

Pro fertility effects:

Potential clinical applications of exogenous regulation of canine reproduction with GnRH analogs include the management of estrous cycles, such as estrus induction or prevention, management of reproductive hormone dependent diseases, and contraception in general. Reversibility of suppression of the pituitary gonadal axis and safety are desired characteristics when either breeding or working dogs are treated.

Taking advantage of the duality of their effects, that is stimulation and inhibition, GnRH agonists have been used both in canine estrus induction and prevention. Native GnRH or short acting formulations of agonists were used for estrous induction but they required either constant infusion or frequent injections (e.g. every 90 min) for 6 to 14 days. These protocols were not clinically useful due to the expense of pulsatile infusion pumps or the need for hospitalization during continuous intravenous infusion (Vanderlip *et al.,* 1987; Concannon, 1989; Cain *et al.,* 1988; Concannon *et al.,* 1997; Rota *et al.,* 2003).

Cinone *et al.* (1996) in a first study of a sustained release formulation of an agonist in dogs, a single buserelin silastic implant was used for estrus induction in 29 anestrous bitches. All the females ovulated and whelped normal litters subsequently, a single injection of a sustained release formulation of the agonist leuprolide acetate (100 μg/kg s/c) followed by the agonist, fertirelin (3μg/kg i/m), as an ovulation induction agent, was used in 18 bitches (days 120[n = 6] or 150 [n = 6] post partum and prepubertal [n = 6]) on the first day of induced estrus. This combined treatment provoked behavioural estrus in all the treated animals and a pregnancy rate of 50, 100 and 83%, respectively, for the three groups. One prepubertal bitch did not become pregnant due to premature luteal failure (Inaba *et al.,* 1998). In 2001, a biodegradable sustained-release subdermal implant containing deslorelin acetate (Ovuplant®, Fort Dodge Animal Health, Overland Park, KS, USA; 2.1 mg), which was licensed for the induction of ovulation in horses, was first used in seven beagle bitches. Although a rapid and synchronous estrus was induced, 2 of 3 bitches that conceived aborted 39 and 51 days after the LH surge and only one remained pregnant to term (Kutzler *et al.,* 2001a, b). As in a previous study (Vickery *et al.,* 1989), the down regulating effect of the long term release GnRH implant shortened diestrus and caused pregnancy interruption. In a subsequent study, the same implants were inserted in the vestibular mucosa of six beagle bitches to facilitate removal after a suitable period of stimulation. Thus, when the deslorelin implants were removed at the time of the preovulatory LH surge and the initial rise in serum progesterone (P4) concentrations, pregnancy rate increased to 50% (Kutzler *et al.,* 2002). More recently, an attempt has been made to prevent premature luteal failure in four deslorelin (1.05 mg) induced bitches using a single dose of human chorionic gonadotrophin (hCG, 150 IU iv) between days 39 and 45 of diestrus (pregnant or not pregnant). Human chorionic gonadotrophin increased serum P_4 concentrations during the first 24 h after treatment, followed by a dramatic decline which reversed later in diestrus only in pregnant bitches. In this previous study, down regulation of hypophyseal GnRH receptors did not fully justify the decreased luteal activity after hCG treatment (Volkmann *et al.,* 2006b). Doses of 2.1 and 1.05 mg of deslorelin implanted beneath the vulvar mucosa could induce estrus in anestrous as well as in diestrous bitches 3 to 5 days after they had been treated with dinosprost (50 to 250μg/kg 12 h apart) for 7 days. Whereas all anestrous bitches ovulated, 1 out of 6 and 3 out of 9 bitches of the 2.1 or 1.05 mg diestrus subgroups, respectively, failed to ovulate. The reason for ovulation failure in these animals requires further study (Volkmann *et al.,* 2006a). Furthermore, only one female of each subgroup became pregnant and the bitch treated with the smaller dose resorbed her only fetus (Volkmann *et al.,* 2006a).

Recently, in preliminary clinical investigations, another slow-release deslorelin product, not approved for sale, has been given to three client owned female Lakeland Terriers at the dose of 1.5 mg. Proestrus occurred in all animals within 3 to 5 days. All animals ovulated, were bred and became pregnant (Kutzler, 2005). Subsequent attempts to induce estrus in nine anestrous beagle bitches with this protocol, administered either once or two or three times always 48 h apart, have not resulted in similar success. In this study, deslorelin serum concentration did not increase consistently in the treated animals (Kutzler *et al.,* 2006).

2.5.3 Time between treatment initiation and the induction of estrus

The time between treatment start and the onset of estrus is fairly constant for all protocols. Cain *et al.* (1989), using infusion pumps delivering GnRH 140ng/kg every 90 minutes for 11 to 12 days, obtained estrus after 5.6 ± 0.53 days in all test bitches. Inaba et al. (1998) , using a leuprolide depot injection of 1 mg/kg, obtained estrus in all 12 bitches in their study on average 10.3 ± 0.9 days post-treatment. Concannon *et al.* (2006), using lutrelin, induced estrus in 89% of treated bitches 4.8 ± 0.2 days after the start of the treatment. All the bitches in the study by Kutzler *et al.* (2002), who used a 2.1mg deslorelin implant, came into heat at the latest 6 days after the start of the treatment, with estrus generally appearing in the 3 to 5 days following implantation (Kutzler, 2005; Volkmann *et al.*, 2006a). Fontaine and Fontbonne (2010) obtained similar results with 4.7 mg deslorelin implants, with heat induction occurring 4.2 ± 1.4 days post implantation.

2.5.4 Ovulation in relation to GnRH

Most of the treated bitches ovulated after induced estrus, but here again the results differ from one study to another. Whereas Concannon (1989) obtained only 18 ovulations out of 24 bitches treated in his protocol with lutrelin in his first study and only 59% ovulations in the second (Concannon *et al.*, 2006), the results obtained in other studies are more encouraging. All the beagle bitches treated with deslorelin by Kutzler *et al.* (2002) ovulated. Seven bitches ovulated between 11 and 15 days after implantation, and 4 after about 11 days. Volkmann *et al.* (2006a), also using deslorelin implants, obtained similar results: the time between implantation and peak LH levels ranged from 9 to 17 days, indicating that ovulation occurred between 11 and 19 days after implantation. 76%

of the 29 bitches implanted by Fontaine and Fontbonne (2010) with deslorelin implants ovulated, this ovulation occurring 12 ± 2 days post implantation.

Inaba *et al.* (1998) and Cain *et al.* (1989), did not determine ovulation time, but simply used the bitches for reproduction. However, the pregnancy rates obtained (14/18 for Inaba *et al.*, 1998; 7/8 for Cain *et al.*, 1989) in these two studies confirm that the bitches had the capacity to ovulate.

2.5.5 Luteal function

The constant administration of GnRH agonists results in hypoluteidism caused by gonadotrophic insufficiency. In this case gestation may not be continued to term and the luteal phase ends between 30 and 40 days after the treatment is started (Volkmann *et al.*, 2006b; Fontaine and Fontbonne, 2010). To avoid this phenomenon, Kutzler *et al.* (2002) suggested removing the 2.1 mg deslorelin implant used in their study after the rise in blood progesterone levels, around the LH peak. On the other hand, Fontaine and Fontbonne (2010) suggested removing the implant after ovulation took place, as they experienced anovulatory cycles when using the former suggestion. The implant was inserted beneath the vulvar mucosa, in reference to practice in mares (Kutzler *et al.*, 2002), or in the umbilical area (Fontaine and Fontbonne, 2010) where it remained, without migration, and was therefore easy to locate and remove. Five bitches treated in this manner became pregnant out of the 8 in the study of Kutzler *et al.* (2002), whereas Fontaine and Fontbonne (2010) obtained pregnancy in 65% of the treated bitches (n = 29). However, both studies reported lost of litters during pregnancy, one between 30 and

37 days postovulation (Kutzler *et al.*, 2002) and one 58 days post ovulation (Fontaine and Fontbonne, 2010).

Subsequently, (Volkmann *et al.*, 2006b) noted that treated individuals absorbed most of the agonist contained in 2.1 mg deslorelin implants in the 10 days following implantation. Implant removal therefore was of little practical use. The bitches induced in this manner saw their blood progesterone levels decrease significantly 35 days later, which may explain why the luteal phase in these induced bitches was not conducive to the maintenance of gestation. Following of the luteal phase is therefore recommended after this kind of treatment, to ensure adequate progesterone replacement therapy if needed (Fontaine and Fontbonne, 2010).

The use of preparations for injection could prove to be more practical. However, the dose-duration effect should in this case be carefully controlled although Inaba *et al.* (1998) obtained 14 pregnant bitches out of 18 with leuprolide 1mg/kg, (Kutzler, 2005) obtained controversial results with a deslorelin preparation for injection. Whereas three pregnancies out of 3 were obtained in Lakeland terriers following deslorelin 1.5 mg i/m administration. On the contrary the 9 beagle bitches treated in the same fashion in an another study by (Kutzler *et al.*, 2006) did not even express estrus.

Materials and Methods

III. MATERIALS AND METHOD

3.1 Animal

Sixty healthy Pugs female dogs presented to the small animal outpatient division of the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bangalore by the pet owners with a specific request to examine reproductive health and recommend the optimum time for mating were utilized for the present investigation. The age of the bitches ranged from 1.5 to 6 years. The animals included for present study had littered atleast once and the number of litters ranged between 1to 4. The litter size in the selected animals during their previous delivery ranged between 1 and 8. Only those animals which were regularly vaccinated, dewormed and in apparently good general health were selected. Animals with previous history of infertility, gestational or parturient disorders were excluded. Animals presented with uncertain history regarding the exact day of the onset of proestrus bleeding were also excluded from present study.

3.2 Experimental design

Sixty healthy Pug female dogs selected for the study were divided into six groups of ten animals in each. The following are the constituent groups

3.2.1Group A: Breeding based on Vaginal Exfoliative Cytologty only

This group comprised of 10 Pugs female dogs in which breeding was recommended based on exfoliative vaginal cytology. The vaginal discharge required for the study of exfoliative cytology was collected using aspiration technique as described by England (1992). A sterile plastic bovine artificial insemination sheath containing 0.5 ml sterile normal saline and attached to a 5 ml syringe by a small piece of rubber adapter was introduced into the vagina carefully directing the sheath upward through the vestibule and then horizontally as far as possible and carefully avoiding the urethral orifice. The normal saline was slowly infused into the anterior vagina. A suction force was applied on the syringe to aspirate the vaginal fluid into the sheath. A sample of the fluid collected was placed on a glass slide and tilted to allow it to spread. The excess fluid was drained off. The smear was dried at room temperature and fixed with methanol for 30 seconds followed by staining with Giemsa staining for 20 minutes. The stained slide was washed in running water, dried and examined microscopically at X400 magnification.

The exfoliated vaginal epithelial cells were classified as parabasal, intermediate and superficial based on the description of the exfoliated canine vaginal cells by Klass (1985). Round or oval cells with a large vesicular nucleus and a somewhat dark stained cytoplasm were considered as Parabasal cells. The intermediate cells were either round or polygonal in shape, the cells size being much larger than the Parabasal cells and had relatively small dark stained nuclei. The superficial cells, otherwise commonly described as cornified or keratinized or anuclear cells were polygonal in shape with the nucleus being either absent or pyknotic.

A total of 200 exfoliated vaginal epithelial cells were counted and the percentage of superficial epithelial cells was calculated. The smears were also examined for the presence of polymorpho nuclear leucocytes, erythrocytes and bacteria.

Vaginal exfoliative cytology was studied from the day 5 of proestrus bleeding and subsequently every other day until the percentage of superficial cells reached a minimum of 95 percent and above and the mating was recommended once on the first day of optimum vaginal cornification and a second mating two days later. This group also served as control group.

3.2.2 Group B: Breeding based on Vaginal Exfoliative Cytologty and administration of GnRH (0.2µg/kg bw i.m) at optimum cornification

Animals in this group were utilised to study whether administration of 0.2µg/kg body weight of GnRH (Busereline Acetate*) intra muscularly at the time of optimum cornification improves the conception rate and litter size. Ten female pugs presented for determination of mating time were subjected to the study of vaginal exfoliative cytology starting from day 5 of proestrus and vaginal exfoliative cytology was repeated every second day on the same bitch until a vaginal cornification index of atleast 95% was observed. Following optimum vaginal cornification, each bitch received a single intra muscular injection of GnRH(0.2µg/kg body weight) and the matings were recommended once at 48 hours and again at 96 hours following GnRH administration.

*Buserelin Acetate (RECEPTAL$^{(R)}$, Intervet)

3.2.3 Group C: Breeding based on Vaginal Exfoliative Cytologty and administration of GnRH (0.4µg/kg bw i.m) at optimum cornification

Ten female pugs in this group were also subjected vaginal exfoliative cytology as in group B and optimum vaginal cornification, each animal received a single intra muscular injection of GnRH at the dose rate of 0.4 µg/kg body weight. Each animal, then received two mating, the 1^{st} at 48 hours and the 2^{nd} at 96 hours following GnRH administration.

3.2.4 Group D: Breeding based on Serum Progesterone (P_4) concentration only

This group consisted of 10 Pug female dogs in which the mating was recommended on the basis of serum Progesterone (P4) concentrations. The presented bitches with the history of proestrus bleeding were initially subjected to vaginal exfoliative cytology and the blood samples were collected for determination of progesterone only when superficial cells was atleast 95 per cent. If the percentage of superficial cells was less than 95 per cent, the owners were advised to reproduce their female dogs 2 days later for a second smear test and procedure were repeated until atleast 95 per cent cornification was reached before blood samples were obtained for the determination of blood serum progesterone concentration.

The blood samples were collected in to a sterile test tube, allowed to clot and the serum was separated and were subjected for determination of serum P4 concentration by using ELFA technique (Enzyme Linked Fluorescent Assay). The sensitivity of the assay was 0.25 ng/ml to 80 ng/ml with a coefficient of variation of 3.8 to 5.7 per cent and the results were obtained within 45 minutes after submitting the serum samples.

Based on serum P_4 concentration mating was recommended as soon as its concentration was 3 ng and above. The owners were recommended to mate the bitches on the same day when the serum p4 concentration reached 3 ng and a second mating 2 days later.

3.2.5 Group E: Breeding based on serum Progesterone concentration and administration of GnRH (0.2µg/kg bw i.m) when P_4 concentration was ≥ 3 ng/ml

This group was utilised to determine if administration of 0.2µg/kg body weight of GnRH at the time when the serum P4 concentration reached 3 ng and above improved the conception rate and litter size in pugs. As in the previous group, P4 concentration in the serum was determined in ten pugs as soon as the vaginal cornification index reached 95 per cent or more and matings were recommended once the P4 concentration in the serum was atleast 3 ng per ml. Each animal also received a single intra muscular injection of GnRH at the rate of 0.2 µg/kg body weight on the first day when the serum P4 concentration was atleast 3ng/ml. The animals were then recommended two mating, once on the day of GnRH administration and again 48 hours later.

3.2.6 Group F: Breeding based on serum Progesterone concentration and administration of GnRH (0.4µg/kg bw i.m) when P_4 concentration was ≥ 3 ng/ml

This group studied the effect of administration of GnRH at the rate of 0.4 µg/kg body weight when serum P_4 concentration was 3ng or more. As in the previous group, serum P4 concentrations were carried out once a cornification index of atleast 95 per cent was observed. Ten animals in this group received a single intra muscular injection of GnRH at the dose rate of 0.4µg/kg bw, as soon as the P4 concentration reached a minimum of 3ng per ml and each animal then received two matings, once on the day of GnRH administration and again 48 hours later.

Following mating, the owners were advised to present their animals for pregnancy diagnosis between 28 to 30 days from the day of last mating. The pregnancy status was ascertained by using a B mode transabdominal multiconvex probe. Animals confirmed pregnant were followed till the completion of pregnancy period and the information on the litter size was obtained.

The data generated for all the six groups were tabulated. Mean and Standard Error were computed. The statistical difference, if any, between the six groups with regard to conception rate and litter size was analysed by one way analysis of variance as per the procedure detailed by Snedecor and Cochran (1989).

Results

IV. RESULTS

4.1 Conception rate and litter size following mating on the basis of vaginal cytology (Group A).

The vaginal smear of ten pug female dogs was examined from the day 5 of proestrus bleeding and subsequently every other day until the percentage of superficial cells reached a minimum of 95 percent and above and the mating was recommended once on the first day of optimum vaginal cornification and a second mating two days later.

Sixty per cent (6/10) of the bitches bred on the basis of the vaginal exfoliative cytology were diagnosed to be pregnant by B mode transabdominal multiconvex ultrasound probe around 28 to 30 days from the last mating, and the litter size ranged from 1 to 6. The mean litter size was determined as of 3.2± 0.73 (Table 1).

4.2 Conception rate and litter size following mating on the basis of vaginal cytology and administration of GnRH (0.2 µg/kg bw) on the day of mating (Group B).

The vaginal smear of ten pug female dogs was examined from the day 5 of proestrus bleeding and subsequently every other day until the percentage of superficial cells reached a minimum of 95 percent and above. The pug female dogs then received a single intra muscular injection of GnRH (0.2µg per kg bw) and the matings were recommended once at 48 hours and again at 96 hours following GnRH administration.

Sixty per cent (6/10) of the bitches which received GnRH at the rate of 0.2 µg/kg bw on the day of mating, mated were diagnosed to be pregnant by B mode transabdominal multiconvex ultrasound probe around 28 to 30 days of last mating, and all of them delivered with litter size ranging from 1 to 8. The mean litter size was determined as of 3.1 ± 0.89 (Table 2)

4.3 Conception rate and litter size following mating on the basis of vaginal cytology and administration of GnRH (0.4 µg/kg bw) on the day of mating (Group C).

The vaginal smear of ten pug female dogs was examined from the day 5 of proestrus bleeding and subsequently every other day until the percentage of superficial cells reached a minimum of 95 percent and above. The pug female dogs then received a single intra muscular injection of GnRH (0.4µg per kg bw) and the matings were recommended once at 48 hours and again at 96 hours following GnRH administration.

Seventy per cent (7/10) of the bitches which received GnRH at the rate of 0.4 µg/kg bw on the day of mating, mated were diagnosed to be pregnant by B mode transabdominal multiconvex ultrasound probe around 28 to 30 days of last mating, and all of them delivered with litter size ranging from 1 to 8. The mean litter size was determined as 3.1± 0.86 (Table 3).

4.4 Conception rate and litter size following mating on the basis of serum progesterone concentration (Group D).

Serum progesterone concentration were assayed, on the day of optimum vaginal cornification and subsequently every other day until its concentration was determined to be a minimum of 3ng/ml then, ten pug female dogs were subsequently mated twice at an interval of two days. The mated animals were examined for pregnancy status 28 to 30 days from the day of last mating.

The (Table 4) shows the mean serum progesterone concentration on the day of first mating recommended and also the conception rate and litter size recorded. The mean serum progesterone concentration on the day of recommendation of mating was determined as 3.16 ± 0.18.

Ninty per cent (9/10) of the bitches mated on the basis of serum progesterone concentration were diagnosed to be pregnant and all of them delivered with litter size ranging from 3 to 6. The mean litter size was determined as 3.9 ± 0.54 (Table 4).

4.5 Conception rate and litter size following mating on the basis of serum progesterone concentration and administration of GnRH (0.2 µg/kg bw) on the day of mating (Group E)

Ten female pugs in this group received a single intra muscular injection of GnRH (0.2 µg/kg bw) on the day when serum progesterone concentration reached a minimum of 3 ng/ml. The mean serum progesterone concentration on the day of GnRH administration was determined as 3.27 ± 0.12 ng/ml.

Ninty per cent (9/10) bitches receiving GnRH at the rate of 0.2 µg/kg bw were diagnosed to be pregnant 28 days later and they delivered with the litter size ranging from 1 to 6 and mean litter size was determined as 3.8 ± 0.33 (Table 5).

4.6 Conception rate and litter size following mating on the basis of serum progesterone concentration and administration of GnRH (0.4 µg/kg bw) on the day of mating (Group F)

Ten female pugs in this group received a single intra muscular injection of GnRH (0.4 µg/kg bw) on the day when serum progesterone concentration reached a minimum of 3 ng/ml. The mean serum progesterone concentration on the day of GnRH administration was determined as 3.14 ± 0.19.

Ninty per cent (9/10) bitches receiving GnRH at the rate of 0.4 µg/kg bw were diagnosed to be pregnant 28 days later and they delivered with the litter size ranging from 1 to 7 and mean litter size was determined as 3.85 ± 0.51 (Table 6).

4.7 Comparison of conception rate between different groups of pugs recommended for mating

The conception rate obtained with different treatment were presented (Table 7 and fig. 1). The conception rate in animals recommended on the basis of exfoliative cytology only determined as 60 per cent. In animals receiving GnRH at the dose level of 0.2 µg/kg bw at the time of optimum vaginal cornification yielded a conception rate of 60 per cent. When the dose level of GnRH was increased to 0.4 µg/kg bw, the conception rate was 70 per cent. However, statistical analysis revealed the administration of either 0.2 or 0.4 µg/kg bw at the time of optimum vaginal cornification did not significantly improve the conception rate as compared to the conception rate of animals mated on the basis of vaginal exfoliative cytology only (Table 7 and Fig. 1). Further, the conception rate between groups of bitches receiving either 0.2 or 0.4 μg/kg bw were also not significantly different ($p > 0.05$).

The conception rate in group of female pugs which were recommended to be mated on the basis of serum progesterone concentration was recorded as 90 per cent. The conception rate in groups of bitches receiving either 0.2 or 0.4 μg/kg bw of GnRH at a time when the serum progesterone concentration was atleast 3 ng/ml was recorded as 90 per cent each (Table 7 and Fig.1). Statistical analysis revealed that the conception rate in groups of bitches mated on the basis of serum progesterone concentration or receiving GnRH either at 0.2 or 0.4 μg/kg bw did not significantly ($p > 0.05$) alter the conception rate. Further the conception rate was also not significantly difference ($p > 0.05$) between any of the groups recommended for mating (Table 7).

4.8 Comparison of litter size between different groups of pugs recommended for mating

The litter size obtained in different treatment group presented in (Table 8 and Fig. 2). The litter size in animals recommended on the basis of exfoliative cytology only was determined as 3.2 ± 073. In animals receiving GnRH at the dose level of 0.2 μg/kg bw at the time of optimum vaginal cornification yielded a litter size of 3.1 ± 0.89. When the dose level of GnRH was increased to 0.4 μg/kg bw, the litter size was 3.1 ± 0.86. However, statistical analysis revealed the administration of either 0.2 or 0.4 μg/kg bw at the time of optimum vaginal cornification did not significantly improve the litter size as compared to the litter size of animals mated on the basis of vaginal exfoliative cytology only (Table 8 and Fig. 2). Further, the litter size between groups of bitches receiving either 0.2 or 0.4 µg/kg bw were also not significantly different.

The litter size in group of female pugs which were recommended to be mated on the basis of serum progesterone concentration was recorded as 3.9 ± 0.54. The litter size in groups of bitches receiving either 0.2 or 0.4 µg/kg bw of GnRH at a time when the serum progesterone concentration was atleast 3 ng/ml was recorded as3.8 ± 0.33 and 3.85 ± 0.51 respectively (Table 8 and Fig. 2). Statistical analysis revealed that, the litter size in groups of bitches mated on the basis of serum progesterone concentration or receiving GnRH either at 0.2 or 0.4 µg/kg bw did not significantly alter the litter size. Further, the litter size was also not significantly difference between any of the groups recommended for mating (Table 8).

Table 1. Conception rate and litter size in pugs mated on the basis of vaginal exfoliative cytology (Group A)

No of animals mated	10
No of animals conceived	6
Per cent conception	60
Mean litter size	3.2± 0.73

Table 2. Conception rate and litter size in pugs mated following administration of GnRH (0.2 µg/kg bw) at the time of optimum cornification (Group B)

No of animals mated	10
No of animals conceived	6
Per cent conception	60
Mean litter size	3.1 ± 0.89

Table 3. Conception rate and litter size in pugs mated following administration of GnRH (0.4 µg/kg bw) at the time of optimum cornification (Group C)

No of animals mated	10
No of animals conceived	7
Per cent conception	70
Mean litter size	3.1± 0.86

Table 4. Conception rate and litter size in pugs mated on the basis of serum progesterone concentration (Group D)

No of animals mated	10
Mean serum P4 concentration (ng/ml)	3.16 ± 0.18
No of animals conceived	9
Per cent of conception	90
Mean litter size	3.9 ± 0.54

Table 5. Conception rate and litter size in pugs mated following administration of GnRH (0.2µg/kg body weight) at the time when serum progesterone concentration was 3ng and above (Group E)

No of animals mated	10
Mean serum P4 concentration (ng/ml) on the day of recommendation of mating	3.27 ± 0.12
No of animals conceived	9
Per cent conception	90
Mean litter size	3.8 ± 0.33

Table 6. Conception rate and litter size in pugs mated following administration of GnRH (0.4 µg/kg body weight) at the time when serum progesterone concentration was 3 ng and above (Group F)

No of animals mated	10
Mean serum P4 concentration (ng/ml) on the day of recommendation of mating	3.14 ± 0.19
No of animals conceived	9
Per cent conception	90
Mean litter size	3.85 ± 0.51

Table 7. Comparison of conception rate between different groups of pugs recommended for mating

Group	Mating on the basis Exfoliative Cytology only	Mating on the basis of Exfoliative Cytology plus GnRH (0.2 µg/kg bw) administered at the time of mating	Mating on the basis of Exfoliative Cytology plus GnRH (0.4 µg/kg bw) administered at the time of mating	Mating on the basis of serum Progesterone concentration only	Mating on the basis of serum Progesterone concentration plus GnRH (0.2 µg/kg bw) administered at the time of mating	Mating on the basis of serum Progesterone concentration plus GnRH (0.4 µg/kg bw) administered at the time of mating
Conception rate (%)	60^{a}	60^{a}	70^{a}	90^{b}	90^{b}	90^{b}

***Conception rate between groups of pugs with different superscripts are significantly different (p<0.05)**

Table 8. Comparison of litter size between different groups of pugs recommended for mating.

Group	Mating on the basis Exfoliative Cytology only	Mating on the basis of Exfoliative Cytology plus GnRH (0.2 µg/kg bw) administered at the time of mating	Mating on the basis of Exfoliative Cytology plus GnRH (0.4 µg/kg bw) administered at the time of mating	Mating on the basis of serum Progesterone concentration only	Mating on the basis of serum Progesterone concentration plus GnRH (0.2 µg/kg bw) administered at the time of mating	Mating on the basis of serum Progesterone concentration plus GnRH (0.4 µg/kg bw) administered at the time of mating
Litter size	3.2 ± 073^{a}	3.1 ± 0.89^{a}	3.1 ± 0.86^{a}	3.9 ± 0.54^{b}	3.8 ± 0.33^{b}	3.85 ± 0.51^{b}

***Litter size between groups of pugs with different superscripts are significantly different ($p<0.05$)**

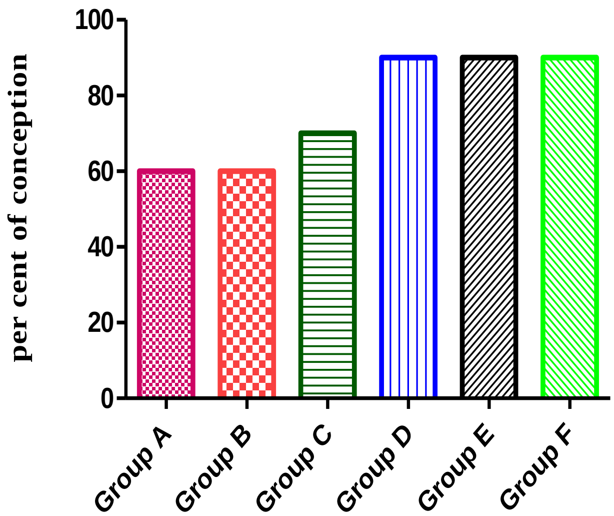

Fig. 1. Comparison of Conception rate between different groups of pugs recommended for matings

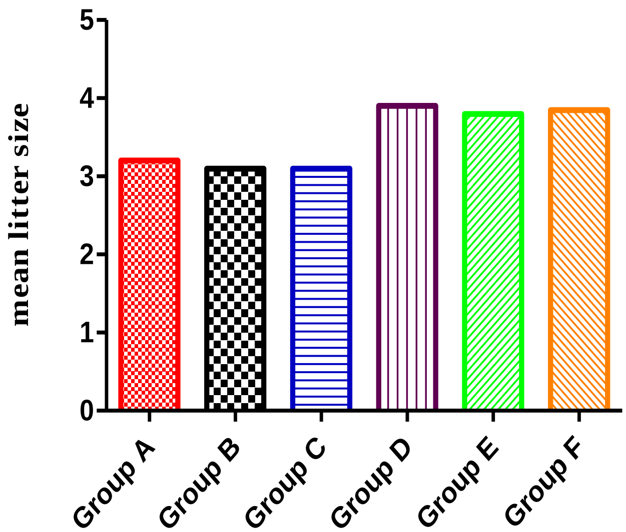

Fig. 2. Comparison of Litter size between different groups of pugs recommended for mating.

Discussion

V. DISCUSSION

Over the last two decades, canine breeding has developed as a highly commercially lucrative industry. Consequently the kennel owners are highly aware of the importance of feeding and management, vaccination, routine health check up and more importantly, the management of breeding dates for maximizing the conception rate. A considerable advance has been made towards determination of optimum breeding date for the individual bitch and the techniques used include vaginal exfoliative cytology (England, 1992 ; Suyash, 2002; Venkatachalapathy, 2005), vaginal endoscopy (Lindsay and Conconnan 1986; Suyash, 2002), and on the basis of serum progesterone concentration (Nothling *et al*., 2003; Venkatachalapathy, 2005). These techniques aim indirectly to determine the LH surge so that matings can be carried out close to the time of LH surge. Nevertheless, the techniques are primarily managemental in nature and do not involve any therapeutic interventions to ensure that the ovulation does occur.

In dairy cattle, Gonadotropin Releasing Hormone (GnRH) are routinely administered at the time of insemination to ensure that the ovulation occurs (Padula and MacMillan, 2002; Peters, 2005). There are also numerous reports of enhanced conception rate following administration of GnRH (Kharche and Srivastava, 2007: Gumen *et al.*, 2011) at the time of artificial insemination in dairy cattle. However, the efficacy of GnRH administered at the time of mating in spontaneously cycling bitches has not been explored. However, an extensive search of the literature revealed that GnRH has not been evaluated for its efficacy for improving the conception rate of spontaneously cycling female dogs, although it has been used as a part of the protocol for induction of estrus in anestrous female dogs (Concannon, 1989; Kutzler, 2007). The present study therefore was designed to assess whether the conception rate and litter size can be improved with the administration of GnRH during estrus as being done in cattle.

5.1 Conception rate and litter size in female pugs recommended on the basis of vaginal exfoliative cytology (Group A).

Vaginal exfoliative cytology is perhaps one of the first techniques developed for detection of mating time in bitches Allen and Doisy (1923).

The vaginal epithelium is one of the several target tissue for ovarian hormones. Characteristic changes in exfoliated vaginal epithelial cells occur as a result of changes in secretory pattern of estrogen. The increasing circulatory levels of estradiol-17β stimulate the growth of vaginal epithelium from a bi-stratified cuboidal epithelium to a stratified squamous epithelium of greater than 30 cells layer with every estrous cycle (Holst, 1986). As the vaginal mucosa develops, number of superficial epithelial cells slough into vaginal fluid. The change in the percentage of superficial cells in a vaginal smear has been used to study the progression of proestrus into estrus and to recommend the appropriate time of mating. **(**Olson, 1989; Wright, 1990; England, 1992; England and Conconnon, 2002**)**

In the present study ten bitches were recommended for mating on the basis of vaginal exfoliative cylology. The vaginal cytology of these bitches were followed from 5th day of proestrus every second day till a cornification index of 95 per cent was observed. The bitches were then advised to be mated twice, once on the day of optimum vaginal cornification and a second mating, 48 hours later.

Following mating on the basis of vaginal exfoliative cytology, 60 per cent were diagnosed to be pregnant (Table1 and Fig. 1). In other reports, the conception rates obtained following recommendation of mating time on the basis of vaginal exfoliative cytology hs range from 30 to 92 per cent (Okkens *et al*., 1985; England *et al.,* 1992; Suyash, 2002; Venkatachalapathy, 2005).

There was a considerable variation between the number of offsprings littered in individual bitches and the litter size 1 to 8 and the average litter size was recorded as 3.1 ± 0.73. There are no reports available in the literature regarding either the conception rate or the litter size in pugs recommended for mating on the basis of vaginal exfoliative cytology

The reliability of vaginal cytology for determining the optimal mating period is controversial. Hiemstra *et al*., (2001) correlated the vaginal cytology of 35 bitches with the endocrine events (plasma progesterone, LH surge) and concluded that vaginal cytology is not useful for determining the optimal mating periods in bitches, since mating according to cytological findings would have resulted in only 28 per cent of the bitches being mated at the proper time. Christie *et al*., (1972) and Bouchard *et al.,* (1991) reported that the initial day of optimum cornification coincided poorly with the onset of LH surge and the peak vaginal cornification occurred between -1 to +7 days after the LH surge and therefore, matings timed on the basis of peak vaginal cornification yields poor result.

5.2 Conception rate and litter size in female pugs receiving GnRH injection at the time of optimum vaginal cornification (Group B and C)

In view of the poor correlation of the vaginal cytology with LH surge, therapeutic induction of LH surge at the time of peak vaginal cornification may be beneficial in terms of induction of ovulation and improvement of conception rate. This hypothesis was tested in two groups of ten female pugs each. In one of the groups GnRH was administered intramuscularly at a dose of 0.2 μg/kg bw on the day of optimum vaginal cornification. In the other group the dose of GnRH was increased to 0.4 μg/kg bw. Animals were advised to be mated once at 48 hours and again at 96 hours after GnRH injection.

Synthetic analogue of GnRH used in the present study (Buserelin acetate) has been shown to be 50 to 170 times more potent than LHRH (Nawito *et al*., 1977) and 20 to 170 times more effective than the natural compound (Snedow *et al.,* 1978). In the bitch progression from proestrus to estrus is characterized by a higher amplitude and larger GnRH pulses (Tani *et al*., 1996), and an increase in pituitary sensitivity to GnRH (Hull, 1987; Jeffcoate, 1993). Pulsatile administration of GnRH at doses of 0.2 to 0.4 μg/kg bw at 90 minute interval was sufficient to obtain increases in LH concentration, similar to the endogenous pulses that normally occur at the end of proestrus (Canconnon, 1993). However, in a clinical setup pulsatile administration is difficult and expensive and may need hospitalization during continuous intravenous infusion.

In the present study, GnRH was administered only once, at the time of peak vaginal cornification, and presumably at the onset of estrus. It was postulated that the animal would have mature follicles around this time and that there may already be an increased number of GnRH pulses characterized by higher amplitude. It was also postulated that the pituitary gland would have become increasingly sensitive to GnRH. Therefore, unlike the frequent administration or continuous administration of GnRH required to induce estrus in anestrous dogs (Kutzler, 2005), a single dose of GnRH may be enough to induce LH surge when administered at the onset of estrus as it could compliment the already existing natural pulses, resulting in increased the magnitude of LH surge and induce ovulation. Further, a higher magnitude of LH surge which could result following GnRH administration may ensure ovulation and significantly improve the ovulation rate.

In the present study, GnRh was administered at two dose levels namely, 0.2 and 0.4 μg/kg bw. The two different dose levels were tested as the dose required to induce LH surge in dogs is not conclusively established. Further, the animals were advised to be mated at 48 and 96 hours after GnRH administration because ovulation in dogs is reported to occur at approximately 2 to 3 days following the preovulatory LH surge (Phemister *et al*., 1973; Canconnon 1977; Wildt *et al*., 1978, Tsutsui, 1989). Canine oocytes are ovulated as primary oocytes and after ovulation they undergo the first meiotic division to become secondary oocytes (Olson and Nett, 1986; Tsutsui,1989; Conconnon, 1997; Jhonston *et al*., 2001). Once the maturation is complete, the viable life span of secondary oocyte is stated to be approximately only 2 to 3 days (Tsutsui, 1989; Conconnon *et al*., 1989; Linde, 1991). The conception rate is reportedly best in bitches bred from 3 days before to 4 days after ovulation (Holst and Phemister 1974).

In the present study, the conception rate of 60 per cent was obtained in female pugs receiving GnRH injection at a dose level of 0.2 μg/kg bw (Table 2 and Fig1). When the dose level was increased to 0.4 μg/kg bw, the conception rate was marginally higher (70 per cent) (Table 3 and Fig1), but this was not a significant improvement over the conception rate obtained with 0. 2 μg/kg bw. Further, the conception rate in GnRH administered groups was also not significantly different from the conception rate obtained following recommendation of mating on the basis of vaginal exfoliative cytology only. This observation suggested that administration of GnRH at the time of peak vaginal cornification at either 0.2 μg or 0.4 μg/kg bw does not significantly improve the conception rate. There is a possibility that these dose levels are not ideal to induce LH surge or that a single dose may not induce sufficient magnitude of LH surge to cause ovulation around the designated time of mating.

The litter size, in the GnRH administered group was also similar to those obtained in pugs mated on the basis vaginal exfoliative cytology. This observation again suggested that GnRH administered at the time of mating did not significantly influence the ovulation rate.

5.3 Conception rate and litter size following mating on the basis of serum progesterone concentration Group D.

The serum progesterone assays focus on using changes in progesterone levels to identify the occurrence of LH surge, ovulation and the fertile period. Elevation of the serum progesterone concentration prior to ovulation is peculiar to the bitches and the source of this elevation is reportedly preovulatory luteinized follicles (Bouchard *et al.*, 1991). Serum progesterone concentrations have been reported to increase gradually from basal values with the advancement of proestrus and exhibit distinct rapid and detectable increase around the time of LH surge (England and Canconnon, 2002). Identification of this rapid distinct rise allows an indirect method of predicting the LH surge, and therefore, the fertile period of the bitch.

The concentration of progesterone in the serum or plasma around the time of LH surge has been a subject of several recent studies (Van *et al*., 1989; Mestre *et al*., 1990; Arbeiter *et al*., 1991; Bouchard *et al*., 1991; Renton *et al*., 1991; Johnston and Root, 1995; Tomaskovic *et al*., 1997; Goodman, 1998; Konuk *et al.*, 2001). In several of these studies, a serum progesterone concentration between 3 and 4 ng/ml corresponded with the time of LH surge. Matings when the serum progesterone concentration is around 4ng/ml, therefore, yield a significantly better fertility rate in bitches. Serum or plasma progesterone concentration may be measured most accurately by RIA, quantitative enzyme linked immuno absorbent assay (ELISA) or enzyme linked immuno fluorescent assay (ELFA). Facilities for determination of serum progesterone concentrations using ELISA and ELFA are available in many human clinical laboratories. The techniques are reliable, cheap and the results of progesterone concentrations are available the results of progesterone concentrations are available the same day.

In the present study, ten bitches were recommended to be bred as soon as the progesterone concentration in serum reached a minimum of 3 ng/ml. Following mating 9 out of 10 (90 per cent) (Table 4 and Fig 1) bitches was diagnosed to be pregnant. The conception rate achieved with matings on the basis of serum progesterone concentration was similar to those reported in free roaming dogs (>90 per cent) (Friedman 1957). A similar conception was also reported by Venkatachalapathy (2005). The conception rate achieved was also a significant improvement over those obtained following recommendation of mating time on the basis of vaginal exfoliative cytology or in bitches receiving GnRH at the time of peak vaginal cornification. Several clinical trials confirmed that recommendation of mating time based on serum progesterone concentration to yield a significantly better fertility than with any other variable techniques (Van *et al.,* 1989; Dietrich and Moller, 1993; Suyash, 2002)

All the 9 bitches delivered with en average litter size of 3.9 ± 0.54 (Table 4), which again a significant over the litter size observed with the other 3 groups. The results of present study clearly showed that serum progesterone concentration was closely related to the fertile period of the bitch.

5.4 Conception rate and litter size following mating on the basis of serum progesterone concentration and administration of GnRH on the day of mating (Group E and F)

Animals in this group received GnRH injection at either 0.2 or 0.4 μg/kg bw at a time when a serum progesterone concentration was 3 ng/ml or more. Therefore, GnRH was administered at the time when the pre ovulatory LH surge had already occurred. In a spontaneously cycling animal, the duration of preovulatory LH surge is reported to vary with a range of 28 to 96 hours (Smith and Mcdonald, 1971; Nett *et al.,* 1975; Conconnon *et al.,* 1977; Reimers *et al.,* 1978; Wildt *et al.,* 1978; Olson *et al.,* 1982). Administration of GnRH at a time when the serum progesterone concentration is around 3ng/ml may further augment the magnitude of LH surge. Further, GnRH injections may also aid in improving the ovulation rate.

In the present study, the conception rate of 90 per cent was obtained when GnRH was administered at the time when the serum progesterone concentration was around 3 ng/ml, irrespective of the dose level employed (Table 5, 6 and Fig.1) the conception rates observed were similar to those obtained in the group of animals mated on the basis only serum progesterone concentration and was not statistically significant ($p>0.05$) (Table 7). The results of the present study suggested that the administration of GnRH at either 0.2 or 0.4 μg/kg bw at a time when the serum progesterone concentration was around 3 ng/ml does not significantly improve the conception rate as compared to the conception rate in bitches mated only on the basis of serum progesterone concentration.

Further, the litter size animals receiving GnRH on the day of when the serum progesterone concentration was around 3 ng/ml was also similar irrespective of the dose level (Table 5 and 6, Fig. 2). Also, the litter size in GnRH treated groups did not differ significantly from the group of animals mated only on the basis of serum progesterone concentration (Table 8).

Therefore, the results obtained in the present study suggest that although, the common practice of injecting GnRH around the time of artificial insemination is beneficial in dairy cattle, the same is not true for female dogs. In fact GnRH administration neither improved the conception rate nor enhanced the litter size.

Summary

VI. SUMMARY

A clinical study was designed to verify the unpublished claims by many canine practitioners that, administration of GnRH prior to mating improved the conception rate and litter size in female dogs. The study was conducted on 6 groups of a single breed of dogs namely Pug, with each group comprising of 10 animals.

In the first experiment, the conception rate and litter size was evaluated in 10 animals following recommendation of mating dates on the basis of vaginal exfoliative cytology. The animals were advised to be mated twice, once on the day of optimum vaginal cornification and a second time 48 hours later. Following mating, 6 out of 10 (60 per cent) animals were diagnosed to be pregnant and the mean litter size was recorded as 3.2 ± 0.73. It was concluded that the poor conception rate observed to matings recommended on the basis of vaginal exfoliative cytology was because of the poor correlation of the technique with the LH surge.

In the second experiment, 10 female pugs received a single intramuscular injection of GnRH at a dose level of 0.2 μg/kg bw at the time of optimum vaginal cornification. The animals were advised to be mated twice, at 48 and 96 hours after the administration of GnRH. Following mating, 6 (60%) animals were diagnosed to be pregnant and the mean litter size was recorded as 3.1 ± 0.89. The conception rate and the litter size observed was not significantly different from those of animals mated on the basis of vaginal exfoliative cytology only.

In third experiment, the dose level of GnRH was increased to 0.4 μg/kg bw which was administered as a single dose at the time of peak vaginal cornification. The schedule of mating was similar as in the previous experiment. Following mating, 7 (70%) animals were diagnosed to be pregnant and the mean litter size was recorded as 3.1 ± 0.86. The conception rate and the litter size observed with 0.4 μg/kg GnRH was similar to those observed with 0.2 μg/kg bw. It was concluded that administration of GnRH at the time of peak vaginal cornification at either 0.2 μg or 0.4 μg/kg bw does not significantly improve the conception rate. There is a possibility that these dose levels are not ideal to induce LH surge or that a single dose may not induce sufficient magnitude of LH surge to cause ovulation around the designated time of mating.

In the fourth experiment, the conception rate and litter size was recorded in 10 female pugs which were recommended to be mated on the basis of serum progesterone concentration. Ten female pugs were advised to be mated as soon as the progesterone concentration in the serum was around 3 ng/ml and the second mating was carried out 48 hours later. Following mating, 9 (90%) out of 10 animals conceived and the mean litter size in these animals was recorded as 3.9 ± 0.54. The conception rate and litter size recorded was significantly higher as compared to the previous 3 experiments. The higher conception rate obtained was attributed to be due to a close correlation of serum progesterone concentration with the LH surge.

In the fifth experiment, GnRH was administered as a single intramuscular injection of 0.2 μg/kg bw on the day when the serum progesterone concentration was around 3 ng/ml and the animals were mated twice, in a schedule, similar to the previous experiment. Following mating, 9 (90%) out of 10 animals conceived and the mean litter size in these animals was recorded as 3.8 ± 0.33. The conception rate and the litter size recorded in this experiment did not differ significantly from those recorded in animals mated only on the basis of serum progesterone concentration.

In the final experiment, the dose of GnRH was increased to 0.4 μg/kg bw which was administered on the day when the serum progesterone concentration was 3 ng/ml. Following mating, 9 (90%) out of 10 animals conceived and the mean litter size in these animals was recorded as 3.85 ± 0.51. The conception rate and the litter size recorded in this experiment did not differ significantly from those recorded in animals receiving a lower dose of GnRH.

The results obtained in the present study suggest that although, the common practice of injecting GnRH around the time of artificial insemination is beneficial in dairy cattle, the same is not true for female dogs. In fact GnRH administration neither improved the conception rate nor enhanced the litter size.

Bibliography

VII. BIBLIOGARPHY

ALLEN, E. and DOISYE, A., 1923. An ovarian hormone. *J. Am. Med. Ass.,* **81** (8): 19-821.

ALLEN, E.W. and ENGLAND, G.C.W., 1990. Reproductive Endocrinology of the bitch, Manual of Small Animal Endocrinology, *BASVA*, 127-129.

ARBEITER, K., DOBRETSBERGER, M., MULLER, E. and HOLZMANN, A., 1991. Indirect detection of ovulation and ova fertilization in dogs by monitoring of plasma progesterone levels. *J. Vet. Med. Series A.,* **38** (9): 696-701.

BADINAND, F., FONTBONNE, A., MAUREL, M.C., 1993. Fertilization time in the bitch in relation to plasma concentration of estradiol, progesterone and luteinizing hormone and vaginal smears. *J. Reprod. Fertil. Suppl.,* **47**: 63–7.

BEACH, F.A., DUNBAR, I.F. and BUCHLER, M.G., 1982. Sexual characteristics of female dogs during successive phases of the ovarian cycle. *Horm. Behav*., **16**: 414-442.

BELL, E.T. and CHRISTIE, D.W., 1971. Duration of proestrus, estrus and vulvar bleeding in the Beagle bitch. *Br. Vet. J.,* **127**: 25-27.

BOUCHARD, G.F., SOLORZANO, N., CONCANNON, P.W., YOUNGQUIST, R.S. and BIERSCHWAL, C,W., 1991. Determination of ovulation time in bitches based on teasing, vaginal cytology and ELISA for progesterone. *Theriogenol.,* **35**: 603-611.

BRUGGER, N., OTZDORFF, C., WALTER, B., HOFFMANN, B. and BRAUN, J., 2011. Quantitative Determination of Progesterone (P4) in Canine Blood Serum Using an Enzyme-linked Fluorescence Assay. *Reprod. Dom. Anim.,* **46**: 870–873

CAIN, J., LASLEY, B., CAIN, G., FELDMAN, E. and STABENFELDT, G. H., 1989. Induction of ovulation in bitches with pulsatile or continuous infusion of GnRH. *J. Reprod. Fertil.*, **39**: 143-147.

CAIN, J.L., CAIN, G.R., FELDMAN, E.C., LASLEY, B.L. and STABENFELDT, G.H., 1988. Use of pulsatile intravenous administration of gonadotropin-releasing hormone to induce fertile estrus in bitches. *Am. J. Vet. Res.*, **49**: 1993–1996.

CATHARINA, L. and INGER, K., 1984. The correlation between the cytology of the vaginal smear and the time of ovulation in the bitch. *J. small Anim. Pract.*, **25**: 7742.

CHRISP, P. and GOA, K.L., 1990. Nafarelin A review of its pharmacodynamic and pharmacokinetic properties, and clinical potential in sex hormone-related conditions. *Drugs*., **39**: 523–551.

CHRISTIE, D.W., BAILEY, J.B. and BELL, E.T., 1972. Classification of cell types in vaginal smears during the canine estrus cycle. *Br. Vet. J.*, **128**: 301-310.

CINONE, M., MINOIA, P., LACALANDRA, G. and DELLAQUILA, M., 1996. Oestrus induction in the bitch with buserelin implant. In: *Proceedings of the Satellite Meeting 13th ICAR, Sydney*., 32.

CONCANNON, P.W., 1980. Effect of hypophysectomy and of LH administration on the luteal phase plasma progesterone level in the Beagle bitch. *J. Reprod. Fert.*, **58**: 407

CONCANNON , P.W., HANSEL,W. and VISEK, W.J., 1975. The ovarian cycle of the bitch: plasma estrogen, LH and progesterone. *Biol. Reprod.*, **13**: 112-121.

CONCANNON, P., TEMPLE, M., MONTANEZ, A. and NEWTON. L., 2006. Effects of dose and duration of continuous GnRH agonist treatment on induction of estrus in beagle dogs: competiting and concurrent up-regulation and down-regulation of LH release. *Theriogenol*., **66**: 1488-1496.

CONCANNON, P.W., 1975. The ovarian cycle of the bitch: Plasma estrogen, LH and progesterone. *Boil. Reprod.,* **13**:112.

CONCANNON, P.W., 1986. Physiology of Reproduction, “In: Small Animal Reproduction and Infertility” Ed: Burke, T. J, Lea and Febiger, Philadelphia., *pp.* 23-77.

CONCANNON, P.W., 1987. The physiology of ovarian cycle, pregnancy and parturition in the domestic dogs. *Proc. of the Society for Theriogenol., pp.* 159.

CONCANNON, P.W., 1989. Induction of fertile oestrus in anoestrous dogs by constant infusion on GnRH agonist. *J. Reprod. Fertil.,* **39**: 149-160.

CONCANNON, P.W., 1989. Induction of fertile oestrus in anoestrus dogs by constant infusion of GnRH agonist. *J. Reprod. Fertil. Suppl*., **39**: 149-160.

CONCANNON, P.W., 1993. Biology of gonadotropin secretion in adult and prepubertal female dogs. *J Reprod Fertil Suppl*., **47**: 3-27.

CONCANNON, P.W., 2005. Estrus induction in dogs: approaches, protocols and applications. In: Proceedings of the CD 30^{th} World Small Animal Veterinary Association Congress (WSAVA), Mexico.

CONCANNON, P.W., 2011. Reproductive cycles of the domestic bitch. *J. Anim. Reprod. Sci.,***124**: 200–210.

CONCANNON, P.W., CASTRACANE, V.D., TEMPLE, M. and MONTANEZ, A., 2009. Endocrine control of ovarian function in dogs and other carnivores. *Anim. Reprod.,* **6:** 72–193.

CONCANNON, P.W., COWEN, R. and HANSEL, W., 1979. LH release in ovariectomised dogs in response to estrogen withdrawal and its facilitation by progesterone. *Boil. Reprod.,* **20**: 523-531.

CONCANNON, P.W., ENGLAND, G., VERSTEGEN, J., FARSTAD, W., LINDE-FORSBERG, C. and DOBERSKA, C., (Eds.) 2001a. Embryo development, hormonal requirements and maternal responses during canine pregnancy. *J. Reprod. Fertil.*, **57**: 159–169

CONCANNON, P.W., HANSEL, W. and MCENTEE, K., 1977. Changes **in** LH, progesterone and sexual behavior associated with preovulatory luteinization in the bitch. *Biol. Reprod.*, **17**: 604-6 13.

CONCANNON, P.W., HANSEL, W. and VISEK, W.J., 1975. The ovarian cycle of the bitch: plasma estrogen, LH and progesterone. *Biol. Reprod*., **13**(1):112–21.

CONCANNON, P.W., LASLEY, B. and VANDERLIP, S., 1997. LH release, induction of oestrus and fertile ovulations in response to pulsatile administration of GnRH to anoestrous dogs. *J. Reprod. Fertil. Suppl.*, **51**: 1–54.

CONCANNON, P.W., McCANN, J.P. and Temple, M., 1989. Biology and endocrinology of ovulation, pregnancy and parturition in dogs. *J. Reprod. Fert. Suppl.*, **39**: 3-25.

CONCANNON, P.W., TSUTSUI, T. and SHILLE, V., 2001b. Embryo development, hormonal requirements and maternal responses during canine pregnancy. *J. Reprod. Fertil. Suppl*., **57**: 159–169.

CONN, P.M. and CROWLEY, W.F., 1991. Review Article: Gonadotropin-releasing hormone and its analogues. *New Eng. J. Med.*, **324**: 93-103.

CRISTINA, G., 2007. New GnRH analogs in canine reproduction. *Anim. Reprod. Sci.*, **100**: 1-13.

DIETRICH, E. and MOLLER, R., 1993. Use of progesterone enzyme immunoassay to determine the optimal mating time in estrus bitches. *J. Reprod. Fert. suppl.*, **47**: 524.

ECKERSALL, P.D. and HARVEY, M.J.A., 1987. The use of bovine plasma progesterone, ELISA kit to measure progesterone in equine, ovine and canine plasma, *Vet. Rec.*,**120**: 5-8.

ENGLAND, G. and CANCONNON, P.W., 2002. Determination of optimum breeding time in bitches: Basic considerations. In: *Recent advances in small animal reproduction,* Canconnon, P.W., England, G., Verstegen III.J. and Linde-Forsberg, C. (Eds.)

ENGLAND, G.C.W., 1992. Vaginal cytology and cervicovaginal mucus arborisation in the breeding management of the bitches. *J. Sm. Anim. Prac.*, **33**: 577-582.

FEDLDMAN, E. C. and NELSON, R. W., 1996. Canine female reproduction, "In: Canine and Feline Endocrinology and Reproduction", Philadelphia, WB Saunders. *pp*. 526-547.

FLOWER, E.H., FELDMAN, M.K. and LOEB, W.F., 1971. Comparison of the histologic features of ovarian and uterine tissues with vaginal smears of the bitch. *Am. J. Vet. Res.*, **32**(2): 327-334.

FONTAINE, E. and FONTBONNE, A., 2010. Use of deslorelin to control fertility in the bitch. In Symposium deslorelin: deslorelin in practice. In: *EVSSAR Congress, 7th, 2010, Louvain la Neuve, Belgium. Louvain la Neuve: EVSSAR., pp*. 15-17.

FRIEDMAN, M.H., 1957. The effect of O-diazoacteyl(Azaserine) on the pregnancy of the dog. J. Vet. Med. Assoc., **130**: 160

GOODMAN, M., 1998. Canine ovulation timing, "In: Canine Reproduction Symposium:, society for Theriogenology., *pp*. 1-6.

GÜMEN, A., KESKIN, A., YILMAZBAS, M.G., KARAKAYA, E., CEVIK, S. and BALCI, F., 2011. Effect of GnRH, $PGF_2\alpha$ and oxytocin treatments on conception rate at the time of artificial insemination lactating dairy cows. *Czech. J. Anim. Sci.*, **56** (6): 279-283.

GUNZEL, A.A., SIEME, H. and HOPPEN, H.O., 1990a: Progesteronbestimmung bei der Hu¨ ndin mittels Mikrotiter – ELISA. *Kleintierpraxis Jahrg*., **35** *pp.* 473–480.

GUNZEL, A.A., SIEME, H. and HOPPEN, H.O., 1990b: Erfahrungen mit einem ‘‘schnellen’’ Progesterontest zur Bestimmung des Bedeckungs-oder Besamungszeitpunktes beim Hund. *Kleintierpraxis Jahrg*., **35**: 301–308.

HEAPE, W., 1990. The sexual season of mammals and the relationship of proestrus to mensuration. Part-I. *Q.J. Microbiol. Sci*., **44**: 1-70

HEGSTEAD, R.L. and JOHNSTON, S.D., 1989. Use of rapid quantitative ELISA technique to determine serum progesterone concentrations in the bitch. *Proc. Sco. Theriogenol., pp.* 277-287.

HERBERT, C.A. and TRIGG, T.E., 2005. Applications of GnRH in the control and management of fertility in female animals. *Anim. Reprod. Sci.,* **88** (1–2): 141–153.

HIEMSTRA, M., SCHAEFERS, O.A.C., TESKE, E. and KOOISTRA, H.S., 2001. The reliability of vaginal cytology in determining the optimal mating time in the bitch. *Tijdschrift. Voor. Diergeneeskunde.* **126** (21): 685-689.

HOLST, P.A. and PHEMISTER, R.D., 1974. Onset of diestrus in the Beagle bitch: Definition and significance. *Am. J. Vet. Res.,* **35**: 401-406.

HOLST, P.A., 1975. Temporal sequence of events in the estrous cycle of the bitch. *Am. J. Vet. Res.,* **36**: 705.

HOLST, P.A., 1986. Vaginal cytology in bitch. “In: Current therapy in Theriogenology”. Ed, Marrow. D.A., **2**: 457

HULL, M.E. and KENIGSBERG, D.J., 1987. Gonadotropin releasing hormone: function and clinical use. *Lab, Manag.,* **25**: 51–8.

HUMKE, V.R., 1977. Treatment results after application of the LH-FSH releasing hormone on maidescensus testis of the male dog. *Kleinterpraxis.*, **22**: 315-322.

INABA, T., TANI, H., GONDA, M., NAKAGAWA, A., OHMURA, M., MORI, J., TORII, R., TAMADA, H. and SAWADA, T., 1998. Induction of fertile estrus in bitches using a sustained-release formulation of a GnRH agonist (leuprolide acetate). *Theriogenol*., **49**: 975-982.

INABA, T., UMERHARA, T., MORI, J., TORRI, R., TANI, H., GONDA, M., NAKAGAWA, A., OHNURA, M., TAMADA, H. and SAWADA, T., 1996. Reversible suppression of pituitary testicular function by a sustained release formulation of GnRH agonist (leuprolide acetate) in dogs. *Theriogenol.*, **46**: 671-677.

JEFFCOATE, I.A., 1993. Endocrinology of anestrous bitches. *J. Reprod. Fertil. Suppl.*, **47**:69-76.

JEFFOCATE, I.A. and LINDSAY, F.E.F., 1989. Ovulation detection and timing of insemination based on hormone concentration, vaginal cytology and the endoscopic appearance of the vagina in domestic bitches. *J. Reprod. Fert. Suppl.*, **39**: 277-287.

JIANG, G.C., STALEWSKI, J., GALYEAN, R., DYKERT, J., SCHTEINGART, C. and BROQUA, P., 2001. GnRH antagonists: a new generation of long acting analogues incorporating para-ureido-phenylalanines at position 5 and 6. *J. Med. Chem.*, **44**: 453–467.

JOHNSTON, S.D. and ROOT, K.M.V., 1995. Serum progesterone timing of ovulation in the bitch. In : Proceedings of Annual meeting of the Scoeity for Theriogenology, San Antonio, TX, September 13-15. Nashville, *Scoeity for Theriogenology*., *pp*. 195-203.

JOHNSTON, S.D., ROOT, K.M.V. and OLSON, P.N., 2001. “In: Canine and Feline Theriogenology”, W.B Saunders, Philadelphia, *pp*. 16-66.

KANITZ, W., SCHNEIDER, F., HOPPEN, H.O., UNGER, C., NURNBERG, G. and BECKER. F., 2007. Pregnancy rates, LH and progesterone concentrations in mares treated with a GnRH agonist. *Anim. Reprod. Sci.,* **97**: 55–62.

KHARCHE, S.D. and SRIVASTAVA, S.K., 2007. Dose dependent effect of GnRH analogue on pregnancy rate of repeat breeder crossbred cows. *Anim. Reprod. Sci.,* **99:** 196-201.

KLAAS, P., 1985. Canine vaginal Cytology during estrous cycle, *Can. Vet. J.,* **26**: 101-104.

KONUK, C.S., KIRSAN, I., GURBULAK, K., TEK, C. and SENUNVER, A., 2001. Determination of ovulation time using the eosinophilic index (EI) of vaginal cytology and serum progesterone levels in the bitch. *Veteriner. Fakultesi. Dergisi.*, **27**: 91-97.

KUTZLER, M.A., 2005. Induction and synchronization of estrus in dogs. *Theriogenol*., **64**: 766–775.

KUTZLER, M.A., 2007. Estrus induction and synchronization in canids and felids. *Theriogenol.,* **68**: 354-374.

KUTZLER, M.A., HUSSNI, O., MOHAMMED, STEPHEN, V.L. and MEYERS-WALLEN V.N., 2003. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. *Theriogenol.*, **60**: 1187–1196.

KUTZLER, M.A., STANG, B., WALDVOGEL, P. and HOLTHOFER, P., 2006. Administration of a single or multiple doses of injectable long acting deslorelin (GnRH analogue) does not induce estrus in bitches. In: *Proceedings of the Annual Meeting Eur. Vet. Soc. Small Anim. Reprod., pp*. 280.

KUTZLER, M.A., WHEELER, R. and VOLKMANN, D.H., 2001a. Canine estrus induction using the GnRH agonist, deslorelin. In: *Proceedings of the Annual Meeting Eur. Vet. Soc. Small Anim. Repro., pp*. 147–148.

KUTZLER, M.A., WHEELER, R. and VOLKMANN, D.H., 2001b. Serum deslorelin concentrations in bitches after Ovuplant® administration. In: *Proceedings of the Annual Meeting Society Theriogenol., pp*. 13.

KUTZLER, M.A., WHEELER, R., LAMB, S. and VOLKMANN, D.H., 2002. Deslorelin implant administration beneath the vulvar mucosa for the induction of synchronous estrus in bitches. In: *Proceedings of the Annual Meeting Eur Vet Soc Small Anim. Reprod., pp*. 96.

LEE, C.N., MAURICE, E., AX, R.L., PENNINGTON, J.A., HOFFMAN, W.F. and BROWN, M.D., 1983. Efficacy of gonadotropin releasing hormone administered at the time of artificial insemination of heifers and postpartum and repeat breeder dairy cows. *Am. J. Vet. Res*., **44:** 2160–2163.

LEEDY, M.G., 1988. Hormonal and Neural control of sexual brhaviour in dogs and cats. “In: Sitsen, JMA, Ed: Handbook of sexology”, The Pharmacology and Endocrinology of sexual function. *Elsevir Science Publisher,* **6**: 231-263.

LINDE, C. and KARLSON, I., 1984. The correlation between the cytology of the vaginal smear and the time of ovulation in the bitch. *J. Sm. Anim. Pract.,* **25**: 77-82.

LINDE, F.C., 1991. Achieving canine pregnancy by using frozen or chilled extended semen. *Vet. Clin. North Amer.,* **21** (3): 467-485.

LINDSAY, F.E.F and CONCANNON, P.W., 1986. Normal canine vaginoscopy, “In: Small Animal Reproduction and Infertility” Ed. Burke, T, J. Lea and Febiger, Philadelphia, *pp*. 112-120.

LINDSAY, F.E.F., 1983. The normal endoscopic appearance of the caudal reproductive tract of the cyclic and non-cyclic bitch, Post uterine endoscopy. *J. Sm. Anim. Pract.,* **24**: 1-15.

MARTEMUCCI, G. and ALESSANDRO, A.G.D., 2010. Estrous and fertility responses of dairy ewes synchronized with combined short term GnRH, PGF2α and estradiol benzoate treatments. *Small. Rumi. Res.,* **93**: 41–47

MELLIN, T.N., ORCZYK, G.P., HICHENS, M. and BEHRMAN, H.R., 1976. Serum profiles of luteinizing hormone, progesterone and total estrogen during the canine estrous cycle. *Theriogenol.,* **5**: 175-187

MESTRE, J., WANKE, M. and SUCHEYRE, S., 1990. Exfoliative vaginal cytology and plasma concentrations of progesterone, luteinizing hormone and estradiol-17β during estrous in the bitch. *J. Small, Anim. Prac.,* **31**: 568-570.

MOHAMED, M., ABDEL, H.S., SAYED, S., WATANABE, G. and TAYA, K., 2002. Induction of Estrous in non breeding season in Eygiptian Baladi goats. *J. Vet. Med. Sci.,* **64** (1): 83-85

NAWITO, M., SCHALLENBERGER, E. and SCHAMS, D., 1977. Gonadoliberin (GnRH) analogue in comparison with gonadoliberlin decepeptide. *Theriogenol.,* **7**: 277-287.

NESTOR, J.J., 1984. Development of agonistic LHRH analogs. In: Vickery, B.H., Nestor, J.J., Hafez, E.S.E. (Eds.), LHRH and its analogues: contraceptive and therapeutic applications. MTP Press, Lancaster, *pp*. 3–10.

NETT, T.M., AKBAR, A.M. and PHEMISTER, R.D., 1975. Levels of Lutenizing Hormone, Estradiol and Progesterone in serum during the estrus cycle and pregnancy in the Beagle bitch. *Proc. Soc. Exp. Biol. Med.,* **148**: 134.

NOTHLING, J.O., IRONS, P.C., GERBER, D. and SCHULMANN, M.L., 2003. The use of plasma progesterone concentration to predict the optimal breeding time in bitches 6 days in advance. *Theriogenol.,* **59**: 229.

OKKENS, A.C., DIELEMEAN, S.J. and VOGEL F., 1985. Determination of the ovulation period in the dog. A comparison of the rapid progesterone assay, Vaginoscopy and Vaginal cytology. "In : Voorjaasdagan, Prc. Nether. Sm. Anim. Vet. Assoc.," pp. 26-27.

OLSON, P. N., THRALL, M.A. and WYKES, S., 1984. Vaginal cytology, Part-I; A usefull tool for stagimg the canine oestrous cycle. *Compend. Contin. Educ. Pract. Vet.,* **6**: 288-297.

OLSON, P.N. and NETT, T.M., 1986. Reproductive Endrocrinology and Physiology of the bitch, In: "*Current therapy in Theriogenology*", Ed. Marrow, D.A, **2**:453-456.

OLSON, P.N., 1989. Exfoliative cytology of the canine reproductive tract. *Proc. of the Annual meeting of the Society of Theriogenol., pp*. 259-263.

OLSON, P.N., BOWEN, R.A. and BEHRENDT, M.D., 1982. Concentration of reproductive hormones in canine serum throughout late anestrus, proestrus, and estrus. *Biol. Reprod.,* **27**:1196-1206.

OLSON, P.N., HUSTEF, P.W. and NETT, T.M., 1983. The management of a successful mating between the bitch and the stud dog. *Kalkan Forum*., *pp*. 15-21.

PADULA, A.M. and MACMILLAN, K.L., 2002. Reproductive responses of early postpartum dairy cattle to continuous treatment with a GnRH agonist (deslorelin) for 28 days to delay the resumption of ovulation. *Anim. Reprod. Sci.,* **70**: 23–36.

PADULA, A.M., 2005. GnRH analogues-agonists and antagonists. *Anim. Reprod. Sci*., **88** (1–2): 115–126.

PATHAK, A.D., WHITMORE, H.L. and BROWN, M.D., 1986. Effect of Gonadotropin releasing hormone and conception in repeat breeding dairy cattle. *Theriogenology*, **26:**605-609.

PETERS, A.R., 2005. Veterinary clinical application of GnRH. *Anim. Reprod. Sci.*, **88**: 155–167.

PHEMISTER, R. D., HOLST, P. A. and SPANO, J. S., 1973. Time of ovulation in Beagle bitch. *Biol. Reprod.*, **8**: 74-82.

REIMER, T.J., PHEMISTER, R.D. and NISWENDER, G.D., 1978. Radioimmunologic measurement of follicle stimulating hormone and prolactin in the dog. *Biol. Reprod.*, **19**: 673-679.

RENTON, J.P., BOYD, J.S., ECKERSALL, P.D., FERGUSON, J.M., HARVEY, M.J.A., MULLANEY, J. and PERRY, B., 1991. Ovulation, fertilization and early embryonic development in bitch. *J. Reprod. Fert.*, **93**: 221-231.

ROTA, A., MOLLO, A., MARINELLI, L., GABAI, G. and VINCENTI, L., 2003. Evaluation of cabergoline and buserelin efficacy for oestrous induction in the bitch. *Reprod. Dom. Anim.*, **38**: 440–443.

RUBION, S., DESMOULINS, P.O., RIVIERE-GODET, E., RUTTEN, F., FLOCHLAY, A. and DRIANCOURT, M.A., 2006. Treatment with a subcutaneous GnRH agonist containing controlled release device reversibly prevents puberty in bitches. *Theriogenology*, **66**: 1651–1654.

RUBION, S., GUERIN, C., RIVIERI-GODET, E., HORSPOOL, L., RUTTEN, F. and DRIANCOURT, M.A., 2003. Treatment with a subcutaneous GnRH agonist containing devise suppresses FSH and ovarian function in bitches. *Reprod. Dom. Anim.*, **79:** 355.

SMITH, M.S. and MACDONALD, L.E., 1971. Serum levels of lutenizing hormone and progesterone during the estrous cycle pseudopregnancy, and pregnancy in the dog. *Endocrinology.,* **94**: 404-412.

SNEDOW, J.O., RECHENBERG, W.V., KOING, W., HAHN, M., JERZABEK, G. and FRASER, H., 1978. In: Hypothalamic hormones chemistry, physiology, and clinical applications. Ed. Tupta, D. and Voelfer, W. Chemi. Publishers, *pp*. 307-326.

STABENFELDT, G.H. and SHILLE, V.M., 1977. Reproduction in the dog and cat. In: H.H. Cole and P.T. Cupps (Editors), Reproduction in Domestic Animals, 3rd edition. Academic Press, New York., *pp*. 499-527.

STERRY, R.A., WALLE, M.L. and FRICKE, P.M., 2006. Treatment with Gonadotropin-Releasing Hormone after first timed artificial insemination improves fertility in noncyclic lactating dairy cows. *J. Dairy. Sci.,* **89**: 4237-4245.

STEVENSON, J.S., FRANTZ, K.D. and CALL, E.P., 1988. Conception rates in repeat-breeders and dairy cattle with unobserved estrus after prostaglandin F2 alpha and gonadotropin-releasing hormone. *Theriogenology*., **29**(2):451-460.

SUYASH, V.B., 2002. Studies on management of mating time in bitches by vaginal exfoliative cytolody and vaginoscopy. *MVSc thesis*, Veterinary College, University of Agriculture Science.

TANI, H., INABA, T., TAMADA, H., SAWADA, T., MORI, J. and TORII, R., 1996. Increasing gonadotropin-releasing hormone release by perfused hypothalamus from early to late anestrus in the beagle bitch. *Neurosci. Lett*., **207**: 1-4.

TARLATZIS, B. and BILI, H., 2004. Safety of agonists and antagonists. *Expert. Opin. Drug. Saf.,* **3** (1): 39–46.

TOMASKOVIC, A., MAKEK, Z., CERGOLJI, M., HERAK, M., KRAMARIC, G. and BARAC. G.I., 1997. Use of serozyme progesterone test in determining the reproductive status of German shepherd bitches. *Veterinarski. Archiv.*, **67** (5): 179-185.

TRIGG, T.E., WRIGHT, P.J., ARMOUR, A.F., WILLIAMSON, P.E., JUNAIDI, A., MARTIN, G.B., DOYLE, A.G. and WALSH, J., 2001. Use of a GnRH analogue implant to produce reversible, long term suppression of reproductive function of male and female domestic dogs. *J. Reprod. Fertil.,* **57**: 255–261.

TSUTSUI, T., 1989. Gamete physiology and timing of ovulation and fertilization in dogs. *J. Reprod. Fert. (Suppl).,* **39**: 269-275.

TSUTSUI, T., TAKAHASHI, F., HORI, T., KAWAKAMI, E. and CONCANNON, P.W., 2009. Prolonged duration of fertility of dog ova. *Reprod. Domest. Anim. Suppl.*, **2**: 231–234.

VAN HAAFTEN, B., DIELEMAN, S. J., OKKEN, A. C. and WILLEMSE, A.H., 1989. Timing the mating of dogs on the basis of blood progesterone concentration. *Vet. Rec.*, **125**: 524-526.

VANDERLIP, S.L., WING, A.E., FELT, P., LINKIE, J., RIVIER, J., CONCANNON, P.W. and LASLEY, B.L., 1987. Ovulation induction in anestrous bitches by pulsatile administration of GnRH. *Lab. Anim. Sci.,* **27**: 459–468.

VENKATKCHALAPATHY, J.N., 2005. Studies on determination of mating time in bitches. *MVSc thesis.* Veterinary College, Bangalore. Karnataka Veterinary Animal and Fisheries Sciences University, Bidar.

VERVERIDIS, H.N., BOSCOS, C.M., STEFANAKIS, A. and KRAMBOVITIS. E., 2002: Use of enzyme immuno assay for oestradiol-17beta and progesterone quantification in canine serum. *Anim Reprod Sci*., **69**: 53–64.

VICKERY, B.H., MC RAE, G.I., GOODPASTURE, J.C. and SANDERS, L.M., 1989. Use of potent LHRH analogues for chronic contraception and pregnancy termination. *J. Reprod. Fertil.*, **39**: 175–187.

VOLKMANN, D., KUTZLER, M.A., WHEELER, R. and KREKELER, N., 2006a. The use of deslorelin implants for the synchronization of estrus in diestrous bitches. *Theriogenol*., **66**, 1497–1501.

VOLKMANN, D., KUTZLER, M.A., WHEELER, R., KREKELER, N., KLEWITZ, J. and LAMB, S.V., 2006b. Failure of hCG to support luteal function in bitches after estrus induction using deslorelin implants. *Theriogenol.*, **66**:1502–1506.

WECKERMAN, D. and HARZMANN, R., 2004. Hormone therapy in prostate cancer: LHRH antagonists versus LHEH analogues. *Eur. Urol.*, **46:** 279–284.

WILDT. D.E., CHAKRABORTYP.K ., PANKO,W .B. and SEAGER,S .W.J., 1978. Relationship of reproductive behavior, serum luteinizing hormone and time of ovulation in the bitch. *Biol. Reprod*., **18**: 561-570.

WRIGHT, P.J. and PERRY, B.W., 1989. Cytology of canine reproduction system. *Vet. Clin. North. Amer.*, **19**: 862-874.

WRIGHT, P.J., 1990. Application of vaginal cytology and plasma progesterone determination to the management of reproduction in the bitch. *J. Sm. Anim. Pract.*, **31**: 335-340.

Abstract

VIII. ABSTRACT

A clinical study was designed to verify the unpublished claims by many canine practitioner that, administration of GnRH prior to mating improved the conception rate and litter size in 6 groups of female pugs, with each group comprising of 10 animals. The conception rate and litter size was recorded as 60 per cent and 3.2 ± 0.73 respectively in animals mated on the basis of vaginal exfoliative cytology which also served as control group for the next two experiments. Administration of a single dose of synthetic analogue of GnRH at a dose level of either 0.2 or 0.4µg/ kg bw at the time of peak vaginal cornification did not significantly improve the conception rate or litter size as compared to the control group.

In another experiment which also served as control group for the subsequent two trails, the conception rate and litter size were recorded following mating when the serum progesterone concentration was around 3 ng/ml. The conception rate and litter size was recorded as 90 per cent and 3.9 ± 0.54 respectively and was significantly higher as compared to those observed with the previous 3 experiments.

In the fifth and sixth trail, GnRH was administered at a dose level of 0.2 and 0.4µg/kg bw respectively, on the day when the serum concentration was around 3 ng/ml. The litter size and conception rate in animals receiving GnRH was similar to the control group.

The results obtained in the present study suggest that although, the common practice of injecting GnRH around the time of artificial insemination is beneficial in dairy cattle, the same is not true for female dogs.