Effect of Different Cardioprotective Methods on Extracorporeal Circulation in Fetal Sheep: A Randomized Controlled Trial

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Abstract

Background Congenital heart disease is one of the leading causes of death in newborns and infants. The development of fetal cardiac surgery is inextricably linked to extracorporeal circulation. We aimed to compare the effects of heart-stopping solutions and extracorporeal circulation on fetal sheep myocardium.

Methods Eighteen pregnant sheep were divided into the non-stop group, St. Thomas stopping solution group, and histidine−tryptophan−ketoglutarate stopping solution group. The three groups underwent the extracorporeal circulation. The right atrial myocardial tissue was removed from the fetal sheep at specific time points, and apoptosis was detected by TUNEL staining. Creatine kinase−muscle band (CKMB), troponin I (cTnI), and troponin T (cTnT) were measured as indicators of myocardial damage.

Results There were no significant differences in the serum cTnT, cTnI, and CKMB concentrations of fetal sheep among the three groups before starting extracorporeal circulation ($P = 0.430$, $P = 0.391$, $P = 0.071$). Changes in the serum cTnI (ng/L) concentrations were not significantly different among the three groups before and during the extracorporeal circulation ($P > 0.05$). The cTnI in the St. Thomas solution group at 1 hour post extracorporeal circulation was significantly higher than prior to it ($P < 0.05$), and cTnI in the non-stop group and histidine−tryptophan−ketoglutarate group after 2 hours was higher than in the pre-bypass value ($P < 0.05$). The cTnI in the histidine−tryptophan−ketoglutarate group at 1 and 2 hours after the extracorporeal circulation was lower than in the St. Thomas solution group. The number of TUNEL-positive cells in the two solution groups was higher than in the non-stop group ($P = 0.001$ and $P = 0.048$, respectively). The number of TUNEL-positive cells in the St. Thomas solution group was higher than in the histidine−tryptophan−ketoglutarate group ($P = 0.007$).

Conclusion Myocardial protection in fetal sheep undergoing extracorporeal circulation was significantly better with non-stop beating than when the beating was stopped. Compared to the St. Thomas arrest solution, histidine−tryptophan−ketoglutarate stopping solution was associated with significantly reduced markers of myocardial damage in fetal sheep. Less cardiomyocyte apoptosis was observed when the beating was not stopped.

Background

Congenital heart disease is one of the leading causes of death in newborns and infants and accounts for about 1% of all congenital malformations[1]. Although there have been impressive advances in pediatric cardiac surgery in the last 20 years, some complex cardiac malformations (left ventricular dysplasia syndrome, pulmonary atresia, etc.) still result in high intrauterine death rates, postnatal mortality, and complications[2-4].

The early fetal heart pattern is formed by the eighth week in utero, and further development of the heart is influenced by the pattern of fetal blood flow[5-6]. Theoretically, if congenital heart disease can be operated on during the fetal period, the transformation of an abnormality into a complex one can be prevented,

complex postnatal cardiac surgery can be avoided, the heart can be given a chance to develop further in utero, and secondary morphologic damage can be avoided[7-9].

The development of fetal cardiac surgery is inextricably linked to extracorporeal circulation (cardiopulmonary bypass, CPB), and fetal CPB is currently being studied in experimental animals, primarily fetal sheep. In this study, we used a small centrifugal pump to establish an extracorporeal circulation model in fetal sheep. We used three cardioprotective methods: non-stop cardiac arrest, St. Thomas fluid perfusion cardiac arrest, and histidine−tryptophan−ketoglutarate stopping solution (HTK solution) fluid perfusion cardiac arrest. We monitored fetal cardiac function and compared the effects of the three cardioprotective methods on fetal sheep, including cardiomyocyte apoptosis and myocardial adenosine triphosphate and lactate levels during and after the surgery, given the clinical applicability of this approach.

Materials And Methods

Study design

Eighteen pregnant sheep (100−120 days gestation, 150 days at term) weighing 25.72 ± 4.29 kg (17.5−32 kg), with 18 fetuses, were used for the study. We divided the fetal sheep into three groups of six: the non-stop group, the St. Thomas protective fluid group (STH group), and the histidine−tryptophan−ketoglutarate stopping solution group (HTK group). In the non-stop group, we established extracorporeal circulation for the fetal sheep for 60 minutes, then stopped it and observed the sheep for 120 minutes. In the STH and HTK groups, the ascending aorta of the fetal sheep was blocked, and the heart was perfused with St. Thomas fluid and histidine−tryptophan−ketoglutarate stopping solution to stop the fetal heart for 30 minutes. The fetal aorta was then reopened to allow both, body and extracorporeal circulation, and after the heart had successfully resumed beating for 30 minutes, the extracorporeal circulation was stopped, and the sheep were observed for two hours.

Preoperative preparation and anesthesia

Pregnant sheep were fasted for 24 hours. Preoperatively, we prepared the skin of the abdomen, back, and neck of the pregnant sheep and administered a preoperative intramuscular injection of atropine 0.5−1.0 mg/kg and ketamine 10−20 mg/kg into the femoral area. After the sheep's conjunctival reflex disappeared, the sheep was lifted onto the operating table, and electrodes were attached to its back. The trachea was intubated, and the sheep were ventilated mechanically with a tidal volume of 10−15 ml/kg and an inhalation oxygen concentration of 40−50%, at a frequency of 15−20 times/minute. A cardiac monitor was attached to the forelimb of the pregnant sheep. The femoral artery was punctured and intubated, and an arterial tester was connected to monitor arterial blood pressure.

Surgical procedures and extracorporeal circulation

The skin of the pregnant sheep's lower abdomen was incised, and the uterus was surrounded with gauze pads moistened with warm saline for support. Anesthetic drugs (fentanyl 25−50 μg/kg and vecuronium bromide 0.1−0.2 mg/kg) were injected directly through the uterine wall into the muscles of the fetus's hind limbs, selecting a site without placental vessels. After the fetal movement disappeared, a 4-6 cm incision was made through the uterine wall in the area without placental blood vessels. Then, the amniotic cavity was opened, the fetal chest exposed, and one upper limb was gently exposed. The axillary artery was exposed and punctured for the placement of a tube to allow monitoring of the fetal arterial blood pressure and heart rate. A mid-thoracic incision was made on the fetal sheep, and the superficial tissue was incised. The space around the aorta and pulmonary artery was dissected.

After the extracorporeal circuit was established, extracorporeal circulation was started. The STH and the HTK groups were perfused with myocardial protective fluid using aortic forceps after starting the extracorporeal circulation, while the non-stop group was not perfused with myocardial protective fluid. The fetal sheep extracorporeal circulation flow rate was above 200 ml/kg/min, and the water tank temperature was kept constant at 40°C. The fetal heart was stopped for 30 minutes in the STH and HTK groups. The ascending aorta was opened to allow simultaneous body and extracorporeal circulation, and the fetal heart was restarted for 30 minutes. The extracorporeal circulation was then stopped, and the sheep were observed for 2 hours. The non-stop group was maintained on the extracorporeal circulation for 60 minutes which was then stopped for 2 hours. After 2 hours, 5 ml of 10% potassium chloride was injected intravenously into the fetal sheep, and the heartbeat was stopped rapidly. A small amount of the right atrial and ventricular myocardium tissue was then removed from the fetal sheep and stored. The fetal sheep were removed and detached from the extracorporeal circulation, and the uterus and abdominal incision of the ewe were sutured. When the ewes awoke from anesthesia, they were returned to the breeding center to continue rearing.

Collection and preservation of specimens

The venous blood of fetal sheep was collected at various time points and centrifuged at 1500g at 4°C for 15 minutes. The supernatant was extracted and transferred to the Eppendorf tubes (approximately 0.5 mL per tube) and stored at -80°C in a refrigerator for further testing. The concentrations of troponin T (cTnT), troponin I (cTnI), and creatine kinase−muscle band (CKMB) in the supernatant were determined by the enzyme-linked immunosorbent assay at five different time points: T1 (before the start of EC); T2 (30 minutes of EC); T3 (60 minutes of EC); T4 (1 hour after EC shutdown) and T5 (2 hours after EC shutdown).

A piece of myocardial tissue of the right atrium of the fetus, with a size of 1 × 1 × 1 × 1 cm, was cut out and placed in a test tube containing 4% formalin solution, sealed, and kept at room temperature. The tissue was dehydrated and embedded in the paraffin wax for slicing. Cells were uniformly stained with TUNEL to facilitate the observation of apoptosis under a 400 × optical microscope; 10 non-overlapping fields of view were randomly selected, and TUNEL-stained cells counted. The apoptosis of cardiomyocytes was detected by combining the morphological characteristics and staining of the

apoptotic cells. Apoptotic cells were stained brown with the TUNEL stain and exhibited chromatin concentration and fragmentation, DNA marginalization and the lysis of the nuclear membrane. The apoptotic index was calculated as follows:

Apoptosis index (AI) = apoptotic cell number/(number of apoptotic cells + number of normal cells) × 100%.

Statistical methods

All data collected in this study were processed using SPSS v.21.0 statistical software (IMB SPSS, Armonk, NY), and the results were expressed as mean ± standard deviation (x ± s). Significant differences between two groups were tested using Student's *t*-test, and comparisons between multiple groups were made using one-way analysis of variance. P < 0. 05 was considered statistically significant.

Results

Preoperative characteristics

Twenty-two pregnant sheep with 22 fetuses were used to develop the extracorporeal circulation direct intracardiac surgery model. Two of the pregnant sheep died of respiratory depression and cardiac arrest during the induction of anesthesia. The remaining 20 ewes were successfully revived after removing the fetuses and were returned to the breeding center. One fetal sheep was intubated into the main pulmonary artery between the outer and middle membranes, which was not detected in time, and it died of cardiac arrest after the start of extracorporeal circulation. Another fetal sheep died due to the failure of cardiac resuscitation, so the experiment was completed with 18 fetal sheep.

There were no statistically significant differences in the gestational age and body weight between the three groups (P > 0.05) (Table 1). The STH group had less stopping-fluid perfusion than the HTK group (P < 0.05), and the STH group required less time to return to a normal heart rate than the HTK group (P < 0.05).

Table 1 Comparison of general information and intraoperative condition of the three groups of fetal sheep (± s)

	non-stop group (n = 6)	STH group (n = 6)	HTK group (n = 6)
Age (d)	123.71 ± l1.98	122.52 ± 12.33	130.10 ± 01.23
Weight (kg)	1.21 ± 0.56	1.24 ± 0.47	1.33 ± 0.70
Stopping-fluid perfusion (ml/kg)	–	47.0 ± 8.4	58.0 ± 0.0*
Time to return to normal heart rate (s)	–	45.0 ± 4.3	61.0 ± 3.5*

Perioperative hemodynamics

The changes in the heart rate and blood pressure in the three groups of fetal sheep between the T1, T4, and T5 points(Table 2) were not statistically different among the groups (P > 0.05). Heart rates were lower in the STH and HTK groups at 60 minutes of transient shut down of extracorporeal circulation (T3) than at T1 point (P < 0.05), and the STH group had lower heart rates than the HTK group (P < 0.05). The heart rate at T4 was lower than the basal values at T1 in the STH and HTK groups (P < 0.05), and that in the STH group was lower than that in the HTK group (P < 0.05). At T3, the mean arterial pressure in the STH group was lower than the basal value at T1 point (P < 0.05) and was lower than that in the HTK group (P < 0.05). At T2, the STH and HTK groups showed higher transient flow than the non-stop group (P < 0.05). There was no statistically significant difference among the groups in the heart rate and mean arterial pressure at T4 and T5 (P > 0.05).

Table 2 Comparison of perioperative hemodynamics in three groups of fetal sheep (x ± s)

	T1	T2	T3	T4	T5
Heart rates (bmp)					
Non-stop Group	173.1 ± 20.10	169.1 ± 17.18	171.7 ± 22.21	171.5 ± 18.58	178.1 ± 10.12
STH Group	175.0 ± 15.12	–	110.3 ± 22.10*	177.5 ± 17.14	173.2 ± 19.56
HTK Group	174.6 ±21.00	–	151.1 ± 19.99*#	174.2 ± 15.11	177.8 ± 11.16
Average Arterial Pressure (mmHg)					
Non-stop Group	57.1 ± 15.67	57.3 ± 17.42	56.9 ± 19.11	57.1 ± 15.12	56.5 ± 18.36
STH Group	56.9 ± 13.81	57.5 ± 13.55	50.0 ± 16.11*	59.5 ± 17.22	57.1 ± 16.39
HTK Group	56.9 ± 16.85	56.6 ± 17.34	58.0 ± 19.73#	58.2 ± 16.55	56.8 ± 15.93
Flow Rate (ml/kg/min)					
Non-stop Group	–	101.5 ± 29.22	–	–	–
STH Group	–	114.9 ± 28.45‡	–	–	–
HTK Group	–	113.3 ± 29.76‡	–	–	–

Note: EC, Extracorporeal circulation.

T1: Before start of EC

T2: Thirty minutes of EC

T3: Sixty minutes of EC

T4: One hour after EC shutdown

T5: Two hours after EC shutdown

* $P < 0.05$, compared with the pre-EC; #$P < 0.05$, compared with the STH group;

‡ P < 0.05, compared with the non-stop group.

Indicators of myocardial impairment at various time points in different groups of fetal sheep

There were no significant differences in the serum concentrations of cTnT, cTnI, and CKMB in fetal sheep before the start of extracorporeal circulation between the three groups (P = 0.430, P = 0.391, P = 0.071, respectively; Tables 3, 4, 5).

Changes in the concentration of cTnI.

There was no statistically significant difference in cTnI among the three groups at T1, T2, and T3 (P > 0.05). The cTnI level in the STH group at T4 was significantly higher than that before the start of extracorporeal circulation (P < 0.05). The concentration of cTnI was lower in the HTK group than in the STH group at T4 and T5 (P < 0.05). The concentration of cTnI (ng/L) was higher in the STH group at T4 and higher in the STH and HTK groups at T5 than in the non-stop group (P < 0.05) (Table 3 and Figure 1).

Table 3: Changes in the concentration of cTnI in the three groups. (± s)

Groups	T1	T2	T3	T4	T5
Non-stop Group	59.03 ± 3.21	58.23 ± 4.21	60.74 ± 2.31	65.74 ± 3.25	73.43 ± 3.37*
STH Group	60.21 ± 3.23	60.38 ± 5.27	61.70 ± 4.51	87.52 ± 4.25* ‡	90.14 ± 5.96* ‡
HTK Group	59.01 ± 2.19	61.27 ± 3.43	65.69 ± 3.24	67.17 ± 4.56#	83.50 ± 4.63* ‡ #

Note: EC, Extracorporeal circulation.

T1: Before start of EC

T2: Thirty minutes of EC

T3: Sixty minutes of EC

T4: One hour after EC shutdown

T5: Two hours after EC shutdown

* P < 0.05, compared with the pre-EC; #P < 0.05, compared with the STH group;

‡ P < 0.05, compared with the non-stop group.

Table 4: Changes in the concentration of cTnT in the three groups (± s)

Groups	T1	T2	T3	T4	T5
Non-stop Group	101.83 ± 12.54	100.11 ± 10.31	104.27 ± 12.11	105.44 ± 9.40	119.85 ± 11.07*
STH Group	103.57 ± 16.32	103.49 ± 10.12	105.96 ± 17.28	117.31 ± 7.75*‡	136.41 ± 9.02*‡
HTK Group	99.40 ± 13.53	103.78 ± 14.56	107.93 ± 10.85	110.62 ± 9.54#	129.69 ± 8.90*#

Note: EC, Extracorporeal circulation.

T1: Before start of EC

T2: Thirty minutes of EC

T3: Sixty minutes of EC

T4: One hour after EC shutdown

T5: Two hours after EC shutdown

* $P < 0.05$, compared with the pre-EC basis; # $P < 0.05$, compared with the STH group;

‡ $P < 0.05$, compared with the non-stop group.

Table 5: Changes in the concentration of CKMB in the three groups (± s)

Groups	T1	T2	T3	T4	T5
Non-stop Group	0.51 ± 0.01	0.51 ± 0.02	0.55 ± 0.01	0.56 ± 0.01	0.59 ± 0.02
STH Group	0.50 ± 0.04	0.50 ± 0.08	0.54 ± 0.08	0.55 ± 0.02	0.66 ± 0.03* ‡
HTK Group	0.53 ± 0.01	0.53 ± 0.04	0.53 ± 0.07	0.54 ± 0.03	0.64 ± 0.06* ‡

Note: EC, Extracorporeal circulation.

T1: Before start of EC

T2: Thirty minutes of EC

T3: Sixty minutes of EC

T4: One hour after EC shutdown

T5: Two hours after EC shutdown

* $P < 0.05$, compared with the pre-EC basis; ‡ $P < 0.05$, compared with the non-stop group.

Discussion

We compared the effects of STH solution, histidine−tryptophan−ketoglutarate stopping solution, and the non-stopping of the heart on the fetal sheep myocardium. Myocardial protection in the fetal sheep undergoing extracorporeal circulation was significantly better with the non-stop beating than when the beating was stopped with the STH or HTK solution. Compared with the STH arrest solution, histidine−tryptophan−ketoglutarate stopping solution had a significantly lower release of the markers of myocardial damage once the beating was stopped, and resulted in less apoptosis.

Research on the theory of fetal extracorporeal circulation began in 1984, with attempts at extracorporeal circulation in fetal sheep first reported by Slate et al. at the University of California [10]. Based on many experiments on fetal sheep and a thorough understanding of fetal sheep physiology, a consensus has been reached on the following points regarding the fetal sheep extracorporeal circulation technique. First, fetal extracorporeal circulation needs to be performed at room temperature; second, the extracorporeal circulation needs to be transferred at a high flow rate; third, the transfer needs pulsatile perfusion to mimic fetal physiological perfusion; fourth, the fetal blood volume is low, and the tubing needs low prefilling during extracorporeal circulation[11]. In the 21st century, the pathophysiological changes in fetal sheep after extracorporeal circulation and, most importantly, the preservation of fetal cardiac function have been studied in greater depth. The introduction of minimally invasive and robotic techniques in cardiac surgery, the development of fetal anesthesia concepts, advances in extracorporeal circulation technology and improvement in extracorporeal circulation equipment have made fetal cardiac surgery possible in the 21st century.

Nevertheless, concerns regarding myocardial protection have been a major hindrance, and the perioperative myocardial injury and postoperative cardiac dysfunction are yet to be addressed [12]. Because of the fetus's unique characteristics, the need for the protection of immature fetal myocardium is much higher than that in the adults. There are significant differences between the immature and mature myocardium in terms of function, ultrastructure, energy metabolism, and tolerance of ischemia and hypoxia. The suitability of adult cardioprotective solutions for immature myocardium is unclear. In order to meet the high level of fetal surgical refinement required, myocardial protective fluid arrest needs to be effective and prolonged. At present, the protection of immature myocardium is still being explored [13]. Thus, the differences and similarities between immature and mature myocardium should be the starting point for studying fetal myocardial protection.

Comparison of the cardiac arrest effects of the two stopping fluids

When the blood flow in the coronary vessels ceases because the heart has stopped beating, cardiomyocytes are no longer supplied with blood and oxygen from the coronary arteries, resulting in the

cessation of cardiomyocyte aerobic metabolism and a decrease in energy production. Cardiomyocytes still need to be supplied with energy to survive, but their metabolism of energy-producing material is lower: when they stop contracting, their metabolic rate is only one-tenth that of the beating state [14]. During prolonged ischemia, the key to the reduction of myocardial injury and the maintenance of cardiac function is the availability of sufficient energy substrates, and the stopping or preserving fluids currently in clinical use do not contain the energy substrates. Therefore, to minimize the energy consumption of cardiomyocytes during the infusion of cardiac arrest fluid, the metabolism, energy and physical integrity of cardiomyocytes are preserved by minimizing the duration of irrigation and arrest. The commonly used clinical cardioprotective solution, St. Thomas stopping solution, is a hyperkalemic arrest solution that effectively reduces myocardial ischemia/reperfusion injury. However, it does not provide complete protection, and some patients may experience reversible myocardial hypocontractility or myocardial tonic depression[15].

The blood-bearing pacing solution can carry oxygen and metabolic substrates and has certain acid-base buffering and oxygen free-radical scavenging ability. However, because of the many erythrocytes in the blood-bearing pacing fluid, its viscosity increases significantly at low temperatures, which affects its effective and uniform perfusion to the myocardium [16]. In addition, CPB-activated leukocytes, which are among the major contributors to the CPB-induced inflammatory response syndrome, are also responsible for myocardial ischemia/reperfusion injury [17]. Our experiments were performed at room temperature (28°C), and the STH solution and HTK stopping solution were used to perfuse the heart downstream. During the room temperature perfusion, the stopping speed is faster with the STH solution and slower with HTK solution. Because the STH solution contains a high concentration of potassium ions, it is possible to stop the heart quickly. After the infusion of STH solution, the myocardial cell membrane is rapidly depolarized due to its high potassium level reaching above the threshold potential of fast sodium channels, resulting in inactivation of these channels and failure to form the ascending branch of the action potential, resulting in rapid cardiac arrest. In contrast, the reason for the slower stopping rate of the HTK stopping solution compared to the St. Thomas stopping solution is its significantly lower concentration of potassium ions. Cardioprotection of fetal cardiomyocytes involves the induction of rapid cardiac arrest, and the STH solution has advantages in this regard. However, the overall protection of cardiomyocytes in fetal sheep should be analyzed further.

Detection of myocardial injury

cTnI is a small molecular weight protein that is only found intracellularly and is released into the bloodstream when the cell membrane of cardiomyocytes is ruptured. cTnI is the most specific marker of myocardial injury [18]. cTnI is also a sensitive marker, and has some diagnostic value if there is mild damage to cardiomyocytes [19].

In cardiomyocytes cTnI plays a major role as a linker between pro-tropomyosin and troponin to form the troponin-pro-tropomyosin complex. There is probably only a very small amount of cTnI in the cytoplasm of a normal organism, and a very small amount of cTnI can be used as an energy substrate during

cardiomyocyte metabolism [20], primarily because the cell membrane is impermeable to cTnI. If a large amount of cTnI is detected in the blood, it indicates that the cardiomyocytes have been damaged, the cell membranes are ruptured, and cTnI from the cytoplasm has entered the body circulation.

During cardiomyocyte contraction, the main function of cTnT is to cooperate with cTnI in a regulatory role. cTnT also enters the humoral circulation after the cardiomyocyte damage, and the current assay requires 2−3 hours to detect elevated cTnT levels, which can persist for up to 2 weeks after myocardial damage.

Another early indicator of myocardial injury is CKMB [21]. CKMB is one of the many isoenzymes of creatine kinase, and CKMB is used as an indicator of the degree of myocardial damage because it is predominantly found in the myocardium, with minimal levels in other tissues. Therefore, the amount of CKMB in the circulation indicates the degree of myocardial cell damage.

CKMB, cTnI, and cTnT were measured as indicators of myocardial damage. In our study, the level of the indicators of the myocardial damage in the non-stop group was lower than that in the HTK and STH groups at all times, and the HTK group fared better than the STH group. The calcium-ion content in the HTK stopping solution is very low, which decreases the amount of calcium ions entering the cells during the ischemia/reperfusion and reduces the occurrence of calcium-ion overload. STH solution has a high concentration of potassium ions, which causes the coronary arteries to spasm and contract when the high potassium concentration of STH solution enters the perfused artery. During the re-entry process, the reperfused blood slows down the overall perfusion velocity due to the constriction of the coronary artery, ultimately causing the cardiac output to be less than before the perfusion [22]. At the same time, high concentration of potassium ions reduces the difference between the potassium ion concentration inside and outside the cell membrane, resulting in a decrease in the negative membrane potential of the vascular endothelium, leading to an increase in vascular permeability, increased inflammation of cardiomyocytes and vascular thrombosis, and further aggravating ischemia and hypoxia in myocardial tissue [23].

During apoptosis, endogenous nucleic acid endonuclease cuts DNA in the nucleolus, exposing the cut end of the DNA, which can be identified using a TUNEL assay. The number of TUNEL-positive cells in the STH group was higher than that in the HTK group, indicating that the protective effect of HTK on cardiomyocytes was stronger than in the STH group.

Our study has some limitations. For example, it is difficult to obtain laboratory animals that are suitable for the conditions, resulting in a small sample size. The number of TUNEL-positive cells in the HTK group was higher than that in the non-stop group, indicating that the protective effect was still limited. In immature myocardium, damage due to the extracorporeal circulation often peaks at 6−8 hours postoperatively, and the degree of apoptosis at 2 hours postoperatively is not necessarily representative of apoptosis at 6-8 hours postoperatively. False-positive results can also occur in TUNEL reactions. Moreover, there are genetic differences between human and sheep immature cardiomyocytes. Therefore,

more experimental evidence is needed to fully evaluate the stopping solutions effects on the fetal myocardium.

Conclusion

This study demonstrated that, in a fetal sheep model of extracorporeal circulation, the non-stop mode of extracorporeal circulation is significantly more protective of the myocardium than the cardiac arrest mode. We also found that the histidine—tryptophan—ketoglutarate stopping solution had a significant advantage over the St. Thomas stopping solution in decreasing the myocardial metabolism and reducing the myocardial injury as evidenced by the decreased myocardial injury markers. Our findings provide a theoretical basis for the protection of fetal myocardium and the future non-stop fetal heart surgery.

List Of Abbreviations

CKMB: Creatine kinase—muscle band

CPB: cardiopulmonary bypass

cTnI: Troponin I

cTnT: Troponin T

HTK: Histidine—tryptophan—ketoglutarate

STH: St. Thomas(solution)

Declarations

Ethics approval and consent participate

All animals were kept in a pathogen-free environment and fed ad lib. The procedures for care and use of animals were approved by the Ethics Committee of the The First Affiliated Hospital of Guangxi Medical University and all applicable institutional and governmental regulations concerning the ethical use of animals were followed.

Our manuscript does not contain data from any individual person，so "consent participate " is "Not applicable ".

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All surgical operations performed by YB Yan on the experimental animals were done under the guidance of BF Lei. S Shi and QB Wu were responsible for collecting samples and testing. With the assistance of JS Cai, YB Yan compiled all the data and wrote this article.

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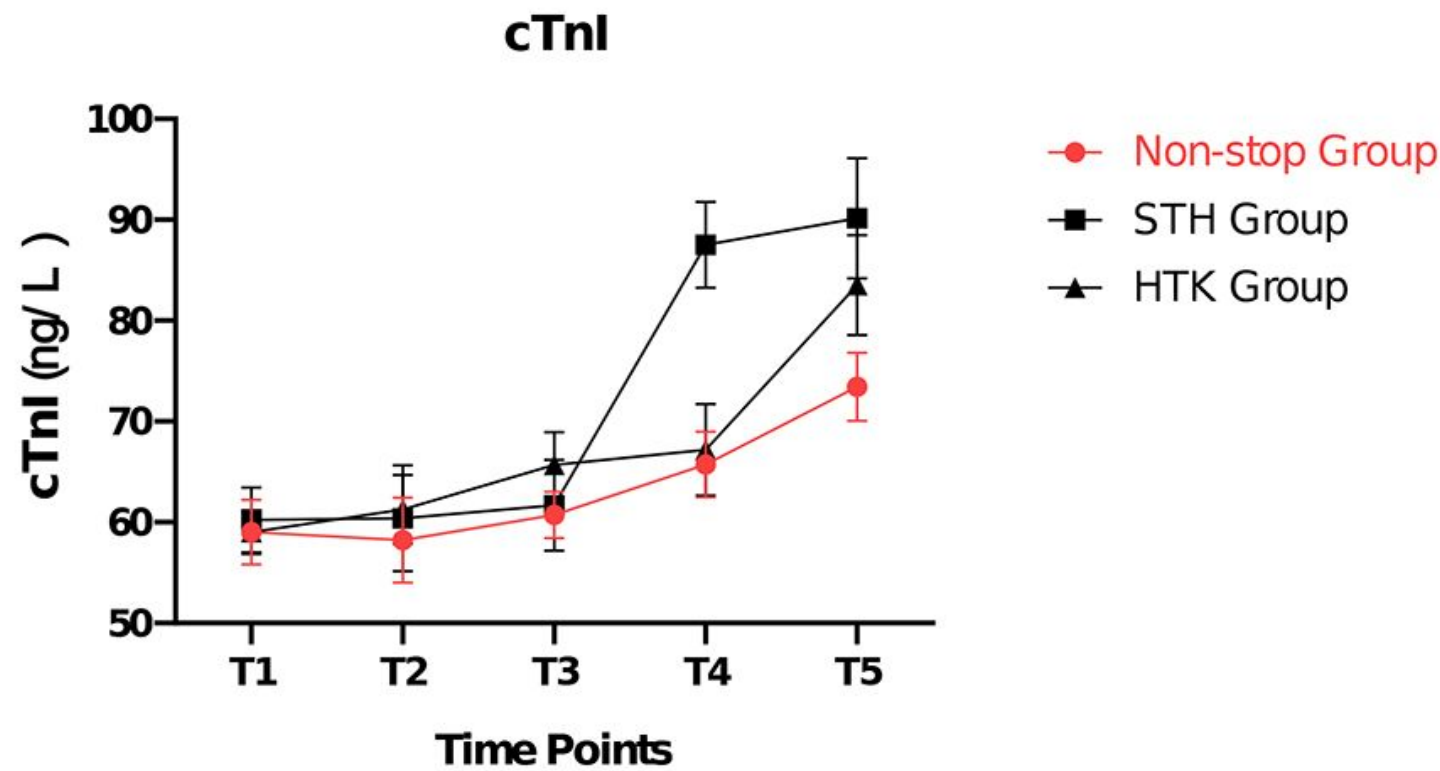
Figures

Figure 1

The concentration of cTnI in the three different groups Changes in the concentration of cTnT. There was no statistically significant difference among the three groups at T1, T2, and T3 (P > 0.05). The cTnT level in the STH group at T4 was significantly higher than that before CPB (P < 0.05). cTnT levels in both the non-stop and HTK groups at T5 were higher than those before CPB (P < 0.05). The concentration of cTnT (ng/L) was lower in the HTK group than in the STH group at T4 and T5 (P < 0.05). The concentration of cTnT (ng/L) was higher in the STH group at T4 and T5 than in the non-stop group (P < 0.05) (Table 4 and Figure 2).
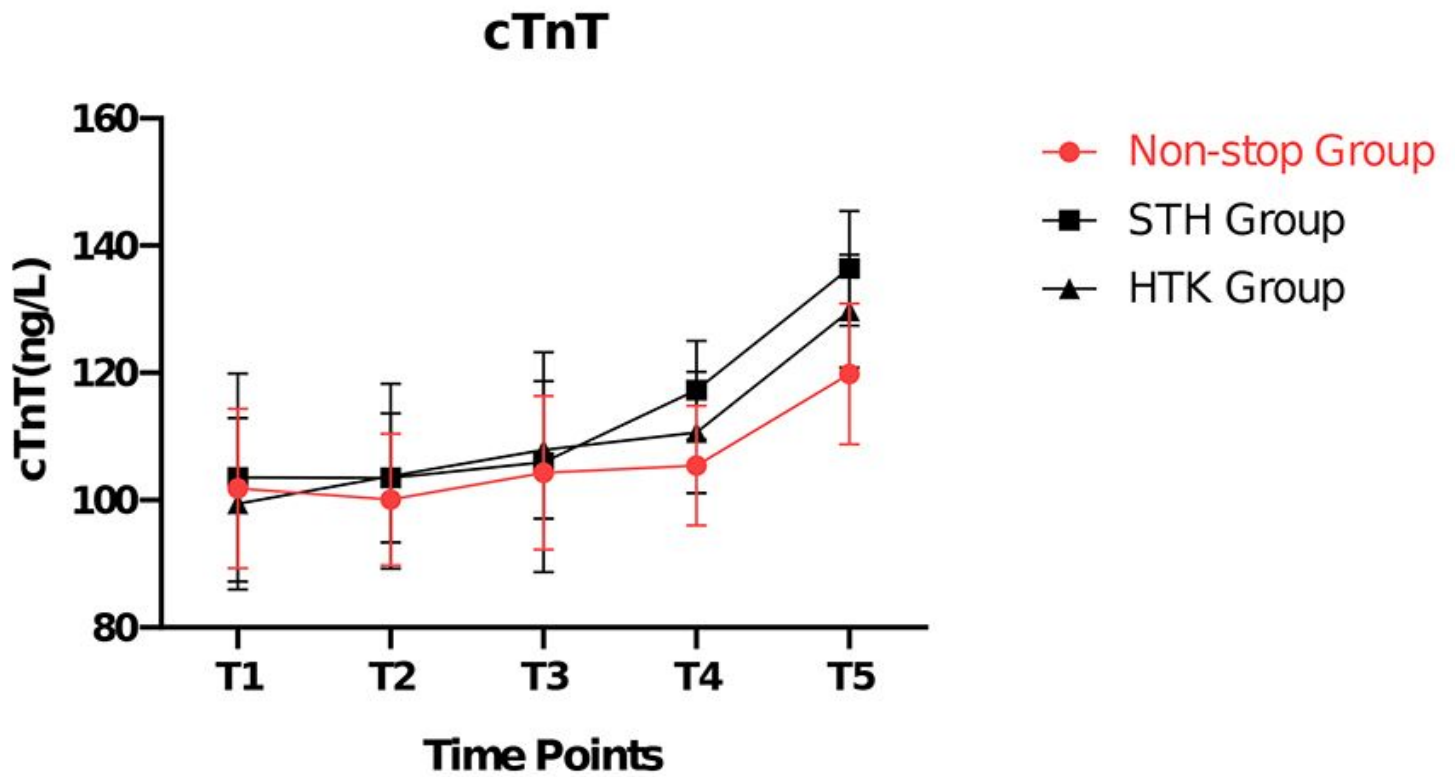
Figure 2

The concentration of cTnT in three different groups Changes in CKMB concentration. There was no statistically significant difference in serum CKMB levels among the three groups at T1, T2, T3, and T4 (P > 0.05). At T5, the CKMB (ng/L) concentration was higher in the STH and HTK groups than in the non-stop group (P < 0.05) (Table 5 and Figure 3).
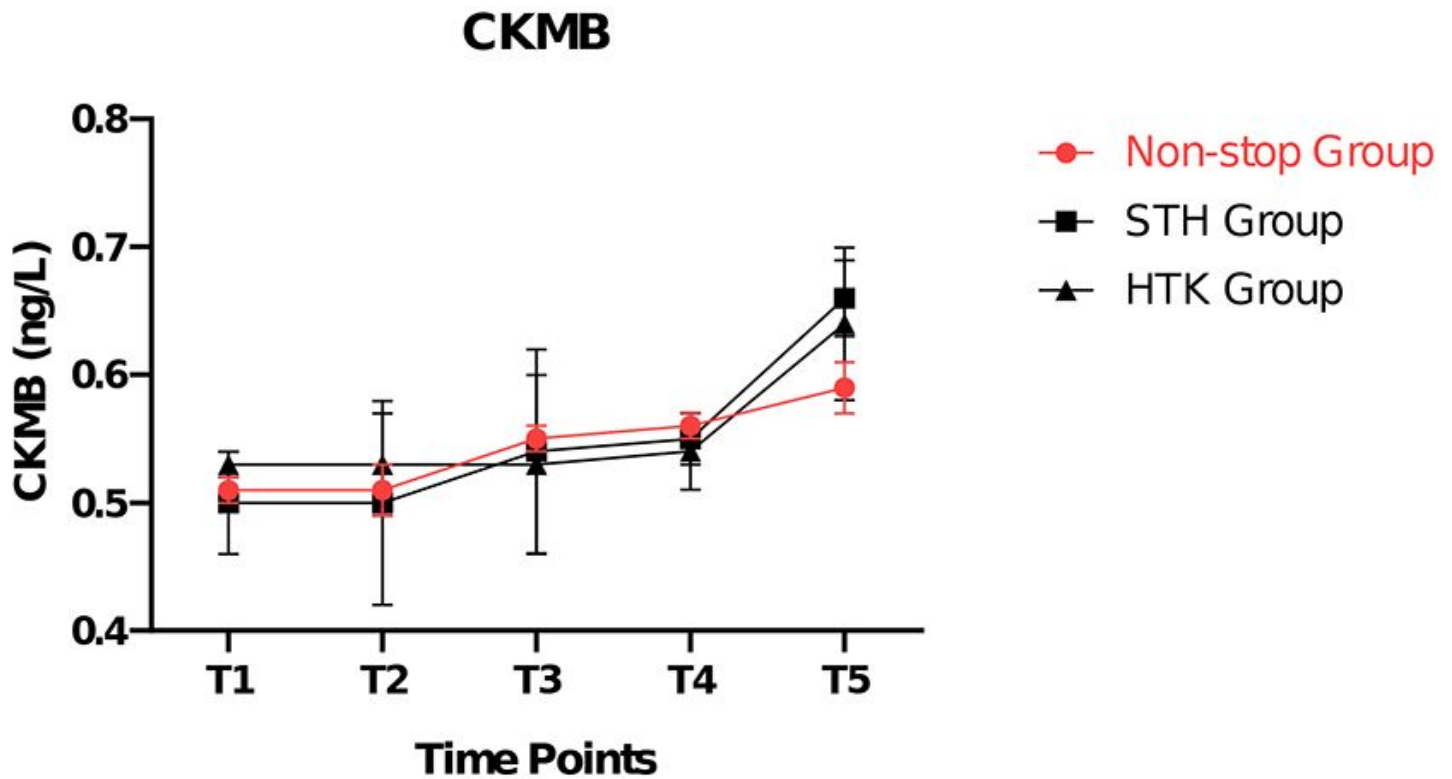
CKMB

Figure 3

The concentration of CKMB in three different group In situ apoptosis detection in cardiomyocytes The apoptotic index was calculated to be 10.2 ± 4.7 in the non-stop group, 34.2 ± 3.7 in the STH group, and 20.2 ± 3.1 in the HTK group (Figure 4). The number of TUNEL-positive cells in the HTK and STH groups was significantly higher than that in the non-stop group ($P = 0.001$ and $P = 0.048$, respectively).The number of TUNEL-positive cells in the STH group was significantly higher than that in the HTK group($P=0.007$).
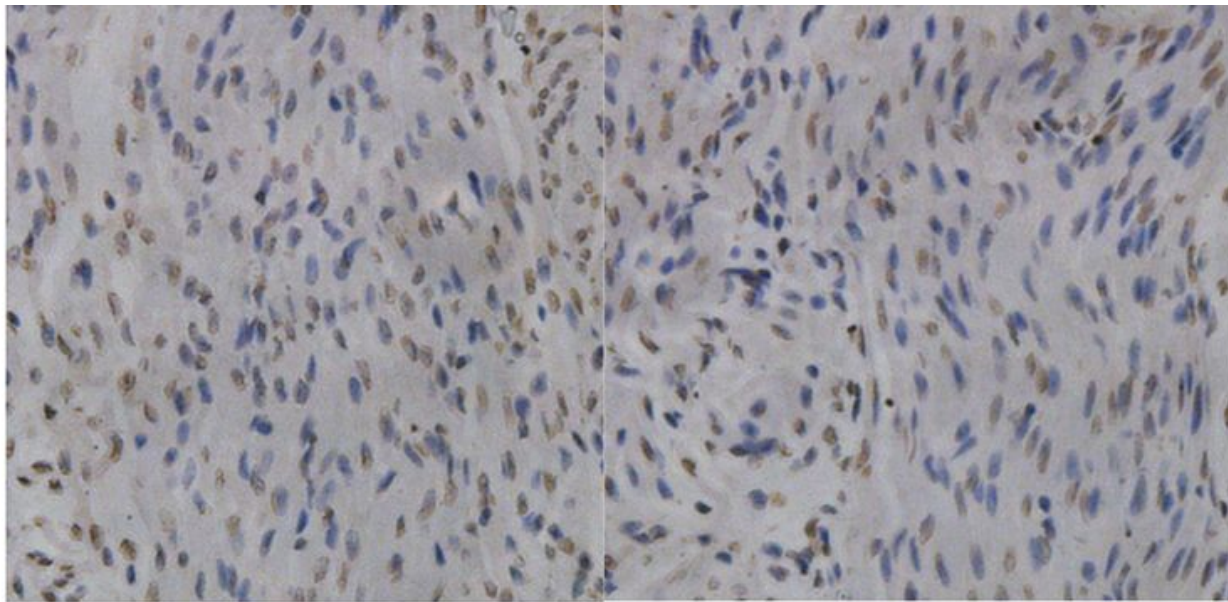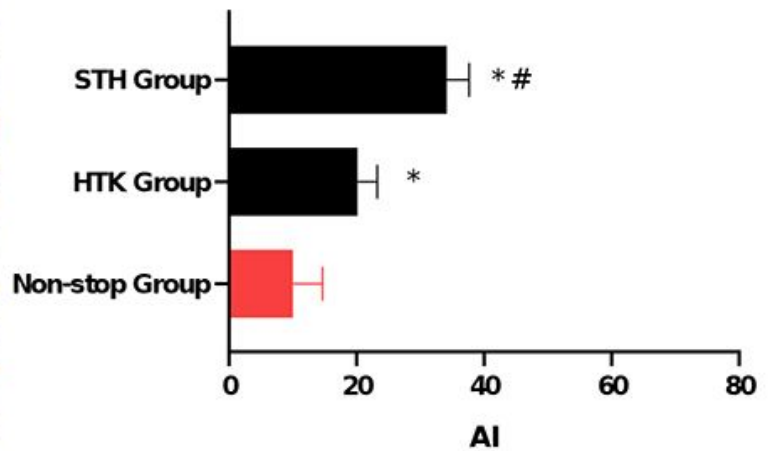
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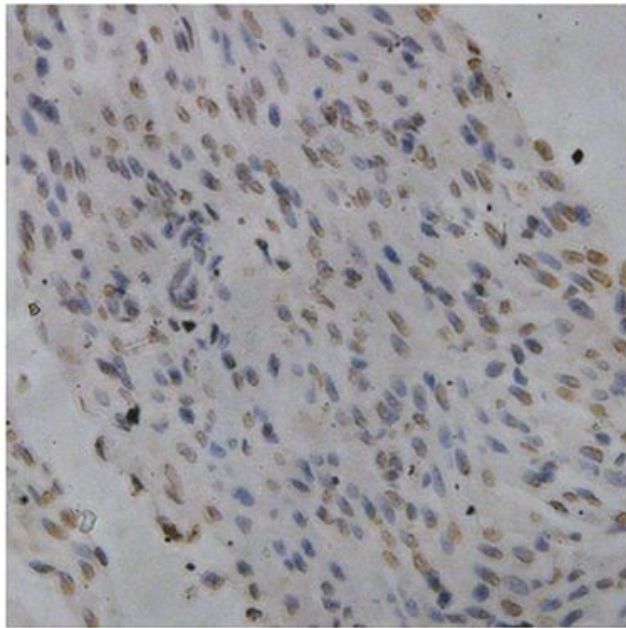
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Figure 4

TUNEL assay to detect cell apoptosis (400×) Note: A: Apoptosis of cardiomyocytes after cardiopulmonary bypass (CPB) in the non-stop group. B: Apoptosis of cardiomyocytes after CPB in the HTK group. C: Apoptosis of cardiomyocytes after CPB in the STH group. D: Comparison of the apoptotic index of the three groups, *P < 0.05 compared with the non-stop group; #P < 0.05 compared with the HTK group.