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CLINIC AND SURGERY

Evaluation of electrosurgery and titanium clips for ovarian pedicle haemostasis in video-assisted ovariohysterectomy with two portals in bitches

Rogério Luizari Guedes[1*] Fernando Wiecheteck de Souza[2] Gabrielle Coelho Freitas[3] Cristiano Gomes[4] Jorge Luiz Costa Castro[5] Francieli Marconato[6] Verônica Souza Paiva Castro[7] Ney Luis Pippi[6] Peterson Triches Dornbusch[1]

[1]Programa de Pós-graduação em Ciências Veterinárias, Universidade Federal do Paraná (UFPR), 80035-050, Curitiba, PR, Brasil. E-mail: rogerioguedes@veterinario.med.br. *Corresponding author.
[2]Departamento de Medicina Veterinária, Universidade Federal de Alagoas (UFAL), Viçosa, AL, Brasil.
[3]Departamento de Medicina Veterinária, Universidade Federal da Fronteira Sul (UFFS), Realeza, PR, Brasil.
[4]Departamento de Medicina Veterinária, Universidade Federal do Rio Grande do Sul (UFGRS), Porto Alegre, RS, Brasil.
[5]Departamento de Medicina Veterinária, Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, PR, Brasil.
[6]Programa de Pós-graduação em Medicina Veterinária, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[7]Hospital Veterinário Batel, Curitiba, PR, Brasil.

ABSTRACT: *This study evaluated the use of bipolar electrosurgery and laparoscopic clipping, and their effects on blood loss and the inflammatory response, during a two portal video-assisted ovariohysterectomy technique (two groups with 10 animals each). Surgical time and blood loss volume were significantly lower in the electrosurgery group. There were no significant changes in haematocrit between groups; however, haematocrit did differ between evaluated times, and decreased 10% from the initial measurement to four hours after the procedure. The inflammatory response was significantly higher throughout the post-surgical period, but without any different clinical signs between the two groups. Both techniques had good application for the two portal video-assisted procedure; however, the bipolar electrosurgery allowed for shorter surgical times, reduced blood loss and a minimal learning curve for the surgeon.*
Key words: *laparoscopy, blood tightening, blood loss, packed cell volume, interleukin.*

Eletrocirurgia bipolar e clipes de titânio para hemostasia em pedículos ovarianos durante ovariohisterectomia videoassistida com dois portais em cadelas

RESUMO: *Este estudo avaliou a utilização da eletrocirurgia bipolar e do clipador laparoscópico em relação à perda sanguínea e resposta inflamatória durante a ovariohisterectomia videoassistida com dois portais (dois grupos com 10 animais). O tempo cirúrgico, assim como o volume de sangue perdido foram significativamente menores no Grupo Bipolar. Não houve mudanças significativas no hematócrito entre os grupos, mas entre os tempos avaliados houve redução de 10% do valor inicial até quatro horas após o procedimento. A resposta inflamatória foi significativamente maior durante todo o período de avaliação após a cirurgia, mas sem manifestações clínicas diferentes daquelas apresentadas pelo Grupo Clipador. Ambas as técnicas têm boa execução pelo procedimento videoassistido, contudo, o uso da eletrocirurgia bipolar permite tempos cirúrgicos menores, perda de sangue mínima e menor curva de aprendizado para o cirurgião.*
Palavras-chave: *laparoscopia, estancamento de sangue, sangramento, hematócrito, interleucina.*

INTRODUCTION

Haemostasis is one of the most important steps in laparoscopic surgery (NEWMAN & TRAVERSO, 2006). It is ideal is create a virtually bloodless surgical site, as in minimally invasive procedures small amounts of blood can interfere with direct visualisation and promote light absorption, making it difficult to identify the dissection planes (FREEMAN, 1998). Through smaller access incisions, laparoscopy limits tissue trauma and decreases parietal bleeding (COLLARD & VIGUIER, 2008).

Laparoscopic haemostasis depends on specific instruments, some adapted from conventional surgical procedures. It involves mechanical methods such as the application of metal clips and plastic and absorbable clips by simple clip appliers (NEWMAN & TRAVERSO, 2006), or linear staplers, pre-tied suture loops, internal ligatures and sutures (BAX, 2003). There are also energy induction methods of

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haemostasis, including electric (by a monopolar or bipolar circuit), the laser beam and also ultrasonic energy (NEWMAN & TRAVERSO, 2006; DUBIEL et al., 2010).

Ovariohysterectomy (OVH) is probably the most studied laparoscopic technique in veterinary (DAVIDSON et al., 2004; MAYHEW & BROWN, 2007; BRUN et al., 2008; SCHIOCHET et al., 2009; BRUN et al., 2011). Nevertheless, there are few data regarding the quality of haemostatic techniques for laparoscopic OVH, and no study has evaluated the laparoscopic clip applier, or its use with the two portals video-assisted technique. For this reason, the goal of this study was to evaluate the effectiveness of haemostasis via applying titanium clips and diathermy by bipolar electrosurgery during OVH.

MATERIALS AND METHODS

Twenty healthy, adult bitches were selected and separated into two groups of 10 animals each. In Group I (GI, 10,62±4,8kg) the haemostasis method was electrosurgery with laparoscopic bipolar forceps (electrosurgical unit Emai BP150, 150W, forceps 42cm/5mm, EDLO S/A, Rio Grande do Sul, Brazil), and in Group II (GII, 11,2±4,5kg) the method was a laparoscopic clip applier with titanium clips (33cm/10mm, Karl Storz Endoskope, Baden-Württemberg, Germany). The patients were submitted to the anaesthetic and fluid therapy protocols as described by Guedes et al. (2015).

The video-assisted OVH technique with two portals followed the indications described by DEVITT et al. (2005), but in this case, due to the use of an endoscope without a working channel, the introduction of the second trocar occurred immediately after the first one was placed, as performed by BRUN et al. (2008). Haemostasis of the ovarian arteriovenous complex (CAVOS) occurred with bipolar electrosurgery in GI patients or by application of titanium clips in GII. Blood removal during surgery was performed by laparoscopic gauze throughout the procedure (from the first access incision to application of the last skin suture), which were weighed on a precision scale for further evaluation of lost blood volume. The gauze was weighed right after use in the surgical procedure to minimise volume loss by evaporation. The difference between the weights of the fresh gauze and used gauze was calculated and provided the mass (in grams) of the patient's blood loss. The conversion factor from grams to millilitres was obtained by weighing 1,0ml of peripheral venous blood from each patient on the same precision scale.

To check serum changes, peripheral venous blood samples were taken for analysis of packed cell volume, total plasma protein, platelet count and white blood cell count. The first sample was from the pre-surgical tests, the second was taken five minutes after premedication (MPA) administration and a third was taken immediately after finishing haemostasis for the second ovary. Three new samples were collected at four, 24 and 72 hours after the procedure. At the same time, samples were separated for serum evaluation of inflammatory cytokines interleukin-1 (IL-1) and interleukin 6 (IL-6), as well the acute phase protein alpha-1-glycoprotein (AGP).

To check if there was a significance difference between the pre- and post-operative samples, as well as between the two different haemostatic techniques used, the values obtained in GI and GII were statistically analysed by analysis of variance, an F test and the Tukey's test. When it was not possible to use parametric tests, the Kruskal-Wallis test was used.

RESULTS

The operative time was 61±14,51min for GI and 85±33,94min for GII, with a significant difference (P=0,05) in the non-parametric Kruskal-Wallis test which was selected since the coefficient of variation normalisation in parametric tests couldn't be achieved. In the procedures conducted in GII, an average of 16,97±6,23 clips were used for the ovarian pedicles. The left ovary required more clips (9,44±6,17) compared to the right ovary (7,67±6,63).

As for trans-operative bleeding, there was significant variation between groups according to the haemostasis technique (P=0,014). The laparoscopic gauzes from GI produced an average of 2,33±2,68g or 1,92±2,25ml, and those from GII produced 13,03±16,11g or 10,86±13,41ml. The serum haematocrit (Ht) went through similar changes in both groups but did not differ statistically; however, it did show significant differences between times (Table 1). Serum Ht levels decreased after MPA application, but after four hours of the procedure, Ht levels began to increase and approached those obtained in the initial evaluation of the patients. The sharpest decrease was of approximately 10%, reaching values below normal for the species. This occurred immediately after haemostasis of the second ovary, and was from the sample collected in the trans-surgical time. Unlike Ht, the values obtained for total plasma protein (PPT) differed significantly between groups and behaved similarly between times, with no reduction of serum levels below normal in the dogs (Table 1).

Table 1 - Average serum levels of packed cell volume (Ht) e total plasma protein (PPT), obtained in Bipolar and Clipador groups, at each evaluation time of the experiment. Pre-surgical evaluation (T1); after preanesthetic medication (MPA); trans-surgical/immediately after hemostasis for the second ovary (T2); after 4 (T3), 24 (T4) and 72 hours from surgical procedures (T5).

Times	----------Ht (%)----------		----------PPT (g/dl)----------	
	GI	GII	GI	GII
T1	43.13a	42.24a	8.42a	8.34a
MPA	37.78ab	40.0ab	8.24a	8.57a
T2	33b	33.89b	7.6a	7b
T3	38.4ab	36.4ab	8.92a	7.74ab
T4	42.60a	41.2ab	8.24a	8.16a
T5	40.9a	39.5ab	8.3a	8.04a
Reference	37-55		5.1-7.8	

a,b - Means followed by different letters differ at 5% of probability by the parametric Tukey test. Values: TILLEY & SMITH, 2000.

The white blood cell count showed changes similar to those occurring with Ht and PPT values, which were highly significant (P=0,0001) between groups and times (Table 2). The white blood cell count remained lower in GI for all times, and 24 hours after the procedure GII presented serum levels above the normal range. Both pre-inflammatory cytokines IL-1 and IL-6, as well as the acute phase protein AGP, also experienced highly significant changes between groups and times (Table 2), characterized by a gradual increase until the last evaluation period at 72 hours postoperative. During all evaluated times, serum concentrations remained lower in GII, with major differences after 24 postoperative hours, and in this same group, IL-6 values persistedafter 72 hours. The values of AGP remained in the normal range for dogs in GI and GII.

DISCUSSION

The variation in surgical times may be influenced by factors such as methods of haemostasis used, the degree of surgical difficulty, surgeon experience and the animal's physical and physiological state before the surgical procedure (VAN GOETHEM et al., 2003). These factors influenced the time differences between the techniques, in a particular amplitude in GII, where procedures ranged from 53-158 minutes. The procedures were shorter in GI, lasting between 39 and 79 minutes. Although surgical time in three animals of GII surpassed the 85 minutes quoted by COLLARD & VIGUIER (2008), these cases fit the time variation found in the study by DAVIDSON et al. (2004), where elective laparoscopic OVH's ranged between 47 and 175 minutes. MAYHEW & BROWN (2007) and GUIZZO-JUNIOR et al. (2015) minimised surgical time using more specialized devices. They used a multiple shot clip applier (which did not need to be removed from the abdominal cavity after each application to exchange clips) as well as an ultrasonic scalpel and a bipolar device that had a cutting blade, thus avoiding exchange with laparoscopic surgical scissors. As described by Van NINWEGEN & KIRPENSTEIJN (2007), deposition of adipose tissue in the mesovarium and morphological/physiological changes in reproductive structures can also increase the operating time by up to 34%, even using a bipolar system with a cutting edge during ovariectomized bitches. However, in all cases regardless of the group, surgical times remained acceptable, with adequate recovery of the patients and without moderate or severe post-operative complications. The authors of this study agree with MAYHEW & BROWN (2007) and SCHIOCHET et al. (2009), in that the ligation and clip techniques are effective, but the bipolar electrosurgery allows for optimal haemostasis, is easy to perform and reduces surgical time, which were all conditions noted by the surgical team during the experimental period.

In a study by MALM et al. (2004), 15 healthy bitches underwent laparoscopic OVH, and nine animals showed no bleeding, four had minor bleeding between 3,8 and 7,4ml and two had severe bleeding of 96 and 140ml. These last two results occurred due to a lesion of the left uterine artery, and the other blood losses agree with those observed in this study, where the bleeding of the CAVOS represented less than 3% of the patient's total blood volume, and in GI that value did not exceed 1%. Empirically, the decrease in Ht was around 10% and was not related to blood loss, but was instead

Table 2 - Average serum levels of total leukocytes, pro-inflammatory cytokines interleukin 1 (IL-1) and interleukin 6 (IL-6), and acute phase protein alpha-1-glycoprotein (AGP) obtained in Bipolar (GI) and Clipador group (GII) at each evaluation time of the experiment. Pre-surgical assessment (T1); after premedication (MPA); trans-surgical/immediately after the second ovary haemostasis (T2); after four (T3), 24 (T4) and 72 hours of surgery (T5).

	-------Leukocytes (mm^3)-------		----------IL-1 (pg/ml)----------		----------IL-6 (pg/ml)----------		----------AGP (µg/ml)----------	
	GI	GII	GI	GII	GI	GII	GI	GII
T1	11.450^{ab}	13.620^{ab}	15.2^{e}	16.3^{c}	25^{f}	26.11^{d}	104.5^{e}	76.6^{d}
MPA	9.378^{cd}	8.774^{c}	25.3^{de}	19.6^{c}	33.7^{e}	31.11^{d}	110.5^{e}	81.6^{d}
T2	5.489^{e}	5.970^{c}	42.4^{d}	33.1^{c}	44.4^{d}	48.67^{c}	129.1^{d}	86.9^{d}
T3	11.960^{bc}	13.935^{ab}	93.7^{c}	68.1^{b}	83.5^{c}	86.89^{b}	159.1^{c}	128.6^{c}
T4	14.459^{a}	19.854^{a}	154.9^{b}	95.6^{b}	172.3^{b}	112.9^{b}	184^{b}	156.1^{b}
T5	11.318^{ab}	15.560^{ab}	203.5^{a}	150.7^{a}	243.5^{a}	163.7^{a}	360.5^{a}	237.6^{a}
Reference	6000 - 17000		1 - 50		2 - 200		<380	

a,b,c,d,e - Means followed by different letters differ at 5% of probability by the parametric Tukey test. Values: TILLEY & SMITH, 2000 (leukocytes); Standard curves performed during the study LABIMED Análises Clínicas, Santa Maria - RS (IL-1, IL-6); RIKIHISA et al., 1994 (AGP).

due to the anaesthetic protocol and fluid therapy, since the values found were very similar to those presented in a study that evaluated only the changes induced by the protocols (GUEDES et al., 2015). This demonstrates that the bleeding that occurred in both groups did not significantly change the Ht, and consequently, did not cause significant physiological changes. But the differences in volume of blood loss influenced the operative difficulty presented in GII, and as suggested by FREEMAN (1998), small amounts of blood can absorb light and interfere with laparoscopic direct vision, making it difficult to identify the dissection planes and therefore leading to inadequate application of clips to blood vessels surrounded by tissues.

An increase in leukocyte numbers and cytokines associated with AGP was expected in both groups, since their responses were directly proportional (JAIN, 1989; CERON et al., 2005). The greater intensity of this response in GI may be due to the electricity generating more aggressive and extensive damage, including cellular and tissue desiccation in the area where the bipolar haemostasis was applied, even having a self-limiting action and minimizing tissue from suffering injuries (FREEMAN, 1998; NEWMAN & TRAVERSO, 2006). In GII, mechanical application of clips potentially injured a relatively smaller area, and the foreign body reaction is also minimal by titanium-based material since it's an inert implant. The hypothesis of a high response due to the need to expand the surgical wound during removal of the ovaries was also considered; however, GI showed fewer cases than GII (n=2/n=3), and thus this variable wasn't attributed to be an inducing agent of the observed inflammatory response.

Although there were significant differences between the groups, this study demonstrated continuous increases of inflammatory parameters at each time evaluated, with the exception of total leukocytes, which differed partially from other studies. Cytokines are considered to be the most faithful indicators of the systemic response to inflammatory and infectious processes. Stimulation of acute phase protein synthesis occurs within six to eight hours after the injury, and the maximum concentration is reached in two to five days. However, the peak and the persistence of the plasma concentrations of these proteins depend on the metabolism, vascular leakage and tissue deposition (PAIM, 2011). According to FREEMAN et al. (2010), the peak of IL-6 activity is found at 24 hours after surgical procedures. FREEMAN et al. (2009) suggested that IL-6 activity peaking at two hours after the procedure, and returning to baseline at 18 hours, being considered as an early indicator of tissue damage. MIRA (2010) stated that the concentration of positive acute phase proteins begins to rise in the early hours after the stimulus, with peaks at 24-48 hours, and remains high while the stimulus persists. The serum concentration of AGP changes significantly after an inflammatory process and increases rapidly, with an average life of approximately 5,5 days (KOGIKA et al., 2003) and potentially persisting up to 12 days (GANROT, 1973). It is believed that due the above reasons it was not possible to observe the decreases in this study, when the last measurements were made at three days after surgery. The persistence of an inflammatory stimulus or infection is a possible explanation for the continued increase in inflammatory markers, but all patients, regardless of the group they belonged to, remained

clinically stable throughout the study period. After 15 days the same clinical conditions were confirmed by telephone with owners or guardians.

CONCLUSION

The evaluated techniques provided good quality haemostasis prevention, but the bipolar system was superior to clip application for the ovarian vessels, as the execution was easier and there was reduced surgical time and less bleeding. Although more effective, bipolar electrosurgery haemostasis also promotes a more exuberant inflammatory response when compared to the application of titanium clips up to 72 hours after surgery, but this was not reflected in clinical changes.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was submitted to the Internal Ethics Committee in Animal Experimentation of the Universidade Federal de Santa Maria (UFSM), with the protocol n°099/2010.

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