

RESEARCH ARTICLE

The Efficacy of Medical Treatment of Pyometra in Queens with Special Reference to Histopathological Changes

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Abstract:

Pyometra is regarded as a serious issue that poses a threat to the life of queens. The risk of pyometra brought on by toxicity and the incidence of septicemia. The most effective and conventional treatment for pyometra is surgical removal of the pus source (the female reproductive tract) which known as spaying operation (ovariohysterectomy). However, medical treatment may be applied if maintaining reproductive ability is desired. In the present study, 30 queens were employed and split into 2 groups. In G1 (n:15), queens received 1µg /kg/24 h of PGF2α intramuscularly and (half tab/day/orally) of Amoxicillin-Clavulanate 1g (chosen based on the result of sensitivity test) for 7 days. Cats in group 2 (n=15) received the same therapy as group 1 with the addition of oral dopamine agonist (5 µg /kg/24 h). The general health condition of the queen and the presence of genital discharge was followed up during the treatment period. the recurrence of this problem was recoded every 4 months. Additionally, the diameter of uterine horns was assessed daily using ultrasound from the beginning of the treatment. After receiving treatment for one week, queens in G1 and G2 no longer had any clinical signs of pyometra or genital purulent discharge. Six cats (40%) in G1 showed their first recurrent pyometra on within 4 months after the start of treatment. But four cats (26.6%) in G2 showed the first recurrence between the 4th and 8th month after the first treatment. The returned cats from the second treatment trial, were exposed to ovariohysterectomy and then apply the histopathological examination. In conclusion, using cabergoline with PGF2α achieve more rapid involution of the uterus and lower recurrence rate than using PGF2α alone.

Key words: Queen, Pyometra, Cabergoline, PGF2α, Ovariohysterectomy.

Introduction:

Cystic endometrial hyperplasia (CEH) and ascending vaginal bacterial infection are two features of pyometra in canines. The pyometra could involve segmental or widespread uterine hypertrophy [1]. The

condition is frequently seen in cats during diestrus due to progesterone dominance, which lasts for around 40 days after sterile mating the condition is most frequently seen in cats during diestrus or pseudopregnancy [2]. The conditions inside the uterus during the luteal phase

are appropriate for microbial development [3]. Progesterone promotes cervical closure, increases uterine secretion, stimulates expansion and multiplication of endometrial glands, and inhibits myometrial contractions. Additionally, there is a reduction in the local leukocyte response in the uterus in response to bacterial infection. In pyometra, circulating levels of estrogen and progesterone are typically not excessively raised, and it is thought that the increased number and higher sensitivity of hormone receptors cause an amplified reaction [4]. There are two forms of pyometra: open pyometra, which is marked by vaginal discharge and mild nonspecific clinical symptoms, and closed pyometra, which is marked by sepsis, peritonitis, and the potential for animal death [5]. The preliminary diagnosis of pyometra is based on the patient's medical history, physical and gynecological examinations, blood biochemistry and hematology tests, and abdominal ultrasound or radiography [6]. Diagnostic imaging by ultrasound is useful for measuring uterine size and excluding alternative causes of uterine enlargement [7]. Even when the uterine diameter is within the normal, ultrasound can identify intrauterine fluid and reveal other pathologic alterations in the uterine tissue [8].

Numerous medical procedures have been tried in the past ten years to deal with both open and closed pyometra [9]. The goals of medical intervention in cases of pyometra are to reduce the effects of progesterone, get rid of the uterine infection, relax the cervix and encourage the discharge of intraluminal pus, and speed up uterine recovery [10]. The commonly used drugs are PGF 2α , dopamine agonists (cabergoline and bromocriptine), and anti-progesterone (aglepristone) [11]. PGF 2α induce

myometrial contractions that cause the uterine contents to gradually leak out over several days. Treatment with PGF 2α is only permitted in cases of open cervix pyometra [12]. Following the injection of prostaglandins, treated cats should be kept in the hospital during the day for observation because numerous adverse effects are frequently seen. Alkaloid substances generated from ergot that operate as prolactin antagonists and exhibit anti-luteotrophic activity may be used to treat pyometra [13]. These medications start to work about 15-20 days after ovulation. Therefore, Anti-prolactin drugs are favored over PGF 2α if a queen exhibits pyometra shortly after oestrus because they are far more successful in causing luteal arrest and luteolysis in early diestrus [14]. To our knowledge, the majority of studies dealing with the treatment of pyometra have used either PGF 2α or anti-prolactin with no previous study had investigated the efficiency of using both PGF 2α and anti-prolactin. Therefore, the current study was designed to evaluate the recovery and the possibility of recurrence after handling cats suffering from pyometra with stable general health conditions with two medical treatment protocols. Additionally, we evaluated the histopathological changes in the genital tract of cats suffering from pyometra.

Materials and methods

Animals

The current study was conducted in the period from January 2020 to January 2022. Thirty nulliparous queens of different breeds (15 Persian cats and 15 cross-breed cats) with ages ranging from 3 to 9 years old (6.11 ± 0.28) were included in this study. These queens were admitted to the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine,

Zagazig University. Animal handling and procedures were approved by Zagazig University Institutional Animal Care and Use Committee with approval number "ZU-IACUC/2/F/317/2022".

Diagnosis and treatment

Cats were thoroughly examined clinically, and they were suffering from inappetence, depression, vomiting, polyuria, abdominal distention, and vulvar discharge. All cats were subjected to

transabdominal ultrasonography using a real-time B-mode ultrasound scanner (esoate Mylab; Netherland) provided with an eight MHz frequency linear transducer. Observation of tubular horns filled with anechoic to hypoechoic fluid was a diagnostic to pyometra (Figure 1). The uterine diameter was measured from the widest area by ultrasound on the day 1, 3, 5, 7, and 9 from the beginning of treatment.

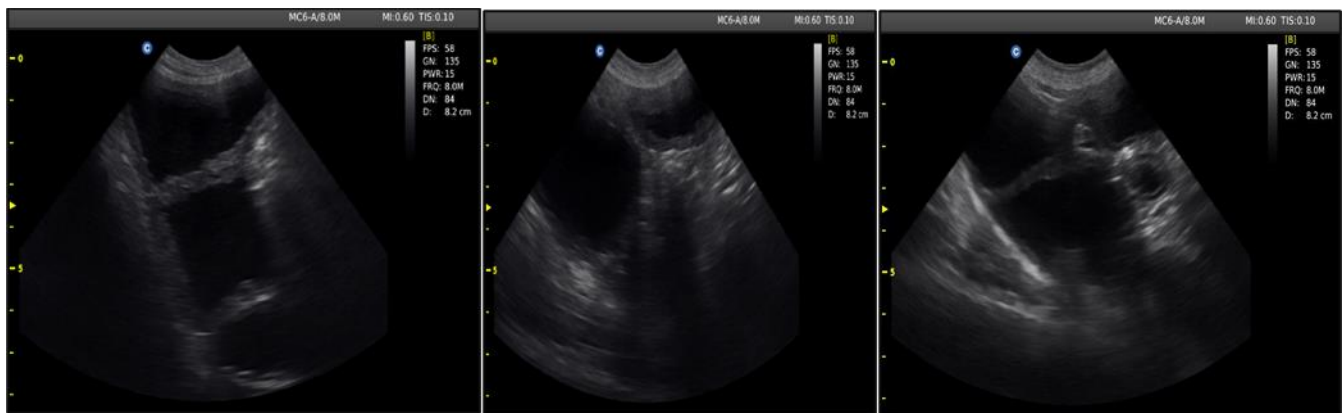


Figure 1. Ultrasonographic image of a uterus with pyometra showed an enlarged uterine segment filled with hypoechoic fluid.

The cats were divided into two treatment groups: group 1 (n=15 cats, 8 Persian and 7 crossbreed) and group 2 (n=15, 7 Persian and 8 crossbreed). In group 1, the cats received 1 µg/kg / 24 h of PGF2α (Estrumate, MSD, USA) intramuscularly for 7 days in combination with Amoxicillin-Clavulanate (Augmentin) 1g (half tab/day/orally) based on the results of sensitivity test (Amoxicillin-clavulanate +++++, cefotaxime +++, gentamicin ++, ampicillin and ciprofloxacin --). While, in group 2, the cats received the same treatment as group 1 plus administration of dopamine agonist (cabergoline, Dostinex, 0.5 mg/tab, Pfizer) 5 µg/kg /24 h/ orally.

Evaluating the efficiency of treatment protocols

Queens in both groups were regularly examined to define the time at which the queen showed signs of recovery. Additionally, queens were examined day after day from day 1 to day 9 of the treatment by using ultrasonography to measure the diameter of the uterine horns at the widest segment. After complete recovery, the condition of the queens was followed up through phone calls with the owner every 4 months or when the cats showed the re-appearance of the signs of pyometra. Queens that showed signs of recurrent pyometra were treated again by using the same protocol, but cats that showed signs of recurrent pyometra after

two successive treatments were subjected to immediate ovariohysterectomy as a surgical interference.

Surgical interference

Cats under ovariohysterectomy were generally anesthetized using a mixture of Ketamine Hcl 5% (Ketalar, Rotex medica, Germany) at a dose of 10mg/kg and xylazine Hcl 2% (Xylaject, Adwia, Egypt) at a dose of 0.5 mg/kg intramuscularly injected. Before anesthesia, fluid therapy as NaCl 0.9% saline solution and antimicrobial (Cefotaxime sodium, 0.5 mg, EPICO, Egypt) at a dose of 50 mg/kg were injected intravenously. Abdominal laparotomy was performed via a ventral midline incision in the hypogastric region. The uterus and ovaries were identified and carefully isolated using a spaying hook. The uterus was gently picked up using

moistened laparotomy towels. The ovarian vessels and broad ligament's vessels were identified and ligated using 2/0 vicryl (Ethicon company). The cervix was ligated using 2/0 vicryl and finally, excision of the ovaries and uterus was done (Figure 2). The residual cervical tissue stump, which is not oversewn, is free of all purulent material. Finally, the abdomen was cleaned with a warm physiologic saline solution and antibiotic (cefotaxime sodium, 0.5 mg) from any remnant of purulent materials [15]. The abdominal incision was sutured with a simple continuous pattern using 2/0 Vicryl, while the skin was sutured with interrupted mattress sutures using 2/0 polypropylene. The cats were injected with an antibiotic at a dose of 0.2mg/cat/intramuscularly for successive 3 days (synulox, Zoetis). Stitches were removed after 10 days postoperatively.

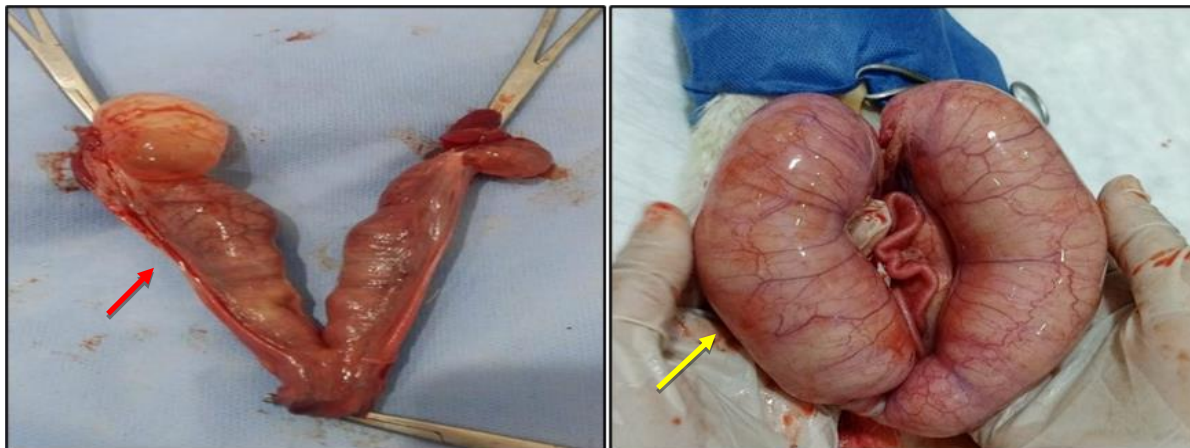


Figure 2. The ovaries and uteri of queen after surgical removal showed different degrees of pyometra (red arrow referred to middle degree, yellow arrow referred to high degree of pus).

Histopathology

Following surgery, the uterine horns were promptly opened, and samples of the ovary and uterus were collected for histopathological examination. The processing and staining methods were performed according to Suvarna *et*

al. [16]. The samples were promptly fixed in 10% buffered neutral formalin for 72 hours after being briefly rinsed in normal saline. After processing, samples were paraffin-embedded. Paraffin slices measuring 5-7 m were cut using a rotary microtome and then exposed to Harris's

Hematoxylin and Eosin (H&E) stain for analysis [17]. The stained sections were examined with standard light microscopy and photographs were taken at (Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University). Quantitative assessment of reported histopathological lesions in both uterus and ovary in all five cats (recurrent cats) were scored in three non-repeated, randomly selected microscopic fields (x40). These scores were according to the following scoring system: (-) absence of the lesion, (+) the lesion was rare, (++) the lesion was medium, (+++) the lesion was severe.

Statistical analysis

The statistical difference in the size of the uterus between different treatments at each point was compared using a T-test.

Table 1. Different Clinical Signs in Diseased Cats and Percent of Each Sign.

Clinical signs	G1	G2
	Number of cats (%)	Number of cats (%)
Anorexia	15 (100%)	12 (80%)
Abdominal enlargement	10 (66.6%)	9 (60%)
Vulvar discharge	13 (86.6%)	10 (66.6%)
Polydipsia	13 (86.6%)	12 (80%)

G1= Cats received 1 µg/kg / 24 h of PGF2α (Estrumate) intramuscularly for 7 days in combination with Amoxicillin-Clavulanate (Augmentin) 1g (half tab/day/orally).

G2= Cats received the same treatment of group 1 plus administration of dopamine agonist (cabergoline) 5 µg/kg /24 h/ orally (Dostinex, 0.5 mg/tab, Pfizer).

The effect of the treatment protocol on the mean diameter of the uterine horns

The mean diameter of the filled uterine horns was 5.22±0.139 cm and 5.4±0.17 cm in G1 and G2, respectively at first examination (day 1). Following the administration of the first dose of the medicine, the amount of pus and uterine

The data were represented as mean ± SE. All data was gathered and analyzed by using SPSS (version 18).

Results

Clinical findings

The duration of clinical signs was ranged from 4 to 21 days before admission to the veterinary hospital and initiation of treatment. These clinical signs were tabulated in Table 1. Following the application of the treatment procedures in G1 and G2, more vulvar discharge was seen over the first 24 hours and the amount of the vulvar discharge decreased on day 5 and day 3 in G1 and G2, respectively. Cats showed signs of complete recovery and the disappearance of all clinical signs at day 7 in both groups.

diameters gradually decreased as noticed in Table 2, where mean±SE of uterine diameter decreased to 2.88±0.17 and 2.2±0.11 in G1 and G2, respectively at day 5. Our findings demonstrated that, when compared to G1, G2 had a significantly smaller uterine diameter on days 5 and 3 (P<0.05), respectively (Table 2). In all cats, uteri could not be

identified by ultrasound on day 12 following the start of treatment.

Table2. Mean \pm SE of The Uterine Diameter Measured by Ultrasound During The Treatment Period.

Days	Diameter of the uterus (Mean \pm SE), cm	
	G1	G2
Day 1	5.22 \pm 0.139	5.4 \pm 0.17
Day 3	4 \pm 0.23	3.2 \pm 0.38*
Day 5	2.88 \pm 0,17	2.2 \pm 0.11*
Day 7	1.2 \pm 0.12	1.1 \pm 0.10
Day 9	1.2 \pm 0.12	0.9 \pm 0.073*

Uterine horn diameters of queens in 2 groups were analyzed by t-test and represented by (Mean \pm SE), P- value < 0.05.G1= Cats received 1 μ g/kg / 24 h of PGF2 α (Estrumate,) intramuscularly for 7 days in combination with Amoxicillin-Clavulanate (Augmentin)1g (half tab/day/orally).G2= Cats received the same treatment of group 1 plus administration of dopamine agonist (cabergoline) 5 μ g/kg /24 h/ orally (Dostinex, 0.5 mg/tab).

The recurrence possibility after using different treatment protocols

In G 1, six out of 15 queens (40%) show recurrent pyometra within 4 months after treatment. But in G2, 4 out of 15 queens (26.6%) show recurrent pyometra within 8 months after the first treatment. The returned cats received the same treatment according to the treatment groups. After the second treatment, 4 out of 6 returned cats in G 1 (66.6%) showed the same clinical signs of pyometra within 4 months. In G 2, 1 out of 4 returned queens (25%) showed recurrency for the second time within 4 months after the second treatment. These 5 queens were subjected to surgical interference. The remaining 9 cats in G1 and 11 cats in G2 that did not revert to pyometra after initial treatment were bred by fertile tom-cats, and 17 of them (85%) conceived. These cats were followed till January 2022 and no recurrence of the pyometra was recorded.

Histopathological findings

The endometrium, myometrium, and perimetrium were the three layers that made up the uterine wall. The luminal epithelium was simple, cuboidal to columnar, and had numerous, deep surface enfolding that led to fingerlike projections of mucosa. The stroma or lamina propria was a loose connective tissue that contained many fibroblast cells. Sometimes destructions of some uterine blood capillaries were observed. The endometrium contained cysts of variable sizes. Uterine glands were straighter and were lined by tall columnar epithelium with many cytoplasmic vacuolations that separate the nuclei from the basal membrane. The deep glands had higher epithelium and more vacuolation than the superficial glands did (hyperplasia in gland epithelial cells). Leucocytic infiltration was prominent between the uterine glands. Congested blood vessels with thickening

in the tunica media (Figure 3A-3I and Table3).

One or two corpus luteum (CL) with an irregularly shaped central chamber and considerable fibrosis was present in the ovaries. Granulosa luteal cells were polygonal or irregular in shape with abundant cytoplasmic vacuolation mainly observed and increased at the periphery of

CL with congested blood vessels. Ovarian follicles at various stages of development were present in several of the studied ovaries as tertiary follicles of diverse sizes, some of which were experiencing atresia as evidenced by collapse, luminal fibrosis, and absence of an ovum (Figure 4A-4F and Table3).

Table3. The Histopathological Lesion Score in Diseased Queens after Ovariohysterectomy

Organ	Lesion	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5
Uterus	*Destructions of uterine blood capillaries	+++	++	++	+++	+++
	*Endometrial cysts	++	-	++	+	++
	*Hyperplasia in gland epithelial cells	-	-	-	+	+
	*Congested blood vessels	+	+++	+++	+++	++
	* Leukocytic infiltration	+	+	-	++	++
Ovary	* Vacuolation of luteal cells	+	++	++	+	+
	* Atretic follicles	+++	+++	++	++	+++
	*Congested blood vessels	+	-	++	++	++

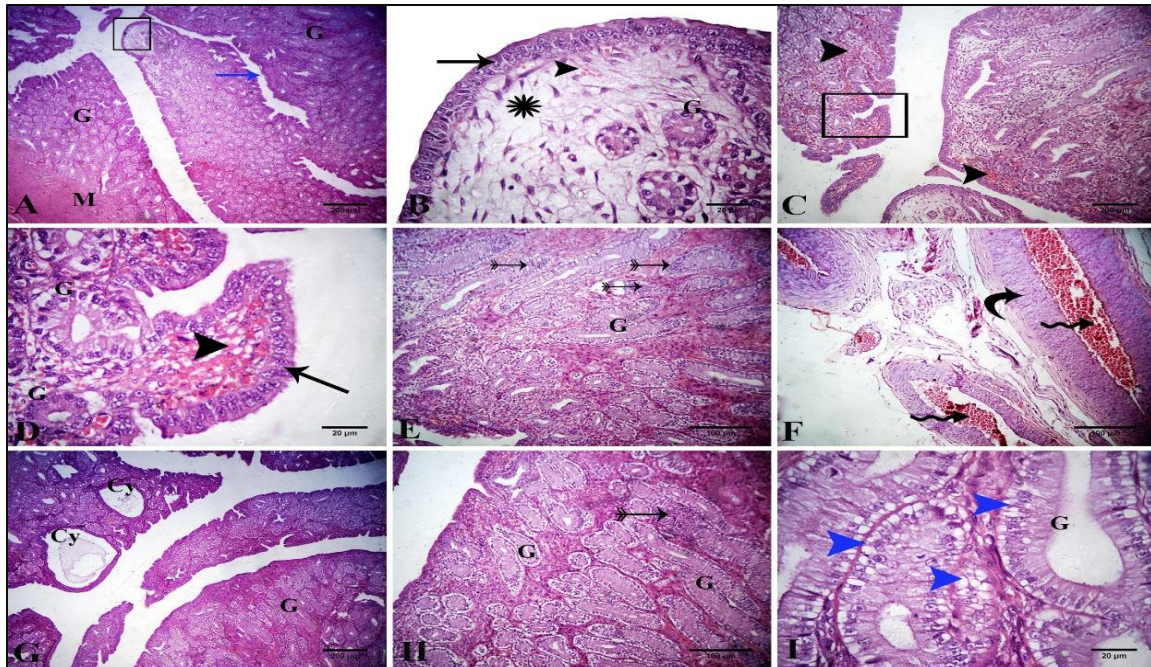


Figure 3. Representative photomicrograph of H&E-stained sections in the uterus of queen uterine glands (**g**), myometrium (**m**), and endometrial cysts (**cy**). the luminal epithelium of endometrium with deep surface infoldings (blue arrow) (**a**). high magnification to the rectangle of fig. a showing the epithelium (arrow) and uterine glands were lined with simple cuboidal to low columnar epithelium, lamina propria (star) with congestion of uterine capillaries (arrowhead) (**b**). destruction of some uterine blood capillaries (arrow heads) (**c**). high magnification to the rectangle of fig. c showing hemorrhage (arrowhead) and luminal epithelium of simple columnar (arrow) (**d**). the deep uterine glands were straighter with many leukocytic infiltrations (tailed arrows) (**e**). congested blood vessels (zigzag arrows) with thickening of their tunica media (curved arrow) (**f**). cysts of variable sizes on the endometrium containing eosinophilic substance (**g**). uterine glands of cystic endometrium were straight with many cytoplasmic vacuolation that increased in the deep than in the superficial glands with leukocytic infiltration (tailed arrow) (**h**). uterine glands are lined by tall columnar epithelium and cell nuclei were separated from the basal membrane by subnuclear vacuoles (blue arrow heads) (**i**).

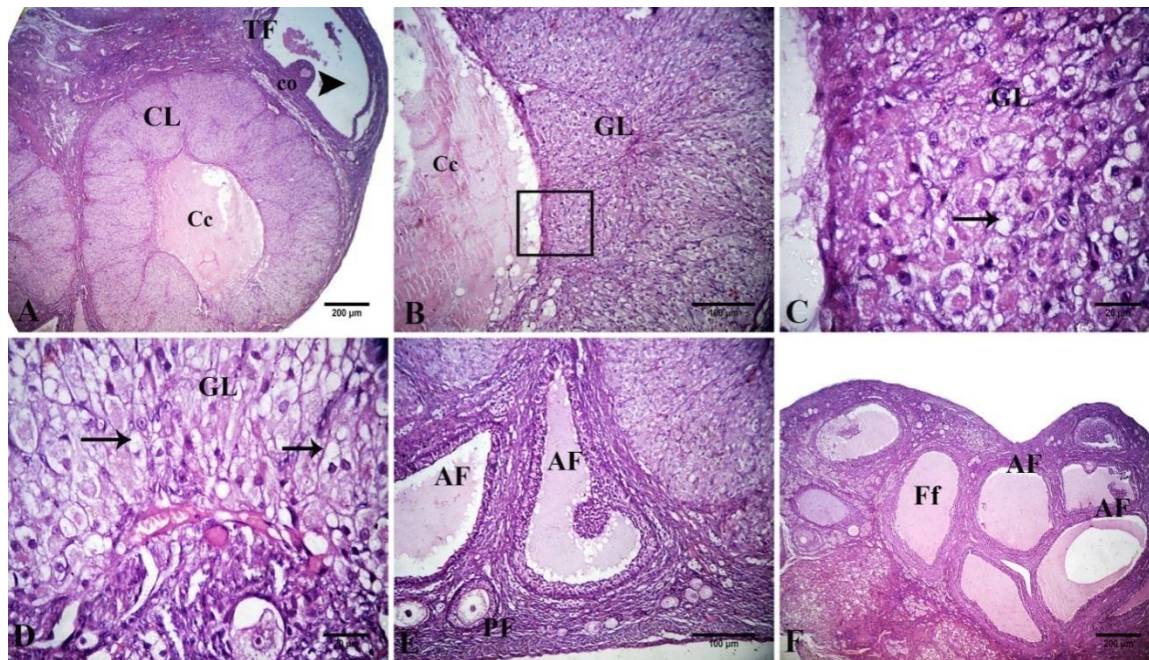


Figure 4. Representative photomicrograph of H&E-stained sections in the ovary of queen. Tertiary follicle (TF), antrum (arrow head), cumulus oophorus (co), corpus luteum (CL), central cavity (Cc), granulosa lutein cells (GL), atretic follicle (AF), primary follicle (PF), follicular fluid (Ff). Ovarian cortex with corpus luteum with tertiary follicle with antrum and cumulus oophorus (A). Granulosa lutein cells of corpus luteum and fibrosis in its central cavity (B). High magnification to rectangle of fig. B showing some apoptotic granulosa lutein cells with vacuolations (arrow) (C). Peripheral cells of corpus luteum were highly vacuolated (arrows) (D). Atretic Graffian follicle (AF) without ova and primary follicle (PF) (E). Atretic follicles (AF) with follicular fluid (Ff) in its lumen (F).

Discussion

Treating a case of pyometra with spaying totally clears the illness, avoids uterine rupture and peritonitis, and almost certainly prevents recurrence [18]. The occurrence of pyometra is not age-related; it can occur shortly after puberty or later in life. However, a high occurrence rate was observed in cats over the age of five who had never had a kitten or had only one or two litters in their lives [19]. The average age at diagnosis is 5-6 years, with a range of 10 months to 20 years, and the incidence rises with age, especially after the age of 7 [20]. In our study, the average age was 6.11 ± 0.28 . However, based on the queen's physical condition and the preference to maintain the ability to

reproduce, a decision to pursue medical treatment may be taken. The medical treatment should be applied only in queens with low systemic compromise and high reproductive value because improvement might take several days and complicate the issue [21]. Additionally, the medical treatment can be harmful to a patient who is seriously afflicted [22], so in difficult and dangerous situations, surgical intervention is the recommended course of action. The medical intervention used in the current study relies on the reduction of progesterone concentration through luteolysis of the corpus luteum and the ejection of the uterine content through relaxation of the cervix and contraction of the uterus. Utilizing $\text{PGF}_{2\alpha}$ in G1 causes the uterus to expel its content due to its ecobolic impact, cervix

relaxation and the vaginal discharge disappeared at D7 after the initiation of the treatment. This is in relation to the result of Ettinger *et al.* [23] which reported that PGF 2α was utilized to induce parturition in dogs and to cause luteolysis in feline and cats and also drainage of purulent material from uterus. As in Von Reitzeinstein *et al.* [24] many side effects followed PGF 2α injection as vocalizing, panting, restlessness, tenesmus, salivation, vomiting, diarrhea, mydriasis, urine, and defecation manifest; so, cats should be hospitalized at least 2 hours after injection. In our study; salivation, defecation, urination and vocalization were recorded as side effects of using PGF 2α . Compared to G1, queens in G2 queens showed a significant reduction in uterine diameter on days 5 and 3 of treatment, respectively. This means that the use of cabergoline in addition to PGF 2α in G2 reduced the diameter rapidly compared to using PGF 2α alone. Due to their anti-prolactin properties, the dopamine agonists such as cabergoline and bromocriptine are excellent luteolytic starting on day 25 post-estrus and have been used in conjunction with PGF 2α to enhance the treatment of pyometra [25]. In one trial, using a combination of cabergoline and a mild dose of cloprostenol caused the sickness to clear up in 90.5% of the 22 treated bitches with pyometra [26]. In comparison to either low-dose cloprostenol alone or a dose of dinoprost, a combination of PGF 2 and aglepriston was likewise proven to be the most efficient [27]. In our study, using of amoxicillin/ clavulanic acid after

sensitivity test, gave excellent results for controlling infection. The relapse rate recorded in G1 was earlier than in G2, with 6 cats relapsed 4 months after first treatment, whereas in G2 4 cats relapsed 8 months after first treatment. This means that using cabergoline together with PGF 2α strengthen uterine evacuation. Our research showed various uterine histopathological observations as cystic endometrium, hyperplasia in gland epithelial cells (CEH), congested blood vessels. Also, the results showed that ovary of cats with pyometra presented some changes as vacuolation of luteal cells and atretic follicles. All these observations that found in feline pyometra as reported previously in Agudelo [28] and Wen-yan *et al.* [29]. In addition, the bitches with pyometra, similar findings were found in Qian and Hou [30] and Demirel *et al.* [31]. Pyometra frequently occurs in luteal phase, correlation between pyometra and corpus luteum presence has been observed, as in about 40-70% of the cases corpora lutea were present [28]. In this feline pyometra case, a corpus luteum was also found. But pyometra has also been found in queens in follicular phase, which is influenced by estrogen besides progesterone [32]. Our study concluded that the using combination of cabergoline and PGF 2α for treatment of pyometra in queens, resulted more rapid evacuation of uterine content and lower recurrence rate after a prolonged period.

Conflict of interest: all authors have not any conflict of interest to declare.

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الملخص العربي

كفاءة العلاج الطبي للصدید الرحمي لدي القطط بالإشارة إلي التغيرات الهستوباثولوجية

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يعتبر الصدید الرحمي قضية خطيرة تشكل تهديداً لحياة القطط من خلال خطر تقيح الرحم الناتج عن السمية والإصابة بتسمم الدم. العلاج الأكثر فعالية وتقليدية لتقيح الرحم هو الاستئصال الجراحي لمصدر القيق (استئصال الجهاز التناسلي الأنثوي) والوقاية من التكرار، علي الرغم من ذلك التدخل الطبي يهدف الي الحفاظ علي القدرة التناسلية لدي القطط، تمت الدراسة علي 30 قطة وتم تقسيمها إلى مجموعتين. في المجموعة الأولى (ن: 15)، تلقت القطط 1 ميكروغرام / كجم / 24 ساعة من $PGF2\alpha$ في العضل لمدة 7 أيام، وبعد إختبار الحساسية، تم إعطاء (نصف جرام / يوم / عن طريق الفم) من أموكسيسيلين-كلافولانات 1 جرام كمضاد حيوي شامل لمدة 7 أيام. تلقت القطط في المجموعة 2 (ن = 15) نفس العلاج مثل القطط في المجموعة 1 مع إضافة ناهض الدوبامين الفموي (5 ميكروغرام / كغ / 24 ساعة) لمدة 7 أيام. بعد تلقي العلاج لمدة أسبوع واحد، لم يعد لدى كل من المجموعة الأولى والثانية أي مؤشرات سريرية علي تقيح الرحم والإفرازات الصديدية. كشفت متابعة معدل التكرار في المجموعتين كل أربعة شهور من بداية العلاج، أن ستة قطط في المجموعة الأولى أظهرت تقيح الرحم المتكرر في المتوسط 4 أشهر بعد بدء العلاج. ولكن بعد 8 أشهر فقط من بدء العلاج، أظهرت أربع قطط أول معدل تكرار في المجموعة الثانية. أخيراً، تعرضت القطط المتكررة غير المعالجة (5 قطط) لإستئصال المبيض والرحم كآخر تعامل بعد تجربتين علاجيتين فاشلتين وتم إجراء الفحص الهستوباثولوجي لهذه الأعضاء التناسلية. وفي النهاية إستخدام كابيرجولين مع $PGF2\alpha$ يحقق رجوع أسرع للرحم ومعدل تكرار أقل من استخدام $PGF2\alpha$ وحده.