OBSTETRICS AND GYNECOLOGY

Original Research

Umbilical cord blood collection through elective cesarean section in an ovine model – a pilot study

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Abstract

Background. Despite advancements in congenital heart surgery, long-term outcomes remain challenging, with many patients developing heart failure and requiring transplants at a young age. Stem cell therapy, particularly using umbilical cord-derived mesenchymal stem cells, is emerging as a promising adjunct treatment. Adult studies suggest functional improvements, but pediatric research remains sparse. This pilot study aimed to establish surgical and anesthetic protocols for elective cesarean section in an ovine model to enable umbilical cord blood collection.

Methods. Three pregnant sheep aged 2–6 years and weighing 40–45 kg underwent elective cesarean section. Gestational age was confirmed using ultrasound and clinical signs. Preoperative preparation involved fasting, dexamethasone administration to prevent respiratory distress, and shaving surgical sites. Local anesthesia and mild sedation (medetomidine) minimized fetal exposure to anesthetics. Two umbilical cord blood collection methods were tested: active aspiration and gravitational collection.

Results. We established efficient anesthetic and surgical protocols for elective cesarean section and described a safe and easy method for umbilical cord blood collection with minimal risk. Elective cesarean section ensures a sterile environment, crucial for stem cell isolation. Local anesthesia with mild sedation proved safe and provided proper comfort for animals and operators. Accurate gestational estimation and effective postnatal care were critical.

Conclusions. This study provides a reliable protocol for elective cesarean section and umbilical cord blood collection in an ovine model, offering a valuable foundation for research in stem cell therapy. However, larger sample sizes are needed to validate these methods and enhance their applicability in translational research.

Keywords: cesarean section, umbilical cord blood collection, ovine model, stem cell

DOI: 10.15386/mpr-2843

Manuscript received: 17.11.2024
Accepted: 19.12.2024

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Background

Congenital heart disease (CHD) is the most common type of congenital malformation. It is characterized by abnormal anatomical development of the heart *in utero* and varies from simple defects to more complex and critical ones, such as transposition of great arteries and hypoplastic left heart syndrome [1]. Despite advances in congenital heart surgery, surgical management remains problematic regarding the long-term outcomes of these patients, with many developing heart failures requiring heart transplantation at a young age [2]. This has prompted the exploration of complementary treatment approaches for patients with CHD. Stem cell therapy has emerged as a promising adjuvant treatment for cardiovascular diseases. While initial studies primarily focused on ischemic adult cardiac disease and proved efficient, recent research has shifted towards CHD, although with less robust evidence [2].

Although multiple stem cell

sources are available, umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) have several advantages. First, they are easily accessible. Second, they can be easily translated into clinical practice, given the existing experience with umbilical cord blood (UCB) stem cell banks and modern *in utero* diagnosis of CHD, which allows for proper preparation for delivery, UCB harvesting, and postnatal care in an experienced center. Third, the age of MSCs matters; "young" MSCs are more plastic and superior to those harvested from adult patients, as they can undergo a significantly higher number of cell divisions before reaching senescence. Moreover, they have a greater ability to expand in culture due to their naive status [3], and they can differentiate into endothelial cells and cardiomyocyte-like cells [2]. Finally, UCB-MSCs possess properties that can target the molecular maladaptation that occurs during right ventricle dysfunction [4].

The hypothesis that stems cell therapy improves ventricular function has led to several experimental and clinical studies in the adult population. However, the available literature regarding the pediatric population is limited [2], and more research is needed to offer a solid foundation for this therapy to become a standard practice and to address the remaining questions. The first step towards achieving this is to chisel the animal model and provide a detailed description of the UCB collection technique, along with a description of the potential obstacles and complications that may arise during this process.

While ovine UCB collection has been described in several studies that used UCB-MSCs for various purposes [5-9], detailed protocols for anesthetic and surgical approaches in elective C-section and UCB collection and thorough descriptions of perioperative challenges and complications are not available. Therefore, this study is the first to offer such a detailed description with an emphasis on perioperative challenges to enhance animal survival, which is critical in the context of autologous stem cell therapy research.

This study aimed to describe the surgical and anesthetic protocol for UCB collection through elective cesarean section (C-section) in an ovine model and to highlight the challenges encountered during the perioperative care and postoperative complications.

Methods

Three pregnant ewes of the "Romanian Tsigaie" breed, aged 2-6 years and weighing 40-45 kg, were purchased from local farmers. Gestation was confirmed by ultrasound examination, and gestational age was estimated based on clinical parameters, such as abdominal shape and size, udder development, colostrum production, and behavioral changes, as well as by ultrasonography.

Elective C-sections were scheduled when significant udder enlargement, colostrum production, vulvar swelling, and typical behavioral changes (such as separation from the flock, seeking an isolated spot, and lying down) occurred, as these changes typically precede lambing.

The animals were initially housed in an outdoor shelter at our experimental facility. One day prior to surgery, they were moved to an indoor shelter, where individual recovery boxes were available for both preoperative preparation and postoperative care. For the first five days after surgery, the operated animal and its lamb were kept in the indoor shelter, separated from the main flock. This indoor facility featured both natural and artificial lighting, along with heating and ventilation systems to maintain optimal conditions. Air circulation, dust, and gas levels were controlled within safe parameters for the animals. Temperature was kept at approximately 25° C, and relative humidity at around 60%, creating a stable environment to support postoperative recovery. After five days, the animals were returned to the outdoor shelter with the rest of the group.

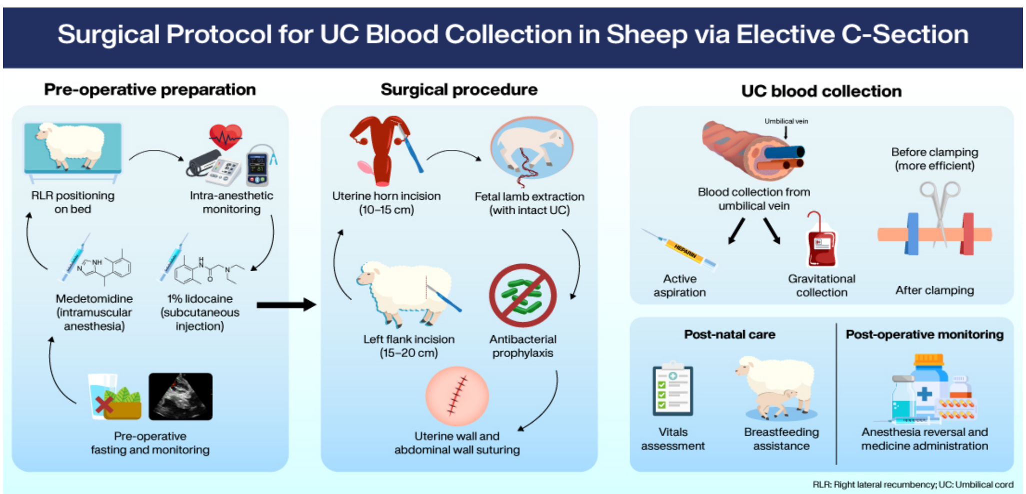

Figure 1. Study methodology.

Study methodology

A graphical abstract presenting the experimental plan is shown in figure 1.

Ethical statement

All procedures and perioperative care of the experimental animals were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" and Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The research was approved by Mures Sanitary-Veterinary and Food Safety Department with approval no. 54/31.08.2022. Ethical approval for this study was obtained from The Scientific Research Ethics Committee of the "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Targu Mures, approval no. 1451/22.07.2021.

This experimental research followed the 3R principle (replacement, reduction, refinement) to the greatest extent possible to enhance animal welfare. The number of animals used was minimized (reduction), and appropriate analgesia was provided during surgery and throughout the postoperative period to ensure minimal suffering (refinement).

Preoperative preparation

One day before surgery, the animals were relocated to an indoor shelter, where their abdominal and forearm areas were shaved and cleaned; the animals were also weighed. Clinical examinations, including heart and lung auscultations, were conducted to rule out any coexisting conditions. Clinical signs of gestational age were reassessed, and the quantity and quality of colostrum production were evaluated. Food and water were withheld for 18–24 hours and 6–8 hours before surgery, respectively. To prevent severe respiratory distress syndrome in the lamb, four doses (6 mg each) of intramuscular dexamethasone were administered 12 hours apart before surgery.

On the day of surgery, preoperative fetal echocardiography was performed to assess fetal heart rate and to confirm gestational age (Figure 2).

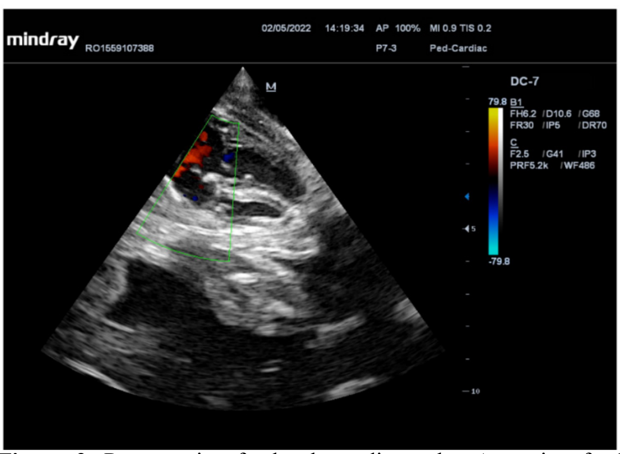

Figure 2. Preoperative fetal echocardiography. Assessing fetal heart rate on the day of surgery.

Anesthetic protocol

To prevent the potential side effects of general anesthesia on the lamb, the anesthetic protocol included sedation with a presynaptic α2 agonist (medetomidine) and local infiltration anesthesia (utilizing the line block technique) with 1% lidocaine.

1. Sedation

30 minutes before surgery, the animals received 0.015 mg/kg body weight of intramuscular medetomidine to facilitate transportation and positioning on the operating table. After achieving a mild sedative effect, the animals were positioned on the operating table in right lateral recumbency and secured with straps.

2. Intra-anesthetic monitoring and vascular access

Intra-anesthetic monitoring consisted of three-lead electrocardiography, non-invasive blood pressure (BP) monitoring using a BP cuff placed on the right forearm, and peripheral oxygen saturation (SpO_2) monitoring using an auricular pulse oximeter.

Vascular access was established by inserting a peripheral venous catheter (18 G) into the cephalic vein of the left forearm (Figure 3A).

3. Anesthesia maintenance

Sedation was maintained by repeated administration of intramuscular medetomidine at 2-hour intervals. Lidocaine (1%) was given to the surgeons for infiltration anesthesia before incision as required, ensuring that the maximum dose of 6 mg/kg was not exceeded.

Intermittent oxygen was administered through a facial mask at a flow rate of 2-8 L/min to maintain the target SpO_2 level above 92%.

Surgical protocol

1. Caesarean section

Local infiltration of 1% lidocaine along the incision line (line block technique) was performed 5–10 minutes before the incision, using a maximum dose of 6 mg/kg (Figure 3B). A 15–20 cm long incision was performed on the left flank (Figure 3C). Following the dissection of the subcutaneous and muscle tissues, the peritoneal cavity was opened, and the uterus was exposed and manually explored while the fetus was palpated. A 10–15 cm long incision was carefully performed on the uterine horn (Figure 3D), and the lamb was exposed (Figure 3E) and extracted from the uterine cavity, with the umbilical cord (UC) remaining intact (Figure 3F).

Following visual and manual exploration of the UC, blood was collected. Two clamps were then placed on the UC, which was subsequently sectioned between the clamps, followed by vascular ligature. The lamb was transferred to a separate team for immediate postnatal care.

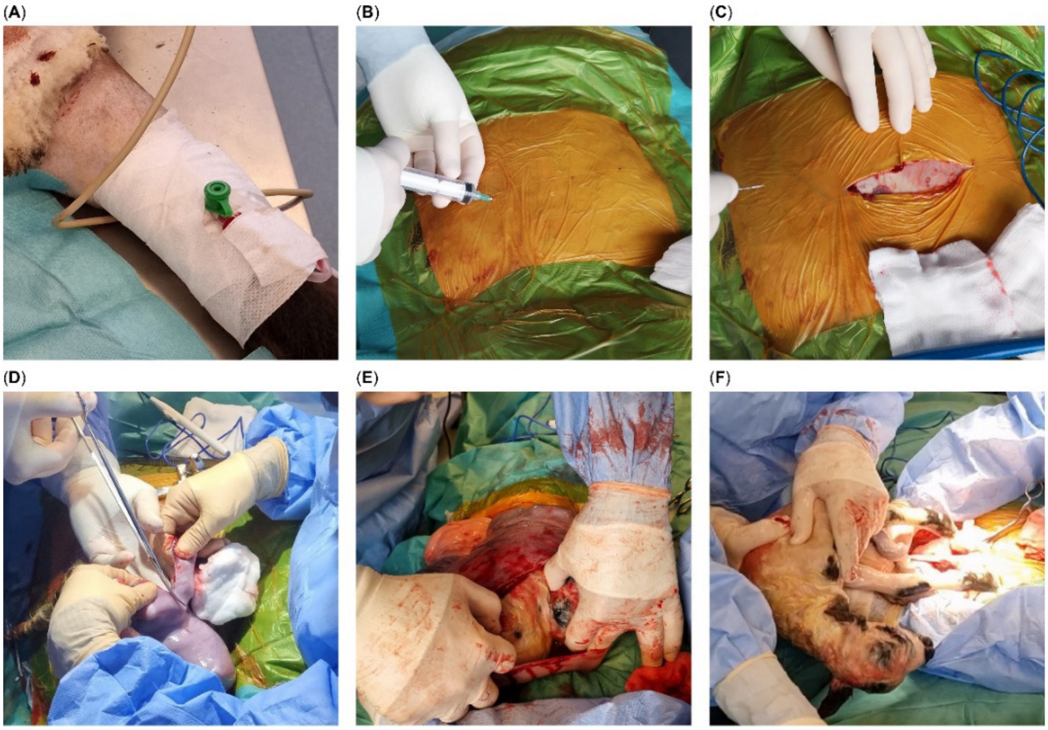

Figure 3. Surgical protocol. (**A**) – Vascular access achieved by inserting an 18 G peripheral venous catheter into the left cephalic vein. (**B**) – Local anesthesia – line block using 1% lidocaine. (**C**) – Skin incision on the left flank. (**D**) – An incision performed on the uterine horn. (**E**) – Exposing the lamb through the uterine incision. (**F**) – Extracting the lamb from the uterine cavity with an intact umbilical cord.

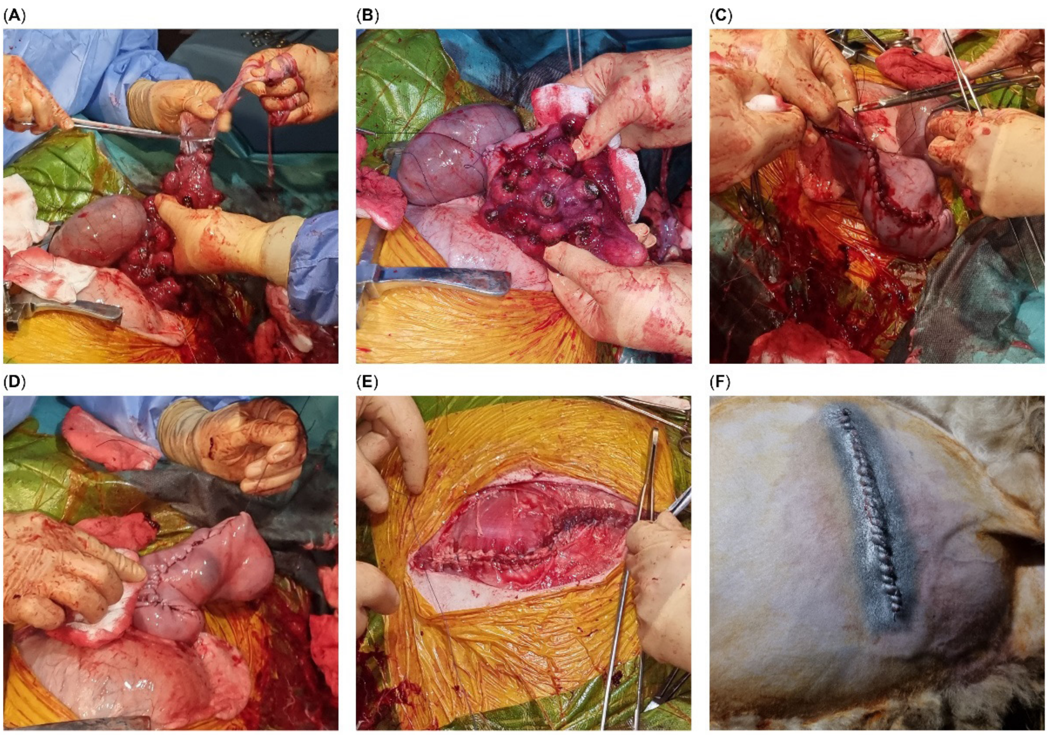

Figure 4. Surgical protocol. (**A**) – Trimming of fetal membranes that separated easily. (**B**) – Uterine caruncles that should not be incised. (**C**) – The first layer of uterine suture – simple continuous suture. (**D**) – The second layer of uterine suture – continuous inverting Cushing's suture. (**E**) – Abdominal wall closure. (**F**) – Skin closure and silver antiseptic spray application.

The uterine cavity was carefully inspected for the presence of other lambs and potential bleeding sources. Fetal membranes that separated easily were trimmed (Figure 4A), whereas the remaining membranes were left in situ, as the sheep would naturally eliminate them during the postoperative period. To prevent significant bleeding, care was taken not to incise the uterine caruncles (Figure 4B). The uterine wall was then closed using a double-layered suture with Catgut 3.0. The first layer consisted of a simple continuous suture (Figure 4C), while the second layer consisted of a continuous inverting Cushing's suture (Figure 4D).

Before complete closure of the uterine incision, 30 mg/kg of ceftriaxone diluted in 10 mL of normal saline was injected into the uterine cavity as an additional method of antibacterial prophylaxis. The abdominal wall was then closed with a double-layered continuous suture using a coated Vicryl (polyglactin 910) thread (Figure 4E), followed by skin closure and application of a silver antiseptic spray (Figure 4F).

Intraoperative antibacterial prophylaxis consisted of intravenous ceftriaxone (30 mg/kg) and metronidazole (10 mg/kg).

2. Umbilical cord blood collection

After the UC was exposed and explored and the umbilical vein was located, two methods of cord blood collection were performed. The first method consisted of active aspiration via venous puncture of 20 mL of cord blood through a 21 G needle into a 20 mL syringe filled with 2500 units of heparin (Figure 5A,B). Alternatively, cord blood was gravitationally collected via venous puncture through a 14 G needle into a special blood collection bag prefilled with 35 mL of citrate phosphate dextrose adenine (CPDA-1) (Figure 5C). The syringes or blood bags were then inverted several times to ensure proper homogenization of the blood and anticoagulant solution. The collected blood was then immediately transported to the lab at room temperature (15–25°C).

Due to the relatively small diameter of the umbilical vessels, blood aspiration after clamping and sectioning the cord was difficult, as the vessels collapsed quickly during active aspiration. Gravitational collection appeared more feasible; however, only a small quantity of blood could be collected using this method. Therefore, blood collection prior to cord clamping proved to be more efficient.

3. Intraoperative and immediate postnatal care of the lamb

After extraction from the uterine cavity, the lamb was thoroughly examined. Muscle tone, the presence of spontaneous respiratory movements, respiratory rate (RR), and heart rate (HR) were assessed. With the UC still intact, the lamb was gently rubbed on the back and actively aspirated in the oral and nasal cavities using a 10 Fr suction catheter (Figure 6A, B). Simultaneously, UCB was collected. After blood collection, the UC was clamped and sectioned, and the lamb was handed over to a separate team that gently stimulated the lamb, repeated aspiration of the oral and nasal cavities, and reassessed HR and RR. When the lamb exhibited sustained normal muscle tone, RR, and HR, it was covered with a warm blanket.

When the lamb exhibited signs of prematurity (very small size, underdeveloped subcutaneous tissue, scarce fur, and complete eyelid closure), hypotonia, bradycardia, or a combination of these, empirical oxygen therapy was administered through a facial mask. Additionally, in case of severe bradycardia (HR < 60/min), 0.3–0.5 mg of intramuscular adrenaline was administered.

To promote the development of maternal behavior and breastfeeding success during the immediate postpartum period, lambs were encouraged to suckle (Figure 6C).

Figure 5. Methods of UCB collection. (**A**), (**B**) – Active aspiration of UCB into a 20 ml syringe. (**C**) – Special blood collection bags used for gravitational UCB collection.

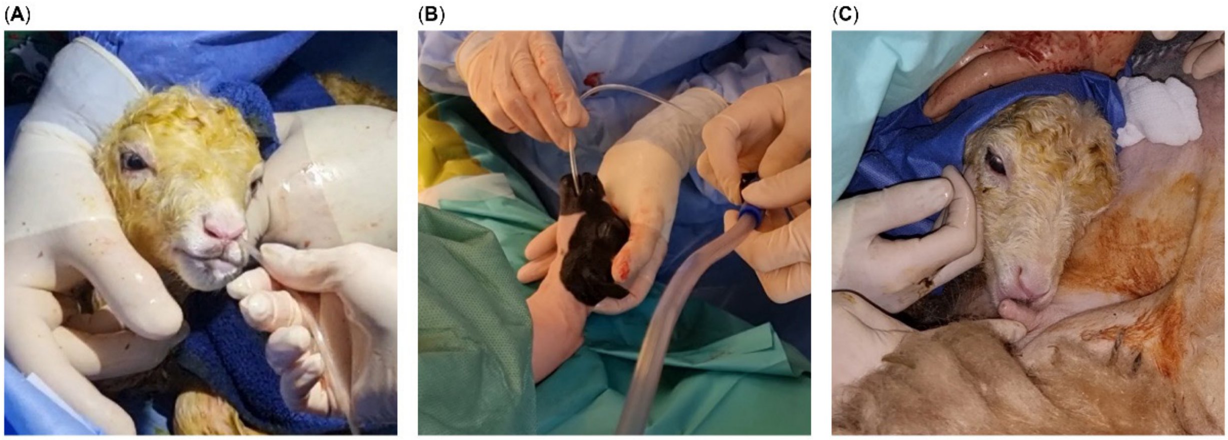

Figure 6. Immediate postnatal care of the lamb. (**A**), (**B**) – Active aspiration of the oral and nasal cavities. (**C**) – Encouraging the lamb to suckle.

Table I. Intraoperative and postoperative drugs, dosages, and routes of administration.

	Intraoperative dose	Route of administration	Postoperative dose	Route of administration
Ceftriaxone	30 mg/kg	IV/intrauterine	-	-
Metronidazole	10 mg/kg	IV	-	-
Metamizole	20 mg/kg	IV	20 mg/kg	IM
Metoclopramide	0.2 mg/kg	IV	0.2 mg/kg	IM
Oxytocin	0.05 IU/kg	IV	0.1 IU/kg	IM
Ceftiofur	-	-	1 mg/kg	IM
ANESTHESIA				
Medetomidine	0.015 mg/kg	IM	-	-
1% Lidocaine	Max. 6 mg/kg	SC infiltration	-	-
ANESTHESIA REVERSAL				
Atipamezole	5 times the medetomidine dose given	IM	-	-

IV: intravenous; IM: intramuscular; SC: subcutaneous.

Anesthesia reversal

Towards the end of surgery, intravenous metamizole was administered to ensure postoperative analgesia. Additionally, intravenous metoclopramide was administered to prevent postoperative nausea and to stimulate lactation. Moreover, intravenous oxytocin was administered as a uterotonic agent to reduce overall blood loss and stimulate lactation. The doses are listed in table I.

Intramuscular atipamezole was administered to reverse the sedative effect of medetomidine and ensure full awakening. The dose of atipamezole was five times that of medetomidine, with a medetomidine/atipamezole dose ratio of 1:5.

When the animal exhibited proper awakening, which typically occurred in approximately 10 minutes, and included signs such as alert state, eye opening, spontaneous movements, and head lifting, it was transferred to the postoperative indoor shelter. Over the following hours, the animal was continuously monitored by assessing alertness, rumination, respiratory pattern, and urine and fecal production, until it rose in orthostatism without unbalance. Post-surgery, water was provided after 2 hours, and food was gradually reintroduced after 4–6 hours.

Postoperative management

During the first five postoperative days, the operated animal and the lamb were housed in an indoor shelter, separated from the rest of the flock. The lamb was encouraged to suckle, and if breastfeeding supplementation was necessary, ovine colostrum replacement formula was used.

The postoperative treatment administered to the sheep during the first five postoperative days included intramuscular administration of metamizole for analgesia, metoclopramide and oxytocin for lactation stimulation, and ceftiofur, a third-generation cephalosporin for veterinary use, as antibacterial prophylaxis. The doses are listed in table I.

After 5 days, the animals were transferred to the outdoor shelter with the rest of the animals.

Results

Complications

Several perioperative complications ranging from mild to severe have been encountered in both sheep and lambs. The complications are presented in table II.

Sheep-related complications

1. Intraoperative desaturation

Significant desaturation was observed in two cases. About 30–40 minutes following medetomidine administration, a decrease in SpO_2 to 55–65% was observed. In one case, oxygen saturation increased easily with 2 L/min of oxygen administered via a facial mask, whereas the second case, which was associated with significant ruminal tympany, required oxygen therapy via a facial mask at a flow rate of 10 L/min, as well as a ruminal puncture to increase oxygen saturation.

2. Intraoperative ruminal tympany

Severe abdominal distension and ruminal tympany were observed in one case, 40–50 min after medetomidine administration. This was associated with significant desaturation (SpO_2 of 55–65%). A ruminal puncture was performed using a 14G peripheral venous catheter (Figure 7A). A significant amount of air was released, followed by a 10–15% increase in oxygen saturation.

Lamb-related complications

1. Neonatal death

In the present study, one neonatal death was recorded. The breeding date was known, and at approximately 130 days of gestation, the sheep presented typical clinical signs of advanced pregnancy and forthcoming labor, including udder enlargement, significant colostrum production, and typical behavioral changes. However, a sudden decrease in udder size and colostrum quantity was observed over the course of 5 days. Consequently, intrauterine distress was suspected, and fetal echocardiography revealed severe fetal bradycardia, leading to an emergency C-section. After lamb extraction and inspection of the UC, before UC puncture, a heavily spiraled cord was noted, and a hematoma measuring approximately 3 × 2 cm was observed near the placental insertion of the UC (Figure 7B). The newborn lamb, weighing 3.5 kg, exhibited generalized hypotonia, subtle sporadic movements, bradypnea, strained breathing, and severe bradycardia. Neonatal resuscitation was attempted with physical stimulation, oral and nasal aspiration, oxygen therapy, and intramuscular adrenaline administration, with no response to these maneuvers.

2. Maternal rejection

One case of maternal rejection was encountered. The lamb was placed near the ewe and encouraged to suckle as soon as the surgical procedure ended. However, the ewe did not exhibit maternal behavior during the first hours postpartum. In the following days, although tolerant and not aggressive towards her offspring, the ewe did not display maternal behavior, which led to a progressive decrease in milk supply.

3. Neonatal diarrhea

One case of neonatal diarrhea was encountered in our study. The lamb was colostrum-deprived due to the absence of maternal acceptance and low milk production. Consequently, the lamb was artificially fed with a colostrum replacer starting on the second day of life. The symptom onset occurred at 6 days of age, characterized by persistent green, watery diarrhea, mild anorexia, and weight loss. The diarrhea persisted for 6 weeks despite treatment, which included oral fluid and electrolyte therapy, oral intestinal adsorbent treatment with diosmectite, and probiotics. No antimicrobial treatment was administered. Artificial feeding consisted of an ovine colostrum replacer supplemented with rice starch. By the age of 2 weeks, the lamb began consuming solid food, and by 6 weeks of age, the diarrhea had resolved, and the lamb presented a good clinical status.

Table II. Perioperative complications, potential causes, and outcome.

Animal number	Resultant lamb	Sheep-related complications	Lamb-related Complication	Potential cause	Outcome
# 1	1	Intraoperative desaturation.	-	Medetomidine. Ruminal tympany.	Improved SpO_2 by O_2 therapy through facial mask and ruminal puncture.
		Intraoperative ruminal tympany.		Medetomidine. Prolonged lateral recumbency.	Associated desaturation. Improved SpO_2 by ruminal puncture.
# 2	1	Intraoperative desaturation.		Medetomidine.	Improved SpO_2 by O_2 therapy through facial mask.
			Maternal rejection.	C-section.	Inadequate milk supply for the lamb.
			Neonatal diarrhea.	Inadequate milk supply for the lamb.	Artificial feeding with colostrum formula. Improved clinical status by oral rehydration, probiotics, and intestinal adsorbent.
# 3	1	-	Neonatal death.	Umbilical cord accident.	Death of lamb.

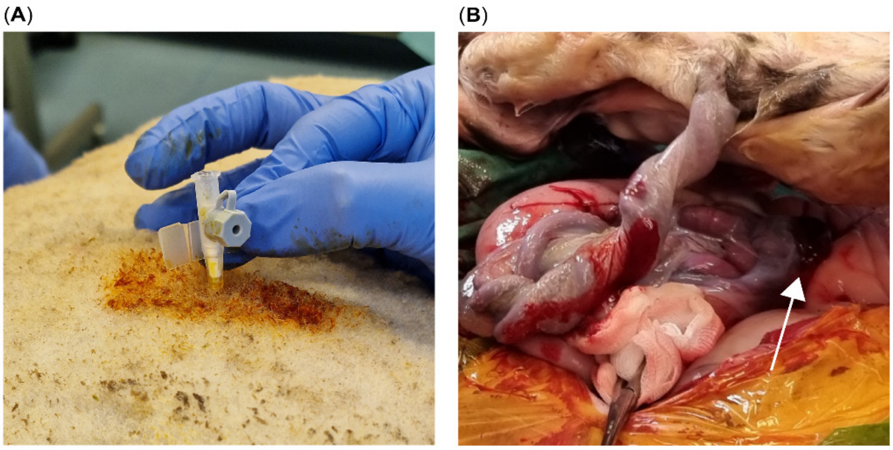

Figure 7. Postoperative complications. (**A**) – Ruminal puncture for intraoperative ruminal tympany in the sheep. (**B**) – Umbilical cord accident – spiraled umbilical cord and umbilical cord hematoma (arrow).

Discussion

Anesthesia for C-section

1. Locoregional anesthesia

Locoregional anesthesia is the primary anesthetic approach for C-sections in small ruminants. Current clinical guidelines on anesthesia and analgesia techniques for C-section in sheep include various methods of local and epidural anesthesia [10]. The technique of choice is typically the line block, although an inverted L-block or paravertebral block can also be used [10,11]. Sedation can be performed to facilitate the procedure [11] (pp. 299-300).

While both the line block and inverted L-block are quick and easy to perform, their main disadvantage is the lack of anesthesia to the peritoneum [10], which can be overcome by combining a systemic analgesic such as an opioid or an α2 agonist. Furthermore, the line block method can cause edema of the incision line, potentially delaying wound healing [11] (pp. 7-23). However, in our experience, this method has proven to be easy and efficient, with no wound complications. The paravertebral block is an effective technique; however, it requires experience, and care must be taken to avoid blocking the fourth lumbar nerve, which can result in postoperative hind limb ataxia, posing a risk for the newborn lamb [12].

Although local infiltration anesthesia is the primary approach for C-section in sheep [10,13], epidural anesthesia may be considered, especially in complicated cases that require extensive manipulation of the uterus [13].

2. Sedation

Sheep usually remain calm when restrained on the operating table. However, slight sedation might be used for additional restraint as well as to facilitate the procedure and increase the comfort of both the animal and operator [10,12]. Several classes of drugs can be used for sedation, including phenothiazines, benzodiazepines, α2 agonists, and opioids [14].

Since their introduction in veterinary use in the late 1960s, α2 agonists have become popular sedatives among veterinarians [15] and are considered among the strongest sedatives in veterinary practice [16]. These agents vary in α2:α1 selectivity as well as potency. While xylazine is the least selective (α2:α1 selectivity ratio of 160:1), medetomidine and dexmedetomidine possess the highest selectivity, with a ratio of 1620:1 [15,17]. Dexmedetomidine is the most potent α2 agonist and is twice as potent as medetomidine [18].

Xylazine is the most popular α2 agonist in current veterinary practice. However, ruminants are particularly sensitive to xylazine, which is 10–20 times more potent in ruminants than in other species [15,16].

While α2 agonists offer excellent sedation and analgesia, given their hemodynamic and respiratory side effects, they should be administered with caution to animals with pre-existing cardiac [16,19,20] and pulmonary [14] disease.

Medetomidine has been used by our team in multiple experiments and proved to be an excellent sedative with few and easily manageable side effects [21,22].

While both phenothiazines and benzodiazepines are useful in the sedation of small ruminants [16], their lack of analgesic effect makes α2 agonists superior in painful procedures.

Opioids can be used in combination with other sedatives to provide analgesia during a procedure and potentiate the sedative effect of other agents, thereby reducing their dose [20]. However, opioids are less commonly used in ruminants compared to other species [23] as they seem to have poor analgesic properties in sheep, making α2 agonists superior to opioids in this species [15,24].

Table III. Advantages and disadvantages of different surgical approaches for C-section [12].

Surgical approach	Advantages	Disadvantages
Left flank laparotomy	Lowest risk of postoperative wound complications. Locoregional anesthesia.	Difficult access to the uterus due to the rumen.
Paramedian laparotomy	Locoregional anesthesia. Better access to the uterus.	Risk of postoperative wound complications (wound can be butted by the lamb while feeding).
Median laparotomy	Excellent access to the uterus.	Highest risk of postoperative wound complications. General anesthesia.

While all the classes of sedatives discussed above have previously been successfully used in C-sections in sheep, it should be noted that any sedation can cause fetal depression [12]; therefore, choosing a sedative or analgesic agent for C-section should be based on comprehensive knowledge of possible side effects and their management, as well as judicious evaluation of the risk-benefit ratio.

Surgical approach

Different surgical approaches for C-section in ewes have been described, including the left flank, paramedian, and median approaches, each one presenting its advantages and disadvantages, as presented in table III; however, the left flank approach is the most commonly used due to anesthesia convenience [10,12] and was therefore the approach chosen in our study.

After lamb extraction, palpation for another fetus in the second uterine horn is important. If present, the second lamb can be extracted either via the same incision, which requires more experience, or by performing a second incision, thereby avoiding additional uterine trauma [10,11].

Fetal membranes can be removed if they separate easily; otherwise, they can be trimmed and the remainder left in the uterus, as they would be naturally eliminated vaginally in the following postpartum days [12]. Fetal membrane separation should not be forced, and caruncles should not be incised, as this might lead to massive bleeding [25].

The use of intrauterine antibiotics to prevent postpartum metritis has been described. A combination of antibiotics, such as benzylpenicillin, streptomycin, and formosulfathiazole (readily available in some countries in the form of pessaries), has been previously described for metritis prevention [12,26]. As an alternative, covering a similar spectrum, we used ceftriaxone, a third-generation cephalosporin, and encountered no cases of postoperative metritis.

Cord blood collection

In this study, two cord blood collection methods were used. The first method involved active aspiration into a heparinized syringe, whereas the second method involved gravitational collection in a blood collection bag. Additionally, cord blood collection was performed both before and after cord clamping.

Fadel et al. reported the surgical collection of 20 mL of ovine cord blood through umbilical artery puncture into heparinized tubes. The animals underwent either C-section or *in-utero* blood collection. In animals undergoing C-sections, blood was collected before UC clamping, and the authors reported no complications regarding the sheep or lambs [5].

Similarly, Li et al. reported ovine cord blood collection by aspiration into heparinized syringes during C-section deliveries of both full- and preterm lambs. The reported total fetal survival rate was 85%, with no significant differences between the full-term and preterm group [27].

In a study by Martins et al., cord blood was collected from goats after natural birth by syringe aspiration. The blood was collected after cord clamping and transported to the laboratory within 2 hours in isothermal boxes with ice [28].

Koch et al., in a study performed on mares, reported harvesting cord blood by puncturing the umbilical vein with a 16 G needle attached to a blood collection bag containing citrate phosphate dextrose adenine as an anticoagulant. The blood was collected immediately after natural birth, before the spontaneous rupture of the UC. Sample volumes varied between 65 and 250 mL. The blood was then transported at room temperature (15–22° C) to the laboratory within 8–24 hours. No complications were registered in either the mares or foals [29].

In our study, we found that aspiration using heparinized syringes was more effective and less traumatic. In larger animals with larger umbilical vessels and when a larger amount of blood is required, gravitational collection into blood bags might be effective; however, in our study, the 14 G needle that was attached to the collection bag was too large for the ovine umbilical vessels, posing a risk for vessel rupture. Although in human practice, UCB collection is performed after clamping the UC and detaching the newborn [30], in this study, blood aspiration after clamping the UC led to a rapid collapse of the vessel, making it impossible to collect the desired amount of blood. In contrast, blood collection before clamping the cord was more permissive, allowing the desired amount of blood to be collected. Aspiration was performed at the maternal end of the UC to minimize potential risks to the lamb.

Complications

The mild sheep-related complications encountered in our study included intraoperative meteorism in one animal and intraoperative desaturation in two animals. These complications were easily managed with oxygen therapy and ruminal puncture.

Alpha2 (α2) agonists can reduce intestinal motility and reticuloruminal contraction in ruminants, potentially leading to abdominal distension [31]. Moreover, ruminal bloat is relatively common in prolonged surgeries with extended periods of lateral recumbency [10].

To minimize the risk of ruminal tympany, a fasting period of 12–24 hours for solid food and 6–12 hours for water is advised before general anesthesia [11] (pp. 7-23). Additionally, a slight head elevation above the rumen is recommended [10]. Furthermore, if the animal is under general anesthesia, the placement of a cuffed endotracheal tube can minimize the risk of regurgitation and aspiration [11] (pp. 7-23). A nasogastric tube can also be inserted to release ruminal gas. Alternatively, a 12 G needle can be inserted into the rumen through the abdominal wall to decrease pressure [16,24].

Mild lamb-related complications, including maternal rejection and neonatal diarrhea, occurred in one lamb. Both complications were manageable, and the lamb presented a favorable outcome.

The first few hours after parturition are crucial for the onset of maternal behavior towards the offspring [32]. The ewe must bond with her lamb within 2–4 hours of parturition to develop Tmaternal behavior [33].

Cervicovaginal stimulation caused by the passage of lambs through the birth canal stimulates the release of oxytocin in the central nervous system, which is a key factor in maternal acceptance and milk production [33,34]. This suggests the importance of natural delivery in the onset of maternal behavior. However, the choice to perform an elective C-section in this study was based on sterility considerations, as blood sample contamination could have compromised the experiment. Moreover, UC rupture, which usually occurs spontaneously after natural delivery, could compromise the blood collection process. Therefore, to stimulate milk production, the postoperative administration of metoclopramide and oxytocin was standardized in all animals.

Neonatal diarrhea is a major cause of neonatal death in lambs [35], accounting for nearly half of lamb mortality [35,36]. It can be caused by infectious or non-infectious factors [36,37]. The most important risk factors include colostrum deprivation and maladaptation to milk substitutes [37]. Treatment typically involves supportive care, including fluid and electrolyte therapy, along with optimized nutritional support. Unless systemic or severe illness is present, antimicrobial treatment is not usually indicated [38].

On the other hand, in our study, severe lamb-related complications occurred in one case, an umbilical cord accident (UCA) (torsion and hematoma). The complication resulted in lamb loss.

Despite being scarcely reviewed in the literature, extrapolating from human patients, UCAs can represent an important cause of fetal distress or death. In humans, UCAs account for 10%–15% of stillbirths. UCAs include UC compression, torsion, entanglement, coiling, knotting, collapse, prolapse, and thrombosis [39].

In humans, UC torsion and hematoma are rare events [40,41]. UC hematomas have an overall perinatal loss rate of 50%, with only a few case reports described in the literature [41].

In contrast to humans, ovine umbilical vessels are not spiraled [42,43], and intrapartum observation of spiraling can signify UC torsion, which can disrupt the blood flow to the fetus to various degrees.

In veterinary practice, only a few cases of UC torsion have been reported, all resulting in fetal loss [44-48].

Both UC torsion and hematoma can lead to blood flow disturbances, resulting in fetal distress. Gardner et al. found that chronic UC compression in sheep led to chronic hypoxemia, decreased oxygen delivery, and increased fatal lactate levels [49]. Conversely, acute and significant UC compression can lead to severe bradycardia [50] and a significant decrease in oxygen delivery in the fetus [51], which can ultimately result in fetal loss.

Study limitations and scope for future studies

This study was conducted with a small sample size of three pregnant ewes. While the protocols developed proved effective, results derived from this limited cohort may not entirely reflect outcomes in a broader or more diverse population of ovine subjects. Future research involving larger groups could provide further validation and increase generalizability. This study was conducted with a limited number of animals, aligned with the ethical guidelines of the 3R principles (Replacement, Reduction, and Refinement) to prioritize animal welfare while achieving the study's objectives. In adhering to the "Reduction" principle, we minimized the number of animals used. While the findings provide initial insights, a larger sample size would enhance the generalizability of these results.

Next, despite rigorous adherence to the described protocols, perioperative challenges such as ruminal tympany and desaturation were observed, highlighting the importance of close monitoring to ensure optimal care and prompt solutions to these challenges.

While the study provided valuable insights into postnatal care, the incidence of maternal rejection and neonatal diarrhea highlights the variability in neonatal outcomes. The success of the protocols may rely heavily on postnatal management conditions, which vary by institution and personnel experience.

The study's use of specific anesthetic and sedative agents was tailored to the conditions available. However, these choices may not be universally applicable, as other facilities may have different drugs or dosages available, potentially affecting the outcomes or replicability of this protocol in different research contexts.

Finally, the two methods of UCB collection presented challenges, particularly in aspirating sufficient volumes due to the small diameter of ovine umbilical vessels. This technical limitation may restrict the applicability of UCB collection in species or contexts requiring larger volumes for research or clinical application. However, in our study, blood collection before clamping the cord was more permissive, allowing the desired amount of blood to be collected.

Conclusions

Anesthetic and surgical approaches should be chosen considering all advantages and disadvantages as well as the risk/benefit ratio. Local anesthesia combined with mild sedation proved to be safe and provided proper comfort for both the animal and the operator.

Elective C-section ensures a sterile environment for cord blood collection, which is crucial for subsequent cell culture and stem cell isolation. Cord blood collection using active aspiration into a syringe before cord clamping is safe, convenient, efficient, and less traumatic, with no significant consequences for sheep or newborn lambs.

We showed that prompt aspiration of pharyngeal secretions immediately after delivery to facilitate breathing and the presence of an experienced resuscitation team are essential for lamb survival.

Postnatal care of the lambs can be challenging, especially in the immediate postnatal period. Encouraging the mother-newborn bond as soon as possible is essential. In the absence of a proper milk supply, ensuring the energetic and hydroelectrolytic needs of the lambs at all times is essential for their survival and well-being.

In conclusion, we suggest that the described anesthetic protocol is safe and effective, ensuring adequate sedation and analgesia without significant complications to the sheep or lambs. The surgical technique is feasible, does not require specialized surgical equipment, and can be performed with minimal surgical complications. The proposed UCB collection technique is efficient and presents no significant risk to the lambs. Perioperative management can be challenging, especially regarding lamb survival, and requires proper immediate and late postnatal care. This study offers a reliable protocol for elective C-section and UCB collection in an ovine model, which can be replicated in larger cohorts of animals. Expanding the study population will enhance the reliability of the results and provide deeper insights into the applicability of these methods in translational research for stem cell therapy.

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