

Hematological, biochemical, and oxidative stress changes in lambs with atresia ani

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Article Info	Abstract
Article history: Received: 08 July 2025 Accepted: 11 October 2025 Available online: 15 July 2026	Anal atresia (AA) is a congenital anorectal malformation in neonatal lambs characterized by failure of the anal membrane to perforate, resulting in the absence of an anal opening. Early diagnosis and surgical correction are essential for improving survival and outcomes. This study evaluated hematobiochemical and oxidative stress parameters in neonatal lambs with non-segmental AA undergoing surgical intervention. Forty-five male lambs diagnosed with non-segmental AA were enrolled and stratified by time of presentation: within 24 hours of birth or 24-72 hours. An additional 45 healthy male lambs served as controls. Hematological and biochemical parameters, including white blood cell count, red blood cell count, hemoglobin, packed cell volume, total protein, C-reactive protein, superoxide dismutase, malondialdehyde, globulin, fibrinogen, and urea were measured at presentation and 3 days post-operatively. All AA lambs underwent surgical correction, with clinical follow-up for 30 days. Significant differences in hematobiochemical indices and oxidative stress markers were observed between AA lambs and healthy controls. Early surgical intervention (within 24 hours) was associated with significantly reduced levels of urea, fibrinogen, and C-reactive protein. It also resulted in lower malondialdehyde concentrations and higher superoxide dismutase activity, indicating decreased oxidative stress and inflammation compared with later intervention. These findings highlight the critical importance of early surgical intervention in neonatal lambs with anal atresia. Timely surgery improves hematological stability, mitigates oxidative damage, and supports better overall recovery.
Keywords: Anal atresia Congenital anomaly Lamb Oxidative stress Rectovaginal fistulae	

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Introduction

Atresia ani, also known as imperforate anus, is a congenital defect that occurs during embryogenesis. In this defect the anal membrane fails to break down and connect to the perineum, resulting in no apparent anal opening after birth.¹ This anorectal anomaly has been reported in humans^{2,3} and numerous animal species,⁴ including domestic animals particularly sheep due to an autosomal recessive gene.⁵ The exact etiology of atresia ani is not well understood, but fetal exposure to environmental teratogens like viral infections and toxic plants during gestation seems to play a vital role.^{6,7} In newborn humans maternal exposure to tobacco, overweight, obesity, and diabetes has been reported to increase the risk of anorectal malformations.³ Four types of atresia ani (AA) have been detected in animal species. In Type I atresia congenital stenosis of the anus exists. In Type II an

imperforate anus as well as persistence of the anal membrane occurs. Type III is also an imperforate anus with more cranially rectum ending than type II. Type IV is defined as the normal ending of the terminal rectum and anus associated with a pouch of cranial rectum in the pelvic region.^{7,8}

Early diagnosis and treatment can reduce the risk of mortality in lambs with atresia ani. Meconium impaction, particularly in male lambs, can lead to severe abdominal pain, systemic absorption of toxic metabolites, and an increased risk of intestinal rupture and septic shock, potentially resulting in death. However, female lambs with concurrent rectovaginal fistulae may exhibit a more favorable prognosis.⁹ Although some forms of the anomaly are not immediately life-threatening, surgical correction should be undertaken as soon as the diagnosis is established.^{1,10,11} The present study aimed to investigate whether meconium impaction in newborn lambs disrupts

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the oxidant-antioxidant balance, thereby affecting selected biochemical and hematological parameters. It was hypothesized that meconium accumulation induces systemic inflammation and oxidative stress, leading to alterations in hepatic function, electrolyte homeostasis, and hematological indices.

Materials and Methods

Animals. From October 2018 to June 2022, a total of 45 newborn male Lori lambs diagnosed with non-segmental anorectal atresia, aged 24 - 72 hr, and were admitted to the Lorestan Veterinary Hospital. Lambs with anorectal atresia were classified into two subgroups according to their age at presentation: The first subgroup consisted of lambs presented within 24 hr of birth (1-day-old [DO]), whereas the second subgroup included lambs presented between 24 and 72 hr after birth (3 DO). In all cases, surgical intervention was performed promptly after diagnosis, regardless of age at presentation. Following clinical examination, 45 healthy male Lori lamb as Control (CT) group lambs, free of congenital defects and neonatal diseases, were selected from the same herds as the affected lambs. These CT lambs were matched with the experimental group based on age, birth conditions, and environmental factors, ensuring minimal confounding variables and maximizing comparability between groups. Postoperative follow-up examinations were performed 30 days post-surgery to evaluate long-term surgical outcomes, including any complications, recurrence of clinical symptoms, or signs of infection

Measurements. Blood samples were collected from the jugular vein into tubes containing EDTA anticoagulant (EDTA/K₂ vacutainer tubes, Tajhiz Gostar, Isfahan, Iran) and into plain tubes without anticoagulant. Samples from healthy lambs were obtained once upon enrollment, whereas samples from atresic lambs were collected at two time points: upon admission and three days after surgical correction. The following hematological parameters were measured including white blood cells (WBC), red blood cells, hemoglobin, packed cell volume, total protein (TP), albumin (ALB), globulin, ALB/globulin ratio, C-reactive protein (CRP), fibrinogen, urea (U). Oxidative stress status was evaluated by measuring superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration, which were used as indicators of antioxidant defense and lipid peroxidation, respectively. Hematological indices were measured via an automated hematology analyzer (Celltac Alpha VET MEK-6450; Nihon Kohden Corp., Tokyo, Japan). Total protein concentration was determined using the Bradford protein assay kit (Zist Shimi Co., Tehran, Iran) at 630 nm wavelength. Albumin concentration was measured using an automated biochemical analyzer according to the manufacturer's protocol. Globulin concentration was calculated by subtracting the measured

ALB value from the total serum protein. Lipid peroxidation was assayed by measuring the MDA concentration by a lipid peroxidation assay kit (Navand Salamat Co., Urmia, Iran) in the serum using thiobarbituric acid reactive substance (TBARS) method at 550 nm in which MDA is formed as a secondary product from oxidation of lipid substrates. The SOD activity was measured by SOD Assay Kit (Navand Salamat Co.) at 405 nm wavelength and room temperature using spectrophotometry. Urea and ALB serum concentration measured with (Zist Shimi Co.). High-sensitivity CRP concentration in serum samples was measured using turbidimetric method (Enison Lab and Pharmaceutical Industries, Tehran, Iran).

Surgical procedure. Lambs were restrained in ventral recumbency, with the hind limbs suspended and the tail roped cranially to the surgical table to facilitate surgical access. The perineum was palpated for a fluctuant swelling, and the rectal pouch was confirmed by aseptic needle aspiration of meconium or gas. The surgical site was prepared aseptically using 10.00% povidone-iodine solution and subsequently draped in a routine sterile manner. Local anesthesia was achieved by infiltration of the skin and subcutaneous tissues with 2.00% lidocaine (2.00 mg kg⁻¹; Rooyan Darou, Tehran, Iran). A full-thickness incision was then made through the perineal tissues to expose the blind-ended distal rectum. Four simple interrupted sutures (Polyglycolate 4-0; Supa, Tehran, Iran) were placed perpendicular to each other at 12-, 3-, 6- and 9- o'clock positions to approximate the rectal edges. Additional sutures were placed to ensure adequate closure. Intramuscular penicillin-streptomycin (10.00 mg kg⁻¹; Norbrook Laboratories, Newry, UK) was administered as postoperative antibiotic therapy for 3 days, and intramuscular flunixin meglumine (1.10 mg kg⁻¹; Rooyan Darou) was administered for 3 days as post-operative analgesia and anti-inflammatory treatment.¹² Skin sutures were removed 14 days after surgery.

Statistical analysis. Data were analyzed using SPSS Software (version 20.0; IBM Corp., Armonk, USA). Data normality was assessed using the Shapiro-Francia test. As the data were non-normally distributed, results are presented as median and quartile range (Median [Q3 - Q1]). Mann-Whitney U test was used to detect significant differences between treatment groups. A *p*-value < 0.05 was considered statistically significant.

Results

Hematological indices. Table 1 indicates CBC and blood biochemical parameters in different study groups. Several hematological and biochemical parameters differed significantly among the study groups. The total WBC count in the AA group was 24.75 (19.00 - 34.50; 1 DO), and 28.25 (20.10 - 36.00; 3 DO) 10³ µL⁻¹, which was higher compared to the CT group (*p* ≤ 0.05).

Table 1. Complete blood count and biochemical parameters in different study groups.

Parameters	Healthy lambs (upon presentation)	Before surgery		3 days after surgery	
		Atresic lambs (1 DO)	Atresic lambs (3 DO)	Atresic lambs (1 DO)	Atresic lambs (3 DO)
RBC ($\times 10^6 \mu\text{L}^{-1}$)	12.40 (11.90 - 12.60) ^a	12.10 (11.60 - 12.50) ^a	12.00 (11.72 - 12.34) ^a	12.05 (11.45 - 12.30) ^a	12.15 (11.00 - 12.40) ^a
PCV (%)	35.25 (34.00 - 36.30) ^a	39.25 (34.90 - 40.30) ^a	42.25 (36.00 - 42.20) ^a	33.25 (33.00 - 35.50) ^a	34.00 (33.80 - 36.50) ^a
Hb (g dL ⁻¹)	12.60 (12.30 - 12.80) ^a	13.50 (12.10 - 13.80) ^a	13.90 (12.50 - 14.00) ^a	12.40 (12.10 - 13.00) ^a	12.30 (12.00 - 13.00) ^a
WBC ($\times 10^3 \mu\text{L}^{-1}$)	14.55 (12.00 - 18.00) ^a	24.75 (19.00 - 34.50) ^b	28.25 (20.10 - 36.00) ^b	18.75 (17.00 - 22.20) ^{ab}	16.40 (15.20 - 21.60) ^{ab}
Neutrophils (%)	0.00 (0.00 - 0.00) ^a	0.00 (0.00 - 0.00) ^a	0.10 (0.00 - 0.60) ^a	0.00 (0.00 - 0.20) ^a	0.00 (0.00 - 0.40) ^a
TP (mg dL ⁻¹)	61.05 (59.80 - 63.50) ^a	66.10 (62.40 - 68.00) ^a	65.90 (63.00 - 67.20) ^a	63.25 (60.50 - 65.40) ^a	64.50 (61.60 - 66.80) ^a
ALB (mg dL ⁻¹)	27.25 (27.10 - 27.50) ^a	28.35 (27.00 - 28.70) ^a	28.65 (27.30 - 28.75) ^a	27.80 (27.10 - 28.00) ^a	27.45 (27.20 - 27.90) ^a
A/G ratio	0.80 (0.76 - 0.84) ^a	0.74 (0.73 - 0.78) ^a	0.76 (0.73 - 0.80) ^a	0.76 (0.72 - 0.82) ^a	0.77 (0.75 - 0.81) ^a
Urea (mg dL ⁻¹)	54.60 (52.00 - 55.90) ^a	68.60 (62.56 - 70.23) ^b	77.50 (72.36 - 85.20) ^c	58.30 (55.35 - 60.20) ^a	59.35 (53.60 - 62.30) ^a
FBS (mg dL ⁻¹)	400 (300 - 600) ^a	600 (500 - 700) ^b	690 (600 - 750) ^b	450 (400 - 580) ^a	510 (480 - 600) ^a
CRP (mg dL ⁻¹)	0.60 (0.50 - 0.80) ^a	1.40 (1.20 - 1.60) ^b	1.90 (1.80 - 2.00) ^c	0.80 (0.70 - 1.00) ^{ad}	1.00 (0.90 - 1.30) ^{bd}
GLB (mg dL ⁻¹)	34.05 (32.50 - 36.10) ^a	38.10 (35.10 - 39.43) ^a	37.25 (35.20 - 39.20) ^a	36.20 (33.30 - 38.15) ^a	35.40 (32.80 - 37.50) ^a

DO: day-old; RBC: red blood cell; PCV: packed cell volume; Hb: hemoglobin; WBC: white blood cell; TP: total protein; ALB: albumin; A/G: albumin/globulin; FBS: Fasting blood sugar; CRP: C-reactive protein; GLB: Globulin.

a-d Various superscript letters show significant difference between groups ($p < 0.05$).

Levels of U was significantly increased in the 1 DO atresic lambs compared to the healthy lambs ($p \leq 0.05$). Moreover, levels of U in the 1 DO atresic lambs was significantly higher than 3 DO group ($p \leq 0.05$) and surgical intervention significantly reduced levels of U in both affected groups ($p \leq 0.05$). Levels of fibrinogen significantly increased in both atresic lamb groups compared to the healthy lamb group and surgical intervention led to a significant decrease in the levels of fibrinogen ($p \leq 0.05$). Concentration of CRP significantly increased in the atresic lamb groups with a more increment in the 3 DO group and surgery significantly reduced CRP in both affected lambs ($p \leq 0.05$).

Oxidative stress indices. Results for oxidative stress biomarkers are shown in Figure 1. Levels of MDA in both atresic groups was significantly increased compared to the CT group ($p \leq 0.05$). Levels of MDA in the three days old lambs was significantly higher than the CT group ($p \leq 0.05$). Surgical intervention of the affected lambs notably reduced MDA levels ($p \leq 0.05$). Atresia ani significantly reduced the levels of SOD compared to the CT group ($p \leq 0.05$). Following surgical correction, SOD activity increased significantly in both affected groups ($p \leq 0.05$).

Discussion

Atresia ani is a relatively common congenital anomaly observed in various animal species, especially in newborn lambs. The etiology is thought to involve a combination of teratogenic factors, viral infections during pregnancy, and genetic predisposition.¹³ In newborns, anorectal atresia leads to meconium impaction, resulting in abdominal distention, colic, and an increased risk of intestinal rupture, which necessitates early surgical intervention.^{1,6} Because prolonged intestinal obstruction may promote systemic intoxication and inflammatory complications, alterations in hematological and biochemical indices are

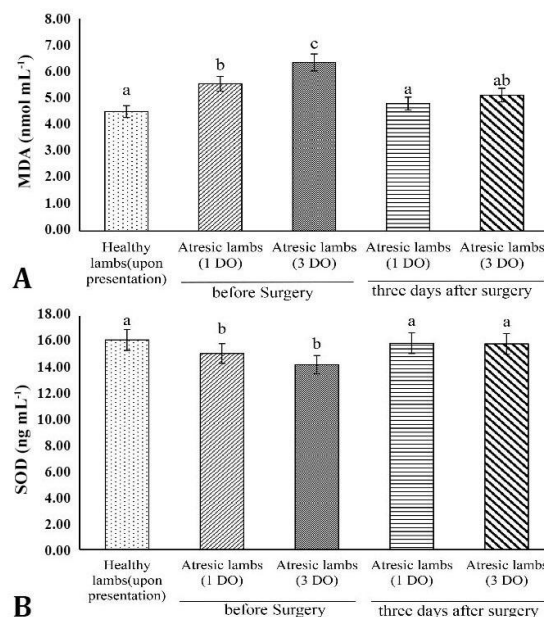


Fig. 1. Concentration of **A)** Malondialdehyde (MDA) and **B)** Superoxide dismutase (SOD) in different studied groups. DO: Day-old. abc Various letters indicate significant difference among different groups ($p < 0.05$).

expected. As highlighted in the results section, several hematological, biochemical, inflammatory, and oxidative stress-related markers (such as MDA and SOD) were significantly altered in atresic lambs compared to healthy CTs. Among the 45 lambs that underwent surgery, 3 (6.67%) required a second procedure due to closure of the newly created anal opening within 1 - 2 months. However, the majority of lambs did not experience serious complications and gained sufficient weight for slaughter after approximately three months.

In neonatal foals, prolonged meconium retention induces intestinal distention, elevated intra-abdominal pressure, and reduced mesenteric perfusion, culminating

in mucosal injury, bacterial translocation, and systemic inflammation.¹⁴ In lambs with congenital AA, continuous meconium retention due to anatomical obstruction creates a comparable ischemic and inflammatory environment. Reduced mesenteric blood flow predisposes the intestine to ischemia-reperfusion injury, which has been shown to drive excessive generation of reactive oxygen species (ROS) via mitochondrial dysfunction and xanthine oxidase activation in experimental and clinical models of intestinal obstruction and mesenteric ischemia.^{15,16} The ROS cause lipid peroxidation, protein oxidation, and nucleic acid damage, thereby impairing enterocyte integrity and further amplifying mucosal barrier disruption.^{17,18}

Concurrently, bacterial translocation across the compromised barrier activates pattern-recognition receptors such as Toll-like receptor-4,¹⁹ initiating nuclear factor kappa B-dependent transcription of pro-inflammatory mediators including tumor necrosis factor- α , interleukin-1 β , and interleukin-6.²⁰ These cytokines promote a further oxidative burst via neutrophil activation and exacerbate tissue injury, thereby establishing a self-perpetuating cycle of inflammation and oxidative stress.²¹ Recent investigations have underscored the central role of these mechanisms in neonatal gastrointestinal obstruction and ischemia models, reinforcing their relevance to AA-associated systemic pathology in lambs.

Our results demonstrated significant changes in hematological and biochemical indices in lambs with AA. Coskun et al. reported that hematological and biochemical parameters in calves with AA remain within normal ranges if the animals are referred for surgery within the first 48 hours and only leukocytosis and elevated ALB have been found that speculated resulted from relevant endotoxemia and bacterial proliferation.²² In agreement with the findings of Coskun et al., our results also showed a significant increase in the WBC count in the affected lambs.

Similarly, Salci *et al.*, reported leukocytosis and increased TP concentrations from calves with intestinal atresia, supporting our findings.²³ However, Bademkiran *et al.*, reported a gradual increase in ALB and TP concentrations one month postoperatively, which was not in line with our results.²⁴ These differences could be due to variations in species, the timing of post-operative measurements, or the specific type of the atresia (e.g., anorectal versus intestinal). Moreover, some authors have suggested that animals with small intestinal or colon atresia may not be suitable candidates for surgery due to the high risk of postoperative complications and the associated economic burden. In humans, similar biochemical changes have been noted in neonates with esophageal atresia, in whom elevated TP and other markers in the amniotic fluid are common.^{25,26}

Notably, a case report describing African buffaloes with AA and concurrent rectovaginal fistulae indicated that

hematological and biochemical parameters remained within normal ranges.²⁷ Our findings, however, contradict these findings, as we found significant biochemical alterations in lambs. This discrepancy may be due to differences in the timing of diagnosis and clinical presentation. In female animals with concurrent rectovaginal fistulae,²⁸ the defect may not be detected until later due to defecation through the vagina, potentially leading to less severe clinical changes. In contrast, male animals may present with more severe defects, such as rectourethral communication, which, may explain the more pronounced hematological and biochemical alterations observed in the lambs included in the present study.

There is limited research on oxidative stress in ruminants with congenital malformations, and to our knowledge, no studies have specifically addressed the effect of anorectal atresia on oxidative stress in lambs. However, studies in lambs,^{29,30} calves,³¹ and rodents,³² have shown increased oxidative stress, consistent with our findings of reduced antioxidant markers (SOD) and increased lipid peroxidation markers (MDA) in affected lambs. These results suggest that oxidative stress may play a key role in the pathophysiology of AA, contributing to the systemic alterations observed in these animals.

The role of oxidative stress in neonatal diseases is well-documented, with researchers identifying it as a potential risk factor for various malformations.^{33,34} Oxidative stress, which occurs as a consequence of inadequate antioxidant defenses during pregnancy, has been linked to congenital malformations in neonates. In human neonates with esophageal atresia, significantly higher MDA levels and lower SOD activity were reported compared to healthy neonates, which aligns with our findings in lambs with AA. This suggests that oxidative stress may be a common pathophysiological mechanism in the pathophysiology of congenital anorectal and esophageal atresia. Furthermore, the elevated MDA levels observed in our study may indicate insufficient antioxidant capacity, a condition that has been observed in human patients with biliary atresia, where oxidative stress markers were significantly elevated compared to healthy CTs.^{35,36}

Our study suggests that early surgical intervention may mitigate oxidative stress and systemic inflammatory responses. Lambs that underwent surgery within the first 24 hr exhibited significantly better hematological outcomes, such as lower WBC counts, U concentration, and CRP levels, compared to those that underwent surgery after 3 days. This could be due to the timely relief of meconium impaction and a reduction in oxidative stress, leading to improved overall health outcomes. Early intervention likely prevents progressive inflammation and oxidative damage, thereby promoting better recovery and reducing the risk of complications.

Furthermore, the increased SOD activity observed in lambs undergoing early surgery suggests that antioxidant

defense mechanisms may be more effective when surgery is performed promptly. This could help reduce ROS and mitigate tissue damage, leading to a better prognosis. Conversely, delayed surgical intervention may allow prolonged exposure to oxidative stress, exacerbating tissue damage and impairing the ability of immune system to mount an effective inflammatory response. Although early surgery has clear benefits, our study also identified complications, as three lambs required a second surgery due to closure of the surgically created anal opening. This highlights the importance of good surgical technique and careful post-operative to minimize the risk of postoperative complications. Early surgical intervention in neonatal lambs with AA is crucial for alleviating the mechanical obstruction and reducing systemic inflammation and oxidative stress. Significant changes in hematological, biochemical and oxidative stress markers highlight the role of oxidative stress in the pathophysiology of this condition. The results suggest that oxidative stress may exacerbate inflammation and tissue damage, which may contribute to increased morbidity and a poorer prognosis in untreated cases.

The present findings, which demonstrate significant elevations in oxidative stress markers and pro-inflammatory cytokines in lambs with AA, underscore the central role of ROS-mediated mucosal injury¹⁵ in the pathophysiology of this congenital disorder. These mechanistic insights highlight targeted antioxidant therapy as a promising adjunct to timely surgical correction, a concept supported by recent experimental studies demonstrating that agents such as N-acetylcysteine and vitamin E can attenuate oxidative burst and modulate inflammatory pathways in ischemic-reperfusion models.^{37,38}

This study has several limitations that should be taken into account when interpreting the findings. Firstly, the sample size of 45 male lambs is relatively small, which may affect the generalizability of the results to other species or female lambs. Moreover, the assessment of hematological, biochemical and oxidative stress markers was conducted at only two time points, which may not fully capture the dynamic changes of recovery over time. The follow-up period of 30 days post-operatively was also limited, and a longer observation period would likely provide more comprehensive insights into the long-term outcomes. Also, potential confounding factors, such as diet or genetic variations, were not accounted for, which could have influenced the results. The study also did not investigate molecular mechanisms, such as gene expression, which could have provided a more detailed understanding of the role of oxidative stress in AA. While some lambs experienced post-operative complications, the small number of cases limits the ability to draw definitive conclusions about factors associated with long-term surgical success. Despite these limitations, this study

provides important insights into the role of oxidative stress and the benefits of early surgical intervention for AA.

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Conflict of interest

The authors declare that there is no conflict of interest.

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