DISCUSSION
V. DISCUSSION

5.1. Incidence of pyometra.

Analysis of clinical data from Jan 2000 to December 2008 indicated a population incidence of 11.23% which was based on the hospital canine attendance record. Varying percentage of incidence of pyometra ranging from 0.7 to 9 % has been reported (Stone et al.1988; Ewald 1961 and Arthur 1996) which is very lower compared to present study. However, Fakuda (2001) reported pyometra up to 15.2% of bitches in a beagle colony and Egenvall et al (2001) and Hagman (2004) reported pyometra in 25% of bitches by 10 years of age.

Highest incidence of 16.51 percent was recorded in the year 2008 and lowest of 5.07 percent in 2000. A gradual increase in the incidence of pyometra was observed from 2005, (Table I) and the probable reasons for the same could be due to increased population of dogs, breeding of bitches for commercial purposes and also due to more awareness about the gynaeco pathological problems of bitches among the owners.

5.1.1. Age wise incidence

Pyometra in the present study was found to be a disease primarily affecting the middle aged to older bitches and the mean age was 7.21± 2.34 years which is in conformity with the reported mean age of 7.25 years by Johnston et.al. (2001).Nearly 66 percent of the animals diagnosed to be suffering from pyometra were over five years of age. (Table II). Other studies (Dow, 1958; 1959; Krook et al. 1960 ; Wheaton et al.,1989;Sevelius et al.,1990 and Niskanen and Thursfield,1998) have also recognized
pyometra to be primarily a disease of middle aged bitches with a mean age of 6-8 years. The greater susceptibility of middle aged and older bitches to pyometra has been attributed to the repetitive exposure to the normal long luteal phase of the estrous cycle (Niskanen and Thrusfield, 1998).

However, pyometra was also observed, although less frequently (7.006%) in animals aged less than two years. Nelson and Feldman (1986), Wheaton et al. (1989) and Bowen et al. (1985), suggested that the hormonal therapies such as progestins for estrus suppression or estrogens for estrus induction or to prevent pregnancy may have a correlation in the development of disease in young animals.

Wheaton et al. (1989) described pyometra in eight bitches under three years of age, five of which had received oestradiol cypionate within six months before diagnosis. Bowen et al. (1985), and Niskanen and Thrusfield (1998) concluded that estrogen is an important factor for the development of pyometra in young animals and that it is probably due to the progesterone priming effects of estrogen. However, in the present study 7.006% of animals in which pyometra was diagnosed were aged under two years with no medical history of previous hormonal treatment suggesting that pyometra can occur even in younger animals inspite of absence of hormonal treatment. It is probable that pyometra in younger animals may be associated with an abnormal response of the uterus to a prolonged progesterone phase rather than repetitive exposures experienced as the bitch gets older.
5.1.2. Breed wise incidence.

Breeds with high risk of pyometra in the present study were Pomeranian followed by German Shepherd, non-descript, Labrador, Spitz and Dalmatian. and Breeds with low risk of pyometra were Golden Retriever, Great. Dane, Doberman, Boxer; Dachshund, Pug, Cocker spaniel, Lhasa apso, Neapolitan Mastiff, Saint Bernard, English bull dog, and Weimerner (Table III).

Predisposition to pyometra in certain breeds had been occasionally reported in the literature. Ewald (1961) recorded a relatively high breed specific prevalence in Collie and Belgium Shepherd dog and low prevalence in Dachshund and Poodle, but no formal statistical procedures were applied to the data. Krook et al. (1960) compared 487 cases of pyometra with a sample of general dog population in Sweden and stated that Chow-chow, French bull dog, Collie, Pointer, Great Dane, Rottweiler, Skye Terrier, Saint Bernard and Swedish haund had a statistically significant predisposition to the development of pyometra where as Boxer, Fox terrier and Poodle had decreased risks. However, in the above studies base population was not considered.

Eganvall, (2001) opined that the occurrence of pyometra differed with age, breed, and geographic location. The risk of developing pyometra was increased (identified using multivariate models) in rough Collies, Rottweilers, Cavalier King Charles Spaniels, Golden Retrievers, Bernese Mountain Dogs, and English Cocker Spaniels compared with baseline (all other breeds, including mixed breed dogs). Breeds with a low risk of developing the disease were Drevers, German Shepherd, Miniature Dachshunds, Dachshunds (normal size), and Swedish Hounds. Survival rates indicate that
an average 23-24% of the bitches in the databases will have experienced pyometra by 10 years of age.

Hagman (2004) in his study, by considering the base population in Sweden, recorded high risk of predisposition to pyometra in Collie (rough-haired), Rottweiler, Bernese Mountain Dog, Cavalier King, and Golden Retriver. Lower risk of developing pyometra has been reported in Swedish Hound, Dachshund and German Shepherd dogs.

Smith (2006) reported that some of the breeds predisposed to pyometra include the Rottweiler, Saint Bernard, Chow Chow, Golden Retriever, Miniature Schnauzer, Irish Terrier, Airedale Terrier, Cavalier King Charles Spaniel, Rough Collie, and Bernese Mountain dog.

Based on the above information it could be inferred that the difference in the present incidence of pyometra in different breeds could be consequent to popularity of a particular breed in different geographical location rather than any breed predisposition. This presumption appears to be well substantiated as Sevelius et al. (1990) also opined that the incidence of pyometra had no bearing on the breed.

5.1.3. Parity wise incidence

The present study has revealed higher frequency of pyometra in nulliparous or maiden bitches (69.63%) as compared to bitches which had whelped at least once (30.36 % Table IV). The higher incidence of pyometra in nulliparous compared to parous bitches in the present study is in confirmation with the case series of Dow (1958) and Frost (1963) in which respectively 75 and 65 percent of the affected bitches were
nulliparous. An increased susceptibility of nulliparous animals for pyometra has also been reported by Niskanen and Thursfield, (1998); Okubo et al. (1995); Dhaliwal et al. (1998) and Chastain et al. (1999).

Based on the epidemiological study of the clinical data of this investigation and observations of previous workers, it could be concluded that the variation in the incidence of pyometra in bitches could be due to variation in the age of the subject studied, hormonal status of the animal, breed susceptibility and the hygienic practices followed in the management of dogs.

5.2. Medical history and Clinical signs

The most common complaint reported by the owner was vaginal discharge (90%), which is suggestive of open type of pyometra. The discharges were purulent in over 40 percent of the affected animals followed by sero-sanguineous (27%), and haemorrhagic (33%) discharge.

Wheaton et al. (1989) reported that vaginal discharge were present in 88% of the cases diagnosed to be suffering from pyometra. Others have also reported that open pyometra are encountered much more frequently than closed pyometra (Dow, 1957; Borresen, 1979, Nelson and Feldman, 1986; Stone et al. 1988, Renton et al. 1993).

Verstegen et al. (2008) has reported that vaginoscopic examination of cranial vagina revealed that in most of the cases some degree of uterine exudate is evident. Cervix may spontaneously open or close during the disease, causing intermittent vaginal
discharge or a sudden deterioration in the clinical status of the bitch. The findings of the present study also revealed some degree of vaginal discharge in all the affected bitches.

The most common complaint reported in this study were anorexia (70%) and depression (85%) which is in line with the observations of Nomura et al. (1984); Stone et al.(1988); Verstegen et al.(2008) and Renton et al.(1993). Other Clinical signs reported in the present study were polydipsia, polyurea, diarrhoea and vomiting. Similar clinical signs in animals affected with pyometra have also been reported by Aranez and Topaico (1955), Dow (1958), Fowler 1964, Hardy (1980), Stone et al.(1988), Smith (2006), Kashinath et al. (2009) and Sevelius et al. (1990). Wheaton et al. (1989) opined that vomiting in affected animals is a poor prognostic sign indicative of a more severe disease process.

Polydipsia and polyurea has been considered as a classical sign of pyometra. Etiopathogenesis of renal involvement is thought to be associated with immunological process due to glomerular antibody interactions of primary or secondary in nature (Ascheim, 1965; Hardy and Osborne, 1974). Bacterial endotoxins originating from the pus filled uterus impair the ability of loop of Henle to reabsorb sodium and chloride and endotoxins also seem to have a specific ability to cause tubular insensitivity to antidiuretic hormone (causing development of a secondary nephrogenic diabetes insipidus) resulting in loss of urine concentrating ability which leads to poly urea and compensatory polydypsia (Verstegen et al.,2008 and Borresen, 1979) and is evident in 85 percent of the animals of present study.
Abdominal enlargement was the most reliable sign of closed pyometra and its absence in nearly 50 percent of open pyometra was expected because of constant drainage of uterine contents. Vulval edema was the other clinical sign exhibited by animals with both closed and open type of pyometra and the cause for the same is not clear. While it can be explained because of constant licking in open pyometra, its presence even in closed pyometra is difficult to explain.

5.3. Onset of pyometra in relation to previous estrous cycle

In the present study, majority of the animals diagnosed for pyometra had a history of being in oestrus at an average of 6.7±0.6 weeks back (Range 1 to 20 weeks). Similarly Pretzer (2008) has reported mean interval of 5.7 weeks to onset of pyometra in relation to previous estrous. Blendinger et al. (1997) have reported that 3/4 of the pyometra cases presented were within 8 weeks of the completion of prior estrus.

Most of the owners in the present study were unable to give a definite history regarding the irregularities of the estrous cycle and the signs of pseudo pregnancy in bitches which developed pyometra. Therefore, in the present study no attempt was made to correlate the estrous cycle abnormalities and pseudo pregnancy with occurrence of pyometra. However, some surveys have suggested that abnormal estrous cycle and pseudo pregnancy increased the risk of pyometra in bitches (Dow 1958, Whitney 1967). On the other hand, in a controlled study on 68 cases and 245 controls, Fidler et al. (1966) found no relationship between the occurrence of pyometritis, estrous irregularities and pseudo pregnancy.
5.4. Bacteriological findings

The results of bacteriologic examination of the vaginal swabs of 60 bitches with pyometra revealed predominance of *Escherichia coli* and are in confirmation with findings of other workers (Asheim, 1965; Renton *et al.*1971; Grindlay *et al.*1973; Sandholm *et al.* 1975; Kivisto *et al.* 1977; Vandesplassche *et al.*, 1991; Dhaliwal *et al.*1998). Other isolates identified were *Klebsiella* sp, *Pseudomonas* sp, *Proteus* sp, *Staphylococcus aureus*, *Hemophilus* sp, *Serratia* sp, and mixed culture. Similar uterine infections associated with these bacterial isolates have also been reported by Coggan *et al.* (2008).

Sandholm *et al.* (1975) opined that *E. coli* adheres to receptors in the progesterone-stimulated endometrium, which might be one explanation for the observed predominance of this bacterium. Grindlay *et al.* (1973) found that certain serotypes of *E. coli*, e.g. 02, 04, 06, and 075, were more commonly associated with pyometra than others. Dhaliwal *et al.* (1998) recorded *E. coli* serotype 032 and 04 as the most commonly observed organisms in the pyometra cases. In addition, they also found that serotypes containing Cytotoxin Necrotizing Factor (CNF) affecting the endometrium more severely than others.

The route of infection of the uterus was suggested to be hematogonic or lymphogonic as well as ascending (Dow, 1959b). The ascending route was not supported by the work of Meyers-Wallen *et al.*(1986), who observed that the type of bacteria isolated from the vagina did not necessarily represent the bacterial species isolated from the uterus in pyometra.
Sandholm *et al.* (1975) showed that cystitis was commonly associated with canine pyometra and the *E. coli* isolated from the urinary bladder and from the uterus showed many similarities. It was suggested that the urinary tract may serve as a bacterial reservoir, and bacteria ascend into the uterus during a susceptible stage of the estrous cycle.

The cultures were tested for antimicrobial susceptibility by the Kirby and Bauer standardized disk diffusion method. Cultures were classified as sensitive, intermediate and resistant considering the diameter of the growth zone of inhibition. The concentrations as well as the criteria used for interpretation were the ones recommended by the “Clinical and Laboratory Standards Institute- CLSI”. More than 75 percent of the samples were sensitive to ofloxacin, lincomycin, clarithromycin and roxythromycin. However, little over 50 percent of the samples were sensitive to chloramphenicol, erythromycin, enrofloxacin, tetracycline, norfloxacin, ciprofloxacin, amoxicillin, clindamicin, amicacin, cefadroxil, cefaclor, azithromycin and cefataxime.

The sensitivity test also revealed that varying percentage of samples were resistant to different antibiotics like Tetracycline, (21.8%), Amicacin,(6.6%), Cephalexin, (5.1%), Gentamicin, (71.6%) , Cefotaxim, (5.1%) , Enrofloxacin, (18.33%) and Azithromycin, 11.6% (Table XI).

**5.5. Effect of different treatment regimens on physiological parameters**

The mean rectal temperature (°F) in Group II (AP), Group IV(P) , Group III(AE) and Group V(S) animals with pyometra was slightly elevated than the normal physiological range (101–102.5 °F ). Sevelius *et al.*(1990), and Wheaton *et al.* (1989) also
recorded elevated temperature in 30 and 43 percent of the pyometra cases, respectively. Slightly higher temperature was also reported by Gandotra et al. (1994), Kashinath et al. (2009). However, the mean rectal temperature observed in Group I (A) was within the normal physiological range before initiation of treatment is in agreement with the findings of Frayer et al. (1991). After initiation of treatment regimes the mean rectal temperature reached normal physiological range on day 4 in all the treatment groups except Group II (AP). The elevated temperature of bitches of Group II (AP) reduced gradually on day 9 after completion of treatment. The mean rectal temperature (F), did not differ significantly between days and groups. Nelson and Feldman (1986) and Fransson (2003) reported that fever was a variable sign and when present was associated with uterine inflammation and secondary bacterial infection as well as septicemia or bacteremia.

It is evident from the present study as well as those made in the past that the animals suffering with pyometra may exhibit normal, subnormal or elevated body temperature. However, body temperature may reflect the severity of infection of the uterus.

In the present investigation, the pulse and the mean respiratory rate in different treatment groups before, during and after completion of treatment were with in the normal physiological range (70-80 and 20-30 ).The present findings are in agreement with those of Frayer et al., (1991); Kayamaz et al., (1999); Troxel et al., (2002); Kashinath et al., (2009). However, Stone et al. (1988), and Hiremath and Sunilchandra (2004) reported tachycardia and tachypnoea which may be due to intercurrent diseases.
5.6 Effect of different treatment regimens on haematological parameters

5.6.1. Haemoglobin, PCV and TLC

The haemoglobin level in clinically healthy dogs is reported to range from 12 to 18 g/dl (Schalm, 1958; Schalm, 1959). In comparison, the mean hemoglobin concentration in bitches with pyometra in different groups before initiation of treatment ranged between 10.83±1.406 in Group III (AE) and 12.74±0.6696 in Group IV (P). The mean hemoglobin concentration tends to be slightly lower in Group III (AE), Group II (AP), Group I(A) and Group V(S) before initiation of treatment. However, there was an increase in the mean hemoglobin concentration during and after completion of treatment compared to pre treatment values. Nevertheless 80 to 85 percent of bitches suffering with pyometra exhibited evidence of anaemia.

The extent of anaemia in bitches with pyometra was also reflected by a mild decrease in packed cell volume (PCV less than 40) in Group III (AE), Group II(MP) and Group V(S) bitches on day 0. The present observations agree with the findings of Sevelius et.al. (1990). The recorded mean Packed Cell Volume (PCV) values increased in animals with pyometra in different groups after initiation and completion of treatment.

In the present study as well as in studies of Sevelius et.al. (1990) and Kashinath.et al.(2009), a large number of anemic patients have been encountered in the affected bitches which is contrary to the findings of Hardy and Osborne (1974) and Borresen (1980). Borresen (1980) opined that it is difficult to determine what the true haematocrit might be in pyometra patients since both dehydration and anaemia might be present concurrently. Anemia has been suggested to be caused by decreased erythropoiesis due to
toxic depression of the bone marrow caused by the toxins produced by bacteria and loss of erythrocytes into the uterine lumen.

Pyometra is a disease of chronic nature in which anemia is caused by a variety of disorders including chronic inflammation, in which lactoferrin and other acute phase reactants mediate an iron sequestration within the myeloid cells in the bone marrow, withdrawing iron from the normal erythropoiesis.

The systemic effects of pyometra are reflected by several laboratory parameters and the most characteristic alteration is an inflammatory leukogram with marked elevation of the total white blood cell count (WBC) and usually a regenerative left shift in the differentiated WBC count (Dow, 1957; Sandholm et al. 1975; De Shepper et al. 1986; Stone et al. 1988; Wheaton et al. 1989 and Sevelius et al. 1990).

The white blood cell count in clinically healthy and normal dogs is reported to vary between 6000 -18000 cells / mm (Schalm,1959). In comparison, a marked leucocytosis as reflected by an increase in mean total white blood cell counts was recorded on day ‘0’ before treatment in all the treatment groups. The mean WBC count in GroupII (AP), Group IV (P), Group I (A), Group III (AE) and Group V(S) was 24054±5966, 38209±5038, 42867±16912, 50753±8678, and 51242±8085 respectively (Table XIV). The marked leucocytosis observed in cases of pyometra is suggested to be due to bone marrow inflammatory response (Sevelius, 1990) and diffused suppurative inflammation of uterus to combat the infection. Leucocytosis had been traditionally considered as a classic sign of pyometra in a bitch. Renton et al. (1971) reported that in
90 percent of cases in their study leucocytosis was always present and concluded that leucocytosis is a useful diagnostic feature of pyometra.

The pretreatment leucocytosis in pyometra has been well documented by Cheri and Johnson (1996), Kochhar et al. (1996), Bartoskova et al. (2007), England et al. (2007) and Kashinath et al. (2009).

It is evident from the mean total leucocyte count (TLC) values recorded on day 4 of the treatment, that in Group II (AP) and Group I(A) the values reached normal physiological range. The TLC significantly (P< 0.05) decreased on day 9 of the treatment in all the groups. Similar results were observed after surgical and medical treatments by Bartoskova et al. (2007); Ucmak and Cagataytek (2008) and Kashinath et al. (2009).

5.6.1. Differential Leucocyte Count

In order to further characterize bone marrow inflammatory response, differential leucocyte count was also carried out. The segmented neutrophilic count in different groups before initiation of treatment ranged between 83.75±3.205 in Group I (A) and 89.00±1.273 in Group V(S) which is indicative of neutrophilia in all the treatment groups. This observation drew support from the findings of Schalm et al. (1991). It was further observed that neutrophilia in these bitches had also exhibited leucocytosis and leucocytosis was due to neutrophilia. The reduction in neutrophilic count in different groups during treatment (on day 4) was evident but statistically not significant compared to day 0. There was a significant (P< 0.005) decrease in the mean segmented neutrophilic count on day 9 which was 83.29±1.629, 78.60±1.360, 79.73±2.166, 81.36±1.330, and 85.33±1.345 in Groups I-V respectively (Table XIV). In Group IV and Group V the
reduction in mean neutrophil count is still higher than the normal physiological range (6 - 17x 10). These findings are in confirmation with the previous reports by Gobello et al. (2003); Hoffman et al.(2000); Trasch et al.(2003) and Fieni (2006).

The normal range of band forms of neutrophils is reported to range from zero to three percent (Schalm 1958, and Schalm 1959). Its count in the present study before initiation of treatment was significantly higher in all the treatment groups than the reported normal values. An increased band cell count together with leucocytosis and neutrophilia may suggest that pyometra is characterised by leucocytosis with degenerative shift to left. Dow (1958) reported that leucocytosis was always due to increase in the neutrophils of which up to 35 percent were immature forms, mostly non lobulated with a few myelocytes. The reduction in the band cell count during and after completion of treatment was statistically significant between days in surgical and different pharmacological groups indicating elimination of toxaemic situation. Similar results of reduction in leucocytosis were observed by Bartoskova et al.(2007) after hysterectomy and medical treatment with antiprogestines by England et al.(2007).

5.6.2. Lymphocyte

In the present study, the pre treatment lymphocyte count was low in all the treatment groups which significantly increased on post treatment day 4 and 9. This pre treatment low lymphocyte count could be due to absolute increase in neutrophil count as a result of severe suppurative inflammation of the uterus. The suppression of lymphocyte activity may be induced by endotoxemia. The bacterial products or components could be
one reason for impaired immune response and human endotoxemia has been associated with decreased production of pro-inflammatory cytokines (Granowitz et al. 1993).

The above observations and inferences explained, appeared to be well founded, since there was a drastic reduction in total leucocyte count and return of differential count to normalcy after different pharmacological and surgical intervention of pyometra cases in this investigation.

5.7 Effect of different treatment regimens on serum biochemical parameters

5.7.1 Blood Urea Nitrogen (BUN)

The mean Blood Urea Nitrogen (BUN) concentration on day 0, 4 and 9 in all the groups was in the normal reference range (10-30 mg%) reported for clinically healthy dogs (Renton et al. 1971) However, in Group I(A), Group III(AE) and Group V(S) the mean Blood Urea Nitrogen (BUN) levels before initiation of treatment slightly exceeded the normal limits. The slightly elevated Blood Urea Nitrogen (BUN) levels was also reported by Jayathangaraj et al. (1994), Ravishankar et al. (2004), Colombo et al. (1988); Schalm et al. (1991) and Cockroft (1995).

Although the measurement of plasma or blood urea level is considered to be a simple renal function test, many factors unassociated with renal lesions can lead to elevation of blood urea nitrogen level. Renton et al. (1971) stated that concentration of urea in the blood was a result of production and elimination levels above normal may be associated with increased production or faulty elimination of urea. The increased production stated to be associated with high protein diets, starvation and fever which are
not relevant to pyometra. The faulty elimination of urea in urine associated with many factors may be pre renal or renal. The pre renal factors are those which lead to the lowering of glomerular filtration rate and impair the efficiency of kidneys while the renal factors associated with specific renal lesions (Renton et al. 1971).

Wheaton et al. (1989) concluded that elevated blood urea nitrogen level seen in some cases in their study were most often a result of pre renal ureamia as there was a lowered incidence of elevated creatinine levels. Nelson and Feldmen (1986) also suggested that pre-renal azotemia may be present if water consumption inadequately compensates for polyurea. It is probable that elevated blood urea nitrogen levels observed in nearly 50 percent of the bitches in the present study was a result of pre renal uremia as dehydration was also present in a little over 50 percent of the affected bitches. Level of BUN was not significantly (P< 0.05) between different groups and days.

A gradual decrease in elevated blood urea nitrogen level during the course of treatment is indicative of positive response of the animal to the treatment and elimination of toxaemia in the affected bitches.

5.7.2. Creatinine

There was no significant variation in mean creatinine values estimated in animals with pyometra in different treatment groups on Day 0, 4 and 9 and were with in the normal reference range (1-2 mg/dl) for clinically healthy dogs (Benjamin,1985). But a slight increase in the level above the normal range in few animals in different treatment groups could be attributed to reduced renal perfusion relating to toxemia (Schalm et al. 1991).
5.7.3. Total plasma protein, albumin and globulin

The total plasma protein concentration in clinically healthy normal dogs is reported to range from 5.3 to 7.3 mg/dl. In comparison, the mean total protein and globulin recorded in the present study before treatment ranged from 7.55 ± 0.23 in Group III (AE) to 8.3 ± 0.26 in Group I (A) and 2.3 ± 0.19 g/dl Group I (A) to 2.8 ± 0.22 g/dl Group V (S) respectively (Table XVI.) Hyper protenemia and hyper globulinemia in the affected bitches has also been reported by Sandholm et al.(1975), Nelson and Feldmen (1986), Wheaton et al.(1989), Hashimoto et al .(1992); Dabhi and Dhami (2007) Kamala Kannan (2001), Kashinath et al. (2009) and was suggested as a consequence to antigenic stimulation of the bitches immune system (Borresen and Skrede, 1980).

After completion of treatment the mean total protein and globulin concentration values recorded ranged between 6.6 ± 0.31 g/dl (Group II) to 7.11 ± 0.31 g/dl (Group IV) and 2.3 ± 0.14 g/dl (Group V) and 2.6 ± 0.28 g/dl (Group I), respectively (Table XVI). The present finding indicates a gradual decrease in total protein and globulin concentration to normal range in all the treatment groups.

The mean albumin level prior to treatment was slightly lower in all the treatment groups compared to normal physiological range but gradually increased after completion of treatment in all the groups.

Hypoalbuminemia has been observed in severe liver insufficiency cases. Serum albumin levels tend to fall in hepatic diseases. It is possible that it is due to accumulation of albumin into the extravascular tissue, because of the rise in capillary permeability or
the expansion of extra vascular tissue. The detection of hypoalbuminemia and the rise in total protein in different treatment groups were in accordance with the findings of other researchers because hypoalbuminemia occurs together with higher total protein levels, in response to acute inflammatory reaction (Kashinath. et al. 2009).

Asheim (1964) suggested that the cause of hyperglobulinemia, concurrent with hypoalbuminemia was due to renal loss of albumin, but later studies have demonstrated only a mild to moderate urinary protein loss (Borresen & Skrede, 1980, Sevelius et al. 1990) and interpreted the changes in serum proteins as part of an acute phase reaction (Borresen and Skrede, 1980).

5.8. Effect of different treatment regimens on serum enzymatic profiles

5.8.1. Serum alkaline phosphatase activity (ALP)

The mean serum alkaline phosphatase activity observed before initiation of treatment in all the groups was almost double than the normal range (97-280) which is in line with the findings of Dabhi and Dhami (2007), Boerresen and Skrede (1980) and Fransson (2003). There was a common tendency for decline in the serum ALP activity with the initiation of different pharmacological treatment and surgical intervention. Sevelius et al. (1990) Ettinger and Feldman (1993) and Van Israel et al.(2002) reported similar findings in case of pyometra. Hagman, (2004) found almost four times increase in serum ALP activity in pyometra cases which was however double in the present study. These findings reflect hepato cellular damage in response to toxemia or diminished hepatic circulation due to dehydration induced by pyometra (Verstegen et al.2008).
The mean concentration of serum ALP decreased significantly (p< 0.05) in all the groups on day 9 and reached very close to normal physiological range with the completion of treatment.

5.8.2. Serum transferases

Measurement of serum alanine amino transaminase concentration is a useful parameter to assess the liver functions in clinically healthy dogs and its concentration averaged 40.4 to 13.0 IU/l (De Schepper et al 1987). In the present study, mean serum alanine aminotransaminase concentration in animals with pyometra in different groups before initiation of treatment, during and after completion of treatment were within the normal physiological range (Table XVII). These findings corroborate well with the results of Bojrab (1985), Kamala Kannan (2001) and Hagman (2004).

Nelson and Feldmen (1986) also reported that only occasionally serum alanine amino transaminase concentrations are mild to moderately increase from hepato-cellular damage caused by septicemia and/or due to diminished hepatic circulation and cellular hypoxia in the dehydrated bitch. However, Wheaton et al. (1989) encountered an increase in serum alanine amino transaminase concentration in 22 percent of the cases in their investigation which suggests that measurements of serum alanine amino transaminase concentrations may be of limited diagnostic and prognostic value in bitches affected with pyometra.
5.9. Effect of different treatment regimens on serum progesterone (P₄)

The pre-treatment serum progesterone concentration recorded in bitches affected with pyometra in surgical and different pharmacological treatment groups were elevated 8 to 10 times compared to post treatment values in all the groups. The present findings corroborated well with the reports of Christie et al. (1972), Hadley (1975), Chauffaux and Thibier (1978) and Austad et al. (1979). However, the value was slightly higher than that reported by Wakankar (1993) and lower than that reported by Austad et al. (1979).

The level of progesterone concentration declined significantly (p<0.05) at day 4 following different treatment regimes and reached almost basal level. The levels in general, were not always higher in pyometra than in normal cases in luteal phase as has been observed by others (Christie et al. 1972; Wakankar 1993). Traditional theories suggest that hormonal changes render the uterus susceptible for infection. This understanding was originally based on work by Teunissen (1952) and Dow (1957, 1958, 1959a, 1959b) who investigated the importance of estrogen and progesterone in the development of endometritis. They found that the cystic changes of the uterus associated with endometritis could be reproduced by injections of estrogen which alone seemed to play a less important role, but appeared to enhance the endometrial response to progesterone, and exogenous estrogen administration, used to terminate pregnancy, has been reported to increase the risk for pyometra (Bowen et al. 1985).

The relevance of the association between pyometra and diestrus was demonstrated by Lesboyries and Berthelon in 1936 who reported that removal of ovaries without hysterectomy in bitches with pyometra was followed by clinical cure in 5–7 days.
Similarly, in a retrospective study in bitches aged more than ten years, Janssens and Janssens (1991) confirmed the evidence of the role of ovarian steroids in the pathogenesis of the disease, as bilaterally ovariectomized dogs never developed pyometra. The importance of progesterone in the pathogenesis of the spontaneous disease is attributed to its suppression of immune responses, stimulation of endometrial gland secretions which provide a suitable environment for bacterial growth, functional closure of the cervix which inhibits drainage of uterine exudates, and mediation of cystic endometrial hyperplasia (Austad et al. 1979; Chauffaux & Thibier, 1978). Considering the role of progesterone, it is interesting to observe that some dogs with pyometra present with basal plasma progesterone concentrations and one would typically expect the pyometra to resolve in such conditions. Failure to resolve may be due to either the inability of the degenerated uterus to contract or failure of the cervix to relax. Another possibility is that although plasma progesterone concentrations are below the sensitivity of standard progesterone assays, even there is enough progesterone produced to inhibit uterine contraction and cervical relaxation, (Verstegen et al. 2008). Although the association between pyometra and diestrus has been well-established, the precise mechanism is still not clear (Borrensen, 1975). Early reports suggested that excessive or prolonged exposure to progesterone was responsible for the susceptibility to pyometra and, indeed, the disease can be induced experimentally by the administration of exogenous progesterone to ovariectomized bitches (Borrensen, 1975). However, more recent studies have not been able to substantiate that either higher concentrations or more prolonged periods of progesterone secretion in bitches resulted in development of pyometra.
5.10. Effect of different treatment regimens on uterine diameter

The uterine diameter recorded in bitches affected with pyometra in different treatment groups on day 0 in groups I, II, III, and IV were 35.17 ± 2.57, 26.83 ± 2.38, 25.42 ± 2.57 and 18.75 ± 2.31 mm respectively (Table-XVIII). The uterine diameters observed in the successfully pharmacologically treated bitches within the physiological limits on days 4 and 9 is in line with findings of others (Breitkopf et al. 1997; Gobello et al. 2003 and Hoffmann et al. 2000)

5.11. Efficacy of different treatment regimens

The efficacy of different regimens employed for treatment of pyometra in bitches was evaluated on the basis of number of animals recovered, relapsed, and died due to the disease.

5.11.1 Group I (A).

Group of bitches that received antiprogestines alone showed a recovery rate of 41.66 percent which is lower to all other treatment groups. Lower recovery and higher relapse rate in the present investigation may be attributed to the inability of this treatment to induce uterine contractions if used alone. Unlike the action of PGF2α, the progesterone antagonists are not expected to induce myometrial contractions (Verstegen et al. 2008).

However, some studies hypothesize that uterine contractions are indirectly induced by the local uterine release of endogenous prostaglandins as a consequence of the endometrial inflammatory process associated with the pyometra (Gobello et al. 2003; Fieni, 2006).
In a study published in 2003 by Trasch et al., 18.9% of the treated dogs relapsed after treatment with aglepristone alone. In most cases of recurrence, there were cystic changes in the ovaries and endometrium. The authors concluded that the recurrence rate can be minimized by the selection of bitches without ovarian cysts and cystic endometrial hyperplasia.

The mortality rate of 33.33 percent observed in this Group may be attributed to jaundice and leucopenia observed in two bitches and concomitant diseases as also observed by Verstegen et al. (2008).

5.11.2. Group II (AP)

The success rate observed in Antiprogestines along with PGF$_2$α was 83.33%. Similar results were reported by Fieni (2006) and Gobello et al. (2003). However, Ucmak and Cagataytek (2008) reported 50 percent success rate.

Progesterone receptor antagonists such as Mifepristone (Hoffman 1989) or Aglepristone (Hoffman, 2001, and Wehrend and Trasch, 2003) bind to the progesterone receptor which they completely block, preventing any biological activity. Progesterone-receptor antagonists competitively prevent progesterone from binding to its receptor to induce transcription and exert all its biological effects at the cellular level. Consequently, the absence of receptor stimulation and activation mimics the effects observed when luteolysis is induced and thereby causes relaxation of the cervix.

The side effects observed with the initiation of PGF$_2$α treatment were salivation, vomiting, straining, diarrhea, pyrexia, some occasional respiratory distress is in line with
the findings of Hubler et al. (1991). In addition, two animals died during the course of investigation after second dose of PGF$_2$α treatment which may be attributed to shock. Berchtold (1997) also reported shock and death as one of the side effect in his findings.

The use of this combination of medication results in both uterine contractions and induction of luteolysis, which in turn prevents all the effects of progesterone on the uterus and on the immune system (Verstegen et al. 2008).

5.11.3. Group III (AE)

In many species, normal relaxation of the cervix at the time of estrus and ovulation is probably the result of the peri-ovulatory changes in reproductive hormones that occur at this time. The increases in estradiol (and possibly oxytocin) receptor concentrations (Shemesh et al., 1997) during the peri-ovulatory period are thought to increase prostaglandin E$_2$ synthesis and receptors (Schmitz et al. 2006), leading to remodeling of cervical extracellular matrix (Stys et al., 1981 and Ledger et al. 1983) which allows relaxation of the cervix. In ewes, Misoprostol has been demonstrated to improve cervical patency and penetrability at the end of estrus (Leethongdee et al. 2007). This effect appears to be related to misoprostol induced increased expression of the mRNA for FSH-R in all cervical layers (Leethongdee et al. 2007).

In the present study, Misoprostol (Prostaglandin E) along with antiprogestins was used in group III bitches with pyometra. Seventy five percent recovery rate was observed with 16.66 percent relapse rate and 8.33 percent mortality rate. The mortality and relapse rate may be attributed to estrogen producing ovarian cysts or from non pathologic
paraovarine cysts or corpora lutea with a fluid filled cavity (England et al. 1993 and Wherend et al. 2002).

5.11.4. Group IV (P)

Fifty percent success rate was observed in bitches treated with PGF \(_2\alpha\). These findings were very low compared to other reports by Gilbert et al. (1993) and Tamturier and Treboz (1985) where they have used higher doses of PGF \(_2\alpha\) for a longer period.

Treatment with PGF\(_2\alpha\), apart from its luteolytic effects, mediates functional opening of the cervix, which permits drainage of exudate, and promotes myometrial contractions, facilitating uterine drainage.

Verstegen et al. (2008) opined that treatment with PGF\(_2\alpha\) at doses of 10 to 50 mg/kg or 10 ug to 250 ug, administered three to five times daily for 3 to 7 days, have been used successfully for treatment of canine pyometra, either solely or in combination with other drugs. Extreme care should be exercised in calculating the dose, as the therapeutic index is relatively small (LD\(_{50}\) in dogs is approximately 5 mg/kg) and side effects are quite severe when high doses are used (more than 100 mg/kg). The drug should be given SC or IM. Since side effects are dose-dependent and are known to diminish with repetition of treatment consist of vomiting, diarrhea, panting of moderate to mild intensity beginning 20–30 min after administration and never lasting for more than 30 min is in confirmation with the present findings.

The relapse rate of pyometra after PGF\(_2\alpha\) treatment was highest (50 percent). Higher relapse rate probably attributed to lower doses of PGF\(_2\alpha\) used in the present
study. Further it was also observed that when these animals were subjected to ovariohysterectomy, the ovaries had multiple ovarian cysts, a condition confirm to result in treatment failures. Meyers-Wallen et al. (1986) described therapeutic success in 10 of 10 treated animals with recurrence in 40 percent of the bitches within 1 year, and 77 percent within 27 months. Johnston et al. (2001) gave an overview of success and recurrence rates after conservative treatment of pyometra with prostaglandins and reported that recurrence rates averaged 10 percent.

5.11.5. Comparison of efficacy of therapeutic group with surgical group

Overall success rate observed in different groups irrespective of surgical group or medical treatment groups were 66.66 percent and both mortality and relapse rate was 16.66 percent. However, success rate observed in medical treatment groups was 62.5 percent compared to 83.33 percent in surgical group. Mortality rate in surgical group and medical treatment groups was 16.66 percent with a relapse rate of 20.83 percent in medical treatment groups (Table XX).

As a conclusion, ovariohysterectomy as a treatment for cystic endometrial hyperplasia-pyometra complex in bitches still maintains its success as a radical and effective solution. It has been determined that carrying out pharmacological treatment with Aglepristone or Aglepristone along with PGF$_2\alpha$, preceded by general, gynaecological, haematological, and ultrasonographical examinations, constitute a safe alternative for bitches in dioestrus, without ovarian cysts.

As for the pharmacological treatment performed in this study, it is believed that the decreases in the ratios of infected uterine diameters from day 1 to give important
clues about the prognostic value of the case. Consequently, this helps in deciding whether to perform an operation or to continue the pharmacological treatment, thus increasing the survival chance of the patient.