

Development of an Artificial Placenta

Introduction

Fetal medicine is a rapidly progressing new field. Numerous fetal abnormalities have been accurately detected prenatally with recently developed technologies such as cardiotocography, ultrasonography and magnetic resonance imaging. It has long been recognized that some of these abnormalities are better treated in utero to achieve favorable outcomes; however, currently available techniques for fetal therapy have been proved to have a limited clinical effectiveness. For example, congenital diaphragmatic hernia in its severe form, which is basically lethal after birth, can now be repaired during fetal life by fetal surgery.¹ This congenital defect causes lung hypoplasia, which is essentially untreatable after birth. Therefore congenital diaphragmatic hernia is a strong candidate for fetal surgery if it is clinically effective. However, due to the difficulty in controlling uterine activity following open fetal surgery, the technique has not yet been recognized as a standard approach for treating the disease. Indeed, uncontrollable uterine activity is one of the most troublesome complications of open fetal surgery in humans because premature delivery immediately after the surgery makes all the obstetrical and surgical efforts meaningless for the prognosis of the affected fetus. For researchers and clinicians in the field of perinatal medicine, it appears that the primate uterus is extraordinarily sensitive to external stimuli compared with that of other species such as the sheep. Therefore, for establishing fetal surgery as a standard clinical technique, it is extremely important to develop a new system for those fetuses unprepared for an extrauterine environment, which were obliged to be born due to a too sensitive uterus following fetal surgery. In this article, an approach for the extrauterine incubation of premature fetuses/the newborn is described.

Incubation of a fetus with an artificial apparatus has been a dream of human beings for very many years. In 1932, in a novel titled *Brave New World*, Aldous Huxley described a kind of artificial placenta:

Mr. Foster described the artificial maternal circulation installed in every bottle at Metre 112; showed them the reservoir of blood-surrogate, the centrifugal pump that kept the liquid moving over the placenta and drove it through the synthetic lung and waste product filter. Referred to the embryo's troublesome tendency to anaemia, to the massive doses of hog's stomach extract and foetal foal's liver with which, in consequence, it had to be supplied.

It appears that Huxley imagined a kind of whole embryo in vitro culture system, which has been shown to be able to incubate the developing mouse fetus up to 11–12 days of gestation (term 19–20 days).² It is the true artificial uterus. Although this approach is extremely attractive, it is not applicable to clinical medicine because, once the placenta is attached to the uterus, it is not detachable as a viable organ. However, Huxley's extraordinary imagination actually described most of the problems experienced by researchers of the artificial placenta during the last four decades. In all the preparations, a blood reservoir, synthetic lung and waste product filter were used. All the fetuses with the artificial placenta did have a tendency to become anemic. In a recently published paper, a centrifugal pump was introduced to the system to minimize the

destruction of blood components by the extracorporeal circuit.³

Actual efforts for the development of a clinically applicable artificial placenta system commenced in the late 1950's. The following papers were a part of the achievements in Europe, Canada and the United States before we started our projects in 1984.

- Harned HS, et al. The use of the pump oxygenator to sustain life during neonatal asphyxia of lambs. *AMA Journal of Diseases of Children Society Transactions*, 94: pp.530 – 531. 1957
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- Alexander DP, et al., Survival of the foetal sheep at term following short periods of perfusion through the umbilical vessels. *J Physiol*, 175: pp.113 – 124. 1964
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- Chamberlain G. An artificial placenta: the development of an extracorporeal system for maintenance of immature infants with respiratory problems. *Am J Obstet Gynecol*, 100:pp.615–626. 1968
- Alexander DP, et al. Maintenance of sheep fetuses by an extracorporeal circuit for periods up to 24 hours. *Am J Obstet Gynecol*, 102: pp.969–975. 1968
- Zapol WM, et al. Artificial placenta: Two days of total extrauterine support of the isolated premature lamb fetus. *Science*, 166: pp.617–618. 1969
- Standaert TA, et al. Extracorporeal support of the fetal lamb simulating in utero gas exchange. *Gynecol Invest*, 5: pp.93–105. 1974

Tremendous efforts by many groups for over 15 years clearly indicated that a short-term extrauterine incubation (for up to 2 days) of viable or previable animal (mainly sheep) fetuses in a warmed fluid container is feasible using extracorporeal circulation mimicking umbilical blood flow and placental oxygenation. However, these efforts were entirely abandoned by 1980. The reason for this was the these groups mainly aimed to use this kind of system (artificial placenta) for the treatment of neonatal respiratory distress syndrome, which was regarded as the life-threatening factor of the premature newborn at that period. For the treatment of this troublesome syndrome, pediatricians developed a completely different approach: continuous positive airway pressure (CPAP) and intermittent mandatory ventilation (IMV) using a mechanical ventilator. Introduction of these new methods dramatically improved the prognosis of the premature newborn with respiratory distress syndrome, although many problems remained. When compared to the successful clinical achievements of CPAP and IMV, the extracorporeal blood circuit of an artificial placenta appeared too complex and dangerous for clinical use. Therefore researchers decided to stop studies for premature neonates.

After an almost 10 year interval, our group headed by the late Prof. Yoshinori Kuwabara

started a new project aimed at developing a new artificial placenta system not only for the treatment of extremely premature neonates untreatable by conventional ventilator methods, but also for the experimental study of fetal physiology.

Methods of extrauterine fetal incubation (EUFI)

The methods presented here were developed based on the results of the preliminary experiments carried out over 6 years.

Animal preparation

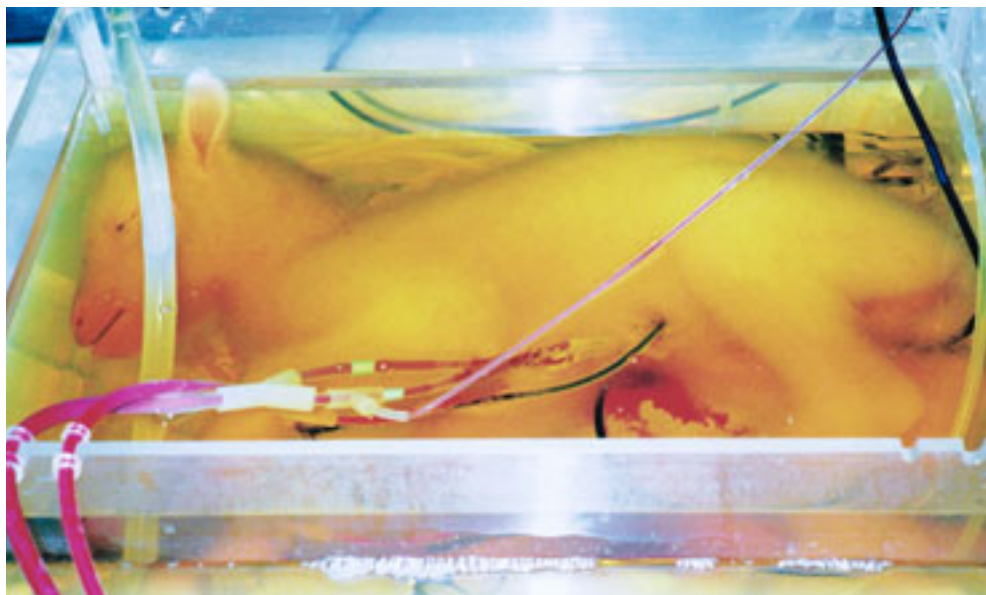
All the experiments were conducted with the approval of our institutional review body. A Cesarean section was performed on pregnant goats with a singleton fetus (term 148 days) and their fetuses were connected to an ECMO circuit. Hysterotomy was performed under general anesthesia with 2% halothane. Fetal hind legs were extracted until the umbilicus was fully exposed. An umbilical artery and vein were isolated. (Goats have two umbilical arteries and two normal veins.) A polyvinyl catheter (length, 20 cm; outer diameter, 10 Fr.) was inserted through an arteriotomy and advanced beyond the bifurcation of the abdominal aorta. Another catheter was inserted into an umbilical vein, with the tip positioned 2 cm beyond the umbilicus. During this procedure, fetal blood-gas exchange was maintained through placental circulation via the remaining umbilical artery and vein. An A-V ECMO commenced immediately after the connection of the catheters to the extracorporeal circuit. The remaining pair of umbilical vessels were cannulated and connected to the circuit. The fetus was transferred to an incubator containing artificial amniotic fluid warmed to 39.5°C. The artificial amniotic fluid consisted of an electrolyte solution (Na^+ 75 mmol/L, K^+ 2.0 mmol/L, Ca^{2+} 0.8 mmol/L, Ca^- 55 mmol/L) based on the analysis of goat amniotic fluid. The total amount of time needed from the hysterotomy until fetal transfer to the incubator was < 30 min.

The incubation system

The extracorporeal circuit consisted of an arterial open-top reservoir (maximum volume 25 ml), a roller pump, a nonmicroporous membrane oxygenator made of hollow silicone fibers, a closed inflatable reservoir and a heat exchanger. The oxygenator had a functional surface area for gas exchange of 0.5 m². The priming volume of the circuit was 200-230 ml. The priming solution consisted of maternal blood anticoagulated with heparin and balanced appropriately for pH, Na^+ , K^+ , and Ca^{2+} . Fetal blood from the umbilical artery catheters drained into the arterial reservoir. Blood flow through the circuit was regulated by a flow-control system to maintain a constant blood volume in the arterial reservoir. The blood was oxygenated with 100% oxygen and returned to the umbilical veins via a closed reservoir and heat exchanger.

The incubator containing sterile artificial amniotic fluid was placed on a clean bench. All the procedures to the fetus were performed with a strict sterile technique to minimize the risk of infection. A heparin solution (400 units/ml) was continuously infused into the circuit to keep the activated coagulation time between 180 and 250 s. The amount of heparin required was 40 to 60 units/kg/h. The fetus was left without anesthesia in the incubator filled with artificial amniotic fluid maintained at 39.5°C.

The length of the umbilical catheters allowed for spontaneous fetal movement. These settings provided the fetus with a physiological, thermal, and unrestrained environment. A solution containing 30% glucose, 3% amino acids, and 1.5% soybean oil was administered to the fetus via extracorporeal circuit at a rate of 2 mL/kg/h, which amounted to 70 kcal/kg/d. Fetal body weight was estimated at the beginning of incubation from a standard curve using crown to rump length.



A goat fetus after 10 days of extrauterine incubation

Extracorporeal circuit blood-gas exchange adjustments

The initial adjustments of the settings of extracorporeal circuit were completed with repeated measurements of fetal arterial blood gases within the first 24 h of incubation. In this system, fetal arterial partial pressure of oxygen (PaO_2) is a function of the inspired oxygen fraction (FIO_2), the fetal oxygen consumption, the extracorporeal blood flow (QEC, mL/min) and the oxygenator performance. Previous studies by other investigators and ourselves revealed the difficulty in maintaining QEC within a physiological range of umbilical blood flow (approx. 200 mL/kg/min) in long-term incubation of exteriorized fetuses using A-V ECMO. This is because a large QEC makes the fetal circulatory condition unstable. We maintained FIO_2 at 1.0 to maximize oxygen delivery with any given QEC which enabled us to stabilize the fetal cardiovascular system by reducing the QEC. The QEC was maintained between 60–130 mL/kg/min by altering the impedance of the arterial portion of the circuit using a tube occluder. Under these conditions, the capacity of the extracorporeal circuit to eliminate CO_2 was determined by the inspired gas flow / QEC ratio. Fetal PaCO_2 was maintained by controlling oxygen flow between 5.3–6.7 kPa (40–50 mmHg).

Measurements and calculations

A catheter was inserted into a carotid artery under local anesthesia with 1% lidocaine, allowing arterial blood pressure (aBP) to be monitored continuously. QEC was determined with an electromagnetic flowmeter, attached to the arterial portion of the blood circuit. The heart rate (HR) was counted from the aBP pulse or the umbilical artery waveform. Fetal aBP, QEC and HR were recorded continuously using a polygraph. Fetal core temperature ($^{\circ}\text{C}$) was determined with a temperature probe chronically implanted into the mediastinal space through an incision made in the neck during the arterial catheter insertion. Blood gas tensions and pH were measured by a pH/blood-gas analyzer calibrated at 37.0°C and oxygen saturation (SO_2) and hemoglobin concentration (Hb) with a hemoxymeter calibrated for goat blood using fully saturated

maternal blood. Blood samples from the sample ports at the venous and arterial side of the circuit were taken simultaneously at 3 and 6 h intervals for measurement of pH, PCO₂, PO₂, SO₂, and Hb throughout the EUFI periods. HR, aBP, QEC, and fetal core temperature were recorded during each blood sampling procedure. Pre-oxygenated (arterial side) and post-oxygenated (venous side) blood oxygen content (CO₂), oxygen delivery by extracorporeal circuit (DO₂), and fetal whole-body oxygen consumption (VO₂) were calculated as follows.

$$\text{CO}_2 \text{ (mL O}_2\text{/L)} = 1.34 \times \text{Hb (g/L)} \times \text{SO}_2 \text{ (\%)} / 100 + 0.03 \times \text{PO}_2 \text{ (mmHg)}$$

$$\text{DO}_2 \text{ (mL O}_2\text{/kg/min)} = \text{post-CO}_2 \text{ (mL O}_2\text{/L)} \times \text{QEC(L/min)} / 1000 / \text{Body weight (kg)}$$

$$\text{VO}_2 \text{ (mL O}_2\text{/kg/min)} = (\text{post-CO}_2\text{(mL O}_2\text{/L)} - \text{pre-CO}_2\text{(mL O}_2\text{/L)}) \times \text{QEC (L/min)} / 1000 / \text{Body weight (kg)}$$

$$\text{VO}_2 \text{ (mmol/kg/min)} = 44.6 \times \text{VO}_2 \text{ (mL O}_2\text{/kg/min)}$$

Results of long-term incubation experiments

Long-term incubation experiment with unrestrained fetal movement.

Long-term extrauterine incubation was conducted with more than 50 goat fetuses. In our first paper,⁴ we emphasized the importance of an arterial reservoir that maintains cardiac afterload constant. After this modification of an extracorporeal circuit (ECC), the incubation time of the exteriorized goat fetus extended up to 165 h. Use of the modified ECC and improved incubation techniques further prolonged the incubation period to 146 + 61 h (mean + SD), the longest being 236 h.⁵ In this second paper, we also described a stable oxygen delivery and consumption during long-term EUFI. The following three studies (6–8) tested fetal conditions during long-term extrauterine incubation in terms of oxygen metabolism, extracorporeal blood flow rate, and changes in fetal stress hormones. These studies revealed that 1) although the oxygen delivery by ECC remained at the sub-physiological level, the oxygen consumption of the fetuses was maintained within normal range by increasing oxygen extraction; 2) Optimal ECC flow rate was revealed to be 100 ml/min/kg which corresponds to approximately 50% of the physiological placental blood flow; and 3) although fetal stress responsive hormones such as adrenaline, noradrenaline, ACTH and cortisol did increase at the initial stage of incubation, during which the fetus was forced to adapt to a completely new environment, these hormones decreased to low levels after 24 hours of incubation. In addition, these low concentrations were maintained until the final 24 h incubation, during which the condition of the fetus gradually deteriorated.

During this series of experiments, we encountered several serious problems which prevented stable long-term incubation. Among these the most annoying was fetal movement. During the incubation, especially when the condition was stable, fetuses showed a variety of movement: eye rolling, mouthing, swallowing, breathing, twitching, body wriggling, body rolling, body stretching, and various limb movements, for example. In one preparation, a fetus tried to stand up and run. Although these movements are physiological behavior during fetal life in the womb and they clearly indicated that the fetuses in our system were active, they caused serious unwanted system malfunctions including catheter problems. The fetus that wanted to stand up and run died due to massive blood loss from the umbilical vessels from which catheters were pulled out. We lost several fetuses in this way due to unexpectedly vigorous fetal movement. Violent body wriggling produced a sudden though temporary decrease in extracorporeal blood flow. These sudden changes in the cardiovascular system, when they occurred repeatedly, gradually affected the activities of the fetus. In addition, vigorous fetal swallowing movement was a serious problem. They actually drank sur-

rounding fluid intermittently. Apparently, this behavior was unrelated to thirst or fetal body water balance. They just drank amniotic fluid intermittently to train their muscles and digestive system. In the womb, fetal body water balance is maintained by the placenta. Swallowing behavior and urine production are not indispensable during fetal life, although, after birth, they are undoubtedly extremely important for survival in a dry air environment. Therefore, the fetuses in the warmed water bath did not care how much fluid they took. As a result, they gradually became edematous. After several days of incubation, excessive water accumulation resulted in manifest ascites, lung fluid and generalized edema. The water accumulation was an additional load to the fetus' cardiovascular system. These two serious problems, catheter malfunctions and water accumulation, caused by the active movement of the fetuses, led us to consider a suppression of fetal activity using sedatives and muscle relaxants.

Long-term incubation experimentation with suppression of fetal movement using a muscle relaxant and a sedative.

In this protocol,⁹ we suppressed vigorous movement on the part of the fetuses by administering a sedative (Diazepam) and a muscle relaxant (Pancuronium bromide) to the fetus during extrauterine incubation. We simply intended to suppress gross fetal movement to prevent unwanted catheter malfunctions and excessive fluid swallowing, therefore peripheral movements including eye rolling, mouthing and breathing were observed throughout the incubation period. With this protocol, fetal conditions were maintained extremely well compared with the previous preparations. Although values of HR, aBP, and CVP were not completely stable, they were within normal fetal range during most of the incubation periods. All of the parameters relating to blood-gas exchange and oxygen utilization were stable throughout the incubation. The presence of consistent blood flow from the pulmonary artery to the descending aorta was confirmed at the ductus arteriosus by ultrasonographic examination including a pulsed-Doppler test.

The incubation periods extended to 494 h and 543 h in two preparations. In our experimental design, the longest extrauterine incubation period with our system was set at three weeks. Since the gestational ages of the two fetuses for this protocol at the start of extrauterine incubation were 120 and 128 days, after three weeks of incubation in the artificial placenta, they were expected to be at term. At 12 h before the end of incubation with our system, we discontinued the administration of those drugs. When the fetus resumed its active movements, it was removed from the incubator and exposed to the air. Following an insertion of a tracheal tube and careful aspiration of airway fluid, the extracorporeal blood circulation was discontinued and lung respiration was stimulated by manual ventilation with 100% oxygen. When spontaneous breathing appeared weak, mechanically assisted ventilation was employed. In both preparations, the respiratory responses were very weak; therefore mechanical ventilation was initiated. With meticulous management including repeated aspiration of endotracheal secretions, changes in body position, careful adjustment of fluid infusion, and nutritional supplementation, the goats maintained physiological stability. Although they showed active movement, spontaneous ventilation with continuous positive airway pressure was unable to sustain stable blood-gas conditions. We repeatedly observed their efforts to rise to their feet that failed. The endotracheal tube was removed after 4 weeks and 1 week of ventilator support. Both goats died within hours due to respiratory insufficiency. The most probable cause of their inability to maintain stable lung respiration following long-term EUFI was muscular weakness due to long-term immobilization.

Further advances in this field

After the long-term incubation protocol, the main interests of researchers shifted to physiological responses and maturation of the fetus during EUFI. The following studies were part of the achievements.

- Fetal metabolic and endocrine reactivity to changes in ambient temperature was confirmed by a cold exposure experiment during long-term EUFI.¹⁰
- The introduction of a closed circuit using a centrifugal pump with a pulsatile flow synchronized with a cardiac cycle achieved long-term incubation up to 237 h at a high flow rate and at low oxygen tension.³
- Lung maturation indicated by increases in lung surfactant and lung weight was achieved following 5 days of extrauterine fetal incubation with tracheal ligation.¹¹
- The behavioral cycle manifested by fetal movement, electroencephalogram and cardiovascular variables that normally exists during late fetal life is maintained during long-term EUFI.^{12,13}

Discussion

Our EUFI system is one of the alternatives for life support of neonates unable to sustain their lives without help. This is a kind of total life support system for disabled or extremely sick babies. It provides a thermally neutral environment with minimal energy requirements to maintain body metabolism, which is important for the small babies to recover from damage or impaired conditions. It also provides a complete respiratory support without using lungs, which is essential for premature babies with hypoplastic or damaged lungs. To date, we have shown that long-term stable EUFI is feasible for more than 3 weeks, which would enable premature fetuses to become mature enough for extrauterine dry, air-breathing life. Although further studies to find optimal nutritional supply and less stressful incubation conditions are required, our results strongly encourage clinical application of this sort of incubation system in future.

We must be extremely careful when considering the potential impact of this kind of technology on the public. Although the idea of EUFI is a simple extension of the pre-existing neonatal intensive care system for the extremely premature newborn, some may regard it as a futuristic style of pregnancy. At this stage of investigation, all we can say is that long-term EUFI using extracorporeal circulation would be destructively expensive as an alternative for natural intrauterine pregnancy.

- double-walled incubator containing artificial amniotic fluid kept at 39.5°C
- heater with thermostat
- umbilical arterial catheter
- tube occluder
- arterial reservoir with blood level detector
- blood pump
- flow controller
- silicone hollow fiber membrane oxygenator
- venous reservoir
- heat exchanger
- umbilical venous reservoir.

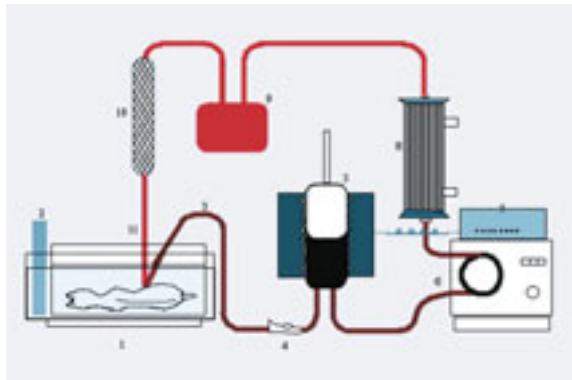


Diagram of extrauterine fetal incubation (EUFI) system

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