The Indian Journal of Veterinary Sciences & Biotechnology ***(2018) Volume 14, Issue 1, 61-66***
ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT
10.21887/ijvsbt.v14i1.13000

Comparative Efficacy of Prolactin inhibitors and $PGF_2\alpha$ analogue in Bitches Affected with Cystic Endometrial Hyperplasia-Pyometra Complex

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Publication Info

Article history:

Received : 13-12-2017
Accepted : 19-04-2018
Published : 20-07-2018

Key Words:

Cystic endometrial hyperplasia-pyometra complex, Bitches, Haematology, Clinical biochemistry, Ultrasonography.

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Abstract

The study was carried out at the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Mhow and in private clinics across Indore on 30 bitches suffering from Cystic Endometrial Hyperplasia-Pyometra Complex (CEH-PC). The animals were randomly subdivided into five groups each of six bitches and treated with bromocriptine (1 μg/kg POx5 days) and cabergoline (5 μg/kg POx5 days) alone and in combination with cloprostenol (5 μg/kg s/c on day 0, 2 and 6) (Gr II to V) together with enrofloxacin (10-20 mg/kg PO, SID×5 days) + combination of metronidazole and povidone-iodine IU (5-10 ml×3 days), the later served as routine control Gr I.The treatment response of animals was evaluated through clinical, ultrasonographical, haematological and biochemical parameters. Blood samples were collected from cephalic or saphenous vein just before treatment (day 0) and after treatment on day 6 and day 14. Treatment approach -III (cabergoline 5 μg/kg POx5 days) was found to be the most effective without side effects with significant decrease in uterine luminal diameter after treatment. The results showed significant improvement in haemato-biochemical attributes in dogs affected with CEH-P complex following treatment IV and V, i.e. with use of bromocriptine and cabergoline in combination with cloprostenol as compared to other groups.

Introduction

The Cystic Endometrial Hyperplasia-Pyometra Complex (CEH-PC) is one of the most serious and common uterine diseases in bitches; defined as acute or chronic, polysystemic, diestrual disorder characterized by hyperplasia of the endometrium and infiltration of inflammatory cells that may involve all layers of the uterus (Zdunczyk *et al*., 2006). Clinical signs that can be observed in bitches with pyometra are anorexia, lethargy, abdominal distension, listlessness, polydypsia, polyuria, vomiting and fever (Bondade, 2006).

Altered haematology and elevated serum creatinine, urea and other metabolites have been documented due to endotoxin induced damage to the vital organs (Dabhi and Dhami, 2006; Shah *et al.,* 2017 and Alok Kumar and Atul Saxena, 2018).

Onclin *et al.* (1999) used dopamine agonists such as bromocriptine or cabergoline with substantial anti-prolactin activities in combination with either natural or synthetic prostaglandins. The combination of dopamine-agonist and prostaglandin potentiates the luteolytic effects of

each drug and results in more rapid luteolysis. Also, reduction in progesterone concentrations induces cervical relaxation and a decrease in uterine secretions. Since, prostaglandins also have a uterine spasmogenic action, it helps in the expulsion of uterine fluid. However, when high doses are used, prostaglandins have also been associated with substantial risk of uterine rupture.

The aim of this study was to assess the therapeutic efficacy of Prolactin inhibitor such as bromocriptine and cabergoline alone and in combination with cloprostenol a synthetic prostaglandin $PGF_2\alpha$ through assessing the changes in the uterine luminal content by ultrasonography and haemato-biochemical profile before and after treatment in CEH-PC affected bitches.

Materials and Methods

The investigation was carried out on 30 clinical cases of CEH-P Complex brought to the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Mhow and in private clinics across Indore. The bitches of variable age brought with the history of vaginal discharge were examined for CEH-P Complex and treated randomly according to the following groups:

Group-I (n=6)	Enrofloxacin (10-20 mg/kg PO, SID×5 days) + Combination of Metronidazole and Povidone-Iodine IU (5-10 ml×3 days)
Group-II (n=6)	Bromocriptine (1 µg/kg PO×5 days) + Enrofloxacin (10-20 mg/kg PO, SID×5 days) + Combination of Metronidazole and Povidone-Iodine IU (5-10 ml×3 days)
Group-III (n=6)	Cabergoline (5 µg/kg PO×5 days) + Enrofloxacin (10-20 mg/kg PO, SID×5 days) + Combination of Metronidazole and Povidone-Iodine IU (5-10 ml×3 days)
Group-IV (n=6)	Bromocriptine (1 µg/kg PO×5 days) + Cloprostenol (5 µg/kg S/C on day 0, 2 & 6) + Enrofloxacin (10-20 mg/kg PO, SID×5 days) + Combination of Metronidazole and Povidone-Iodine IU (5-10 mlx3 days)
Group-V (n=6)	Cabergoline (5 µg/kg PO×5 days) + Cloprostenol (5 µg/kg s/c on day 0, 2 & 6) + Enrofloxacin (10-20 mg/kg PO, SID×5 days) + Combination of Metronidazole and Povidone-Iodine IU (5-10 ml×3 days)

Side effects if any of the drugs used were observed during and after treatment and were recorded. Uterine luminal diameter was measured by using ultrasound machine of Famio 5 (SSA – 510) with the help of micro convex transducer (3.5 MHz) at day 0 and then twice a week up to two weeks.

Blood samples were collected from cephalic or saphenous vein just before treatment (day 0) and after treatment on day 6 and day 14. The samples collected in heparinized vacutainer were used for haematological examination and samples collected without heparin were allowed to clot in test tube for serum separation and stored at –20°C until biochemical analysis. The haematological parameters were evaluated and indices were calculated as per Scham *et al.* (1975). The serum biochemical constituents, viz., creatinine, alkaline phosphatase (ALP) and blood urea nitrogen (BUN) were estimated using standard procedures with Erba Manheim diagnostics kits on auto-biochemistry analyzer.

The group- and period-wise means and standard errors were calculated as per standard formulas. Data were analyzed by applying completely randomized design (CRD) to know the significant differences, if any, between various treatments (Snedecor and Cochran, 1994).

Results and Discussion

Haematological Alterations:

The mean haematological values observed in different groups before and after treatment are presented in Table 1.

Table 1: Haematlogical parameters before and after various treatments in bitches affected with pyometra

Elements and their normal values*	Days	Group I	Group II	Group III	Group IV	Group V
Haemoglobin (g/dl) 12-19	0	10.28±0.57	12.10±0.53	13.37±0.38	11.25±0.60	11.33±0.55
	6	10.58±0.58	12.53±0.53	12.00±0.76	11.70±0.47	11.63±0.47
	14	11.15±0.36	12.65±0.55	13.37±0.57	11.83±0.52	11.80±0.46
Total leucocyte count ($x10^3$/cmm) 5.0-14.1	0	12.63±1.26	$14.28±1.04^{b}$	$19.38±2.91^{c}$	$22.02±0.65^{b}$	$21.68±0.97^{b}$
	6	11.73±0.88	$9.65±0.18^{a}$	$15.52±1.78^{b}$	$20.12±0.50^{ab}$	$18.87±0.62^{ab}$
	14	10.18±0.35	$7.78±0.29^{a}$	$9.12±1.28^{a}$	$18.50±0.40^{a}$	$16.97±0.69^{a}$
Neutrophil count (%) 58-85	0	$74.66±3.14^{b}$	65.00±2.52	66.33±2.43	$68.00±2.99^{b}$	$68.17±3.57^{b}$
	6	$70.50±2.51^{b}$	59.83±4.62	65.83±1.62	$63.33±2.98^{ab}$	$63.17±2.71^{b}$
	14	$57.50±2.93^{a}$	57.17±2.93	62.17±1.50	$57.33±1.98^{a}$	$52.17±2.33^{a}$
Lymphocyte count (%) 8-21	0	$18.83±2.98^{a}$	29.67±1.33	30.00±2.68	29.33±2.99	$27.17±3.12^{b}$
	6	$21.50±2.98^{a}$	32.83±2.93	29.67±1.66	24.00±1.64	$24.83±2.31^{a}$
	14	$33.17±3.11^{b}$	35.17±2.86	30.17±1.57	31.00±2.63	$35.83±1.64^{c}$
Monocyte count (%) 2-10	0	4.00±0.68	3.17±0.79	2.17±0.65	$1.33±0.33^{a}$	$2.50±0.42^{a}$
	6	5.50±0.68	3.17±1.02	2.33±0.76	$7.50±0.93^{b}$	$7.67±1.48^{b}$
	14	4.67±0.72	4.17±0.47	3.83±0.61	$7.17±0.61^{b}$	$6.67±1.33^{b}$
Eosinophil count (%) 0-9	0	2.50±0.85	2.17±0.55	1.50±0.42	$1.33±0.33^{a}$	$1.83±0.55^{a}$
	6	2.50±1.00	4.17±1.44	2.00±0.51	$5.17±0.79^{b}$	$4.33±1.08^{b}$
	14	4.67±0.67	4.50±1.00	3.83±0.91	$4.67±0.49^{b}$	$5.33±1.46^{b}$

Day 0 = Just before treatment, day 6 & 14 after treatment; Means having different superscripts within column for a trait differ significantly between days (P<0.01). *Latimer *et al.* (2003)

The variations in mean levels of haemoglobin were found to be non-significant between groups and between days of treatment in the present study. The present mean value of Hb is in close agreement with report of Hagman *et al.* (2006) in CEH-PC affected bitches.

The difference in mean total leucocyte count (thousand/cu.mm) was found to be highly significant (P<0.01) between days in all the treatment groups, and a significant decrease towards normalization was observed to be faster in treatment II and III. Leucocytosis has been considered as a classical sign of pyometra in the bitch. Marked elevation of the total white blood cell count has been recorded by several authors. Sevelius*et al.* (1990) suggested that leucocytosis could be due to bone marrow inflammatory response and diffused suppurative inflammation of uterus to combat the infection.

Highly significant (P<0.01) differences were found between days or time intervals within each group in the mean neutrophil count. The neutrophil count which was increased on day 0 declined significantly in treatment group I, IV and V on day 14. The neutrophilia recorded in the present investigation may be attributed to the defense mechanism of the uterus in response to the invading microorganisms. In contrast, significant (P<0.01) increase in lymphocyte count was noticed by day 14 in treatment group I, II and V. The increase in mean monocyte and eosinophils count (%) on day 6 and 14 was statistically highly

significant (P<0.01) over day 0 values in treatment IV and V. Similar findings were reported by Baithalu *et al.* (2010) and Shah *et al.* (2017).

Biochemical Alterations:

The mean values of serum creatinine levels (mg%) observed on day 0 in different groups declined by day 6 and 14 of treatment within each group, with statistically highly significant (P<0.01) differences between day 0 and day 14 only in treatment group IV & V, while values for day 6 were intermediate in all groups (Table 2). The present observation supports the findings of Nak *et al.* (2001) and Dabhi and Dhami (2006) who reported the mean creatinine values of 2.5 mg% and >1.5 mg% in pyometric bitches, respectively. The elevated creatinine level in blood might be due to massive destruction of tissue elements coupled with urinary insufficiency indicating renal failure (Sahoo *et al*., 2012).

The mean levels of serum alkaline phosphatase in different groups also declined gradually on day 6 and 14 of treatment over 0 day values, with significant differences in group III, IV and V only. Verstegen *et al*. (2008) found elevated serum alkaline phosphatase in approximately 50-75% of cases affected with CEH-PC. These changes reflect hepato-cellular damage in response to toxemia or diminished hepatic circulation due to dehydration.

The mean levels of BUN (mg/dl) were observed to be elevated (upto 40 mg/dl) in CEH-PC affected bitches. They were found to be significantly higher before treatment and decreased to the normal levels after treatment. Gayakwad *et al.* (1999) found that the increased value of BUN in pyometric bitches was because of the inefficiency of kidneys to remove nitrogenous waste from the circulation. Similar findings were also reported by Shah *et al.* (2017).

Table 2: Serum creatinine, alkaline phosphatase and BUN levels before and after various treatments in bitches affected with pyometra

Elements and their normal values*	Days	Group I	Group II	Group III	Group IV	Group V
Creatinine (mg/dl) 0.5-1.7	0	1.45±0.23	1.50±0.23	1.45±0.42	2.58±0.31^{b}	2.80±0.38^{b}
	6	1.25±0.15	1.37±0.17	0.72±0.05	1.84±0.26^{ab}	2.23±0.34^{ab}
	14	1.15±0.14	1.20±0.15	0.82±0.10	1.15±0.14^{a}	1.43±0.23^{a}
Alkaline phosphatase (IU/L) 1-114	0	69.33±11.42	76.17±4.20	117.83±5.91^{b}	85.00±6.03^{b}	84.17±9.46^{b}
	6	73.00±6.17	71.67±3.70	66.83±9.15^{a}	64.17±3.42^{a}	69.00±6.10^{ab}
	14	72.00±2.91	65.83±2.99	54.83±6.48^{a}	49.33±3.37^{a}	55.50±6.22^{a}
BUN (mg/dl) 8-28	0	24.67±2.27	40.83±6.35^{b}	22.17±3.24	26.67±2.50^{b}	34.33±5.41^{b}
	6	33.67±2.78	36.50±5.40^{ab}	20.00±2.50	18.42±0.92^{ab}	25.22±2.93^{ab}
	14	33.17±1.73	26.92±2.17^{a}	22.33±5.02	13.09±1.39^{a}	16.33±0.89^{a}

Day 0 = Just before treatment, day 6 & 14 after treatment; Means having different superscripts within column for a trait differ significantly between days (P<0.01). *Latimer *et al*. (2003)

Treatment V, IV and III were found to be more efficient in reducing/normalizing the altered haematology and elevated serum creatinine, alkaline phosphatase and BUN in bitches affected with CEH-PC as compared to other groups.

Uterine Ultrasonogrpahy:

The differences in mean values of uterine luminal diameter (mm) on day 0, 5 and day 10 of treatment were found to be statistically highly significant (P<0.01) in each of the five treatment groups (Table 3). The present findings are in agreement with England *et al.* (2007) who reported that the combination of a prolactin inhibitor and a prostaglandin appears to have been effective in rapidly terminating the luteal

phase and promoting uterine evacuation which holds true in the present study. Pretzer (2008) also observed a reduction in the diameter of the uterus after treatment. Verstegen *et al*. (2008) stated that ultrasonography has made definitive diagnosis of pyometra much easier. In pyometra, the uterine wall is usually thickened and the uterus is distended to a variable extent with serous to viscid heterogenic fluid often presenting flocculation as seen in the present finding.

Table 3: Uterine Luminal Diameter (mm) before and after various treatments in bitches affected with pyometra

Normal condition*	Days	Group I	Group II	Group III	Group IV	Group V
<10 mm in diameter.	0	12.92±1.34^{b}	19.52±1.26^{c}	21.62±1.40^{c}	18.77±2.04^{c}	16.22±2.14^{c}
	5	10.47±1.70^{ab}	12.98±1.30^{b}	10.02±1.61^{b}	9.30±0.62^{b}	7.07±0.74^{b}
	10	10.33±1.70^{ab}	12.50±1.05^{b}	9.60±1.20^{b}	9.00±0.52^{b}	6.82±0.75^{ab}
	14	8.30±0.91	7.87±0.82	5.00±0.64	3.83±0.27	4.00±0.37

Day 0 = Just before treatment, day 6 & 14 after treatment; Means having different superscripts within column differ significantly ($p<0.01$). *Mannion (2006)

Side Effects:

Side effects observed in the present study were vomition, panting, restlessness and hyperpnoea. 50% (3/6) animals suffered from side effects in treatment II, 100% (6/6) in treatment IV and 83.33% (5/6) in treatment V. The similar side effects of prostaglandin analogue administration were observed in the study of Bondade (2006) and Dash *et al*. (2014). Davidson (2006) concluded that $PGF_2\alpha$ treatment should never be used in extremely ill dogs.

Conclusions

In this study, Group III (cabergoline+ enrofloxacin + combination of metronidazole and povidone-iodine IU) was considered the most effective without side effects with significant decrease in uterine luminal diameter after treatment. Prostaglandin injection although effective has a lot of side effects and should be administered under low doses with constant supervision with emergency drugs.

Acknowledgement

Authors are thankful to the hon'ble Vice Chancellor, NDVSU, Jabalpur and Dean, College of Veterinary Science and AH, Mhow for providing facilities to undertake this study.

Conflict of Interest:

All authors declare no conflict of interest.

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