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Effect of Intraoperative PEEP Application on Colonic Anastomoses Healing: An Experimental Animal Study

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This study aimed to assess the effect of intraoperative positive end–expiratory pressure (PEEP) intervention on the healing of colonic anastomoses in rabbits. A total of 32 New Zealand type male rabbits were divided into 2 groups of 16 animals each. Following ventilation with tracheostomy, colonic resection and anastomosis were performed in both groups. Although 10 cm of H_2O PEEP level was applied in group 1 (PEEP), group 2 [zero end-expiratory pressure (ZEEP)] was ventilated without PEEP throughout the surgery. Half of both the PEEP and ZEEP group animals were killed on the third postoperative day, whereas the remaining half were killed on the seventh. Anastomotic bursting pressures, the tissue concentrations in hydroxyproline, and histologic assessments were

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performed. Intraoperative oxygen saturation and postoperative arterial blood gas parameters were also compared. On the first postoperative day, both arterial oxygen tension (PO_2) and oxygen saturation (SO_2) in the PEEP group were significantly higher than in the ZEEP group. On the seventh postoperative day, the bursting pressures of the anastomoses were significantly higher in the PEEP group; however, the hydroxyproline content was significantly lower in the PEEP group than in the ZEEP group. At day 7, the PEEP group was significantly associated with increased neoangiogenesis compared with the ZEEP group. The anastomotic healing process is positively influenced by the intraoperative PEEP application.

Key words: Colon anastomosis – Positive end–expiratory pressure – Neoangiogenesis – Anastomotic bursting pressure

Abnormalities in gas exchange during anesthesia are generally caused by atelectasis, and such alterations are more pronounced with early postoperative hypoxemia, even in healthy individuals.[1] The extent to which postoperative complications are caused by a respiratory dysfunction during anesthesia is still not clear. Serious postoperative respiratory side effects can lead to tissue hypoxia involving deterioration of the ventilation/perfusion ratio (V/Q).[2] Also, systemic hypoxia may be due to a deficiency in oxygen that affects collagen synthesis and deposition directly, and impairs anastomotic healing in the colon.[3] Indeed, ischemia is a well-known cause of leakage from colonic anastomoses.[3–8]

Atelectasis impairs oxygenation by reducing lung compliance. Positive end–expiratory pressure (PEEP) leads to improved lung compliance, decreased shunting, and higher arterial oxygen pressure (PO_2).[1,2] In the data, analysis has shown that an increase in the PO_2/fraction of inspired oxygen (PO_2/FiO_2) ratio and atelectasis are rarely observed during the postoperative period following intraoperative PEEP application.[2]

The efficacy of intraoperative PEEP intervention in relation to postoperative oxygenation and systemic effects is discussed in the literature, although this subject was not found in any studies evaluating colonic anastomosis healing. This study aimed to assess the postoperative effect of intraoperative PEEP on colonic anastomoses. To our knowledge, this is the first controlled experimental study investigating this issue.

Materials and Methods

Ethics approval for the experimental protocols was received from the Animal Research Committee at Ankara University, Ankara, Turkey. All procedures were performed in accordance with the Guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals.

Animals and experimental design

Thirty-two New Zealand type male rabbits with a weight average of 3134 ± 483 g were used in this study. All research animals were kept individually in stainless steel cages at a constant temperature (22°C) and humidity, and in 12-hour light-darkness cycles. The animals were fed standard chow, and water was given *ad libitum*, except for an overnight fast before surgery and on the first postoperative day, when only water was given. The rabbits were randomly divided into 2 groups of 16 animals, namely the experimental (PEEP group, or group 1) and control [zero end-expiratory presure (ZEEP) group, or group 2] groups. The rabbits were killed in groups of 8 animals on the third and seventh postoperative days in both the experimental and control groups.

Operative procedure

Antibiotics (cefazolin sodium 20 mg/kg, intraperitoneally) were given preoperatively, and all procedures were performed under standard aseptic conditions. The animals were warmed to maintain a constant body temperature of 37°C. The rabbits were anesthetized using a mixture of isoflurane and oxygen while breathing spontaneously through a mask. After confirming the sufficiency of the anesthesia, the rabbits were secured to the operating table in the supine position. The right marginal ear vein was cannulated using a 24-G catheter for drug and fluid administration, while in the left ear arterial cannulation was used for arterial blood pressure and heart rate evaluation. The arterial cannula was left in place for blood samples on the first postoperative day. Both the anterior neck and abdomen hair were removed, and the sites were prepared with 10% povidone-iodine. A 2-cm horizontal incision through the skin was made 1 cm below the cricoid cartilage, and the subplatysmal flaps were elevated. The strap muscles were separated by blunt dissection. The pretracheal fascia was then dissected, and the tracheal cartilage was exposed. Following the tracheostomy, the same 3-mm outer diameter cannulae (Satis Medical Co, Ankara, Turkey) were introduced before the carina in both groups. Ventilation was set with a tidal volume of 0.08 mL/g body weight, a respiratory rate of 60/min, and an FiO_2 of 0.3 (SMS VENT V, Security Medical Services, Ankara, Turkey). The abdomen was opened by a 4-cm–long midline incision for sufficient exposure of the colon and rectum. A 1-cm segment of colon was resected approximately 5 cm above the peritoneal reflection in both groups. Colonic continuity was restored via 8 interrupted and inverting end-to-end anastomosis using 5/0 prolene (Ethicon). The abdominal fascia and skin were closed with 3/0 silk (Ethicon). Then, the endotracheal tube was removed and the tracheal stoma closed with 6/0 prolene (Ethicon). The fascia, subcutaneous tissue, and skin were separately closed with interrupted sutures. Arterial blood gas samples were taken from all rabbits on the first postoperative day.

The animals were killed by exsanguination under anesthesia after colonic reconstruction on either the third or seventh postoperative day. A 4-cm colonic segment including the anastomosis at the center was resected and taken to measure the bursting pressure. To measure the bursting pressure, normal saline was continuously infused at a rate of 2.0 mL/min via the catheter. The maximum pressure was recorded immediately before a sudden loss of pressure, at which any leakage of saline or gross rupture was noted. Thereafter, the anastomosis site was cut longitudinally and divided into two equal segments. One half was used for histopathologic assessment and the other half for biochemical evaluation.

Determination of tissue hydroxyproline levels

The anastomotic hydroxyproline (HP) content was analyzed according to the method used by Jamall *et al*.[9] Briefly, tissue samples were hydrolyzed in 6 M hydrochloric acid at 105°C for 18 hours and then allowed to evaporate to dryness. The HP levels were analyzed via colorimetric methods with Ehrlich reagent (10 g of *p*-dimethylaminobenzaldehyde, 11 mL perchloric acid). The HP content was measured against standard concentrations and is expressed as microgram per gram of tissue.

Histopathologic evaluation

The histopathologic evaluation was performed by a pathologist who was blind to the group assignment. Colonic segments were fixed in 10% neutral-buffered formalin, and, after a routine follow-up, samples were embedded in paraffin blocks. The paraffin blocks were cut into 4-μm sections that were stained with hematoxylin-eosin and Masson trichrome. The evaluation was performed using the scoring system proposed by Verhofstad *et al*.[10] This scale was modified by uniting lymphocyte and macrophage infiltration as mononuclear cell infiltration. The degrees of granulation tissue formation, neoangiogenesis, fibroblasts, and collagen deposition in each sample were also assessed. The fibroblastic activity, neoangiogenesis, and collagen deposition grading scale was used as previously described by Ehrlich and Hunt.[11] As shown in Table 1, this scale, which was designed on a numeric system by Philips *et al*,[12] was adopted for our study.

Statistical analysis

The statistical analyses were conducted using the SPSS 15.0 (SPSS Inc, Chicago, Illinois) software package. The differences between colon rupture pressures, HP levels, and histopathologic scores were analyzed using Kruskal-Wallis variance analysis. Statistical significance was based on a value of $P < 0.05$. The Mann-Whitney U test was used to identify the group causing the difference when significance was detected for any parameter. The differences between the PEEP and ZEEP groups in terms of the blood gas results on the first postoperative day were also investigated using the Mann-Whitney U test. Statistical significance was set at a Bonferroni correction of $P < 0.0125$ in order to increase the reliability of the test results. The descriptive characteristics of the data were expressed as median-minimum-maximum for each variable. The correlations between the variables were analyzed using Spearman rho correlation coefficient.

Table 1 Histopathologic parameters in decision-making related to wound healing

	Score			
Variables	0	1	2	3
Necrosis	Absence	Small patches	Some patches	Massive
PMNs	Normal number	Mild increase	Marked infiltration	Massive infiltration
MNLs	Normal number	Mild increase	Marked infiltration	Massive infiltration
Edema	Absence	Some	Marked	Intense
Mucosal epithelium	Normal glandular	Normal cubic	Deficient cubic	None
Submucosal and muscular layer	Well bridging	Medium bridging	Poor bridging	No bridging
Granulation	Absence	Some	Marked	Intense
Neoangiogenesis	Absence	Mild	Moderate	Marked
Fibroblast	Absence	Mild	Moderate	Marked
Collagen deposition	Absence	Mild	Moderate	Marked

MNLs, mononuclear cells; PMNs, polymorphonuclear leukocytes.

Results

Postoperative period

All animals survived without complications until they were euthanized. The rupture pressure of 1 rabbit in group 1 (PEEP) could not be measured. Also, in 1 rabbit from group 1, the pH, HCO_3, and arterial oxygen saturation (SO_2) could not be measured during the postoperative blood gas analysis, for technical reasons.

Anastomotic bursting pressure and HP levels

The bursting pressures were measured in order to determine the strength of colonic anastomoses. Although the colon mostly burst at the site of anastomosis, on the seventh day ruptures happened away from the anastomosis in 2 of the rabbits in both the control and PEEP groups. On the third day, there was no statistical difference in tensile strength between the groups. The bursting pressures of the anastomoses were statistically higher in the PEEP group [78.5 mmHg (minimum-maximum, 69–82 mmHg)] than the ZEEP group [69 mmHg (minimum-maximum, 47–144 mmHg)] on the seventh postoperative day ($P < 0.0125$).

On day 3, the difference between the anastomotic tissue HP levels was not statistically significant between the groups. However, the HP content was significantly lower in the PEEP group [272 μg/g (minimum-maximum, 241.4–331.5 μg/g)] than in the ZEEP group [325 μg/g (minimum-maximum, 295.8–367.2 μg/g)] at day 7 ($P < 0.0125$). The bursting pressures and HP levels are summarized in Table 2. The correlation between the HP levels and the bursting pressures of the anastomoses was investigated using Spearman rho correlation coefficient, and the result was not statistically significant ($P > 0.05$).

Histologic evaluation

Examples of the hematoxylin-eosin–stained histologic sections are shown in Fig. 1. At day 3, the scores for each histologic parameter were found to be similar between the ZEEP and PEEP groups, and so the differences were statistically insignificant. The edema, granulation tissue, neoangiogenesis, and fibroblast scores were significantly increased in the PEEP group on day 7 compared with day 3 ($P < 0.05$; Fig. 2).

At day 7, the neoangiogenesis score in the PEEP group was significantly higher than that in the ZEEP group ($P < 0.05$). Although not statistically significant ($P > 0.05$), the granulation tissue and fibroblast accumulation scores were also higher in the PEEP group than in the ZEEP group on day 7. Interest-

Table 2 Comparison of the bursting pressures and HP levels between the PEEP and ZEEP groups

	Bursting pressure, mmHg, median (minimum-maximum)			HP, μg/g tissue, median (minimum-maximum)		
	PEEP	ZEEP	*P* value	PEEP	ZEEP	*P* value
Day 3	47 (32–74)	66 (47–97)	>0.0125	270.3 (251.6–312.8)	298.35 (266.9–311.1)	>0.0125
Day 7	78.5 (69–82)	69 (47–144)	<0.0125	272 (241.4–331.5)	325.55 (295.8–367.2)	<0.0125

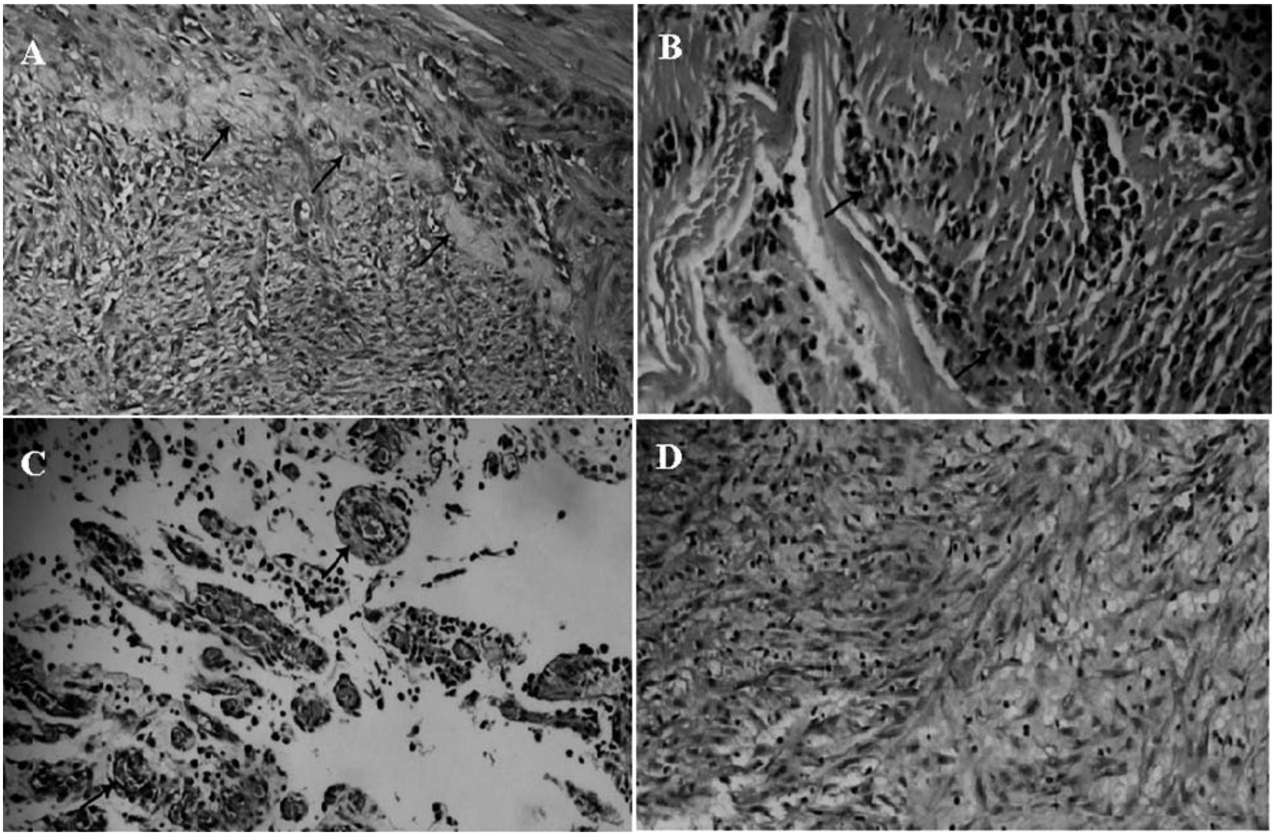

Fig. 1 Anastomotic histology with hematoxylin-eosin staining in the colon. (A) Distinctive collagen deposition in PEEP group at day 7 (×20). (B) Massive polymorphonuclear leukocyte (PMN) infiltration in ZEEP group at day 3 (×40). (C) Typical example obtained at day 7 in PEEP group representing neoangiogenesis (×20). (D) Severe inflammatory granulation tissue in PEEP group at day 7 (×40). Black arrows in panels A, B, and C denote regions of collagen deposition, PMN infiltration, and neoangiogenesis, respectively.

ingly, the anastomotic edema was severe and collagen deposition tended to decrease in the PEEP group compared with the ZEEP group on day 7, but the differences between the groups were not statistically significant ($P > 0.05$). The histopathologic scores are summarized in Table 3.

Comparison of the SO_2 in the groups according to the time interval during surgery

As can be seen in Fig. 3, there were significant differences in terms of the intraoperative saturation values between the 0 minute and the 15th, 30th, and 45th minutes in the PEEP group ($P < 0.05$). However, the differences were insignificant in the ZEEP group ($P > 0.05$). The observed SO_2 was significantly higher in the PEEP group than in the ZEEP group animals at each time interval except for minute 0 ($P < 0.05$).

Comparison of the arterial blood gases on the first postoperative day

At day 1, the differences between both groups in terms of arterial blood gas parameters were also evaluated. As shown in Table 4, both arterial PO_2 and SO_2 in the PEEP group were significantly higher than in the ZEEP group ($P < 0.05$). However, the arterial carbon dioxide partial pressure (PCO_2), pH, and HCO_3 values in the PEEP group were lower than those in the ZEEP group ($P < 0.05$). No significant differences were found regarding the hemoglobin and hematocrit values between the groups ($P > 0.05$).

Discussion

In the present study, we investigated the effect of intraoperative PEEP on colonic anastomotic healing in experimental animals. The study resulted in

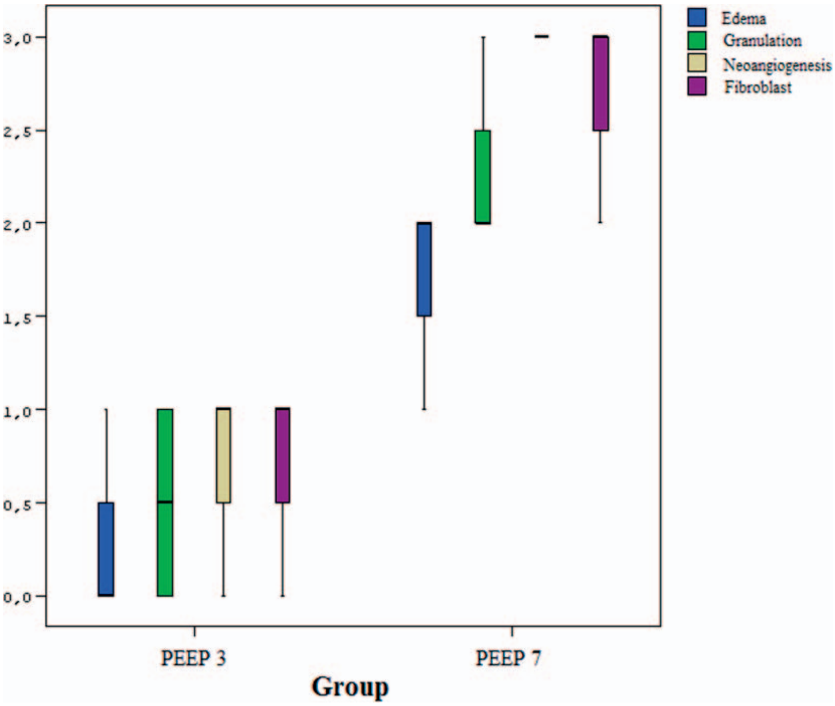

Fig. 2 Subgroups of PEEP differ between day 3 and day 7 in terms of edema, granulation tissue, neoangiogenesis, and fibroblast scores. $P < 0.05$ was considered significant.

Table 3 Anastomotic healing process on postoperative day 3 and day 7[a]

		Groups, median (minimum-maximum)		
Variables	Day	PEEP	ZEEP	*P* value
Necrosis	D3	3 (3–3)	3 (2–3)	NS
	D7	3 (2–3)	3 (2–3)	NS
PMNs	D3	2 (1–3)	2.5 (2–3)	NS
	D7	3 (2–3)	2.5 (1–3)	NS
MNLs	D3	0 (0–1)	0.5 (0–1)	NS
	D7	0.5 (0–1)	0.5 (0–1)	NS
Edema	D3	0 (0–1)	1 (1–2)	NS
	D7	2 (1–2)	1 (1–3)	NS
Mucosal epithelium	D3	3 (2–3)	3 (2–3)	NS
	D7	2 (2–2)	2.5 (2–3)	NS
Submucosal and muscular layer	D3	3 (3–3)	3 (2–3)	NS
	D7	3 (3–3)	3 (3–3)	NS
Granulation	D3	1 (0–1)	1 (1–1)	NS
	D7	2 (2–3)	1 (1–3)	NS
Neoangiogenesis	D3	1 (0–1)	1 (0–1)	NS
	D7	3 (3–3)	1.5 (0–2)	<0.05
Fibroblast	D3	1 (0–1)	1 (1–1)	NS
	D7	3 (2–3)	2 (1–3)	NS
Collagen deposition	D3	0 (0–1)	1 (1–1)	NS
	D7	1 (1–1)	1.5 (0–2)	NS

D, day; MNLs, mononuclear cells; NS, not significant; PMNs, polymorphonuclear leukocytes.

[a]$P < 0.05$ was considered statistically significant according to the Kruskal-Wallis test for comparison between groups on different days.

several key findings: (1) on the first postoperative day, better responses were linked to systemic oxygenation in the PEEP group, while increased PO_2 and SO_2 were observed compared with the ZEEP group; (2) bursting pressure was significantly higher in the PEEP group compared with the ZEEP group on the seventh postoperative day; and (3) increased neoangiogenesis was significantly demonstrated at the anastomotic site in the PEEP group compared with the ZEEP group. In light of these findings, intraoperative PEEP administration appears to be an effective modality for promoting the anastomotic healing process.

The functional residual capacity is impaired during surgery and general anesthesia with mechanical ventilation. Thereby, the normal V/Q ratio is disturbed and the intrapulmonary shunt fraction is increased, leading to arterial hypoxemia.[13] Airway closure and a low V/Q ratio can only be prevented when the functional residual capacity is raised through PEEP or similar methods. The application of PEEP is widely accepted in cases of hypoxemia caused by reduced functional residual capacity.[13–15] Previous studies suggest that an adequate PEEP ventilation mode improves pulmonary functions and thus results in increased arterial and peripheral tissue oxygenation.[16–18] However, no measurements were recorded postoperatively in these earlier studies. Indeed, studies evaluating the

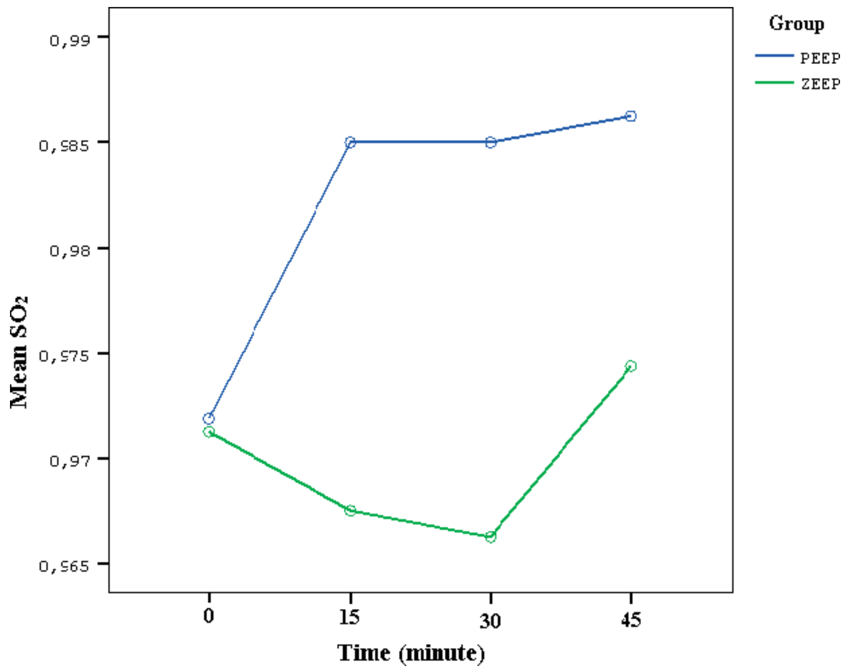

Fig. 3 The Mann-Whitney *U* test demonstrates that the arterial SO_2 positively correlates with PEEP during surgery ($P < 0.05$).

PO_2 levels in the PEEP and ZEEP groups during the postoperative period are limited in number. Yakaitis *et al*[14] reported that the highest mean PO_2 levels were detected in patients to whom 10 cm of H_2O PEEP was applied during the surgery, although this beneficial effect was not maintained after the operation. Conversely, a significantly higher PO_2/FiO_2 ratio on the first postoperative day in the PEEP group has been revealed by two trials.[19,20] These results are compatible with those in our study, because significantly higher PO_2 levels were obtained on the first postoperative day in the PEEP group in comparison with the ZEEP group.

Mild to moderate hypoxemia (SO_2 86%–90%) is observed in 53% of patients who undergo elective surgery and receive general anesthesia. Severe hypoxemia (SO_2 <81%) develops in 20% of these patients.[21] Although high inspiratory oxygen concentrations might reduce the risk of hypoxemia, such concentrations can also induce atelectasis.[21,22] This condition is called *preoxygenation*. Preoxygenation is the most important cause of atelectasis during the induction of anesthesia. Thus, a moderate FiO_2 (*e.g.*, 0.3–0.4) during PEEP seems plausible.[21,22] The application of 10 cm of H_2O PEEP reduces the area affected by atelectasis as well as the intrapulmonary shunts, and improves the arterial oxygenation during general anesthesia without causing any abnormal hemodynamic states.[21–25] Further, 10 cm of H_2O PEEP has been assumed to be the limit for splanchnic blood flow reduction, and levels of 15 to 20 cm of H_2O PEEP have frequently been found to be associated with reduced splanchnic circulation in both animals and humans.[26–28]

Table 4. Comparison of the arterial blood gas study components between the groups on the first postoperative day[a]

Group	pH	PO_2	SO_2	PCO_2	HCO_3	HTC	HGB
PEEP							
Median	7.30	96.85	0.98	24.95	13.90	0.36	10.80
Minimum	7.25	64.40	0.97	18.60	9.10	0.32	9.90
Maximum	7.42	104.00	0.99	31.20	21.30	0.39	12.80
ZEEP							
Median	7.41	72.90	0.95	33.40	20.60	0.35	10.60
Minimum	7.34	61.60	0.93	31.40	16.80	0.30	9.70
Maximum	7.44	80.70	0.96	40.70	23.90	0.44	14.50
P value	<0.05	<0.05	<0.05	<0.05	<0.05	NS	NS

HGB, hemoglobin; HTC, hematocrit; NS, not significant.

[a]According to the Mann-Whitney *U* test, $P < 0.05$ was considered statistically significant.

Moreover, the perfusion is reportedly maintained without disturbance even at these levels, through fluid support and simultaneous inotropes or vasopressors.[26,29] Recently, it was revealed that elevated intra-abdominal pressure related to the PEEP level had no negative effects on oxygenation or hemodynamics in an experimental rat study.[30] Therefore, 10 cm of H_2O PEEP during the surgery can be safely applied without risk of mesenteric and hepatic oxygen consumption and hepatosplanchnic circulation.[31,32] In order to comply with the literature, we have also applied 0.3 FiO_2 with 10 cm of H_2O PEEP to the rabbits in our study to prevent preoxygenation and hemodynamic changes during general anesthesia.

The beginning and duration of the PEEP during general anesthesia remain unclear. Still, the literature suggests that it should be initiated immediately after or up to the 30th minute following induction and then continued throughout the operation.[2,33] In our study, the mean duration of the surgery was 55 $\pm$ 4.59 minutes for each rabbit. PEEP was initiated at induction and was continued throughout the surgery.

Atelectasis causes decreased lung compliance and impaired oxygenation, and is the main cause of hypoxemia during the postoperative period.[1] Although the extent of the postoperative complications due to respiratory dysfunction has not yet been clarified, based on the data at hand, PEEP might improve postoperative atelectasis and oxygenation. Several studies measured atelectasis using computed tomography in the postoperative period and concluded that both the extent and the frequency of the atelectasis are smaller in the PEEP groups compared with the ZEEP groups.[2,24] It is well known that systemic hypoxia has a direct impact on collagen synthesis and deposition, and thus disturbs the healing of the anastomosis.[3] If PEEP is able to improve the postoperative oxygenation, its influence on the healing of the anastomosis must then be investigated.

Blood and tissue oxygenation are vital in the healing of anastomoses. PO_2 below 40 mmHg is associated with failed mature collagen formation.[5] Additionally, perianastomotic tissue oxygen levels of 20 mmHg or lower are predictive of anastomotic failure in human patients.[34] However, evidence of systemic PO_2 level compatibility with tissue perfusion is controversial. Systemic hypoxia was found to be a direct cause of local tissue hypoxia as well as to affect the healing of anastomosis by disturbing angiogenesis, collagen synthesis, and collagen deposition.[3] Various studies have indicated the involvement of oxygenation in peripheral tissues, including the conjunctiva and transcutaneous and subcutaneous tissues, being measured at different PEEP levels through superficial oximetry, and a correlation has been observed between local perfusion and PO_2 levels.[35,36] Local intestinal oxygenation is also said to be independent of systemic PO_2.[27] On the other hand, higher systemic PO_2 levels are also reported to improve tissue oxygenation and increase collagen deposition.[37,38] Increased PO_2 improves tissue oxygenation and enhances wound healing. For this purpose, hyperbaric oxygen therapy has been experimentally shown to improve healing in colon anastomoses.[4] However, the high cost of hyperbaric oxygen therapy, the need for frequent sessions, the difficulty of continuing medical support, and the need for special units are all limiting factors regarding its clinical use on a large scale. Also, the positive effect of high-pressure and systemic pure PO_2 on wound healing is still controversial.[39]

Gastrointestinal anastomoses lose much of their strength within the first 2 to 3 days.[5] Therefore, bursting pressure is lowest during the first 3 days, which is consistent with our results. In fact, all wounds exhibit localized tissue hypoxia in the early phase, and the early inflammatory phase requires less oxygen than the proliferative phase.[4,37,40] Also, there is a consistent relationship between tissue oxygen tension and the mechanical strength of colon anastomosis.[37] Hunt and Pai[41] reported that collagen synthesis and accumulation were roughly proportional with arterial PO_2 level. It has been consistently demonstrated that systemic hypoxia leads to a significant reduction in anastomotic bursting pressure.[3] In the present study, the higher colon bursting pressures in the PEEP group on the seventh day may be explained with better systemic oxygenation through PEEP in the postoperative period and the reflection of this systemic effect on the perianastomotic tissue. The improved neovascularization in the PEEP group during later phases also supports this idea.

Interestingly, in the present study the HP tissue level was higher in the ZEEP group than in the PEEP group on the seventh postoperative day. The healing of the anastomosis depends on the complex balance between collagenolysis, collagen synthesis, collagen maturation, or cross-linking capacity.[42,43] Also, oxygen is required for the activities of 3 enzymes involved in the cross-linking of collagen (lysyl hydroxylase, lysyl oxidase, and prolyl hydroxylase).[44] An incomplete cross-link of collagen does not maintain the tensile strength of anastomosis.[3] As in the current study, no correlation has ever been demonstrated between bursting strength and the collagen concentration of the colonic wall.[3,8,43] Thus, the quality of the collagen, including the degree of cross-linking present and the architecture, is more accurate in determining the wound strength than the actual amount of collagen.[42] Evaluation of the anastomotic healing primarily depends on mechanical parameters; therefore, bursting pressure is especially important.[43,45]

Neoangiogenesis is the development of new capillaries from preexisting vessels and is essential for the healing process.[46] In the present study, there was a significant increase in neoangiogenesis at the anastomotic site in the PEEP group compared with the ZEEP group. Although not statistically significant, we also recognized more granulation tissue and fibroblast accumulation at the anastomotic site in the PEEP group. As was previously understood, fibroblasts play a key role in the production of collagen and in providing the structural extracellular matrix. Adequate tissue oxygenation is necessary for the normal oxidative function of neutrophils, as well as for leukocyte activation, fibroblast production, angiogenesis, and reepithelialization.[4] Increased neoangiogenesis is also valuable for restoring the tensile strength of anastomosis.[47] Hypoxia Inducible Factor (HIF-1) is a transcription factor that regulates oxygenation in the tissue and provides a metabolic adaptation to hypoxia. The effect is brought about via the activation of angiogenic factors, such as VEGF.[3,6] Although hypoxia may stimulate neovascularization during the early phase, it is not sustainable. Ultimately, angiogenesis cannot proceed because of a lack of response to the angiogenic factors.[3,39] Thus, it can be interpreted that increased neoangiogenesis in the PEEP group at the seventh day may be the result of better tissue oxygenation around the anastomosis due to improved arterial oxygen saturation. The current study is limited by factors that may need to be addressed in future trials. The number of samples was too low to be interpreted broadly. Despite some indirect evidence being obtained concerning the systemic effect of PEEP, direct measurement of tissue-wound oxygenation and perfusion failed, for technical reasons.

In conclusion, the intraoperative administration of 10 cm of H_2O PEEP leads to significant improvement in terms of arterial PO_2, oxygen saturation, and PCO_2 reduction. The anastomotic healing process is positively influenced as a consequence of improved systemic arterial oxygenation.

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