



Assessment of Human-originated Medroxyprogesterone Acetate (MPA) as Effective Contraceptive in Domesticated Queens (*Felis catus*) in Iraq

Mushtaq A Alabodi^{*1} , Imad M Almeeni² 

¹Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Kufa, Kufa, Iraq,

²Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

A B S T R A C T

Domestic cats (*Felis catus*) pose a significant challenge for pet owners during the mating season, necessitating effective contraceptive strategies to manage feline populations. This study investigated the use of medroxyprogesterone acetate (MPA), precisely the human formulation Depo-Provera®, as a potential solution for feline contraception. Ten adult female cats were included in a randomized controlled trial, with the treatment group receiving a single intramuscular injection of 25 mg/cat MPA. Clinical observations, vaginal cytology, and hormonal analysis were conducted to assess treatment efficacy. Results demonstrated that MPA treatment effectively suppresses estrus behaviours, prolongs interestrus duration, and modulates hormonal profiles (estrogen, progesterone, and prolactin). This indicates its promise as a safe and practical contraceptive option for managing feline populations. This study provided a viable, non-invasive alternative for feline population control. Further research is recommended to optimize dosing regimens and evaluate long-term effects on reproductive health.

Keywords: domestic queens, estrus suppression, medroxyprogesterone acetate

*Correspondence:

Mushtaq.alabodi@uokufa.edu.iq

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INTRODUCTION

Cats are highly efficient reproduction animals (1), achieving sexual maturity by 6 to 9 months (2). The recent increase in cat ownership within Iraq has presented many challenges for pet owners, particularly during the mating season when female cats exhibit estrus-related behaviors (3). These behaviors can be distressing for pets and their owners, leading to a significant rise in veterinary consultations (4). The two prevalent solutions—surgical interventions and hormonal contraceptives—each have their drawbacks (5). A surgical procedure to remove the gonads, which is effective, involves potential health risks, e.g., bleeding, ovarian remnant syndrome, ethical concerns (6,7), or the administration of contraceptives (8). In the context of contraceptives, there are notable challenges that

merit attention. Firstly, a diverse array of contraceptive products is available in the Iraqi market; each of these available medications recommends different doses, which leads to confusion and inconsistency in treatment protocols. Secondly, there is a problematic pattern of unsuitable dosage administration, characterized by numerous cases of overdose administration. In some cases, doses as high as 100 mg per cat have been reported, significantly exceeding the recommended levels. Besides that, the administration of the contraceptive products is performed without evaluating the estrus cycle stage, as it is a rule of thumb that the administration of such intervention must be within the interestrus phase (9,10). This practice deviates from recommended veterinary guidelines mentioned in different published works (11,12). It raises concerns about the potential health risks of excessive and

non-clinical based-timing dosages, including mammary tumors, abnormal growth, and uterine pathology (13). Thirdly, and perhaps most critically, is the indiscriminate use of both veterinary and non-veterinary (human) contraceptive products (14). This lack of standardization and reliance on non-veterinary products poses significant risks to feline health and wellbeing, as these products are not formulated for feline physiology. These issues collectively underscore the need for a more standardized, safe, and practical approach to feline contraception (15). These substances are commonly used to regulate the reproductive cycle of female domestic animals. Their primary contraceptive action is achieved by suppressing the visible signs of estrus and inhibiting ovulation (16).

Pharmacologic techniques, including progestins, androgens, gonadotropin-releasing hormone (GnRH) analogs, immunological contraception, and melatonin implants, can be used to provide reversible reproduction control. Despite their efficacy as contraceptives, they can induce side effects that may be life-threatening (17).

Medroxyprogesterone acetate (MPA) (17 α -hydroxy-6 α -methyl progesterone acetate) is a highly strong artificial progesterone commonly used as a progestin. It is available for oral or injectable use in human and veterinary medicine (18). Prolonged suppression of estrus in queens with MPA using a single intramuscular injection of 25–100 mg to be recurrently every 4–6 months. However, based on a review study by Munson (2006) and a recently published paper (19), this contraceptive, which was developed for humans, is not yet approved by the Food and Drug Administration to be used in cats and has only been tested for efficacy and safety in laboratory animal species (rodents), the domestic dog, or non-human primates (20,21).

In light of these concerns, this study aimed to explore a standardized approach to feline contraception. It focuses on administering a regular high dose of medroxyprogesterone acetate MPA (Depo-Provera®, Pfizer [New York, NY]) for human use in cats as a contraceptive medication. MPA is chosen for its efficacy and potential for fewer side effects compared to surgical alternatives or other contraceptive methods. The study proposed a 25 mg/cat safe, high dosage, determined based on existing veterinary guidelines and literature (22, 23). This dosage ensured the treatment's effectiveness while minimizing the risk of harmful side effects commonly associated with higher or unregulated dosages (24). Through this study, we sought to establish a more reliable and safer contraceptive protocol for feline population management, contributing to the overall wellbeing of pets and easing the concerns of pet owners.

MATERIALS AND METHODS

Ethical Approval

This study was conducted with the approval of the local Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad (Approval Number: 14/P.G. dated 3/1/2024).

Animals and Management

The research involved 10 adult crossbred domestic female cats aged 1.5 to 3 years and weighing 3 to 4 kg, all in the interestrus phase (25). Additionally, three healthy tomcats, each 3 years old, were included as teaser males and isolated in a metal cage within the room to stimulate estrous behavior.

The queens were obtained from various owners in different areas of Iraq, including Shirazi (3 cats), Persian (1 cat), Angora (2 cats), Himalayan (2 cats), and local Iraqi breeds (2 cats). The animals were verified to be healthy and fertile based on medical history records, with criteria of no detectable reproductive system issues, including previous pregnancies, regular estrus cycles, and normal physical exams (26). Before starting the experiment, the cats' reproductive status was evaluated. This involved using ultrasound (Mid-Ray, German) to confirm the absence of pregnancy and assess the condition of the uterus. Simultaneously, vaginal swabs were collected to determine the particular phase of their estrus cycle and monitor the presence of estrus-related behavioral signs in the female cats.

The animals were transported and housed in a dedicated room (6×5 m) in the Animal Facility at the College of Veterinary Medicine, University of Baghdad. One month for adaptation was provided to the animals, during which they experienced a natural light photoperiod, 20–23°C temperature, a standard commercial diet twice daily, and ad libitum access to water (27).

Experimental Design

The experimental design based on the randomization technique allocates the queens to the control and treatment groups, thus minimizing potential biases. Each group consisted of 5 cats, ensuring a balanced and impartial approach to estimating the effects of the treatment. The treatment group received a single intramuscular injection of 25 mg/cat MPA (Medroxyprogesterone acetate, Depo-Provera®, Pfizer, USA). The treatment was administered during the interestrus phase as an MPA group, and a dose was determined through a critical evaluation of existing veterinary practices and literature reviews (11,24,28).

Clinical Observations and Vaginal Cytology

Clinical observations and vaginal cytology played pivotal roles in monitoring the estrus cycle and assessing the efficacy of MPA treatment (29). Throughout the seven months of the experiment, a daily two-hour observation period involved systematically observing feline behavior, physiological cues, and external signs of estrus (20). The methodology for vaginal cytology, including the Diff-Quik staining technique. The stains were subjected to microscopic analysis using stained slides and evaluated at 40X and 100X magnifications for establishing and enumerating intermediate, parabasal, and superficial cells. The estrus cycle stage was identified according to the percentage and type of vaginal cells (30). Providing cytological findings with figures or extra photos would enhance understanding of the cellular changes that occur

throughout the estrus period and the impact of MPA treatment. Daily detailed records of the estrus phase in each queen before and after treatment were kept, and the interval between estrus during treatment was carefully noted.

Blood Collection and Hormonal Analysis

The critical roles of estrogen, progesterone, and prolactin hormones in the feline estrus cycle informed their selection for hormonal analysis. The blood collected from each cat on day zero (before treatment), fourteen days, thirty days, and sixty days was approximately 2-3 mL at each time point (31). The cats were fasted overnight, then anesthetized with ketamine (15 mg/kg; Alfasan Woerden, Holland) and xylazine (0.15 mg/kg; VMD Livestock Pharma, Belgium) administered intramuscularly, based on estimated body weight (BW) (32). The middle to lower part of the Jugular vein area was sterilized with Alcohol 70%. A sterile 3-mL disposable syringe with 23-G was used for blood collection. The blood samples were evacuated in plain gel tubes, left for 30 min at room temperature, and centrifuged at 3000 rpm for 15 min. The serum was aliquoted into the Eppendorf tube and kept frozen at -20°C until used for hormonal analysis (33,34). Blood samples were collected for hormonal analysis, and estrogen, progesterone, and prolactin were selected for their pivotal roles in the estrus cycle.

Hormonal Analysis

An automated enzyme immunoassay system analyzed the serum to measure the concentration of estrogen E2 and progesterone. Estrogen E2 hormone was quantified using the Antibodies of estrogen CALSET ESTRADIOL II kit, using the Cobase-analyzer (Roche, Germany).

Progesterone was quantified using the Antibodies of Progesterone Elecsys III kit, which was analyzed using the Cobase-analyzer that Roche manufactured in Germany.

The estimation of prolactin hormone was evaluated using commercially available Enzyme Linked Immuno Sorbent Assay (ELISA) kits for prolactin. The quantitation was done using an Antibodies of Prolactin (PRL) Elecsys II

kit (Cobase- analyzer Roche – Germany). The serum samples were utilized to measure the concentration of (estrogen E2, progesterone, and prolactin) by an automated enzyme immunoassay system using the Cobase-analyzer (Roche, Germany).

Statistical Analysis

The Chi-square (χ^2) test was used to compare response rates. The durations of interestrus and estrus were analyzed using One-Way Analysis of Variance (ANOVA), followed by the Least Significant Difference (LSD) post-hoc test to identify significant differences between groups at $P \leq 0.05$. Serum levels of progesterone, estrogen, and prolactin hormones were analyzed using Two-Way ANOVA. The model included fixed factors of day (0, 14, 30, and 60), treatment (control vs. MPA), and their interaction (day \times treatment). All data were analyzed using JMP Pro 16.0.0 software (SAS Institute Inc., Cary, NC, USA) (35).

RESULTS

Clinical Observation

Daily clinical observations were conducted to closely monitor female cats' behavioral and physical cues, focusing on identifying their entry into the estrus phase. These observations revealed distinct behavioral and physiological changes, such as increased attraction to male cages and interactions, greater sociability with persons and other objects, and unique walking patterns. They also heightened vocalization during estrus. When palpated by hand, raising the hind quarter and tail, the signs of estrus were observed.

Vaginal Cytology Examination

During the anestrus phase in feline queens, vaginal smears exhibited a predominant presence of parabasal and small intermediate vaginal epithelial cells (Figure 1 A and B). However, during interestrus, most cells were intermediate with few basal or parabasal and keratinized nuclear cells. The transition to estrus was marked by increased superficial cells with nuclear characteristics (Figure 1B).

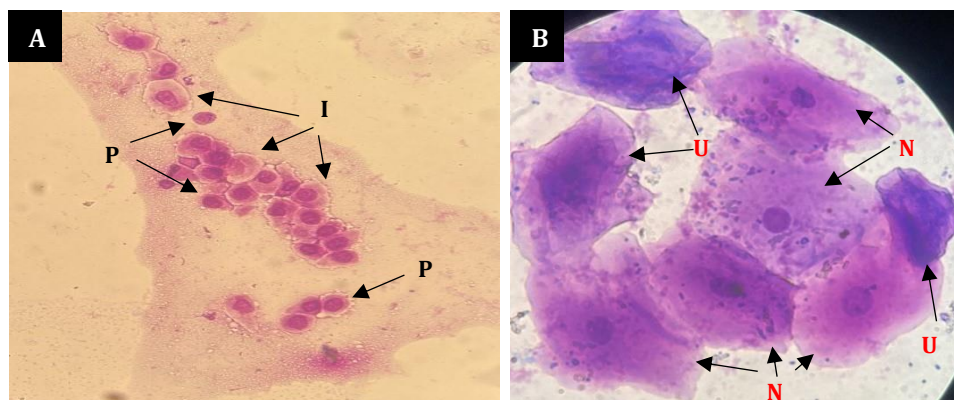


Figure 1. Vaginal cytology smear in interestrus period. **(A)** showing intermediate cells (I) and parabasal cells (P)(40 \times), and estrus period. **(B)** showing nucleated superficial cells (N) and unnucleated superficial cells (U) (100 \times)

Animal Response to MPA Treatment

The study's core investigation centered on the impact of progesterone on estrus suppression. Despite all females entering the estrus phase during the study, differential responses to progesterone treatment were noted (Table 1). The variability in estrus returns rates between control group (100%) and MPA treated group (80%) was not statistically significant ($P>0.05$), suggesting progesterone's temporary contraceptive effect.

Table 1. Effect of medroxyprogesterone acetate (MPA) treatment on estrus suppression in domestic cats (animal response) six months post-treatment

Groups	Responded animals		χ^2	P-value
	No. of animals	Rate		
Control group	5	100%	2.14	0.340
MPA treatment	5	80%		

Effect of MPA on the Estrus and Interestrus Duration in Domestic Queens

As shown in Table 2, the Control group returned to estrus after an average of 28 ± 8.38 days, while the MPA Treated group showed a significantly longer return to estrus, with an average of 153 ± 6.08 days ($P<0.05$).

Table 2. Effect of medroxyprogesterone acetate (MPA) on estrus and interestrus duration in domestic cats

Day	Factors	Treatment	Hormones		
			Progesterone (ng/mL)	Estrogen (pg/mL)	Prolactin (pg/mL)
0		Control	0.907 ± 0.152	11.6 ± 4.00 ^{ab}	0.440 ± 0.016
		MPA	0.820 ± 0.331	8.42 ± 1.19 ^{ac}	0.466 ± 0.005
14		Control	1.210 ± 0.097	7.62 ± 1.47 ^{ac}	0.392 ± 0.024
		MPA	1.300 ± 0.558	4.56 ± 0.75 ^c	0.414 ± 0.014
30		Control	1.050 ± 0.298	20.9 ± 9.22 ^{ac}	0.446 ± 0.009
		MPA	1.930 ± 0.658	6.97 ± 1.56 ^{ac}	0.402 ± 0.024
60		Control	0.948 ± 0.090	23.10 ± 9.93 ^a	0.422 ± 0.022
		MPA	2.180 ± 1.120	6.02 ± 0.95 ^{bc}	0.430 ± 0.024
Main Effect					
Day					
0			0.864 ± 1.721	9.99 ± 2.038	0.453 ± 0.009
14			1.251 ± 0.267	6.09 ± 0.929	0.403 ± 0.013
30			1.490 ± 0.370	8.80 ± 1.479	0.424 ± 0.014
60			1.564 ± 0.567	9.40 ± 2.013	0.426 ± 0.015
Treatment					
Control			1.029 ± 0.087 ^Y	10.6 ± 1.435 ^X	0.425 ± 0.009
PMA			1.556 ± 0.354 ^X	6.49 ± 0.620 ^Y	0.428 ± 0.010
P-values					
Day			0.551	0.338	0.083
Treatment			0.168	0.014	0.826
Day x Treatment			0.553	0.824	0.230

Values are means ± SEM, n = 5. a-c Means followed by different lowercase letters in a column are different from each other in interaction effect Day × Treatment. A-C Means followed by different uppercase letters in a column are different from each other in the Day factor. X-Y Means followed by different uppercase letters in a column are different from each other in the Treatment factor

In this study, there was no significant interaction between treatment and time on serum estrogen levels between groups ($P=0.824$). Similarly, there was no significant main effect of time ($P=0.338$) on serum estrogen levels. However, the main effect of treatment was

Additionally, the interestrus duration was non-significant in the MPA Treatment group and the control group ($P>0.05$). These findings highlight the substantial impact of MPA treatment on the studied subjects' estrus and interestrus periods

Table 2. Effect of medroxyprogesterone acetate (MPA) on estrus and interestrus duration in domestic cats

Groups	No. of animals	Return to estrus (days)	Duration of estrus (days)
Control	5	28±8.38 b	8.80±0.96
MPA treatment	4	153±6.08 a	7.00±0.70
P-value		<0.001	0.193

Hormonal Analysis

The impact of MPA treatment on serum hormone levels was assessed across multiple time points (Table 3). In terms of serum level of progesterone, there were no significant main effects of time ($P=0.551$) and MPA treatment ($P=0.168$) on progesterone levels. Additionally, there was no significant interaction effect between MPA treatment and time on serum progesterone levels ($P=0.553$) (Table 3).

significant on estrogen levels ($P=0.014$). In 30 and 60 days, the control group's estrogen levels significantly increased ($P<0.05$) compared with the MPA group, as in (Table 3).

The study found no significant interaction effect between the main effect of treatment and time between

MPA treatment and the control group in serum prolactin levels over time ($P=0.230$). Additionally, treatment had no significant main effect on prolactin levels ($P=0.826$). However, prolactin levels in the MPA group remained relatively stable throughout the study. Among groups, no statistically significant main effects changes were seen at any time ($P=0.083$) suggesting that the administration of MPA did not significantly impact prolactin levels compared to the control group.

DISCUSSION

Contraception in cats is reversible to block fertility, while estrus suppression inhibits estrus behavior by blocking or suppressing the follicular phase (36). Medroxyprogesterone acetate (MPA) is a potent synthetic progesterone widely used to prevent or suppress estrus in cats (12).

This study demonstrated that a single injection of human-originated MPA successfully suppressed folliculogenesis, ovulation, and estrus behavior in local domesticated queens for up to 6 months. Only 1 out of 5 cats treated with MPA did not show signs of estrus during the six months (table 1-2), compared to the other cats in the same group that displayed estrus signs within the six months (37), compared to 100% of control cats showing estrus within the first months. The MPA group significantly delayed Return to estrus by over 5 months. The prolonged anestrus period indicates that MPA suppresses the hypothalamic-pituitary-gonadal (HPG) axis, preventing the gonadotropin surges necessary for follicular growth, estrogen production, and ovulation (16, 18). MPA exhibits potent anti-gonadotropic and gestagenic properties with a modest antiestrogenic effect (16). It undergoes slow metabolism in the liver, inhibiting the estrous cycle for six months when administered once during the proestrus phase. Some queens have reported a lack of heat for up to 26 months following application (38).

During the estrus phase in feline queens, superficial cells with nuclear characteristics were primarily observed in Figure 1B, consistent with prior investigations (30,39). These studies describe vaginal cytology during estrus as consisting mainly of cornified, anuclear superficial cells, with more than 75% cornified epithelium, though intermediate cells may remain visible (40). Cornification may not reach 100%, as, in the dog, the nucleus does not disappear altogether (41,42). This pattern agrees with findings that increased estrogen concentration during estrus results in more cell layers, keratinization, and shedding in the vaginal epithelium (43,44).

The exact mechanism of MPA's action is debated (12). It likely involves negative feedback at the hypothalamus and pituitary, inhibiting GnRH release and suppressing FSH and LH release, leading to arresting folliculogenesis, and, therefore, no ovulation will occur (45). The low estrogen concentrations support failed follicular maturation and ovulation, as estrogen suppression prevents the LH surge and ovulation (43). Progesterone may also directly inhibit gonadotropin receptors in ovarian follicles (46), but the primary effect is via the hypothalamic-pituitary axis,

suppressing estrus behaviors mediated by estrogen. This aligns with studies showing that progestins prevent the rise of estradiol and LH peak (20).

The efficacy and duration observed align with previous reports using MPA and other progestins for estrus suppression in felines (47, 48). Doses of 25-100 mg intramuscularly or subcutaneously can prevent estrus for six months to a year (21), varying based on individual sensitivity and metabolism of the progestin. Since a follicular phase can still progress before the progestin takes effect, the interestrus interval may be shorter after the first dose than subsequent doses. This may explain the return to estrus in one progesterone-treated cat after only 5 months.

Estrogens regulate ovarian follicular growth and ovulation, rising levels during follicular recruitment and maturation under FSH stimulation (49). Estradiol from developing follicles induces the LH surge, leading to ovulation (38). In this study, MPA-treated cats had estrogen concentrations below 20 pg/mL, significantly lower than the 20-100 pg/mL in control cats during the follicular phase as in (Table 3). This suggests that progesterone prevented FSH-dependent follicle growth, reducing estradiol synthesis and blocking the LH surge needed for ovulation (45). Similar persistent low estrogen levels have been observed with other progestins and GnRH agonists (19). The maintained low estrogen levels in MPA-treated cats confirmed the prevention of follicular maturation and ovulation (16).

In cats, progesterone levels remain low throughout anestrus, estrus, and proestrus, rising just before the LH surge and plateauing at 5-20 ng/mL during the luteal phase (49, 50). The corpus luteum secretes progesterone during pseudopregnancy or pregnancy. In treated cats, sustained baseline progesterone levels indicated no luteal activity or ovulation, as GnRH/LH surges were suppressed, preventing follicle rupture and luteinization (16). Progesterone levels were raised slightly due to the administered exogenous progesterone, while endogenous levels remained within standard range during the anestrus phase, confirming no corpora lutea formation. Other studies also show progestins block the estrogen rise needed for the LH peak, resulting in anovulation and inhibited luteinization (10). This supports the idea that progesterone treatment effectively suppresses estrus cycles.

Prolactin levels can provide insights into cats' luteal function and ovarian activity. During anestrus, prolactin remains below 1 ng/mL, rising to ~3 ng/mL during the luteal phase with increasing progesterone from the corpus luteum (50,51). It continues to rise throughout pseudopregnancy and pregnancy, reaching 10-25 ng/mL at parturition. This study's lack of significant prolactin changes in progesterone-treated cats indicated suppressed cyclic ovarian function. Since these cats did not ovulate or form corpora lutea, they lacked the progesterone rise needed to stimulate prolactin secretion. This maintained baseline prolactin confirmed that folliculogenesis was arrested, preventing luteinization and pseudopregnancy (16, 52). Persistent low prolactin levels indicate effective inhibition of ovulation and luteal activity over six months.

Prior research also shows unchanged prolactin levels when GnRH and LH surges are suppressed by progestin contraceptives (53). Overall, the unchanged prolactin levels, low estrogen and progesterone, and prolonged interestrus intervals confirm the successful suppression of ovarian cyclicity by progesterone treatment (24).

Initially, hormonal measurements were conducted only during the first two months of the study to confirm the absence of estrus in the treated cats. However, this method was discontinued after the initial period due to the high costs of continuous hormonal analysis. Instead, vaginal cytology and clinical observation of estrus signs were employed as reliable and cost-effective alternatives, so vaginal smears were taken three times a week, and daily observations were made with males present in the cages to ensure accurate detection of estrus. These methods used concurrently with hormonal analysis during the initial two months, validated the hormonal results, confirming their effectiveness in monitoring the reproductive status of the cats.

Longer treatment duration studies on repeat dosing at 4-6 months intervals are still needed. The effects were reversible as all cats eventually resumed normal cyclicity—no adverse reactions aligned with previous findings using low-dose progestins (54). However, larger-scale studies should monitor potential risks such as pyometra, diabetes, and mammary tumors associated with high doses or prolonged use (55).

The administration of a single 25 mg/cat dose of progesterone (medroxyprogesterone acetate) effectively suppresses estrus in domestic queens for six months without significant side effects or alterations in estrus duration post-treatment. This regimen maintains basal hormonal levels, inhibiting ovarian activity (folliculogenesis and ovulation), thereby confirming progesterone's utility in feline contraceptive management.

While this study elucidates progesterone's role in estrus suppression, future research should explore long-term effects, potential side effects with repeated dosing, and comparisons with other contraceptive methods to fully understand progesterone's utility and safety in feline reproductive management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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تقييم أسيتات ميدروكسي بروجسترون (MPA) ذات الاصل البشري كوسيلة فعالة لمنع الحمل في القطط المستأنسة في العراق (*Felis catus*)

مشتاق عليم العبودي^١، عماد مجيد العمعيني^٢

^١ فرع الجراحة و التوليد، كلية الطب البيطري، جامعة الكوفة، الكوفة، العراق، ^٢ فرع الجراحة و التوليد، كلية الطب البيطري، جامعة بغداد، بغداد، العراق ،

الخلاصة

تشكل القطط المنزلية (*Felis catus*) تحدياً كبيراً لأصحاب الحيوانات الأليفة خلال موسم التزاوج، مما يستلزم استراتيجيات فعالة لمنع الحمل لإدارة مجموعات القطط. تبحث هذه الدراسة في استخدام أسيتات ميدروكسي بروجسترون (MPA)، وتحديدًا الصيغة البشرية Depo-Provera®، كحل محتمل لمنع الحمل في القطط. تم تضمين عشر قطط إناث بالغة في تجربة، حيث تلقت مجموعة العلاج حقنة عضلية واحدة من ٢٥ مجم / قطة MPA. تم إجراء الملاحظات السريرية ومسح الخلايا المهبلية والتحليل الهرموني لتقييم فعالية العلاج. تظهر النتائج أن علاج MPA يثبط بشكل فعال سلوكيات الشبق (estrus cycle)، ويطيل مدة بين الشبق والآخر، ويعدل التراكيز الهرمونية، يشير ذلك إلى وجود خيار آمن وعملي لمنع الحمل لإدارة مجموعات القطط. توفر هذه الدراسة بديلاً قابلاً للتطبيق وغير مؤذي للتحكم في أعداد القطط. يوصى بإجراء مزيد من البحوث لتحسين نظم الجرعات وتقييم الآثار طويلة الأجل على الصحة الإنجابية.

الكلمات المفاحية: القطط المستأنسة، قمع دورة الشبق، البروجسترون