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Effects of three steroidal compounds on oestrus suppression in queens

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Summary

Pregnancy prevention raises much concern to many pet owners. Female cats usually show undesirable behaviour during oestrus. Nowadays, researchers try to explore the reliable and effective ways to prevent oestrus signs in queens. The results of their studies prove that hormone therapy is probably one of the most reliable methods for this purpose. The aim of our study was to compare the effect of three steroidal preparations on oestrus suppression in queens. Twenty clinically healthy adult female cats and three healthy adult male cats with natural libido were used in the present study. The female cats were randomly divided into 4 groups (n = 5). The male cats were caged separately and maintained near females for sexual stimulation. The photoperiod was regulated artificially to induce oestrus in cats (14 h light and 10 h dark). The experimental cats were kept in a roofed room for about 3 months for adaptation and confirmation of oestrus detection. After this period, group I (control group) did not receive any treatment, group II received 12.5 mg methyltestosterone/week orally for 90 days, group III received one tablet of contraceptive LD/day (0.15 mg levonorgestrel + 0.03 mg ethinyloestradiol) orally for 90 days and group IV received 3 mg medroxyprogesterone acetate intramuscularly. The cats were monitored clinically every day (4 h in the morning and 4 h in the afternoon). The number of queens observed in oestrus and the number of oestrous cycles were recorded during the study. The results of the present study showed that in group I (control) oestrus was detected twice in 2 out of 3 cats. In group II oestrus was detected 3 times in 3 cats, which was not statistically different to that of the group I ($P>0.05$). In group III oestrus was detected 13 times (more than the other groups). All 5 cats of this group showed oestrus, which was statistically increased compared to group I ($P<0.05$). In group IV no oestrus was detected, which was statistically decreased compared to group I ($P<0.05$). According to our results, methyltestosterone prevented the oestrus for only 1 month so that it can be used for oestrus suppression for a short time; contraceptive LD unexpectedly increased the frequency of oestrus in queens, therefore this drug can not be advised for prevention of the oestrus in queens; however, medroxyprogesterone acetate completely prevented the oestrus during the treatment. Therefore, Medroxyprogesterone acetate is reliable, and only one injection is sufficient to prevent the occurrence of the oestrous cycle.

Key words: Cat, Oestrus prevention, Levonorgestrel, Methyltestosterone, Medroxyprogesterone acetate

Introduction

There are important incentives for controlling the oestrous cycle of cats; the world-wide feral, domestic cat (*Felis catus*) population is in the billions and they are responsible for wide scale ecological damage and human health risks (Olson and Moulton, 1993). On the other hand, oestrous queens have physiologic manifestations and behaviours that can be problematic for the owners (Johnston *et al*., 2001). The options available for preventing oestrus or pregnancy in queens remain limited. Ovariohysterectomy is the most obvious approach if permanent sterility is desired. That is relatively expensive, invasive and permanent procedure. Thus, temporary suppression of oestrus is sometimes desired by owners (Nelson and Couto, 2003). When comparing with surgical contraception, chemical contraception has not the risk and pain associated with surgery (Tamada *et al*., 2003). In dogs, administration of available

synthetic progestogens or androgens can be used for prevention of oestrus and ovulation. In many cases methods that have been developed for use in dogs have been untested or might pose problems in cats (Concannon and Meyers-Wallen, 1991). Thus, designing contraceptives that are effective and reversible, yet safe, has been the primary challenge for felids (Munson, 2006). In women, most contraceptive regimens (including contraceptive LD) involve both an estrogen and a progestin and they both acting by negative feedback on gonadotrophins. Such combination pills or progestin only treatment in women maintains gonadotrophin levels below concentrations normally observed in the early follicular phase of the cycle. Estrogen-progestin combinations have not been studied in dogs and cats (Concannon, 1993). The aim of the present report is to describe the effects of three steroidal compounds, methyltestosterone, contraceptive LD and medroxyprogesterone acetate (MPA) on oestrus suppression in queens.

Materials and Methods

Twenty adult female cats weighed 2.37 ± 0.23 kg (Table 1) and 3 adult male cats were used in the study. Five female cats were randomly assigned to each of the 4 treatment groups (Table 2).

All cats were given a complete physical examination and were treated for internal and external parasites. All cats were housed in the research wards of the Small Animal Clinic, School of Veterinary Medicine, Shiraz University, Iran. The females were numbered and released into the room; males were kept in individual cages in the same room. Fresh water was supplied *ad libitum* and chicken head was fed once daily. The animals were exposed to artificial light 14 h daily. The cats were maintained in this condition for about 3 months for adaptation and confirmation of oestrus detection.

Cats in group I (control) had no treatment, in group II they received 12.5 mg methyltestosterone weekly PO for 90 days (13 weeks), in group III they were given one tablet of contraceptive LD (0.15 mg levonorgestrel + 0.03 mg ethinyloestradiol) daily PO for 90 days, and in group IV a single IM injection of 3 mg MPA was used (Burke, 1982; Baldwin *et al*., 1994).

Each female was observed every day for signs of oestrus (4 h in the morning and 4 h in the afternoon) (Burke *et al*., 1977). Lordosis, treading with the feet, dorsolateral placement of the tail to expose external genitalia, characteristic vocalizations and circling around the male cages were considered to be the signs of oestrus. Queens were not permitted to copulate throughout the study (day 0 through 90). Oestrus activity was recorded for each queen during treatment. Physical examination was performed during the experiment for observation of the adverse effects of drugs. Special attention was directed to external genitalia and mammae. All cats were humanely euthanized and necropsied on day 97, and all organs including uterus, ovaries and clitoris were examined macroscopically.

Table 1: Mean body weight of female cats in different groups

Groups	Mean ± SD (kg)	Mean ± SD (kg)
	Before treatment	After treatment
I (Control)	2.10 ± 0.17	2.13 ± 0.25
II (Methyltestosterone)	2.52 ± 0.28	2.94 ± 0.34
III (Contraceptive LD)	2.44 ± 0.15	2.94 ± 0.26
IV (Medroxyprogesterone acetate)	2.34 ± 0.16	2.42 ± 0.15
Total	2.37 ± 0.23	2.66 ± 0.41

Table 2: Experimental design

Groups (n = 5 female cats each)	Dose of drugs
I (Control)	-
II (Methyltestosterone)	12.5 mg/cat weekly PO
III (Contraceptive LD)	1 tablet (0.15 mg levonorgestrel + 0.03 mg ethinyl oestradiol) daily PO
IV (Medroxyprogesterone acetate)	3 mg/cat, once, IM

Statistical analysis

The oestrous cycles and days in oestrus in treatment groups were compared with the control group using nonparametric Mann-Whitney U test. A p-value less than 0.05 was considered statistically significant.

Results

General and necropsy examinations proved no abnormality of genital as well as other organs in all treated female cats, and there were no noticeable changes in body weight (Table 1), behaviour and frequency or mode of micturition. Oestrous activity during treatments is shown in Table 3.

Cats in all treatment groups cycled during the study. Two out of 3 cats in group I (control) had a total of 2 oestrous cycles with the average of 0.67 per animal (2 cats of this group died in the early stage of study and were omitted in calculations). The mean duration of oestrus phase of each cycle in this group was 1 day.

Three out of 5 cats in group II (methyltestosterone) showed oestrus signs during treatment. They had 3 cycles during this period. In this group the average of 0.60 oestrous per animal was detected. The mean duration of oestrus phase of each cycle in this group was 2.33 days.

All cats in group III (contraceptive LD) came into oestrous during treatment. They had 13 cycles during this study with the average of 2.6 cycles per cat. The mean duration of oestrus phase of each cycle was 2.69 days.

Oestrous cycles were not observed in the cats of group IV (MPA).

Discussion

There are many reasons for controlling reproduction in the queen. Owners may want a safe and efficacious method for preventing oestrus temporarily. In the last few decades, non-surgical methods for sterilizing an animal have been sought. These methods must be safe, effective, easy and convenient to administer, and inexpensive (Olson *et al*., 1986). The most important advantages of non-surgical methods are that they are less invasive than surgical methods and are potentially reversible (Johnston *et al*., 2001).

Various steroid hormones have been shown to suppress normal ovarian cyclicity in bitches during the course of administration. They include the natural steroids, progesterone and testosterone, and a variety of synthetic steroids derived from testosterone and progesterone (Concannon and Meyers-Wallen, 1991).

Weekly intramuscular injections of 110 mg of testosterone propionate have been used to prevent oestrus in greyhound dogs. Oral dosing with 25 mg methyltestosterone at weekly intervals for up to 5 years has also been used to prevent ovarian cyclicity in racing greyhounds (Burke, 1982). In our study oral administration of 12.5 mg of methyltestosterone at weekly intervals for up to 3 months could not prevent oestrus activity in cats ($P>0.05$). The oestrous cycles occurred in 2 cats in 30 days after beginning of the treatment. With the exception of non-significant increase in body weight, there were no clinical signs of abnormality in the queens since the beginning of the treatment. There is less information about the use of methyltestosterone in oestrus suppression in queens. The present study showed that the selected dose of this compound is not effective in suppressing the oestrus in queens and further studies are required to find the minimal effective contraceptive doses.

Table 3: Oestrous activity in queens during treatments

Group	No. of Cats	No. cycling	Total cycles	Average cycles in group (Mean ± SD)	No. of days in oestrous in 90 days	Average days in oestrus (Mean ± SD)
I	3	2	2	0.67 ± 0.58	2	1 ± 0
II	5	3	3	0.60 ± 0.55	7	2.33 ± 1.15
III	5	5	13	2.60 ± 0.89*	35	2.69 ± 1.49α
IV	5	0	0	0**	0	0

*The number of cycles significantly increased compared to control ($P = 0.021$). **The number of cycles significantly decreased compared to control ($P = 0.049$). αAverage days in oestrus significantly increased compared to control ($P = 0.03$)

In the present study unexpectedly levonorgestrel (LNG) caused an increase in oestrous cycles compared to the control group (P = 0.021). Baldwin *et al*. (1994) in a study investigated the contraceptive effects of levonorgestrel in the domestic cat and reported that silastic implants of levonorgestrel completely prevented the oestrus activity. The compound used in the present study was a combination of 0.15 mg levonorgestrel and 0.03 mg ethinyloestradiol (contraceptive LD) which is routinely used in human for contraception. It seems that the presence of ethinyloestradiol in this compound is the cause of these results. Diaz *et al*. (1982) found no pregnancies in 101 women implanted with LNG for five years.

Plotka and Seal (1989) reported that the LNG implants were ineffective as contraceptive in deer, but did not measure the LNG concentrations. White *et al*. (1994) evaluated the silastic implants containing levonorgestrel as a contraceptive in captive white-tailed deer. Five of the six implanted adult females had normal oestrous cyclicity, three of them became pregnant in the first year. The results of the present study are somehow similar to White *et al*. (1994). Jordan *et al*. (1993) and Pelican *et al*. (2005) reported that levonorgestrel exerts some estrogenic activity in cats. It seems that the biological activity of contraceptive steroids may vary widely between species because of different physiology and metabolic pathways (Phillips *et al*., 1987; Goodman, 1989). On the other hand, using ethinyl-oestradiol associated levonorgestrel in the present study might cause the increases in oestrous cycles. Thus, we do not recommend the use of this compound (contraceptive LD) for oestrus suppressing in cats.

A case-control field study on cats revealed that progestagens used for oestrus prevention or treatment of dermatological problems considerably increased the risk of mammary carcinoma if given regularly, but not if given irregularly. The results of a study indicate a dose-related tumorogenic effect of progestagens and development of mammary tumours in dogs and cats (Misdorp, 1991). Progestin administration is intended to produce an artificial luteal phase (i.e. circulating progestin mimicking the normal post-oestrus profile of progesterone); during this period a new ovarian cycle will not occur and then a normal anoestrus period is reinitiated (Concannon, 2004).

Medroxyprogesterone acetate is poorly soluble in aqueous solutions and is commercially available as a repositol (depot) injectable product used mainly as a contraceptive drug for dogs and cats. A single injection of depot MPA forms a compound that is capable of maintaining effective circulating concentrations for several months (Plumb, 1999). In some countries, there are no licensed products to prevent or suppress the oestrus in female cats (Noakes *et al*., 2001). Nevertheless, in other countries, parenterally administered steroids are still available in the market for these purposes (Concannon and Meyers-Wallen, 1991). The MPA is used as a human and canine contraceptive in many countries. High dose of MPA in the dogs may cause cystic endometrial hyperplasia, mammary tumours, adrenal suppression and acromegaly (Concannon *et al*., 1980; Concannon and Meyers-Wallen, 1991). The minimal effective contraceptive dose of MPA is a single IM injection of approximately 2 mg/kg every 3 months, or 3 mg/kg every 4 months. A single injection of MPA in human can provide highly effective contraception for more than two months (IPPF, 1982).

Medroxyprogesterone acetate has also been used in cats and its side effects have been ascribed including a potential increase in the incidence of mammary adenocarcinoma (Hernandez *et al*., 1975).

In the present study, a single IM injection of MPA at a dose of 3 mg completely prevented the oestrus activity for 3 months. In one cat in the late period of study, pyometra was observed but we could not confirm if it is related to drug used or other factors.

The female domestic cat is a seasonal breeder when exposed to natural photoperiod, with ovarian activity ceasing under decreasing photoperiod and resuming with increasing photoperiod (Leyva *et al*., 1989). Therefore, contraception may be necessary only during the breeding season. However, it is unknown that postponing of reproductive functions, lead to fertility (after contraception withdrawal) and the potential

birth of offspring during a less optimal season of the year (Wildt *et al*., 1998). Thus, it seems that one IM injection of 3 mg of MPA before the beginning of oestrus activity and repeating it 3 months later prevent the oestrus activity in breeding season, then the non-cycling season for cat will start. This method may permanently prevent the oestrus activity while decreases the side effects of MPA. However, further studies are required to confirm this recommendation.

Hernandez *et al*. (1975) have used 25 mg depot-MPA injections every 3 months for an average period of 5 years in 5 cats and reported that 2 cats developed adenocarcinoma of the breast at 2 and 4 years after the last injections. Histologically, the tumours resembled human cancers. These tumours are rare in cats.

Loretti *et al*. (2004) reported that depot-MPA therapy in cats causes feline mammary fibroadenomatous change (FMFAC). They suggested that the noxious effects of MPA were probably enhanced by other endogenous hormones, e.g. estrogen, progesterone, mammary growth hormone, insulin-like growth factor-I and adrenocortical sexual steroids.

The adverse effects of single depot-MPA injections could also be associated with dosing during follicular phase when circulating estrogen concentrations are increasing or during pregnancy when progesterone levels are high. FMFAC associated with one single injection of synthetic progestins has been reported only in rare occasions. Normally, repeated doses of synthetic progestins during months to years are required to produce FMFAC in older animals (Hayden and Johnson, 1986).

According to our results, methyl-testosterone prevented the oestrus for only 1 month so that it can be used for oestrus suppression for a short time; contraceptive LD unexpectedly increased the frequency of oestrus in queens, therefore this drug can not be advised for prevention of oestrus in queens; the MPA completely prevented the oestrus during the treatment. This drug is reliable, and only one injection is sufficient to prevent the occurrence of the oestrous cycle.

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