A fetal goat model of cardiopulmonary bypass with cardioplegic arrest and hemodynamic assessment

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Objective: Increasing evidence shows that some cardiac defects may benefit from fetal interventions, including fetal cardiac surgery. We attempted to develop an in vivo animal model of fetal cardiopulmonary bypass with cardioplegic arrest.

Methods: Operations were performed on 14 pregnant goats. The extracorporeal circulation circuit consisted of a centrifugal pump, silicone tubings with an inner diameter of 6 mm, a roller pump, and a reservoir. The placenta was the sole oxygenator. Cardiopulmonary bypass was maintained at a mean flow rate of 344 ± 68 mL/kg/min, including 30 minutes of cardiac arrest and 15 minutes of reperfusion. Mean arterial blood pressure and heart rate were monitored. Arterial blood samples were analyzed. The pulse index and resistance index of the fetal umbilical artery were monitored.

Results: Experiments were completed in 11 cases (79%), with the fetuses weighing 0.65 to 1.8 kg. Fetal mean arterial blood pressure and heart rate remained stable throughout the experiments. A decrease in partial pressure of oxygen with concomitant increase in carbon dioxide partial pressure was noted, but trends were relatively stable. Metabolic acidosis was recognized during and after cardiac bypass. The pulse index and resistance index of the umbilical artery increased significantly after 2 hours off bypass.

Conclusions: We confirmed the technical feasibility of establishing an in vivo model of fetal cardiac bypass with cardioplegic arrest. This fetal goat model provides reproducible data and is suitable to study clinically relevant problems related to fetal cardiopulmonary bypass, myocardial protection, and hemodynamics. (J Thorac Cardiovasc Surg 2011;142:1562-6)

Some severe fetal cardiac disease is progressive and involves secondary damage to the rapidly growing pulmonary bed and ventricles,¹⁻³ affecting postnatal treatment options and prognosis, and may require palliative rather than definitive surgical procedures. Some fetuses with cardiac abnormalities may also develop cardiac failure and fetal hydrops, leading to a higher rate of intrauterine fetal demise or early neonatal death. Theoretically, in utero therapy might improve the outcome. There is increasing evidence that some of these defects may benefit from fetal interventions, including fetal cardiac surgery.⁴⁻⁶ If the primary lesion is corrected in the fetal period, secondary

damages that might be irreversible and result in single ventricle physiology can be avoided.

There is no safe and effective method to gain access to perform open surgery in the fetus. A motionless and bloodless field is absolutely necessary to permit the meticulous intracardiac repair. Furthermore, little is known about the pathophysiologic effect of cardiopulmonary bypass (CPB) with cardioplegic arrest on the developing fetus.

The feasibility of safely supporting the fetus during bypass has been demonstrated, when placental dysfunction was prevented by overcoming the detrimental response of the placenta to bypass.⁷⁻⁹ Studies on fetal myocardial protection have been carried out, but research is limited and principally relies on isolated heart models.^{10,11} The requisite techniques to safely arrest and protect the fetal myocardium during cardiac interventions need to be developed.

By using existing beating-heart models of CPB in fetal goats, we developed a clinically relevant model of performing open surgery that allows administration of antegrade cardioplegia and crossclamping in the fetus. We also assessed the hemodynamics of fetal-placental circulation after fetal cardiac bypass. This novel model enables us to study the various pathophysiologic effects of CPB with cardioplegic arrest on hemodynamics and myocardial protection in vivo. Defining these mechanisms has significant

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Abbreviations and Acronyms

CPB	= cardiopulmonary bypass
EDV	= end-diastolic velocity
HR	= heart rate
MAP	= mean arterial pressure
PI	= pulse index
RI	= resistance index
SPV	= systolic peak velocity
TAMAX	= time-averaged maximum velocity

implications for the successful translation of fetal cardiac surgery in vivo.

MATERIALS AND METHODS

Animals

The study protocol was approved by the Animal Research Committee of Guangdong Provincial People's Hospital. All animals were handled in accordance with the Guidelines of Guangdong Provincial People's Hospital for Laboratory Animal Studies. Operations were performed on singleton and twin fetuses carried by mixed-breed, time-dated pregnant goats (110–130 days, term = 150 days).

Anesthesia and Monitoring

Pregnant goats ($n = 14$) were anesthetized by intramuscular injection of 10 to 20 mg/kg ketamine (Gutian Pharmacy Co, Fujian, China) and then intubated endotracheally. Arterial oxygen saturation was maintained at 100%, and arterial carbon dioxide tension was maintained at approximately 30 mm Hg. Catheters were placed in the goats' femoral artery and jugular vein for measurement of blood gases and delivery of intravenous fluids, respectively. Maternal arterial pressure and heart rate (HR) were monitored. Anesthesia was maintained with 5 mg/kg intravenous fentanyl per hour (Renfu Pharmacy Co, Yichang, China) and 0.1 mg/kg intravenous vecuronium per hour (Xianjun Pharmacy Co, Zhejiang, China).

Operation

The uterus was exposed through a midline laparotomy. The number and orientation of the fetus were determined, and 50 mg fentanyl and 0.2 mg vecuronium were administered intramuscularly to the selected fetus. A hysterotomy was performed to expose a forelimb in which the axillary artery was cannulated to monitor fetal arterial blood pressure and HR. Anesthesia was maintained with ketamine 5 mg/h via the axillary vein.

Fetal Cardiac Pulmonary Bypass

The extracorporeal circulation circuit consisted of a nonpulsatile centrifugal pump (BP50; Medtronic, Inc, Minneapolis, Minn), silicone tubing with an inner diameter of 6 mm for the arterial and venous line (length 90 cm), another silicone tubing coupled to a roller pump (Stockert Instrumente GmbH, Munich, Germany), and cardiotomy reservoir with filter via a Y connector into the venous line of the extracorporeal circuit to confirm the left heart venting (Figure 1). The placenta was the sole oxygenator. Approximately 50 mL of the heparinized maternal blood containing 10 mg heparin, 50 mg methylprednisolone, and 5 mL 5% sodium bicarbonate was used to prime the pump circuit.

A midline fetal sternotomy was performed, and the pericardium was opened. Heparin (10 mg; Shanghai No 1 Biochemistry Pharmacy Co, Shanghai, China) was administered intravenously before cannulation. A fetal cardiac bypass was instituted after cannulation of the main pulmonary

FIGURE 1. Extracorporeal circuit consisted of centrifugal pump (A), flow probe (B), arterial cannula (C), venous cannula (D), and silicone tubing with an inner diameter of 6 mm for the arterial and venous lines. Another silicone tubing coupled to a roller pump and reservoir with filter was inserted via a Y connector (E) into the venous line of the extracorporeal circuit for left heart venting (not shown).

artery using an 8F DLP arterial cannula (Medtronic, Inc) and the right atrium using a 16F straight venous cannula (Medtronic, Inc). After ensuring the absence of any air bubbles within the circuitry, CPB was initiated. Because of the need for high flows when the placenta is incorporated into the circulation for oxygenation, pump flows were maintained at the maximum achievable flow in each animal (the mean value for a stable pump flow is 344 ± 68 mL/kg/min). Fetal body warming was ensured with an infrared lamp and gauze soaked in warm saline. Fetal body temperature was satisfactorily maintained during and after bypass, and the maximum gradient between pre-bypass and bypass temperatures did not exceed 3°C .

Fetal Cardiac Arrest

Immediately on reaching stable target bypass flow rates (2–3 minutes), the aorta was crossclamped and cardioplegic arrest was induced by cold modified St Thomas cardioplegia (4°C) without calcium solution to a total of 25 mL/kg estimated fetal body weight. Topical cooling using ice slush was used. A left heart venting catheter through the left auricle coupled to a reservoir and roller head pump was used for decompression of the fetal heart. After 30 minutes of myocardial ischemia, the aortic crossclamp was released and the heart was reperfused. After clamp removal, the fetal heart spontaneously resumed normal sinus rhythm. Bypass continued for 15 minutes before the animals were weaned from CPB. Heparin was not antagonized routinely. After cessation of CPB, the animals were decannulated. The fetuses were then monitored for 2 hours. After the completion of study, the fetuses were killed for autopsy and measurement of fetal weight.

Ultrasonographic Measurements

For ultrasonographic examinations, the ultrasound system GE LOGIQ book XP (GE, Wuxi, China) equipped with a (4–10 MHz) linear transducer probe for B-mode and color-coded and pulsed Doppler measurements was used. The umbilical artery was first visualized by means of color-coded Doppler. Then a pulsed Doppler flow velocity waveforms sample volume was placed exactly in the center of the color-coded blood flow, and the waveforms of at least 3 consecutive cardiac cycles were recorded; thus, the measurements of 3 different waveforms for each vessel were taken. The systolic peak velocity (SPV), end-diastolic velocity (EDV), time-averaged maximum velocity (TAMAX), pulse index (PI), and resistance index (RI) were automatically calculated for each waveform ($\text{PI} = \text{SPV} - \text{EDV}/\text{TAMAX}$, $\text{RI} = \text{SPV} - \text{EDV}/\text{SPV}$).

TABLE 1. Fetal hemodynamic data and results of blood gas analysis ($\chi^2 \pm$ standard deviation)

	Before bypass	On bypass	Cardiac arrest	1 h off bypass	2 h off bypass
HR (beat/min)	177 $\pm$ 25	129 $\pm$ 24	0 $\pm$ 0	149 $\pm$ 29	139 $\pm$ 25*
MAP (mm Hg)	64.9 $\pm$ 7.8	63.6 $\pm$ 16.9	51.2 $\pm$ 14.9	60.6 $\pm$ 7.8	59.6 $\pm$ 10.7
PACO ₂ mm Hg	37.9 $\pm$ 9.5	43.5 $\pm$ 15.9	41.6 $\pm$ 12.1	46.2 $\pm$ 12.3	49.0 $\pm$ 14.7*
PaO ₂ mm Hg	29.6 $\pm$ 5.5	23.4 $\pm$ 13.6	29.9 $\pm$ 11.5	24.3 $\pm$ 8.8	23.3 $\pm$ 7.7*
Base excess (mmol/L)	-5.4 $\pm$ 4.3	-4.7 $\pm$ 3.9	-3.8 $\pm$ 3.6	-14.5 $\pm$ 3.8*	-15.5 $\pm$ 4.4*
Lactic acid (mmol/L)	3.1 $\pm$ 1.6	6.2 $\pm$ 2.5*	5.8 $\pm$ 1.4*	10.6 $\pm$ 2.4*	10.9 $\pm$ 2.7*
pH	7.33 $\pm$ 0.11	7.30 $\pm$ 0.13	7.32 $\pm$ 0.15	7.11 $\pm$ 0.12*	7.08 $\pm$ 0.17*
Hematocrit (%)	33 $\pm$ 7	24 $\pm$ 6*	20 $\pm$ 6*	25 $\pm$ 6*	23 $\pm$ 7*

* $P < .05$ vs before bypass values.

Data Acquisition

Maternal and fetal HRs and arterial blood pressure were monitored continuously during the experiments. Maternal and fetal arterial blood was collected for blood gas and metabolite analysis immediately on gaining arterial access, on cardiac bypass before arrest, after 15 minutes of arrest, and 60 and 120 minutes after weaning from CPB. The PI and RI of the fetal umbilical artery were monitored after heparin administration and 60 and 120 minutes after weaning from CPB.

Statistical Analysis

Data are presented as the mean $\pm$ standard deviation. Data were subjected to repeated-measure analysis of variance. Statistical analysis was performed with the SPSS 13.0 software package (SPSS Inc, Chicago, Ill).

RESULTS

Experiments were completed in 11 cases (79%), with the fetuses weighing from 0.65 to 1.8 kg (1.2 ± 0.36 kg). Pump flow varied from 290 to 480 mL/min with a mean flow rate of 344 ± 68 mL/kg/min at steady state. All experimental animals were hemodynamically stable throughout the study period. Fetal body temperature decreased to 36°C during bypass but slowly warmed to baseline (39°C) after weaning from CPB. Only 1 fetus did not survive to cardioplegic arrest. The cause of fetal demise was tearing of the fragile right atrial tissue during aortic crossclamping. One fetus could not be weaned from CPB because of insufficient cardiac activity most likely due to air embolism to the coronary arteries. One fetus was weaned from bypass for approximately 1 hour but developed low cardiac output with severe low blood pressure. We reinstituted extracorporeal circulation, but the fetus could not be weaned from CPB again. The cause of cardiac failure was attributed to air embolism to the coronary arteries.

Fetal mean arterial pressure (MAP) remained stable throughout the experiments at each time point, and there was no difference between corresponding time intervals. Fetal HR was well maintained. However, HR was significantly decreased at 120 minutes after weaning from cardiac bypass compared with the baseline value (HR, 177 $\pm$ 25 to 139 $\pm$ 25 beats/min; Table 1).

Fetal blood gas analysis is shown in Table 1. A stable decrease in partial pressure of oxygen (PO₂) with a concomitant stable increase of carbon dioxide partial pressure

(PCO₂) was noted. At 120 minutes after weaning from cardiac bypass, the PACO₂ (37.9 ± 9.5 to 49.0 ± 14.7 mm Hg) and PaO₂ (29.6 ± 5.5 to 23.3 ± 7.7 mm Hg) were significantly different from that of the baseline ($P < .05$ for both), suggesting impairment of the placental function. Metabolic acidosis was also noted during and after cardiac bypass, manifested by the alterations in lactic acid, pH, and base excess. These deteriorative responses progressed after cessation of cardiac bypass; however, no significant difference was seen 1 to 2 hours after cardiac bypass. The hematocrit decreased significantly on initiation of CPB (pre-CPB 33 ± 7 ; on CPB 24 ± 6) because of fetal hemodilution and surgical blood loss, but remained stable for the remaining period of the experiment.

The PI and RI of the umbilical artery remained the same as at baseline 1 hour after off CPB but were significantly elevated 2 hours after off bypass, suggesting there was an increase in placental vascular resistance and that placental dysfunction was a time-dependent process (Table 2).

DISCUSSION

The present study describes a fetal goat model of CPB including cardioplegic arrest and ischemia, weaning from CPB, and assessment of hemodynamic. The model provides reproducible stable hemodynamics 120 minutes after CPB and offers a reproducible and reliable method to study in-vivo effects regarding hemodynamic and myocardial performance after fetal cardiac arrest. A number of fetal goat CPB models have demonstrated that it is technically possible to place the fetal goat on CPB and separate from CPB. Furthermore, Fenton and colleagues⁷ and Reddy and colleagues¹² reported their experiences with fetal goat bypass models that subsequently delivered normal full-term fetuses. Of note, all the different experimental settings have been performed on beating-heart CPB. To date, no

TABLE 2. Pulse and resistance indexes of umbilical artery ($\chi^2 \pm$ standard deviation)

	Before bypass	1 h off bypass	2 h off bypass
PI	0.69 $\pm$ 0.16	0.64 $\pm$ 0.13	0.89 $\pm$ 0.06*
RI	0.49 $\pm$ 0.10	0.48 $\pm$ 0.09	0.58 $\pm$ 0.04*

* $P < .05$ vs. 1 h off bypass values.

successful fetal model of CPB with aortic crossclamp and cardioplegic arrest has been developed, and little is known about the pathophysiologic effect of CPB including cardiac arrest on the developing fetus. Malhotra and associates^{10,11} used an isolated fetal heart preparation in an attempt to examine fetal myocardial protection. To our knowledge, the only model of in vivo fetal CPB with cardioplegic arrest to evaluate the various ways of myocardial protection was reported by Petrucci and associates,¹³ but their experimental animal could not be separated from CPB. An animal model of CPB including cardiac arrest and ischemia, weaning from CPB, on an experimental basis is essential for future investigation of fetal intracardiac intervention.

Of the 14 experiments we conducted, 11 animals survived at least 120 minutes post-CPB. In the 3 fetuses that did not survive to weaning from bypass, the main cause of failure was tearing of the fragile right atrial tissue and insufficient cardiac activity most likely secondary to coronary air embolism. Our circuit design is a closed system that has no mechanism for preventing air embolism from the venous cannulation site if the venous line pressure becomes subatmospheric in case of tearing of the right atrium. By incorporating a pericardial pledget into the atrial purse-string as described,^{14,15} this technical fault is preventable in future experiments.

To obtain higher fetal bypass flow rates, after trials with straight or angled venous cannulae ranging from 12F to 16F, we found that the 16F straight venous cannula is the best fit for a fetus of this size, with the greatest ease of insertion and adequate drainage. Despite the suitable venous cannulae and verification of right atrial emptying during bypass, poor decompression of the fetal heart was observed. We noted the patent foramen ovale was unusually positioned distally toward the inferior vena cava. The patent foramen ovale opens like a valve, allows most of the blood entering the right atrium from the inferior vena cava to pass into the left atrium, and prevents passage of blood in the opposite direction because the septum primum closes against the relatively rigid septum secundum. A left heart vent catheter through the left auricle coupled to a reservoir and roller head pumps solved this particular problem and provided stability during bypass. In addition, this approach had the added advantage of saving blood by allowing its recirculation from the surgical field through the roller pump.

Our group elected to use the autoplacenta for fetal gas exchange to simplify the circuit and avoid activation of a stress response associated with exposure to foreign surfaces (artificial membrane lung, CPB tubing).^{16,17} The results of blood gas analysis showed that PO_2 and $PACO_2$ were all adversely affected, but within an acceptable range, and represented good gas exchange effects of the autoplacenta. Our results suggested the modulating effect of the autoplacenta on fetal gas exchange mechanism, at least for a short arrest

period of 30 minutes and a total CPB time not more than 1 hour.

The present study also showed that cold modified St Thomas cardioplegia excluding any calcium solution, which is most commonly used in neonatal surgery in Guangdong Provincial Cardiovascular Institute, provided quick arrest of the fetal heart. Previous work has demonstrated that hypocalcemic cardioplegia solutions offer better protection in neonates¹⁸ and in vitro studies examining the effect of cardioplegia calcium content on fetal hearts.¹⁰ In our study, immediate return to regular sinus rhythm and restoration of preoperative hemodynamics presented further indirect evidence that the cardioplegic regimen we used provided optimal preservation of fetal myocardial function.

Despite our successful fetal support during bypass and the ease of weaning from the pump and the fetal goats survived (fetal HR and MAP remained stable during and after CPB), fetal gas exchange deteriorated (Table 1). In particular, mixed metabolic and respiratory acidosis developed after weaning from bypass. Blood gas values were used as an index of placental function because conditions of the mother remained stable throughout the procedure. We ascribe fetal acidosis to placental dysfunction from hysterotomy, technical limitations, stress associated with CPB¹⁹⁻²¹ and cardiac arrest, or perhaps the exposure of such fragile fetuses. This study also showed that the immature fetus is susceptible to increase in placental vascular resistance (Table 2) and less capable of compensating for metabolic demands.^{22,23} Furthermore, inadequate flows (344 ± 68 mL/kg/min) during CPB and cardiac arrest also may have played a role in poor gas exchange because previous investigators have reported better placental gas exchange with higher flows (400–600 mL/kg/min).²⁴ In our experiments, there was no significant difference in the hemodynamic and metabolic status between 1 and 2 hours after cardiac bypass, suggesting that the deleterious effects of fetal CPB may not be progressive after the first hour off bypass.

CONCLUSIONS

This study confirmed the technical feasibility of establishing an in vivo model of fetal cardiac bypass with cardioplegic arrest and successful weaning from CPB. The data show that this fetal goat model provides reproducible data and is suitable to study clinically relevant problems related to fetal CPB with cardioplegic arrest, myocardial protection, and hemodynamics. Placental dysfunction was generally not prevented in this study. Further experimental evaluation including bypass circuit design, pharmacologic or other means to alleviate the adverse effects of CPB-induced inflammatory response on placental function, and comprehensive fetal perioperative monitoring will be necessary to move forward to the clinical application of this challenging surgical intervention.

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References

1. Michelfelder E, Gomez C, Border W, Gottliebson W, Franklin C. Predictive value of fetal pulmonary venous flow patterns in identifying the need for atrial septoplasty in the newborn with hypoplastic left ventricle. *Circulation*. 2005; 112:2974-9.
2. Rychik J, Rome JJ, Collins MH, DeCampi WM, Spray TL. The hypoplastic left heart syndrome with intact atrial septum: atrial morphology, pulmonary vascular histopathology and outcome. *J Am Coll Cardiol*. 1999;34:554-60.
3. Taketazu M, Barrea C, Smallhorn JF, Wilson GJ, Hornberger L. Intrauterine pulmonary venous flow and restrictive foramen ovale in fetal hypoplastic left heart syndrome. *J Am Coll Cardiol*. 2004;43:1902-7.
4. Allen BS. Fetal cardiac surgery: simplicity versus success in a new frontier. *J Thorac Cardiovasc Surg*. 2003;126:1254-6.
5. Hanley FL. Fetal cardiac surgery. *Adv Card Surg*. 1994;5:47-74.
6. Park HK, Park YH. Fetal surgery for congenital heart disease. *Yonsei Med J*. 2001;42:686-94.
7. Fenton KN, Zinn HE, Heinemann MK, Liddicoat JR, Hanley FL. Long-term survivors of fetal cardiac bypass in lambs. *J Thorac Cardiovasc Surg*. 1994;107: 1423-7.
8. Reddy VM, Liddicoat JR, Klein JR, McElhinney DB, Wampler RK, Hanley FL. Fetal cardiac bypass using an in-line axial flow pump to minimize extracorporeal surface and avoid priming volume. *Ann Thorac Surg*. 1996;62:393-400.
9. Lubbers WC, Baker RS, Sedgwick JA, Lam CT, Schenbeck JL, McNamara JL, et al. Vacuum-assisted venous drainage during fetal cardiopulmonary bypass. *ASAIO J*. 2005;51:644-8.
10. Malhotra SP, Thelitz S, Riemer RK, Reddy VM, Suleman S, Hanley FL. Fetal myocardial protection is markedly improved by reduced cardioplegic calcium content. *Ann Thorac Surg*. 2003;75:1937-41.
11. Malhotra SP, Thelitz S, Riemer RK, Reddy VM, Suleman S, Hanley FL. Induced fibrillation is equally effective as crystalloid cardioplegia in the protection of fetal myocardial function. *J Thorac Cardiovasc Surg*. 2003;125:1276-82.
12. Reddy VM, Liddicoat JR, Klein JR, Wampler RK, Hanley FL. Long-term fetal outcome after fetal cardiac bypass: fetal survival to full term and organ abnormalities. *J Thorac Cardiovasc Surg*. 1996;111:536-44.
13. Petrucci O, Baker RS, Lam CT, Reed CA, Duffy JY, Eghtesady P. Fetal right ventricular myocardial function is better preserved by fibrillatory arrest during fetal cardiac bypass. *Ann Thorac Surg*. 2010;90:1324-31.
14. Crucean A, Murzi B, Giorgi A, Burchielli S, Trivella M, Coceani F. Cardiopulmonary bypass in ewe's fetus: advances and setbacks in our learning curve. *ASAIO J*. 2005;51:649-53.
15. Lombardi J, Sedgwick J, Schenbeck J, Lubbers W, Ferguson RE, Gardner A, et al. Cardiopulmonary bypass in the immature fetus through novel use of a mini-centrifugal pump. *Perfusion*. 2006;21:185-91.
16. Sabik JF, Heinemann MK, Assad RS, Hanley FL. High dose steroids prevent placental dysfunction after fetal bypass. *J Cardiovasc Surg*. 1994;107:116-24.
17. Bradley SM, Hanley FL, Duncan BW, Jennings RW, Jester JA, Harrison MR, et al. Fetal cardiac bypass alters regional blood flows, arterial gasses, and hemodynamics in sheep. *Am J Physiol*. 1992;263:919-28.
18. Bolling K, Kronon M, Allen BS, Ramon S, Wang T, Hartz RS, et al. Myocardial protection in normal and hypoxically stressed neonatal hearts: the superiority of hypocalcemic versus normocalcemic blood cardioplegia. *J Thorac Cardiovasc Surg*. 1996;112:1193-201.
19. Sabik JF, Assad RS, Hanley FL. Prostaglandin synthesis inhibition prevents placental dysfunction after fetal cardiac bypass. *J Thorac Cardiovasc Surg*. 1992; 103:733-42.
20. Fenton KN, Heinemann MK, Hickey PR, Klautz RJ, Liddicoat JR, Hanley FL. Inhibition of the fetal stress response improves cardiac output and gas exchange after fetal cardiac bypass. *J Thorac Cardiovasc Surg*. 1994;107:1416-22.
21. Su Z, Zhou C, Zhang H, Zhu Z. Hormonal and metabolic responses of fetal lamb during cardiopulmonary bypass. *Chin Med J (Engl)*. 2003;116:1183-6.
22. Reddy VM, McElhinney DB, Rajasinghe HA, Liddicoat JR, Hendricks-Munoz K, Fineman JR, et al. Role of the endothelium in placental dysfunction after fetal cardiac bypass. *J Thorac Cardiovasc Surg*. 1999;117:343-51.
23. Vedrinne C, Tronc F, Martinot S, Rajasinghe HA, Robin J, Allevard AM, et al. Better preservation of endothelial function and decreased activation of the fetal renin-angiotensin pathway with the use of pulsatile flow during experimental fetal bypass. *J Thorac Cardiovasc Surg*. 2000;120:770-7.
24. Hawkins JA, Clark SM, Shaddy RE, Gay WA Jr. Fetal cardiac bypass: improved placental function with moderately high flow rates. *Ann Thorac Surg*. 1994;57:293-7.