

A Method for Making an Animal Model for Fetal Asphyxia in the Goat

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Summary. During the period between 1991 and 1993, an animal model for fetal asphyxia caused by umbilical cord compressions in utero was made using 12 pregnant goats of the Japanese Saanen strain. In fetal research, sheep have generally been used in making fetal preparation. In Japan, however, sheep are difficult to obtain as an experimental animal. In addition, the reproductive period of goats is longer than that of sheep. These factors led us to evaluate, goats as superior to sheep as an fetal experimental models.

The goat fetuses were removed from the uterus at a gestational age of 110 to 130 days. After performing cannulation of the carotid arteries with placement of triolar cardioelectrodes and a balloon catheter around the umbilical cord, the fetuses were returned to the uterus to complete the gestational period.

The time remaining until the termination of the postoperative pregnancy and available for various intra-uterine monitoring procedures varied from 60 to 96 h. We observed fetal bradycardia by compressing the umbilical cord by inflating the cuff with saline. The fetal arterial pH value fell down from 7.38 ± 0.07 to 7.12 ± 0.16 . The continuation of the postoperative gestation for longer than 3 days indicated a return to the physiological state, as evidenced by the measured PO_2 , PCO_2 and pH values.

The results of this study show that we can deduce human fetal physiological events from the results of these experimental models using Japanese Saanen Goats. This animal experimental model using goats permits us study biochemical and biophysical measurements of the fetus in an asphyxiated condition in utero.

INTRODUCTION

Details about the human fetal physiological events in an asphyxiated condition in utero have yet to be elucidated. In the recent years, significant progress in this field has been made quite quickly with rapid advances in obstetrics especially as a result of devel-

opment in fetal heart rate monitoring.^{1,2)} However, in normal clinical practice, it has been quite difficult to make noninvasive physiological studies on the fetus. Therefore, the present-day general practice is to study fetal physiology in animals.³⁻⁵⁾ To our knowledge, sheep have been used in making chronic fetal preparation. There have been no reports concerning fetal physiological research using goats. Goats of the Japanese Saanen strain were used in the present study and made physiological experimental models by cannulation, application of tripolar cardioelectrodes and returning the fetus to the uterus to be carried till term. Biochemical and biophysical measurements were made in these animal models.

MATERIALS AND METHODS

Twelve Japanese Saanen goats were included in this study. To allow the gestational age to be correctly determined, the "marking harness method" was used. This method involves putting a marking harness on the male goat and letting him mate upon confirming his female counterpart to be in heat. After intercourse, the markings on her back were observed. Echographic examinations were performed to assess fetal development and well being.

To allow the female goat to be used in the experimental environment, each animal was transferred to the experimental room to be kept and fed for 2 days prior to the operation. Animals were monitored daily to check their general condition including urine volume and feces discharged and food intake. They were fasted for 24 h prior to the operation to avoid vomiting and difficulties during the operation due to a full stomach.

The operation was performed under halothane general anesthesia. Induction was performed with



Fig. 1. The head of a goat fetus extracted from the uterus and prepared. No initial breathing was observed after extraction from the uterus.

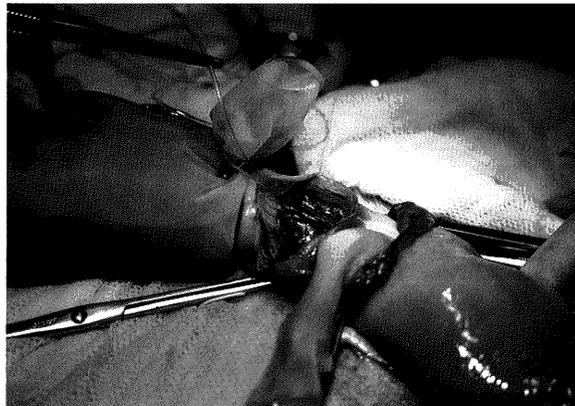


Fig. 2. Carotid artery of goat fetus in sight before cannulation. This is called the exteriorization method or window technique.



Fig. 3. A balloon catheter placed around the umbilical cord of the fetus. Five milliliter of saline water was used to compress the cord.

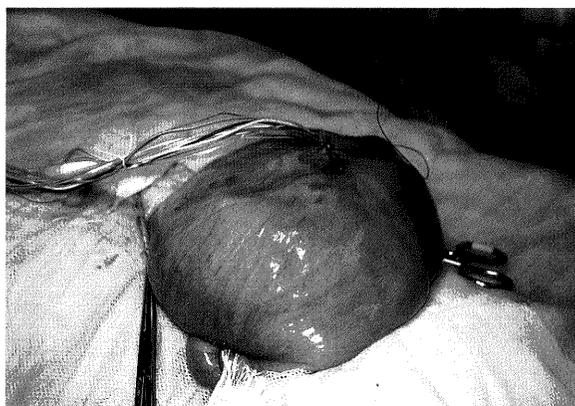


Fig. 4. Suturing of the uterine muscle and a two layer suture of the uterine muscle performed with cut gut.

thiamylal sodium 300 mg. i.v. and intubated 9.5 Fr polyvinyl trachea tube with balloon cuff.

After general anesthesia, the animal was placed on the operating table in a supine position. Following brushing and cleaning with soap, the operation site was disinfected with povidone-iodine and draped with a sterile disposable sheet. After a midline incision was made, the uterus was palpated to establish the fetal presentation. A 1-2 cm incision was made in the myometrium of the uterus. The head of the fetus was pulled out of the wound and wrapped with a rubber glove containing with sterile saline (Fig. 1). A T-shaped hookless forceps was applied to the edge of the wound. An incision was also made in the neck of fetal skin and the trachea was identified by separating the two transverse sternohyoid muscles and transecting them for cannulation. Through the same

incision, access was gained to the carotid artery and the jugular vein. Loose connective tissue was dissected and the adjacent vagus nerve was carefully dislocated, and then a 4-Fr polyvinyl catheter filled with sterile saline was advanced into the carotid artery and the jugular vein. The distal end of this catheter was fixed to the skin with 3-0 silk (Fig. 2). Tripolar cardioelectrodes were placed on the chest of the fetus. The chest of the fetus was extracted to apply the electrodes under the skin. Three electrodes were attached one each on either side of the heart and a third on the flank of the fetus. A balloon catheter for compressing was placed around the cord of the fetus (Fig. 3). It was necessary to pull the upper half of the fetal body out of the uterus for application of the equipments. Upon completion of these procedures, the fetus was returned to the uterus. The uterine

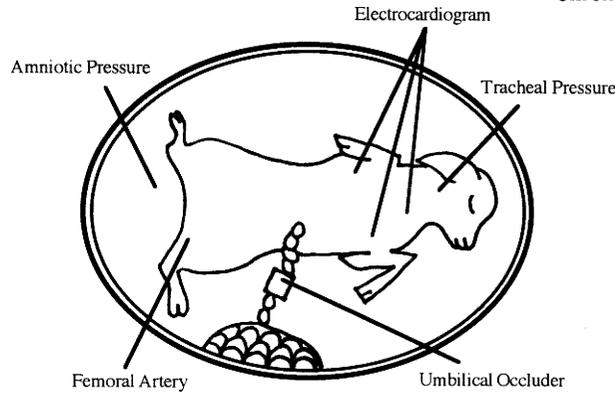


Fig. 5. Schematic illustration of the operation of chronic preparation of the goat fetus.

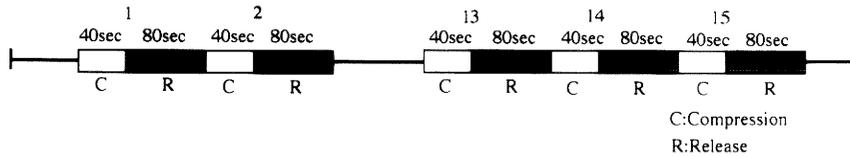


Fig. 6. The design of experiment on asphyxia caused by cord compressions in goat fetuses.

muscle and amniotic membrane were bundled together with the amnion and sutured using 2-0 cut gut. The tubes and cords were led outward without being put together and fastened from the end of the aperture made in the uterine muscle. A Z-figure suture made in the uterine wall allowed us to minimize leakage of the amniotic fluid. The tubes and cords from the wound were gathered and connected to the analyzer (Fig. 4). A schematic illustration of the operation on the goat fetus is shown in Fig. 5.

Postoperative treatment was performed daily, with food intake, stool, and urine output recorded. Blood gas analysis with fetal arterial blood was carried out and fetal well-being was assessed. Antibiotics were administered once a day to the mother and fetus during the postoperative period.

According to the protocol shown in Fig. 6, the experiment was performed two to three days after the operation, because it was necessary for the fetus to recover from the effect of operative intervention. Blood gas values from the fetal aorta were measured with an automated analyzer (CORNING 168 Corning Glass Modified, Mass U. S. A. 02052) and corrected for goat body temperature 39°C. Fetal heart rate and electrocardiogram were monitored with a monitoring telemeter. The data were recorded continuously on a polygraph (RTA-1200, Nippon Kohden, Japan). We also recorded amniotic fluid pressure.

In analyzing the data, the mean and standard deviation were calculated. The level of significance adopted was $p < 0.05$.

RESULTS

The gestational age at the time of operation was 110

Table 1. Biographic data on goat fetuses in the experimental model.

No.	Fetal weight	Single/Twin	Gestational age	Duration of preparation
1.	2950	Twin	116	192
2.	3150	Single	120	96
3.	4800	Twin	135	72
4.	3300	Single	125	120
5.	2860	Single	123	72
6.	2630	Twin	118	72
7.	1750	Twin	128	96
8.	2600	Single	134	96
9.	2400	Twin	128	96
10.	1700	Twin	109	60
11.	2500	Twin	138	72
12.	3400	Single	125	72

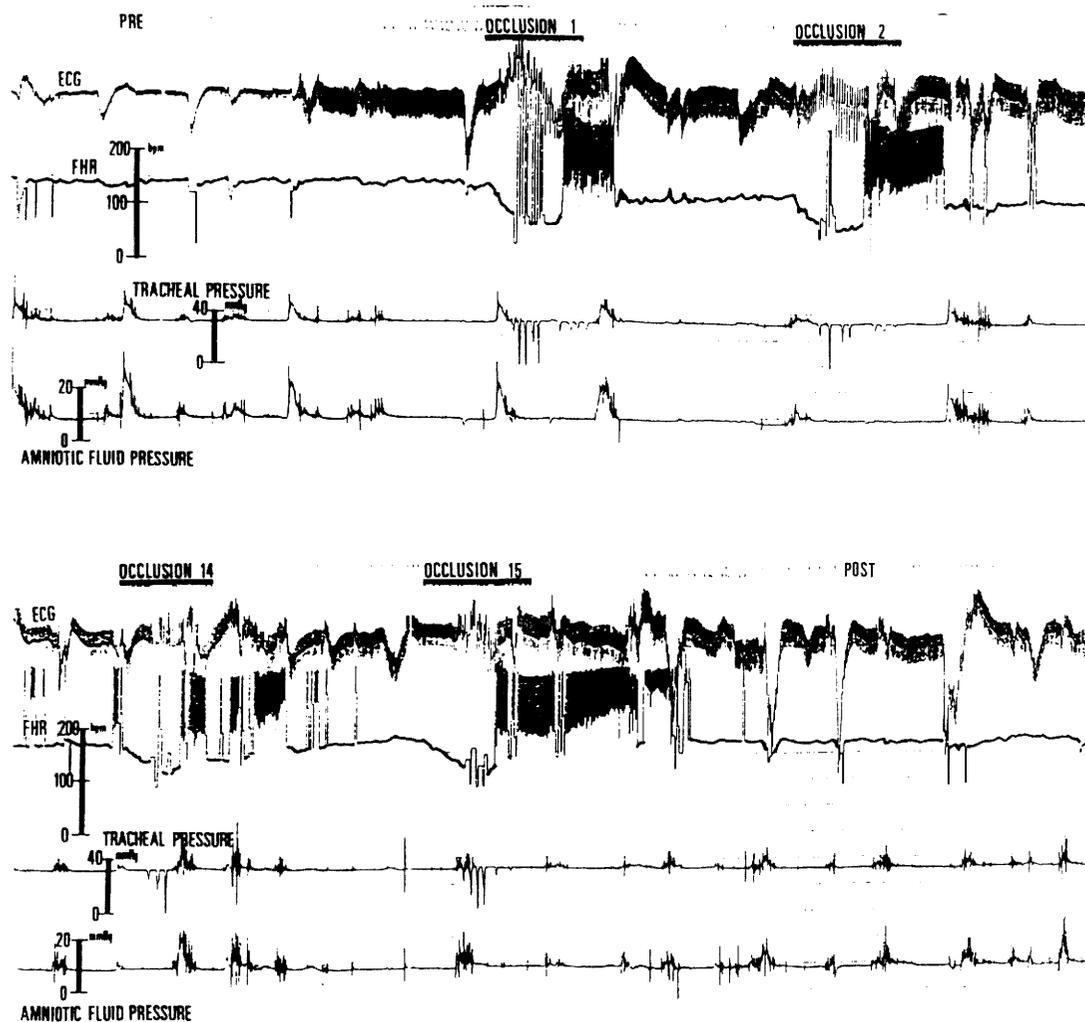


Fig. 7. Recording of fetal heart rate, fetal electric cardiogram, intertracheal pressure and amniotic pressure. Recording chart of the asphyxiated condition of the goat fetus by compressions of umbilical cord with a balloon catheter. Fetal heart rate decelerations of 80 to 90 bpm were noted after cord compressions. Negative pressure spikes observed in an intertracheal pressure tracing indicating fetal gasping movement during asphyxiated condition. Paper speed was 3 cm per min.

to 130 days (averaging 124.9 ± 8.4). Fetal survival period after the operation ranged from 60 to 192 h (averaging 93 ± 35.5 h). Five cases out of 12 were singleton, the others being twin pregnancies. After the experiment, 4 kids were born by spontaneous vaginal delivery, and 12 by cesarean section. The birth weights varied between 1,700 and 4,800 g (averaging 2836.7 ± 818.7 g). Biographic data of the goat fetuses included in this study are summarized in Table 1.

Fetal heart rate (FHR), intratracheal pressure

(ITP) and intrauterine pressure (IUP) were measured in this study. A recording chart of the asphyxiated condition of the goat fetus by cord compressions with a balloon catheter is shown in Fig. 7. FHR accelerations indicating fetal well being^{6,7)} were detected before the experiments. After performing repeated cord compressions, fetal heart rate decelerations were observed. Also, negative spikes of ITP indicating fetal gasping movements were observed. When maternal body movements or uterine contractions occurred, IUP changed synchronously.

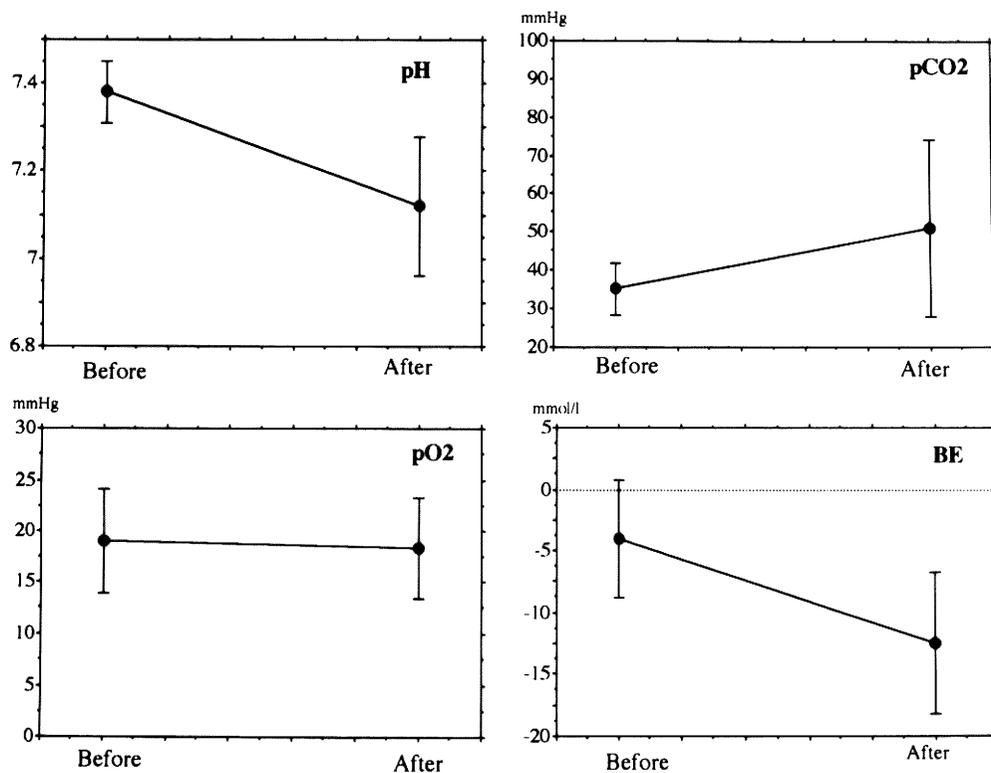


Fig. 8. Fetal arterial blood gas values before and after asphyxia in 20 experiments in 12 goat fetuses.

The pH values of the arterial blood of the goat fetuses that survived for more than three days in utero were 7.38 ± 0.07 , and partial pressure values for PO₂ and PCO₂ were 18.9 ± 5.1 and 35.1 ± 6.7 , respectively (Fig. 8). After performing repeated cord compressions, the mean arterial pH value changed to 7.12 ± 0.16 , while corresponding the values for PO₂ and PCO₂ were 18.3 ± 4.9 and 51.1 ± 23.1 , respectively. These figures were in good agreement with those of human fetuses after 30 min of fetal distress.

DISCUSSION

Despite the increase in human fetal physiological research in recent years, great restrictions are imposed on studies in this particular field by the fact that any invasive procedure may produce disastrous consequences.⁸⁻¹¹⁾ In order to gain direct information concerning the fetal heart rate, respiratory movement, eye movement, gastro-intestinal movement and other fetal physiological activities, it is necessary to use animal models. We have used Japanese Saanen goat fetus for this experiment. The reason for this

selection is that the fetal weight in this species is roughly the same as that of human fetus.

Confirmation of the gestational age of the pregnant goat is the most important and challenging problem to be solved. In the present study, we used a marking harness method for this purpose. However, the marks on the back of female goat do not always indicate completion of intercourse. Ultrasonic examination was carried out to assess fetal growth. In humans, it is easy to examine any part of the fetal body, especially the head, chest, abdomen and legs. On the contrary, it is difficult to observe parts of a goat fetus because the mother's breast is located just in front of the uterus.

As regards anesthesia, some investigators have reported that epidural anesthesia is superior to general anesthesia because of a smaller decrease in placental circulation and a lesser degree of suppression of the maternal breathing movements it produces.^{12,13)} We used halothane general anesthesia because it has a relaxant effect on the uterus. During operation, mother goats were deeply anesthetized and no body movement was observed. We anticipated that the mother goat's movements would prevent the

operator from skillfully performing fine procedures of fetal surgery.

We performed the fetal operation by the window technique or the partial exteriorization method in preparing the fetus. With this method, there is only a slight loss of amniotic fluid and blood. On entering the uterus, an incision was made where no cotyledon was found. Separation of cotyledons caused by the operation will diminish fetomaternal circulation and result in abnormal physiological conditions in utero.¹⁴⁾

The deceleration of FHR observed in this animal model suggests that a similar deteriorating condition might be induced by repeated cord compression in the human fetus as well.¹⁾ This model system using Japanese Saanen strain goats affords the same physiological conditions as in human fetuses and provides a useful model for research work on the fetal asphyxiated condition in utero caused by repeated cord compressions.

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