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Protective and Therapeutic Effects of Bovine Amniotic Fluids Collected in Different Trimesters on the Epidural Fibrosis After Experimental Laminectomy in Rats

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■ BACKGROUND: **The aim of this study was to investigate the protective and therapeutic effects of bovine amniotic fluid (BAF) on the inhibition of epidural fibrosis (EF) after experimental laminectomy.**

■ METHODS: **Forty female Sprague Dawley rats were used. The amniotic fluids were collected from each trimester of a pregnant cow. The rats were divided into 5 groups. Whereas no laminectomy was applied to the control group, animals in the sham group underwent laminectomy. Laminectomy was performed in the animals in other groups and the operation area was closed by dripping 1 mL of BAF collected in 3 trimesters of pregnancy. Animals were killed 28 days after the operation.**

■ RESULTS: **Compared with control, VEGF gene expression levels were downregulated approximately 5-fold in BAF-2. Whereas IL-6 was upregulated approximately 8-fold in the sham, it was downregulated 5-fold and 3-fold in BAF-1 and BAF-2, respectively. There was downregulation in BAF-2 and BAF-3 in terms of CD105 gene expression levels. TGFβ1 was upregulated approximately 2-fold in the sham group and downregulated in BAF-1 and BAF-2. Although histopathologic alterations including EF grade and fibroblast cell density were found to increase in the sham group, all BAF treatment decreased those of alterations. The highest CD105 immunoreactivity was detected in the sham group. All BAF treatment markedly aggravated fibrosis via decreasing CD105 immunoreactivity. In terms of grading parameters, almost the closest grades to the control were determined in the BAF-2. BAF collected in the second trimester is most effective in healing of scar tissue and preventing fibrosis via decreasing microvessel and fibroblast densities.**

■ CONCLUSIONS: **The results indicate that BAF may be used as a potential protective agent to prevent EF.**

INTRODUCTION

Degeneration of the intervertebral disc is a physiologic process that develops with aging. Surgical intervention is required in cases of disc herniation that does not respond to medical treatment or in which clinical symptoms gradually worsen.[1] In these cases, a laminectomy operation, defined as a surgical operation to remove the back of ≥1 vertebrae for decompression of the spinal cord, is frequently used.[2] Laminectomy is widely used to reach the epidural space in the surgical treatment of spinal disorders.[2] On the other hand, epidural fibrosis (EF) might develop after laminectomy. EF is characterized by excessive accumulation of extracellular matrix components such as collagen, fibronectin, and dermatan sulfate and severely decreased tissue cellularity in the operated region.[3,4] After laminectomy, fibroblasts and endothelial cells are stimulated as a result of epidural bleeding. In addition, the

Key words
- Bovine amniotic fluids
- Epidural fibrosis
- Inflammation
- Laminectomy

Abbreviations and Acronyms
BAF: Bovine amniotic fluid
EF: Epidural fibrosis
HA: Hyaluronic acid
HASA: Hyaluronic acid—stimulating factor
IL-6: Interleukin 6
PBS: Phosphate-buffered saline
TGF-β1: Transforming growth factor β_1
VEGF: Vascular endothelial growth factor

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expression of inflammatory cytokines, angiogenic markers, and various growth factors are increased after laminectomy.[5-7]

Although many clinical and experimental studies have been conducted to prevent EF, which has a complex etiopathogenesis, an effective and definitive solution has not yet been suggested.[1,8] Recently, the therapeutic effects of various agents such as steroids, biological fluids/membranes, antiinflammatory agents, and hyaluronan in the treatment of experimentally created EF models have been investigated.[1,4]

Amniotic fluid contains high levels of hyaluronic acid (HA), HA-stimulating factor (HASA), extracellular macromolecules such as fibronectin, laminin, and many growth factors (epidermal growth factor, fibroblast growth factor, insulinlike growth factor).[9] It has been reported in some studies that amniotic membrane and its fluid have potential in the inhibition of EF.[10-12] On the other hand, it is known that the use of human amniotic fluid is not common because of both legal procedures and the insufficient number of donors available for mass production. The aim of this study was to investigate the protective and therapeutic effects of bovine amniotic fluid (BAF) on the inhibition of EF after experimental laminectomy in rats. In addition, the protective and therapeutic effects of amniotic fluids collected from each trimester (first, second, and third) of a pregnant cow were compared at the molecular, biochemical, and histopathologic levels.

METHODS

This study was carried out in the Experimental Animals Laboratory of the Physiology Department of Dokuz Eylul University, Turkey. The study was ethically approved by the animal experiments local ethic committee of Dokuz Eylul University (decision number 38/2022). Forty female Sprague Dawley rats weighing between 200 and 250 g were used in the study. During the study, the animals were kept in a room isolated from sound and other environmental factors, with an ambient temperature of 24°C ± 1°C, relative humidity of 60% ± 10%, and a light–dark cycle of 12 hours light–12 hours dark. In addition, there was no restriction on the feed and water intake of the animals. The rats were divided into 5 groups ($n = 8$). Although no laminectomy or any other treatment was applied to the first group and the animals were kept in normal cages under laboratory conditions (control group), animals in the second group underwent only laminectomy (sham group). Laminectomy was performed in the animals in other 3 groups, and immediately after, the operation area was closed by dripping 1 mL of BAF. The difference between these groups was that the BAF used was taken in 3 different trimesters of pregnancy (BAF-1, BAF-2, and BAF-3). Detailed information about the groups is presented in **Table 1**.

Table 1. Information About Groups of the Study

Control group (control)	No laminectomy or any other treatment was applied to this group and the animals were kept in normal cages under laboratory conditions
Sham group (sham)	Only laminectomy was performed on animals in the sham group
Laminectomy + BAF-1	Laminectomy was performed in the animals in this group, and immediately after, the operation area was closed by dripping 1 mL of BAF, which was taken from the first trimester of a pregnant cow and sterilized by γ radiation method
Laminectomy + BAF-2	Laminectomy was performed in the animals in this group and immediately after the operation area was closed by dripping 1 ml of BAF, which was taken from the second trimester of a pregnant cow and sterilized by γ radiation method
Laminectomy + BAF-3	Laminectomy was performed in the animals in this group and immediately after the operation area was closed by dripping 1 mL of BAF, which was taken from the third trimester of a pregnant cow and sterilized by γ radiation method

BAF-1, bovine amniotic fluid first trimester; BAF-2, bovine amniotic fluid second trimester; BAF-3, bovine amniotic fluid third trimester.

Collection and Measuring of HA Levels of BAF

Amniotic fluid was collected from a pregnant cow by ultrasound-guided amniocentesis on day 80, 160, and 240 of pregnancy. On day 80 of pregnancy, ultrasound-guided amniocentesis was performed from the vaginal route as described by Garcia and Salahaddine[13] and Kamimura et al.[14] After antisepsis of the perineal region was provided with iodinated antiseptic solution, a transvaginal 5-MHz convex probe with a 21G needle was advanced to the cervix placed in the vagina and contacted with the vaginal wall lateral to the portio vaginalis of the cervix. After the ultrasonographic image was evaluated and the fetus and amniotic sac monitored, while the probe was held in a fixed position, the needle placed at an angle of 30° was advanced by an assistant to enter the amniotic sac and 50 mL of amniotic fluid was aspirated into a sterile Falcon tube (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). On day 160 and 240 of pregnancy, amniocentesis was performed transabdominally under ultrasound guidance. In this method, the right abdominal wall was shaved starting from the fossa paralumbar area through to the paramedian to remove the hair on the skin. An assistant applied pressure on the opposite side of the abdominal wall to bring the uterus and fetus as close to the right abdominal wall as possible. The fetus and amniotic sac were visualized ultrasonographically using a 3.5-MHz convex probe. After determining the area to be amniocentesis, the region was disinfected with iodine antiseptic solution. A sterile 30-cm needle was inserted in the amniotic sac with ultrasound guidance and 50 mL of amniotic fluid was aspirated into a sterile Falcon tube.

Amniotic fluid samples were centrifuged at 3000 rpm for 15 minutes and stored at −20°C until used. HA levels of amniotic fluids taken from all 3 trimesters before being used in the study were measured using commercial enzyme-linked immunosorbent assay kits containing bovine HA antibodies. Then, these liquids were sterilized by the γ radiation irradiation method at a dose of 25 kGy and made ready for use. HA levels of the collected amniotic fluids were measured by commercial kit (catalog number E4616-

Table 2. Forward and Reverse Sequences of Primers

Genes	Forward and Reverse Sequences	References
CD105	F: 5′-TGAGTCTCCAGCCCACTTCT-3′ R: 5′-GCCTTATCCGGTGTCCAGAG-3′	*
IL-6	F: 5′-GCCCTTCAGGAACAGCTATGA-3′ R: 5′-TGTCAACAACATCAGTCCCAAGA-3′	Wang et al., 2010[16]
TGFb1	F: 5′-ATTCCTGGCGTTACCTTGG-3′ R: 5′-CCTGTATTCCGTCTCCTTGG-3′	Rezaii et al., 2019[17]
VEGF	F: 5′-CAGCTATTGCCGTCCAATTGA-3′ R: 5′-CCAGGGCTTCATCATTGCA-3′	Alghzzawy et al., 2020[18]
ACTB	F: 5′-TGACAGGATGCAGAAGGAGA-3′ R: 5′-TAGAGCCACCAATCCACACA-3′	Yang et al., 2021[19]
GAPDH	F: 5′-TGGGTGTGAACCACAAGAAA-3′ R: 5′-GTGGCAGTGATGACATGGAC-3′	Motawea et al., 2020[20]

*Designed in this study.

100; Biovision, Milpitas, California, USA) with an enzyme-linked immunosorbent assay method. According to the manufacturer's declaration, intra-assay coefficient of variation (CV) was <8%, interassay CV was <10%, and the detection range was 1.563–100 ng/mL.

Surgical Procedure

For general anesthesia, 10 mg/kg of xylazine hydrochloride (intramuscular) (Alfazyne 2% injection 50 mL [EGE-VET, Izmir, Turkey]) and 50 mg/kg ketamine hydrochloride (intramuscular) (Alfamine 10% injection 50 mL [EGE-VET]) were administered to rats in all groups undergoing surgical intervention. After the operation areas of the rats were shaved and disinfected with povidone iodine under ketamine and xylazine anesthesia, a midline skin incision was made on L3-L5. Paravertebral muscles were dissected subperiosteally, and the L3-L5 laminae were observed before a total laminectomy was performed along with the excision of ligamentum flavum and epidural adipose tissue.[15] Then, total laminectomy was performed at the L3 level, but the integrity of the dura mater was not compromised. After the surgical procedure, intracardiac blood of rats was collected under deep anesthesia and the animals were killed on the 28th day. After the rats were killed, L1-L5 vertebral columns were removed as a block.

Total RNA Isolation and Complementary DNA Synthesis

After euthanasia, samples were quickly collected under sterile conditions and kept in RNA stabilizer solution (catalog number E0280; Fix RNA, Poland). Then, samples were transferred in cold chain to −80°C until molecular analysis. Total RNA isolation from the samples was performed according to the protocol of TRItidy G (catalog number A4051; PanReac Application, Germany). For this purpose, 1 mL of TRItidy G was used for approximately 50 mg of tissue. After the chloroform-isopropyl alcohol-ethyl alcohol stages, obtained RNA pellets were dried at room temperature for about 10 minutes and then diluted with 30–50 μL of nuclease free water.

Purity (A260/280 ratio) and concentration values of isolated RNAs were determined by nucleic acid meter (Merinton SMA-1000, China). In addition, RNA integrities of the samples were evaluated electrophoretically on 1% agarose gel (100 V and 30 minutes). To eliminate possible genomic DNA contamination, DNA digestion protocol was performed in the samples with DNase I Kit (DNase I, catalog number EN0521; RNase-Free Thermo Fisher Scientific, USA). After DNA digestion, complementary DNA synthesis was performed in the samples with High-Capacity cDNA Reverse Transcription Kit (catalog number 4368814; Thermo Fisher Scientific, USA). In thermal cycler (Bio-Rad, T100, USA), the complementary DNA synthesis protocol was as follows: after 10 minutes at 25°C, 20 minutes at 37°C, and 5 minutes at 85°C, respectively. After the reaction, the samples were completed to 200 μL with nuclease free water and stored at −20°C until gene expression analysis.

Reverse Transcription Quantitative Polymerase Chain Reaction Application

Amplifications of *CD105* (endoglin), *IL-6* (interleukin 6), *VEGF* (vascular endothelial growth factor), *TGFβ1* (transforming growth factor β_1) genes were investigated in the study. The SYBR Green I dye containing kit (Power SYBR Green PCR Master, catalog number 4367659; Thermo Fisher Scientific, USA,) was used for the amplifications in reverse transcription quantitative polymerase chain reaction (Rotor Gene Q, MDx 5 plex HRM, Qiagen). Each sample was studied in triplicate. The reaction was arranged 10 minutes at 95°C, followed by 15 seconds at 95°C, 60 seconds at 60°C, and 40 cycles. *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *ACTB* (β actin) were used as housekeeping genes. Primers used in amplification of genes were checked by Primer Blast and Primer3Plus (National Center for Biotechnology Information, National Library of Medicine, Bethesda, Maryland, USA) (Table 2).

Histopathologic Examinations

Fixation of the vertebral column tissue samples was performed in 10% formaldehyde solution for 3 days, and then those of tissues were decalcified in EDTA (ethylenediaminetetraacetic acid) solution for 4 weeks. After routine histologic procedures, tissue

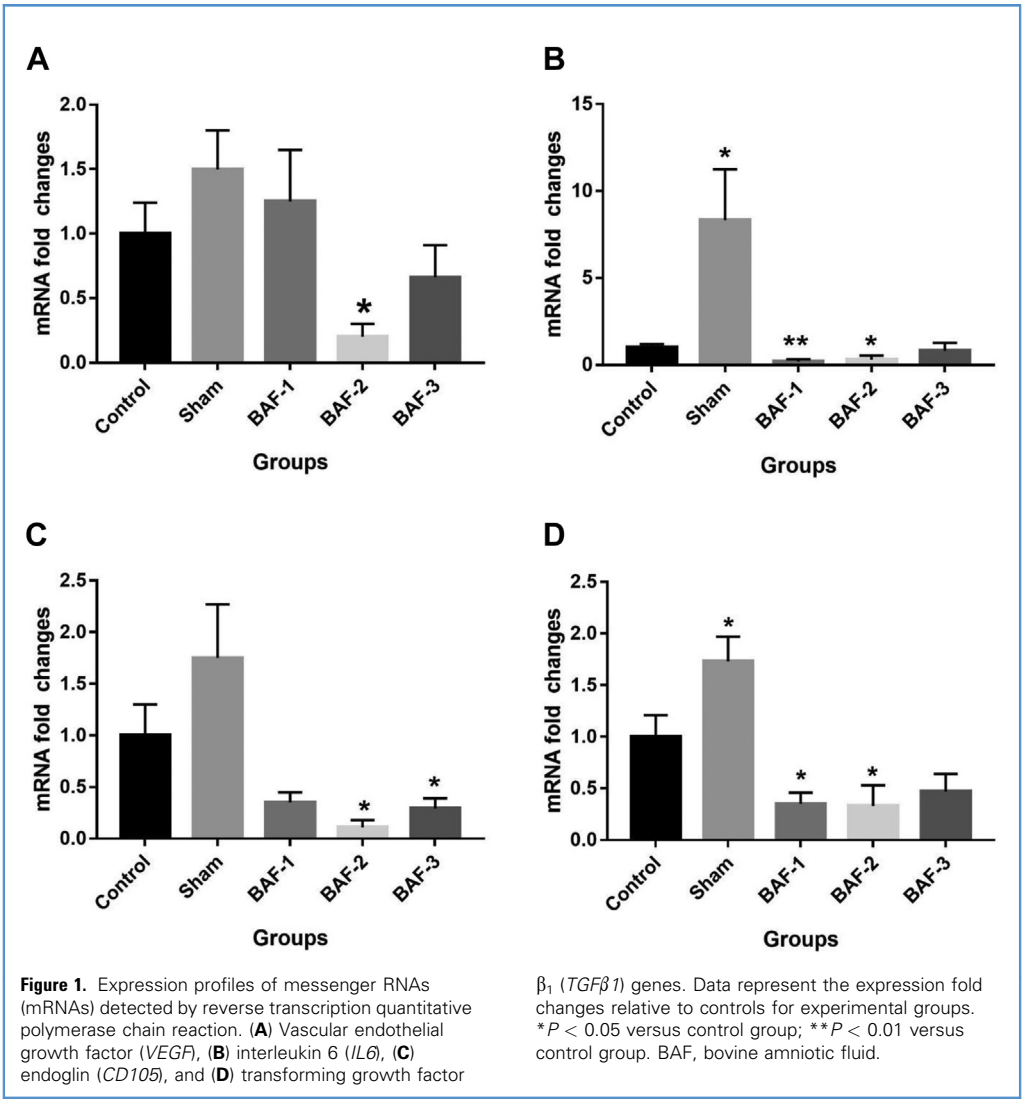


Figure 1. Expression profiles of messenger RNAs (mRNAs) detected by reverse transcription quantitative polymerase chain reaction. (**A**) Vascular endothelial growth factor (*VEGF*), (**B**) interleukin 6 (*IL6*), (**C**) endoglin (*CD105*), and (**D**) transforming growth factor β_1 (*TGFβ1*) genes. Data represent the expression fold changes relative to controls for experimental groups. $^*P < 0.05$ versus control group; $^{**}P < 0.01$ versus control group. BAF, bovine amniotic fluid.

samples were embedded in paraffin blocks. The tissues were stained with hematoxylin-eosin and Masson's trichrome stain and cellular intensity and fibrosis were scored to show connective tissue differences. EF was evaluated as follows: grade 0, no scar tissue in dura mater; grade 1, then fibrosis bands present between scar tissue and dura mater; grade 2, adhesions holdings 2/3 of laminectomy defect present; and grade 3, affecting more than two thirds of the laminectomy defect.[21]

Fibroblasts were counted at 100× magnification from 3 areas of each section, including 1 area in the middle and 2 at the laminectomy sides. EF was scored by 2 blinded histologists. The number of fibroblasts was evaluated as follows: 1) <100 fibroblasts; 2) 100–150 fibroblasts; and 3) >150 fibroblasts.[4]

Immunohistochemical Examination

Microvessel density was determined immunohistochemically according to degree of staining anti-CD105 antibody (catalog number BS-4609R; Bioss, USA). Sections were mounted on poly-L-lysine-coated slides. The streptavidin-biotin-peroxidase assay was performed using the primary antibodies against anti-CD105. After deparafinization, sections were treated with tripsin (TA-125-TR [Thermo Fisher]) for 15 minutes and endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxidase in phosphate-buffered saline (PBS) at room temperature for 10 minutes. Then, primary antibodies were applied for 2 hours at 4°C and washed in PBS. After washing, the secondary antibody (85–9043, Invitrogen) was applied for 30 minutes, followed by washes in PBS. The peroxidase activity was visualized with diaminobenzidine (Roche, Penzberg, Germany). Slides were counterstained with Mayer hematoxylin, dehydrated, and cleared, and they were analyzed on a light microscope.[22] For the analysis, 3 different areas with maximum neovascularization were selected. At 400× magnification, single endothelial cells or interconnected endothelial cell clusters that stained brown,

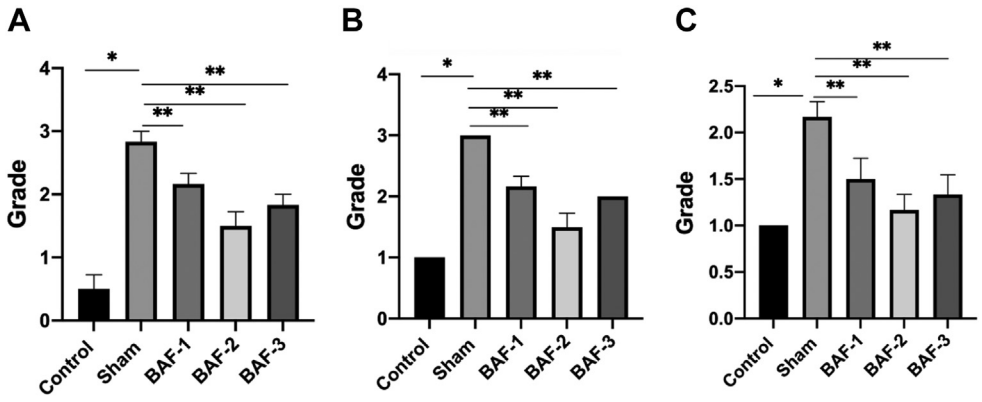


Figure 2. Mean width of the (**A**) epidural fibrosis grade, (**B**) fibroblast density, and (**C**) CD105 positive cells in all groups. *$P < 0.05$ versus control group, **$P < 0.05$ versus sham group. BAF, bovine amniotic fluid.

which discriminated these from other connective tissue elements, regardless of whether they comprised a lumen, were accepted as a countable microvessel. For immunohistochemical staining, the number of vessels was counted at 400× magnification. Those with ≤3 vessels were considered as score 1, those between 4 and 6 were considered as score 2, and those ≥7 were considered as score 3. Vessels with a muscle layer were not considered as a new vessel and were not included in the counting. The mean number of vessels counted from 3 areas was calculated and this number was recorded as the microvessel intensity.[4]

Statistical Analyses

Data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, New York, USA). Values are presented as mean ± standard error of the mean. Because the data obtained from the study were inconsistent with normal distribution and the sample was small, we used the nonparametric statistical methods. Differences between all groups were examined with Kruskal-Wallis test and the 2 groups were examined with the Mann-Whitney U test, with findings of $P < 0.05$ considered significant. GraphPad Prism 6 (GraphPad, San Diego, California, USA) was used for drawing bar charts.

Gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ methods and geometric mean of *GAPDH* and *ACTB* housekeeping genes Ct values was used for expression analyzes. The results were presented as fold changes.[23]

RESULTS

Expression Profile of Messenger RNAs in Vertebral Column Tissue Samples of Laminectomy Rats

The levels of HA in different trimesters were detected as 16.75, 19.87, and 18.01 ng/mL, respectively. Purity (A260/280 = 1.78 ± 0.01) and concentration (185.44 ± 15.60 ng/μL) values of isolated RNA from samples were appropriate for gene expression analysis. Compared with the control group, *VEGF* gene expression levels were downregulated approximately 5-fold in the BAF-2 group ($P < 0.05$) (Figure 1A). Whereas *IL6* was upregulated approximately 8-fold in the sham group, it was downregulated 5-fold and 3-fold in the BAF-1 and BAF-2 groups, respectively ($P < 0.01$; $P < 0.05$) (Figure 1B). It was determined that there was significant downregulation in BAF-2 and BAF-3 groups in terms of *CD105* gene expression levels ($P < 0.05$) (Figure 1C). In addition, the *TGFβ1* gene was upregulated approximately 2-fold in the sham group and downregulated approximately 3-fold in the BAF-1 and BAF-2 groups ($P < 0.05$) (Figure 1D).

Histopathologic Results

The sections from the control group were normal in histologic appearances. Histopathologic alterations including EF grade and fibroblast cell density were found to increase in the sham group compared with controls. EF grade and fibroblast cell density markedly decreased in all BAF-treated groups compared with the sham group ($P < 0.05$, for all comparisons, Figure 2A and B). In particular, those parameters detected in the BAF-2 group were similar to those of controls (Figures 3 and 4). Grade 1–3 EF was observed in both BAF-1 and BAF-3 groups (Figures 3 and 4).

Microvessel density in scar tissue was conducted by CD105 antibody in all groups. The highest CD105 immunoreactivity score was detected in the sham group compared with controls ($P \leq 0.05$). Scores markedly decreased in all animals in the BAF-treated group ($P \leq 0.05$, Figure 2C, Figure 5). When all scoring parameters were examined, almost the closest scores to the control were determined from the BAF-2 group. According to histopathologic results, it seems that BAF from the second trimester is most effective in healing of scar tissue and preventing fibrosis via decreasing microvessel and fibroblast densities ($P > 0.05$, for both).

DISCUSSION

EF is one of the most important complications of laminectomy and can cause postlaminectomy syndrome.[12,24-26] It has been

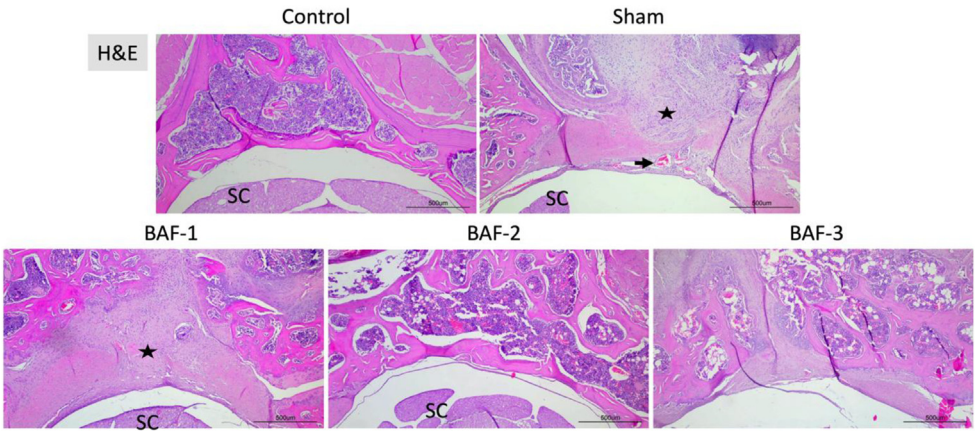


Figure 3. Histologic sections of the laminectomy sites in all groups. Control group appears normal. Grade 3 epidural fibrosis is shown in the sham group. Note that microvessels in scar tissue (*arrow*) and fibroblasts accumulated within the scar tissue (*asterisks*) are clearly seen in the sham group. Grade 1 and grade 2 epidural fibrosis are seen in BAF-1 (bovine amniotic fluid trimester 1) and BAF-3 groups, respectively. Laminectomy site is generally normal in the BAF-2 group. The BAF from the second trimester markedly improved histopathologic findings. H&E, hematoxylin-eosin; SC, spinal cord.

reported that attempting a reoperation to prevent EF carries a high risk because of possible complications such as dural laceration, root injury, and epidural bleeding.[26] Therefore, operative intervention is avoided in the treatment of EF after laminectomy. Medical and other alternative treatment methods that may show more permanent effects are recommended.[27]

Amniotic fluid was used in the treatment of many inflammatory diseases in both experimental and clinical studies.[9,28,29] Amniotic fluid is one of the biological fluids and it contains water, electrolytes, proteins, peptides, carbohydrates, lipids, hormones, HA, HA-stimulating factor (HASA), fibronectin, laminin, epidermal growth factor, fibroblast growth factor, and insulinlike growth factor. The composition of amniotic fluid varied according to different periods of gestation.[9,28] The therapeutic effects and HA, HA-stimulating factor (HASA) concentrations of amniotic fluids collected from the 3 trimesters of pregnancy were evaluated

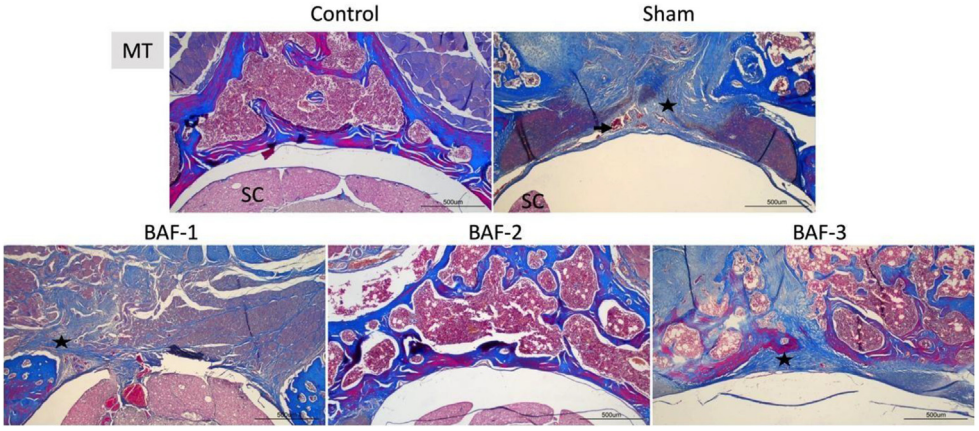


Figure 4. Histologic sections of the laminectomy sites in all groups. Control group appears normal. Scar tissue increased the laminectomy side in the sham group. Grade 3 epidural fibrosis is seen in the sham group. Grade 2 epidural fibrosis is seen in both BAF-1 (bovine amniotic fluid trimester 1) and BAF-3 groups. Laminectomy site is generally normal in the BAF-2 group. MT, Masson trichrome stain; SC, spinal cord; *arrow*, micro vessels; *asterisks*, fibroblasts.

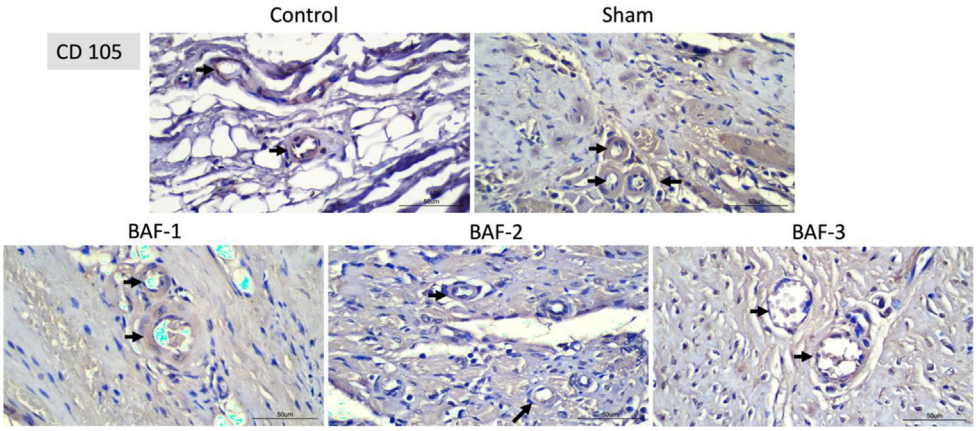


Figure 5. Immunohistochemical detection of CD105. Endoglin expression was observed around the vessels. *Arrows* show CD105-positive cells. BAF-1, bovine amniotic fluid trimester 1.

comparatively. Our measurements showed that the highest HA concentration was measured in the second trimester, similar to that of human amniotic fluids.[30-33]

Effects of human amniotic membrane and fluid against EF after spinal laminectomy were investigated in experimental rat and canine models.[10-12] It was shown in previous studies[10-12] that human amniotic membrane implantation and human amniotic fluid administration significantly reduced amount of scar tissue, epidural scar adhesion, inflammatory cell infiltration, and fibroblast proliferation induced by spinal laminectomy in both rat and canine models.

IL-6 plays an important role in acute and chronic inflammations including EF.[26] In the current study, *IL6* gene expression levels were significantly upregulated in the sham group. All BAF treatment showed an antiinflammatory effect via downregulating *IL-6* gene expression. It was believed that the possible reason for downregulation of *IL6* might be related to the period of BAF composition.

CD105 is a membrane glycoprotein expressed by activated endothelial cells and regulates TGF-β1 signaling.[34] CD105 can be evaluated as a marker in different stages of EF developing after laminectomy because of its role in fibrosis and angiogenesis activities.[4,35] VEGF is a potent angiogenic cytokine that is effective in the formation of fibrosis and associated adhesions and contributes to angiogenesis in postoperative wound healing.[36,37] Inhibition of *VEGF* is related to negative regulation of *TGFβ1*, which is one of the major molecular biomarkers for the detection of fibrosis in tissue and organs.[27,38,39] In the current study, *VEGF*, *CD105*, and *TGFβ1* gene expression in the sham group was upregulated almost 2-fold compared with controls. Those genes were downregulated in BAF-treated groups. As expected, parallel to the downregulation of *VEGF* gene expression levels, *TGFβ1* gene expression level was significantly downregulated in both BAF-1 and BAF-2 groups. Although similar downregulation trends were shown in BAF-treated groups, the highest downregulation of *VEGF*, *CD105*, and *TGFβ1* genes were obtained in BAF-2 (approximately 5-fold) group.

EF causes reparative inflammation, unnatural extensive scarring, and subsequent adhesions as a result of the fibrotic process.[40-43] In this study, severe histopathologic damages including EF, increase in fibroblast density, and scar tissue formation after laminectomy were observed in the sham group. All BAF treatment improved histopathologic alterations via decreasing EF and fibroblast density grades.

Microvessel density measurement is an evaluation criterion to determine the level of angiogenesis in fibrotic tissue. The increase in the number of microvessels indicates intense angiogenesis.[43] The expression of CD105, a protein found in the membrane of endothelial cells, is used in the determination of angiogenesis. Anti-CD105 specifically binds to endothelial cells in angiogenic tissues, and, therefore, it is a superior marker in the determination of neovascularization.[35,44,45] We found that CD105 immunoreactivity was significantly higher in the sham group than in other groups. These results were compatible with EF and fibroblast density grades detected in that group. In all BAF-treated groups, especially the BAF-2 group, EF and fibroblast density grades decreased compared with the sham group.

In our literature review, it was determined that the effect of BAF on EF developing after laminectomy has not been investigated previously. To the best of our knowledge, this is the first study of treatment of experimental EF with BAFs conducted in rats. According to the results, BAF collection in the second trimester of the pregnancy is better for the treatment of EF and complications. As a result, BAFs, which are more readily available and less expensive than human amniotic fluids, may be an option for prevention of EF. On the other hand, more study is needed at the molecular levels for the investigation of cellular mechanisms of EF.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Ziya Yurtal: Conceptualization, Investigation, Writing — original draft, Writing — review & editing. **Kadri Kulualp:** Conceptualization, Investigation, Writing — original draft, Writing — review & editing. **Huseyin Ozkan:** Investigation, Data curation, Writing — original draft, Writing — review & editing. **Serap Cilaker Micili:** Investigation, Writing — original draft, Writing — review & editing. **Halef Doğan:** Investigation, Writing — original draft, Writing — review & editing. **Ali Riza Sisman:** Formal analysis, Writing — original draft, Writing — review & editing. **Nevin Ersoy:** Investigation, Writing — original draft, Writing — review & editing. **Ceren Kizmazoglu:** Investigation, Writing — original draft, Writing — review & editing. **Akin Yakan:** Investigation, Data curation, Writing — original draft, Writing — review & editing.

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