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Autologous serum in surgical wound healing of dogs (Canis lupus familiaris): preliminary efficacy data

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Abstract: This study evaluated the efficacy of autologous serum (AS) in promoting the healing of primarily closed abdominal surgical wounds in female dogs using a modified Bates-Jensen Wound Assessment Tool (BWAT). Thirty dogs were equally divided into Control, Treatment 1 (once-daily AS), and Treatment 2 (Twice-daily AS) groups. Wounds were assessed every other day for 10 days postoperatively, focusing on parameters including epithelialization, exudate, skin color, edema, necrosis, and wound edges. Findings revealed a strong positive correlation between AS application frequency and epithelialization rate, with treatment 2 showing a significant difference compared with the other groups. Repeated measures ANOVA confirmed a highly significant difference among groups ($p < 0.001$). These results indicate that autologous serum not only accelerated wound healing but also promoted a more efficient inflammatory response without increasing the risk of complications. This study highlights Autologous Serum as a biologically active, cost-effective adjunct to conventional wound management in veterinary surgery, offering practical benefits for clinical application.
Keywords: Autologous serum, modified Bates-Jensen Wound Assessment Tool, wound healing.

1. Introduction

Surgical wound healing is a vital aspect in veterinary medicine, particularly in facilitating the dog's recovery and well-being following surgical procedures. There are four major stages of wound healing. The first stage is hemostasis, in which blood vessels constrict (vasoconstriction) to reduce blood flow, and platelets gather to form a temporary plug at the wound site, initiating clot formation. The second is inflammation, which begins shortly after hemostasis is complete and is characterized primarily by vasodilation (dilation of blood vessels) that facilitates the recruitment of immune cells and bioactive molecules to the wound site. During these phase, leukocytes, particularly neutrophils and macrophages, along with various enzymes and inflammatory mediators, infiltrate the wound area to eliminate pathogens, remove cellular debris, and release cytokines that regulate subsequent healing phases; the third stage is proliferation wherein the temporary wound matrix that emerges during the hemostasis phase is then replaced by granulation tissue, characterized by a substantial presence of granulocytes, fibroblasts, blood vessels, macrophages, together with collagen bundles and this process also serves to partially restore both the structure and functional aspects of the wound; lastly, the remodeling stage as the collagen fibers that were recently deposited shift along the tension lines (Winker, 2023).

Autologous serum (AS) and platelet-rich plasma (PRP) are effective and are widely used across various fields of medicine. In musculoskeletal conditions, autologous serum has been used to mediate inflammation, pain, and tissue destruction. Moreover, studies using autologous serum in eye drops have demonstrated variable efficacy in treating ocular surface disease (Evans et al., 2016).

Several studies have highlighted the therapeutic role of blood-derived products in promoting wound repair. Growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor-β (TGF-β) play important roles in stimulating fibroblast proliferation, collagen synthesis, and epithelial cell migration during tissue repair. Experimental studies have shown that PDGF and EGF significantly enhance epithelial and keratocyte proliferation, both of which are essential for wound healing (Haber et al., 2003). Similarly, research on platelet-derived biological products has demonstrated improved wound-healing outcomes through increased angiogenesis, collagen deposition, and reduced inflammation. For example, a study in veterinary regenerative medicine reported that platelet-rich fibrin significantly accelerated skin wound healing by enhancing growth factor expression and collagen formation, while simultaneously reducing inflammatory cytokine levels (Zhang et al., 2024).

Furthermore, experimental work in canine wound models showed that growth factor-enhanced stromal cell therapies promoted faster epithelialization and increased granulation tissue formation compared with control treatments, highlighting the role of platelet-derived growth factors in improving tissue repair and regeneration (Kim et al., 2020).

Hence, the potential of autologous serum for surgical wound healing is well-supported by studies exploring its therapeutic applications across diverse medical contexts, and the established biological properties of AS may promote faster, safer recovery following routine surgical procedures.

2. Materials and Methods

A total of 30 female dogs, aged between 7 months and 2 years, scheduled for elective or routine procedures (such as spaying or a scheduled cesarean), were selected to participate in the study. Each group, treatment 1, treatment 2, and the control group, consisted of 10 female dogs. All selected dogs were required to be apparently healthy, weigh at least 5 kilograms, and have no pre-existing conditions that could affect wound healing. As part of pre-surgical assessment, each dog underwent a physical examination and a complete blood count. Before surgery, 5–10 mL of blood was collected from each patient via venipuncture into red-top tubes. The blood was allowed to clot at room temperature for 20 minutes, then centrifuged at 300 × g for 10 minutes. The resulting serum was collected, transferred to a sterile tube, and stored at 4°C. Since refrigerated autologous serum remains viable for up to 7 days, repeat blood collection, preferably on the 5th to 6th day after the initial collection, was necessary to ensure a consistent, fresh supply of serum throughout the 10-day treatment period.

The data gathering form served as the primary instrument for collecting information and records for this study. It included sections to document patient signalment, deworming and vaccination status, history of allergies or concurrent diseases, tick/flea preventives, and diet. The form incorporated survey topics from Hill et al. (2006), covering clinical signs, etiological categories, diagnoses, diagnostic procedures, and prescribed treatments for skin disorders.

Surgical animals were administered specific medications for pain management, antibiotics, and pre-anesthetic purposes. Atropine sulfate was used as the pre-anesthetic, while Zoletil (a combination of Zolazepam and Tiletamine) served as the anesthetic agent. Before surgery, food was withheld for 8–12 hours. The ventral abdomen area was clipped and aseptically prepared for the procedure. The length of the surgical incision was recorded.

Thirty dogs undergoing elective or abdominal surgery were randomly assigned to two treatment groups that received autologous serum and a control group that received the conventional method. An adequate amount of autologous serum, approximately 3–5 drops (0.15–0.25 mL), was applied to the surgical wounds of animals in treatment groups 1 and 2 for ten consecutive days. Co-amoxiclav was administered twice daily for seven days as the antibiotic of choice. For pain management, prednisone was given once daily for three days. Postoperative care was primarily managed at home by the pet owners, while follow-up treatments and evaluations were conducted at Whizler Animal Clinic. To ensure consistent daily use of autologous serum, the owners were regularly monitored and guided through follow-up via chat.

Wound healing was assessed using the modified Bates-Jensen assessment tool. The primary modification involved the "Size" characteristic (Item 1). Unlike open wounds, which are measured by area (length x width in cm²), primarily closed surgical incisions maintain a relatively constant linear dimension throughout the healing process, provided there are no complications such as dehiscence. Therefore, "Size" was redefined to specifically measure the longest linear length of the incision in millimeters (mm). Subsequently, the standard BWAT depth scale was adapted to more precisely reflect the anatomical layers encountered in abdominal wounds and to better distinguish between various levels of tissue involvement in a surgical incision or potential dehiscence.

Furthermore, the granulation tissue category, which assesses the presence and quality of granulation tissue filling a wound bed, is primarily relevant for wounds healing by secondary intention (open wounds). In a healthy, primarily closed surgical incision, significant external granulation tissue is not expected and, if present, would indicate a complication such as infection or partial dehiscence (Johnston & Tobias, 2017). Therefore, this item was omitted from the modified assessment tool as it did not provide relevant information for the specific wound type under investigation. In primarily closed surgical incisions, epithelialization is the process by which epidermal cells migrate across the approximated wound edges to re-establish the skin barrier. Rather than assessing the "percentage of wound covered," as is typically done for open wounds, this item was modified to quantify the length of the apposed (healed) wound edge along the incision line. This measured length was then converted to a percentage of the total incision length, serving as a direct approximation of the extent of epithelial healing along the surgical incision. The scoring was designed to reflect the increasing proportion of the incision length that was successfully epithelialized. This method aligns with the principles validated by Bloemen et al. (2012), suggesting that well-structured subjective clinical assessments can reliably reflect the progress of wound healing. While digital tools provide precise measurements, the study concluded that clinical evaluation remains a valid, practical, and time-efficient method for assessing epithelialization in both clinical and research contexts. Therefore, the use of a modified, length-based scoring approach to assess epithelialization in this study is not only appropriate for surgical wounds but also supported by validated methodologies in the existing literature.

Wound depth was excluded from the modified assessment tool due to the practical limitations in evaluating this parameter in primarily closed surgical wounds. Once sutured, the wound edges are approximated, concealing internal layers, and making direct depth measurement impractical and unreliable during early healing. Moreover, the biological timeline of tissue repair further supports this decision. According to Gurtner et al. (2008), while the skin and subcutaneous tissues typically achieve visible closure within 10 to 14 days through epithelialization and initial collagen deposition, deeper structures such as the rectus abdominis muscle heal more slowly. Muscle tissue undergoes fibrous scar formation rather than true regeneration, with significant functional recovery occurring over four to six weeks and full scar maturation requiring two to four months. Given that the current study spanned only 10 days, meaningful changes in muscle layer healing would not yet be apparent. Therefore, excluding depth from the assessment is both methodologically sound and biologically appropriate.

The remaining BWAT categories (edges, undermining, necrotic tissue type, necrotic tissue amount, exudate type, exudate amount, skin color surrounding wound, and peripheral tissue edema) were retained with their original scoring rubrics, as they provide valuable insights into the inflammatory response, integrity, and potential complications of surgical wounds.

Statistical Analysis

All analyses were performed using Microsoft Excel and R software version 4.4.2. Descriptive statistics, such as frequency, mean, and standard deviation, were used to summarize the data. One-way repeated-measures Analysis of Variance (ANOVA) was used to test differences among means across multiple time points and treatment variables. This analysis was used since the observations were measured at more than two time points. If the equality of means for treatment and/or time variables was rejected, the Bonferroni Test was used for pairwise comparison of means. All analyses were performed at the 5% significance level.

3. Results

This study was conducted at Whizler Animal Clinic from January 2024 to January 2025. The selected animals met the criteria for elective surgery, and all the dogs passed the physical exam; they are all apparently healthy.

Table 1. Mean wound length and standard deviation among treatment groups

VARIABLE	CATEGORY	MEAN		STANDARD DEVIATION
Treatment	Control	51.20		10.91
	Treatment 1	47.73		11.45
	Treatment 2	52.20		11.50
F			0.405	
p-value			≤0.673	
Day	Day 0	51.2		11.40
	Day 2	51.2		11.40
	Day 8	51.2		11.40
	Day 10	51.2		11.40
F			1.000	
p-value			≤0.429	

Table 1 – shows that wound length remained consistent across all treatment groups and time points. Mean values for the control (51.20 mm), treatment 1 (47.73 mm), and treatment 2 (52.20 mm) groups showed minimal variation, with no statistically significant difference (F = 0.405, p = 0.673). Likewise, wound length stayed constant from day 0 to Day 10 (F = 1.000, p = 0.429), confirming no significant change over time.

VARIABLE	CATEGORY	MEAN	STANDARD DEVIATION
Treatment	Control	41.66[a]	36.38
	Treatment 1	46.60[b]	36.33
	Treatment 2	52.16[c]	38.65
F		*89.119*	
p-value		*≤0.001* (if 2 decimal places, <0.01*)*	
Day	Day 0	0	0.00
	Day 2	8.427	1.45
	Day 4	20.474	1.62
	Day 6	46.783	3.85
	Day 8	74.267	3.40
	Day 10	100	0.00
F		4187.383	
p-value		*≤0.001* (if 2 decimal places, <0.01*)*	
Control Group	Day 0	0.00[ac]	0.00
Control Group	Day 2	8.4[b]	1.45
Control Group	Day 4	20.47[ac]	1.62
Control Group	Day 6	46.78[ac]	3.85
Control Group	Day 8	74.27[ac]	3.40
Control Group	Day 10	100.00[ac]	0.00
Treatment 1	Day 0	0.00[a]	0.00
Treatment 1	Day 2	10.26[b]	1.15
Treatment 1	Day 4	33.06[bc]	1.53
Treatment 1	Day 6	56.98[bc]	5.62
Treatment 1	Day 8	79.30[abc]	7.39
Treatment 1	Day 10	100.00[ac]	0.00
Treatment 2	Day 0	0.00b[a]	0.00
Treatment 2	Day 2	10.95[b]	1.15
Treatment 2	Day 4	39.69[ac]	1.80
Treatment 2	Day 6	71.63[ac]	4.36
Treatment 2	Day 8	90.67[abc]	8.70
Treatment 2	Day 10	100.00[ac]	0.00
F		33.46	
p-value		≤0.01*	

p-values are from repeated measures ANOVA. Superscript letters are based on the of Bonferroni test. Means with the same letters are not significantly different (p-value > 0.05)

*indicates statistical significance at 0.05 level (p-value ≤0.05).

Table 2 – mean wound epithelialization and standard deviation by treatment and day post-operation.

The results presented in Table 2 indicate a statistically significant enhancement in epithelialization among dogs treated with autologous serum, with the most notable improvement observed in the treatment 2 group. Although all groups achieved complete epithelialization (100%) by day 10, the rate of progression varied substantially across groups. The mean epithelialization percentages over the 10 days were highest in treatment 2 (52.16%), followed by treatment 1 (46.60%), and lowest in the Control group (41.66%). Analysis of variance (ANOVA) confirmed a highly significant difference among treatments ($F = 89.119$, $p < 0.001$), indicating that increased serum application frequency positively influenced epithelialization.

Temporal analysis revealed a consistent upward trend in epithelial coverage beginning on day 2. Epithelialization advanced to 46.78% by day 6 and reached 74.27% by day 8, demonstrating that epithelial cell migration and wound surface coverage were well underway before complete closure. This progression was also statistically significant across time points ($F = 4187.383$, $p < 0.001$), underscoring a sustained and accelerating healing response.

Wound edge assessment provides insight into how well the wound margins are approximated and whether healing by primary intention is taking place. As shown in Figure 2, all treatment groups, control, treatment 1, and treatment 2 consistently demonstrated a wound edge score of 1 across all days of observation. This uniformity suggests that surgical closure was properly achieved and that the incision margins remained intact and closely apposed throughout the healing process. This finding is consistent with expectations for surgical wounds closed by primary intention, in which the wound edges are intentionally aligned using sutures, staples, or surgical adhesive.

From days 0 to 6, a notable amount of exudate was present across all treatment groups. This finding aligns with the expected early inflammatory response following surgical incision. The volume of exudate gradually decreased by day 8 and was minimal or absent by day 10, suggesting progression into the proliferative and remodeling phases of healing.

As for the exudate type, it was primarily serosanguinous during the first few days, indicating a healthy wound environment with minimal bleeding and no signs of infection. In some instances, sanguineous or slightly bloody exudate was observed within the first 24–48 hours, particularly in wounds with greater tissue disruption or vascularity. This transitioned appropriately to a clearer, more fluid state as healing progressed. No cases of purulent or infected exudate were recorded throughout the observation period, supporting the conclusion that all wounds were healing in an orderly, non-infected manner.

All treatment groups had a skin color rating of 2 (erythema <50%) on days 0 and 2, indicating the expected inflammatory response immediately following surgery. By day 4, some wounds began to improve, particularly in the treatment 2 group, where some animals already had a rating of 1, indicating resolution of erythema and reduced inflammation. From day 6 onward, all groups showed complete normalization of skin color (score 1 across all treatments), indicating that the acute inflammatory phase had subsided and that the wounds were progressing into the proliferative phase of healing. This pattern is consistent with the normal progression of surgical wound healing, as the inflammatory phase usually lasts 2 to 4 days postoperatively, depending on wound severity and management.

All groups, including the Control, Treatment 1, and Treatment 2, consistently scored 1 for peripheral tissue edema from Day 0 through Day 10. This score denotes no visible edema, suggesting that swelling around the wound site was either absent or minimal throughout the monitoring period. This is particularly relevant in this study, where standardized surgical procedures were employed, and healing occurred by primary intention, reducing the likelihood of excessive fluid accumulation in the peri-wound tissues.

The results for a variable that consistently yielded a mean of 0.00 with a standard deviation of 0.00 across all treatment groups (control, treatment 1, and treatment 2) and observation days (day 0 to day 10). This indicates that there was no variation in this variable during the study. As a result, ANOVA was not applicable, and therefore, no F-values or p-values were generated. The uniformity of the data suggests that the variable in question remained constant, unaffected by either the treatment administered or the passage of time. This outcome may reflect the absence of the specific condition or characteristic being measured in all cases, or a uniform response across all subjects and conditions.

Throughout the study, both the type and amount of necrotic tissue consistently registered scores indicating the absence of necrosis in all surgical wounds across all treatment groups.

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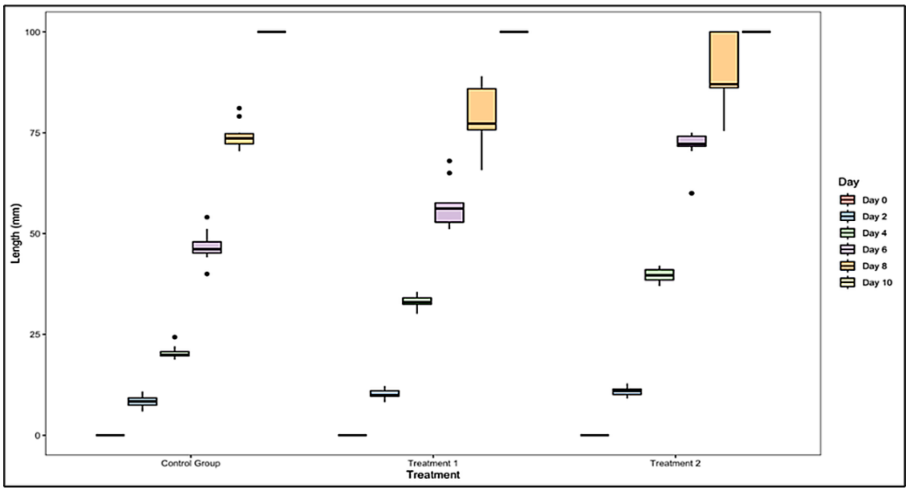

Figure 7 – Boxplot of treatment and time variable of wound healing.

The width of the boxplot from its center indicates the data's variability (dispersion). A wider boxplot indicates greater variability, suggesting that the data are more widely distributed. In contrast, a narrower boxplot suggests that the data values are more closely clustered. In the present study, the granulation (% of wound covered) remained at 0 at the initial time point and progressively increased over the subsequent days.

Notably, for treatments 1 and 2, greater variability was observed on day 8, as indicated by the wider boxplot spread compared to other days. This visual representation is supported by the corresponding standard deviation values, with day 8 showing the highest (3.40), although day 6 recorded the highest overall value. On days 0 and 10, the boxplots appear as flat lines, indicating zero variability because the data values are uniform across all observations.

The central line in each boxplot denotes the median, the midpoint of the dataset when values are arranged in ascending or descending order. This measure provides a reliable indication of the central tendency at each observation point.

4. Discussion

In surgical wound healing, particularly with primary closure techniques, the wound length remains relatively constant throughout the healing process. Measuring wound length alone is not a sensitive indicator of healing, as it does not reflect underlying biological processes such as granulation or epithelialization. In surgically closed wounds, the dimensions remain largely unchanged throughout healing, making length a static parameter. Instead, assessing the quality and extent of tissue regeneration, particularly through granulation and epithelialization, provides a more accurate and clinically relevant measure of healing progression (Landa et al., 2014).

The results of the study support the hypothesis that autologous serum accelerates wound repair, primarily by enhancing epithelialization. This effect was especially noticeable in the group that received treatment twice daily, suggesting that both the frequency and consistency of application play important roles in improving healing outcomes. The likely reason behind this benefit is the presence of naturally occurring biological components in autologous serum, such as growth factors, cytokines, and immunoglobulins. These substances are known to stimulate keratinocyte activity, support tissue regeneration, and help create a balanced wound environment that promotes faster and more effective healing. Comparable results were reported by Jee et al. (2014), who observed improved epithelialization and more organized collagen formation in dogs treated with platelet-rich plasma. This product also comes from the animal's own blood. By day 14, they noted significant improvements in wound closure and tissue structure. Together, these findings strengthen the growing body of evidence showing that autologous blood-derived products, including autologous serum, can be effective, natural options for supporting wound healing in veterinary patients.

Granulation tissue formation was not evaluated in this study because the surgical wounds were closed primarily with sutures. In wounds healing by primary intention, the wound edges are closely approximated with minimal tissue loss, thereby reducing the biological requirement for granulation tissue formation. Healing in such cases primarily occurs through rapid epithelialization and collagen deposition beneath the approximated skin edges, rather than through the development of granulation tissue on the wound surface. The absence of visible granulation tissue in these wounds is consistent with the expected pattern of healing by primary intention. The presence of granulation tissue on the surface of the sutured might indicate delayed closure, a wound starting to reopen, or even a local infection, any of which could lead the wound to shift toward secondary healing. These findings suggest that both the administered treatment and the duration of the healing process had a significant effect on the measured outcome, with treatment 2 showing the most favorable response and a consistent progression of values over time.

When such closure is performed correctly and without disruption, the edges remain well-approximated until epithelialization is complete, as supported by Fossum (2019). Furthermore, Guo and DiPietro (2010) emphasized that minimal edge separation and early epithelialization are indicators of proper inflammatory control and early transition into the proliferative phase of healing. The results of this study reinforce the findings that no complications, such as wound dehiscence, tension, or infection, disrupted the integrity of closure in any group. The presence of exudate during the early inflammatory phase of wound healing is a normal and essential response. Following tissue injury, increased vascular permeability allows fluid, proteins, and immune cells to enter the wound site. This exudate maintains moisture, aids in debridement, delivers nutrients and oxygen, and supports immune defense to prevent infection (Spear, 2012).

The wound depth parameter was excluded from the modified assessment tool due to inherent limitations in measuring it in primarily closed surgical wounds. Following suturing, the wound edges are approximated, and the internal layers are no longer visible or readily accessible for evaluation, making direct depth measurement impractical and unreliable in the early postoperative period. Moreover, the biological timeline of tissue repair further supports this decision. According to Gurtner et al. (2008), the skin and subcutaneous tissues typically exhibit visible closure within 10 to 14 days through epithelialization and initial collagen deposition. However, deeper structures such as the muscle layer, specifically the rectus abdominis, undergo a much slower healing process. Muscle tissue heals predominantly through fibrous scar formation rather than true regeneration, with initial repair occurring within 10 to 14 days but functional restoration taking approximately four to six weeks. Full scar maturation may take 2 to 4 months. Given that the present study monitored healing over 10 days, it is expected that substantial remodeling and tissue deposition in the deeper layers, particularly muscle, would not yet have occurred. As such, depth would remain largely unchanged during this time frame. In contrast, more dynamic and observable parameters, such as epithelialization and granulation tissue formation, are more appropriate indicators of early healing progression. Therefore, the exclusion of depth from the wound assessment is both methodologically and physiologically justified.

5. Conclusion

This study demonstrates that autologous serum significantly improves epithelialization in primarily closed abdominal surgical wounds in female dogs. A dose-dependent effect was observed, with more frequent applications yielding better outcomes. The absence of infection across all groups suggests additional potential in supporting wound hygiene. Overall, autologous serum shows promise as a practical and accessible adjunct to enhance wound healing in veterinary surgical practice.

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