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Comparative efficacy in the conservative treatment of canine pyometra

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List of abbreviations:

CEH – Cystic endometrial hyperplasia

OHE – Ovariohysterectomy

OVE – Ovariectomy

ET – Endotoxins

DIC – Disseminated intravascular coagulopathy

CRP – C-reactive protein

UTI – Urinary tract infection

PGF2α – Prostaglandin F2α

PRL – Prolactin

PO – Per os

1. Introduction

Pyometra is a potentially life threatening and common disease of middle-aged to older intact bitches. Pyometra is considered to be a disease of the luteal phase, where a hormonal imbalance of estrogen and progesterone, in combination with a secondary bacterial infection is believed to be the origin behind the disease. Ovariohysterectomy is the most common treatment option for pyometra. The surgery removes the entire reproductive tract, prevents any further recurrence and leads to a rapid recovery of the disease. In order to maintain fertility or avoid the risks of surgery, the medical treatment with drugs such as prostaglandins, progesterone receptors blockers and dopamine agonists, has shown to be an effective way of treating both open and closed-cervix pyometras.

Having always wanted to practice small animal veterinary medicine, particularly within the field of reproduction and obstetrics, the choice was easy when choosing a department to work with. Pyometra proves a relevant question to be addressed in this field of veterinary medicine, and is therefore an interesting topic to work with. Even though continuous research on this topic has been carried out, to date, the complete pathophysiological and etiological background of pyometra is still not clear. This retrospective study aims to collect and review information from recent studies regarding the medical management of pyometra and to compare the efficacy and approach of the different treatment options available. My thesis is based on previously published articles, and this body of work is put together as a literature review. In addition to looking at the effectiveness of the different drugs used in the conservative treatment of pyometra, I will also look into the background, predisposing factors, diagnostic confirmation and clinical relevance of pyometra.

My main goal with this thesis is to determine whether treating pyometra with a conservative approach is ideal, and if so, which methods provides the most useful results. I will evaluate the advantages and disadvantages present for using a medical approach as treatment. Furthermore I will appraise the different drugs used for the medical therapy of pyometra and their results, both when administered individually or together.

2. Canine Pyometra

2.1 Etiology

Pyometra is a bacterial uterine infection, and a term used to describe the pathological accumulation of pus in the uterus (Smith, 2006). It is often described as one of the major uterine diseases, as it has been reported to develop in nearly one fourth (19%) of female dogs, before reaching 10 years of age. In Scandinavian countries like Sweden were elective spaying is not routinely performed; the proportion of intact bitches is high. These female dogs are at a higher risk of developing diseases associated with the reproductive organs, such as pyometra (Egenvall *et al.*, 2001). Pyometra most often occurs 4 weeks to 4 months after the onset of estrus and is considered a to be a disease of the luteal or diestrus phase of the estrous cycle (Smith, 2006). During this phase a hormonal imbalance will cause morphological changes in the uterus, making it prone to an infection by secondary opportunistic bacteria. Depending on the severity, several clinical symptoms can be seen. Even though the mortality rate in pyometra is relatively low (3-4%), the disease may result in a life threatening condition, which requires immediate medical attention (Egenvall, 2001; Hagman, 2017). Work by Dow (1959) outlined and classified the four stages of pyometra, based upon the stages of endometrial hyperplasia and the presence of a secondary bacterial infection (Table 1).

	CYSTIC ENDOMETRIAL HYPERPLASIA	PLASMA CELL INFILTRATION	ENDOMETRITIS (SECONDARY BACTERIAL INFECTION)
STAGE I	X		
STAGE II	X	X	
STAGE III	X		ACUTE
STAGE IV	X		CHRONIC

Table 1: The four stages of pyometra (Dow, 1959)

2.2 Pathogenesis

Pyometra is a complex condition, which includes several components. Even though the etiology and pathogenesis behind the disease is not completely understood, the basic fundamental factors are thought to be the interaction between the hormonal and bacterial components (Dow, 1959). Cystic endometrial hyperplasia (CEH) is the name used to describe the atypical response of the uterus to hormonal changes. CEH is not necessarily associated with any clinical symptoms and may in fact not even lead to pyometra. It is however understood to be a crucial predisposing factor to the disease. Therefore pyometra might occur only when the uterus is under hormonal influence and the uterine lumen contains susceptible bacteria. Currently there is still no conclusive evidence that elucidates the true pathogenesis of pyometra. What we do know is that CEH and pyometra often occurs independently. Findings indicate that these two conditions may in fact have two separate entities: a cystic endometrial hyperplasia complex and an endometritis-pyometra complex. However, if both components are present, they are known as the CEH- pyometra complex (De Bosschere *et al*., 2001).

A study performed later by De Bosschere *et al*., (2002), supported this suggestion. The study examined the expression of hormonal receptors in the uterus of bitches with CEH and pyometra. The results showed a distinct difference in the expression patterns of estrogen-α and progesterone receptors in the uterus of bitches with CEH, opposed to bitches with pyometra. The progesterone and estrogen receptors expressed in uterus of bitches with pyometra were significantly lower compared to uterus of bitches with CEH. These findings indicate that the two conditions might have two different pathogeneses, and that CEH and pyometra is indeed two separate entities.

2.2.1 The Hormonal Component

Hormonal changes during the estrous cycle in the bitch are especially thought to have an important role concerning the pathogenesis. During the diestrus phase of the estrous cycle the uterus will undergo several morphological changes due to the influence of estrogen and progesterone. The prolonged and repeated effect of estrogen replaced by the long intervals of progesterone dominates the changes in the uterus (Smith, 2006). Progesterone is an essential feature to the pathogenesis of CEH. The repeated stimulation and high levels of this hormone during the luteal phase is considered to be one of the initiating steps in the development of the

CEH-pyometra complex (De Bosschere *et al*., 2001). The plasma levels of progesterone and estrogen change during the estrous cycle (Table 2), and may be a useful indicator when determining the status of the estrous cycle in the bitch. In addition, it can also be used to check the effectiveness of drugs used in the conservative therapy of pyometra (Breitkopf *et al*., 1997; Hoffmann *et al*, 2000; Fieni, 2006; Gobello *et al*, 2013).

	Progesterone ng/ml	*Estradiol-17β pg/mL*
Anestrus	<0.5	<25
Proestrus	>1	>25
Estrus	>1	>25
Metestrus	>0.5	<25

Table 2: Progesterone and estrogen levels during the estrous cycle
(De Bosschere et al., 2001)

Progesterone influences the uterus in several ways. The hormone causes the uterine glands to proliferate and increase in size, leading to a thickened, hyperplastic endometrium with a cystic appearance. The endometrial glands' secretory ability also increases. This leads to the accumulation of fluid in the glands and in the lumen of the uterus, which is known as mucometra or hydrometra. Progesterone also closes the cervix, and suppresses the contractions of the myometrium. The uterus is therefore unable to contract and expel unwanted fluid. This makes the uterus susceptible to bacterial overgrowth and further infections (De Bosschere *et al*., 2001).

Despite the age of the study, Teunissen, (1952) reported that when injecting dogs with exogenous estrogen and progesterone, morphological changes of the uterus would occur based on the influence of the hormones. Progesterone showed the most significant changes, which included the increased secretion, proliferation and dilatation of the endometrial glands. Estrogen administered alone only showed some mild morphological changes, such as glandular hyperplasia and proliferation resulting in a cystic appearance. However, when estrogen was administered prior to progesterone, the study showed that the effect of progesterone was potentiated and that the response of the endometrium to progesterone would be enhanced.

2.2.2   The Bacterial Component

Eschericia coli is the most common infective agent isolated in bitches diagnosed with pyometra. In addition, other pathogens such as *Streptococci, Staphylococci and Klebsiella* organisms, are occasionally isolated, but to a lesser extent (Fransson & Ragle, 2003). The infection route of the bacterium is still not fully understood, however studies have shown a connection between *E.coli* isolated from the infected uterus of bitches with pyometra and *E.coli* isolated from the urogenital or fecal origin of the same bitches (Wadas *et al*., 1996; Chen *et al*., 2003). Even though the primary origin of infection is not fully understood, these findings indicate that an ascending route of infection from the urinary tract or from the anogenital region into the uterus is the most likely route of infection.

Other research has shown that *E.coli* strains isolated from dogs with urinary tract infections was identical to strains isolated from the infected uterus, indicating that an important infection route for *E.coli* is in fact the urinary tract (Sandholm *et al*., 1975). Another explanation for the predominance of *E.coli* is the unique receptors of the bacterium produced in the endometrium. During diestrus, progesterone stimulates the endometrium to produce suitable receptors for *E.coli*, which increase the ability of the bacteria to adhere to the uterine endometrium. The adherence occurs with the help of K-antigens, which theoretically enhances the colonization of the pathogen during the development of pyometra (Sandholm *et al*., 1975).

3.   Predisposing Factors to Pyometra

There are several predisposing factors that may lead to pyometra. The two most important factors is the age and breed of the bitch, (Egenvall *et al*., 2001) but the increased risk of developing pyometra has also been associated with the administration of hormonal compounds, such as estrogens and progestins (Niskanen & Thrusfield, 1998; Whitehead, 2008). Furthermore, pregnancy has shown to have a protective effect from the disease, as nulliparous bitches have been associated with a higher risk of developing pyometra, compared to multiparous bitches (Niskanen & Thrusfield, 1998). However, a recent study found that previous pregnancies only had protective effect in some breeds, but not all (Hagman, 2011).

3.1   Breed and Age Related Risk of Pyometra

During the past several years, studies have been conducted to investigate the age and breed-related differences with respect to the occurrence of developing pyometra. Egenvall *et al*., (2001), investigated this connection, where the aim of the study was to evaluate the occurrence of pyometra in Swedish dogs up to 10 years of age, as related to breed and age. The study was based on the database of Agria Insurance Company, and included 200,000 dogs and 30 different breeds. The data was collected during the time period of 1995 and 1996. The result of the study showed that 1756 (14,3%) bitches in 1995 and 1710 (13,7%) bitches in 1996, were diagnosed with pyometra.

The mean age of bitches developing pyometra was 6.5 and 6.9 years in 1995 and 1996 respectively. The percentage of reported mortality was relatively low with 4.3% in 1995 and 4.2% in 1996. Based on the results, it was estimated that 23–24% of the bitches in the databases would have experienced pyometra by 10 years of age. Of the 30 breeds represented in the research, the breeds with increased risk of developing pyometra were rough Collies, Rottweilers, Bernese Mountain Dogs, Cavalier King Charles Spaniels, Golden Retrievers and English Cocker Spaniels. Breeds with a lower risk of developing the disease were Drevers, German Shepherd Dogs and Miniature Dachshunds (Table 3).

	% Survival		
	4<5	6<7	9<10
Rough Collies	93	79	54
Rottweilers	91	72	50
Bernese Mountain Dogs	89	77	46
Cavalier King Charles Spaniels	95	85	59
Golden Retrievers	96	88	65
English Cocker Spaniels	99	93	73
Drevers	98	92	83
German Shepherd Dogs	95	91	75
Miniature Dachshunds	99	96	87

Table 3: Estimated percentage of survival of females <10 years of age diagnosed with pyometra (Egenvall et al., 2001)

When considering the results of this study, some limitations should to be taken into account, as the study only examined a limited amount of breeds and during a short time interval. The occurrence of pyometra is therefore missing in several dog breeds, and more breeds should be added to make the result more accurate. More recent studies are however present and gives a more detailed picture of the prevalence of pyometra regarding age and breed.

Jitpean *et al*., (2012) continued investigating the incidences of pyometra in Swedish dogs, related to breed and age. In addition, the occurrence of mammary tumors was also investigated. During this study, the incidence of pyometra and mammary tumors were explored in 260,000 female dogs of 110 mixed breeds, up to 10 years of age. The mean age of bitches developing pyometra was 7.0 years. This finding shows a correlation between the presence of pyometra and the age of the animal. The proportion of the bitches that developed pyometra at 10 years of age was 19 % in all breeds, which is slightly lower than the 23-24%, which was previously reported by Egenvall *et al*., (2001). This result may reflect the fact that in the 110 breeds investigated in the present study, breeds with a lower risk of developing pyometra, were included.

However, like previously shown by Egenvall *et al*., (2001), this study also confirmed that the breed of the dog is important when predicting the risk for developing pyometra. According to the present study, it was also proven that the occurrence of the disease varied greatly among the different breeds. The study showed that large or giant breed dogs are the ones most likely to develop the disease. Similar to previous studies, (Egenvall *et al*., 2001) the Bernese Mountain dog and Rottweiler were both represented among the breeds most likely to develop pyometra.

According to Jitpean *et al*., (2012) the 10 breeds with the highest risk of developing pyometra included the following: Bernese Mountain Dog, Great Dane, Leonberger, Rottweiler, Irish Wolfhound, Staffordshire Bull Terrier, Keeshond, Bull Terrier, Bouvier des Flandres and Newfoundland. Whereas the breeds with the lowest risk of developing pyometra were the following: Finnish Spitz, Norrbotten Spitz, Coton de Tulear, Maltese, Gordon Setter, Laika, Saluk, Tibetan Terrier, Lancashire Bull Terrier and Norwich Terrier (Table 4). These findings indicate that genetic factors may predispose for the development of pyometra. The results from this study may be valuable for future breeding programs or genetic studies aimed to decrease the prevalence in high-risk breeds.

Breed at high risk at < 10 years of age	% Pyometra
Bernese Mountain Dog	66
Great Dane	62
Leonberger	61
Rottweiler	58
Irish Wolfhound	58
Staffordshire Bull Terrier	54
Keeshond	52
Bull Terrier	52
Bouvier des Flandres	50
Newfoundland	50

Breed at low risk at < 10 years of age	% Pyometra
Finnish Spitz	3
Norrbotten Spitz	4
Coton de Tulear	5
Maltese	8
Gordon Setter	8
Laika	8
Tibetan Terrier	10
Lancashire Bull Terrier	10
Norwich Terrier	11

Table. 4: Proportion of bitches that had developed pyometra at <10 years of age

(Jitpean et al., 2012)

3.2 Hormonal Therapy

Hormonal therapy including both estrogens for the induction of estrus or terminating unwanted pregnancies or progestins for the suppression of estrus, may partly explain the reason why pyometra occurs in young bitches (Pretzer, 2008), and will be discussed in more detail below.

3.2.1 Estrogen Therapy

For the prevention of pregnancy after unintentional copulation, the administration of an estrogen or a progestin compound is frequently used. There are several types of synthetic estrogen hormones used for this purpose, including estrogen cypionate and estradiol benzoate. These types of estrogens have been reported to prevent pregnancy at low doses, but further research is necessary to establish the minimum effective dosage and timing of administration (Tsutsui *et al*., 2006). The physiological effect of estrogen is thought to initiate and enhance the stimulatory effect of progesterone on the uterus, making the uterus prone to pyometra or pseudopregnancy (Niskanen and Thrusfield, 1989). Several studies have been performed to further investigate the relationship between estrogen treatment of mismated dogs, and the side effects including the potential development of pyometra. These studies all indicated to some degree, that the administration of exogenous estrogen to prevent conception might be a predisposing factor to pyometra (Bowen *et al*., 1985; Wheaton *et al*., 1989; Niskanen and Thrusfield, 1998).

Bowen *et al*., (1985) found that when administrating both low and high doses of estradiol cypionate, (22 to 44 μg/kg) during early diestrus, pyometra occured in two out of the eight bitches examined. However no change occurred when injecting the same drug during late pro-estrus or estrus. Wheaton *et al*., (1989) described the occurrence of pyometra after administrating high doses of estradiol cypionate in eight bitches less than three years of age. Within the six months before diagnosis, five of the bitches had received estradiol cypionate. These findings suggested that high dosage of estrogen would increase the risk of pyometra developing in young bitches.

A case study of pyometra in Finland, Niskanen and Thrusfield, (1998) reported that a high dosage injection of 0,1 mg/kg estradiol benzoate in bitches less than four years of age would significantly increase the risk of pyometra occurring. It was suggested that there might be a significant relationship between the administration of estrogen and the incidence of pyometra occurring in bitches between one and four years of age, but not in older bitches. Recent studies by Whitehead *et al*., (2008) investigated the risk of bitches developing pyometra when treated for mismating with both high and low doses of estradiol benzoate. 104 bitches were treated, with an age range from 9 months to 13.3 years. Out of 104 bitches treated with the synthetic steroidal hormone, nine bitches developed pyometra within four months of the treatment. These bitches developed a so-called treatment-associated pyometra. The study concluded that both high and low dose estrogen treatments would considerably increase the risk of pyometra, like the previous studies suggested (Bowen *et al*., 1985; Wheaton *et al*., 1989; Niskanen and Thrusfield, 1998).

3.2.2 *Progesterone Therapy*

As previously outlined in the body of work, progesterone is an important hormone in the pathogenesis of pyometra. Progesterone exerts a negative feedback on the hypothalamic-pituitary axis, thus preventing the gonadotropin secretion. Progesterone is therefore able to suppress estrus in both the bitch and queen, when administered exogenously. Several progestins are available on the market, including megestrol acetate, proligestone and medroxyprogesterone acetate (Noakes *et al*., 2009). Medroxyprogesterone acetate is a synthetic progesterone hormone used for the postponement of estrus or to suppress pseudopregnancy in the bitch (Withers *et al*., 1967; Hagman *et al*., 2011). Withers *et al*., (1967) carried out a study where 86 bitches were given up to 5 injections with MPA during a 6 months time interval. One bitch developed pyometra and two bitches developed a cystic hyperplasia of the endometrium.

Teunissen (1952) carried out an experimental study in 15 bitches up to five years of age. The aim of the experiment was to check the effect of progesterone when administered alone during various stages of the dog's estrous cycle and to investigate the correlation between progesterone and the development of pyometra. In seven of the fifteen dogs tested, pyometra was successfully induced. It was therefore argued that the administration of exogenous progesterone might increase the risk of developing pyometra. Dow (1959) tried to repeat these

findings a few years later, but was unsuccessful to achieve the same results when progesterone was administered alone. However when administrating estrogen before high levels of progesterone, the development of pyometra occurred. The connection between estrogen and progesterone was proven to have a relevant connection to the development of pyometra. As previously discussed, a high level of progesterone during the luteal phase is considered to be the initiating steps in the development of the CEH- pyometra complex (De Bosschere *et al*., 2001). However, if progesterone is stimulated by the presence of estrogen first, the endometrial hyperplasia will be more pronounced. Thus if administrating exogenous estrogen when progesterone levels are high during diestrus, the bitch may be predisposed to pyometra (Noakes *et al*., 2009).

4.   Clinical Significance of Pyometra

4.1   The Typical Clinical Portrait of Canine Pyometra

Pyometra may present itself with several characteristic clinical findings. The findings are generally determined based on the patency of the cervix and whether endotoxaemia or systemic inflammation is present (Pretzer, 2008; Hagman *et al*., 2009). During the physical examination, the bitch may present with a localized vaginal discharge or with non-specific generalized signs such as lethargy, depression, fever, polyuria, polydipsia, lack of appetite, and gastrointestinal disturbances such as vomiting and diarrhea (Hagman *et al*., 2009; Pretzer 2008). These findings have been supported later by Hagman *et al*., (2011), when several of the same symptoms were detected. In the group of bitches tested, 84% presented with vaginal discharge, 83% with depression and a poor general condition, 72% with decreased appetite, 64% with increased thirst and 43% with severe dehydration.

Vaginal discharge is one of the most typical clinical findings seen when diagnosing a dog with pyometra, and its presence is based upon the patency of the cervix (Figure 1). The patency is classified into two categories depending if the cervix is open (with vaginal discharge) or closed (without vaginal discharge). Vaginal discharge is seen in the case of an open-cervix pyometra, when the cervix is open, allowing the drainage of the purulent fluid from the uterus. The vaginal discharge is often foul smelling and may have a sanguineous to a

mucopurulent consistency (Smith, 2006; Pretzer, 2008; Jitpean *et al.*, 2017). In case of a closed-cervix pyometra, the cervix is functionally closed and the pus remains in the uterus.

Bitches with closed-cervix pyometras generally presents with more severe generalized signs, and in addition a distended abdomen sometimes may be seen. (Pretzer, 2008) They are also more systemically affected than bitches with open-cervix pyometras. This may be due to the fact that without vaginal discharge, it is harder to detect the early stages of the disease, which contributes to a more severe disorder. Therefore, pyometra should always be considered as a differential diagnosis if any intact bitch is ill, and presents with a poor general condition. (Pretzer 2008; Smith, 2006)

Figure 1: Vaginal discharge in a bitch, indicating an open-cervix pyometra
(Enpevet, no date, accesses 9th of November 2017)

Jitpean *et al.*, (2017) performed a study investigating the connection between the patency of the uterus and the presence of vaginal discharge. The aim of the study was to examine the severity and outcome of bitches with open and closed cervix pyometras. The result showed that sepsis was diagnosed more frequently in bitches with closed cervix pyometra compared to open cervix pyometra. In 59% of the bitches tested, sepsis was detected. Also the bitches presented with more severe clinical signs than the bitches with an open cervix pyometra. Without the presence of vaginal discharge, it can be harder to recognize the disease at an early stage when other clinical signs are unspecific. The disease is therefore allowed longer time to progress, contributing to a more severe illness. Results of this study support the theory that

dogs with closed cervix pyometra are more severely ill than dogs with open pyometra and vaginal discharge. The outcome however was not concluded to be poorer.

4.2   Endotoxaemia and Systemic Effects of Pyometra

The clinical signs of pyometra are not only limited to the genital tract. The disease is also associated with systemic signs due to endotoxemia and sepsis (Fransson & Ragle, 2003; Hagman *et al*., 2009). Gram-negative bacteria, such as *E.coli*, consist of a lipopolysaccharide cell wall component. During bacterial growth or destruction, endotoxins are released from the cell wall and released into the circulation. Endotoxins are thought to be responsible for the systemic symptoms of pyometra, such as fever, vomiting and depression. This occurs when inflammatory mediators are stimulated, inducing a systemic inflammatory response syndrome (SIRS), also known as sepsis. SIRS is often associated with any serious infection such as canine pyometra. Sepsis and endotoxaemia may also have more severe effects, such as multi-organ failure, DIC, shock and death (Fransson & Ragle, 2003; Hagman *et al*., 2006; Jitpean *et al*., 2017).

5.   The Diagnostic Evaluation of Pyometra

Pyometra is a severe and life-threatening disease. According to recent studies, a mortality rate of approximately 10% has been confirmed, (Jitpean *et al*., 2014) which is higher than what was previously reported (3-4%) by Egenvall *et al*., (2001). As discussed earlier, pyometra has several typical clinical symptoms, which can be further used in the diagnostic process of the disease. In order to secure a favorable result and increase the chances for survival, early identification and correct treatment of the disease is essential in attaining a better outcome (Hagman, 2017).

5.1   Anamnesis and Physical Examination

A dog entering the veterinary clinic with a suspected case of pyometra, should be evaluated based on several criteria. The anamnesis, also know as the history of the patient, is often the first step in diagnosing the disease, and is based on the owner's own observations of clinical

symptoms. Even though the onset of clinical signs may occur gradually, the owner often might detect abnormal changes in the dog's behavior quickly. The most regular observations include vaginal discharge, depression, inappetance, polydipsia, polyuria and vomiting (Verstegen *et al.*, 2008). Furthermore, a history of the dog's recent estrous cycle is of great importance. The highest chance for developing pyometra occurs approximately 4 weeks to 4 months after the onset of estrus, (Smith, 2006) thus is an important factor to include when taking the anamnesis. Other predisposing factors such as age, breed and exogenous hormonal therapy should also be included (Niskanen & Thrusfield, 1998; Egenvall *et al.*, 2001; Whitehead, 2008). In addition to the poor general condition of the dog, several typical signs for pyometra may be detected during a physical examination. The most important ones include hyperemic and/or pale mucous membranes, dehydration, abdominal pain upon palpation and an enlarged, palpable uterus (Jitpean *et al.*, 2014). Based on whether a secondary bacterial infection or septicemia is present, fever may also be associated with the disease. It is however a variable physical parameter, and might not always be present (Jena *et al.*, 2013a). Based on the anamnesis and physical examination alone, pyometra may already be suspected. For a complete diagnosis, there are other additional methods available. Laboratory tests and diagnostic-imaging techniques are the most frequently performed methods for a complete diagnosis of pyometra (Hagman, 2017).

5.2   Laboratory Diagnostic Methods

As previously outlined in the body of work, endotoxaemia and SIRS is associated with the systemic effects of pyometra. The systemic signs will be evident when performing laboratory tests and subsequent results further contribute to the diagnostic workup of pyometra. Laboratory diagnostic methods include haematological and biochemical investigations, both of which could yield important information during a diagnosis procedure (Fransson & Ragle, 2003).

5.2.1   Haematological Laboratory Results

Several changes are observed in the haematological parameters of a dog with pyometra. A marked leukocytosis characterized by neutrophilia with a left shift, monocytosis and anaemia, are the most frequently observed changes (Jitpean *et al.*, 2017). The most consistent haematological finding is the marked leukocytosis. This finding is associated with the

ongoing systemic infection, and the increased stress on the body's defense mechanism (Jena *et al*., 2013a). Neutrophilia with a regenerative left shift is also a typical feature in the haematological laboratory result, and might be due to the retention of purulent exudates in the uterus. There is also a connection to be made between the toxins released from the uterus, and their degenerative effect on the neutrophils. Many affected bitches also show a decreased number of haemoglobin, PCV, total erythrocyte count and lymphocyte count, indicating a normocytic, normochromic anaemia. This reflects the chronic nature of the disease and the toxic suppression on the bone marrow (Verstegen *et al*., 2008; Jena *et al*., 2013a). Another useful parameter for detecting pyometra is the C-reactive protein (CRP). CRP is a major acute phase protein in dogs, and has shown to be an informative marker for systemic inflammation (Cerón *et al*., 2005). Although there are few published studies about CRP in dogs, the concentration of CRP has shown to be elevated in bitches with pyometra, and may be used in predicting the presence of pyometra (Fransson *et al*., 2004).

5.2.2   *Biochemical Laboratory Results*

From the biochemical laboratory results, the alterations of kidney and liver function are most often detected (Verstegen *et al*., 2008). When investigating the liver function, the most consistent finding is the increased serum alkaline phosphatase (ALP), which is present in about 50-75% of pyometra cases. Serum alanine aminotransferase (ALT) concentrations are also occasionally mildly elevated. These alterations reflect the function of the liver, including hepatocellular damage due to toxaemia or the impaired hepatic circulation due to dehydration (Verstegen *et al*., 2008). In addition, increased bilirubin and serum cholesterol might be observed. This indicates an intrahepatic cholestasis, which may be a consequence of the ongoing endotoxaemia (Borresen & Skrede, 1980; Fransson & Ragle, 2003).

Impaired renal function has been mentioned as a common feature of canine pyometra. As a consequence of dehydration, pre-renal azotemia will occur. This condition is reflected in the increased concentrations of serum creatinine and blood urea nitrogen (BUN). Proteinuria and hyperproteinemia is also seen as a response to dehydration (Smith, 2006; Jitpean *et al*., 2017). The alterations seen in kidney function may be a result of a tubulointerstitial inflammation or an immune complex associated glomerulonephritis. Both of which might result in a tubular and glomerular dysfunction (Heiene *et al*., 2007; Maddens *et al*., 2011).

5.3 Diagnostic Imaging Techniques & Pyometra Confirmation

Diagnostic imaging techniques, including ultrasonography and radiographs, are often used for a complete and definite diagnosis of pyometra. A lateral abdominal radiograph most often identifies a fluid filled tubular organ, with a sausage-like appearance (Smith, 2006). However, ultrasonography is the imaging technique preferred when suspecting pyometra. With ultrasound, the same findings can be seen as in a radiograph, but in addition it provides more reliable and detailed information (Figure 2). This includes the information about the size of the uterus, the thickness of the uterine wall and the appearance of fluid accumulation in the uterus. Also it includes the presence of proliferative changes of the endometrium in case of CEH or any signs of ovarian disease (Hagman *et al.*, 2006; Smith, 2006; Hagman, 2017). Additionally, the uterine blood-flow is possible to detect with ultrasound. This may aid in the differentiation of pyometra versus mucometra, since not all accumulation of fluid in the uterus in due to pyometra (Hagman, 2017).

Figure 2: Uterus of a bitch with pyometra, performed by ultrasonographic diagnostic technique. (K. Trasch et al., 2003)

6. Treatment of Pyometra

6.1 Surgical Treatment of Pyometra

When diagnosing a bitch with pyometra, the safest and most common treatment option is surgery, which includes the removal of the ovaries and uterus (Smith, 2006; Verstegen *et al.*, 2008). Previous research has suggested that the surgical treatment of pyometra leads to a fast recovery in the animal's hematological and biochemical disturbances, which in turn leads to the disappearance of any clinical signs (Bartoskova *et al.*, 2007). In bitches there are two basic surgical methods where the aim is to remove the gonads: ovariectomy (OVE) and ovariohysterectomy (OHE). During an OVE only the ovaries are removed, whereas the entire reproductive tract is removed during an OHE (Reichler, 2009). When treating pyometra surgically, the only option is however the OHE, as the surgery allows for the immediate removal of the uterus with its purulent contents, and inhibits any further release of endotoxins (Fieni *et al.*, 2014).

The incidence of diseases such as pyometra is significantly decreased in countries where gonadectomy, also known as routine spaying, are performed (Reichler, 2009). In Norway however, it is illegal by law to perform ovariohysterectomy on healthy dogs for preventative measures. According to the Law on Animal Welfare, LOV-2009-06-19-97, §9. "Medicinal and surgical treatments; surgical interventions and removal of body parts on animals without a valid reason, should not be performed (Translated from lovdata.no, (2009) Accessed: 30.10.17). Accepted reasons for spaying a dog may be the following: pyometra, pseudo pregnancy or extreme behavioral issues related to sex hormones.

The stabilization of the patient prior to surgery with fluid and antibiotic therapy is recommended. Fluid therapy is administered intravenously to correct dehydration and to treat the animal's state of shock. Antibiotics are given to prevent septicemia in bitches with a poor general health (Fieni *et al.*, 2014). The supportive measures should last during, but also after the surgery (Verstegen *et al.*, 2008). Although the results of the surgeries often are successful and the bitches recover quickly, there are some drawbacks with this type of treatment. Typical complications reported include the increased risk of anesthesia due to the poor general health of the animal. As well as peritonitis, hemorrhages, incomplete removal of the ovaries, wound swelling and infection (Wheaton *et al.*, 1989).

6.2 Conservative Treatment of Pyometra

6.2.1 Aims of Conservative Therapy

Whenever surgery is not an alternative, for example if the bitch affected is old and surgery or anesthesia is considered with high risk for serious complications or death, the conservative treatment of pyometra is another alternative. Other reasons for using the medical treatment option instead of surgery can either be due to the owner's economy or the wish to improve the general condition of a patient prior to surgery (Fieni *et al.*, 2014). In bitches intended for breeding, the medical approach might be a better alternative, as it preserves the fertility of the bitch (Jena *et al.*, 2013b).

6.2.2 Patency of the Uterus

Before initiating the conservative treatment option of pyometra, the patency of the uterus should be established. The patency of the uterus is either classified as an open or a closed pyometra. In case of an open pyometra, the cervix is open and a vulvar discharge might be seen. The vulvar discharge may vary from having a sanguineous to a mucopurulent consistency. Closed pyometra often presents without any vaginal discharge, as the purulent fluid is accumulating inside the lumen of the uterus (Pretzer, 2008). Bitches with closed-cervix pyometras often presents with more severe clinical signs, and research has shown that this condition may lead to a seven-fold increased risk of having a prolonged postoperative hospitalization. In addition, closed-cervix pyometras have been associated with the increased risk of uterine rupture resulting in a secondary peritonitis (Jitpean *et al.*, 2014).

Before initiating a medical treatment protocol, certain precautions should be made to ensure the best efficacy of the drugs. The clinical presentation of the bitch in addition to the patency of the uterus is important to determine the severity of the illness and the best treatment option (Smith, 2006). If the bitch presents with a closed-cervix pyometra and a poor general condition, surgery is the safest treatment option, as some authors believe that the medical treatment in this case, is contraindicated due to the risk of uterine rupture and secondary peritonitis (Nelson *et al.*, 1982; Smith, 2006).

Furthermore, it has been suggested, that the most successful outcome of the conservative treatment of pyometra can be seen in young bitches that presents with a good general condition and with an open-cervix pyometra (Fieni, 2006; Jurka *et al.*, 2008).

6.3 Drugs Used in Conservative Treatment of Pyometra

When treating pyometra conservatively, the main aim of the treatment is to remove or inhibit the effects of progesterone. The development of pyometra is associated with progesterone due to its inhibitory effect on uterine contractions and that it is responsible for the closure of the cervix. Furthermore, it stimulates the cystic endometrial development and increases uterine secretion (Verstegen *et al.*, 2008). Drugs frequently used in the conservative treatment of pyometra include prostaglandins, progesterone antagonists, dopamine agonists or a combination of these drugs (Corrada *et al.*, 2006; Fieni, 2006; Gobello *et al.*, 2003). Together with these drugs, broad-spectrum antibiotics are used to inhibit bacterial growth (Verstegen *et al.*, 2008). Although the drugs have different protocols, they are all trying to achieve the same goals for a successful result. Preventing the circulating effect of progesterone, also allows the relaxation of the cervix and the uterine contractions. Preventing the effect of progesterone is achieved either with the help of luteolysis or by preventing the binding of progesterone to its receptors.

The drugs responsible for this are the prostaglandins or the progesterone-receptor blockers. These drugs may inhibit progesterone either directly or indirectly. Prostaglandins will directly inhibit progesterone through the destruction of the corpus luteum, causing luteolysis. The progesterone-receptor blockers on the other hand, have an indirect effect on progesterone. Due to their high affinity to the progesterone-receptors, they will bind to the receptors instead of progesterone (Verstegen *et al.*, 2008).

7. Prostaglandins

Prostaglandins, also know as prostaglandin-F2 alpha or PGF2α, is derived from the fatty acid arachidonate, and has several functions in the body (Burke, 1982). In several countries, prostaglandins are only approved for the treatment of livestock, including estrus synchronization or to treat chronic endometritis. In dogs it can only be given off label, and if the owner approves it (Verstegen *et al.*, 2008). Due to the luteolytic and uterotonic properties of prostaglandins, the drug is indicated as a conservative treatment option of pyometra or metritis. The luteolytic effect of prostaglandins is activated approximately five days into the estrous cycle, during the diestrus phase, ensuring the destruction of the corpus luteum. Luteolysis is responsible for the decline of serum progesterone level, which in turn leads to the opening and relaxation of the cervix (Gobello *et al.*, 2003; Fieni *et al.*, 2014). Prostaglandins also have uterotonic properties. Due to its contractile effect on the smooth musculature, including the myometrium, PGF2α stimulates the motility of the uterus, further facilitating the uterine drainage. The increased intrauterine pressure causes the uterine contents to move towards the already opened cervix. The relaxed and open cervix allows the drainage of exudate from the uterus to occur (Verstegen *et al.*, 2008; Jena *et al.*, 2013b).

7.1 Side effects of Prostaglandins

Even though prostaglandins has shown to be helpful in the treatment of pyometra, several side effects, both mild and more severe, have been observed after injecting the animals with the drug. They appear within minutes and may last for approximately 1 to 1.5 hours (Fieni *et al.*, 2014). The side effects observed are mainly due to prostaglandins action on the smooth musculature. When the smooth muscles of the gastrointestinal tract, myometrium and bladder contracts, and the result may be seen as short-term side effects. Such side effects include increased frequency of defecation, vomiting and salivation. Furthermore, depression, excitation or hypothermia may occur (Jena *et al.*, 2013b; Fieni *et al.*, 2014). The drug may also elicit more severe side effects. As mentioned earlier, the use of prostaglandins in closed cervix pyometra is contraindicated. Because the drug it is associated with the risk of uterine rupture and peritonitis, care should be taken before administrating the drug. A study done by Nelson *et al.*, (1982), found that prostaglandins should not be administered in severely ill bitches with closed cervix pyometra. In one of the bitches treated with prostaglandins, the general condition rapidly declined and an OHE was performed. During the surgery, vaginal

discharge was discovered in the abdominal cavity. Peritonitis is thought to either occur as a result of the uterus rupturing, or if purulent fluid enters the peritoneal cavity through the ovarian bursa (after the forceful passage through the uterine tubes) (Nelson *et al*. 1982).

7.2 Efficacy of Prostaglandins

7.2.1 Natural PGF2α

Natural forms of prostaglandins results in solid uterine contractions and causes rapid luteolysis (Jena *et al*., 2013b). Dinoprost, a natural prostaglandin, is approved in several countries as a prescription drug. According to the Norwegian drug formulary the drug is indicated for the supportive treatment of open-cervix pyometras in dogs and cats whose general condition is satisfying. Based on this protocol, the drug should be administered subcutaneously once daily, for a time period of seven days. On the first day of treatment, 0.1 mg/kg should be administered, on the second day 0.2 mg/kg and on day 3-7, 0.25 mg/kg should be given (Translated from the Norwegian Drug Formulary, felleskatalogen.no, (no date) Accessed: 13.10.17). There are however other protocols available for the dosage calculation of PGF2α. Doses of 10-50 µg/kg, given five times daily for 3 to 7 days have shown successful results for treating pyometra, either alone or in combination with other drugs. When calculating the doses, caution should be made due to the small therapeutic index. Actually the LD50 in dogs is about 5mg/kg. Because side effects of the drug are thought to be dose-dependent, it is recommended to start with the lowest dosage possible to avoid severe side effects, and then slowly increase the dosage after 2-3 days (Verstegen *et al*., 2008). Several studies have been completed, focusing on the treatment of pyometra with natural prostaglandins. The main findings can be seen in a summary below.

In a study performed by Arnold *et al*., (1988), the effect of low doses PGF2α was investigated in ten bitches with pyometra. Dinoprost was administered at a low dose of 20 µg/kg, intramuscularly, three times daily. The treatment was continued up to eight days, in combination with antimicrobials for four weeks. On the 8th day, ultrasonic examination revealed that seven out of ten bitches (70%) had responded well to treatment. The uterus of three dogs was however still enlarged, and these bitches underwent ovariohysterectomy. The uterus of these bitches was diagnosed histologically with CEH.

The result showed that a low dose of prostaglandins might be sufficient in the medical treatment of certain cases of pyometra. However, the success rate of the treatment could be better.

Jena *et al*., (2013b), performed a study investigating the therapeutic effect of natural and synthetic prostaglandins in the treatment of canine pyometra. The result when administrating synthetic prostaglandins will be summarized later. Seven bitches were treated with the natural prostaglandin, dinoprost, at a high dose of 100 µg/kg subcutaneously once daily, for seven days. The recovery rate of the treated group was 100%, as all of the seven bitches were treated successfully with the natural PGF2α. The therapeutic efficacy was judged based on side effects, post treatment reproductive status and recurrence rate.

The recurrence rate was 43%, as three bitches suffered from recurrence of pyometra within four months after treatment. One bitch recurred with pyometra one month after treatment. In all the bitches where pyometra reoccurred, the typical clinical signs included vaginal discharge, polydipsia, polyuria and lethargy. In addition, severe side effects were reported. Panting, restlessness, vomiting, urination, defecation and hyperpnoea were side effects observed. Salivation however was not observed during this study. The reason for this may be the fact that 10-15 minutes prior to the treatment, atropine sulphate was given, in addition to withholding food and water 4-6 hours before administrating the drug. Furthermore, the animals were allowed to take short walks after the administration of PGF2α, in order to facilitate the metabolism and early excretion of the drug. Side effects occurred few minutes after the administration and disappeared within 1 to 1.5 hours later.

Side effects might have a connection with the high dose of PGF2α used, as no adverse signs were reported in studies performed with low doses of prostaglandins (Arnold *et al*., 1988). During the following 10 months, none of the bitches showed any signs of recurrence. The post treatment reproductive status was checked to see if the bitches returned to estrus and if conception was possible. Six bitches (85,71%) came into estrus within two months. These bitches were mated in order to prevent recurrence. Out of the six bitches, four bitches conceived.

Gabor *et al*., (1999) tried to determine if the intravaginal infusion of natural prostaglandins would be useful for the treatment of pyometra or metritis in the bitch. Seventeen bitches of different breeds, diagnosed with pyometra were included in the study. Natural prostaglandins, at doses of 150 µg/kg were administered intravaginally, once or twice daily. Amoxicillin or gentamycin was given intramuscularly as well. By the time period ranging from 3 to 12 days, fifteen bitches (88%) had recovered. Ovariohysterectomy was performed in two bitches with deteriorating general condition. Within 12 months of the treatment and at the next estrus, no recurrence was observed. Furthermore none of the bitches treated showed any side effects in the months following the treatment. The study was based on a relatively small number of cases, and therefore needs further approval and verification before being suggested as a treatment option. It did however show that prostaglandins could be successfully used for treating bitches with pyometra

7.2.2 Synthetic PGF2α

The synthetic types of prostaglandins, also known as prostaglandin analogues, have less reported side effects than the natural form of the drug. However, the drug causes weaker contractions of the uterus, which results in a slower evacuation from the uterus (Verstegen *et al*., 2008). Cloprostenol (Estrumat) is the prostaglandin analogue most frequently used (Corrada *et al*., 2006). These drugs may be given off label to treat pyometras in dogs with normal liver and kidney function and who present with an open-cervix pyometra (Fieni *et al*., 2014).

A study previously discussed, (Jena *et al*., 2013b) investigated the therapeutic effect of natural and synthetic prostaglandins in the treatment of canine pyometra. Seven bitches were injected with the synthethic prostaglandin (cloprostenol), subcutaneously at a low dose of 1µg/kg once daily, for seven days. No side effects were observed and all of the treated bitches recovered, which demonstrates a 100% short-term success rate. However the recurrence rate revealed that 86% (6/7) of the bitches developed pyometra again. In addition, a low conception rate was reported, as only one of the two bitches that came into estrus conceived. This result demonstrates that even though low doses of prostaglandins are administered to help reduce side effects, there is a correlation between the doses used and the high recurrence rate and lower conception rates.

As a conclusion, the effectiveness when treating pyometra with PGF2α seemed to vary based on which type and dose of the PGF2α were used in the different studies. In the studies administrating high doses of natural prostaglandins (dinoprost), a higher success rate and lower recurrence rate was reported. Compared to the higher recurrence rate and low conception rate after using synthetic prostaglandins (cloprostenol) However more severe side effects were observed when using high doses natural prostaglandins, compared to the non-existing side effects reported when using low doses of synthetic prostaglandins (Jena *et al*., 2013b). A summary of the most significant studies concerning results after administrating natural and synthetic PGF2α can be seen below (Table 5).

AUTHOR	NUMBER OF TREATED BITCHES	PROTOCOL	SUCCESS RATE (n/%)	TIME FOR RECOVERY	RECURRENCE RATE (%)	SIDE EFFECTS
Arnold *et al*., (1988)	10	Dinoprost, low dose: 20µg/kg IM Three times daily, for 8 days	**70%** (7/10)	8	-	None
Jena *et al*., (2013b)	**GROUP I:** 7 NATURAL PGF2A	Dinoprost, high dose: 100 µg/kg SC Once daily, for seven days	**100%** (7/7)	7	**43%** (n=3) 4 months	Severe
	GROUP II: 7 SYNTHETIC PGF2A	Cloprostenol, low dose: 1µg/kg SC Once daily, for 7 days	**100%** (7/7)	7	**86%** (n= 6)	None
Gabor *et al*., (1999)	17	Dinoprost, high dose: 150 µg/kg intravaginally, once or twice daily	**88%** (15/17)	3-12	**0%** 12 months	None

Table 5: Summary of significant studies concerning the administration of prostaglandins in bitches with pyometra (Arnold et al., 1988; Gabor et al., 1999; Jena et al., 2013b)

8. Progesterone receptor blockers

The main aim of the conservative treatment of pyometra is to prevent the effects of progesterone. This can be achieved either by luteolysis or by blocking the binding of progesterone to its receptors (Verstegen *et al*., 2008). Progesterone is a natural steroid hormone, which acts by binding to the progesterone receptors in the endometrium. When progesterone binds to these receptors, a group of physiological effects will be induced. These effects include closure of the cervix, cessation of estrogenic activity, induction of endometrial secretion and inhibition of myometrial contractility. The same effects are responsible for maintaining pregnancy and producing optimal conditions for the fetus. In addition they are all included in the etiology behind pyometra (Pesch, 2017).

8.1 Aglepristone

Drugs used to block the binding of progesterone to its receptors are known as progesterone receptor blockers, antiprogestins or progesterone antagonists (Gobello *et al*., 2003; Verstegen *et al*., 2008; Fieni, 2014). There are several progesterone receptor blockers on the market, including aglepristone, mifepristone and onapristone. Aglepristone is known under the brand name Alizin, (Figure 3) and is the only drug registered for veterinary use today (Hoffmann and Schuler, 2000). Aglepristone is registered in several continents and countries such as Europe, Asia, South Africa, Australia and New Zealand. The drug is approved for the induction of parturition or the termination of unwanted pregnancy up to 45 days in the bitch. Furthermore the drug is used off label for the conservative treatment of pyometra (Hoffmann and Schuler, 2000; Pesch, 2017). Aglepristone is a synthetic steroid, which binds completely to the progesterone receptors with high affinity. Consequently, no stimulation and activation of the receptors will occur and the biological activities of progesterone are blocked (Gobello *et al*., 2003; Verstegen *et al*., 2008).

By blocking the effect of progesterone, the drug mimics the effects seen during luteolysis, which causes the relaxation and opening of the cervix, which in turn leads to the evacuation of uterine contents (Verstegen *et al*., 2008; Fieni, 2014). Aglepristone's affinity to the progesterone receptors varies between species. In dogs the fixating rate is three times higher than that of progesterone, whereas in the queen the affinity is even nine times higher, making the drug highly efficient (Pesch, 2017).

Figure 3: Aglepristone under the brand name Alizin vet
(Virbac, no date, accessed 20th of October 2017)

8.2 Treatment Protocol of Aglepristone

For the conservative treatment of pyometra with aglepristone, several treatment protocols have been described in the literature. Hoffmann *et al*, (2000) introduced the classical treatment protocol of aglepristone. This type of protocol involves the administration of three subcutaneous doses of 10 mg/kg on day 1, 2 and 7. The first dose should be given on the day of diagnosis and the second dose should be given 24 hours later. To achieve complete recovery, another dose is given on day seven together with weekly injections if needed. Another protocol included the single subcutaneous injection of 10 mg/kg on days 1, 2 and 8. If the uterine lumen could still be seen after day 8, another injection was given on day 15 (Fieni, 2006).

Recently, a more modified treatment protocol has been introduced for the treatment of pyometra with aglepristone. The bitches treated with this type of protocol, are injected with four subcutaneous injections of 10 mg/kg, on day 1, 3, 6 and 9 (Contri *et al*., 2015). This treatment protocol proved to be more successful than the other protocols described, as it was not associated with the recurrence of the disease over a 24-month period. Suggesting that this type of protocol has a higher and better long-term efficacy.

8.3 Side effects of Aglepristone

When treating pyometra with progesterone receptor blockers, the risk of developing severe side effects is lower and not as severe, compared to when using prostaglandins (Pesch, 2017). In fact, several authors described the successful treatment of pyometra using aglepristone, with no side effects observed (Hoffmann *et al*, 2000; Gobello *et al*., 2003). However some side effects when using aglepristone have been reported. These side effects include anxiety, loss of appetite, polydipsia, diarrhea, abdominal cramps and panting (Ros *et al*., 2014). In addition, a local pruritic reaction may occur at the injection site, which normally resolves within a week (Fieni, 2006). It is therefore advised to rub this area gently after administrating the drug (Pesch, 2017). Aglepristone also has a tendency to influence the interestrus interval. In 19 to 43% of the cases a shortening interval was described, (Trasch *et al*., 2003; Jurka *et al*., 2008; Contri *et al*., 2015) and a prolonged interval was observed in 20% of the cases (Trasch *et al*., 2003). However no effect on the following fertility has been established.

8.4 Efficacy of Aglepristone

8.4.1 Aglepristone

Aglepristone may be used alone or combined with other drugs, such as prostaglandins and systemic antibiotics (Gurbulak *et al*., 2005; Verstegen *et al*., 2008). By blocking the progesterone receptors, aglepristone is able to decrease the intrauterine progesterone concentration, which in turn leads to the cervical relaxation and opening. This will further induce the emptying of large amounts of purulent discharge from the uterus, often leading to the immediate improvement of the bitch's general condition (Fieni, 2014; Pesch, 2017). The aim of the following studies was to determine the efficacy, safety, recurrence rate, fertility and side effects after administrating aglepristone in the medical treatment of pyometra.

Breitkopf *et al*., (1997) demonstrated the use of low doses of aglepristone in seven bitches diagnosed with pyometra. The plasma progesterone concentration was above 3.2 nmol/l in all bitches, which confirmed the diestrus status of the animals. 5-6 mg/kg of aglepristone was administered subcutaneously on day 1, 3, 4, 8, 12 and 16. Within 28 days all bitches recovered, and during the following estrus, no recurrence was observed. Two dogs were later mated and produced healthy puppies. The results from this study proved that aglepristone

could be used successfully in the treatment of pyometra, but as only seven bitches were treated in this study, the results should be interpreted with caution.

The classical treatment protocol of aglepristone, used most frequently today, was first introduced in a study done by Hoffmann *et al*, (2000). The drug was injected subcutaneously in 22 bitches, with a dose of 10 mg/kg on day 1, 2 and 7. In addition, amoxicillin-clavulinic acid or enrofloxacin was administered at the same time. Bitches in diestrus with progesterone levels above 3.2 nmol/l and with normal ovarian function, showed a successful treatment result. A rapid improvement of the general condition was also seen and the increased vulvar discharge was observed shortly after administrating the drug. 21 of the 22 successfully treated bitches could be further examined during an observation period of 14 months. Only one of these developed pyometra again. The other bitches on the other hand, showed signs of normal cyclicity and two were later bred. No side effects were observed.

Trasch *et al*., (2003) investigated the efficacy of aglepristone in 52 bitches diagnosed with pyometra. The treatment protocol used during this study was the same as the classical treatment protocol introduced by Hoffmann *et al*, (2000). 10 mg/kg was given subcutaneously on day 1, 2 and 7, together with antibiotic treatment, either amoxicillin clavulinic acid or enrofloxacin, for a minimum of seven days. Before initiating the treatment protocol, a general physical examination was performed, in addition to checking the blood parameters. Uterine rupture was also excluded in all bitches with ultrasonography before initiating the conservative treatment. Of the 52 bitches included in the study, four bitches did not undergo treatment, due to findings observed after admittance. One bitch died due to acute renal failure, one bitch was in an extremely poor condition and two bitches had too high renal parameters. Eight patients out of the 52 were diagnosed with a closed-cervix pyometra.

The result of this study showed that the recurrence rate of pyometra depends on the time period after administrating the drug. Within 21 days, 48 out of the 52 bitches treated had a complete recovery, which corresponds to a 92.3% short-term success rate. Within 90 days after the treatment, recurrence occurred in four bitches (9.8%). In these bitches, OHE was performed. In three of the bitches that underwent surgery, cystic changes of the ovaries and the endometrium were observed. This indicated that the result of the treatment might be unsuccessful in patients with hormonal abnormalities prior to the administration of the drug. One year after treatment seven bitches suffered from recurrence of pyometra. With a long-

term recurrence rate of 18.9%, it appears that this type of treatment was not as successful in the long term. After completing the study, the reproductive status was only available in 37 bitches. In 22 bitches the next heat started in the normal time period. However, five of these animals developed pyometra in the following diestrus period. A prolonged interestrus interval was observed in 20% of the cases, where the following heat was delayed with six months. Out of these, one bitch developed pyometra. The fertility after treatment proved to be successful, with a whelping rate of 83.4%, where six bitches were mated and delivered healthy litters. In addition, this study revealed an interesting finding. The presence of cystic changes of the ovaries and endometrium was observed, which indicates that there might be a connection between the efficacies of the drug if given to a bitch with a hormonal imbalance prior to treatment. Before initiating the treatment of aglepristone, bitches with cystic appearance of the ovaries and endometrium should be excluded, so that the efficacy of treatment is improved. It is also worth mentioning that all bitches with a closed cervix pyometra were successfully treated.

Ros *et al.*, (2014) performed a retrospective study during a 9-year period, focusing on the long-term outcome after administrating aglepristone in 28 bitches diagnosed with pyometra. 10 mg/kg was administered subcutaneously, combined with antibiotics such as amoxicillin clavulinic acid or enrofloxacin. Aglepristone was given on day 1 and 2, thereafter on day 7 to 8, 14 to 15 and every 7th to 8th day until the end of treatment. All bitches showed signs of vaginal discharge before treatment, which indicated an open cervix pyometra. Out of the bitches treated, 75% (21/28) had a successful recovery. Throughout the study seven bitches did not recover, six of which underwent OHE and one was euthanized. Cystic ovaries were observed during ultrasonography in the bitches before and after treatment, but did not affect the success rate as suggested in other studies (Trasch *et al.*, 2003). The bitches that were still intact at the end of the study were continuously examined from 1.5 to 6 years.

During this time period 48% (10/21) of the successfully treated bitches showed recurrence of pyometra. Based on the age of the bitches, the recurrence rate was greater in bitches older than 5 years (86%), compared to the bitches younger than 5 years. Out of the 10 bitches, seven were ovariohysterectomized or euthanized. 17 to 72 months after the first treatment, the remaining three bitches also had a secondary recurrence. The bitches' reproductive status after the treatment showed a pregnancy rate of 69%. This indicates that the fertility of the bitches was not affected on a long term. Side effects including loss of appetite, anxiety,

polydipsia, diarrhea, abdominal cramps and panting were also observed during the study. In conclusion, the medical treatment with aglepristone in combination with antimicrobial therapy was successful in 75% of the bitches, with a recurrence rate of 48%. The long-term result of the study showed that out of the 28 bitches treated, eight bitches remained intact, 11 bitches were ovariohysterectomized and nine was euthanized.

Contri *et al*., (2015) recently introduced a study focusing on the effectiveness of a more modified treatment option for pyometra. 73 bitches diagnosed with pyometra were divided into two test groups, where two different treatment protocols were provided. 26 bitches were assigned to the classical treatment group, where four subcutaneous injections of 10 mg/kg, were administered at day 1, 2 and 7. 47 bitches were assigned to the modified treatment group, in which the same dosage was given at day 1, 3, 6 and 9. In addition amoxicillin-clavulinic acid was given for 5 consecutive days. Within 24 hours after administrating the drug, most animals showed an improvement in the general condition. Vaginal discharge was present in 44.7% of all the bitches on the day on diagnosis, in 93% of the bitches after 24 hours and in 100% after 36 hours. This correlates with the significant decrease in the size of the uterine lumen and horns at day seven. Both groups showed recovery within 14 days. Bitches treated with the classical treatment protocol had a success rate of 88.5% (23/26) Two of the 26 bitches underwent OHE, and one bitch got one more administration of the drug, which further led to a complete healing. Four bitches showed signs of recurrence of pyometra, (17.4%) either at the first or second estrus after treatment. Bitches treated with the modified treatment group had a 100% success rate, and no signs of recurrence were observed within 24 months after treatment.

This indicates that the bitches treated with the modified treatment group had a higher success rate and a lower risk of developing pyometra again, compared to the classical treatment protocol. When examining the bitches 12 months after finishing the treatment, the reproductive status of the bitches treated with the classical treatment protocol revealed that 19 out of the 23 bitches showed signs of estrus. Seven of these bitches were mated, and had a subsequent pregnancy rate of 85.7%. 42 out of the 47 bitches treated with the modified treatment protocol showed signs of estrus, and 23 of these were mated, with a following pregnancy rate of 78.3%. This indicates that the pregnancy rate in both treatment groups were acceptable. Furthermore, both groups had a reduced interestrus interval. The results of this study confirm the previously reported findings for the classical treatment method, (Trasch *et*

al., 2003) although better results were recorded during this study when using the modified treatment protocol.

Jurka *et al*., (2008) evaluated the efficacy of aglepristone for the treatment of pyometra in bitches with different ages, ranging from 8 months to 9.5 years. 24 bitches were treated with 10 mg/kg subcutaneously on day 1, 2, 7 and 14 in addition to the administration of amoxicillin clavulinic acid for seven days. The bitches were separated into two groups according to their age. Group I included animals up to 5 years of age and group II consisted of bitches older than 5 years of age. The results were assessed based on the clinical recovery, recurrence and fertility after treatment. The short-term success rate within 21 days, in both young and old bitches was 100%. During the two following estrous cycles, ranging from 6 to 12 months, the animals were examined to look for the recurrence of pyometra. The recurrence rate of younger bitches was 0%, compared the bitches in the older group, where 30% had a recurrence of pyometra. These bitches underwent OHE, where ovarian cysts and cystic endometrial hyperplasia were observed during the operation. The length of the interestrus period in both groups was shortened in some of the animals. The fertility of the bitches was observed up to 54 months after treatment, and all bitches were naturally mated during the first estrus. All the young bitches in group I were mated, where eight had a full term pregnancy, which correlates to a pregnancy rate of 57.1%. None of the bitches that were mated in group II became pregnant. The result of the study showed a clear correlation between the success rate and the age of the animal. The risk of developing pyometra again after treatment was higher in bitches over 5 years of age compared to the bitches less than 5 years of age.

As a conclusion, based on studies performed during the past several years, it has been shown that the efficacy and success rate when using aglepristone as the single drug in the treatment of pyometra, may vary based on several factors that have been shown to influence the efficacy of the treatment. Such factors include the age of the patient and the presence of cystic ovaries and endometrium. The result of the treatment might be ineffective in patients where hormonal imbalance is present prior to the administration of the drug. Excluding the bitches with ovarian cysts and cystic endometrial hyperplasia may therefore minimize the recurrence of pyometra. However the presence of ovarian cysts is not easy to detect, even with ultrasonography. Furthermore the differentiation of estrogen producing cysts from non-pathologic cysts is impossible (Verstegen *et al*., 2008). A summary of the most significant studies investigated can be seen in table 6.

AUTHOR	PROTOCOL	NUMBER OF TREATED BITCHES	SUCCESS RATE (n/%)	TIME FOR RECOVERY (Days)	RECURRENCE RATE (%) + follow-up period
Breitkopf *et al*., (1997)	5-6 mg/kg Day 1, 3, 4, 8 and 12	7 Open: 2 Closed: 5	**100%** (7/7)	28	**0%** -
Hoffmann *et al*, (2000)	10 mg/kg Day 1, 2 and 7	22	**100%** (22/22)	-	**4.55 %** 14 months
Trasch *et al*., (2003)	10 mg/kg Day 1, 2 and 7	52	**92.3%** (48/52)	21	**18.9%** (n=37) 1 year
Ros *et al*., (2014)	10 mg/kg Day 1, 2, 7 or 8, 14, 15 + every 7th to 8th day	28 Open: 28	**75%** (21/28)	-	**48%** (n=21) 9 years
Contri *et al*., (2015) **Classical**	10 mg/kg Day 1, 2 and 7	26 Open: 12 Closed: 14	**88.5%** (23/26)	14	**17.4%** (n=23) 24 months
Contri *et al*., (2015) **Modified**	10 mg/kg Day 1, 3, 6 and 9	47 Open: 20 Closed: 27	**100%** (47/47)	14	**0%** (n=47) 24 months
Jurka *et al*., (2008)	10 mg/kg Day 1, 2, 7 and 14	24	**100%** (24/24)	21	<5 years: **0%** (n=14) >5 years: **30%** (n=9)

Table 6: A summary of all significant studies concerning the administration of aglepristone in bitches with pyometra. (Breitkopf et al., 1997; Hoffmann et al, 2000; Trasch et al., 2003; Jurka et al., 2008; Ros et al., 2014; Contri et al., 2015)

Common for all studies was the improvement in general condition of all the bitches, due to the rapid uterine emptying within 4 to 38 hours after the first injection. The conservative treatment with aglepristone did not seem to affect the subsequent fertility. Several long-term follow up periods reported pregnancy rates from 69% to 85%, followed by the whelping of healthy litters. However age seems to influence the success of mating after medical treatment with aglepristone. Jurka *et al.*, (2008) argued that age seems to influence the success of fertility after mating, as none of the bitches older than 5 years became pregnant, compared to the 57% pregnancy rate seen in the bitches younger than 5 year.

8.4.2 *Aglepristone in combination with cloprostenol*

The use of aglepristone as the primary drug in the treatment of pyometra has been investigated in several studies. Although these studies have shown to be successful, the drug's ability to induce effective uterine contractions when administered alone, is still not fully understood, as aglepristone is not expected to induce myometrial contractions. It has therefore been suggested to combine the drug with prostaglandins to induce stronger uterine contractions and to promote a more sufficient uterine emptying (Verstegen *et al.*, 2008). When combining aglepristone and prostaglandins a synergic effect is expected. The antiprogestin action of aglepristone and the luteolytic and uterotonic action of PGF2α is thought to lead to a more effective result. These results have been reported in a few studies, and will be summarized below (Gobello *et al.*, 2003; Fieni, 2006; Thirumurugan *et al.*, 2011).

Gobello *et al.*, (2003) evaluated the efficacy and safety when combining aglepristone and cloprostenol for the treatment of open cervix pyometra. 15 bitches were assigned into two treatment groups. In group I, eight bitches were injected subcutaneously with 10 mg /kg aglepristone on days 1, 3, 8 and 15 if necessary. In addition 1 μg/kg of cloprostenol was administered subcutaneously, followed a period of fasting, on day 3 and 8. In group II, seven bitches got the same treatment of aglepristone as in group I, and the same dosage of cloprostenol was given. However cloprostenol was administered on days 3, 5, 8, 10, 12 and 15 if necessary. Amoxicillin clavulinic acid and fluid therapy was given together with the drugs. The success rate of both treatment protocols was 100%. All of the 15 bitches recovered either after 15 (10/15) or 29 (5/15) days. No local or systemic side effects were observed, and vulvar discharge increased in all bitches within 24 to 48 hours after the first administration of aglepristone. The recurrence rate at the next estrus occurred in 3/15 bitches (21%), which is

higher than the 13% recurrence rate, later reported by Fieni, (2006) Although further work is necessary to determine the optimal administration when combining these drugs, the study proved that the combination was efficient and safe in the treatment of open-cervix pyometra.

Fieni, (2006) also evaluated the safety and efficacy when using aglepristone alone or in combination with low doses of cloprostenol. The study involved 67 bitches with metritis (15) and bitches with open or closed cervix pyometra (52). The bitches were divided into three groups based on their clinical presentation. Group I, included 15 bitches with metritis. These bitches only showed signs of vaginal discharge, but no enlargement of the uterine lumen was detected when performing ultrasonography. Group II included 35 bitches with open-cervix pyometra, where vaginal discharge and enlargement of the uterine lumen was present. Group III included 17 bitches with closed cervix-pyometra, where an enlargement of the uterine lumen was present, but no vaginal discharge was seen. Group I was injected with 10 mg/kg of aglepristone once daily on days 1, 2 and 8. In addition to this treatment, 1 μg/kg of cloprostenol was injected subcutaneously daily from day 3 to 7, in groups II and III. If pyometra did not resolve, additional doses of aglepristone were administered on day 14 and day 28. All bitches were examined on days 8, 14, 28 and 90. In addition, follow-up data was available in 23 bitches after 24 months following the beginning of treatment. The long-term recovery rate could therefore be evaluated in this study, in comparison to the short-term recovery rates reported by Gobello *et al*., (2003) and Thirumurugan *et al*., (2011). The recurrence rate of pyometra after 12 months was 13% (3/23) and 19% (4/21) after 2 years. At the first estrus after treatment, five bitches were mated and four conceived. No side effects were reported during this study.

When combining aglepristone and cloprostenol, the success rate at day 90 significantly improved compared to when aglepristone was used alone. In the bitches where aglepristone and cloprostenol was administerd together the success rate was 84.4% (54/67). In comparison to 60% (12/20) when the bitches were treated with aglepristone alone. This indicated that the additional administration of low doses of cloprostenol may significantly increase the success rate, and that the drug's uterotonic activity will lead to a faster decrease in the diameter of the uterine lumen. In addition its luteolytic activity may cause a rapid decrease in the mean plasma progesterone concentration, which in turn is responsible for opening the cervix. Bitches with metritis, treated with aglepristone alone showed a 100% recovery rate within 90 days. Furthermore the drug induced cervical opening within 48 hours, in the bitches with

closed-cervix pyometra. This indicates that aglepristone may be used effectively in the treatment of metritis and that the drug may lead to the opening of closed cervix pyometras.

Thirumurugan *et al*., (2011) investigated the efficacy of different drugs in the conservative treatment of pyometra in 18 bitches with open cervix pyometra. The bitches were randomly assigned into three groups, with 6 bitches in each group. Group I was injected with 5 μg/kg PGF2α, subcutaneously once daily. Group II got injections with aglepristone at 10 mg/kg subcutaneously on day 1, 2 and 7. Group III was injected subcutaneously with 10 mg/kg of aglepristone in combination with 1 μg/kg of prostaglandins, on day 1, 2 and 7. In addition group III and I got another dose of aglepristone on day 14 if the bitches had not recovered yet. All groups were treated with antibiotics and fluids, and were regularly examined during a follow-up period of two months. Any observed recurrence after this time was not reported. A summary of the results of the study can be seen below (Table 7).

Treatment group	Total number of bitches	Day 6	Day 7	Day 8	Day 10	Day 21	Treatment response on day 21
Group I	6	-	1 (16.66%)	3 (50%)	2 (33.33%)	-	(6/6) 100%
Group II	6	-	-	3 (50%)	-	3 (50%)	(6/6) 100%
Group III	6	1 (16.66%)	1 (16.66%)	3 (50%)	-	1 (16.66%)	(6/6) 100%

Table 7: Treatment response in bitches treated for open cervix pyometra in different treatment groups (Thirumurugan et al., 2011)

The recovery rate of the drugs was considered based on a 21 days period, and the results varied based on which treatment option had been used. In group I, a 100% success rate was reported within ten days. One bitch recovered on day seven (16.66%), three bitches showed complete healing on day 8 (50%) and 2 bitches recovered within ten days (33.33%). In group II, all bitches recovered within 21 days. 50% (3/6) recovered by day eight, and the rest recovered by day 21. Bitches from group III also showed a 100% success rate within 21 days (Table 3). Side effects including panting, salivation, vomiting, defecation and urination, were reported in both groups where prostaglandins was injected. However they showed a tendency to decrease from day 4 to 9 after treatment. In the 12 bitches treated with aglepristone, one bitch had a local swelling at the site of injection, which disappeared within a week.

As a conclusion, the combination of aglepristone and cloprostenol seems to be more effective in the medical treatment of open and closed cervix pyometra than with aglepristone alone. The results of these studies showed that the success rate varied from 80.6% to 100%. The recurrence rate also differed from other studies where aglepristone was the only drug used. Fieni, (2006) reported a 0% recurrence rate at day 90 when combining the two drugs, compared to Trasch *et al.*, (2003) who reported a recurrence rate of 9.8% for the same time interval when using aglepristone alone. However the presence of cystic ovaries and endometrium was observed in these bitches, and may explain the recurrence rate reported in this study.

Furthermore, Fieni, (2006) had repeated administrations until day 28 if needed, whereas Trasch *et al.*, (2003) only administered three doses of aglepristone on day 1, 2 and 7. In order to evaluate the recurrence rate, more long-term studies should be carried out, as the majority of these studies focused on the short-term recovery rate. Even though the use of prostaglandins is normally associated with side effects, the low doses (1μg/kg) used in the studies combining aglepristone and cloprostenol seemed to have a positive correlation, since no severe side effects were reported. A summary of all the studies can be seen in table 8.

AUTHOR	NUMBER OF TREATED BITCHES	PROTOCOL	SUCCESS RATE (%/N)	TIME FOR RECOVERY (Days)	RECURRENCE RATE (%)
Gobello *et al.*, (2003)	GROUP I: 8	Cloprostenol: 1μg/kg Day 3 and 8	**100%** (8/8)		
	GROUP II: 7	Cloprostenol: 1μg/kg Day 3, 5, 8, 10, 12 ± 15 + Aglepristone: 10mg/kg Day 1, 3, 4, 8 and 12 ± 15 and	**100%** (7/7)	15-29	**21%** (n=15)
Fieni, (2006)	32	Aglepristone: 10mg/kg Day 1, 2, 8 and 12 ± 14 ± 28 Cloprostenol: 1μg/kg Day 3, 4, 5, 6 and 7	**84.4%** (27/32)	14-90	12 months: **13%** (n=3) 2 years **19 %** (n=21)
Thirumurugan *et al.*, (2011)	6	Aglepristone: 10 mg/kg Day 1, 2 and 7 ± *14* Cloprostenol: 2μg/kg Day 1, 2, 7 ± *14*	**100%** (6/6)	8-21	-

Table 8: A summary of all studies where aglepristone and cloprostenol was administered in bitches with pyometra (Gobello et al., 2003; Fieni, 2006; Thirumurugan et al., 2011)

8.4.3 Agleptistone and intrauterine antibiotics

Gurbulak *et al*., (2005) performed a study comparing the efficacy of bitches treated with aglepristone alone or in combination with intrauterine antimicrobials. 24 bitches were divided into two groups. In the first group, 13 bitches, 7 with open and 6 with closed-cervix pyometra, were given 10 mg/kg of aglepristone on day 1, 2, 7 and 14. The success rate after treatment was 46.1% (6/13). Of these, one bitch with closed-cervix pyometra recovered. In the second group, 11 bitches were given the same dose of aglepristone in combination with intrauterine antibiotics on day 1, 2, 4, 6 and 8. Of these bitches, 6 presented with an open-cervix pyometra, and 5 with a closed-cervix pyometra. The success rate was 81.8% (9/11). Of these, 4 bitches with closed cervix pyometra recovered. Even though the recovery process was more difficult in bitches with closed cervix pyometra in both groups, the study showed that these bitches, when treated with aglepristone combined with intrauterine antimicrobials, had a higher success rate, compared with the bitches with closed cervix pyometra only treated with aglepristone.

The results of this study suggest that bitches treated with aglepristone and intrauterine antimicrobials had a quicker and higher recovery rate. In addition the patency of the cervical canal also affected the success rate of therapy. Bitches from group two with a closed cervix pyometra, had a higher success rate than those in group one. This suggests the use of intrauterine antimicrobials may result in a better treatment result. Furthermore 58.3% (14/24) bitches examined showed signs of estrus and three bitches from group two became pregnant after the end of the study.

9. Dopamine agonists

Dopamine agonists are a group of drugs with anti-prolactin effects. The two main drugs are bromocriptine and cabergoline. Cabergoline (Galastop) is the drug of choice, as it has less side effects and needs fewer administrations compared to bromocriptine (Corrada *et al.*, 2006; Verstegen *et al.*, 2008). Dopamine agonists are ergotine-derivative alkaloid compounds that have a direct effect on the D2-dopamine receptors of the lactotrophic cells of the anterior pituitary gland. This direct effect inhibits the secretion of prolactin (PRL). When the secretion of PRL is stopped, the milk production is inhibited, which makes cabergoline the drug of choice for bitches with pseudopregnancy (Gobello, 2006). Furthermore, prolactin is the most important luteotropic hormone regulating the corpus luteum, during the second half of the canine luteal phase (Corrada *et al.*, 2006). Using cabergoline will inhibit PRL and the luteal action of progesterone, making the drug useful in the treatment of pyometra and for pregnancy termination (Gobello, 2006). Due to prolactin's luteotropic support, the hormone is necessary to maintain pregnancy in dogs. With repeated administrations of cabergoline for more than 25 days after ovulation, a rapid reduction in the plasma progesterone concentration may be seen, which further leads to the termination of any unwanted pregnancy (Onclin *et al.*, 1993).

9.1 Cabergoline in combination with cloprostenol

The combination of cabergoline and prostaglandins is an option when treating pyometra conservatively. This combination enhances the luteolytic effect of each drug, which results in a rapid luteolysis and a decline in the serum progesterone concentration (Verstegen *et al.*, 2008). When combining these drugs, lower doses of prostaglandins can be administered, which has shown to be an important factor in reducing unwanted side effects. Furthermore, the uterotonic effect of prostaglandins helps to promote uterine emptying (Corrada *et al.*, 2006). Below follows three studies where cabergoline and cloprostenol where administered in bitches with pyometra (See the summary in table 9).

Corrada *et al.*, (2006) tested the efficacy and safety when administrating cabergoline and cloprostenol in bitches suffering from pyometra. 29 bitches, either with endometritis (15) or pyometra (14) was included in the study. All bitches presented with vaginal discharge, which indicated an open-cervix pyometra. During a time period of seven days, all bitches were

treated once daily with 5μg/kg of cabergoline orally and 1μg/kg of cloprostenol subcutaneously. In addition to supportive therapy of oral antibiotics, cloprostenol was given in additional doses until day 14 if no response to the treatment was observed. Within seven days, 69% (20/29) of the bitches had recovered. The success rate increased by day 14, when 83% (24/29) of the bitches showed complete recovery. The five remaining bitches that failed to respond to the medical treatment underwent OHE on the same day. The vulvar discharge increased within 36 to 48 hours, similar to what's been previously reported by Fieni, (2006). During the following estrus, recurrence of pyometra was observed in 25% (6/24) of the bitches treated. Two bitches were mated, out of which one conceived. This confirms that it is possible to maintain fertility after this kind of treatment. Side effects, including vomiting and diarrhea, were observed in 31% (9/29) of the bitches after being treated with cloprostenol. The low doses of cloprostenol used during the study are most likely responsible for the mild side effects observed.

In one of the previously mentioned studies, Jena *et al.* (2013b) proved that the combination of cabergoline and cloprostenol could be used as an effective treatment option in the conservative therapy of pyometra. seven bitches were treated with 5μg/kg of cabergoline PO and 1μg/kg of cloprostenol subcutaneously, once daily for seven days. The high success rate (100%) and low recurrence rate (29%) indicates that this type of treatment might be effective in the treatment of pyometra. Although some mild side effects were observed in up to 43% of the bitches, including vomiting, panting, restlessness and hyperpnoea, the overall results of this study shows that this type of treatment was safe to use in bitches with pyometra. All of the bitches came into estrus out of which five conceived after mating. This indicates that the fertility after treatment was present.

England *et al.*, (2007) presented one of the most recent results regarding the efficacy when combining cabergoline and cloprostenol. 22 bitches were included in the study, where three presented with closed and 19 with open-cervix pyometra. 16 of the bitches presented with high plasma progesterone concentrations, whereas six of the bitches presented with low circulating concentrations of progesterone and all bitches showed typical clinical signs. All bitches were treated once daily for ten days with 5μg/kg cabergoline PO and 5μg/kg cloprostenol every third day subcutaneously. Potentiated sulphonamides were given twice a day during the duration of the treatment. 19 of the bitches had a complete recovery by day nine. These bitches got the last doses of cabergoline on day ten and cloprostenol on day nine

as planned. Two bitches required further treatment until day 13 and one bitch underwent surgery due to ineffective treatment on day 12. By day three, a rapid clinical improvement was reported in all bitches. An increase in vulvar discharge was observed and the progesterone concentration had decreased in all bitches. After administrating cabergoline, no side effects were reported. However, after the first injection of cloprostenol, all bitches showed signs of retching or vomiting, mild abdominal straining, diarrhea and panting. The incidence of side effects was reduced after each successive dose. After the second treatment 77% (17/22) and after the third injection 41% (9/22) of the bitches showed signs of side effects. After the first estrus following treatment, recurrence rate of pyometra was 18% (4/22), which is lower compared to the results reported by Corrada *et al*., (2006) and Jena *et al*. (2013b). At the next estrus, 11 bitches were mated and 64% (7/11) produced healthy litters.

In conclusion, the synergic effect of cabergoline and cloprostenol showed to be effective in rapidly terminating the luteal phase and promoting uterine evacuation. The success rate varied from 83% to 100%, which indicates that this type of treatment is effective and safe in the conservative treatment of pyometra. In two studies (Corrada *et al*., 2006; Jena *et al*., 2013b) where low doses of cloprostenol (1µg/kg) were administered for seven days, mild side effects were observed in 31% to 43% of the bitches, and the recurrence rate varied between 25-29%. England *et al*., (2007) on the other hand administered higher doses of cloprostenol (5µg/kg) for nine days, which resulted in more severe side effects, but a lower risk of recurrence (18%). The side effects were however reduced after subsequent injections. This confirms what Verstegen *et al*., (2008) recommended, when it comes to the dose dependent administration of prostaglandins.

These findings indicate that even though low doses of cloprostenol are administered to help reduce side effects, there might be a correlation between the low doses of prostaglandins and the higher recurrence rate. The conception rate in the studies was not influence by the treatment, as several bitches produced healthy litter. This shows that it is possible to maintain fertility after the treatment of cabergoline and cloprostenol. The results of this study shows that the treatment might be useful for conserving the future breeding capacity of bitches and that it might be useful in stabilizing bitches with high anaesthetic risk prior to surgery.

AUTHOR	NUMBER OF TREATED BITCHES	PROTOCOL	SUCCESS RATE (n/%)	RECOVERY TIME (days)	RECURRENCE RATE (%)	SIDE EFFECTS
Corrada et al., (2006)	29 Open: 29	Cabergoline: 5μg/kg, PO Cloprostenol: 1μg/kg, SC Once daily, for 7 days	83% (24/29)	14	25% (n=6)	31% (Mild)
England et al., (2007)	22 Open: 19 Closed: 3	Cabergoline: 5μg/kg, PO Once daily for 10 days Cloprostenol: 5μg/kg, SC Every third day	95% (21/22)	9-13	18% (n=4)	41-77% (Mild)
Jena et al., (2013b)	GROUP III: 7	Cabergoline: 5μg/kg, PO Cloprostenol: 1μg/kg, SC Once daily for seven days	100% (7/7)	7	29%	43% (Mild)

Table 9: A summary of all studies where cabergoline and cloprostenol was administered in bitches with pyometra. (Corrada et al., 2006; England et al., 2007; Jena et al. 2013b

10. Discussion

Based on this literature review, the conservative treatment of pyometra has shown to be an effective way of treating both open and closed-cervix pyometras. When treating pyometra with PGF2α, the effectiveness varied based on the type and dose of prostaglandins used. When comparing the administration of natural and synthetic prostaglandins, Jena *et al*., (2013b) found that the side effects and recurrence rate were in correlation with the dose administered. When injecting high doses of natural prostaglandins (100 μg/kg) and low doses of synthetic prostaglandins (1μg/kg) the recurrence rate varied between 43% and 86% respectively. However, bitches treated with high doses of natural prostaglandins presented with more severe side effects, compared to the non-existing side effects reported when using low doses of synthetic prostaglandins. These findings suggest that the administration of prostaglandins are dose dependent, and that a high recurrence rate, less side effects are expected when administrating lower doses of synthetic prostaglandins, such as cloprostenol (Table 10). Jena *et al*., (2013b) made the same discovery when combining cabergoline and low doses of cloprostenol in the same study. Mild side effects and high recurrence rate (29%) were reported, compared to more severe side effects and a low recurrence rate (18%) when using higher doses of cloprostenol (England *et al*., 2007).

	GROUP I – NATURAL PGF2α	GROUP II – SYNTHETIC PGF2α	GROUP III - CABERGOLINE
Recovery rate (%)	100 (7/7)	100 (7/7)	100% (7/7)
Conception rate (%)	57.15	14.28	71.43
Recurrence rate (%)	42.85 (3/7)	85.72 (6/7)	28.57 (2/7)
Side effects	Severe	None	Mild

Table 10: Summary of the efficacy when treating bitches with natural and synthetic prostaglandins, in addition to cabergoline (Jena et al., 2013b)

When Gobello *et al*., (2003) and Fieni, (2006) combined low doses of cloprostenol with aglepristone, less severe side effects were reported and no recurrence of pyometra was observed. The use of aglepristone, either alone (Trasch *et al*., 2003) or combined with cloprostenol (Fieni, 2006) showed the most effective results and the least side effects, in the conservative treatment of pyometra. When using the classical treatment protocol of aglepristone, three doses were given on day 1, 2 and 7 (Trasch *et al*., 2003) or 8 (Fieni, 2006), which showed variable effectiveness (92.3% and 60% respectively). When adding cloprostenol, the success rate at day 90 increased from 60% to 84.4% (Fieni, 2006). In addition, no signs of recurrence was reported by day 90, compared to Trasch *et al*., (2003) who reported a recurrence rate of 9.8% for the same time interval when using aglepristone alone. However, the presence of cystic ovaries and endometrium were detected during the study, which might explain the less successful result.

Furthermore, Fieni, (2006) administered additional doses until day 28 if needed, whereas Trasch *et al*., (2003) only administered three doses of aglepristone on day 1, 2 and 7. These findings argue that the additional administration of low doses of cloprostenol in addition to the repeated administration of aglepristone may considerably increase the success rate and efficacy of the treatment. In order to further evaluate the efficacy of the drugs used in the conservative therapy of pyometra, additional long-term studies should be carried out, as the majority of the studies examined only focused on the short-term recovery rate of the disease. It is likely that the recurrence rate would be higher in these studies had they been followed for a longer time period. The result obtained by Ros *et al*., (2014) confirms this, where a high recurrence rate of 48% was reported during a time period up to 6 years. Despite results of this study reporting the lowest success rate (75%), it is more informative compared to studies where only the short-term success rate was investigated, since it gives a more correct picture regarding the risk of recurrence of the disease.

The successful treatment with aglepristone has been demonstrated in diestrus bitches selected with a progesterone concentration above 3.2 nmol/l. By reducing the plasma progesterone level, a subsequent recovery was detected (Breitkopf *et al*., 1997; Hoffmann *et al*, 2000). However, recent studies have shown the same result when administrating aglepristone in bitches with low circulating progesterone concentrations. These findings suggest that the decrease in intrauterine progesterone levels is determined based on the sensitivity and numbers of P4 receptors, and not based on the circulating plasma progesterone concentration

(Fieni, 2006; Gobello *et al*, 2013). Recently, Contri *et al*., (2015) compared the efficacy between the classical treatment of aglepristone with a new, modified treatment protocol. In the modified treatment protocol, where four doses of aglepristone were administered, the success rate significantly increased, compared to what was previously reported in the classical treatment protocol. (Trasch *et al*., 2003; Fieni 2006) The modified protocol showed a 100% efficacy, compared to the 87% efficacy when the classical protocol was used. In addition, the recurrence rate of the modified protocol after 24 months was not associated with any relapses, suggesting that this type of treatment has a higher long-term efficacy.

Based on previous literature, this retrospective study found that several factors may influence the success rate of the drugs used in the treatment of pyometra. Such factors included the age of the patient, where the chance for a successful treatment seemed to be lower and the risk for recurrence higher in bitches older than 5 years. Jurka *et al*., (2008) Furthermore, the importance of the open or closed-cervix in the success rate of therapy needs further research, as the number of animals used in the studies was too low to prove the sufficient and safe emptying of the uterus with the different drugs. In addition, the presence of cystic ovaries or endometrium should be ruled out, and the selection of candidates for each study should focus on bitches with a better general condition for the most effective treatment result for pyometra.

There might be a correlation between when the first signs of symptoms began and when treatment was initated. Bitches treated early in the course of the disease might have a better chance of recovering, compared to the bitches treated later on. Furthermore, some studies only included a low number of bitches, and is therefore not representative enough. As the study might give a false high or a false low result depending on the individuals selected for the study, the results of these studies should be evaluated with caution. The studies that showed the most significant results are presented in table 11.

AUTHOR	NUMBER OF TREATED BITCHES	PROTOCOL	SUCCESS RATE (%/n)	TIME FOR RECOVERY (Days)	RECURRENCE RATE (%) + Long term follow-up period
Gobello *et al*., (2003)	GROUP I: 8	**10 mg Aglepristone + Cloprostenol**: 1µg/kg Day 3 and 8	**100%** (8/8)	15-29	**21%** (n=15)
	GROUP II: 7	**Cloprostenol**: 1µg/kg Day 3, 5, 8, 10, 12 ± 15	**100%** (7/7)		
Fieni, (2006)	32	**Aglepristone**: 10mg/kg Day 1, 2, 8 and 12 ± 14 ± 28 **Cloprostenol**: 1µg/kg Day 3, 4, 5, 6 and 7	**84.4%** (27/32)	14-90	90 days: **0%** 12 months: **13%** (n=3) 2 years: **19 %** (n=21)
Trasch *et al*., (2003)	52	**Aglepristone**: 10 mg/kg Day 1, 2 and 7	**92.3%** (48/52)	21	90 days: **9.8%** 1 year: **18.9%** (n=37)
Ros *et al*., (2014)	28 Open: 28	**Aglepristone**: 10 mg/kg Day 1, 2, 7 or 8, 14, 15 + every 7th to 8th day	**75%** (21/28)	-	9 years: **48%** (n=21)
Contri *et al*., (2015)	**AGLEPRISTONE:** 26 Open: 12 Closed: 14	**CLASSICAL:** 10 mg/kg Day 1, 2 and 7	**87%** (23/26)	14	2 years: **17.4%** (n=23)
	47 Open: 20 Closed: 27	**MODIFIED:** 10 mg/kg Day 1, 3, 6 and 9	**100%** (47/47)		2 years: **0%** (n=47)

Table 11: Summary of the most significant studies when treating bitches with pyometra (Gobello et al., 2003; Trasch et al., 2003; Fieni, 2006; Ros et al., 2014; Contri et al.,

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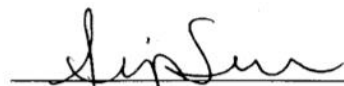
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