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Extrauterine Incubation of Fetal Goats Applying the Extracorporeal Membrane Oxygenation via Umbilical Artery and Vein

The fetus is an unstable subject for an isolated physiological and biochemical study. To study the fetus in a controlled and stable environment, a trial was done using 12 goat fetuses. Extrauterine incubation system was devised using an extracorporeal membrane oxygenation system. The system consisted of a venous reservoir with a servo-controlled roller pump and a membrane oxygenator. The extracorporeal circuit and membrane oxygenator were primed with the maternal whole blood of 200 mL. Fetal umbilical cords was exposed by Cesarean section. Fetal umbilical arterial blood was drained via the drainage cannula. The drained blood was perfused to the oxygenator by the roller pump. The highly oxygenated and decarboxylated blood was returned to an umbilical vein via the perfusion catheter. The blood flow rate was controlled manually using a roller pump. Fetal heart rate, blood pressure, and electrocardiogram were continuously recorded. Gas analysis of drained and perfused bood was performed hourly. With this system, the fetuses were able to survive under fairly stable physiological condition for periods of up to 34 hr. The extrauterine incubation system used in this study could therefore be a encouraging future experimental model in researching the artificial placenta for premature fetuses.

Key Words : *Extracorporeal Membrane Oxygenation; Fetus; Oxygenation; Decarboxylation; Goats*

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INTRODUCTION

The premature detachment of the fetus from its placental connection and its maintenance by an artificial oxygenator may be directly investigated and may have an application in the management of the premature infant. Immaturity of the newborn from preterm labor and delivery still remains to be a major contributor to perinatal morbidity and mortality. The recent disenchantment with the available tocolytic agents and the doubts about their effectiveness have increased the interest in the newer approach for the immature infants. Extrauterine fetal incubation system using arteriovenous extracorporeal membrane oxygenation (AV ECMO) is one of those approaches. Since the successful implementation of an artificial uterus or artificial placenta during the 1960s (1-3), a stride has been lengthened into the long-term extrauterine support with recent advances in ECMO. There are more durable oxygenators, better cannulas, and more sophisticated machineries supporting the ECMO apparatus.

Since fetoplacental circulation consists of an AV shunt, the importance of flow rate and oxygen tension should be taken into account to prevent the constriction of the ductus arteriosus and interruption of fetal circulation. According to Sakata et al. (4), the goat umbilical blood flow accounted for 41% of the combined output of both ventricles. They succeeded in keeping the extrauterine support of goat fetuses up to 237 hr by cannulating two umbilical arteries (UAs). However, in Unno et al.'s system (5), the pump flow consisting of the ECMO drainage obtained from two UAs was low between 60 and 130 mL/min/kg with an open top blood reservoir to provide a constant afterload to the fetal heart.

We hypothesized that an extracorporeal flow rate can be obtained by cannulating an UA using the conventional ECMO system which allows a height difference for the easy drainage of blood. With the aim of establishing an experimental model for an isolated fetus, the first step in the development of an extracorporeal incubation system using goat fetuses is reported.

MATERIALS AND METHODS

Extracorporeal membrane oxygenation circuit

The extracorporeal closed circuit consisted of a roller pump (HPM-15, Nikkiso, Ltd., Tokyo, Japan), a closed reservoir, and a membrane oxygenator (Menox 2000, Kurare Co, Osaka, Japan) with a functional surface area for gas exchange of 0.3 m^2 (Fig. 1). A silicon tube of 3/16 was used for the entire circuit and two 10 F polyvinyl cannulas were adapted for the drainage and perfusion catheterization. Prior to the experiments, the entire extracorporeal membrane oxygenation circuit was prefilled with 200 mL of heparinized maternal blood. The blood was warmed to between 38°C and 39°C by flushing heated gas into a plastic box.

Surgical preparation

This study was approved by the Animal Care and Use Committee of Seoul National University College of Medicine. Twelve pregnant goats weighing between 33 to 40 kg (37.5 ±3.1 kg) were used. They were adapted to the research facility for a couple of days before surgery. The goats were sedated with an injection of Rompun (Xylazine hydrochloride, Bayer Korea Ltd., i.m., 25 mg/kg) and the abdominal wall was clipped before being placed supine.

Goats were subjected to cesarean sections. The surgery was performed around day 120-130 of gestation (term 150±2 days). The gestational age was calculated from the date of mating. Under general anesthesia with halothane, a 10 cm midline laparotomy was done exposing the uterus. Through a small incision of the uterine wall, fetal hind limbs were extracted, and the umbilical cord was completely exposed. Papaverine was injected around the umbilicus to prevent vascular constriction. For a baseline blood gas analysis, about 1 mL of umbilical arterial and venous blood samples were collected before catheterization. Out of four, one umbilical artery and one umbilical vein were used for cannulation. A 10 F polyvinyl catheter (length 15 cm) for drainage was inserted into the umbilical artery and advanced beyond the bifurcation of the abdominal aorta. Another catheter was inserted into the umbilical vein with the tip positioned 2 cm beyond the umbilicus. Then the catheters were connected to the extracorporeal circuit, and the AV ECMO began. The fetus was isolated from the placenta and transferred into the incubator chamber filled with 38°C artificial amniotic fluid. The fluid was a balanced electrolyte solution (Na^+, 75 mEq/L; K^+, 2.0 mEq/L; Ca^+, 1.5 mEq/L; Cl^- 55 mEq/L) containing albumin (2.2 g/L), glucose (130 mg/L), and antibiotics (aminobenzyl penicillin, 1.0 g/L).

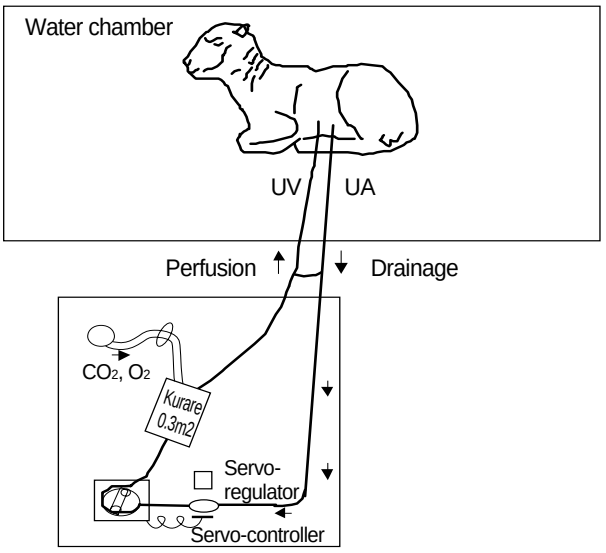

Fig. 1. The extracorporeal blood circuit of the extrauterine fetal incubation system. Blood from the umbilical artery (UA) is drained by the height difference in a reservoir bag. Servo-controller operated by an infrared light on the reservoir regulates the revolution of the roller pump. The drained blood is pumped through the membrane oxygenator and returned to the fetal umbilical vein (UV).

Blood oxygenation was done using a gas blender of 100% oxygen and air through the oxygenator for fetal oxygen tension of 20 mmHg. Glucose (50% solution) was infused to maintain arterial blood glucose level at more than 70 mg/dL. Activated coagulation time was kept around 200 sec by monitoring with a Hemochron (Model 801, International Technidyne Corp., N.J., U.S.A.) with the heparin infusion at 1 U/min/kg. Fetal blood from the umbilical arteries drained into the arterial reservoir by the pressure and height differences. The blood flow rate was controlled manually using a roller pump to maintain the blood volume in the arterial reservoir constant. The amount of blood flow was determined by the revolution frequency of the roller pump. The blood was then returned to the umbilical veins via the perfusion cannula.

Fetal monitoring

For fetal monitoring, blood samples of umbilical artery and umbilical vein were frequently collected from the sampling port of the circuit for hematological, electrolyte, and blood gas analysis. PO_2, PCO_2, pH, SO_2, and base excess were determined by a Critical Care Analyzer (AVL, U.S.A.). Fetal blood pressure and pulse rate were monitored through the cannula from the umbilical artery. The extracorporeal circulatory blood (ECC) flow (mL/min) was determined by an electromagnetic blood flowmeter which was attached to the arterial portion of the blood circuit. Basically, the experiment was continued until fetal death. Following fetal death, the body weight and the cause of death were determined. On autopsy, the patency of ductus arteriosus and foramen ovale were examined.

Data analysis

All values were reported as the mean±standard deviation.

For comparison of data from blood gas analysis between umbilical artery and vein, Student t-test using the SPSS software (version 10.0, 2001) was applied. The following formulas (5) were implemented for the calculation of oxygen delivery (OD, mL/min/kg), oxygen consumption (OC, mL/min/kg), and oxygen extraction rate (OER, %) to determine the fetal oxygen metabolism. Data used for this analysis were available only in 5 animals that survived over 23 hr.

$$OD = (postCO_2 \times ECC\ flow)/100/dry\ body\ weight$$
$$OC = (postCO_2 - preCO_2) \times ECC\ flow/100$$
$$OER = (OC/OD) \times 100$$

RESULTS

The baseline data for blood gas analysis are shown in Table 1. As expected, the carbon dioxide tension in umbilical venous blood was lower than in arterial blood and vice versa for the oxygen tension with significant differences ($p<0.05$).

Table 1. Blood gas analysis of the umbilical artery and umbilical vein before the catheterization for ECMO

	Umbilical Artery	Umbilical Vein
pH	7.35±0.09	7.35±0.11
PO_2 (mmHg)*	20.53±2.54	31.03±13.03
PCO_2 (mmHg)*	54.35±14.55	48.72±13.19
SO_2 (%)*	46.61±18.14	71.56±15.39
HCO_3*	23.32±4.03	21.85±3.74
Na^+	140.67±3.25	140.51±4.29
K^+	5.33±0.71	5.52±0.63
Ca^{2+}	1.35±0.24	1.39±0.26

Values are expressed as mean±SD from twelve goat fetuses. PO_2: oxygen tension, SO_2: oxygen saturation, PCO_2: carbon dioxide tension. *Significant differences between two umbilical vessels at the p-value level of 0.05.

The venous oxygen saturation and bicarbonate ion were significantly higher and lower, respectively, than their counterparts ($p<0.05$), since the oxygenator was a part of the circulatory system. Sodium, potassium, and calcium ions including pH, however, did not show any differences ($p>0.05$) between umbilical artery and vein.

Data from the 12 goat fetuses that underwent AV ECMO are summarized in Table 2. The incubation period ranged widely with a maximum of 34 hr 30 min. The major cause of fetal death was circulatory failure as shown in 8 cases in this study. Hypoxia due to blood clot in the circuit was noted in 1 case, while catheter malfunction in 3 cases. Based on autopsy findings, other factors were related with pleural effusions, ascites, subcutaneous edema, intraperitoneal hemorrhage, and intraabdominal petechiae. Even in some of the cases in which fetal conditions had been excellent at the beginning of the

Table 2. Summary of experiments*

	Body weight (g)†	Incubation (min)	Cause of death	Autopsy
1	820	1,410	circulatory failure	pleural effusion
2	1,225	1,440	circulatory failure	
3	2,329	2,070	circulatory failure	ascites
4	1,900	360	circulatory failure	
5	1,745	1,380	circulatory failure	intraperitoneal hemorrhage
6	1,430	210	hypoxia‡	
7	1,410	180	catheter problem	
8	1,115	1,440	circulatory failure	intraabdominal petechiae
9	1,111	240	catheter problem	
10	1,250	560	circulatory failure	
11	1,180	490	circulatory failure	
12	1,850	1,390	catheter problem	

*Total 6 animals survived over 23 hr. The main cause of death was circulatory failure, †At the end of experiment (1447.5±428.3 g), ‡Due to coagulation in extracorporeal circulatory blood flow.

Table 3. Blood gas analysis of the umbilical artery and umbilical vein

	Time (hr) after start of extracorporeal membrane oxygenation						p value
	1 hr	4 hr	8 hr	12 hr	18 hr	24 hr	
In the umbilical artery							
pH	7.37±0.04	7.33±0.17	7.45±0.92	7.40±0.13	7.38±0.26	7.28±0.12*	0.04
PO_2 (mmHg)	25.2±20.6	23.0±9.4	21.0±5.1	27.9±9.7	37.8±6.9*	36.3±6.5*	0.008
PCO_2 (mmHg)	38.9±18.0	34.0±15.2	31.0±4.2	29.7±7.4	31.5±7.4	27.4±7.6*	0.002
SaO_2 (%)	43.2±5.6	45.0±24.8	49.5±27.2	65.7±10.3*	59.0±10.1	68.6±11.7*	<0.001
BE (mEq/L)	-1.4±0.9	-7.6±6.6*	-3.3±5.6	-4.7±3.3*	-2.2±1.7	-7.0±4.8*	<0.001
In the umbilical vein							
pH	7.39±0.05	7.38±0.17	7.34±0.08	7.39±0.03	7.42±0.07	7.34±0.07	0.05
PO_2 (mmHg)	36.8±18.4	41.3±15.8	42.6±13.1	50.6±29.8*	64.6±25.7*	74.1±27.2*	0.002
PCO_2 (mmHg)	36.1±10.6	30.7±17.7	29.2±2.4	29.0±5.2	30.4±10.9	26.2±2.0*	0.045
SaO_2 (%)	80.2±3.7	77.9±25.2	86.0±11.3	92.5±3.0	100.2±8.2*	100.8±3.6*	0.002
BE (mEq/L)	-1.0±2.5	-2.9±6.5	-4.4±3.6*	-0.8±3.1	-1.3±4.7	-5.5±4.3*	0.001

Values are expressed as mean±SD, n=6. Statistical test was done using by repeated measured ANOVA followed by multiple comparisons. *: $p<0.05$ compared with the values of 1 hr. PO_2: oxygen tension, SaO_2: arterial oxygen saturation, SvO_2: venous oxygen saturation, PCO_2: carbon dioxide tension, BE: base excess.

Table 4. Blood hemoglobin, glucose, pump flow rate, and oxygen utilization

	Time (hr) after start of extracorporeal membrane oxygenation						*p* value
	1 hr	4 hr	8 hr	12 hr	18 hr	24 hr	
Hemoglobin (g/dL)	7.8±1.8	11.4±4.7*	12.5±3.7*	13.3±1.3*	11.0±3.1*	12.4±3.5*	<0.001
Blood glucose (g/dL)	105.0±19.0	92.0±57.0	133.0±123.0	112.0±47.0	94.0±4.0	84.0±17.0	0.162
Oxygen delivery (mL/min/kg)	15.3±1.4	14.1±3.4	16.3±2.4	22.4±2.1*	14.5±3.4	16.8±1.4	0.025
Oxygen consumption (mL/min/kg)	5.8±1.4	4.0±1.2*	3.9±0.8*	6.5±0.9	7.0±1.4	5.1±1.8	<0.001
Fetal O_2 extraction (%)	35.4±5.2	30.0±3.2*	22.1±4.0*	28.5±7.0*	39.1±7.1*	27.2±1.3*	<0.001
Pump flow rate (mL/min/kg)	176.0±15.0	90.0±13.0*	103.0±17.0*	135.0±17.0	135.0±27.0	115.0±18.0*	<0.001

Values are expressed as mean±SD, n=6. Statistical test was done using repeated measured ANOVA followed by multiple comparisons. *; $p<0.05$ compared with the values at 1 hr.

incubation, signs of cardiac failure soon appeared and became progressively worse.

Blood gas analysis data from six fetuses survived over 23 hr are listed in Table 3. The flow rate of the perfusate was kept in the range of 65-220 mL/min/kg (average 176.3±61.9 mL/min/kg, Table 4). In this condition, oxygen tension values of the UA were between 21.0±5.1 mmHg at 8 hr and 37.8±6.9 mmHg at 18 hr. Oxygen tension values of the UV was between 36.8±18.4 mmHg at 1 hr and 74.1±27.2 mmHg at 24 hr. Arterial oxygen saturation in both UA and UV increased as time passed. The mean PCO_2 values were 32 mmHg and 30 mmHg in UA and UV, respectively. The pH values were in the normal range. During the fetal handling, metabolic acidosis frequently associated with hypothermia was not observed.

During the AV ECMO support, the oxygen was continuously supplied to the fetus from 14.1±3.4 to 22.4±2.1 mL/min/kg with an average consumption rate at 3.9±0.8 to 7.0±1.4 mL/min/kg (Table 4). The oxygen extraction ratio was between 22.1±4.0 and 39.1±7.1%. The fetal blood hemoglobin concentration was 11.4±2.0 g/dL and the blood glucose level was maintained above 70 g/dL.

DISCUSSION

We report our first step in the development of an extracorporeal incubation system using the conventional AV ECMO in goat fetuses. An extrauterine fetus is supposed to go through extraordinary environmental hardships during the transition period (6). It is an ideal approach to provide the fetus with an appropriate condition to maximize the survival time. Therefore, the optimal settings of the system should be determined for stable long-term incubation of exteriorized premature fetuses. An AV bypass is used for hemodialysis in renal failure and for extracorporeal lung support in ventilatory insufficiency associated with severe CO_2 retention. A relatively small amount of blood flow through the extracorporeal circuit is needed to improve the above clinical conditions. However, fetal AV ECMO requires a large amount of bypass flow for the total support of fetal oxygenation. Therefore, the control of extracorporeal blood flow is vital to maintain the premature fetus for a long period in good conditions.

Since it has been reported that placental blood flow is greater than 200 mL/min/kg in the sheep fetus (7), many studies attempted to develop a suitable method for controlling blood flow for long-term extrauterine incubation of premature fetuses. Unno et al. (5) cannulated two UAs and UVs and inserted an open top blood reservoir into the extracorporeal circuit between the UA catheter and the roller pump to minimize circulatory resistance changes. They concluded that approximately 100 mL/min/kg of extracorporeal blood flow was desirable to assure the long-term incubation of an exteriorized goat fetus. However, Sakata et al. (4) used a centrifugal pump and removed the blood reservoir to minimize the priming blood volume as low as 95 mL. They also cannulated the two arterial catheters to maintain the arterial drainage blood flow at 170 mL/min/kg. Therefore, the optimal condition of the pump flow rate and oxygenation in the artificial placenta is the issue to be addressed.

The aim of the this study was to establish an experimental model for an isolated goat fetus to be incubated as stable as possible. The present method of fetal catheterization was based on those of Unno et al. (5) and Zahraa et al. (8) with further modification. A drainage catheter was inserted into one UA to be located in the lower part of the abdominal aorta and another UA was catheterized for the fetal blood pressure monitoring. By using the drainage system of the conventional ECMO system which allows the height difference from the animal, blood could flow into the reservoir as much as in the other system using a centrifugal pump. By removing the open top arterial reservoir, the priming volume could be reduced and the chance of blood and air interaction was eliminated.

Under the intrauterine physiologic condition, highly oxygenated umbilical blood passes through the ductus venosus to the heart, the brain, and to the upper body. Poorly oxygenated blood passes through the ductus arteriosus to the lower body and to the umbilical cords, resulting in placental circulation. PO_2 values of UA and UV in the goat fetus are known to range from 17.3 to 24 mmHg and from 30 to 33 mmHg, respectively. Fetal baseline data shown in Table 1 are in agreement with those of previous studies (4, 9). PCO_2 values were higher than normal values probably due to the higher CO_2 production in the room air outside the uterus.

Oxygen consumptions of the fetuses were camparable to those of the previous study (4). However, oxygen delivery was higher and oxygen extraction ratio was lower than the values by Kuwabara et al. (10) probably due to the high pump flow rate.

Previous experiments on an artificial placenta have tried to keep UA PO_2 at about 20 mmHg to prevent constriction of the ductus arteriosus. Prostaglandin E_2 was supplemented to reduce ductal constriction. In our study, the pump flow was maintained as maximum as possible with one drainage catheter, and blood oxygenation was done by controlling the oxygen concentration between 0.6 and 1.0 in the gas flow to the oxygenator. The PO_2 of UA was kept in normal range for 12 hr and after 18 hr ECMO run, the pump flow became irregular followed by an increase of the PO_2 level. Since an increase of the PO_2 level causes vascular constriction, especially of ductus arteriosus, the advantage of prostaglandin infusion has been suggested. Sakata et al. (4) used prostaglandin to prevent ductal constriction at the initiation of artificial circulation, and reported a prolonged incubation. However, in our study no ductal closure was found on autopsy in all fetuses. The cause and effect of the PO_2 level could not be evaluated.

Our artificial placentation made the goat fetuses survive over a wide range of period. The most common cause of death was circulatory failure. The same cause of death was experienced in long-term incubation models. Kuwabara et al. (10) hypothesized that an increase of extracorporeal blood flow may depress the fetal peripheral circulation, resulting in a reduced blood flow to a certain level. They succeeded in minimizing the water retention by the addition of a dialyzing system to the circuit. In our experiment, sodium and potassium concentrations were maintained in the normal physiological ranges. However, the levels of blood urea nitrogen and creatinine were increased significantly from 21 ± 2 to 29 ± 1 and from 1.3 ± 0.5 to 1.8 ± 0.3, respectively, in 3 fetuses after 24 hr of incubation. Therefore, excessive fluid load by the circuit or immature renal function could have caused the water retention, resulting in a circulatory insufficiency. Since the duration of incubation has a great significance for fetal growth and development, an inflatable reservoir located before the infusion of oxygenated blood via the UV catheter to reduce pulsation of blood flow after the roller pump was suggested to relieve the burden on the fetal heart by maintaining a constant blood flow. Because the blood from the umbilical artery is drained by the suctioning power of the roller pump, it might be desirable to compensate the burden by placing a postoxygenator reservoir.

Neither blood coagulation from insufficient heparinization or hemorrhage due to over-heparinization was detected in our study, while they have been reported elsewhere (11, 12) as the other major causes of fetal death. However, it is important to maintain the physiologic fetoplacental circulation condition in an extrauterine fetus with a continuous monitoring of blood gases since its surroundings are still quite different from those of an intrauterine fetus. As far as a proper fetal circulation and fetal blood gas profile are guaranteed, a fetus can maintain its optimal physiological condition outside the uterus. This system could affect extrauterine fetal lung growth and maturation through production of surfactant as previously reported (13).

In summary, we showed that an isolated goat fetus could survive with the support of extracorporeal membrane oxygenation, implying the application of this system for the immature neonates. The simplicity of our extrauterine fetal incubation system requires much improvement. In the next stage of our study, the exact relationship between the oxygen delivery and its consumption with fetal oxygen extraction is expected to be defined because oxygen metabolism can determine the survival time of extrauterine fetuses. In addition, the nutrition of extrauterine fetuses should be taken into account to prevent the fluctuations of acid-base balance. Further investigation into the condition of extrauterine fetuses will surely give us more information for the improvement of the system. By setting up the fetal research model, one could foresee the clinical usage of this kind of approach in premature neonates with severe lung hypoplasia and as a backup system for fetal operations.

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