MEDROXYPROGESTERONE ACETATE AS AN AID TO BIRTH CONTROL PROGRAMME IN STRAY DOGS

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

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DECLARATION

I hereby declare that this thesis, entitled **"MEDROXYPROGESTERONE ACETATE AS AN AID TO BIRTH CONTROL PROGRAMME IN STRAY DOGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"MEDROXYPROGESTERONE ACETATE AS AN AID TO BIRTH CONTROL PROGRAMME IN STRAY DOGS"** is a record of research work done independently by D.K. DEEPAK MATHEW, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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ACKNOWLEDGEMENT

It is with immense pleasure that I record my sincere and heartfelt gratitude to the Chairman of the Advisory Committee **Dr. *Joseph Mathew***, *Associate Professor, Department of Livestock Production Management for his meticulous guidance, personal attention, keen interest, affectionate encouragement, persuasion and incessant help offered to me from the initiation of work to the ship shaping of the manuscript. Without his strong support and co-operation the successful completion of this work would not have been possible.*

To the greatest degree, I am grateful to **Dr. *P.C. Saseendran***, *Professor and Head, Department of Livestock Production Management, Member of the Advisory Committee, for his generous encouragement, inspiration, kindness and personal guidance in the pursuit of this work.*

I am sincerely thankful to **Dr. *K. S. Anil***, *Assistant Professor, Department of Livestock Production Management and* **Dr. *G. Ajit Kumar***, *Assistant Professor, Department of Animal Reproduction, Members of the Advisory Committee for their whole-hearted co-operation, words of inspiration, incessant encouragement and affection rendered during the entire period of research work.*

I am grateful to the **Dean**, *College of Veterinary and Animal Sciences, Mannuthy and Kerala Agricultural University for the facilities provided for this research work.*

I remember with gratitude the help extended to me by **Dr. *K.S. Sebastian***, *Professor (Research Co-ordination) and* **Dr. *Francis Xavier***, *Associate Professor, Department of Livestock Production Management during the course of study.*

My sincere thanks to **Dr. *Viswanathan***, *Professor and Head, Department of Animal Nutrition and* **Dr. *Sharadamma***, *Department of Veterinary Surgery and Radiology for granting permission to use their animal houses.*

I wish to extend my thanks to **W.S.P.A, *Switzerland***, *for sending me articles and videocassettes on stray dog population control.*

I express my gratitude to **Dr. *Ramkumar*** (former member of V.C.I.), ***Antonio Gonzalez*** *(Researcher on the evolution of dogs) and* **Mark *Goullet***, *for providing me articles and guidance on the relevant subject.*

I am in short of words to express my deep sense of gratitude to my great friends ***Drs. Subash, Roshin, Anton, Laiju, Cyel, Ratheesh, Jayant, Rajesh*** *and* ***Ajith,*** *without whose tireless help, unconditional support and constant encouragement the successful completion of this research work would not have been possible.*

The generous help, assistance, guidance and support rendered to me by my beloved seniors ***Drs. Cijo*** *and* ***Sada*** *A bouquet of thanks to friends and junior colleagues* ***Drs. Acty*** *and* ***Biya*** *for their help and co-operation.*

Words possess no enough power to reflect my thankfulness for the invaluable help, moral support and affection rendered by my great friends ***Drs, Senthil., Jegaveera, Rishi, Prejit, Balaji*** *and* ***Muthu.***

I sincerely acknowledge the staff of our department ***Mr. Gopi, Mr. Sajeev*** *and* ***Mrs. Sarada*** *for their timely help.*

I wish to thank ***Dr. Suresh Kumar,*** *Assistant Professor, Radiotracer Laboratory, Vellanikkara for his help in progesterone analysis. I am thankful for the wholehearted help rendered for statistical analysis of data by* ***Smt. Sujatha,*** *Assistant Professor and Head,* ***Smt. K.A. Mercy,*** *Assistant Professor, Department of Statistics.*

No phrase or words in any language can ever express my deep sense of love and gratitude to my beloved ***Parents,*** *beloved brothers* ***Dileep*** *and* ***Dibin.***

Upon whom I relied – the Lord of my heart- for getting done everything that seemed impossible in my path to the completion of this record, to him I deliver the core of my effort.

D. K. Deepak Mathew

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INTRODUCTION

The dog was the first animal to be domesticated. The ability to understand and respond to human cues as well as the availability of food and companionship must have encouraged the dog to remain dear and near to man. Companion animals can provide the physical and psychological support needed to move a person towards independence, and somewhat improve their daily life. They also decrease feelings of anxiety stress and increase self-esteem and respect for all living beings. All these have led to the dog being accepted as a pet and even as a family member.

Though society had began to accept dog even as a family member, the number of stray dogs continue to increase day by day. Absence of responsibility from the part of the owner in controlling the unwanted pregnancies and non-availability of reliable, easily available and easy to use birth control techniques, were the main reasons attributed for it. This coupled with food in the form of garbage generated by urbanization lead to unrestrained increase in stray animals. The uncontrolled breeding and media hyped craving for pet animals has generated a great demand for pets, a major chunk of which are later relinquished to the streets. Absence of proper control programme on the part of the government had made the situation dismal. The growing propinquity of large number of stray dogs with large number of people produces a menace, which is still met by inefficient and inhumane methods of eliminating dogs.

Surgery, though the most effective tool for birth control was not acceptable to many who are not ready to put their animals through the pain of surgery. Also it was impractical to use surgery over a wide area within a short period of time due to both lack of qualified personnel and high cost of implementation. It is also impractical to kill all the stray animals or continue doing surgical sterilization on a few animals when a majority of them still breed freely increasing the burden.

In India majority of stray animals are community dogs, where people feed them and had little control over their movements, any attempt to remove these animals from the community thus creates a hue and cry from the community. Any method of control of stray dogs should therefore require their active participation and co-operation.

The development of nonsurgical alternative for birth control with easy application that could be used by the common man with minimum technicalities is the need of the hour. There had been several studies since the development of the contraceptive pill for humans to apply a similar one in his pet animals ever since the emergence of pet overpopulation and relinquishment of pets has started creating a problem in the West. The increased interest in the welfare of animals also had created a demand for an alternative to the euthanasia of relinquished pets. All these paved the way for the development of non-surgical birth control methods for pets.

Though several non-surgical alternatives are available including the immunological control, none of these are available for use by the common man neither are they cheap. The present study was aimed at trying a drug, which was easily available, easy to administer and affordable to common man.

Medroxyprogesterone acetate is a progestagen and was first marketed in the early 1960's for use in humans as a contraceptive. The long duration of it's contraceptive effect made it widely acceptable as a contraceptive for pet animals. The drug was widely used in humans and hence was easily available at the market, which made it an ideal choice for the present study.

The objectives of this study include:

1) To evaluate the efficacy of medroxyprogesterone acetate in the prevention of oestrus in stray dogs.

2) To evolve non-surgical methods for prevention of oestrus in dogs.

REVIEW OF LITERATURE

2.1 STRAY DOGS

In India stray dogs were considered as a menace to public health but disposal of such animals by killing was not preferred on sentimental grounds (Chatterjee and Kar, 1968).

In Baltimore, Beck (1975) observed that stray dogs originated from, (1) pets released in early mornings and evenings for daily unsupervised runs, (2) pet escapes (3) pets abandoned to streets when families move out of the area, (4) pets that run away or are released after being stolen by children and (5) birth (under porch steps and in wood lots), in order of magnitude. He found that smaller groups of dogs usually two in number either both males or one male and one female are common and that larger groups form and dissolve within minutes to days, the latter true when forming around a bitch in oestrus.

Two general characteristics of the urban dog system were indicated by simulation results: (1) the system was characterized by potentially exponential growth of both pet and stray populations, and (2) the stray population was dependent upon pet abandonment and immigration for continued survival (Heussner and Grant, 1978).

The number of females spayed in the Manhattan population reached a frequency of 66 per cent in 1979. Considering only present age-dependent birth and survival rates, the population could not maintain itself and would have decreased rapidly in size. It was found, however, that the population was maintained by a high

net rate of immigration, with about 75 per cent of all dogs under 6 months old coming from outside (Nassar and Mosier, 1980).

Dog bite cases occurred throughout the year. The number of cases per month was correlated with the breeding cycle of dogs. Transmission of rabies from dog to dog was considered most likely to occur during oestrus when the amount of fighting was greatest, and also when the bitch had to protect its litter by fighting (Narayan, 1985).

Font (1987) observed that stray dogs occasionally formed groups with dominance hierarchies and communal defence of a territory. He concluded from the stability of these groups that long-term affiliative bonds apparently existed among group members. This finding conflicted with the accepted notion that urban stray dogs are asocial and do not form stable social groups. It was suggested that stray dogs, like most canids possessed, remarkable behavioural plasticity allowing them to adjust their social system to prevailing ecological constraints.

Straying pair or group of dogs included an "initiator", a dog that actively sought the companionship of one or more others and then lead them off on long excursions. Very often this leader teamed up with a dog of the opposite sex (Coman and Robinson, 1989).

According to Kneafsey and Condon (1995), all dogs were social animals and possessed an inherent pack instinct. They were excited to frenzy by the smell and taste of blood. Once an attack had started, in a pack situation, other dogs join in. Pack attacks have a greater probability of resulting in serious injury than attacks by a single dog.

The natural population of dogs always multiplies to that number which can be sustained by its environment. So the females during the heat cycle gave birth to an additional number of dogs which thereby replaced the vacuum (Anon, 2002).

Butler and Bingham (2000) pointed out that though high juvenile mortality occurs among dogs, the population of dogs increased, probably due to human derived waste food and due to influx from the owned dogs.

House soiling was the most frequently listed behavioural reason for relinquishment of both dogs and cats among mixed reasons for relinquishment. It was also the most frequently listed reason when behavioural reasons alone were provided for relinquishment of cats. However for dogs, aggressive and destructive behaviours were more frequently recorded than house soiling when behavioural reasons were listed (Salman *et al.* 2000).

The primary benefit of licensing animals would be identification, should the animal become lost. Licensing also ensured rabies vaccinations, allowed quick identification in case of a bite incident, and provided revenue to help offset the costs of administering the animal control program. An effective program could be a source of reliable demographic data as well. A well resourced animal control agency was vital for public health and safety within any community (Beaver, 2001).

Free-roaming dogs and cats had long been causing major public-health problems and animal-welfare concerns in many countries. Free-roaming dogs have been considered to be more of a problem than cats for several reasons. Free-roaming cats were becoming more of an issue in countries where free-roaming dog problems were coming under control. The change in perception of pets, beyond their value as a commodity, had also contributed to the increase in concern and attention focused on free-roaming dogs and cats (Slater, 2001).

Many modern pet owners evidently were ignorant of the difficulties associated with keeping dogs in spatially restricted urban housing. Unwanted pets were the inevitable outcome of this process. In addition, majority of the dogs were sexually intact, leading to an overproduction of puppies and the general devaluation of dogs because of an excess of supply over demand. When all these factors were combined with specific religious and cultural traditions that discouraged the killing of unwanted animals while promoting their abandonment, the stray dog population explosion was not entirely surprising (Hsu *et al.*, 2003).

Kato *et al.*, (2003) reported that the increased stray dog population in Kathamandu could be due to the availability of food, including the presence of garbage scattered in the streets and tendency of some dog lovers to feed dogs.

2.2 REPRODUCTIVE CYCLE IN BITCH

The prolonged period of anoestrus or sexual quiescence between successive oestrus periods irrespective of whether they had become pregnant or not was an attribute that distinguished bitches from other polycyclic species. (Shille *et al.*, 1984)

2.2.1 Proestrus

According to Wildt *et al.*, (1978) proestrus averaged 8.7 ± 0.7 days in duration with a range of 3-17 days. This period commenced with onset of serosanguinous vulval discharge and vulval edema and cease with onset of receptivity.

The average duration of proestrus was found to be nine days in natural oestrous cycle. (Johnston, 1980; Olson *et al.* 1987; England and Allen, 1989; England, 1998).

However variations of two days to four weeks was observed by Concannon and Digregario (1986).

Inaba *et al.*(1998) noted that proestrus varied from six to eight days in postpartum bitches and 4.2 days in prepubertal bitches.

Chakraborty *et al.* (1980) found the duration of proestrus as 7.7 ± 0.5 days in the second reproductive cycle while it was only 3.9 ± 0.7 days in the first cycle of the bitch, indicating the duration of proestrus was longer in the second cycle.

2.2.3 Oestrus

The length of oestrus averaged 11.4 ± 0.9 days and was also quite variable with a range of 3-21 days (Wildt *et al.*, 1978).

Chakraborty *et al.* (1980) found that the durations of oestrus to be 11.0 ± 1.1 days in the second reproductive cycle while it was only 5.4 ± 0.4 days in the first cycle of the bitch, indicating that the duration of estrous cycle was longer in the second cycle.

Oestrus is the period of receptivity and ovulation. The behavioural signs became very conspicuous and the duration averaged nine days (Johnston, 1980 and England, 1998).

Variations in duration of oestrus was observed as three days to three weeks or more (Concannon and DiGregario, 1986; Concannon *et al.*, 1989).

2.2.4 Dioestrus

This period is the phase of progesterone dominance after oestrus and begins with the refusal for coitus, lasts for about 60 days and terminates when circulating progesterone concentrations become minimum (England, 1998).

2.2.5 Anoestrus

This is the period of reproductive quiescence between whelping or dioestrus and next proestrus. The duration varied from one to 10 months (Concannon and DiGregario, 1986; Concannon *et al.*, 1989).

According to England, (1998) the length of anoestrus averaged about four months.

2.3 REPRODUCTIVE BEHAVIOUR

When estrogen primed bitches were injected with progesterone, the stimulus value of their vaginal secretions was greatly enhanced and it was highest four hours after progesterone treatment. Effects of progesterone administered after several injections of estrogen consist primarily of increasing the female's excitatory value, at least a part of which could be due to a change in vaginal secretions, which serve as an important source of stimulation for the male (Beach and Merari, 1968).

The increasing levels of plasma estrogen during proestrus depressed the normal behaviour pattern of bitch, culminating in complete passivity at end of proestrus when estrogen levels are maximal. Moreover since the reflex behaviour elements characteristic of oestrus appeared coincident with or immediately following the LH peaks, it is possible that they were affected by the initial preovulatory increase in plasma progesterone found at this time (Concannon *et al.*, 1975).

Reproductive hormones affected chemical communication in several ways. They increased the frequency and distribution of urine within the environment, and they heightened the interest of individuals for odors of members of opposite sex. Such close relationships between endocrine factors and external chemical secretions appeared to provide canids with an efficient system for communicating fundamental

biological information necessary for the stability of the group and continuation of the species (Anisko, 1976).

Proestrus behaviours, was associated with increasing plasma oestradiol concentration while the onset of standing oestrus theoretically coincided with a marked increase in plasma progesterone concentration caused by preovulatory leutinisation (Concannon *et al.*, 1977).

The length of proestrous and estrous behaviour varied considerably between individual bitches suggested that the steroid and gonadotropin hormone interrelationships (the controlling factors for ovulation) during these stages of the estrous cycle were quite complex (Wildt *et al.*, 1978).

Maximal sexual receptivity was maintained by combined estrogen-progesterone treatment and throughout the following week after estrogen was removed. Initial treatments with progesterone alone caused no morphological changes. One week after the estrogen was superimposed upon progesterone only signs characteristic of proestrus were observed. When progesterone was removed estrous behaviour was lost and, during the week when only estrogen implants remained (Concannon *et al.*, 1977).

2.4 STRAY DOG POPULATION CONTROL

Carding (1969) suggested discouragement of the keeping of dogs in general, encouragement of the keeping of males or spayed females, encouragement of destruction of all but one male pup per litter within a few hours of birth, encouragement of surrender of unwanted pups and adults, prohibition of abandonment of dogs and a system for the capture of stray dogs as measures for stray dog population control.

Four schemes evaluated significantly decreased the stray population: (1) imposition of fines for abandoning animals, (2) encouragement of proper pet ownership through public education, (3) encouragement of pet neutering through the establishment of differential licensing fees and free neutering clinics, and (4) increased effort to impound strays (Heussner and Grant, 1978).

Methods to prevent or abort unwanted pregnancies would have been effective in reducing pet overpopulation only if they were readily used by the pet owner or guardian. Any surgical or nonsurgical method of preventing pregnancy to eliminate overpopulation was to be used on most pets, prior to the birth of any litter. (Olson and Johnson 1993)

Mechler (2002) suggested that the sterilisation of dogs was a better alternative to killing the dogs for population control and that due to the large number of animals to be sterilised and cost of surgery, nonsurgical contraception is the only answer if cost and delivery are manageable.

The rabies control programme in Phetchabun province (Thailand), targeted elimination of rabies through several strategies including monitoring the dog population and implementing vaccination and sterilisation programmes. The dog population was controlled by contraception and sterilisation, with stray dogs and community dogs that lived around temples and schools particularly targeted (Kamoltham *et al.* 2003).

Efforts to overcome stray dog problem should be focused on increasing the value of dogs by enforcing registration fees, especially for unsterilized animals. The creation of low cost subsidized neutering schemes would have been beneficial (Hsu *et al.*, 2003).

The proper garbage disposal and prohibition of giving feed to stray dogs deprived the animals of nutrition resulting in reduced lifespan and lowered reproductive ability and this could play a role in reduction in the number of stray dogs (Kato et al., 2003).

The general public should have been made aware of all problems connected with irresponsible dog ownership, the importance of dog spay/neuter programmes as carried out in many countries. Such concerted activities would have resulted in fewer abandoned and unwanted animals and their relinquishment to shelters (Nemcova and Novak, 2003).

2.5 REPRODUCTIVE CONTROL IN DOGS

Suppressive dosage of a contraceptive agent would normally be administered at the time of first pro-oestral bleeding or soon after, but this term could also apply where an animal was dosed when attractive to male but with no evidence of pro-oestral blood loss. Prevention is a term that was used to describe the administration of a contraceptive agent to a normal bitch when it was not in season. The term delay was used to indicate the interval between the administration of a preventive dosage and the occurrence of the next normal heat (Murray and Eden, 1952).

Megestrol acetate was found to be effective in postponing oestrus when given orally at a dosage of 2.2 mg/kg per day for 8 days in early proestrus while in anoestrous it had to be given at the dose rate of 0.55 mg/kg per day for 32 days (Burke and Reynolds, 1975).

Vanos and Oldenkamp (1978) in their studies in bitches using proligesterone concluded that proligesterone could be administered in any stage of the cycle to prevent or suppress the next oestrus. The time of treatment in dioestrus or anoestrus had no influence on the duration of the effect.

Infertility in bitches immunized with porzine zona pellucida may have been due to prevention of zona penetration, because their antisera inhibited zona penetration of oocytes by spermatozoa in vitro. However, alterations in ovarian function preventing ovulation and luteinization could be involved in high-titered bitches (Mahi-Brown *et al.*, 1985).

There are some situations in which practitioners have no choice but to use chemical control; for example in bitches that are already in heat, in animals for which anesthesia may be more risky and for which the owner is uncertain about the future breeding plans or is frightened of surgery (Evans and Sutton, 1989).

The concentrations of chlormadinone acetate (CAP) in the individual bitches indicated that the lowest concentration effective in preventing oestrus is 0.7 ng/ml. Plasma progesterone levels remained low throughout the period of oestrus prevention, indicating a close correlation with the effect of CAP. (Sahara, *et al.* 1993).

Oral treatment with dexamethasone had been used to terminate pregnancy in bitches, but in some cases the withdrawal of treatment after 8 days resulted in retention of live pups and required a further treatment or the use of another abortifacient. (Wanke, *et al.*, 1997)

Prepubertal gonadectomy, often referred to as early-age neutering, had increased in popularity, here puppies and kittens were neutered as early as 7 weeks of age. Early-age neutering was one technique that was used to combat pet overpopulation, a problem where millions of unwanted healthy dogs and cats were euthanased each year. (Olson, *et al.*, 2001)

Trigg *et al.,* (2001) suggested that a single implant using deslorelin (GnRH analogue) postponed oestrus in bitches and suppressed the reproductive function in dogs by suppressing the function of pituitary gonadal axis.

Lou (2002) indicated the possibility of a permanent contraceptive vaccine using porcine zona pellucida.

According to Palmer and Post (2002), a major source of unwanted dogs was accidental mating bitches whose owners were unwilling to pursue surgical sterilisation. He also suggested that the total cost of any nonsurgical treatment for preventing unwanted births, should not be less than that of ovariohysterectomy, so that this, or any other method of pregnancy prevention (termination) would not become an alternative to permanent sterilization.

In studies conducted by Phillips *et al.* (2003) Exogenous testosterone (testosterone cypionate) was found to be moderately effective in suppressing oestrus in Beagle bitches when given during the dioestral period.

2.6 PROGESTINS

It was Murray and Eden (1952) who reported that oestrus could also be controlled in the bitch, using progesterone and so led the way to the evaluation of progestational steroids.

Since the discovery, in 1937, that injections of progesterone will inhibit ovulation in the rabbit, many derivatives have been synthesized in a quest for greater potency, slower excretion and oral activity. These synthetic compounds mimic the actions of progesterone, were called progestagens (or less commonly, progestins). Basically they were divided into two main groups, oestranes and pregnanes. Of the

pregnanes, megesterol acetate and medroxyprogesterone acetate have been mostly used in the bitch (Evans, 1976).

The development Progestagens started in early 1930's and they mimicked physiologic actions of natural progesterone. Some were used in Veterinary practice for control of oestrus in small animals and the synchronization of ovulation in large animals. They were also used for treatment of military dermatitis in cat, for false pregnancy in bitches and to control hyper sexuality in dogs. Experiments in various species indicated their general action as 1) Antigonadotropic in that they suppress follicular development and formation of corpora lutea; 2) antioestrogenic in that they control vaginal bleeding; 3) Antiandrogenic in that they interfere with sperm transport and desynchronize those events which needed to be critically timed if pregnancy was to result from mating; and 5) Progestagenic in that they maintained pregnancy and produced a secretary endometrium. The minor difference in molecular configurations brought about variations in pharmacological effects (Evans and Sutton, 1989).

Late metoestrus and subclinical proestrus, i.e. days 120 to 170 of the cycle, was the most suitable period of the cycle to initiate suppression of the cycle, i.e. prevention of the next oestrus, by using progestins with the least tendency for side effects (Jochle, 1987).

Progestogens stimulated synthesis and release of growth hormone (GH) in dogs, which in turn was the major stimulant (with progestogens) of mammary growth and tumors. (Johnson, 1989).

2.7 MEDROXYPROGESTERONE ACETATE

Eighty six bitches were given injections of medroxyprogesterone acetate to prevent or suppress oestrous. This procedure was entirely successful in 96.5 per cent

bitches and partially successful or unsuccessful in remaining. Forty bitches received 250 mg medroxyprogesterone acetate to suppress false pregnancy and the results were satisfactory in 86 per cent of the 36 bitches where a follow up history was obtained (Withers and Whitney, 1967).

Medroxyprogesterone as an injection was used at a dosage of 50 mg by Withers and Whitney. Oral dosages of medroxyprogesterone administered ranged from 0.01mg/kg/day in the long term study of Harris and Wolchuk to an average of 0.9 mg/kg/day by Sokolowski. while Moltzen used 5 mg daily irrespective of body weight (Christie and Bell, 1970).

In experiments conducted by Bryan (1973) it was found that medroxyprogesterone at a dose of 50 mg when given by subcutaneous route showed oestrus 295 days after treatment while intramuscular and intraperitoneal routes produced oestrus at 148 days and 225 days respectively.

The original problems associated with the use of medroxyprogesterone stemmed from over dosage, use in dogs that were in proestrus or oestrus, and use in dogs with genital disease. The recommended dosage had been later established as 50 mg for dogs weighing less than 40 kg. and 50 to 75 mg., for dogs weighing more than 40 kg. The 50mg. dosage resulted in an average delay in return to oestrus of 12.5 months. The suggested regimen for oral use of medroxyprogesterone acetate was one 5 mg. tablet daily for 2-3 weeks. If suppression of oestrus was intended it was important to begin early in proestrus (Stabenfeldt, 1974).

During a four year study of contraceptive safety in mammals, no increased incidence of mammary tumors were noted in rats, mice, or monkeys but an increased incidence of mammary dysplasia was, noted in dogs. Medroxyprogesterone acetate and progesterone produced similar incidence rates,

types, and numbers of mammary nodules per animal. The incidence of mammary nodules was dose-related (Frank, *et al.* 1979).

A single injection of 50 mg medroxyprogesterone acetate was given to 12 mongrel bitches in anoestrus or metoestrus. Blood progesterone concentrations remained at less than 1 ng/ml throughout the 6-month experiment. Vaginal smears indicative of anoestrus occurred within 7 days of treatment. The ovaries and oviducts were inactive (Koowatanatawarn *et al.* 1986).

Romagnoli and Concannon (2003) suggested medroxyprogesterone acetate at a dosage of 2 mg/kg body weight for every three to four months and three to five mg for every five to six months. They recommended the use of lowest possible effective dosing regimen to reduce the side effects.

2.8 EXFOLIATE VAGINAL CYTOLOGY IN BITCHES

The ovarian hormones act on vaginal epithelium and with rising levels of oestrogen, the vaginal epithelium changes from a two to four cell layers into a multilayered epithelium. These changes included proliferation, differentiation and exfoliation. The characteristic cellular pattern in each stage of oestrus cycle can be used to evaluate the stage of the cycle in bitch and therefore the effectiveness of reproductive control (Holst, 1986).

2.8.1 Collection of exfoliated vaginal cells

Schutte (1967a) had used glass micro slides to obtain cellular material from the floor and walls of posterior vagina after manually opening of the vulval lips.

Fowler *et al.* (1971) gently irritated the cranial portion of the vaginal mucosa with sterile physiological saline solution and aspirated it with a sterile aspirator to obtain vaginal cells.

Phemister *et al.* (1973) collected vaginal smears by flushing the vagina with physiological saline solution in a pipette, and running a drop of the aspirated fluid across a glass slide.

Dore (1978) used a speculum to bypass the vestibule and vaginal smears were obtained from the cranial vagina using cotton swab. He reported that the changes in cell population from the vestibule were not at all found responsive to hormonal changes.

Allen and Dagnall (1982) had collected the exfoliated vaginal cells by flushing out the anterior vagina with normal saline using a sterile glass pipette with a rubber bulb at the distal end.

2.8.2 Staining of vaginal smear

Phemister *et al.* (1973) fixed the preparation in methanol and stained with Giemsa stain for cytological study.

Dore (1978) fixed the smears in 70 per cent alcohol, stained with haematoxylin and then with Shorr's single staining, Barret (1976) used Wright staining procedure for exfoliative vaginal cytology.

Allen (1985) stained the vaginal smears using Leishman's stain to demonstrate the abnormal vaginal cytology in a bitch. Post (1985) used Wright Giemsa stain to evaluate the vaginal smear during estrous cycle. Allen (1986) observed that Leishman's staining was simple and effective for canine vaginal cytology.

2.8.3 Vaginal Cytology during different stages of oestrous cycle

Phemister, *et al.* (1973) observed that in early in proestrus the majority of cells were intermediate or precornified epithelial cells, but some cornified cells were

present. The numbers of cornified cells increased until they predominated in the smears in oestrus. Leukocytes and parabasal cells were not seen during oestrus. There were great individual variation in the time that full cornification was achieved before the onset of oestrous.

Vaginal smears during early proestrus had high erythrocyte concentration, leucocytes and cells from the deeper layers. The percentage of keratinized cells had increased and leukocytes disappeared in late proestrus. Oestrous phase had more number of anuclear superficial cells, superficial cells with pyknotic nuclei and large intermediate cells. During metoestrus, very high concentration of leukocytes, predominance of cells from deeper layers and metoestrus cells were observed. There was a decrease in leukocyte concentration during anoestrus, which had remained more or less constant (Schutte, 1967b).

Allen (1986) observed erythrocytes neutrophils and an increase in number of intermediate cells during proestrus. At the beginning of oestrus, 60 to 80 per cent cells were polygonal squamus cells. At the beginning of metoestrus, the smear changed dramatically to more number of intermediate and parabasal cells with neutrophils.

Concannon and Di Gregario (1986) described that in normal proestrus there is a progressive decline in the relative number of parabasal cells and small intermediates, a transient increase in large intermediates and a progressive increase in superficial cells.

Bouchard *et al.* (1991) defined day one of diestrus as a drop of 20 per cent or more in the total number of superficial cells.

2.9 PROGESTERONE PROFILE IN BITCHES

According to Phemister *et al.* (1973) Serum progesterone was 0.5 ± 0.5 ng/ml during proestrus, which increased to 4.6 ± 1.2 on the day of LH peak and had increased to 10.8 ± 0.2 by the end of oestrus.

Graf (1978) noted a marked increase in progesterone level during late proestrus and oestrus. During early metestrus the progesterone concentration reached maximum and remained high for about 20 days before declining slowly.

Progesterone increased rapidly throughout oestrus, reached 19.1 ± 2.5 ng/ml on day ten and a maximum of 22.9 ± 2.7 ng/ml on day 25, and remained elevated until Day 30 at 19.9 ± 2.7 ng/ml. Levels declined gradually after day 30. (Concannon *et al.*, 1975)

In studies by Edqvist *et al.* (1975) in Greyhound and German Shepherd bitches, the mean progesterone level was found to be very low, around or under 1 ng/ml, until 1-4 days after the oestradiol peak after which the level gradually increased to around 10 ng/ml at the start of oestrus. In the pregnant bitches there was a significant drop of the progesterone level at parturition.

Hadley (1975) observed an increased progesterone level of 18 ± 1 ng / ml after 16 days of end of oestrus.

Levels of progesterone were 1.7 ± 0.3 ng/ml during proestrus, 3.5 ± 0.3 ng/ml during early oestrus and 23 ± 2.8 ng/ml on day 5 after the LH peak. Progesterone levels remained elevated through day 28 of diestrus and pregnancy. Progesterone levels decreased significantly between day 28 of pregnancy and one day prior to whelping (3.3 ± 1.2 ng/ml, with a further decrease on the day of whelping (1.1 ± 0.2 ng/ml) (Nett *et al.*, 1975).

Austad *et al.* (1976) observed plasma progesterone level in oestrus cycle to increase to a maximum concentration of 20 to 55 ng/ml. Plasma progesterone levels decreased in late pregnancy and in one in three bitches showed low or undetectable levels ten days before parturition.

Mellin *et al.* (1976) noticed the increase in serum progesterone levels from less than or equal to 5 ng /ml during late proestrus to 46 ± 6 ng/ml six days afterwards.

Plasma progesterone levels did not increase markedly during proestrus. At oestrus, progesterone values increased and maximal concentrations, which varied from about 20 to about 55 ng/ml, were reached within a few days of the oestradiol peak (Austad, *et al.*, 1976).

Estrous behaviour was first observed only after an increase in plasma progesterone and the initial increase in progesterone occurred either just prior to, or coincident with, initiation of the LH surge. Initial treatments with progesterone alone caused no morphological or behavioural changes. The reflex sexual behaviour characteristic of oestrus is affected by the initial preovulatory increase in plasma progesterone. (Concannon, *et al.*1977).

Coster *et al.* (1979) observed that progesterone levels were maximal (40 to 50 ng/ml) at the end of oestrus and decreased progressively to reach an average basal level of 0.64 ng/ml in 80 to 100 days in non-pregnant bitches.

Progesterone gradually increased for 8 days after the LH peak, remained elevated, but then fluctuated. Oestrogen in the bitch served as a priming agent to facilitate receptivity upon the addition of progesterone. The initial elevations in progesterone associated only with the coincident rise in LH. Consequently, progesterone release commenced at a precise time during the cycle. A behavioural

mechanism has evolved in the bitch in which progesterone aids in the expression of sexual receptivity (Wildt, *et al.*1979).

Serum progesterone concentration during the week before Day 0 (day of LH peak) was greater in the second cycle (1.6 ± 0.1 vs 1.0 ± 0.3 ng/ml) but the mean progesterone level during the second week following Day 0 was higher in the first cycle 15.8 ± 2.0 vs 9.4 ± 0.8 ng/ml. The data suggested that in general, serum hormone concentrations were elevated during the second cycle compared with the first cycle. (Chakraborty, *et al.* 1980)

The ability of pubertal dogs to display normal reproductive relationships appeared to be related to age, because animals that exhibited normal sexual behaviour and endocrine profiles tended to be older than females that produced aberrant patterns. Reduced preovulatory estradiol-17 beta concentrations and a delayed increase in circulating progesterone were also associated with a lack of sexual receptivity. (Wildt, et al., 1981)

Olson *et al.* (1982) observed that the mean concentration of progesterone remained less than 1 ng/ml during late anoestrus, but increased to greater than 1 ng/ml on the day of the maximum concentration of LH (preovulatory surge).

Linde and Karlsson (1984) observed the maximum keratinization when peripheral plasma level of progesterone increased to 5.44 ± 0.93 ng/ml

Reimers *et al.*, (1984) observed progesterone values of 0.41 ± 0.1 during anoestrus, 0.87 ± 0.6 during proestrus and 20.10 ± 7.6 during dioestrus.

Concannon and Digregario (1986) observed that serum progesterone levels during proestrus rose from 0.4 to 0.6 ng/ml to reach 0.8 to 1.2 ng/ml at the time of peak oestrogen levels. Progesterone levels then increased rapidly throughout the LH

surge to reach a level of 4 ng/ml which further increased continuously to reach a peak level of 15 to 80 ng/ml between 15 and 25 days after the LH peak.

Arnold *et al.* (1989) observed the progesterone concentration of less than 3.5 ng/ml (average 1.2 ng/ml) in anoestrus bitches.

At completion of functional luteolysis the mean progesterone fell from a maximal value of 58.31 ± 11.0 ng/ml at secretory stage to 1.97 ± 1.4 ng/ml. At structural luteolysis progesterone values were minimal and many cases undetectable, the mean being 0.91 ± 0.55 ng/ml and represented anoestrus (Dore, 1989).

Arbeiter (1993) observed anovulatory ovarian cycles in 14 of 1152 dogs examined. There was an increase in progesterone concentration to 6.4 to 9.5 nmol/L followed by a sudden decrease to the basal rate of 0.64 nmol/L in bitches with anovulatory ovarian cycles.

On the day after the maximum concentrations of oestradiol were recorded, plasma progesterone began to rise rapidly, reached a plateau after approximately two weeks, then declined gradually after a further two weeks. At the height of the luteal phase, peak levels of 12.6-70.1 ng/ml were measured. The time of occurrence of the initial rise in the progesterone concentration during oestrus presumably indicated that preovulatory luteinization had taken place. During anoestrus the basal concentration of progesterone was generally less than 1 ng/ml and that of oestradiol less than 9 pg/ml. (Weilenmann *et al.*, 1993).

Romagnole *et al.* (1996) observed a progesterone concentration of 26.1 ± 6.6 ng/ml at 8 to 19 days of dioestrus.

During anestrous phase, plasma levels of progesterone was 0.14 + 0.03 ng/ml, whereas in proestrus it was 0.97 ng/ml and during oestrus it increased to 6.2 + 1.83 ng/ml. (Ibuki *et al.*, 1997).

The progesterone level on the ovulation day was 1.88–2.81 ng/ml, with an average of 2.34 ng/ml. The progesterone level on the day before ovulation was 0.8–1.56 ng/ml, (1.12 ng/ml on an average). Assuming that ovulation occurred two days after the LH peak, the progesterone level was 2.12–4.06 ng/ml (2.78 *ng/ml* on an average). The elevation of the progesterone levels after the LH peak was related to the ovulation. Daily measurement of the progesterone level was a useful parameter for the prediction of ovulation in dogs (Hase *et al.*, 2000).

According to Hay *et al.* (2000) Day zero, was the first day in which fecal progestins reached a sustained rise above 100 ng/g feces. He demonstrated that sequential changes in domestic dog serum ovarian steroid concentrations are paralleled in the feces, and that it is feasible to non-invasively monitor individual progestin changes in the periovulatory interval using fecal hormone analysis.

According to Naokes *et al.* (2001) the main feature that distinguishes bitches from other species is the prolonged luteal phase illustrated by high progesterone levels in peripheral blood.

Intact female dogs had greater concentrations of progesterone compared to ovariectomised dogs. (Frank *et al.*, 2003)

The day of preovulatory rise in serum progesterone was defined as the day that progesterone concentration rose to greater or equal to 1.5 ng/ml and was at least twice the baseline concentration. The predicted parturition date, 65 days following the day of preovulatory rise in serum progesterone was compared to actual parturition date, the day the first pup was delivered. The mean progesterone concentration at day zero was determined to be 2.02 ± 0.18 ng/ml. (Kutzler *et al.*, 2003).

In bitches, the onset of pyometra, characteristically occured in the first half of the diestrous stage in the estrous cycle, in which the blood concentration of progesterone peaks and that of estradiol is lowest. In the first half of the diestrous stage, suppressed activity of cellular immunity resulted from increasing progesterone concentration and minimal estrogen release. This marked decrease of immune resistance allowed the expansion of *E. coli*, which entered the uterine cavity through the loosened cervical canal during oestrus, leading to pyometra onset (Sugiura, *et al.*, 2004).

MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Animals for the study consisted of 18 healthy adult bitches, housed under prescribed conditions during the period of the study from November 2004 to April 2005. The experimental animals were randomly divided into two groups, Group A and Group B. Six healthy bitches, formed the control group- Group C. Vaginal cytology of all the animals were regularly taken to asses the onset of proestrus.

3.2 SUPPRESSION OF REPRODUCTION IN BITCHES USING MEDROXYPROGESTERONE ACETATE

Group A

Bitches in group A were given a single subcutaneous injection of medroxyprogesterone acetate (Depo-Povera. inj.[1]) at the rate of 50 mg total dose at the fag end of proestrus. The animals were closely observed for the signs of oestrus. Vaginal cytology and serum progesterone levels confirmed proestrus and oestrus.

Vaginal smears and serum samples were collected before treatment and subsequently every fortnight for a period of three months post treatment.

[1] Depo-Provera inj. (Pharmacia N.V./S.A.,Belgium) : Contains medroxyprogesterone acetate, a derivative of progesterone, The chemical name is pregn-4-ene-3,20-dione,17-(acetyloxy)-6-methyl-,(6α). Depo-Provera is supplied in 150 mg/ml vials and disposable syringes, each containing 1ml medroxyprogesterone acetate sterile aqueous suspension. Each ml contains, medroxyprogesterone acetate (150 mg), polyethylene glycol 3350 (28.9 mg), polysorbate 80 (2.41 mg), sodium chloride (8.68 mg), methylparaben (1.37 mg), propylparaben (0.150 mg), water for injection.

Group B

Bitches in this group were administered medroxyprogesterone acetate (Meperate[2]) orally at the rate of 10 mg per day for four days followed by 5 mg per day for another 12 days starting from the fag end of proestrus.

Vaginal smears and serum samples were collected as detailed in Group A.

Group C

Six bitches, which had come to proestrus, acted as control animals. The stages were confirmed using vaginal cytology and progesterone assay. Vaginal smears and serum samples were collected as detailed for Group A animals.

3.3 BEHAVIORAL RESPONSES

The following parameters were observed to asses the stage and intensity of the cycle for each dog: vulval discharge vulval edema, interest towards male dogs, tail deviation reflex and frequency of urination. Vulval discharge, vulval edema and interest towards male was graded as high medium low or absent. Tail deviation reflex was scored as present or absent. Frequency of urination was graded as increased or normal.

The behavioural responses were observed and scored based on the findings of Goodwin *et al.* (1979), Wildt *et al.* (1981), Johnston (2001) as furnished in Table 3.1

[2] Meperate (Serum International, Pune, India) : Contains medroxyprogesterone acetate, a derivative of progesterone, The chemical name is pregn-4-ene-3,20-dione,17-(acetyloxy)-6-methyl-,(6α). Meperate is supplied as 10 mg tablets each strip containing 10 such tablets. Each tablet contains 10 mg medroxyprogesterone acetate.

Scoring pattern of behavioural responses

Parameter	Score
Vulval discharge	High Medium Low Absent
Intensity of vulval edema	High Medium Low Absent
Interest towards male	High Medium Low Absent
Tail deviation reflex	Present Absent
Frequency of urination	Increased Normal

3.4 EXFOLIATE VAGINAL CYTOLOGY

3.4.1 Preparation of Vaginal Smear.

Vaginal smears were collected by the technique described by Allen and Dagnell (1982). A small column of normal saline (approx. 0.2ml) was taken in a sterile glass pipette with a rubber bulb fitted at the distal end. The animal was controlled in the standing position and the pipette was carefully introduced into the vagina, directing the pipette cranio-dorsally to the vestibule and on reaching the vestibulo vaginal junction, it was redirected cranially to reach the anterior vagina. The rubber bulb was squeezed several times to pickup up the exfoliated vaginal cells with the fluid column. The pipette was then withdrawn carefully. A small drop of aspirate was kept on a slide, a thin smear was prepared and air dried. Four such smears were prepared each time.

3.4.2 Staining of Vaginal Smears.

The smears were stained using Wright-Giemsa's stain (Post, 1985)

3.4.2.1 *Composition of Wrights- Giemsa stain*

Wright stain powder	---	300 mg.
Giemsa stain powder	---	30 mg.
Absolute methanol	---	100ml.

3.4.2.2 *Wright-Giemsa's staining procedure*

1) Prepared the smear and air dried
2) Kept the slide in a staining rack
3) Poured Wright Giemsa stain on the smear
4) Allowed the stain to act for 30 second
5) Poured neutral water on the slide and mixed it with the stain by gentle blowing
6) Allowed the stain water mixture to act for 1 minute
7) Poured of the stain water mixture
8) Washed the slide in tap water and air dried the smear

Examined the smears first under low power and then under high power of a microscope.

3.4.3 Cell Types

a) Epithelial cells

They were classified as into three major categories: 1)Superficial cells, 2)Intermediate cells and 3)Parabasal cells

1) Superficial cells

These are polygonal cells and are of four types based on nuclear appearance: large polygonal anucleated cells, large polygonal cells with intact nuclear membrane, large polygonal cells with small nuclear remnant and large polygonal cells with pycknotic nuclei.

2) Intermediate cells

These include a wide range of sizes and types as they represent all stages of maturation between parabasal and fully mature superficial cells They become more angular, enlarge and flatten as they mature. The relative size of the nucleus decreases as the cells enlarge The less mature small intermediate cells are small and polygonal with a relatively large nucleus

3) Parabasal Cells

These cells are round or oval and are the smallest epithelial cells commonly observed in smears. The diameter of their nucleus ranges between 45 and 90 per cent of the cells diameter.

b) Blood Cells

Neutrophils and erythrocytes are the two blood cells found in smears, rarely lymphocytes and eosinophils can occur

c) Spermatozoa

These are found in smears taken after mating has occurred.

3.4.4. Superficial Cell Index (SCI)

It is the per cent of superficial cells and large intermediate cells evaluated against the number of small intermediate cells and parabasal cells. It was calculated using the formula,

$$SCI = \frac{\text{Number of cells from superficial layer}}{\text{Number of cells from deeper layers}} \times 100$$

3.5 PROGESTERONE ASSAY

3.5.1 Collection of Serum

Approximately 5 ml blood was collected aseptically from the saphenous vein using a 20 G sterile needle and syringe to a test tube. The tubes were kept at an angle for clotting. After an hour, the tubes were kept inside a refrigerator for an hour. Later the tubes were centrifuged at 1000 rpm for 10 minutes, the serum was aspirated from the top and stored in cryovials in deep freezer at –20° C till assay was carried out.

3.5.2 Assay of Progesterone

Progesterone in the serum sample were assayed quantitatively using I^{125} labeled progesterone RIA kit [3]

[3] Progesterone RIA kit (Radim, Pomezia (Roma), Italia: Contains Calibrators. Radioactive conjugate, Coated tubes and Control serum.

3.5.2.1 *Test Principle*

Competition between a radioactive and a non-radioactive antigen for a fixed number of antibody sites. The amount of I^{125} labeled progesterone bound to the antibody is inversely proportional to the concentration of unlabelled progesterone present.

3.5.2.2 *Measuring Range*

Approximately 0 to 60 ng/ml

3.5.2.3 *Components*

A) Calibrators : Vials containing approximately 0, 0.3, 1.0, 5.0, 20.0, and 60.0 ng. of progesterone in serum with sodium azide as preservative.

B) Radioactive conjugate containing < 5 μ Ci (185 kBq) of [I^{125}]- labeled progesterone in a protein buffer with sodium benzoate as preservative.

C) Coated tubes: 100 plastic tubes with rabbit anti-progesterone immunoglobulin immobilized to the inside wall of each tube.

D) Control serum (Lyophilized) Two vials containing low and high concentration of progesterone in serum with sodium azide as preservative.

3.5.2.4 *Assay procedure*

All reagents were allowed to attain room temperature (`20° C) and mixed the liquid reagents by gentle inversion. Reconstituted the calibrators and control sera and mixed thoroughly. Calibrators, control sera and unknowns were assayed in duplicate.

1. Labeled two plain (uncoated) tubes for Total Counts. Labeled and arranged the anti-progesterone-coated tubes in duplicate.

2. Added 25 μL of the calibrators, control sera or unknowns to appropriate tubes.

3. Added 500 µL of radioactive conjugate each tube

4. Mixed by racking the test tubes gently by hand.

5. Incubated all the tubes in a water bath at 37 ± 2° C for 60 to 70 minutes.

6. Decanted all tubes except the total count tubes, by simultaneous inversion with a sponge rack into a radioactive waste receptacle. Stroked tubes sharply on absorbent material to facilitate complete drainage and then allowed them to drain on absorbent paper for a minimum of two minutes. Blotted the tubes to remove any droplets adhering to the rim before returning them to upright position.

7. Counted all tubes in a gamma counter for one minute

3.5.2.5 *Calculation of Results*

The results were calculated using a linear log curve

A) Calculated the mean counts per minute (cpm) for each Calibrator, Control serum and unknown. Calculated the per cent bound (%B/T) for each calibrator, control serum and unknown as follows :

$$\%B/T = \frac{\text{Mean Sample Counts}}{\text{Mean Total Counts}} \times 100$$

B) Plotted a curve of % B/T for the Calibrators (y axis) against the progesterone concentration (x axis) on semi log graph paper. Drawn a calibration curve through the mean of the duplicate points.

C) From the calibration curve, determined the progesterone concentrations of unknowns and controls sera from the means of the duplicate counts.

D) Any sample reading greater than the highest calibrator was diluted appropriately with 0 ng/ml Progesterone calibrator and reassayed.

E) Any sample reading less than the lowest calibrator was reported as such.

RESULTS

The results obtained in the study are furnished in Tables 1 to 13 and Figures 1 to 4

4.1 SUPPRESSION OF REPRODUCTION IN BITCHES USING MEDROXYPROGESTERONE

Data presented in Tables 1 to 2 and Fig. 1 indicate the response of animals after treatment with medroxyprogesterone acetate.

4.1.1 Suppression of reproductive cycle and duration of suppression

Reproductive cycle was suppressed in all six animals of group A at a mean of 3.17 ± 0.3 days from the day of treatment, while in group B animals it was suppressed at a mean of 2.67 ± 0.33 days from the day of treatment. One animal in group A returned to cycle 52 days after treatment.

In group A, animals had a mean duration of suppression of 83.3 days while in group B it was 90 days. One animal in group A returned to cycle after 52 days of treatment.

4.2 BEHAVIORAL RESPONSE

The behavioral responses observed during the experimental period before and after treatment, the data are furnished in Tables 3 to 5

Before treatment during proestrus in group A, 66.6 per cent animals had high vulval discharge while 33.4 per cent animals had medium discharge. The intensity of vulval edema was high in 83.3 per cent animals while 16.7 per cent animals had medium edema. Tail deviation reflex was present in 83.3 per cent animals and absent in 16.7 per cent animals. Interest towards male was high in 66.6 per cent animals

while 16.7 per cent animals animals had medium and 16.7 per cent animals animals had low interest towards male. Increased frequency of urination was found to be there in 83.3 per cent animals while it was normal in 16.7 per cent animals.

After treatment complete cessation of vulval discharge was noted in 83.3 per cent animals while it was low in 16.7 per cent animals. Vulval edema was absent in all the animals (100 %). There was absence of interest towards male in 83.3 per cent animals after treatment while 16.7 per cent animals maintained a low level of interest in the male. Frequency of urination and tail deviation reflex was absent in all animals (100 %) following treatment.

In group B, before treatment and during proestrus, 83.3 per cent animals had high vulval discharge while, it was medium in 16.7 per cent animals. Vulval edema was high in 83.3 per cent animals and low in 16.7 per cent animals. All the animals (100 %) showed tail deviation reflex. Only 83.3 per cent animals had increased frequency of urination.

After treatment vulval discharge, vulval edema, interest towards the male and tail deviation reflex was absent in all the animals (100 %). Frequency of urination also remained normal during the period following treatment.

Among group C (control) animals, 83.3 per cent animals showed high vulval discharge and 16.7 per cent animals showed medium discharge. All the animals (100 %) had high vulval edema. Interest towards male was high in 83.3 per cent animals whereas 16.7 per cent animals showed medium interest. Tail deviation reflex was present in all the animals (100 %). Only 83.3 per cent animals showed an increased frequency of urination.

The behavioural parameters remained same in group C (control) except for a higher degree during estrus along with a change in the type of discharge from

serosanguinous to a clear one during estrus. Frequency of urination was found to be normal in two animals but still elevated in 66.6 per cent animals as in proestrus. All the behavioural changes gradually reduced to very low level during dioestrus and was totally absent during anoestrus.

4.3 EXFOLIATE VAGINAL CYTOLOGY

The exfoliate vaginal cytology of bitches in which estrus was controlled were studied before and after the treatment and was compared to that of the control animals. The results are represented in Table 6 to 7 and Fig. 2 to 3.

4.3.1 Cellular Changes

There was a predominant change from presence of erythrocytes and superficial and large intermediate cells characteristic of proestrous stage to parabasal and small intermediate cells characteristic of anoestrous after treatment with medroxyprogesterone acetate indicating the passage to anoestrous from proestrus. The mean percentage of various cells before and after treatment in groups A and B and during the normal cycle in the group C control bitches are given in the Tables 6 and 7

The mean percentage of parabasal cells, small intermediate cells, large intermediate cells and superficial cells in Group A animals were 18.17 ± 3.14, 23.14 ± 0.86, 30.67 ± 1.23 and 28.17 ± 1.33 respectively during proestrus, before treatment, 62.50 ± 1.92, 16.17 ± 0.64, 11.83 ± 0.82 and 9.50 ± 1.47 respectively on 14^{th} day after treatment, 63.17 ± 0.59, 19.00 ± 0.84, 13.00 ± 0.62 and 4.83 ± 1.09 respectively on 28^{th} day after treatment, 63.17 ± 0.47, 18.17 ± 0.96, 12.33 ± 0.33 and 6.33 ± 1.33 respectively on 42^{nd} day after treatment, 64.40 ± 0.46, 16.40 ± 0.46, 11.40 ± 0.46 and 7.20 ± 0.77 respectively on 56^{th} day of treatment, 65.40 ± 0.61, 18.80 ± 0.66, 10.60 ± 0.67 and 5.20 ± 0.59 respectively on 70^{th} day of treatment and

65.20 ± 0.59, 18.80 ± 0.77, 10.40 ± 0.83 and 5.60 ± 0.36 respectively on 84th day of treatment.

In Group B, the mean percentage of parabasal cells, small intermediate cells, large intermediate cells and superficial cells were 16.67 ±1.42, 23.00 ± 0.72, 31.17 ± 1.30 and 29.17 ± 1.06 respectively during proestrus, before treatment, 61.83 ± 1.85, 16.83 ± 0.69, 11.67 ± 0.75 and 9.67 ± 1.12 respectively on day 14th of treatment, 62.50 ± 0.75, 19.50 ± ±0.70, 12.83 ± 0.59 and 5.17 ± 0.96 respectively on 28th day of treatment, 63.17 ± 0.69, 18.50 ± 0.97, 12.00 ± 0.57 and 6.33 ±1.06 respectively on 42 day of treatment, 64.50 ± 0.61, 16.83 ± 0.93, 11.17 ± 0.64 and 7.50 ± 0.61 respectively on 56th day of treatment, 62.33 ± 1.03, 20.17 ± 0.89, 11.33 ± 0.48 and 6.17 ± 0.59 respectively on 70th day of treatment and 64.17 ± 0.99, 18.67 ± 0.79, 10.67 ± 0.79 and 6.50 ± 0.97 respectively on 84th day of treatment.

The mean percentage of parabasal cells, small intermediate cells, large intermediate cells and superficial cells in Group C were 18.83 ± 0.86, 22.50 ± 0.66, 30.67 ± 1.23 and 28.00 ± 1.19 respectively during proestrus, 3.67 ± 0.33, 5.83 ± 0.47, 12.33 ± 0.79 and 78.17 ± 0.99 respectively during oestrus. During dioestrus the mean percentage of parabasal cells, small intermediate cells, large intermediate cells and superficial cells were 57.50 ± 0.94, 15.17 ± 0.69, 16.00 ± 0.57 and 11.33 ± 0.41, 59.67 ± 0.48, 18.50 ± 0.42, 12.00 ± 0.62 and 9.83 ± 0.47, 60.83 ± 0.53, 11.67 ± 0.33 and 7.67 ± 0.65, 62.17 ± 0.30, 20.50 ± 0.61, 10.50 ± 0.42 and 6.83 ± 0.78, respectively at an interval of 14 days. The mean percentage of parabasal cells, small intermediate cells, large intermediate cells and superficial cells were 65.00 ± 1.16, 20.67 ± 0.33, 9.17 ± 0.64 and 5.17 ± 0.64 respectively during anoestrus.

4.3.2 Superficial Cell Index

The data of superficial cell index before and after the treatment in the groups A and B and during the cycle in group C are presented in Tables 8 to 10

The mean superficial cell index in Group A treated animals were 151.05 during proestrus before treatment and 16.00 after treatment while in Group B it was 137.79 and 14.27 respectively. Control animals showed mean SCI values of 148.24 during proestrus, 955.66 during oestrus, 34.74 during dioestrus and 13.08 in anoestrus.

4.4 SERUM PROGESTERONE PROFILE IN BITCHES

Serum progesterone profile in bitches treated with medroxyprogesterone acetate and control groups are presented in Tables 11 to 13 and Fig 4.

4.4.1 Serum progesterone profile in treated and control groups

Animals in group A showed mean serum progesterone level of 0.53 ± 0.08 ng/ml during the beginning of proestrus. Following the injection of 50 mg medroxyprogesterone acetate, the mean serum progesterone level had rose to 27.83±1.83 ng/ml, on day 14 of treatment, followed by 30.50 ± 2.92 on day 28, 31.17± 4.25 ng/ml on day 42, 19.12 ± 6.08 ng/ml on day 56, 17.73 ± 7.85 on day 70 and then decreased to a level of 0.82 ± 0.18 on day 84 of the treatment.

Animals in group B showed a mean serum progesterone level of 0.57±0.10 ng/ml. during the beginning of proestrus. After the oral treatment with medroxyprogesterone acetate mean serum progesterone level were 0.55 ± 0.06 ng/ml on day 14 of treatment followed by, 0.45 ± 0.08 ng/ml on day 28 of treatment, 0.38 ± 0.04 ng/ml on day 42 of treatment, 0.33 ± 0.02 ng/ml on day 56, 0.40 ± 0.04 ng/ml on day 70, and 0.45 ± 0.08 ng/ml on day 84 of the treatment.

Animals in group C showed a mean serum progesterone level of 0.45 ± 0.09 ng/ml during the beginning of proestrus, followed by 4.43 ± 0.33 ng/ml on during estrus, 28.17 ± 1.91 ng/ml, 39.83 ± 1.94 ng/ml and 49.00 ± 2.07 ng/ml during dioestrus and 0.80 ± 0.11 ng/ml during anoestrus

Table 1. Time taken (days) for suppression of proestrus and duration of suppression in group A animals(medroxyprogesterone acetate administered parenterally) *

Animal No	Time taken for Suppression of proestrus (days)	Duration of suppression (days)
1	3	90
2	4	90
3	2	50
4	3	90
5	4	90
6	3	90
Mean ± SE	3.17± 0.3	83.33 ± 6.53

Table 2. Time taken (days) for suppression of proestrus and duration of suppression in group B animals (medroxyprogesterone acetate administered orally)*

Animal No	Time taken for Suppression of proestrus (days)	Duration of suppression (days)
1	2	90
2	4	90
3	2	90
4	3	90
5	3	90
6	2	90
Mean ± SE	2.67 ± 0.33	90 ± 0.0

* Animals in the group C (control group) continued in the oestrous cycle without any interruption.

Table 3. Behavioural response in Group A animals before and after the treatment

Parameter	Score	No. of animals (%)	
		Before treatment	After treatment
Vulval discharge	High	4(66.6 %)	-
	Medium	2 (33.4%)	-
	Low	-	1(16.7%)
	Absent	-	5(83.3%)
Intensity of vulval edema	High	5(83.3%)	-
	Medium	1(16.7%)	-
	Low	-	1(16.7%)
	Absent	-	5(83.3%)
Interest towards male	High	4(66.6%)	-
	Medium	1(16.7%)	-
	Low	1(16.7%)	1(16.7%)
	Absent	-	5(83.3%)
Tail deviation reflex	Present	5(83.3%)	-
	Absent	1(16.7%)	6(100%)
Frequency of urination	Increased	5(83.3%)	-
	Normal	1(16.7%)	6(100%)

Table 4. Behavioural response in Group B animals before and after the treatment

Parameter	Score	No. of animals (%)	
		Before treatment	After treatment
Vulval discharge	High Medium Low Absent	5(83.3%) 1(16.7%) - -	- - - 6(100%)
Intensity of vulval edema	High Medium Low Absent	5(83.3%) 1(16.7%) - -	- - - 6(100%)
Interest towards male	High Medium Low Absent	5(83.3%) 1(16.7%) - -	- - - 6(100%)
Tail deviation reflex	Present Absent	6(100%) -	- 6(100%)
Frequency of urination	Increased Normal	4(66.6%) 2(33.4%)	- 6(100%)

Table 5. Behavioural responses in Group C animals

Parameter	Score	No. of animals (%)	
		Proestrus	Oestrus
Vulval discharge	High Medium Low Absent	5(83.3%) 1(16.7%) - -	5(83.3%) 1(16.7%) - -
Intensity of vulval edema	High Medium Low Absent	6(100%) - - 	6(100%) - - -
Interest towards male	High Medium Low Absent	5(83.3%) 1(16.7%) - -	5(83.3%) 1(16.7%) - -
Tail deviation reflex	Present Absent	6(100%) -	6(100%) -
Frequency of urination	Increased Normal	5(83.3%) 1(16.7%)	4(66.6%) 2(33.4%)

Table 6. Cytological changes in vaginal smears of animals treated with medroxyprogesterone acetate

Type of Cells	Day of examination of vaginal smear													
	0		14		28		42		56		70		84	
	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
PBC Mean ±S.E.	18.17 ±1.14	16.67 ±1.42	62.50 ±1.92	61.83 ±1.85	63.17 ±0.59	62.50 ±0.75	63.17 ±0.47	63.17 ±0.69	64.40 ±0.46	64.50 ±0.61	65.40 ±0.61	62.33 ±1.03	65.20 ±0.59	64.17 ±0.99
SIC Mean ±S.E.	23.17 ±0.86	23.00 ±0.72	16.17 ±0.64	16.83 ±0.69	19.00 ±0.84	19.50 ±0.70	18.17 ±0.96	18.50 ±0.97	16.40 ±0.46	16.83 ±0.93	18.80 ±0.66	20.17 ±0.89	18.80 ±0.77	18.67 ±0.79
LIC Mean ±S.E.	30.67 ±1.23	31.17 ±1.30	11.83 ±0.82	11.67 ±0.75	13.00 ±0.62	12.83 ±0.59	12.33 ±0.33	12.00 ±0.57	11.40 ±0.46	11.17 ±0.64	10.60 ±0.67	11.33 ±0.48	10.40 ±0.83	10.67 ±0.79
SPC Mean ±S.E.	28.17 ±1.33	29.17 ±1.06	9.50 ±1.47	9.67 ±1.12	4.83 ±1.09	5.17 ±0.96	6.33 ±1.33	6.33 ±1.06	7.20 ±0.77	7.50 ±0.61	5.20 ±0.59	6.17 ±0.59	5.60 ±0.36	6.50 ±0.97

PBC –Parabasal cells
SIC – Small intermediate cells
LIC – Large intermediate cells
SPC – Superficial cells

Table 7. Cytological changes in vaginal smears of control animals

Type of Cells	Proestrus	Oestrus	Onset of dioestrus	Early dioestrus	Mid dioestrus	Late dioestrus	Anoestrus
PBC Mean ± S.E	18.83±0.86	3.67±0.33	57.50±0.94	59.67±0.48	60.83±0.53	62.17±0.30	65.00±1.16
SIC Mean ± S.E	22.50±0.66	5.83±0.47	15.17±0.69	18.50±0.42	19.83±0.59	20.50±0.61	20.67±0.33
LIC Mean ± S.E	30.67±1.23	12.33±0.79	16.00±0.57	12.00±0.62	11.67±0.33	10.50±0.42	9.17±0.64
SPC Mean ± S.E	28.00±1.19	78.17±0.99	11.33±0.41	9.83±0.47	7.67±0.65	6.83±0.78	5.17±0.64

PBC – Parabasal cells

SIC – Small intermediate cells

LIC – Large intermediate cells

SPC – Superficial cells

Table 8. Superficial cell index (SCI) before and after suppression of oestrus cycle in Group A animals

Animal No	Superficial cell index	
	Proestrus	After suppression
1	156.66	13.46
2	136.16	16.76
3	134.74	12.54
4	167.18	22.16
5	148.17	14.63
6	163.36	16.46
Mean ± SE	151.05 ± 5.60	16.00 ± 1.40

Table 9. Superficial cell index (SCI) before and after suppression of oestrus cycle in Group B animals

Animal No	Superficial cell index	
	Proestrus	After suppression
1	127.67	15.43
2	156.64	12.64
3	124.57	16.67
4	126.68	12.45
5	143.56	14.76
6	147.64	13.67
Mean ± SE	137.79 ± 5.44	14.27 ± 0.68

Table 10. Superficial cell index (SCI) in Group C animals during different stages of the oestrus cycle

Animal No	Superficial cell index			
	Proestrus	Estrus	Dioestrus	Anoestrus
1	166.67	673.87	32.34	14.26
2	134.24	465.76	26.27	12.23
3	124.46	1489.76	36.46	11.12
4	157.48	658.64	43.24	14.46
5	158.27	756.34	28.67	13.56
6	168.32	1689.56	41.43	12.87
Mean ± SE	148.24 ± 7.36	955.66 ± 205.89	34.74 ± 2.80	13.08 ± 0.52

Table 11. Serum progesterone level in Group A animals

Day of estimation (post treatment)	Serum Progesterone level (ng/ml.)						Mean ± SE
	Animal No						
	1	2	3	4	5	6	
0	0.6	0.6	0.3	0.3	0.2	0.6	0.53 ± 0.08
14	34	28	22	26	32	25	27.83 ± 1.83
28	36	29	24	24	42	28	30.50 ± 2.92
42	28	26	27	24	52	30	31.17 ± 4.25
56	26	24	32	0.2	0.5	32	19.12 ± 6.08
70	22	20	4.2	53	3.7	3.5	17.73 ± 7.85
84	1.2	0.8	0.4	0.5	1.5	0.5	0.82 ± 0.18

Table 12. Serum progesterone level in Group B animals

Day of estimation (post treatment	Serum Progesterone level ng/ml. Animal No						Mean ± SE
	1	2	3	4	5	6	
0	0.6	0.8	1	0.3	0.6	0.7	0.57± 0.10
14	0.6	0.5	0.9	0.3	0.6	0.4	0.45 ± 0.4
28	0.5	0.6	0.8	0.4	0.5	0.5	0.55± 0.06
42	0.3	0.4	0.5	0.3	0.3	0.5	0.38± 0.04
56	0.3	0.3	0.3	0.3	0.4	0.4	0.33± 0.02
70	0.5	0.5	0.4	0.4	0.3	0.3	0.40± 0.04
84	0.4	0.8	0.3	0.5	0.3	0.4	0.45± 0.08

Table 13. Serum progesterone level in Group C animals

Day of estimation (from onset of proestrous)	Serum Progesterone level ng/ml.						Mean ± SE
	Animal No						
	1	2	3	4	5	6	
0	0.8	0.2	0.3	0.3	0.5	0.6	0.45± 0.09
14	4.2	5.5	3.5	3.7	5.2	4.5	4.43± 0.33
28	46	38	44	37	43	46	42.33± 1.61
42	55	42	46	53	46	52	49.00± 2.07
56	43	32	42	36	42	44	39.83± 1.94
70	34	22	27	24	32	30	28.17± 1.91
84	1.2	0.8	0.8	0.5	1	0.5	0.80 ± 0.11

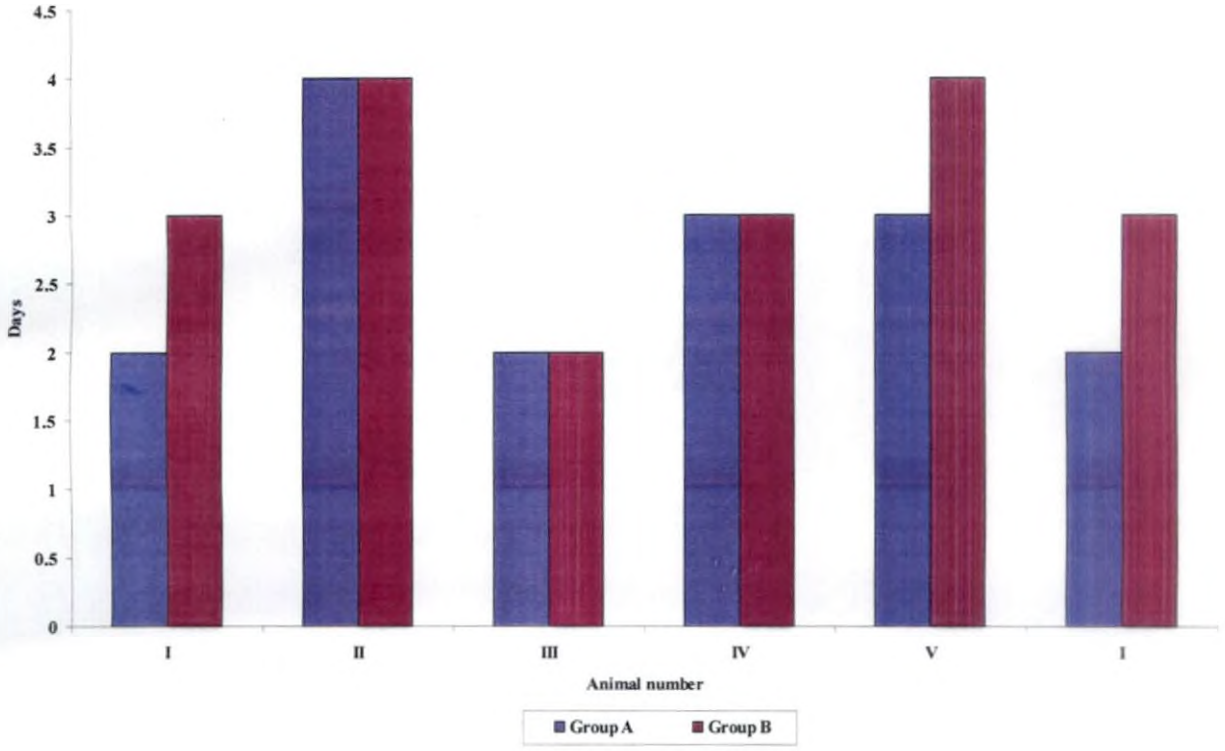
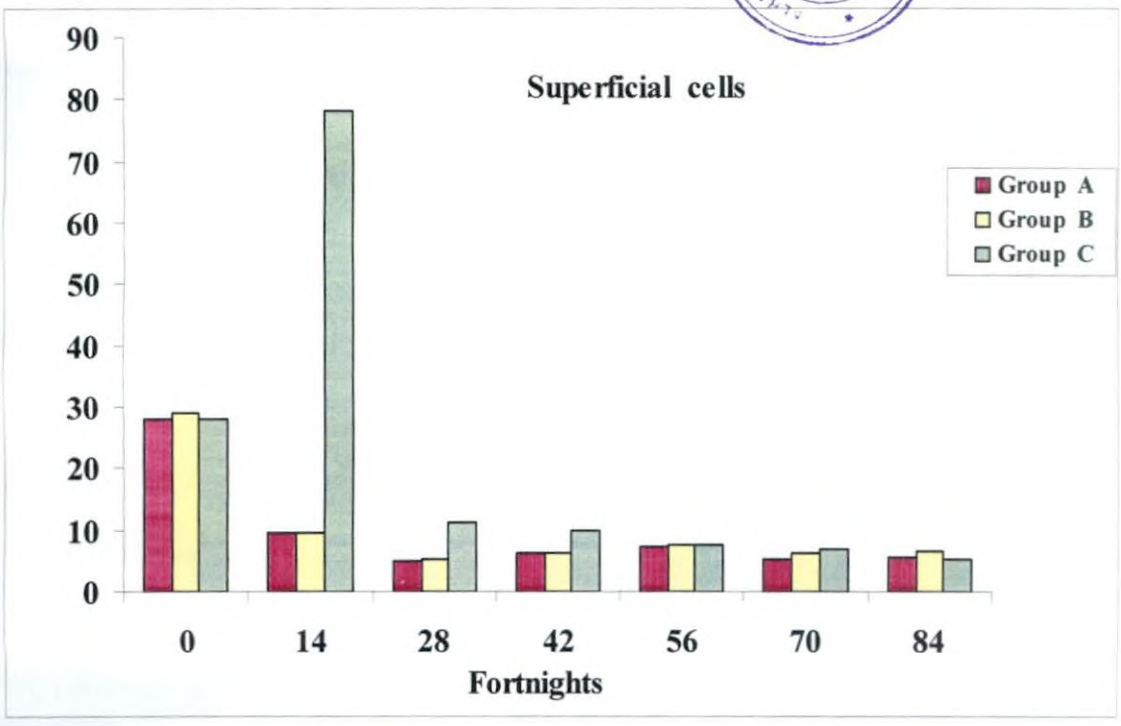


Fig.1 Time taken (days) for suppression of proestrus.



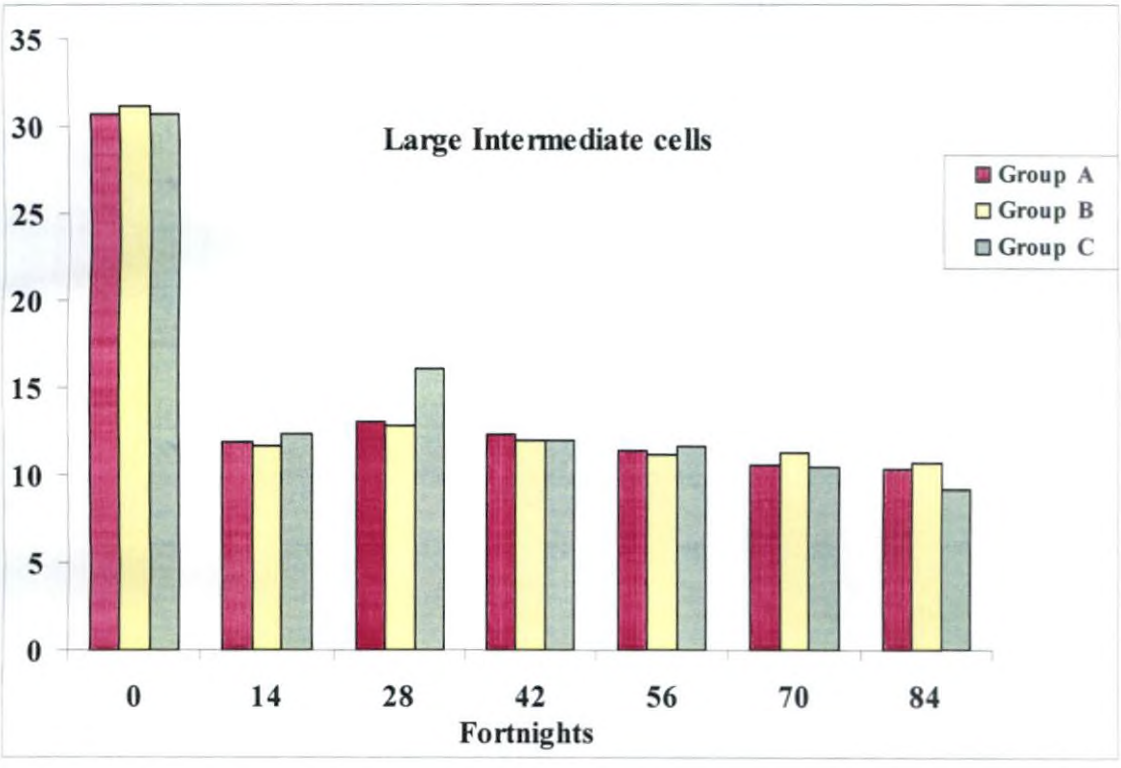
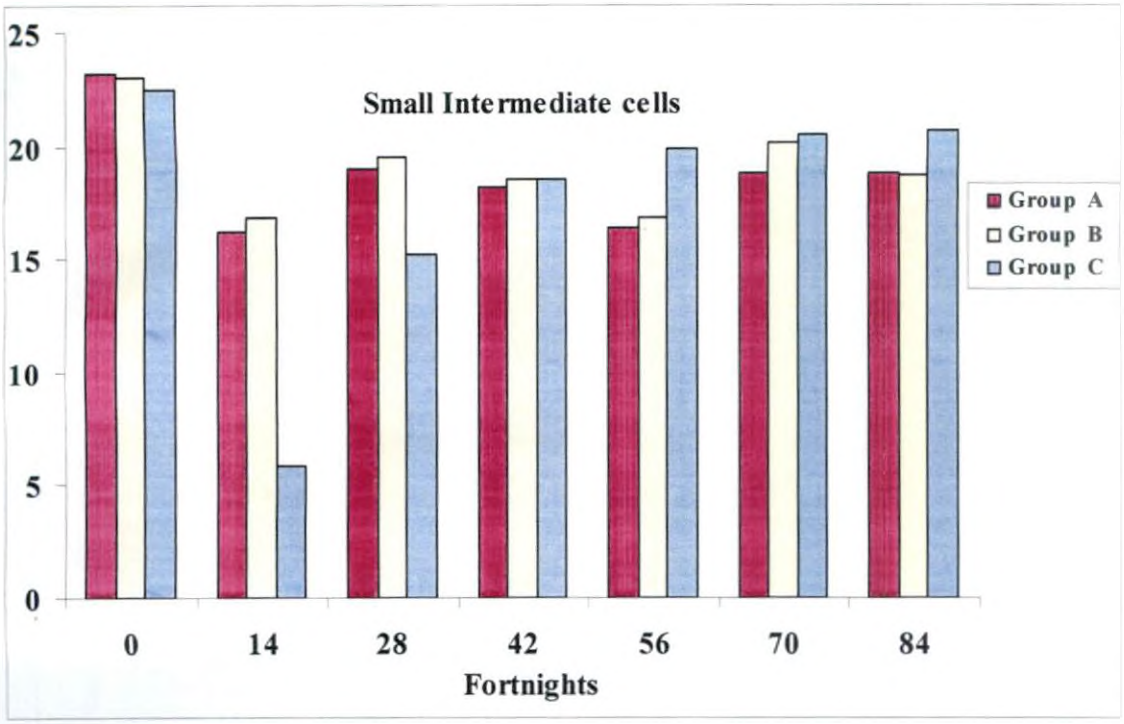


Fig. 2 Cytological changes in vaginal smears of treatment and control animals: 1 (Superficial cells and Large Intermediate cells)



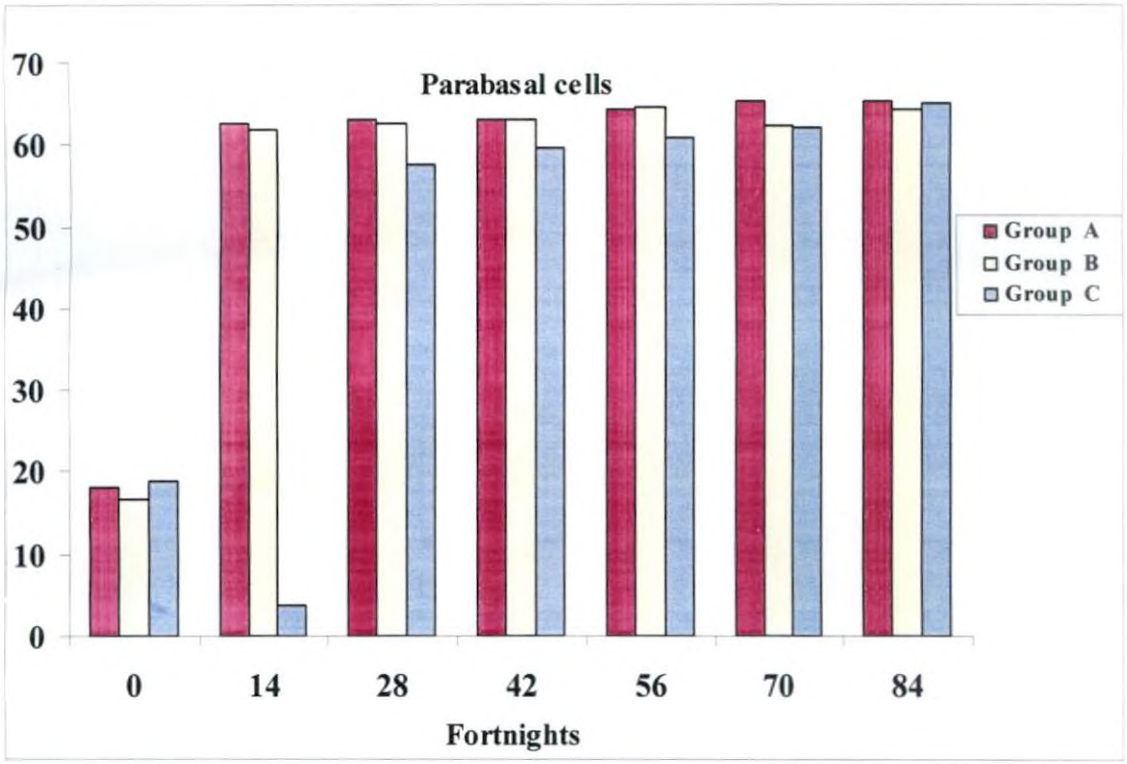


Fig. 3 Cytological changes in vaginal smears of treatment and control animals : 2 (Small Intermediate and Parabasal cells)

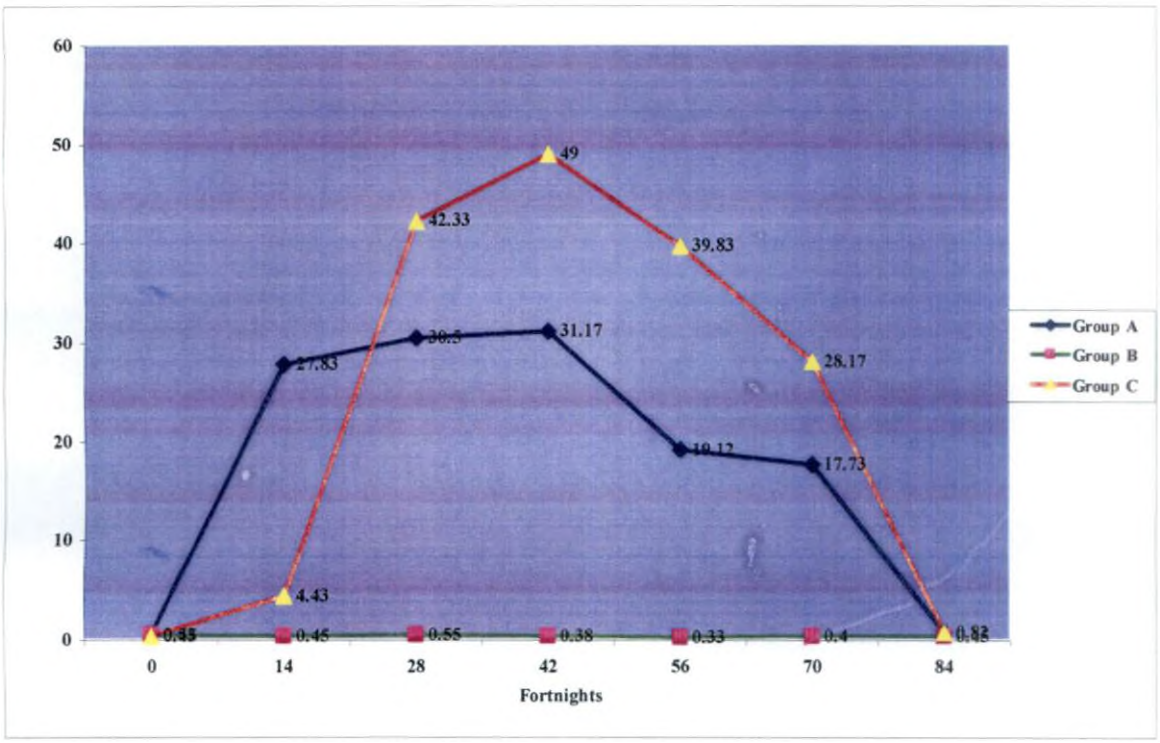


Fig. 4 Serum progesterone level in treatment and control animals

Discussion

DISCUSSION

The results obtained in the study are discussed here under.

5.1 SUPPRESSION OF REPRODUCTION IN BITCHES USING MEDROXYPROGESTERONE ACETATE

From the Table 1 and 2 it could be seen that the reproductive cycle in animals in group A (medroxyprogesterone administered parenterally), was controlled in 3.17 ± 0.33 days and that in group B (medroxyprogesterone administered orally) was controlled in 2.67 ± 0.3 days with animals in group C, continuing in oestrous cycle without interruption. There was no significant difference in the time taken for suppression of proestrus between animals in group A and group B, but a trend for lower time duration (2.67 days) in group B when compared to group A (3.17 days) indicated that medroxyprogesterone acetate when administered orally had a rapid action controlling oestrous. The above findings are in agreement with that of Mathieu and Rambaud (1968), Magnusson and Ottander (1969) and Withers and Whitney (1967)

The rapid control of reproductive cycle by medroxyprogesterone acetate by oral and parenteral routes may be attributed to the feed back effect of medroxyprogesterone acetate on the hypothalamo-hypophyseal-gonadal axis. Apparent arrest of oestrous cycle within four days of administration of medroxyprogesterone acetate in both oral and parenteral routes throws light into the possibility of using the drug for effective control of oestrous in bitches.

5.2 BEHAVIORAL RESPONSE

The details of behavioral responses are represented in tables 8, 9 and 10

During proestrus in group A, majority of the animals had characteristic signs of proestrus like high vulval discharge (66.6 % animals) high intensity of vulval edema (83.3 %), high interest towards male (66.6% animals), presence of tail deviation reflex (83.3 % animals) and increased frequency of urination (83.3

% animals). The majority of group B animals also had a high degree of behavioural signs indicative of proestrous like high vulval discharge (83.3 % animals), high intensity of vulval edema (83.3 %) high interest towards male (83.3 % animals), presence of tail deviation reflex (83.3 % animals) and increased frequency of urination (100% animals). In control animals of group C, 83.3 per cent animals showed high vulval discharge, all animals (100%) had high vulval edema, interest towards male was high in 83.3 per cent animals, tail deviation reflex was present in all animals (100%) and increased frequency of urination was present in 83.3 per cent animals. It is supposed that the increased frequency of urination helps in the distribution of pheromones, which in turn help in attracting males. According to Concannon *et al.* (1975), proestrus is chracterised by swelling of vulva and serosanguinous discharge. Goodwin et al (1979) had observed increased frequency of urination and increased interest in male with tail deviation reflex in proestrus. Wildt *et al.* (1981) reported distinct vulval swelling and vaginal discharge during the onset of proestrus. According to Johnston (2001), the lateral deviation of tail or flagging occurs in proestrus along with winking of the vulva. The observations in the present study were in agreement with the previous findings.

Following treatment in group A, vulval discharge was absent in 83.3 per cent animals while it was low in 16.7 per cent animals. Vulval edema was absent in all the animals (100 %). No interest towards male was shown after treatment by 83.3 per cent animals while 16.7 per cent animals maintained a low level of interest in the male. Frequency of urination and tail deviation reflex was absent in all animals following treatment. After treatment vulval discharge, vulval edema, interest towards the male and tail deviation reflex were absent in all the animals (100 %). Frequency of urination also remained normal during the period following treatment. The behavioral parameters remained same except for a higher degree during oestrous along with a change in the type of discharge from serosanguinous to a clear one during oestrous. Frequency of urination was found to be normal in two animals but remained elevated in 66.6 per cent animals as in

proestrus. The absence or decrease in vulval discharge, vulval edema and interest in male was usually characteristic of dioestrus or anoestrus phase. This is due to the decrease in the circulating amount of oestrogen, which is responsible for the characteristic vulval edema and discharge during proestrus and oestrous.

The results in group A and B clearly indicated a shift from the oestrous to another stage, which was probably metoestrus or anoestrus as there was a lack of interest towards the male along with the change of conspicuous behavioural expressions of proestrus and oestrous which included vulval edema and discharge and the tail deviation reflex. The vulva lost its swollen appearance and became more or less dry. There was total absence of tail deviation reflex. One animal had low level of interest even after treatment along with low level of vulval discharge. Though there are generalisations about the various aspects of canine reproductive physiology and behaviour, there are very many variables, which could occur between breeds and across different ages. The present study included nondescript dogs, which could be an admixture of several different breeds with variable inheritance. So it is obvious that certain extend of variation could occur with respect to behaviour.

The control animals in group C had shown characteristic behavioural signs during various stages of their reproductive cycle. Following proestrus, oestrous was characterised with the change of vulval discharge from serosanguinous to clear. The vulval edema was present in all animals except that it was medium in one animal. The tail deviation reflex was still present. The animals showed greater interest for males. Frequency of urination was still high in four animals while it became almost normal in one more animal. Concannon *et al.* (1977) observed that estrous behaviour in the bitch was elicited by a synergism between falling estrogen levels and rising progesterone levels. The present study also revealed similar results.

The observation on the behaviour elements of treatment and control animals indicated that the animals administered medroxyprogesterone either oral

or parenteral routes showed ethological manifestation of changes from oestrous to anoestrus while control animals showed a progress in the oestrous cycle which may lead to breeding and conception.

The overall results in the study indicate that medroxyprogesterone acetate when administered in the right dose at right time is an effective tool for suppressing oestrous and unwanted pregnancies in bitches and thus can be effectively used in strategic approaches for stray dog population control measures.

5.3 CELLULAR CHANGES DURING DIFFERENT STAGES OF THE OESTROUS CYCLE

These findings are in consistency with that of Schutte (1967a). There was a characteristic change from the prescence of erythrocytes, superficial and large intermediate cells to parabasal and small intermediate cells after treatment with medroxyprogesterone acetate indicating the transition from anoestrus to proestrus. The mean per cent of other cells, small intermediate cells, large intermediate cells and superficial cells varied significantly at different stages of oestrous cycle, ($P \leq 0.05$). The mean per cent of large intermediate cells in the treatment groups A and B was significantly higher than the control animals during the third fortnight while the mean of small intermediate cells was significantly higher during the second third and fifth fortnights. The mean of superficial cells were significantly lower during the second, third and fourth fortnights. The per cent of parabasal cells was significantly higher in all the stages of oestrous cycle except during proestrus and anoestrus in the animals administered with medroxyprogesterone acetate.

The increase in proportion of parabasal cells and small intermediate cells reflect low level of oestrogen and are indicative of anoestrus condition. This finding is in accordance with that of Prabhakar *et al.* (1992) and indicate the effect of medroxyprogesterone acetate on different types of exfoliate vaginal cells. The increase in oestrogen during oestrous cycle lead to conversion of

deeper layers of cells (parabasal and small intermediate) to large intermediate and superficial cells, resulting in increased number of cells from the superficial layers. In animals treated with oral and parenteral medroxyprogesterone acetate, there was an increase in parabasal cells and small intermediate cells, which indicated a transition from oestrous to anoestrus state.

5.3.1 Superficial Cell Index.

The superficial cell index before and after medroxyprogesterone acetate treatment in group A and B and during various stages of oestrous cycle in group C are presented in Tables 8 to 10 indicating that the mean index values in group A and B were 151.05 and 137.79 during proestrus before treatment, and 16.00 and 14.27 after treatment respectively, but the control animals showed the mean superficial cell index value of 148.24 during proestrus, 955.66 during oestrous, 34.74 during dioestrus and 13.08 in anoestrus.

There was a decrease in SCI values following medroxyprogesterone acetate treatment, in group A and B, indicated lower number of superficial layer cells compared to deeper layers which may be due to suppression of gonadotropin secretion by medroxyprogesterone acetate. These observations are in agreement with Schutte (1967c) and Post (1985)

5.4 SERUM PROGESTERONE PROFILE IN BITCHES

Serum progesterone profile during different stages of oestrous cycle in medroxyprogesterone acetate treated and control animals are presented in Tables 8 to 10

In treatment group of animals (Group A and Group B) and control animals (Group C) the mean levels of progesterone observed during proestrus before treatment were 0.53, 0.57 and 0.45 respectively. The progesterone levels during the proestrus and before treatment was similar and was in agreement with earlier studies of Edqvist *et. al.* (1975)) and Olson *et al.* (1982) who observed the mean progesterone level to be very low less than or equal to 1 ng/ml during early

proestrus while Concannon and DiGregario (1986) observed values of 0.4 to 0.6. Nett *et al.* (1975) had observed mean values of 1.7 ± 0.3 ng/ml during proestrus that was slightly higher than the values obtained during the present study. Vermeirsch (2000) observed mean serum progesterone values of 0.44 ± 0.09 ng/ml during proestrus

During the first fortnight the mean progesterone value in Group A was 27.83 ± 1.83 ng/ml while in group B it was 0.45 ± 0.4 ng/ml. Control animals in Group C had mean values of 4.43 ng/ml during the same period. The high values obtained in Group A animals could be due to the effect of single depot preparation of progesterone while the decreased values of serum progesterone in Group B suggested a stage similar to anoestrus on continuous oral administration. Group C control animals showed mean progesterone values of 4.43 ± 0.33 ng/ml suggestive of early oestrous. Nett *et al.*, (1975) had observed mean progesterone values of 3.5 ± 0.3 ng/ml during early oestrous. The progesterone values had increased from 2.3 ng/ml before LH peak during oestrous to 4.4 ng/ml on day of LH peak. (Wildt *et al.*, 1979). Vermeirsch (2000) noted mean serum progesterone values of 3.38 ± 2.55 during oestrous. The mean progesterone concentration at day zero (day of ovulation) was determined to be 2.02 ± 0.18 ng/ml by Kutzler *et al.*, (2003) Austad, *et al.*, (1976) had observed that progesterone values increased and reached maximal concentrations, which varied from about 20 to about 55 ng/ml, within a few days of the oestradiol peak which was probably in late oestrous.

The progesterone levels in group A animals from second fortnight onwards varied between 30.50 ± 2.92 to 17.73 ± 7.85 until it finally reduced to 0.82 ± 0.18 on day 84 (Table 8). The values observed were found to be lower than that of control animals. The animals probably had some amount of progesterone secretion which might have been at a lower threshold level to exhibit ominous signs of oestrous. The progestagens are antioestrogenic in that they control vaginal bleeding (Evans and Sutton, 1989), this activity may have been responsible for the control of the reproductive behavior along with it's

antigonadotropic actions. Dhaliwal *et al.* (1999) in their studies observed that progesterone values in three bitches injected with medroxyprogesterone acetate changed from 7.3 to 7.5, 8.2 to 17.5 and 0.6 to 50 ng/ml after about four days post treatment.

The progesterone levels in group B animals during the following fortnights varied from 0.55 ± 0.06 to 0.40 ± 0.04 ng/ml until it reached 0.45 ± 0.08 ng/ml on day 84. The low progesterone level observed throughout the post treatment period could have been due to the longer duration of treatment with oral medroxyprogesterone acetate for a continuous period of 16 days. This might have suppressed the cycle through the antigonadotropic action and antioestrogenic action. (Evans and Sutton, 1989)

The control animals had normal estrous cycle following the proestrus with peak progesterone values of 49.00 ± 2.07 during dioestrus. The progesterone values finally reduced to 0.80 ± 0.11 ng/ml during anoestrus. Austad *et al.* (1976) had observed plasma progesterone level at oestrous to increase to a maximum concentration of 20 to 55 ng/ml. Concannon *et al.* (1975) noted maximum mean level of 22.9 ± 2.7 ng/ml on day 25 of LH peak. Mellin *et al.* (1976) noticed the increase in serum progesterone levels to a maximum of 46 ± 6 ng/ml. Concannon and Rendano, (1983) recorded initial peaks of 15 to 90ng/ml progesterone in serum by 15-30 days after LH peak. Vermeirsch (2000) noted mean serum progesterone values of 14.79 ± 3.64 during early dioestrus and 1.14 ± 0.84 in late dioestrus and 0.60 ± 0.56 in anoestrus. According to Butinar *et al.* (2004) progesterone levels were less than 1ng/ml during anoestrus. The present findings were in agreement with the above findings but were higher than that reported by Vermeirsch (2000).

A higher serum level of progesterone in group A (medroxyprogesterone acetate administered parenterally) was not in agreement with previous works. The high values are indicative of dioestrus but there was absence of behavioural signs, which made it unsuitable for breeding. In animals administered with

medroxyprogesterone acetate orally, resulted in a lower serum progesterone level compared to parenteral administration, keeping a level below 1ng/ml indicative of physiological anoestrus. In oral administration of medroxyprogesterone acetate the feedback effect of high level of progesterone was enough to suppress gonadotropins rendering the animal anoestrus. In both cases, oral as well as parenteral medroxyprogesterone acetate rendered the animal in physiological anoestrus state thereby preventing breeding and conception.

The findings in the study also calls for an application of the technology in field level, identifying the bottlenecks, rectifying the defects if any so that the limitations in the present study like number of animals, long term consequences, and other related parameters can also be analysed and an effective animal birth control system can be developed; which is cheap, with minimum pain inflicted to the animals, user friendly and suitable for massive application.

Summary

Summary

Non surgical contraception gained tremendous momentum in the past. Though several non surgical alternatives are available including the Immunological control, none of these are available for use by the common man neither are they cheap. The present study was aimed at trying a locally available and cheap drug for controlling reproduction in stray dogs.

Eighteen proestrus bitches of varied age group and parity formed the material for the study. The proestrus bitches were randomly divided into three groups of six animals each – Group A, Group B and Group C. Group A animals were administered with a single subcutaneous injection of 50 mg medroxyprogesterone acetate. Group B animals were administered medroxyprogesterone acetate orally at the rate of 10 mg per day for four days followed by 5 mg per day for another 12 days starting from the end of anoestrus. Group C animals formed the control group. Vaginal cytology of all the animals were regularly taken to asses the onset of proestrus. Vaginal smears and serum samples were collected after treatment subsequently every fortnight.

Vaginal smears were stained using Wright-Giemsa's stain. Superficial cell index was derived during the different stages after treatment and during the natural oestrus cycle. The levels of progesterone were estimated in serum samples using RIA technique. Serum progesterone level was estimated in the animals treated with medroxyprogesterone and also the control animals.

All animals in Group A and Group B responded to the treatment though one animal in Group A had returned to cycle in about 50 days. Reproductive cycle was controlled in all six animals of group A at a mean of 3.17 days and a mean of of 2.67 days in group B. Group A animals had a mean duration of control of 83.3 days while in group B it was 90 days. There was significant difference between treatment groups in the control of reproduction.

Before treatment during proestrus in group A, 66.6 per cent had high vulval discharge while 33.4 per cent animals had medium discharge. The intensity of vulval edema was high in 83.3 per cent animals while 16.7 per cent animals had medium edema. Tail deviation reflex was present in 5 83.3 per cent animals and absent in 16.7. Interest towards male was high in 66.6 while one animal 16.7 per cent animals had medium and 16.7 per cent animals had low interest towards male. Increased frequency of urination was found to be in 83.3 per cent animals while it was normal in 16.7 per cent animals.

In Group A, after treatment vulval discharge was absent in 83.3 per cent animals while it was low in 16.7 per cent animals. Vulval edema was absent in all the 100 per cent animals. 83.3 per cent animals showed no interest towards male after treatment while 16.7 per cent animals maintained a low level of interest in the male. Frequency of urination and tail deviation reflex was absent in all animals following treatment.

In group B, before treatment and during proestrus, 83.3 per cent animals had high vulval discharge while, it was medium in 16.7 per cent animals. Vulval edema was high in 83.3 per cent animals and low in one animal (16.7 per cent animals). All the 100 per cent animals showed tail deviation reflex. Only 83.3 per cent animals had increased frequency of urination.

After treatment vulval discharge, vulval edema, interest towards the male and tail deviation reflex was absent in 100 per cent animals. Frequency of urination also remained normal during the period following treatment.

In group C animals, 83.3 per cent animals showed high vaginal discharge and 16.7 per cent animals showed medium discharge. All the 100 per cent animals had high vulval edema. Interest towards male was high in 83.3 per cent animals whereas 16.7 per cent animals had medium interest. Tail deviation reflex was present in 100 per cent animals. 83.3 per cent animals showed an increased frequency of urination.

The behavioral parameters remained same except for a higher degree during estrus along with a change in the type of discharge from serosanguinous to a clear one during estrus. Frequency of urination was found to be normal in two animals but still elevated in 66.6 per cent animals as in proestrus.

The details of vaginal cytology during the various stages of the cycle were studied using Wright-Giemsa's stain. Superficial Cell Index (S.C.I.) was derived. The mean superficial cell index in Group A treated animals were 151.05 during proestrus and before treatment and 16.00 after treatment while in Group B it was 137.79 and 14.27 respectively. Control animals showed mean SCI values of 148.24 during proestrus, 955.66 during oestrus, 34.74 during dioestrus and 13.08 in anoestrus.

In treatment group (Groups A and B) during the proestrus phase there was a predominance of superficial cells and large intermediate cells. The erythrocyte concentration was comparable. There was a change from the presence of erythrocytes and superficial and large intermediate cells to parabasal and small intermediate cells after treatment with MPA indicating the transition to anoestrus from proestrus. There was significant difference in the mean percentage of parabasal cells between Groups A and B after treatment and during different stages of the oestrus cycle of the Group C up to end of dioestrus. The significance of the mean percentage of other cells varied at different stages.

Animals in group A, B and C showed mean serum progesterone concentrations of 0.53 ng/ml, 0.57 ng/ml and 0.45 ng/ml during the beginning of proestrus. There was significant difference in the progesterone values following treatment between the groups. The mean progesterone in serum following treatment elevated in Group A to 27.83 ng/ml, while it maintained a low level of 0.55 ng/ml in group B and had a value of 4.43 ng/ml in group C which indicated oestrus phase. The progesterone level reached maximum concentration of 31.17 ng/ml on day 42 in group A and 49.00 ng/ml during dioestrus in group C. The values remained low in group B.

In the present study, it was observed that medroxyprogesterone acetate could effectively control reproduction in bitches, though the oral form was more suitable and required least technical assistance in administration. Vaginal cytology and progesterone profile enabled the detection of the stage of reproductive and proper timing of administration of the medication.

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MEDROXYPROGESTERONE ACETATE AS AN AID TO BIRTH CONTROL PROGRAMME IN STRAY DOGS

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Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

The utility of Medroxyprogesterone acetate as an aid in the birth control programme of stray dogs was studied.

Eighteen early proestrus bitches were randomly allotted to three equal groups (Group A Group B and Group C). Group A animals were administered with a single subcutaneous injection of 50 mg medroxyprogesterone acetate. Group B animals were administered medroxyprogesterone acetate orally at the rate of 10 mg per day for four days followed by 5 mg per day for another 12 days starting from the end of anoestrus. Six bitches of Group C acted as control animals.

All animals in Group A and Group B responded to the treatment though one animal in Group A had returned to cycle in about 50 days. Reproductive cycle was controlled in all six animals of group A at a mean of 3.17 days and a mean of of 2.67 days in group B. Group A animals had a mean duration of control of 83.3 days while in group B it was 90 days.

Vulval edema, vulval discharge, tail deviation reflex and interest in male decreased following treatment in Groups A and B while it seemed to be not changed or high in Group C animals.

Detailed vaginal cytology was studied using Wright- Giemsa's stain. Cellular changes was characterized by a change from predominance of parabasal cells following treatment in Groups A and B while changes characteristic of oestrus cycle occurred in the control animals. There was significant difference in the mean percentage of parabasal cells between Groups A and B after treatment and during different stages of the oestrus cycle of the Group A up to end of dioestrus.

Serum progesterone was estimated in all animals at fortnightly intervals. Animals in group A, B and C showed mean serum progesterone concentrations of

0.53 ng/ml, 0.57 ng/ml and 0.45 ng/ml during the beginning of proestrus. The mean progesterone in serum following treatment elevated in Group A to 27.83 ng/ml, while it maintained a low level of 0.55 ng/ml in group B and had a value of 4.43 ng/ml in group C which indicated oestrus phase. The progesterone level reached maximum concentration of 31.17 ng/ml on day 42 in group A and 49.00 ng/ml during dioestrus in group C. The values remained low in group B. Towards the end of experiment all animals had basal concentration of progesterone.

It is concluded that reproductive control can be achieved effectively in bitches treated with medroxyprogesterone acetate by both oral and parenteral routes. Oral administration demand less technicalities and hence is more suitable for use in the field.