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RESEARCH ARTICLE

The Effects of Progesterone Hormone Applications Used for Suppression of Estrus on Mammary Glands in Queens [1] [2]

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Summary

The effects of single dose medroxyprogesterone acetate (MPA) applications on serum estradiol 17-β, progesterone, growth hormone levels and their receptors in mammary glands, development of feline mammary fibroepithelial hyperplasia and the efficacy of progesterone hormone for suppression of first estrus in queens were investigated. Crossbred 20 queens were divided into two equal groups. Initiation and the end of the first heats were detected by clinical signs and vaginal smears. At the 1st day of estrus (Day -1) blood and mammary gland tissue samples were taken from the cats. Following day (Day 0), treatment (MPA) group received 100 mg MPA, control group received same dose physiologic saline solution intramuscularly. Blood samples were collected at intervals of 3 day during the first heat and at 10 day intervals thereafter for a period of 4 months. Two other mammary gland samples were collected at intervals of 45 days after Day 0. Serum progesterone levels were significantly ($P<0.05$) higher on days 2, 30, 50, 60 70, 90 for treated group than control group cats; serum estradiol 17-β levels were significantly ($P<0.05$) higher on days 20, 30, 40, 50, 70, 100 for control than MPA group of the study. Growth hormone could not be detected throughout the study both in serum and in receptors of the mammary tissue samples. Clinically feline mammary fibroepithelial hyperplasia occurred in a cat and ultrasonography was performed when the lesion first noticed. Tissue samples had significantly higher estrogen receptor labelling for control than MPA group. Highly extensive and dense progesterone receptor positive labelling were observed in all tissue samples. It was concluded that MPA was effective to suppress estrus in cats. The incidence of feline mammary fibroepithelial hyperplasia (FMFH) was small. There can be higher incidence of occurring FMFH when used more cat population.

Keywords: *Feline mammary fibroepithelial hyperplasia, Progestin, First estrus suppression, Receptor, Queen*

Kedilerde Östrusu Baskılamak İçin Kullanılan Progesteron Hormonu Uygulamalarının Meme Bezi Üzerine Etkileri

Özet

Çalışmada kedilerde ilk kızgınlığı baskılamak için kullanılan tek doz medroksiprogesteron asetatın (MPA), serum östradiol 17-β, progesteron, büyüme hormonu seviyelerine ve meme bezinde bu hormonlara ait reseptörler üzerine olan etkileri, kedi meme fibroepitelyal hiperplazi oluşumu ve uygulanan progesteron hormonunun etkinliği araştırıldı. Yirmi adet dişi melez kedi iki eşit gruba ayrıldı. Kedilerin ilk kızgınlıklarının başlangıcı ve bitişi klinik belirtilere ve vaginal smearlara göre tespit edildi. Kızgınlığın ilk günü (Gün -1) kan ve meme dokusu örnekleri kedilerden alındı. Takip eden gün (Gün 0), tedavi (MPA) grubu kedilere 100 mg MPA, kontrol grubu kedilere ise aynı doz fizyolojik tuzlu su intramusküler uygulandı. Kan örnekleri ilk kızgınlık boyunca 3 günde bir ve kızgınlık sonrası 10 günde bir olmak üzere 4 ay boyunca toplandı. Diğer iki meme dokusu örneği 0.günden itibaren 45 gün ara ile toplandı. Serum progesteron seviyeleri çalışmanın 2, 30, 50, 60, 70, 90. günlerinde kontrol grubuna göre tedavi grubunda istatistiki olarak önemli ($P<0.05$) bulundu. Serum östradiol 17-β seviyeleri 20, 30, 40, 50, 70, 100. günlerde kontrol grubunda tedavi grubuna göre istatistiki olarak önemli ($P<0.05$) bulundu. Büyüme hormonu çalışma boyunca hem serumda hem de meme dokusu reseptörlerinde tespit edilemedi. Klinik olarak bir kedide kedi meme fibroepitelyal hiperplazisi ultrasonografi ile de lezyon ilk farkedildiğinde tespit edildi. Doku örneklerinde MPA grubuna göre kontrol grubunda istatistiki olarak daha yüksek östrojen reseptör işaretlenmesi bulundu. Yüksek yoğunlukta ve yaygınlıkta progesteron reseptör işaretlenmesi tüm doku örneklerinde saptandı. Kedilerde MPA'nın kızgınlığı baskılamada etkili olduğu saptandı. Kedi meme fibroepitelyal hiperplazisi (KMFH) insidensi düşük bulundu. Daha fazla kedi popülasyonu ile KMFH insidensinin daha fazla olabileceği düşünüldü.

Anahtar sözcükler: *Kedi meme hiperplazisi, Progestin, İlk kızgınlık baskılanması, Reseptör, Kedi*

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INTODUCTION

Progestins are used in small animal practice for variable purposes ranging from dermatologic to behavioral problems, but the most common purpose of use is the control of reproductive cycle; such as suppression of sexual behaviors, ovarian cycles and supplementation of endogenous progesterone in inadequate luteal function [1].

Various side effects of progestin in small animals are; increased incidence of uterine pathology, mammary gland hyperplasia and tumors, increased secretion of prolactin and growth hormone, acromegalic changes, diabetes mellitus, masculinization of female fetuses, adrenocortical suppression, delayed onset of parturition, local skin alterations, behavioral modifications [1] and increase incidence in mammary adenocarcinoma [2].

Feline mammary fibroadenomatosis is also known as; fibroadenomatous hyperplasia, feline mammary hypertrophy, feline fibroadenomatous change, feline mammary fibroepithelial hyperplasia (FMFH) or fibroadenoma. The condition is a non-neoplastic progesterone responsive situation which is characterized by rapid proliferation of mammary stroma and duct epithelium in one or more mammary glands. It is reported to occur in young estrous cycling cats, sexually intact queens at the time of puberty, in pregnant or pseudopregnant female cats, in old intact female cats and castrated male cats following a progestagen treatment [3-5]. High levels of endogenous progesterone cause an exaggerated proliferative effect of the glandular tissue of the mammary gland [6].

Growing of the mammary gland could be rapid, expansive or could be slow and progressive including of one, several or all mammary glands in this condition. The mammary gland could be soft, fluctuant or firm. The overlying skin could be ulcerative, erythematous, necrotic, alopecic, hemorrhagic and moist. The mammary masses could be sharply circumscribed with clearly defined borders. The animal should not show clinical signs or signs of systemic illness such as; fever, depression and anemia could be observed [5].

Clinical symptoms, ultrasonic examination, histopathologic evaluation of fine needle aspiration or biopsy samples, immunohistochemical labelling of estrogen and progesterone receptors, serum hormone assays are required for the diagnosis of feline fibroadenomatous hyperplasia [3,5,7,8]. Feline mammary fibroepithelial hyperplasia (FMFH) is one of the side effects of progestins in cats. This study was designed in order to determine one dose effect of depot progestin to mammary glands in a short duration in cats.

The aim of this study is to determine whether single dose of depot medroxyprogesterone acetate (MPA) can suppress first estrous activity in queens and whether this treatment causes variations at serum hormone concentrations, the extensity of estrogen, progesterone and growth hormone receptors (ER, PR, GHR), respectively in mammary glands and the occurrence of feline mammary fibroepithelial hyperplasia.

MATERIAL and METHODS

A total of 20 healthy crossbred queens which were at their first estrous cycle were randomly divided into two equal groups. Throughout the study each cat was maintained in an individual cage, fed kitten food and given *ad libitum* water.

Initiation and the end of the first heat were detected by means of clinical signs and vaginal smears. We composed a daily log of estrus which contained the behaviors presented daily by each queens which were appropriate to estrous phase such as; lordosis, rolling, vocalizing, deviating the tail to one side, inappetence, when stroking the back lowering the forequarters with elbows on the ground [9]. Throughout the first estrus, vaginal smears were collected every 2-3 days and stained with methylene blue. Smears were examined at 40x magnification under light microscope. Clean background, cell populations with the majority of the superficial cells, keratinized superficial cells with picnotic nuclei or keratinized cells without nuclei were detected in the smears at estrus of the queens. Subsequent heat periods were detected by observation of clinical signs.

After the detection of first estrous cycle, jugular blood samples and initial mammary gland samples (right inguinal mammary lobe) were taken on that day (Day -1) under general anesthesia. The following day (Day 0), treatment (MPA) group queens received 100 mg Medroxyprogesterone acetate (MPA) intramuscularly (Farlutal Depot 500®; IM, Deva, Turkey, identical with Depot-provera) [10-12] and control group queens received same dose of placebo (physiologic saline solution). Further blood samples were collected at intervals of 3 day during the first heat and at 10 day intervals thereafter for a period of 4 months. Collected blood samples were centrifuged 10 min at 3.000 x g and serum was stored at -20°C until the analysis. Serum progesterone (P4), estradiol 17-β (E2) and growth hormone (GH) assays were performed using DSL-38000 RIA, DSL-43100 RIA and DSL-1900 RIA (Diagnostic Systems Laboratories, INC. Webster, USA) kits, respectively.

After day 0, two different mammary gland tissue samples were collected with 45 days of intervals; second tissue samples were collected from left inguinal lobe and 3rd tissue samples were collected from right caudal abdominal lobe and submitted to pathology for immunohistochemical and histopathologic evaluations. After each operation 10 days of postoperative care was taken, till the removal of sutures. Collected mammary gland tissue

samples were fixed in 10% buffered formalin solution for 24 h, processed through routine procedures, embedded in paraffin and were collected in Poly-L-Lysine (PLL) covered slides and stored at + 4-8°C till use. From all paraffin blocks 2 samples were sectioned; one of them was stained with hematoxylin-eosin (HE) and the other one is labeled with immunohistochemistry. For immunohistochemistry after routine procedures sections were treated with heated citrate buffer solution using with microwave oven (~70°C, 4x5 min + 20 min cooling inside same solution) for the retrieval of the antigens. Then slides were washed three times with phosphate-buffered saline (PBS) solution and incubated for 7 min with UV block (Lab Vision, USA, Cat. No. TP-125-HL) for protein blocking. Subsequently they were treated with primary antibodies as detailed in *Table 1*. As negative control instead of primary antibody PBS was applied to the tissue sections used for positive control. After rinsing three times with PBS solution, sections were treated with a horseradish-peroxidase-labelled streptavidin-biotin kit (Biotinylated Goat Anti Polyvalent, TP-125-BN, Lab Vision Corporation, Fremont, CA, USA) according to manufacturer's instructions. Then 3.3' Diaminobenzidin (DAB) (TA-125-HD, Lab Vision Corporation, Fremont, CA, USA) was applied to the sections and incubated for 5-15 min at room temperate and washed 3 times with distilled water. Finally the slides were counterstained with Mayer's haematoxylin, dried, covered and examined under light microscope. From each section, 10 random areas were examined under light microscope and scored (none, +: low, ++: medium, +++: high). Two individuals examined and scored the extensity and density of immunohistochemical staining separately and the mean value of their scores were taken as final results. The histological evaluation of mammary tissue sections were made through the criteria which are applied for domestic animals mammary lesions and stated by World Health Organization (WHO) [13].

For this study permission from "Istanbul University Veterinary Faculty Ethic Committee" was taken (verdict number: 2005/127).

RESULTS

The mean first estrus age of the cats was 6.2 months in both groups. Clinically, excessive expression of estrous behaviors was helpful for the detection of estrous phase in the queens. Also vaginal smears were used to detect the estrus of both groups of cats. In estrus the vaginal smears of queens demonstrated; superficial, anuclear superficial and picnotic superficial cells. The duration of the first estrous period was 12.0±0.58 d for control and 8.9±0.9 d for MPA treatment groups. The mean duration of the first estrous period between control and MPA treated cats was found statistically significant ($P<0.05$). Cats treated with MPA did not show any symptoms of estrus, while in all the control animals estrus occurred at intervals of 2 to 3 weeks.

Serum progesterone levels were always higher for treated than control cats after Day 0 and significantly ($P<0.05$) higher on Days 2, 30, 50, 60, 70 and 90 of the study. Conversely, serum estradiol 17-β levels were always higher for control cats than MPA treated cats and the difference was significant ($P<0.05$) on Days 20, 30, 40, 50, 70 and 100 of the study *(Fig. 1)*. MPA group cats' first estrus was finished earlier than control group cats so at Day 8 there was no blood collection from these animals (*Fig. 1A* and *1C*).

Regular serum growth hormone could not be detected throughout the study because of the pulsatile GH secretion and the long interval applied for collecting blood samples from the queens.

Table 1. *Clones, dilutions, incubation periods and control tisssues for ER, PR and GHR antibodies used*

Tablo 1. *Kullanılan ER, PR ve GHR primer antikorları için klon, dilüsyon, inkübasyon süresi ve kontrol dokuları*

Antibody	Clone	Dilution	Incubation Period	Control Tissues
Estrogen Receptor (ERα) Signet 660, Monoclonal	6F11	1:1	1 hour	Normal uterus and mammary gland
Progesterone Receptor (PR) Signet 521, Monoclonal	1A6	1:1	1 hour	Normal uterus and mammary gland
Growth Hormone Receptor (GHR) Biogenesis 4750, Polyclonal	-	1:1	1 hour	Normal Pituitary gland, mammary gland

Statistical analyses were carried out using independent t-test with SPSS 10.0 packet program. For comparison of vaginal smears and individual behavioral differences correlation analyzes were performed. The significance level for all statistical analyses accepted as ($P<0.05$). Ultrasonography (Medison CO; LTD; Seoul- KOREA) with a 6.5 MHz microconvex (SA-600V) probe was performed to the cat which presented clinical feline mammary fibroepithelial hyperplasia.

Twenty nine days after MPA administration, clinically feline mammary fibroepithelial hyperplasia occurred in two asymmetric mammary lobe of a cat and regressed in 36 days spontaneously. In physical examination the masses were in body temperature, mobile, fluctuant and painless in palpation. Macroscopically these masses were bordered and oval, uninflamed and rigid. Necrosis, ulcer and milk production did not observe in affected mammary glands. Ultrasonography was performed when the lesion first

noticed *(Fig. 2)*. In the ultrasonography homogenity resembling typical mammary gland was determined, the borders of the mass were distinct. Around the lobes, hyperechoic areas were determined.

Serum progesterone values of the cat were measured as 3.25 ng/ml, 1.65 ng/ml and 3.39 ng ml respectively, at 50th, 60th, 70th days during the mammary gland hyperplasia. The cats that received MPA did not demonstrate any signs of discharge which represents pyometra. The other side effects of MPA were not determined in this study. All cats were spayed at the end of the study.

Histologically inactive mammary gland structures were observed in control group animals and MPA group's primary mammary gland tissues *(Fig. 3A)*, while active

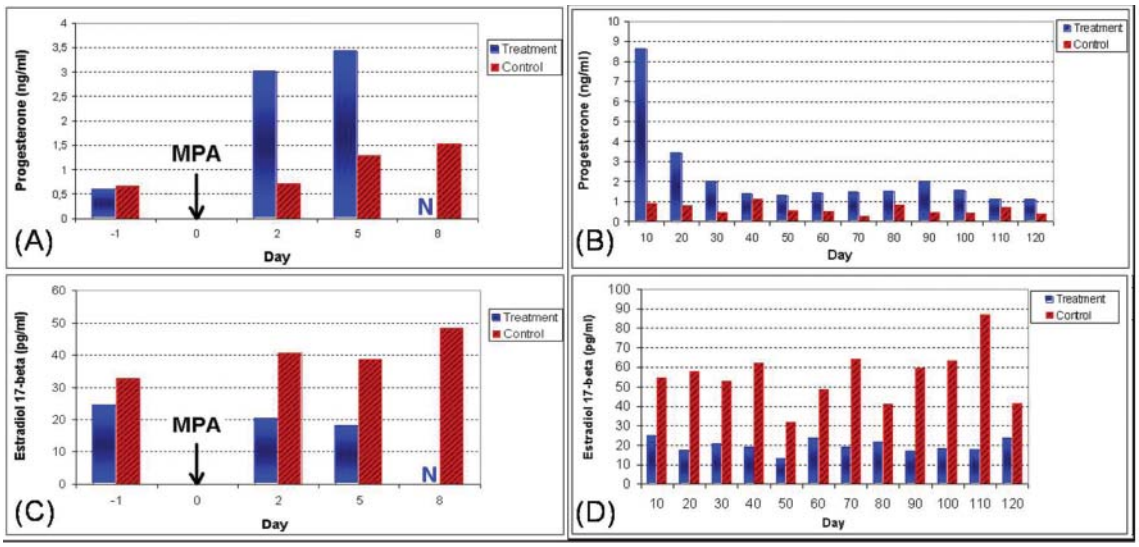

Fig 1. (A) Mean serum progesterone levels during the first estrous period of cats when placebo (control) or 100 mg medroxyprogesterone acetate (MPA) was administered IM one day (Day 0) after the beginning of estrus (Day -1). **(B)** Mean serum progesterone levels after the first estrous period of cats when placebo (control) or 100 mg medroxyprogesterone acetate (MPA) was administered IM one day (Day 0) after the beginning of estrus (Day -1). **(C)** Mean serum estradiol 17-beta levels during the first estrous period of cats when placebo (control) or 100 mg medroxyprogesterone acetate (MPA) was administered i.m. one day (Day 0) after the beginning of estrus (Day -1). **(D)** Mean serum estradiol 17-beta levels after the first estrous period of cats when placebo (control) or 100 mg medroxyprogesterone acetate (MPA) was administered i.m. one day (Day 0) after the beginning of estrus (Day -1). (**N:** No blood sampling, MPA group, day 8)

Şekil 1. (A) Plasebo (kontrol) veya 100 mg medroksiprogesteron asetat (MPA) IM uygulandığı bir gün için (Gün 0) ve kedilerin ilk kızgınlık periyodu esnasında ortalama serum progesteron seviyeleri, östrusun başladığı gün sonrası (Gün -1). **(B)** 100 mg medroksiprogesteron asetat (MPA) veya placebo (kontrol) i.m. uygulandığı kızgınlığın başladığı günden (Gün -1) sonraki gün (Gün 0) kedilerin ilk kızgınlık periyodu sonrası ortalama serum progesteron seviyeleri. **(C)** Plasebo (kontrol) veya 100 mg medroksiprogesteron asetat (MPA) IM uygulandığı bir gün için (Gün 0) ve kedilerin ilk kızgınlık periyodu esnasında ortalama serum östradiol 17-beta seviyeleri, östrusun başladığı gün sonrası (Gün -1). **(D)** 100 mg medroksiprogesteron asetat (MPA) veya placebo (kontrol) i.m. uygulandığı kızgınlığın başladığı günden (Gün -1) sonraki gün (Gün 0) kedilerin ilk kızgınlık periyodu sonrası ortalama serum östradiol 17-beta seviyeleri. (**N:** Kan toplanmayan gün, MPA grubu, 8. gün)

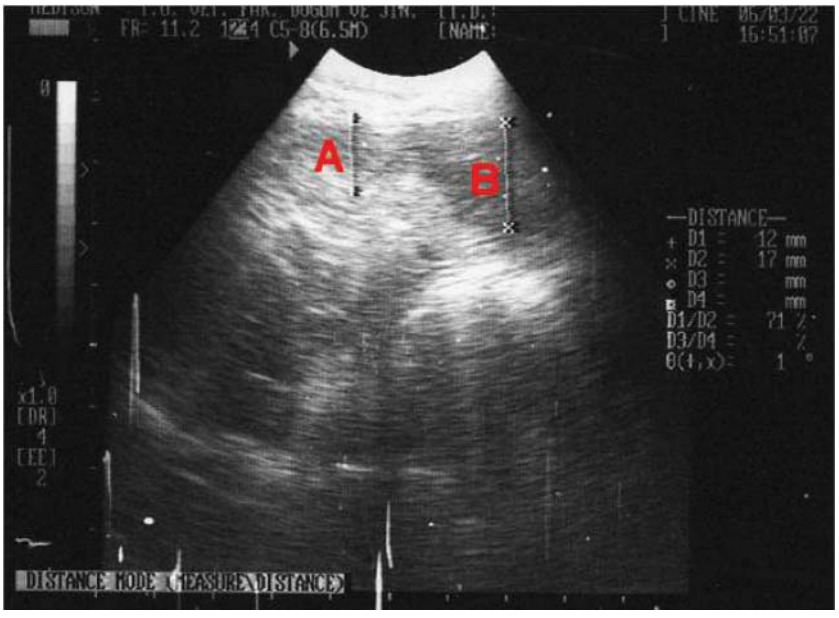

Fig 2. Ultrasonographic image of feline mammary fibroepithelial hyperplasia that occured in a cat 29 d after treatment with 100 mg medroxyprogesterone acetate (MPA) (A = 12 mm; B = 17 mm)

Şekil 2. Bir kedide 100 mg medroksiprogesteron asetat (MPA) uygulamasından 29 gün sonra oluşan kedi meme fibroepitelyal hiperplazisinin ultrasonografik görüntüsü (A = 12 mm; B = 17 mm)

mammary gland structures were observed in 2nd and 3rd mammary gland tissue samples of MPA group queens. Twenty nine days after the MPA administration only one queen (no 2) demonstrated tumoral alterations, which clinically resemble fibroadenomatous changes but histopathologically this outlook was an active mammary gland tissue which goes into hyperplastic changes. Besides that, chronic mastitis was another histopathologic finding, determined both in control and study group queens (20%) in different times of the study.

Immunohistochemically, in positive control sections superficial endometrial and glandular epithelial and myometrial smooth muscle cells stained densely *(Fig. 3B)*. In mammary gland sections, highly extensive and dense PR positive labelling was observed in all tissue samples, especially in control group *(Table 2)*. Positive labeling were located in the nuclei of basal and suprabasal cells of ductal glandular and aciner epithelium, no labelling were observed in luminal cells and myoepithelial cells *(Fig. 3C-E)*.

Declining positive Estrogen Receptor (ERα) labelling, in ductal glandular and aciner epithelial nuclei were stated in all control group tissues throughout the study and primary mammary gland tissue samples displayed very little positivity in MPA group *(Table 2)*.

ER positivity generally observed in basal and suprabasal nuclei of mammary glands, in some rare cases weak and aberrant labelling were stated in luminal nuclei *(Fig. 3F)*. Myoepithelial cells were labelled negatively with ER. Other than those luminal labeling, the intensity of all labeling were dense. It is stated that the dissociation of immunoreactive products of PR antibody was rather higher than ER antibody but no staining was observed in luminal secretion products. The extensity of estrogen and progesterone labeling showed differences between groups *(Fig. 4)*. ER and PR positive results of 2nd and 3rd tissue samples of the cat (no 2) who was clinically FMFH were similar to the MPA group.

Growth Hormone (rabbit anti Growth Hormone) positive control tissue samples *(Table 1)* which were labelled in correspondence with negative control tissue samples did not show immunopositive labelling in this study.

A cat from the control group died 56 days after the initiation of the study. Thus the last eight blood samples and the last mammary gland tissue sample could not be collected from this cat. The necropsy of this cat did not show any differential lesions for an infection.

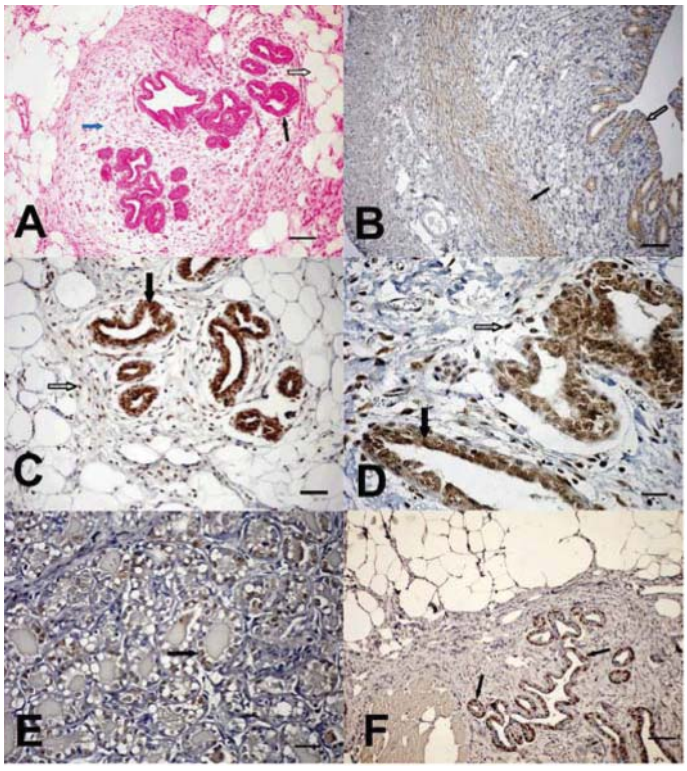

Fig 3. (A) MPA group (no.3/1). Inactive mammary gland. Ductal tubule *(black arrow),* lipoid tissue (white arrow) and fibrose stroma surrounding ductal tubules *(blue arrow)* HE, 100x. (Bar=100 µ). **(B)** Positive control tissue (cat uterus). PR positive staining in superficial endometrial and glandular epithelial *(white arrow)* and myometrial smooth muscle cells *(black arrow)*. Labelled Streptavidin-Biotin Peroxidase, Mayer's Hematoxylin, 200x (Bar=50 µ). **(C)** MPA group (no.5/3, mammary gland) inactive mammary gland. PR positive labelling in ductal basal and suprabasal epithelial cells *(black arrow)* and stromal cells *(white arrow)*. Labelled Streptavidin-Biotin Peroxidase, Mayer's Hematoxylin, 200x (Bar=50 µ). **(D)** MPA group (no. 5/3, mammary gland) inactive mammary gland. PR positive labelling in ductal basal and suprabasal epithelial cells *(black arrow)* and stromal cells *(white arrow)*. Labelled Streptavidin-Biotin Peroxidase, Mayer's Hematoxylin, 400x (Bar=20 µ). **(E)** MPA group (no. 3/3, mammary gland). Active mammary gland. PR positive labelling in alveolar gland epithelial cells (Black arrow). Labelled Streptavidin-Biotin Peroxidase, Mayer's Hematoxylin, 400x (Bar=20 µ). **(F)** Control group (no.1/3, mammary gland). Inactive mammary gland. ER positive labelling in ductal basal and suprabasal epithelial cells *(black arrow)*. Labelled Streptavidin-Biotin Peroxidase, Mayer's Hematoxylin, 200x (Bar=50 µ)

Şekil 3. (A) MPA grubu (no 3/1). İnaktif meme bezi. Duktal tubül *(siyah ok),* yağ doku *(beyaz ok)* ve duktal tubüllerin etrafını saran fibröz stroma *(mavi ok)* HE, 100x. (Bar=100 µ). **(B)** Pozitif kontrol dokusu (kedi uterusu). Süperfisiyel endometriyel ve glandüler epitelyel *(beyaz ok)* ve miyometriyal düz kas hücrelerinde *(siyah ok)* PR pozitif işaretlenmesi**.** Labelled Streptavidin-Biyotin Peroksidaz, Mayer's Hematoksilen, 200x (Bar=50 µ). **(C)** MPA grubu (no.5/3, meme bezi) inaktif meme bezi. Duktal bazal ve suprabazal epitelyal hücrelerinde *(siyah ok)* ve stromal hücrelerde (beyaz ok) PR positif işaretlenmesi. Labelled Streptavidin-Biyotin Peroksidaz, Mayer's Hematoxylin, 200x (Bar=50 µ). **(D)** MPA grubu (no. 5/3, meme bezi) inaktif meme bezi. Duktal bazal ve suprabazal epitelyal hücrelerinde *(siyah ok)* ve stromal hücrelerde (*beyaz ok)* PR positif işaretlenmesi. Labelled Streptavidin-Biyotin Peroksidaz, Mayer's Hematoxylin, 400x (Bar=20 µ). **(E)** MPA grubu (no. 3/3, meme bezi). Aktif meme bezi. Alveoler bez epitelyal hücrelerinde *(siyah ok)* PR positive işaretlenmesi. Labelled Streptavidin-Biyotin Peroksidaz, Mayer's Hematoxylin, 400x (Bar=20 µ). **(F)** Kontrol grubu (no.1/3, meme bezi). İnaktif meme bezi. Duktal bazal ve suprabazal epitelyal hücrelerde ER positive işaretlenmesi *(siyah ok)*. Labelled Streptavidin-Biyotin Peroksidaz, Mayer's Hematoxylin, 200x (Bar=50 µ)

Table 2. *Extensity of immunohistochemical staining in control and MPA groups*
Tablo 2. *Kontrol ve MPA gruplarında immunohistokimyasal boyanmanın yaygınlığı*

	Control Group											
	1st Tissue Samples				2nd Tissue Samples				3rd Tissue Samples			
Extensity*	None	+	++	+++	None	+	++	+++	None	+	++	+++
ER (%)	12.5	25	50	12.5	-	12.5	75	12.5	-	57.1	-	42.9
PR (%)	-	-	50	50	-	-	-	100	-	-	100	-
	MPA Group											
	1st Tissue Samples				2nd Tissue Samples				3rd Tissue Samples			
Extensity*	None	+	++	+++	None	+	++	+++	None	+	++	+++
ER (%)	71.4	-	28.6	-	90	-	-	10	100	-	-	-
PR (%)	-	-	25	75	-	-	-	100	-	-	16.7	83.3

** extensity of labeling in tissue section; none: no staining, +: low, ++: medium, +++: high*

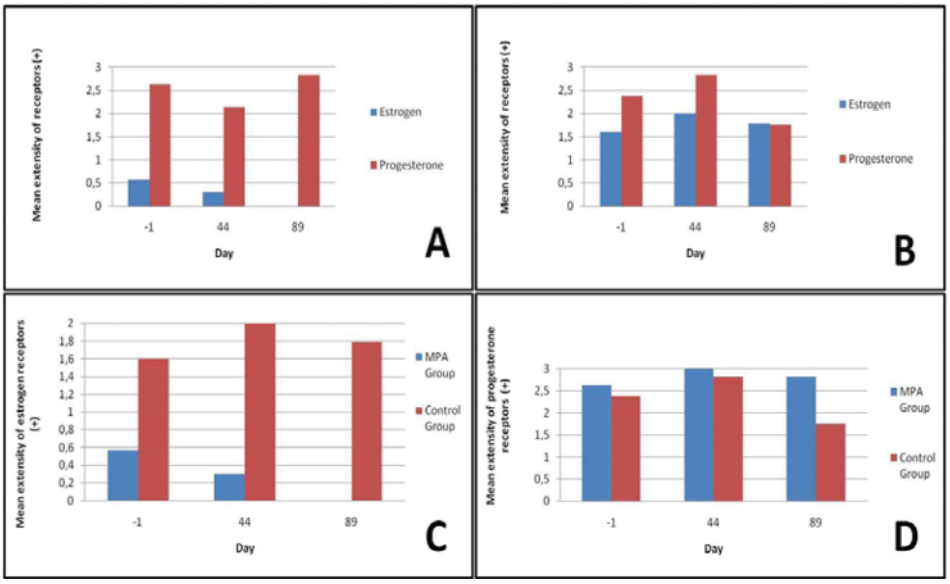

Fig 4. (A) Mean receptor extensity of the mammary tissue of MPA group cats'. **(B)** Mean receptor extensity of the mammary tissue of control group cats'. **(C)** Immunohistochemical extensity of estrogen receptors in mammary glands of MPA and control group cats. **(D)** Immunohistochemical extensity of progesterone receptors in mammary glands of MPA and control group cats

Şekil 4. (A) Çalışma grubu kedilerin meme dokusu ortalama reseptör yaygınlıkları. **(B)** Kontrol grubu kedilerin meme dokusu ortalama reseptör yaygınlıkları. **(C)** Çalışma ve kontrol grubu kedilerin meme bezinde östrojen reseptörlerinin immunohistokimyasal olarak yaygınlığı. **(D)** Çalışma ve kontrol grubu kedilerin meme bezinde progesteron reseptörlerinin immunohistokimyasal olarak yaygınlığı

DISCUSSION

Side effects of progestins in queens have been known for a long time [1]. The primary action of the synthetic hormones also include medroxyprogesterone acetate, probably involves suppression of gonadotropic hormone secretion, resulting in suppression of ovarian cyclicity [2]. In previous reports it is stated that suppression of the first estrus especially with a single dose of MPA in healthy queens could cause mammary hyperplasia [5,6,14]. Also, possible cause of mammary hyperplasia by the long term use of synthetic progestins such as MPA and megestrol acetate (MA) in spayed or unspayed adult female or male cats was previously reported [4].

Medroxyprogesterone acetate was recommended as a contraceptive with the dosage of 25-100 mg IM or 2.5-5 mg per os, 6 to 8 days at the estrous phase in queens [11] and Munson [12] reported that the same dose of the injectable formula suppressed estrus for 2-4 months. A single dose of depot MPA injection can cause effectively circulating concentrations of progesterone for several months [5]. In this study for the suppression of estrus we used IM MPA with the dosage of 100 mg and did not observe the estrous symptoms for 4 months after the administration.

Many researchers [5-7,15-18] investigated about the development mechanism of feline mammary hyperplasia in young, healthy queens' spontaneous cycle or a short time after the application of MPA for suppressing the estrus. In unspayed healthy young queens following the SC application of one dose (10.8-25 mg/kg) MPA for suppression of the estrus about 2 to 90 days interval, clinically feline mammary hyperplasia was detected [5,6]. In this study IM application of 100 mg MPA to young queens suppressed the first estrus and 29 days after the MPA application, mammary hyperplasia was determined only in one cat.

In this study serum P4 concentration were measured as 3.25 ng/ml, 1.65 ng/ml and 3.39 ng/ml respectively on days 50, 60, 70 at the time of feline fibroepithelial hyperplasia. Hayden et al.[19] measured serum P4 concentration as 6.7 ng/ml. This finding was high when compared to our

results. Nak et al.[20] and Loretti et al.[6] measured serum P4 concentration results at the time of feline fibroepithelial hyperplasia were lower than ours. This difference was likely to be welded from the sampling times which Loretti et al.[6] performed later then our study. Also results that were reported in those studies [6,19,20] belonged to single cases with application of progesteron products several times.

Graham and Clarke [21] described that progesterone acts in lobulo-alveolar development for preparation of milk secretion in the mammary glands of the mammals. Corresponding to that we histologically determined inactive mammary gland in 1st tissue samples and all tissue samples of control group while we determined active mammary gland tissue in 2nd and 3rd mammary gland samples of the cats (32.7% of all cats) which received progestin.

Martin de las Mulas et al.[7] stated that in 18 cats which have adenomatous hyperplasia, 10 of them demonstrate ER receptors while all of them demonstrate PR receptors. In this study, immunoreactive products were observed in 4 cell populations: basal, suprabasal, epithelial lumen cells which were located luminally, fibroblast in stromal areas and lobule like units. Millanta et al.[22] determined ER positive staining in 1 of 8 and PR positive staining in all of feline fibroadenomatous change cases. In this study, the labelling was observed in same cell populations. In control group PRs were determined in small numbers in 1st and 2nd mammary gland tissues and MPA group's all tissue samples in an increasing manner. In first tissue samples of all control groups and a small percent of MPA group, ERs were determined in a decreasing manner. Immunostaining of positive control tissue components were compatible with literature [3].

Loretti et al.[6] determined positive immune labelling of progesteron receptors more than 60%, respectively 5-20% and 20-60% of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in the cytoplasm of ductal epithelium, but no estrogen receptors. They also stated that fibroblasts and myoepithelial cells were consistently unreactive for the immunohistochemical methods used in all their cases. Ordas et al.[17] detected IGF-I positive immune labelling only in ductal branching areas, in 77% of their cases while they determined PR and GHR in all lesions. In this study no positive reaction in GH receptors was observed. This different observation may have been due to differences in the source or clone of the GH antibody used. Besides Selman et al.[23] stated that only long term exogenous progestin receiving dog's mammary gland tissues were positively labelled with GH antibodies, hyperplasia and tumoral lesions could be occurred in mammary gland.

Loretti et al.[6] reported that an injection of MPA to a queen soon after spontaneous ovulation would cause mammary hypertrophy as the queen experiences serum progesterone concentration much higher than normal. Administration of recommended doses of MPA during the estrus the cats may not produce side effects eg. cystic endometrial hyperplasia, feline mammary fibroepithelial hyperplasia. Higher sensitivity of the mammary tissues of some individuals to estrogen and progesterone hormones, genetic and age-related factors could be included at the occurrence of feline mammary fibroepithelial hyperplasia [6]. High dose MPA for suppressing estrus was administered at the puberty as described by Loretti et al.[6]. In this study there were no high serum progesterone values. Because of this we thought that we only obtain one mammary hyperplasia. Maybe if we would have administered MPA later in the estrous period, because of the higher estrogenic effect there would be more progesterone receptors which would possibly lead to development of more cases.

Görlinger et al.[24] and Loretti et al.[6] could not answer why fibroadenomatous hyperplasias were observed in very limited number of cat populations and why generally younger populations were affected after progestagen administrations. They thought that those can be dependent to; individual differences in tissue sensibility, genetic or age related factors, differences in the responses to progesterone during life time, differences between the cellular concentrations and plasma concentration of hormones, mammary tissues ability to regulate its own endocrine metabolism, changes in density and susceptibility of progesterone receptors in altered cells, modified sensibility to circulating progesterone even in same plasma progesterone concentrations and puberty. In this study the hypothesis of development of fibroepithelial hyperplasia under one year old cats after the application of MPA was compatible with their findings. Also its difference from the mammary tumors as clinical appearence and character was confirmed.

Treatment of the fibroadenomatous hyperplasia can vary from observation to surgical removal of the affected mammary glands and/or ovariohysterectomy, ovariectomy and administration of antiprogestins [3,5,19,24,25]. In this study non extirpated lobe of the mammary fibroepithelial hyperplasia resolved spontaneously.

In summary, following MPA application, no signs of estrus in cats were detected during the study period. While serum P4 levels were higher in MPA group throughout the study, E2 levels were higher in control group depending on cyclic period. Histopathologically hyperplastic active mammary glands, decreased estrogen receptor numbers and increased progesterone receptor numbers were detected in MPA group. In control group throughout the study both ER and PR receptors were detected, while growth hormone receptors could not be labelled in both groups of cats.

Mammary hyperplasia occurred in only 10% of treatment group cats, we assumed that a larger population could provide more possibilities of developing fibroepithelial hyperplasia.

It was concluded that MPA treatment was an effective means of suppressing estrus in cats over the duration of this study and the incidence of mammary fibroepithelial hyperplasia was small. There can be higher incidence of occurring FMFH when used more cat population.

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REFERENCES

1. Romagnoli S, Concannon PW: Clinical use of progestins in bitches and queens: A review. **In**, Concannon PW, England G, Verstegen J (Eds): Recent advances in small animal reproduction. International Veterinary Information Service, (www.ivis.org) 2003. Document No. A1206.0903, *Accessed:* 03 March 2007.

2. Concannon PW, Meyers-Wallen VN: Current and proposed methods for contraception termination of pregnancy in dogs and cats. *JAVMA*, 198 (7): 1214-1225, 1991.

3. Wehrend A, Hospes R, Gruber AD: Treatment of feline mammary fibroadenomatous hyperplasia with a progesterone-antagonist. *Vet Rec*, 17, 346-347, 2001.

4. Hayden DW, Barnes DM, Johnson KH: Morphologic changes in the mammary gland of megestrol acetate-treated and untreated cats: A retrospective study. *Vet Pathol*, 26, 104-113, 1989.

5. Loretti AP, Ilha MRS, Breitsameter I, Faraco CS: Clinical and pathological study of feline mammary fibroadenomatous change associated with depot medroxyprogesterone acetate therapy. *Arq Bras Med Vet Zootec,* 56 (2): 270-274, 2004.

6. Loretti AP, Ilha MRS, Ordas J, Martin de las Mulas M: Clinical, pathological and immunohistochemical study of feline mammary fibroepithelial hyperplasia following a single injection of depot medroxyprogesterone acetate. *J Feline Med Surg,* 7, 43-52, 2005.

7. Martin de las Mulas J, Millan Y, Bautista MJ, Perez J. Carrosco L: Oestrogen and progesterone receptors in feline fibroadenomatous change: An immunohistochemical study. *Res Vet Sci*, 68, 15-21, 2000.

8. Mac Dougall LD: Mammary fibroadenomatous hyperplasia in a young cat attributed to treatment with megestrol acetate. *Can Vet J*, 44 (3): 227-229, 2003.

9. Feldman EC, Nelson RW: Feline Reproduction. **In,** Feldman EC, Nelson RW (Eds): Canine and Feline Endocrinology and Reproduction. 3rd ed., pp. 45-60, W.B. Saunders Co, Philadelphia, 2004.

10. Wildt E: Estrous cycle control-induction and prevention in cats. **In,** Morrow DA (Ed): Current Therapy in Theriogenology 2 (Diagnosis, Treatment and Prevention of Diseases in Small and Large Animals). pp. 808- 812. W.B. Saunders Co, Philadelphia, 1986.

11. Kırşan İ: Dişi kedilerde jinekolojik endokrinoloji. **In,** Şenünver A, Horoz H, Kılıçarslan R, Ekici H, Konuk C, Kırşan İ (Eds): Veteriner Jinekolojik Endokrinoloji. s. 95-102, Masaüstü Yayımcılık, İstanbul, 1997.

12. Munson L: Contraception in felids. *Theriogenology*, 66, 126-134, 2006.

13. Misdrop W, Else W, Hellmen E, Lipscomb TP: Histological classification of mammary tumors of the dog and the cat. **In,** WHO International Histological Classification of Tumors of Domestic Animals. Second Series. Vol. VII., p. 17, Washington DC: Armed Forces Institute of Pathology, 1999.

14. Vitasek R, Dendisova H: Treatment of feline mammary fibroepithelial hyperplasia following a single injection of proligestone. *Acta Veterinaria BRNO,* 75, 295-297, 2006.

15. Mahler M, Nautrup CP, Schulze C: Fibroepithelial hyperplasie der mamma mit spontaner regression bei einer katze. *Kleintierpraxis,* 39, 869-875, 1994.

16. Bastan A, Özenc E, Yagcı İP, Beceriklisoy HB. Mammary hyperplasia in a cat. *Ankara Univ Vet Fak Derg,* 51, 79-82, 2004.

17. Ordas J, Millan Y, Espinosa de los Monteros A, Reymundo C, Martin de las Mulas J: Immunohistochemical expression of progesterone receptors, growth hormone and insulin growth factor-I in feline fibroadenomatous change. *Res Vet Sci,* 76, 227-233, 2004.

18. Güvenç K, Toydemir S, Apaydın S, Kırşan İ: Bir kedide ilk östrustan sonra karşılaşılan fibroepithelyal meme hiperplazisi olgusu. *KHVHD,* 2 (1): 36-39, 2006.

19. Hayden DW, Johnston SD, Kiang DT, Johnson KH, Barnes DM: Feline mammary hypertrophy/fibroadenoma complex: Clinical and hormonal aspects. *AJVR,* 42 (10): 1699-1700, 1981.

20. Nak D, Nak Y, Seyrek-İntaş K, Kumru İK: Treatment of feline mammary fibroadenomatous hyperplasia with Aglepristone. *Aust Vet Pract,* 34 (4):161-162, 2004.

21. Graham JD, Clarke CL: Physiological action of progesterone in target tissues. *Endocrine reviews,* 18 (4): 502-519, 1997.

22. Millanta F, Calandrella M, Bari G, Niccolini M, Vannozzi I, Poli A: Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues. *Res Vet Sci,* 79, 225- 232, 2005.

23. Selman PJ, Mol JA, Ruteman GR, van Garderen E, Rijnberk A: Progestin-induced growth hormone excess in the dog orginates in the mammary gland. *Endocrinology,* 134 (1): 287-292, 1994.

24. Görlinger S, Kooistra HS, van den Broek A, Okkens AC: Treatment of fibroadenomatous hyperplasia in cats with Aglepristone. *JVIM,* 16, 710-713, 2002.

25. Meisl D, Hubler M, Arnold S: Treatment of fibroepithelial hyperplasia (FEH) of the mammary gland in the cat with the progesterone antagonist Aglepristone (Alizine). SAT, 145 (3): 130-136, 2003.