CLINICO-HEMATOLOGICAL EVALUATION OF INTRAOSSEOUS ADMINISTRATION OF BLOOD IN ANEMIC GOATS

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ABSTRACT: This study investigates the clinical viability and hematological assessment of intraosseous (IO) transfusion of whole blood in goats suffering from severe anemia. This is an alternative route in those animals where traditional and commonly used intravenous access is challenging or delayed due to hypovolemia, small body size, or other anatomical constraints. Intraosseous access is already established in humans, while it is still utilized in small animals. The present study was conducted on six anemic goats aged 2-6 months at the teaching hospital of Riphah College of Veterinary Sciences, Lahore. Whole blood was collected from healthy donor goats, and after compatibility testing via minor and major cross matching and administered through the proximal humerus using a Jamsheedi IO needle. Clinical and hematological parameters were recorded before and 24 hours post-infusion. There were statistically significant improvements observed in pack cell volume (PCV), hemoglobin concentration (Hb), and red blood cell (RBC) count, which indicates the successful restoration of oxygen-carrying capacity. However, notable changes were also observed in red cell indices, such as a mild decrease in mean corpuscular volume (MCV) and a significant increase in mean corpuscular hemoglobin concentration (MCHC), which shows the administration of normocytic normochromic donor cells. There was mild leukocytosis and slightly increased platelet counts, which reflect little immunologic response post transfusion. Clinically, heart rate, respiration rate, and capillary refill time (CRT) showed substantial improvement, and body temperature was also normalized post transfusion. In goats, intraosseous blood transfusion at 1–5 mL/min (20–100 drops/min) was slower than the IV rate (10–12 mL/min) but remained safe and effective without any complications. These findings support the hypothesis that IO transfusion provides a rapid, effective, and safe alternative to IV infusion, especially under field conditions or in emergency scenarios. This study lays the groundwork for further exploration into standardizing IO transfusion protocols in veterinary practice.

Key Words: Intraosseous administration, Blood transfusion. Hematology.

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INTRODUCTION

Blood transfusion is a preferred treatment for animals suffering from severe anemia. It is necessary to use whole blood in conditions like severe anemia, which occurs through acute hemorrhage via trauma, blood loss during surgery, blood parasitemia, red blood cell destruction through heavy metals, hypersensitivity reactions, and neonatal isoerythrolysis (Tocci, 2010). Blood transfusion is an ancient medical procedure that has become more popular over time, mostly as a result of significant technological advancements, including the use of anticoagulants during the collection and analysis of individual differences to select the donor. Hemotherapy is frequently used in veterinary medicine, and for it to be successful, consideration must be given to the donor's preference, search criteria for collection, processing, and storage of blood bags, and the setting of quality control parameters that show whether the blood is viable for use (Kumar, 2017).

Research across various animal species has demonstrated that storage duration can significantly alter blood gas levels, lipid peroxidation, antioxidant enzyme activity, as well as hematological and biochemical parameters. There are many factors that are considered before the transfusion of blood, such as pathogen transmission, adverse transfusion reactions, expenditures, and difficulties in transfusion. Certain indicators, such as oxygen saturation in blood, packed cell volume, and hemoglobin concentration, are analyzed before coming to the conclusion of blood transfusion (Luethy *et al.*, 2017). There are a few diseases that can potentially be transmitted from donor to recipient, such as heartworm disease, babesiosis, brucellosis, and anthrax, which can be fatal for the recipient. Blood cross-matching is another major issue for compatibility in transfusion, as five blood

groups are recognized in goats, which are quite similar to the RBC antigens of sheep (Patel, 2014). During blood transfusions in **veterinary medicine**, zoonotic diseases that can be transmitted through blood are an important concern. Pathogens like *Anaplasma* spp., *Babesia* spp., *Brucella* spp., *Ehrlichia* spp., *Leishmania* spp., and *Trypanosoma* spp may be present in the blood of healthy animal. These infections may be prevalent in many areas and oftenly remain subclinical, increasing the challenges of transfusion-transmitted diseases. Consequently, strict donor screening, proper diagnostic testing, and vector control are crucial to decrease the risk of zoonotic diseases transmission.

IV administration of fluid is very important in transfusion in emergency cases. However, IV administration is severely compromised in serious patients, such as patients with severe hypovolemic shock, as well as in infants, in which IV continuous access is difficult to achieve. (Jackson *et al.*, 2011). When the IV route is unavailable, the IO blood transfusion method can be preferred and lifesaving for a patient. The common locations for IO administration are the humerus (proximal site), tibia, wing of the ischium, iliac crest, and femur (Mader *et al.,* 2010*)*.

Although different researchers studied the effects of different drugs through the route of IO, there are not many studies established on the use of this route for blood transfusion (Borron *et al.*, 2009; Cameron *et al.*, 1989; Okrasinski *et al.*, 1992). In their study, Jackson *et al.* (2011) used an IO catheter device for the first time in goats to see the major side effects of long-term usage of an IO catheter on bone tissue damage and observed no damaging effects on bone tissue.

By placing a needle straight into a bone's medullary cavity, the IO route enables fluids and blood components to pass through the venous sinusoids of the bone and into the central circulation. This approach has a long history in human health and is becoming more popular in veterinary care, particularly for small ruminants and neonates, where venous access is technically difficult. The clinical safety and hematological effects of intraosseous whole blood administration in goats are still poorly understood, despite its increasing use (Lee *et al.*, 2021).

Goats are employed extensively in veterinary education, agriculture, and research. Intra-Venous access is complicated due to their distinct physiology & behavior. So, it becomes critical to explore other substitutes, for example, intraosseous infusion, for both typical transfusions and resuscitation. Blood profiling and clinical examination are noteworthy to figure out procedure interpretations in significant bone injury, hemolysis, infection, or physiological stress (Ermilio and Smith, 2011). The feasibility of Intraosseous blood transfusion in general and critical treatments can be evaluated by estimating clinic-hematological parameters after the procedure. The safety and efficiency of the intraosseous approach can be interpreted from blood parameters like Complete Blood Profile, heart rate, respiration rate, body temperature, and indications of localized issues like infection & inflammation (Borron *et al.*, 2009).

Vascular administration of infusions and medicines is an important component of critical care. Nevertheless, attaining intravenous access proves to be tough in emaciated animals in a situation of severe hypovolemia. IV access can be technically tough & tedious for novice clinicians, still making it tough to attain access. Even though some drugs can be given via the endotracheal route, but has constraints regarding suitable medication and uncertainty of their bioavailability, absorption, and distribution rates (Orlowski *et al.*, 1990).

In human emergency medicine, intraosseous catheterization has got more importance. Previously employed only in pediatrics, its applications in emergency care or adults, and military ops have sparked curiosity. The EZIO® (Vidacare, San Antonio, TX) is one of the various IO catheterization devices that have been established. This powerful device can be placed at various anatomic landmarks regardless of the use of local anesthetics, allowing for control and little tissue trauma. The FDA has approved the EZ-IO® for 24-hour use in the human tibia and humerus with availability in various sizes (Ong *et al.*, 2009; Leidel *et al.*, 2009).

These needles aid in rapid insertion; multiple studies have shown their efficiency and can be accurately placed regardless of the operator's experience level (Leidel *et al.*, 2009).

Lately, the EZ-IO® device has been made available for use in veterinary medicine. As of now, its use is limited to companion animals. We believe that this automated drill system could also offer significant advantages in large animal species of all ages, especially in situations where manual placement of intraosseous catheters is difficult and time-consuming. Thus, our research aimed to study extended term safety and productivity of this device to comprehend its potential applications in all species (Mader *et al.*, 2010).

Analysis of both homologous and autologous blood transfusions in bovines has shown that specific physiological changes can happen in response to transfusion. Particularly, blood bags used in veterinary medicine are actually the same as those used in humans, but the citrate phosphate dextrose adenine-1 anticoagulant bags are normally favored for use in goats because of their compatibility and efficacy. (Tavares *et al.*, 2019).

In case vascular access is hard or unrealistic, IO cannulation acts as a substitute for the quick administration of fluids and medicines, specifically in emergencies. In small ruminants like goats, establishing

Intravenous access can be particularly difficult because of their size, stress responses, and environmental conditions. The IO route for giving drugs directly into the bone marrow provides a feasible solution, as the medullary cavity contains a rich vascular bed. Although it has been applied well in human and lab animal models, its clinical and hematological applications are not well understood. Thus, assessment of hematological changes, clinical tolerance, and potential complications following IO administration is critical to determine its safety and efficacy in veterinary practice. In this study, we monitored the efficacy of IO blood transfusion as an emergency alternative to the vascular route in animals with anemia and hypovolemia.

In conclusion, even if the proximal tibia is a generally preferred site for IO access due to its ease, the proximal humerus is greatly predominant in terms of infusion rates, proving to be a precious substitute in emergencies requiring quick fluid infusions. These results showcase the space for further research in human patients and emphasize the need of advancing clinical guidelines for intraosseous site selection dependent on specific emergencies. (Tan *et al.*, 2012).

MATERIAL AND METHODS

Study Area: This study was performed in the teaching hospital of Riphah College of Veterinary Sciences, Lahore.

Animals: Six goats (mixed breed), aged 2-6 months, suffering from anemia, assessed by hematological evaluation, were used in our study. Hematological parameters include PCV, RBC count, and Hb concentration, as well as RBC indices was measured for inclusion in our study. Clinical parameters like heart rate, pulse rate, respiration rate, and capillary refill time were measured before collection and infusion in both the recipient and the donor.

The donors were selected from healthy adult goats present at RCVets farm.

Blood collection from healthy donor goats: The amount of blood volume for infusion was calculated by:

$$\text{Total volume (ml)} = \frac{\text{Desired PCV} - \text{Actual PCV}}{\text{Donor PCV}} * \text{BW (kg)} \times 0.076$$

A healthy donor animal from the RCVetS farm was selected for blood collection after minor cross-matching. In minor cross-matching, donor plasma was separated by centrifugation at 1500 rpm for 10 minutes. Recipient blood was centrifuged at 2000 rpm for 10 minutes, and the bottom layer containing RBCs was separated. Mixed 0.02 ml of recipient RBCs with 0.98 ml of normal saline. Then, one drop of donor plasma and one drop of recipient RBC suspension were mixed on a clean glass slide. The slide was incubated at 37°C for 15 minutes and observed under a microscope for agglutination. Absence of any visible reaction was considered a compatible match, and the donor was approved for collection and transfusion to a match. The goat was placed in lateral recumbency, and the jugular groove was shaved for jugular puncture. The amount of blood was calculated by the aforementioned formula and drawn in 10 minutes directly into a blood collection bag, which contains citrate phosphate dextrose and adenine solution, and kept at 4 °C (Balcomb and Foster, 2014).

Intraosseous transfusion: For the IO method, the proximal part of the humerus was shaved and disinfected. The animal was in right lateral recumbency, limb will be palpated for administration. The landmarks for the proximal humerus were greater and lesser tubercles, deltoid tuberosity, and bicipital groove (Popesko *et al.*, 1992). The IO access was gained by administering the Jamsheedi IO needle. Blood was infused in the humerus with a flow rate of 1-2 ml/kg/hour and then increased to 5-10ml/kg/hour.

Post-Transfusion Monitoring: Complete blood count parameters, including packed cell volume, hemoglobin concentration, red blood cell count, and other hematological indices, as well as clinical parameters like heart rate, pulse rate, and respiration rate was measured after 24 hours of blood transfusion (Najarnezhad *et al*., 2016).

Statistical Analysis: The statistical analysis was performed on Statistical Package for Social Sciences (SPSS) version 20. The paired sample t-test was applied for comparison of hematological as well as clinical parameters before and after reinfusion of blood. $p<0.05$ was considered significant.

RESULTS

Hematological Evaluation: The findings of our study indicate an improvement in most hematological parameters before and after 24 hours of transfusion. There was significant improvement in packed cell volume, hemoglobin concentration, and red blood cell count after 24 hours of post transfusion. PCV increased 14.92% to 23.98%, which indicates a major restoration of circulating RBCs, which is also confirmed by an increase in hemoglobin concentration that increased significantly. Total leukocyte counts and platelet levels also increased after 24 hours of transfusion. These mild increases are likely physiological responses to transfusion stress or mild immune system activation.

Clinical Evaluation: The heart rate showed a statistically significant reduction after 24 hours of blood transfusion. A similar trend was noticed in the respiration rate, which

also decreased significantly after 24 hours of blood transfusion. CRT improved from 3 seconds to 1.72 seconds, which indicates a major improvement in circulatory volume in the body. However, the rectal temperature increased significantly from 100.73 ^{o}F to 101 ^{o}F, suggesting normalization of body temperature to the normal physiological range. Overall, the clinical parameters show a rapid and significant improvement in systemic stability.

Table 4.1: Hematological evaluation in recipient goats before and after blood transfusion

Parameter	Mean Before	Mean After (24h)	Mean Difference	p-value	Interpretation
PCV (%)	14.92	23.98	+9.07	0.00	Significant ↑
Hb (g/dL)	5.20	8.92	+3.72	0.00	Significant ↑
RBCs ($\times10^{12}$/L)	5.18	8.78	+3.60	0.00	Significant ↑
MCV (fL)	29.45	27.98	-1.47	0.04	Significant ↓
MCH (pg)	10.06	10.16	+0.10	0.58	Non-significant
MCHC (g/dL)	33.77	36.42	+2.65	0.00	Significant ↑
WBC ($\times10^{9}$/L)	8.35	9.02	+0.67	0.00	Significant ↑
Platelet ($\times10^{9}$/L)	280.5	291.0	+10.5	0.00	Significant ↑

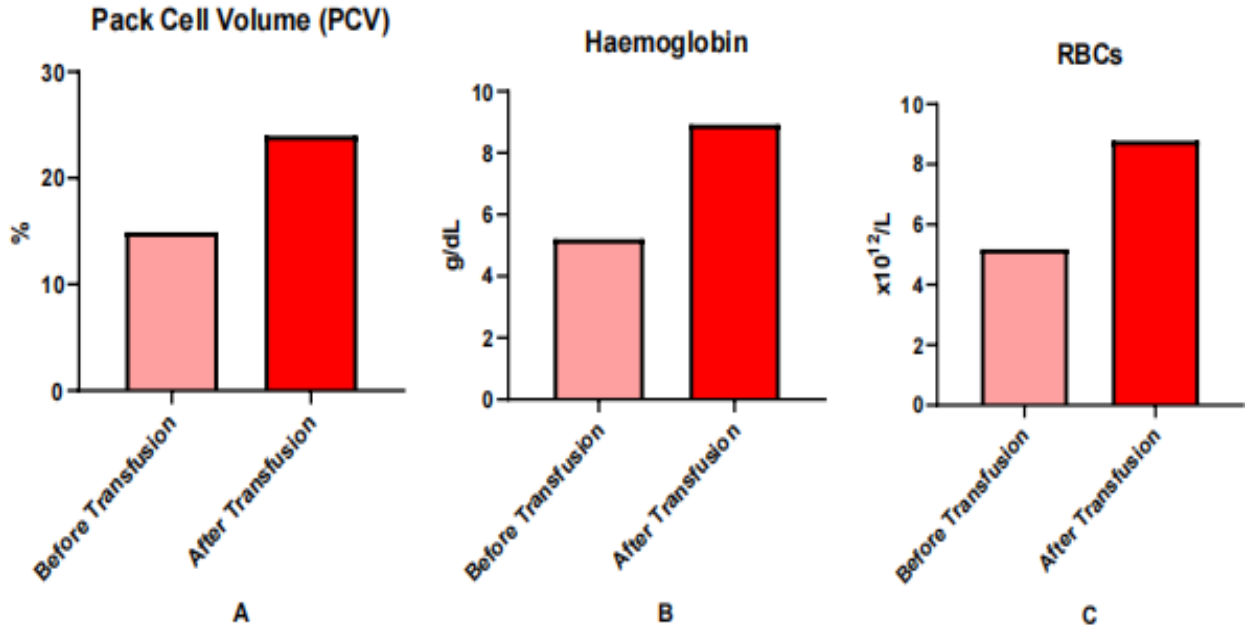

Figure 1. Comparison of key hematological parameters before and after introasseous blood transfusion in anemic goats (A) \pack Cell Volume (PCV), (B) Hemoglobin concengration (Hb), (C) Red Blood Cell (RBC).

Table 4.2: Clinical parameters evaluation in recipient goats before and after blood transfusion

Parameter	Before Transfusion (Mean ± SD)	After Transfusion (Mean ± SD)	Mean Difference	p-value	Interpretation
Heart Rate (bpm)	136.0 ± 5.10	98.17 ± 5.08	37.83	0.00	Significant decrease
Respiration Rate (bpm)	52.67 ± 2.94	38.67 ± 2.16	14.00	0.00	Significant decrease
Capillary Refill Time (sec)	3.03 ± 0.33	1.72 ± 0.25	1.32	0.00	Significant improvement
Temperature (^{o}F)	100.73 ± 0.10	101.75 ± 0.13	-1.01	0.00	Significant improvement

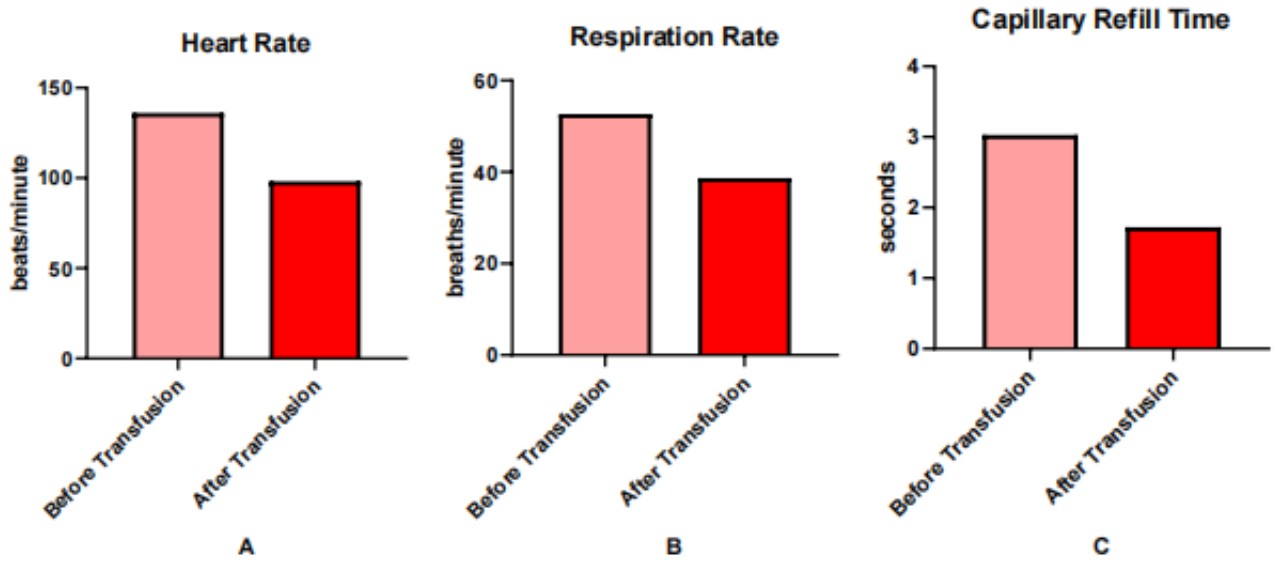

Figure 2. Comparson of key clinical parameters before and after intraosseous blood transfusion in anemic goats (A) Heart Rate (HR), (B) Respiration Rate (RR), (C) Campillary Refill Time (CRT).

DISCUSSION

The present study evaluated the clinical and hematological effects of intraosseous blood transfusion in anemia-suffering goats. This study is unique because of its choice of species, which often has challenges during traditional intravenous access due to its small and very difficult to locate peripheral veins, particularly in the conditions of hypovolemia and in neonates. The present investigation in this study reinforces the hypothesis that IO blood transfusion is clinically viable and an effective alternative method to IV access, which offers rapid restoration of physiological and hematological homeostasis in emergency conditions.

The result shows statistically significant improvements in key red cell parameters following IO, that includes increase in packed cell volume, hemoglobin concentration, and RBC count. These outcomes are very important because they directly reflect an increase in oxygen-carrying capacity, which is very critical to reverse the adverse effects of anemia on the body (SHAH *et al.*, 1982). The average PCV increases from 14.92% to 23.98%, Hb rises from 5.20 to 8.92 g/dl, and RBC increases from 5.18 to 8.78 $\times 10^6/\mu L$ all of this aligns with previous studies (Hunt and Wood, 1999; Nazifi and MASHHADI, 2005; Becht, 2009). The rapid rise in PCV and Hb enhances the physiological effectiveness of blood administered through the medullary cavity, which ultimately validates the IO route as an alternative route for whole blood transfusion in anemic goats. PCV increases shows rise of red blood cells, which aligns with an increase in hemoglobin concentration.

However, there was a notable statistically significant reduction in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). MCV is a measure to find the average volume or size of a single red blood cell. It is one of the critical key red blood cell indices used to detect different types of anemia. The recipient goats show a decrease in MCV values, which might be due to a compensatory response in goats; it might result in reticulocytosis or larger immature cells. Transfusing mature donor RBCs results in a slight reduction in average MCV due to dilution of larger native RBCs. MCH tells about the average hemoglobin content per red blood cell, and it did not significantly alter after intraosseous transfusion. This may be due proportional increase in both the hemoglobin and RBC count, which maintained the constant MCH values before and after intraosseous transfusion. The donor red blood cells were normochromic (normal amount of hemoglobin), and our recipient goats were normochromic or might have mildly hypochromic anemia. Therefore, transfusion did not introduce a major shift in hemoglobin content per cell. This finding ultimately indicates that while the total oxygen carrying capacity was improved due to the number of RBCs and hemoglobin, the average hemoglobin load per cell remained stable, which suggest and effective and balanced integration of donor red cells into the recipient's circulation. The significant increase in MCHC observed after 24 hours of blood transfusion likely shows the addition of normochromic donor red blood cells into a population of slightly hypochromic recipient cells. This resulted in an overall higher hemoglobin concentration per unit volume of red cells. The hemoglobin raised slight better relative to red blood cells, which further contributed to the elevation in MCHC.

In our study, there was a significant increase in white blood cell count 24 hours after the intraosseous

transfer of whole blood from a healthy donor into anemic recipient goats. This leukocytosis reflects a mild immune response following homologous transfusion, even though minor cross-matching was performed to reduce the chances of incompatibility. A study by Najarnezhad, Mohammadi, and Velayati (2016) also noted a rise in WBCs after 24 hours, even using autologous blood transfusion, which suggests that even well-matched transfusions can stimulate a mild inflammatory response. This pattern also aligns with a study by Maier (2000) that transfusion can stimulate inflammatory reactions, particularly in the early phase of transfusion. The study by Najarnezhad *et. al.* 2016) shows WBC levels drop to normal after 96 hours in recipient goats.

Platelet count is significantly improved following intraosseous blood transfusion. The elevation in levels of platelets can be mainly due to transfusion of whole blood, which contains platelets that ultimately increase the recipient platelet circulatory pool.

From a clinical point of view, the main evidence supporting the IO transfusion is derived from significant improvement in vital parameters like heart rate, temperature, and respiration rate. The heart rate dropped from an average of 136 bpm to 98.17 bpm, and the respiration rate from 52.67 to 38.67 breaths per minute. However, there was an insignificant rise in heart rate in donor goats after blood donation. It may be due to sympathetic effects. In human beings and animals, hypovolemia enhances the sympathetic responses, which ultimately stimulate beta receptors on the heart and causes increase in heart rate as well as cardiac output (Yokusoglu *et al.*, 2007). But after blood infusion in recipient goats, it shows that blood replenishment decreased sympathetic drive and returned toward homeostasis following improved oxygen delivery. The capillary refill time (CRT) improved significantly, decreasing from 3.03 to 1.72 seconds. CRT is a critical indicator of peripheral perfusion as well as of cardiovascular circulatory efficiency(Constable *et al.*, 2017).

A rapid CRT improvement after transfusion indicated that transfused blood not only increased systemic volume but also actively reached distal capillary beds. This is a major positive outcome as it shows both central and peripheral circulation restored successfully. Body temperature increased significantly from 100.73 ^{o}F to 101.75 ^{o}F, which is consistent with a previous study conducted on pigs, which shows severe hypothermia in anemic pigs (Heinius *et al.*, 2002). The temperature rise is due to increased metabolic activity and systemic stabilization.

During intraosseous (IO) blood transfusion, the infusion was initiated slowly at a rate of 1–2 mL/min (approximately 20–40 drops/min) to ensure proper flow establishment and prevent excessive pressure at the site. Once the flow was confirmed to be smooth and well-tolerated, the rate was gradually increased to 4–5 mL/min (about 80–100 drops/min).

In comparison, the standard intravenous (IV) infusion rate for whole blood in goats under gravity is approximately 10–12 mL/min. Thus, the IO infusion rate was about 36% lower than the IV rate but remained sufficient to complete the transfusion within the experimental period.

No flow obstruction, extravasation, or leakage was observed in any of the animals during or after the infusion, indicating that the IO route provided an effective and reliable means of administering whole blood.

Conclusion: The findings of this study will help veterinary practitioners, especially working in the field or in emergency conditions and offer a new protocol for addressing acute anemia. IO transfusion is not just a rescue therapy but can be used with full confidence when IV access is not available.

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