



Cytokine Profile of Dogs Undergoing OSH

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Abstract – The aim of the present study was to access the cytokine profile (IL-6, TNF- α and TGF- β) of 30 female dogs undergoing ovariosalpingohysterectomy using autologous bone marrow. The animals were randomly divided into three groups: GI (control); GII (conventional) and GIII (experimental). In order to access the cytokines pre and post-operative, 5 ml of blood was collected at moments M0 (before surgery) M1 (six hours after surgery), M2 (48h), M3 (120h), M4 (168h). In addition, 0.5 ml of bone marrow was collected using a myelogram. The results showed that IL-6 exhibited a significant difference between the lowest average (50.05 ± 58.35) in the experimental group and the highest average (124.30 ± 217.45) in the conventional group. In the analysis of TNF- α , there was only a significant difference between the experimental group with the lowest average (40.53 ± 44.03) and the control group with the highest average (113.63 ± 99.94). With regards to TGF- β 1, a significant difference was found between the experimental group (563.46 ± 405.24) and the conventional group in terms of the lowest average (347.16 ± 405.25). The myelogram showed no differences between the groups analyzed. Thus, the intramuscular application of autologous bone marrow could be a therapeutic option for female dogs undergoing ovariosalpingohysterectomy.

Keywords – Cytokine, Profile, Dog, OSH, Bone Marrow.

I. INTRODUCTION

Animal overpopulation is a concern to both government and health professionals. The presence of errant dogs and cats is a problem especially in urban and peripheral areas, since these places often do not have appropriate sanitation, which can facilitate the spread of diseases and zoonosis.

On the other hand, owners are more informed about the need for population control, and sterilization has become the preferable birth control method for dogs. Surgery is the most common method of animal sterilization and is also considered the most rational, since it avoids the mass sacrifice performed in several countries. It also decreases the risk of diseases that occur with the indiscriminate use of contraceptive drugs¹.

In female dogs, surgical sterilization is the most widely used and least controversial method of reproductive control. It is definite, safe, efficient and benefic. It is also the most suitable for the majority of animals raised for non-reproductive purposes². In veterinary practice, drugs for pain and inflammation management are administrated after this kind of procedure. Therefore, the aim of the

present study was to access the cytokine profile in female dogs undergoing ovariosalpingohysterectomy using autologous bone marrow and tramadol.

II. MATERIALS AND METHODS

The present study was approved by the Ethics Committee on Animal Experimentation (CEUA/UEMA) under protocol number 025/2011. After receiving prior authorization from the owners, 30 clinically healthy adult female dogs of different races were selected from the serial cases of the Veterinary Hospital of the State University of Maranhão.

The animals were randomly divided into three groups: the control group (G1), which received analgesia with tramadol; the conventional group (G2), which received benzathine penicillin (40,000UI/kg) and the experimental group (G3), which received tramadol plus 10 ml of bone marrow, collected from the proximal metaphysis of the humerus and immediately applied into the biceps femoris muscle.

A physical examination was performed before the surgical procedure to measure the rectal temperature, heart rate and respiratory rate. A clinical examination was also performed to measure the blood count and search for hemoparasites. The indirect immunofluorescence assay (IFA) was used to access leishmaniasis.

The analysis of the cytokines interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α) and transforming growth factor (TGF- β 1) was performed using ELISA. For this purpose, 5 ml of blood was collected from the cephalic vein at five different moments: M0 (preoperative); M1 (6 hours after surgery); M2 (48 hours after surgery); M3 (120 hours after surgery) and M4 (168 hours after surgery).

For the myelogram, 0.5 ml of bone marrow was collected, stretched on eight slides (four slides from M0 and four from M4) and stained with Giemsa. This enabled a comparison of the granulocyte, erythroid and myeloid proportions.

All animals underwent the same anesthetic protocol, using 1% acepromazine as a premedication (0.1 mg/kg) and an intravenous combination of ketamine (5 mg/kg) and diazepam (0.5 mg/kg) for anesthetic induction. The animals were intubated using sevoflurane diluted in 100% oxygen.

The animals were kept in venoclysis with the

administration of Ringer's lactate solution (10 ml/ kg/hour during the intra-operative and post-operative period) until the return of the physiological parameters and anesthesia. The traditional surgical technique was adopted in all groups.

Post-operatively, the animals were kept in appropriate individual cages where they received water and a commercial diet ad libitum, with daily cleaning of the wound with sodium chloride 0.9% for seven days. They were given back to their tutors immediately after the point's removal.

Data were tabulated and submitted to ANOVA®, followed by the Student-Newman-Keuls test (SNK) to compare the means of different observation times within the same group and between groups, considering a significance level of 5% ($p < 0.05$).

III. RESULTS

The results of the analysis of cytokines showed that

serum IL-6 exhibited a significant difference between the lowest average in the experimental group (G3) and the highest average in the conventional group (G2) (Table I). However, when performing the analysis considering the five different times, there were no significant differences between groups. There was also a statistical difference between the same cytokine in the experimental group, where M1 had a higher mean value in relation to the other moments, with the exception of M4.

When analyzing TNF- α serum, significant differences were only found between GIII, with the lowest average (40.53 ± 44.03), and GI, with the highest average (113.63 ± 99.94). No significant differences were observed in relation to the moments.

The levels of TGF- β 1 revealed significant differences between the experimental group and the conventional group, which also produced the lowest average. In the analysis between moments, there were only significant differences in GII, where M0 was lower than all the other moments (Table I).

Table 1 – Mean and standard deviation for measurements of IL-6, TNF- α and TGF- β 1 in female dogs undergoing ovariosalpingohysterectomy.

Cytokines	Groups	MOMENTS					MÉAN ± S. D	Min. Value	Max. Value
		M ₀	M ₁	M ₂	M ₃	M ₄			
IL-6	G1	89,03 ^{Aa} ± 126,30	125,23 ^{Aa} ± 90,25	132,29 ^{Aa} ± 161,23	62,75 ^{Aa} ± 91,65	76,25 ^{Aa} ± 92,97	96,85 ^{AB} ± 114,45	0	494,33
	G2	150,40 ^{Aa} ± 309,62	230,62 ^{Aa} ± 303,47	113,19 ^{Aa} ± 183,87	66,48 ^{Aa} ± 65,37	52,54 ^{Aa} ± 51,26	124,30 ^B ± 217,45	0	1049,88
	G3	31,86 ^{Aa} ± 57,01	104,50 ^{Ab} ± 72,20	31,36 ^{Aa} ± 44,72	37,56 ^{Aa} ± 44,10	45,81 ^{Ab} ± 43,13	50,05 ^A ± 58,35	0	210,00
TNF- α	G1	77,74 ^{Aa} ± 70,10	96,20 ^{Aa} ± 98,73	136,90 ^{Aa} ± 165,91	102,3 ^{Aa} ± 108,18	84,77 ^{Aa} ± 119,95	99,94 ^A ± 113,63	0	527,22
	G2	67,93 ^{Aa} ± 61,82	63,26 ^{Aa} ± 47,97	139,40 ^{Aa} ± 229,90	54,93 ^{Aa} ± 47,11	33,79 ^{Aa} ± 25,51	72,28 ^{AB} ± 112,56	5.000	774,33
	G3	49,73 ^{Aa} ± 62,36	33,46 ^{Aa} ± 52,64	36,21 ^{Aa} ± 47,04	36,74 ^{Aa} ± 29,00	47,61 ^{Aa} ± 26,24	40,53 ^B ± 44,03	0	171,22
TGF- β 1	G1	701,52 ^{Aa} ± 331,68	552,87 ^{Aa} ± 179,88	482,22 ^{Aa} ± 274,51	474,00 ^{Aa} ± 376,41	726,23 ^{Aa} ± 352,05	576,33 ^A ± 318,13	0	1237,50
	G2	119,90 ^{Ab} ± 306,85	339,26 ^{Aa} ± 441,83	606,86 ^{Aa} ± 449,37	225,93 ^{Aa} ± 185,64	468,09 ^{Aa} ± 503,09	347,16 ^B ± 405,25	0	1556,25
	G3	799,02 ^{Aa} ± 400,95	555,73 ^{Aa} ± 387,76	527,50 ^{Aa} ± 294,24	610,19 ^{Aa} ± 525,07	293,14 ^{Aa} ± 40,02	563,46 ^A ± 405,24	0	1546,00

Averages presented without data transformation. To perform the analysis of variance, values were transformed by the square root of x. Mean (\pm SD) followed by same letters in lowercase and uppercase line in column do not differ by SNK $p > 0.05$. Test for Normality of errors:

Kolmogorov-Smirnov: D = 0.09, P = 0.089.

The myelogram analysis revealed no statistically significant differences in the variables studied, either between groups or moments (Table II).

Table 2 – Mean and standard deviation of the myelogram in dogs undergoing ovariosalpingohysterectomy: granulocytic, erythrocyte and myeloid series.

Moments	GI	GII	GIII
M ₀ Granulocytic series	51,80±7,03 ^{Aa}	54,60±4,84 ^{Aa}	48,80±7,60 ^{Aa}
M ₀ Erythrocytic series	39,30±4,7 ^{Aa}	40,10±5,04 ^{Aa}	37,30±5,90 ^{Aa}
M ₀ Myeloid proportion	1,35±0,34 ^{Aa}	1,24±0,25 ^{Aa}	1,52±0,45 ^{Aa}
M ₄ Granulocytic series	55,30±5,01 ^{Aa}	60,30±6,27 ^{Aa}	60,30±5,39 ^{Aa}
M ₄ Erythrocytic series	35,90±5,10 ^{Aa}	34,40±4,00 ^{Aa}	32,60±3,23 ^{Aa}
M ₄ Myeloid proportion	1,57±0,3 ^{Aa}	1,79±0,36 ^{Aa}	1,87±0,30 ^{Aa}
Overall average (Gs)	53,55±6,25	54,55±8,03	57,45±7,05
Overall average (Es)	37,60±5,09	37,25±5,31	34,95±5,22
Overall average (Mp)	1,46±0,34	1,51±0,41	1,70±0,41

Means followed by different letters, capital letters in the same row and lowercase in the same column, different by SNK test at 5% significance between groups and same parameter respectively.

IV. DISCUSSION

Although ovariosalpingohysterectomy be a relatively simple surgical procedure, any surgical intervention promotes tissue damage and as a consequence to an injury, trauma or inflammation of tissue, it develops a complex series of reactions that aims to eliminate the offending agent and aid in tissue repair.³

The acute phase response is part of the innate defense system of animals, protecting it from trauma, infection and inflammation. During this process, a wide range of mediators are released by the tissue macrophages and blood monocytes, including cytokines, particularly TNF- α and the interleukins IL-1 and IL-6, which are the principal inducers in the synthesis of acute phase proteins.⁴

The lowest rates observed in the levels of IL-6 and TNF- α in the experimental group showed that there was a decrease in the inflammatory reaction, which is common in surgical procedures, even considering the doses of tramadol and bone marrow used to stimulate the synthesis of opioid receptors. These were able to prevent the elevation of IL-6 and TNF- α , except in the conventional group, without delaying the healing process, which occurred normally in all groups.^{5,6}

With regards to the dosages of TGF- β 1, there was a similarity between the experimental and control groups, demonstrating that the healing process occurred satisfactorily. Furthermore, this cytokine may also have decreased the level of cytotoxicity produced by T cells, thereby reducing the expression of other cytokines or their production.^{7,8}

In the present work, bone marrow plays a role as immunomodulator since it is capable to produce several kinds of cytokines and growth factors, which contribute in the healing process as is described by⁹. In Veterinary Medicine, autologous bone marrow transplantation has been used mainly in wound healing¹⁰ and bone repair¹¹.

V. CONCLUSION

The autologous bone marrow used in animals can be applied immediately before the animals are submitted to ovariosalpingohysterectomy (OSH). Pro and anti-inflammatory cytokines exhibited balanced actions during the ovariosalpingohysterectomy of female dogs.

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REFERENCES

- [1] B. V. Goethem, A. Schaeffers-Okkens and J. Kirpenstejn (2006, February 10). Making a rational choice between ovariectomy and ovariohysterectomy in the dog: a discussion of the benefits and effects. *Veterinary Surgery* [Online]. Available: <http://onlinelibrary.wiley.com/doi/10.1111/j.1532-950X.2006.00124.x/epdf>
- [2] W. P. Concannon (1997). Endocrinologia reprodutiva, contracepção e terminação da gestação em cães. In: J. S. Ettinger and C. S. Feldman (eds): *Tratado de medicina interna veterinária* (ed 4). São Paulo, Manole.
- [3] P. D. Eckersall (2000). Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Revue de Médecine Vétérinaire*. Vol. 151, no. 7, pp. 577-584. [Online] Available: http://www.revmedvet.com/2000/RMV151_577_584.pdf
- [4] C. Cray, J. Zaia, N. H. Altman (2009, December). Acute Phase Response in Animals: A Review. *Comparative medicine*, v. 59, n. 6, p. 517-26. [Online] Available: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2798837/pdf/cm2009000517.pdf>
- [5] TR Ulich, J del Castillo, IK McNiece, ES Yi, CP Alzona, SM Yin, KM Zsebo (1991, October 15). Stem cell factor in combination with granulocyte colony-stimulating factor (CSF) or granulocyte-macrophage CSF synergistically increases granulopoiesis in vivo. *Blood Journal*, Vol. 78, No. 8, pp 1954-196. [Online] Available: <http://www.bloodjournal.org/cgi/reprint/78/8/1954.pdf>
- [6] C. R. Lee, D. Mctavish and E. M. Sorkin (1993). Tramadol. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in acute and chronic pain states. *Drugs*. Vol. 46, pp. 313-340.
- [7] J. H. Curfs, J. F. Meis and J. A. Hoogkamp-Korstanje (1997, October 1). A primer on cytokines: sources, receptors, effects, and inducers. *Clin. Microbiol. Rev.* vol. 10 no. 4, pp. 742-780. [Online] Available: <http://cmr.asm.org/content/10/4/742.full.pdf>
- [8] J. M. Zhang and J. An (2007, November 30). Cytokines, inflammation, and pain. *Int Anesthesiol Clin*. Spring vol.45 no. 2, pp. 27-37. [Online] Available: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2785020>
- [9] Y. Liu, D. S. Dulchavsky, X. Gao, D. Kwon, M. Chopp, S. Dulchavsky and S. C. Gautam. (December, 2006). Wound repair by bone marrow stromal cells through growth factor production. *J. Surg. Res.* Vol. 136, Issue 2, pp. 336-341.
- [10] A. Akela, S. K. Nandi, D. Banerjee, P. Das, S. Roy, S. N. Joardar, M. Mandal, P. K. Das and N. R. Pradhan (2011, December 14). Evaluation of autologous bone marrow in wound healing in animal model: a possible application of autologous stem cells. *Int. Wound. Journal*, Vol. 9, Issue 5, pp. 505-516. [Online] Available: <http://onlinelibrary.wiley.com/doi/10.1111/j.1742-481X.2011.00909.x/pdf>
- [11] R. Hobbenagh, P. Mahboob, S. Saifzadeh, J. Javanbakht, J. Y. Y. Manesh, R. Mortezaee, S. R. Touni, E. Hosseini, S. Aghajanshakeri, M. Moloudizargari and S. Javaherypour (2014). Histopathological features of bone regeneration in a canine segmental ulnar defect model. *Diagn. Pathol.* Vol. 9, no. 59. [Online] Available: <http://www.biomedcentral.com/content/pdf/1746-1596-9-59.pdf>

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