Supplementary file: Influence of Circadian Rhythm on the Surgical Stress Response in Bitches Undergoing Elective Ovariohysterectomy

Table S1. Padronization of the surgical time and procedures in bitches undergoing ovariohysterectomy in the morning (GAM) or at night (GPM).

Pre-operative	
Procedure	**Duration (minutes)**
Application of preanesthetic medication	15
Trichotomy	10
Cephalic vein access and fluid therapy	5
Induction/intubation	4
Epidural anesthesia	10
Animal positioning and antisepsis	3
Total time	**50**
Surgery	
Procedure	**Duration (minutes)**
Positioning and securing of surgical drapes	3
Skin, subcutaneous and muscular incision	2
Localization of the right ovary, administration of lidocaine, rupture of the ligament, and cauterization of the arteriovenous complex	4
Localization of the left ovary, administration of lidocaine, rupture of the ligament, and cauterization of the arteriovenous complex	4
Localization of the uterine body and cauterization	2
Examination of the abdominal cavity and omentalization	1
Muscle layer closure	4
Closure of the subcutaneous	2
Skin closure	3
Total time	**25**

CLINICAL AND LABORATORIAL DATA

Table S2. Mean and standard deviation (M ± SD) of the PAS (mmHg) in bitches undergoing ovariohysterectomy in the morning (GAM) or at night (GPM). Columns present the results of each group; lines present the different times of evaluation.

Tempo	**GAM** **M ± SD**	**GPM** **M ± SD**	**P *value***
Basal	150 ± 27.3	159 ± 21.0	0.47
2h	143 ± 19.5	160 ± 27.9	0.14
6h	163 ± 29.4	171 ± 36.2	0.62
12h	155 ± 23.4	161 ± 22.2	0.55
24h	152 ± 17.8	151 ± 23.2	0.96
48h	152 ± 23.3	156 ± 15.2	0.67
14 dias	168 ± 31.2	167 ± 20.9	0.92
P *value*	0.40	0.71	

Table S3. Mean and standard deviation (M ± SD) of the heart rate (bpm) in bitches undergoing ovariohysterectomy in the morning (GAM) or at night (GPM). Columns present the results of each group; lines present the different times of evaluation. Lowercase letters indicate statistical differences ($p < 0.05$).

Tempo	**GAM** **M ± SD**	**GPM** **M ± SD**	**P *value***
Basal	106 ± 18.50abc	106.4 ± 20.41a	0.96
2h	89.8 ± 12.18abc	83.1 ± 15.85ab	0.33
6h	90.8 ± 9.81abc	92.8 ± 16.42ab	0.74
12h	91.6 ± 12.61abc	96 ± 9.38ab	0.36
24h	90.2 ±10.02abc	82.7 ± 11.83b	0.16
48h	86.2 ± 13.28ab	85.6 ± 15.11ab	0.92
14 dias	111.6 ± 29.15ac	158 ± 23.81c	**0.003**
P *value*	**0.009**	**<0.001**	

Table S4. Mean and standard deviation (M ± SD) of the rectal temperature (ºC) in bitches undergoing ovariohysterectomy in the morning (GAM) or at night (GPM). Columns present the results of each

group; lines present the different times of evaluation. Lowercase letters indicate statistical differences ($p < 0.05$).

Tempo	GAM M ± SD	GPM M ± SD	P *value*
Basal	38.3 ± 0.41a	38.5 ± 0.33ac	0.14
2h	37.4 ± 0.34b	37.9 ± 0.55bc	**0.046**
6h	38.1 ± 0.47ab	38.3 ± 0.32abc	0.26
12h	38.2 ± 0.37a	38.0 ± 0.50abc	0.35
24h	38 ± 0.37a	38 ± 0.38abc	0.90
48h	37.8 ± 0.38ab	37.9 ± 0.39ab	0.69
14 dias	38.5 ± 0.52a	38.6 ± 0.47ac	0.65
P *value*	**<0.001**	**0.001**	

Table S5. Mean and standard deviation (M ± SD) of the Lymphocytes (cells/µL) of bitches undergoing ovariohysterectomy in the morning (GAM) or at night (GPM). Columns present the results of each group and the lines the different times of evaluation.

Tempo	GAM M ± SD	GPM M ± SD	P *value*
Basal	2756 ± 933	2043 ± 950	0.12
6h	2251 ± 700	1785 ± 673	0.18
12h	2296 ± 840	1893 ± 426	0.27
24h	2772 ± 1156	2489 ± 989	0.59
48h	2850 ± 1163	2672 ± 936	0.71
14 dias	2894 ± 764	2387 ± 759	0.27
P *value*	0.59	0.19	

Table S6. Mean and standard deviation (M ± SD) of the Eosinophil (cells/µL) of bitches undergoing ovariohysterectomy in the morning (GAM) or at night (GPM). Columns present the results of each group and the lines the different times of evaluation..

Tempo	GAM M ± SD	GPM M ± SD	P *value*

Basal	1459 ± 774	1465 ± 671	0.96
6h	1041 ± 843	1128 ± 748	0.81
12h	698± 841	1498 ± 1107	0.14
24h	1115 ± 1083	1680 ± 1026	0.25
48h	988 ± 439	1612 ± 819	0.08
14 dias	1837 ± 1228	1460 ± 1099	0.57
P *value*	0.06	0.74	

Table S7. Mean and standard deviation (M ± SD) of the erythrocyte substances reactives of the thiobarbituric acid (TBARS) (nmol MDA/mL erythrocytes) of bitches undergoing ovariohysterectomy in the morning (GAM) or at night (GPM). Columns present the results of each group and the lines the different times of evaluation.

Tempo	**GAM** **M ± SD**	**GPM** **M ± SD**	**P *value*****
Basal	18.6 ± 4.56	17.7 ± 3.64	0.65
6h	20.0 ± 4.58	17.4 ± 2.83	0.14
12h	18.8± 3.21	19.5 ± 3.54	0.62
24h	17.7 ± 3.01	20.1 ± 4.32	0.16
48h	17.4 ± 2.97	19.9 ± 3.80	0.12
14 dias	17.7 ± 4.41	19.5 ± 2.29	0.28
P *value*	0.73	0.38	