

A New Surgical Approach in Bitches with Vaginal Fold Prolapse

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ABSTRACT

The aims of present study were to investigate the efficiency of new surgical approach by clinical (complete blood count, operation time) and to determine the response of oxidative stress, immun system and acute phase proteins with total antioxidant status, total oxidant status, oxidative stress index, nitric oxide, interleukin 1, 6, 8, 10, tumour necrosis factor alpha, haptoglobin, C-reactive protein and serum amyloid A in bitches with vaginal fold prolapse. The seven bitches with vaginal fold prolapse were conducted as a study group and operated vaginal amputation or submucosal resection with a new technique by Reverdin needle. The six bitches with vaginal fold prolapse were planned as a control group and treated by routine vaginal amputation or submucosal resection. The blood samples were collected to just before operation and after operation two hours, seven days, fourteen days in two groups. The mean weight of the excised prolapsed fold in the study group was 157.31 ± 44.46 g with an operative time of 39.28 ± 6.74 min. In comparison, the removed vaginal tissue in the control group weighed 165.17 ± 36.82 g and took 45.67 ± 7.87 min ($P>0.05$). In the groups, the above parameters were evaluated preoperatively and postoperatively (after 2 hours, 7 and 14 days) and were not find difference except for few blood findings in both groups and interleukin 1 in the control group. In the intergroup comparison, the study group showed statistically higher interleukin 1, 8, haptoglobin, and C-reactive protein levels on postoperative day 14 compared to the control group, while its total oxidant status value was found to be lower. The result of this study, the new technique used did not demonstrate a clear superiority over the routine operative procedure. However, it can be chosen to shorten the operation time and press of all tissue.

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INTRODUCTION

In the bitches, masses protruding from the vulva may occur due to many reasons such as vaginal fold prolapse (VFP), true vaginal prolapse, vaginal neoplasia, clitoral enlargement or uterine prolapse (Sontas et al., 2010). VFP occurs often at the peak of estrogen concentration in intact female dogs which are usually young and during between first and third estrous cycle (Schutte, 1967; Post et al., 1991; Johnston et al., 2001; Nak and Kasikci, 2013; Singh et al., 2022; Antonov and Ivanova, 2023). VFP is a result of the vaginal mucosa overreacting to estrogens during prooestrus or early oestrus. On the other hand, true vaginal prolapse is the protrusion of the entire vaginal mucosa through the vulva. This rarely occurs after copulation, during pregnancy and before or after parturition due to increased oestrogen levels, or as a result of increased intra-abdominal pressure, abdominal trauma in a pregnant bitch, vaginal tumours (transmissible venereal tumour), or constipation. In the past, VFP is also named vaginal hyperplasia, vaginal prolapse, vaginal hypertrophy, estrual hypertrophy, vaginal eversion and vaginal protrusion. Today, the more common term VFP is used to describe this

condition, which is different from other species (Sontas et al., 2010; Ozenc et al., 2016 and 2017).

There are three degrees of VFP in bitches. Slight to moderate eversion of vaginal floor with no observation this tissue through the vulvar lips is classified as Type I. In Type II; prolapse tissue of the cranial floor and lateral walls of the vagina is observed through the vulvar lips like a tongue or pear. Type III, the vaginal wall is completely prolapsed as a doughnut shaped (Johnston et al., 2001; Kurt et al., 2019; Anya et al., 2020; Bucci et al., 2022; Antonov et al., 2023; Methai et al., 2023).

Slight cases can be spontaneously resolved during diestrus stage without any treatment (Semacan, 2007; Singh et al., 2022; Antonov et al., 2023). However, in the majority of spontaneous regressing cases will be recurrence at the subsequent estrous cycle (66-100%) (Johnston et al., 2001; Antonov et al., 2023). Ovulation may be induced by GnRH or hCG in affected bitches that are not yet during preovulatory stage with serum progesterone level below 2 ng/ml (Johnston et al., 2001; Nak and Kasikci, 2013).

Hysteropexy can be tried, if the prolapsed vagina tissue is not returned or stabilized its normal position. Ovariohysterectomy can be applied in chronic cases (Semacan, 2007). If the prolapsed vagina tissue prevents urination, there is a possibility of infection or impair of vagina and uterine circulation, serious necrosis and it becomes persistent, it must be excision by operation (Nak and Kasikci, 2013; Bucci et al., 2022). Submucosal resection of vagina and vaginal amputation can be performed in Type II and III, respectively (Nak and Kasikci, 2013).

Wound healing processes consist of cellular and biochemical events that begin with trauma and result in new tissue formation. Many factors affect this recovery (Gonzalez et al., 2016). Antioxidants play a role in wound healing by removing reactive oxygen species that damage wounds due to their effects on cells and tissues (Kumar et al., 2007). Cellular immunity also plays an important role in the regulation of wound healing. Proinflammatory cytokines such as interleukin 1 (IL-1), IL-6 and tumour necrosis factor alpha (TNF- α) help protect against infections, prepare injured tissue for repair and increase the migration and activation phagocytes (Lowry, 1993). It had been reported that IL-8 modulates the functions of many inflammatory cells during wound healing (Konstantinova et al., 1996). Acute phase proteins are used to assess the immune system against infection, inflammation or trauma. These blood proteins are synthesized in the liver and their secretion is regulated by proinflammatory cytokines, especially IL-6 (Tiftik, 1996; Murata et al., 2004; Petersen et al., 2004). They play a role in limiting microbial growth and reorganizing hemostasis in animals exposed to infection, inflammation, surgical trauma or stress (Eckersall, 2004; Eckersall and Bell, 2010).

The aims of present study were to investigate the efficiency of new surgical approach by clinical (complete blood count, operation time) and to determine the response of oxidative stress, immune system and acute phase proteins with total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NO), oxidative stress index (OSI), IL-1, IL-6, IL-8, IL-10, TNF- α , haptoglobin (Hp), C-reactive protein (CRP) and serum amyloid A (SAA) in bitches with VFP.

MATERIALS AND METHODS

Groups

The thirteen female Turkish-Anatolian Shepherd Dogs with VFP, ranging in age from 8-36 months, were used in the present study. Seven of these animals were in the study group (applied new surgical technique) (Figure 1) and six bitches were in the control group (applied routine surgical technique). The bitches with VFP were taken groups sequentially. Operations of all animals were performed by the same operators.

Study design

Amoxicillin (Vilamoks-LA®) (15 mg/kg, SC) was administrated before xylazine-ketamine anesthesia. Anesthesia was induced with xylazine (Basilazin®) (2 mg/kg, IM), followed by ketamine (Ketasol®) (10 mg/kg, IM) ten minutes later. The bladder was catheterized following anesthesia. Operation time was recorded from the placement of surgical drape to end of the operation. In addition, the weight of the excised tissue was measured.

Operation in the study group (n=7). Figure 1 was showed operations of study group in Type III (n=5).

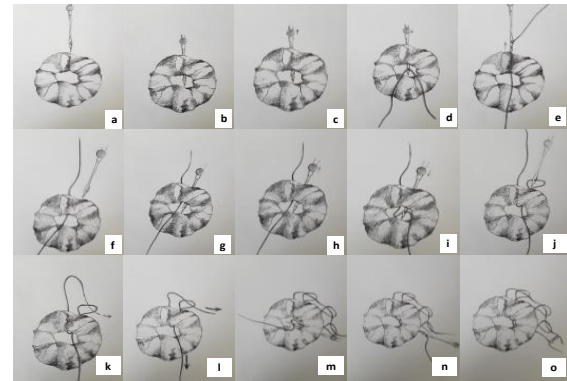


Figure 1. Operation technique applied to bitches with Type III vaginal fold prolapse in the study group (up to 4 o'clock). a-b: Direction and application of Reverdin needle (without suture material). c: Opening the eye of Reverdin needle. d: Threading the suture material into the eye of Reverdin needle and closing the needle's eye. e: Pulling out the half of the suture material. f-g-h: Re-application of Reverdin needle at another angle. i: Threading the suture material (part of inside) into the eye of Reverdin needle and closing the needle's eye. j: Forming a loop by pulling out the Reverdin needle. k: Passing the suture material (part of outside) through this loop. l: Tightening the inner and outer ends of the suture. m: Repeating of this technique. n-o: Ligation of the suture.

All of prolapse tissue was surrounded using this suture technique (ligated at 4, 8 and 12 o'clock) in Type III.

This suture technique was also applied in Type II (n=2) without interruption. The Reverdin needle was passed from right to left through the base of the prolapsed tissue without a suture material. The eye of the Reverdin needle was then opened, threaded with the suture material, and closed. Half of the suture material was pulled through from left to right. Next, the Reverdin needle was passed through the tissue again, directly underneath, without the suture material. The left part of the suture material was threaded into the needle's eye, and the eye was closed. A loop was formed by drawing the Reverdin needle back to the right, and the right end of the suture was passed through this loop. Both ends of the suture were then tightened. This technique was repeated along the entire length of the prolapsed tissue. Upon reaching the end of the tissue, the left end of the suture was pulled to the right using the Reverdin needle, and the suture was ligated.

In both types, the prolapsed tissue was removed just in front of the suture line.

Operation in the control group (n=6). Submucosal resection and vaginal amputation were applied to the bitches with Type II (n=1) and Type III (n=5) in the control group, respectively (Nak and Kasikci, 2013).

Sample collection and preparing for analysis

The blood samples were collected just before induction of anesthesia and 2 hours, 1 week and 2 weeks after operation in two groups. Blood samples were collected into serum, EDTA and heparinized test tubes by *V. cephalica antebraichium*. The sera and plasma samples were obtained in the blood sample collected into the sera (3000 rpm, 10 min) and heparinized tubes (3000 rpm, 10 min, at 4°C) by centrifugation (Hettich, Germany), respectively. The sera and plasma samples were stored at -80°C until biochemical analysis.

Hematological parameters

Into the EDTA tubes, blood samples were used to determine white blood cell (WBC), erythrocyte (RBC), lymphocyte (LYM), monocyte (MON), neutrophil (NEU),

eosinophil (EOS), basophil (BAS), hemoglobin (HGB), hematocrite (HCT) and platelet (PLT) count by using commercial test kits using blood counter (Abacus Junior Vet 5, Austria). All samples were evaluated on the same day.

Cytokines and acute phase proteins, nitric oxide levels

Blood samples were individually collected into serum test tubes for cytokines, acute phase proteins and NO. IL-1 (ECA0030), IL-6 (ECA0013), IL-8 (ECA0014), IL-10 (ECA0012), TNF- α (ECA0020), CRP (ECA0006), HP (ECA0008), SAA (ECA0029) and NO (EH4229) levels were determined by an ELISA reader (Biotek, USA) with Canine ELISA diagnostic kits (Fine Test, China).

Oxidative stress parameters

Blood samples were individually collected into heparinized test tubes for TOS and TAS were determined by an ELISA reader (SIRIO S, Indonesia) with test kits (Rel Assay Diagnostics, Türkiye). The ratio of TOS to TAS is defined as OSI. The units of TOS and TAS were equated to calculate the index. Results were expressed in Arbitrary Units (AU). OSI (arbitrary unit) = TOS ($\mu\text{mol H}_2\text{O}_2$ Eq/L)/TAS ($\mu\text{mol Trolox Eq/L}$) $\times 100$ (Kosecik et al., 2005).

Statistical analysis

Data processing was performed with the SPSS 15.0 (SPSS, Inc., Chicago, IL, USA). The normality of all data was assessed by the Shapiro-Wilk test. In the blood samples taken before the operation, on the 2nd hour, 7th day, and 14th day after the operation, repeated measurements and comparisons between groups, variance analysis (General Linear Model) was used for parameters with normal distribution, and Friedman's test was used for non-normally distributed parameters. In the statistical analysis of whole blood findings, TAS, TOS, OSI, acute phase proteins, and cytokine levels of dogs in the study and

control groups, one-way ANOVA test was performed in repeated measurements. In the test, $p < 0.05$ was accepted as important. RBC, PLT (study group) and RBC, LYM, NEU (control group) counts, IL-1, IL-6, IL-10, TNF- α , HP, SAA, NO (study group) and IL-6, SAA (control group) levels were tested using the Friedman test and $p < 0.0083$ in the Wilcoxon test with a Bonferroni adjustment to determine which of the 4 groups differed from the others. When comparing the control and study groups; Normality assessments for intergroup comparisons (Shapiro-Wilk test) revealed that IL-6 and SAA levels (preoperative, postoperative hour 2, days 7 and 14), TNF- α levels (preoperative and postoperative days 7 and 14), HP levels (preoperative and postoperative hour 2), and NO levels (postoperative days 7 and 14) exhibited a non-parametric distribution. Accordingly, the Mann-Whitney U test was used to compare the data at these specific time points, and statistical significance was evaluated at a Bonferroni-adjusted $p < 0.025$. Independent Samples T Test was performed on the other parameters. In the test, $p < 0.05$ was accepted as important.

RESULTS

The bitches were 17 ± 3.66 and 11.67 ± 2.54 months old in the study and control groups, respectively. There was no statistically difference between groups ($p > 0.05$) (Table 1). According to the owners, eight bitches (study group=3, control group=5) were at first estrous period, four bitches (study group=3, control group=1) were at second estrous period and one bitch (study group) was at third estrous period. However, it was learned that all of the bitches, which were at second and third estrous periods, were also observed at the previous estrous periods by anamnesis. The owners stated that vaginal fold prolapsed were lasting for 17.28 ± 7.25 and 13.67 ± 3.67 days in the study and control groups, respectively (Table 1). There was no statistically difference between groups ($p > 0.05$).

Table 1. Age and lasting of vaginal fold prolapse of the bitches in the study and control groups.

Groups	Study (n=7)			Control (n=6)			p
	Mean $\pm$ SE	Median	Mean Rank	Mean $\pm$ SE	Median	Mean Rank	
Age (month)	17 ± 3.66	12	8.79	11.67 ± 2.54	9	4.92	NS
Lasting of vaginal fold prolapse (day)	17.28 ± 7.25	10	7	13.67 ± 3.67	12.50	7	NS

NS: not significant and n: number of animals.

The weight of the extracted vagina tissue from the bitches was 157.31 ± 44.46 and 165.17 ± 36.82 g in the study and control groups, respectively. There was no statistically difference between groups ($p > 0.05$). The mean operation time of the bitches with VFP was 39.28 ± 6.74 and 45.67 ± 7.87 min in the study and control groups, respectively. There was also no statistically significant difference between the groups ($p > 0.05$).

The results of complete blood count of preoperative and postoperative (hour 2, days 7 and 14) in the study group are shown in Table 2.

In the study group, the count of WBC ($p < 0.01$), MON ($p < 0.05$) and NEU ($p < 0.001$) were significantly increased 2nd hour after the operation compared to the 14th day after the operation. Other hematological parameters did not differ statistically among the preoperative and

postoperative (hour 2, days 7 and 14) ($p > 0.05$). The results of complete blood count of preoperative and postoperative (hour 2, days 7 and 14) in the control group are shown in Table 3.

In the control group, the count of WBC ($p < 0.05$) and NEU ($p < 0.01$) were significantly increased 2nd hour after the operation compared to the 14th day after the operation. Other hematological parameters did not differ statistically among the preoperative and postoperative (hour 2, days 7 and 14) ($p > 0.05$).

No statistically significant difference was found when complete blood count findings were compared between the groups (Table 4).

The levels of IL-1, IL-6, IL-8, IL-10, TNF- α , Hp, CRP and SAA of preoperative and postoperative (hour 2, days 7 and 14) in the study group are shown in Table 5.

Table 2. Preoperative and postoperative (hour 2, days 7 and 14) hematological parameters in the study group (n=7).

Parameters	Preoperative	Postoperative (hour 2)	Postoperative (day 7)	Postoperative (day 14)	P
WBC (10 ⁹ /l)	17.59±2.79 ^{ab}	21.52±2.89 ^a	15.07±1.47 ^{ab}	10.57±1.49 ^b	<0.01
LYM (10 ⁹ /l)	1.03±0.19	0.92±0.23	1.80±0.35	1.80±0.34	NS
MON (10 ⁹ /l)	0.78±0.14 ^{ab}	0.94±0.08 ^a	0.57±0.09 ^{ab}	0.41±0.05 ^b	<0.05
NEU (10 ⁹ /l)	14.46±2.39 ^{ab}	18.68±2.65 ^a	11.86±1.37 ^{ab}	7.31±1.41 ^b	≤0.001
EOS (10 ⁹ /l)	1.10±0.51	0.78±0.36	0.59±0.08	1.00±0.18	NS
BAS (10 ⁹ /l)	0.22±0.05	0.20±0.06	0.21±0.02	0.15±0.02	NS
RBC (10 ¹² /l)	7.13±0.44	6.06±0.40	5.91±0.60	6.18±0.48	NS
HGB (g/dl)	6.83	5.87	6.41	6.13	NS
HCT (%)	14.41±0.67	12.51±0.44	12.16±0.96	12.40±0.71	NS
PLT (10 ⁹ /l)	40.19±1.59	33.75±0.90	33.23±2.47	34.87±1.78	NS
	495.29±44.71	407.57±69.52	556.57±46.37	567.71±73.03	NS
	449	478	533	532	

Mean values within the same row with different superscripts letter a, b are significantly different (p<0.05; p<0.01; p≤0.001). Parametric data were expressed as mean ± SE. Nonparametric values were expressed as mean ± SE and median. NS: not significant and n: number of animals.

Table 3. Preoperative and postoperative (hour 2, days 7 and 14) hematological parameters in the control group (n=6).

Parameters	Preoperative	Postoperative (hour 2)	Postoperative (day 7)	Postoperative (day 14)	P
WBC (10 ⁹ /l)	22.70±2.82 ^{ab}	25.30±2.90 ^a	16.74±1.54 ^{ab}	13.73±1.05 ^b	<0.05
LYM (10 ⁹ /l)	1.69±0.31	1.43±0.39	2.39±0.74	2.63±1.03	NS
MON (10 ⁹ /l)	1.7	1.23	2.00	1.88	NS
NEU (10 ⁹ /l)	0.62±0.11	0.84±0.11	0.56±0.07	0.55±0.10	NS
EOS (10 ⁹ /l)	19.71±3.01	22.50±3.20	12.66±1.75	8.24±0.58	<0.01
BAS (10 ⁹ /l)	17.47 ^{ab}	20.19 ^a	11.07 ^{ab}	8.29 ^b	
RBC (10 ¹² /l)	0.76±0.23	0.37±0.10	0.96±0.31	2.14±0.83	NS
HGB (g/dl)	0.25±0.07	0.15±0.04	0.17±0.03	0.17±0.02	NS
HCT (%)	7.09±0.54	6.43±0.46	5.99±0.40	6.75±0.45	NS
PLT (10 ⁹ /l)	7.01	6.83	6.31	6.98	NS
	14.70±1.36	13.58±1.31	12.60±1.13	13.75±1.00	NS
	41.87±4.11	38.18±4.06	35.75±3.83	39.67±3.65	NS
	504.50±79.62	508.50±58.32	531.33±92.29	438.67±66.52	NS

Mean values within the same row with different superscripts letter a, b are significantly different (p<0.05; p<0.01). Parametric data were expressed as mean ± SE. Nonparametric values were expressed as mean ± SE and median. NS: not significant and n: number of animals.

Table 4. Comparison of hematological parameters between the control (n=6) and study (n=7) groups at preoperative and postoperative (hour 2, days 7 and 14).

	Preoperative		Postoperative (hour 2)		Postoperative (day 7)		Postoperative (day 14)	
	control	study	control	study	control	study	control	study
WBC (10 ⁹ /l)	22.70±2.82	17.59±2.79	25.30±2.90	21.52±2.89	16.74±1.54	15.07±1.47	13.73±1.05	10.57±1.49
LYM (10 ⁹ /l)	1.69±0.31	1.03±0.19	1.43±0.39	0.92±0.23	2.39±0.74	1.80±0.35	2.63±1.03	1.80±0.34
MON (10 ⁹ /l)	0.62±0.11	0.78±0.14	0.84±0.11	0.94±0.08	0.56±0.07	0.57±0.09	0.55±0.10	0.41±0.05
NEU (10 ⁹ /l)	19.71±3.01	14.46±2.39	22.50±3.20	18.68±2.65	12.66±1.75	11.86±1.37	8.24±0.58	7.31±1.41
EOS (10 ⁹ /l)	0.76±0.23	1.10±0.51	0.37±0.10	0.78±0.36	0.96±0.31	0.59±0.08	2.14±0.83	1.00±0.18
BAS (10 ⁹ /l)	0.25±0.07	0.22±0.05	0.15±0.04	0.20±0.06	0.17±0.03	0.21±0.02	0.17±0.02	0.15±0.02
RBC (10 ¹² /l)	7.09±0.54	7.13±0.44	6.43±0.46	6.06±0.40	5.99±0.40	5.91±0.60	6.75±0.45	6.18±0.48
HGB (g/dl)	14.70±1.36	14.41±0.67	13.58±1.31	12.51±0.44	12.60±1.13	12.16±0.96	13.75±1.00	12.40±0.71
HCT (%)	41.87±4.11	40.19±1.59	38.18±4.06	33.75±0.90	35.75±3.83	33.23±2.47	39.67±3.65	34.87±1.78
PLT (10 ⁹ /l)	504.50±79.62	495.29±44.71	508.50±58.32	407.57±69.52	531.33±92.29	556.57±46.37	438.67±66.52	567.71±73.03

Parametric data were expressed as mean ± SE. n: number of animals.

Table 5. Preoperative and postoperative (hour 2, days 7 and 14) cytokine and acute-phase protein levels in the study group (n=7).

Parameters	Preoperative	Postoperative (hour 2)	Postoperative (day 7)	Postoperative (day 14)	P
IL-1 (pg/ml)	13.31±0.77	14.09±1.34	14.33±1.03	13.41±0.57	NS
	13.00	12.50	14.33	12.96	
IL-6 (pg/ml)	5123.72	5151.08	5238.20	5382.23	NS
IL-8 (pg/ml)	84.28±0.62	83.82±0.49	83.38±0.54	83.20±0.59	NS
IL-10 (pg/ml)	152.91±18.82	322.96±83.59	179.17±25.67	304.72±108.75	NS
	129.08	212.65	197.99	183.22	
TNF-α (pg/ml)	12.38±2.48	13.99±2.77	7.99±2.56	17.89±5.60	NS
	10.93	13.99	6.07	12.58	
Hp (ng/ml)	1.64±1.22	0.98±0.66	0.35±0.09	0.24±0.02	NS
	0.22	0.22	0.23	0.24	
CRP (ng/ml)	3.35±0.94	3.49±0.79	2.88±0.62	3.88±0.36	NS
SAA (ng/ml)	0.14	0.24	0.19	0.21	NS

Parametric data were expressed as mean ± SE. Nonparametric values were expressed as mean ± SE and median. NS: not significant and n: number of animals.

The levels of IL-1, IL-6, IL-8, IL-10, TNF- α , Hp, CRP and SAA did not differ statistically significant between the preoperative and postoperative (hour 2, days 7 and 14) in the study group ($p > 0.05$). The levels of IL-1, IL-6, IL-8,

IL-10, TNF- α , Hp, CRP and SAA of preoperative and postoperative (hour 2, days 7 and 14) in the control group are shown in Table 6.

Table 6. Preoperative and postoperative (hour 2, days 7 and 14) cytokine and acute-phase protein levels in the control group (n=6).

Parameters	Preoperative	Postoperative (hour 2)	Postoperative (day 7)	Postoperative (day 14)	p
IL-1 (pg/ml)	11.93 $\pm$ 0.15 ^{ab}	12.46 $\pm$ 0.30 ^a	12.16 $\pm$ 0.25 ^{ab}	11.62 $\pm$ 0.10 ^b	<0.05
IL-6 (pg/ml)	5411.10	5385.09	5571.57	5319.39	NS
IL-8 (pg/ml)	82.78 $\pm$ 0.62	82.17 $\pm$ 0.55	82.28 $\pm$ 0.32	81.57 $\pm$ 0.19	NS
IL-10 (pg/ml)	158.43 $\pm$ 22.59	166.52 $\pm$ 28.48	175.05 $\pm$ 22.63	114.62 $\pm$ 24.93	NS
TNF- α (pg/ml)	23.42 $\pm$ 1.52	13.36 $\pm$ 1.00	18.32 $\pm$ 2.52	24.97 $\pm$ 2.01	NS
Hp (ng/ml)	0.16 $\pm$ 0.00	0.16 $\pm$ 0.00	0.16 $\pm$ 0.00	0.17 $\pm$ 0.00	NS
CRP (ng/ml)	1.99 $\pm$ 0.62	2.18 $\pm$ 0.63	2.44 $\pm$ 0.45	2.61 $\pm$ 0.39	NS
SAA (ng/ml)	0.14	0.14	0.14	0.14	NS

Mean values within the same row with different superscripts letter a, b are significantly different ($p < 0.05$). Parametric data were expressed as mean $\pm$ SE. Nonparametric values were expressed as median. NS: not significant and n: number of animals.

The levels of IL-1, IL-6, IL-8, IL-10, TNF- α , Hp, CRP, and SAA were compared between the groups (Table 7). In the preoperative period, the control group exhibited significantly higher TNF- α levels than the study group ($p < 0.05$). This statistically significant difference was observed on postoperative day 7. At postoperative hour 2 and day 14, IL-8 levels were significantly higher in the study group than in the control group ($p < 0.05$). Furthermore, IL-1, Hp, and CRP levels were also found to be higher in the study group compared to the control group on postoperative day 14 ($p < 0.05$).

In the control group, the levels of IL-1 were significantly increased 2nd hour after the operation compared to the 14th day after the operation ($p < 0.05$). The levels of NO, TOS, TAS and OSI of preoperative and postoperative (hour 2, days 7 and 14) in the study group are presented in Table 8.

There were no statistically significant changes in terms of NO level, TOS, TAS and OSI between preoperative and postoperative 2nd hour and 7th and 14th days in the study group ($p > 0.05$). The levels of NO, TOS, TAS and OSI of preoperative and postoperative (hour 2, days 7 and 14) in the control group were given in Table 9.

When comparing the oxidative stress parameters of the control and study groups, only the TOS value on postoperative day 14 in the study group was found to be statistically significantly lower than that of the control group ($p < 0.05$). The comparison of oxidative stress parameters between the groups is summarized in Table 10.

Recurrence was not observed any bitches. Gestation information was obtained from five bitches in the study group and three bitches in the control group and all were found to have delivered.

Table 7. Comparison of preoperative and postoperative (hour 2, days 7 and 14) cytokine and acute-phase protein levels between the control (n=6) and study (n=7) groups.

	Preoperative		Postoperative (hour 2)		Postoperative (day 7)		Postoperative (day 14)	
	control	study	control	study	control	study	control	study
IL-1 (pg/ml)	11.93 $\pm$ 0.15	13.31 $\pm$ 0.77	12.46 $\pm$ 0.30	14.09 $\pm$ 1.34	12.16 $\pm$ 0.25	14.33 $\pm$ 1.03	11.62 $\pm$ 0.10	13.41 $\pm$ 0.57*
IL-6 (pg/ml)	5411.10	5123.72	5385.09	5151.08	5571.57	5238.20	5319.39	5382.23
IL-8 (pg/ml)	82.78 $\pm$ 0.62	84.28 $\pm$ 0.62	82.17 $\pm$ 0.55	83.82 $\pm$ 0.49*	82.28 $\pm$ 0.32	83.38 $\pm$ 0.54	81.57 $\pm$ 0.19	83.20 $\pm$ 0.59*
IL-10 (pg/ml)	158.43 $\pm$ 22.59	129.08	166.52 $\pm$ 28.48	212.65	175.05 $\pm$ 22.63	197.99	114.62 $\pm$ 24.93	183.22
TNF- α (pg/ml)	23.42 $\pm$ 1.52*	12.38 $\pm$ 2.48	13.36 $\pm$ 1.00	13.99 $\pm$ 2.77	18.32 $\pm$ 2.52*	7.99 $\pm$ 2.56	24.97 $\pm$ 2.01	17.89 $\pm$ 5.60
	24.48	10.93	12.51	13.99	19.18	6.07	27.13	12.58
Hp (ng/ml)	0.16 $\pm$ 0.00	1.64 $\pm$ 1.12	0.16 $\pm$ 0.00	0.98 $\pm$ 0.66	0.16 $\pm$ 0.00	0.35 $\pm$ 0.09	0.17 $\pm$ 0.00	0.24 $\pm$ 0.02*
	0.16	0.22	0.16	0.22				
CRP (ng/ml)	1.99 $\pm$ 0.62	3.35 $\pm$ 0.94	2.18 $\pm$ 0.63	3.49 $\pm$ 0.79	2.44 $\pm$ 0.45	2.88 $\pm$ 0.62	2.61 $\pm$ 0.39	3.88 $\pm$ 0.36*
SAA (ng/ml)	0.14	0.19	0.14	0.24	0.14	0.19	0.14	0.21

Parametric data were expressed as mean $\pm$ SE. Nonparametric values were expressed as mean $\pm$ SE and median. n: number of animals. * $p < 0.05$

Table 8. Preoperative and postoperative (hour 2, days 7 and 14) oxidative stress parameters in the study group (n=7).

Parameters	Preoperative	Postoperative (hour 2)	Postoperative (day 7)	Postoperative (day 14)	P
NO (ng/ml)	0.27 $\pm$ 0.02	0.27 $\pm$ 0.03	0.29 $\pm$ 0.04	0.33 $\pm$ 0.04	NS
	0.25	0.22	0.26	0.30	
TOS (μ molH ₂ O ₂ /l)	5.32 $\pm$ 1.28	5.35 $\pm$ 0.99	6.21 $\pm$ 0.86	4.18 $\pm$ 0.75	NS
TAS (mmol Trolox Equiv/l)	0.92 $\pm$ 0.22	0.58 $\pm$ 0.08	0.51 $\pm$ 0.10	0.83 $\pm$ 0.28	NS
OSI (arbitrary unit)	0.83 $\pm$ 0.22	0.98 $\pm$ 0.16	1.52 $\pm$ 0.30	1.13 $\pm$ 0.42	NS

Parametric data were expressed as mean $\pm$ SE. Nonparametric values were expressed as mean $\pm$ SE and median. NS: not significant and n: number of animals.

Table 9. Preoperative and postoperative (hour 2, days 7 and 14) oxidative stress parameters in the control group (n=6).

Parameters	Preoperative	Postoperative (hour 2)	Postoperative (day 7)	Postoperative (day 14)	P
NO (ng/ml)	0.26±0.04	0.25±0.03	0.28±0.02	0.30±0.02	NS
TOS (μmolH ₂ O ₂ /l)	7.12±0.53	4.72±1.31	6.56±0.84	6.93±0.65	NS
TAS (mmol Trolox Equiv/l)	0.45±0.13	0.41±0.13	0.26±0.07	0.49±0.13	NS
OSI (arbitrary unit)	2.26±0.55	1.92±0.79	3.21±0.76	1.91±0.43	NS

NS: not significant and n: number of animals.

Table 10. Comparison of preoperative and postoperative (hour 2, days 7 and 14) oxidative stress parameters between the control (n=6) and study (n=7) groups.

Parameters	Preoperative		Postoperative (hour 2)		Postoperative (day 7)		Postoperative (day 14)	
	control	study	control	study	control	study	control	study
NO (ng/ml)	0.26±0.04	0.27±0.02	0.25±0.03	0.27±0.03	0.28±0.02	0.29±0.04	0.30±0.02	0.33±0.04
	0.23	0.25	0.24	0.22	0.27	0.26	0.30	0.30
TOS (μmolH ₂ O ₂ /l)	7.12±0.53	5.32±1.28	4.72±1.31	5.35±0.99	6.56±0.84	6.21±0.86	6.93±0.65	4.18±0.75*
TAS (mmol Trolox Equiv/l)	0.45±0.13	0.92±0.22	0.41±0.13	0.58±0.08	0.26±0.07	0.51±0.10	0.49±0.13	0.83±0.28
OSI (arbitrary unit)	2.26±0.55	0.83±0.22	1.92±0.79	0.98±0.16	3.21±0.76	1.52±0.30	1.91±0.43	1.13±0.42

Parametric data were expressed as mean ± SE. Nonparametric values were expressed as mean ± SE and median. n: number of animals. *p<0.05

DISCUSSION AND CONCLUSION

Vaginal fold prolapse is observed in young bitches which were not neutered and usually occurred during between first and third estrous cycle (Schutte, 1967; Post et al., 1991; Johnston et al., 2001). Schutte (1967) reported that 22 bitches with vaginal prolapse were between 9 and 40 months (approximately 22 months) and they were during between first and forth estrous cycle. Trafer (1970) found this average as 18 months (Post et al., 1991). In the present study, it was determined that the bitches were young and average age was 14.53 months (8-36 months). It was also determined that eight of these bitches were at the first estrus period, four bitches were at second estrous period and one bitch was at third estrous period. However, it was learned that VFP was observed at the previous estrous periods in five bitches, which were at second and third estrous periods. These findings were similar to the results of the researchers.

Mild VFP cases can be spontaneously resolved during diestrus stage without treatment (Semacan, 2007; Singh et al., 2022). GnRH or hCG may be used for hormonal treatment (Nak and Kasikci, 2013). Surgical treatment can be choose including purse-string sutures, circumferential excision of prolapsed vaginal tissue, hysteropexy (Johnston et al., 2001), cervicopexy (Ozturkler et al., 2001) and ovariectomy (Alan et al., 2007). While submucosal resection of vagina and vaginal amputation, which are based on horizontal mattress suture, are used as the standard methods (Wykes and Olson, 2002; Kalkan and Alacam, 2007; Nak and Kasikci, 2013; Anya et al., 2020), modified new techniques have been tried (Post et al., 1991; Antonov et al., 2009; Bucci et al., 2022; Singh et al., 2022). Canoglu (2013) reported that a new technique, which was modified by Antonov et al. (2009) and based on passing through each other ligature, was started to be used by researchers. Bühner vulvar suture, which was modified for the bitch, has been performed by Bucci et al. (2022) and Singh et al. (2022). Anovulvar cleft and vaginal prolapse-hyperplasia in a bitch was treated with perineoplasty (Mostachio et al., 2007). A new technique was tried in the present study. This technique is

original and it is not a modification of any suture or methods. It was examined for alternative of other methods. This technique is a continuous suture performed by Reverdin needle and it can pressure the whole prolapsed vaginal tissue.

In the present study, submucosal resection of vagina and vaginal amputation, which are standard methods and based on horizontal mattress suture (Wykes and Olson, 2002; Kalkan and Alacam, 2007; Nak and Kasikci, 2013), were compared the new technique. There was no statistically significant difference between the groups in parameters that could affect results such as age (study group: 17±3.66 months; control group: 11.67±2.54 months), prolapse duration (study group: 17.28±7.25 days; control group: 13.67±3.67 days) and weight of prolapsed tissue (study group: 157.31±44.46 g; control group: 165.17±36.82 g).

The complete blood count is used for determination of inflammatory, prognosis and recovery (Turgut, 2000). In this study WBC, monocyte and neutrophil counts were significantly higher in postoperative 2 hours of complete blood count as compared to postoperative day 14 of complete blood count in the study group (p<0.05). WBC and neutrophil counts were also significantly higher in postoperative 2 hours of complete blood count as compared to postoperative day 14 of complete blood count in the control group (p<0.05). The neutrophil count increases of postoperative hour 2 in both groups were might be due to induced endogenous corticosteroids by stress. The monocyte count increase of postoperative 2 hours was considered due to its role in tissue repair and regeneration (Turgut, 2000). The increase in WBC count was result of the increase in neutrophil and monocyte in the study group and the increase in neutrophil in the control group. Decreasing of these values on the postoperative day 14 was considered as treatment of infection. On the other hand, the lack of a statistically significant difference in the comparison between the groups suggests that the new technique does not offer an advantage over the standard method in terms of infection.

Trauma and wound healing cause pro and anti-inflammatory cytokines to rise significantly (Uluisik and Keskin, 2012; Hismiogullari et al., 2013). In particular, cytokines such as IL-1, IL-6, IL-10, TNF- α and CRP as an acute phase protein are markers used to monitor healing and traumatic inflammation in both human and veterinary medicine (Schijns and Horzinek, 1997; Reikeras et al., 2014; Ok et al., 2015). Ozcinar et al. (2014) found that IL-1 and TNF- α levels increased after thyroid operation in humans. It has been reported that pre-operative IL-6 and IL-10 concentrations decrease after 3 days and 10 days after operation in bitches with pyometra (Dabrowski et al., 2015). It was stated that there were change in acute phase response and biosynthesis of pro and anti inflammation cytokines during inflammation (Fransson et al., 2007; Kjellgaard-Hansen et al., 2007; Karpinski et al., 2009). Kandir and Keskin (2016) determined that serum IL-1, IL-6, IL-10 levels of the rats which had thyroid operation increased slightly compared to control group, but only the increase in TNF- α level was statistically significant. In this presented study, it was determined that in both control (excluding IL-1) and study groups pro and anti inflammation cytokines and acute phase proteins levels difference were not statistically significant. Despite there was statistically significant difference in IL-1 levels of control group, it was noted that values were close to each other. In our study, the significantly higher levels of IL-1, IL-8, Hp, and CRP on postoperative day 14 in the study group (Table 7) may suggest a prolonged inflammatory response. This extended elevation could be attributed to a higher degree of tissue manipulation or a subclinical tissue reaction associated with the procedures of the new technique. However, these increases in values were not reflected in hematological parameters (Table 4).

Oxidative stress is defined as an imbalance between the production of reactive oxygen species and antioxidant defense system (Sies, 1997). While TAS reflects the defense system of metabolism against oxidative stress, TOS is a marker of oxidative stress. The OSI indicates the tolerance level of the oxidant compounds by the antioxidant compounds (Erel, 2004). Nitric oxide is responsible for providing hemostasis in physiological conditions and in many pathological conditions. In cases of tissue damage, excessive amounts of NO are produced (Kuyumcu et al., 2004). Lee and Kim (2014) performed laparoscopic and laparotomic ovariectomy in dogs. After the surgery, there was an increase in TOS value in both techniques while a decrease in TAS value was detected. In the presented study, it was determined that the preoperative TAS, TOS, NO, and OSI values of the animals in both groups showed no significant intra-group differences across the postoperative time points. On the other hand, in the inter-group analysis, while oxidative stress markers were similar between the groups preoperatively, at postoperative hour 2, and on day 7; a significant decrease in TOS levels was observed on postoperative day 14 in the study group compared to the control group. This delayed divergence suggests that the new technique does not alter the initial, unavoidable surgical inflammatory response, but accelerates the resolution of oxidative stress during the late healing phase.

In conclusion, the new technique used did not demonstrate a clear superiority over the routine operative procedure, except for the lower TOS value on day 14. However, it is believed that it can be preferred due to the shorter operation time, easy application to thick tissues and providing pressure to the entire tissues.

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Ethical Declaration

This study was approved by the Local Ethics Committee of Laboratory Animals (14/50).

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authorship Contributions

Concept: H.C.M., Design: H.C.M., H.K., Data Collection or Processing: H.C.M., H.K., Analysis or Interpretation: M.Ç., Literature Search: H.C.M., H.K., M.Ç. Writing: H.C.M., H.K., M.Ç.

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