Cannulation for a Bovine Fetus in Late Gestation under Regional Anesthesia

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Abstract. This experiment was carried out to collect fetal blood and fluid via catheters fitted to elucidate interaction between the mother and her fetus during a comparatively long term. Fifteen cows in late gestation were used for this study. Under regional anesthesia, an incision was made in the left paralumbar fossa in the standing position. A catheter for fetal blood was inserted into a vein of the fetus and catheters for fetal fluid were inserted into amniotic and allantoic sacs respectively. Four types of catheters were used for collecting fetal blood. The best of these catheters for fetal vein was the angiographic catheter covered with spring tube. Fetal blood samples were able to be collected for more than seven days constantly from four fetuses until parturition. Fetal fluid could be collected daily from three fetuses until parturition perfectly. Parturition occurred at the end of a normal gestation period. The concentration of fetal and maternal cortisol decreased to the basal level within 24 hours (P<0.05) after operation. The changes in fetal and maternal cortisol during periparturition were the same as previously reported. These results suggested that this method would be beneficial for fetal cannulation and the sample collected by this method was useful for endocrine study during the perinatal period in cattle.

Key words: Cannulation, Bovine fetus, Late gestation, Regional anesthesia

To elucidate the mechanisms of bovine parturition, fetal immune system development and function, fetal nutrition and the interaction between mother and fetus, it is important to examine the concentration of bioactive substances in the blood of both mother and fetus. For this purpose, the collection of fetal blood and amniotic and allantoic fluid are useful. Several reports [1–3] have illustrated the changes in fetal and maternal hormones through the perinatal period. In these reports, fetal cannulation was done under general anesthesia, but this technique is required the special facilities and technicians. From this viewpoint and further considering the influences of the anesthesia on animal health [4, 5], cannulation under local or regional anesthesia is convenient. However, there are few reports on fetal cannulation under regional anesthesia. In addition, details of catheters were not given; for example, materials, length, etc. We have undertaken to fit catheters to fetal veins and maternal uteri under regional anesthesia and examined the availability of the method for the operation and material suited to catheters for collecting fetal blood. Furthermore, to confirm the utility of the samples collected, the cortisol concentrations in maternal and fetal plasma were measured.
Materials and Methods

**Animals, surgical procedure and after care**

Fifteen cows (Japanese Black; 8, Japanese Black × Holstein Friesian crossbred; 6, and Holstein Friesian; 1) in late gestation were used for this study. Details of the cows and state of perioperation are shown in Table 1. The fetal cannulation procedure is outlined briefly in Fig. 1. Operations were performed on days 239 to 262 of gestation. Feed was withheld for 48 hours and water for 24 to 16 hours before operation. The cows were preloaded with an intramuscular injection of 20 mg xylazin one hour before operation for sedation. Under regional anesthesia (caudal epidural anesthesia or lumbar anesthesia, and infiltration anesthesia) by injecting a mixture of 2% procaine hydrochloride with bupivacaine hydrochloride (0.5% Marcain, Fujisawa Pharmaceutical Co.) in the proportion of two to three, a 25 to 30 cm incision was made through the skin and muscle layers in the left paralumbar fossa, and a fetal limb was exposed through an incision in the uterine wall. Catheters were then inserted into veins in the chorion or fetal limb and fetal sacs. Details of these catheters and procedure for fixing the catheter to the fetus are described in the following section, Catheters, methods of fixing and maintenance. Ritodrine hydrochloride (about 4 µg/min/kg, Sigma-Aldrich Japan Co.) with glucose Ringer solution was infused into the maternal jugular vein during the operations as a uterine relaxant. Antibiotics (Penicillin and Streptomycin, 150,000 I.U. and 0.15 g, respectively) were added to the amniotic fluid and allantoic fluid to prevent intrauterine infection, and then the chorionic and allantoic membranes were closed. Antibiotic (Ampicillin) was administered to both dams (1800 mg/18 ml) and fetuses (200 mg/2 ml) via catheters for two or three days after operation. Ketoprofen (400 mg) as an anodyne was administered to the dams for care after operation. The cow was kept loosely tied with rope in a pen (about 3 m × 3 m) and fed adequate feed and water. All animals received humane care as outlined in the Guide for the Care and Use of Experimental Animals, surgical procedure and after care
Animals (Animal Care Committee, National Institute of Grassland Science).

Catheters, methods of fixing and maintenance

Four types of catheters were examined for drawing off fetal blood. On four fetuses, type “A” catheters were used. These were polyvinyl chloride tubes and the outside x inside diameter (mm) was 1.5 × 0.97 or 1.2 × 0.7, 70 cm long, an indwelling catheter kit for humans. On one fetus, a type “B” catheter, medical tube (outside x inside diameter was 1.27 × 0.86, 100 cm long. Intramedic Polyethylene Tubing, Becton & Dickinson Ltd.) was used. Type “C” catheter, 4 or 5 Fr. (outside x inside diameter was 1.33 × 0.95, 150 cm long. angiographic catheter (Medikit Ltd. Japan) was used on three fetuses. On seven fetuses, Type “D” catheter, 5 Fr. (1.33 × 0.95), 1.5 m long angiographic catheter (Medikit Ltd. Japan) covered with stainless spring tube (Sawane Spring Ltd. Japan), except for approximately 25 cm from the tip of the catheter, was used. A fixing tool (Unitika Ltd. Japan), reinforced by nylon mesh, was tied to the catheter by means of suture ligature with instant adhesive. This catheter was then inserted into the vein of the fetuses. The depth of insertion of the catheter was approximately 25 cm and it was stitched onto the fetal limbs with a fixing tool after fixing with instant adhesive (Fig. 2, 3).

The catheter for fetal fluid was a 14 G 1.4 m long polyurethane tube for indwelling in human vessels. The top of the catheters were attached to a tool to prevent tube obstruction by sucking in chorion (Fig. 4). And the fixing tool (Unitika Ltd. Japan) was placed about 25 cm from the top of the catheter with instant adhesive and suture ligature and catheter was inserted into the amniotic and allantoic sacs, respectively. The fixing tool to prevent the catheter from slipping out was positioned just inside the amnion, the allantoic membrane or both. These were fixed outside the fixing tool with suture ligature before the uterine wall was closed. (Patents have been applied for for these catheters and the methods of fixing to the fetal limb, amniotic and allantoic sacs. No. 2000–372724 Japan, No. 2000–372725 Japan).

Each after sampling, the fetal blood catheter was washed by 2 ml of sterile physiological saline with antibiotics added (penicillin and streptomycin, 100 I. U./ml and 100 µg/ml, respectively) and continuously filled with sterile physiological saline with heparin and antibiotics added (penicillin and streptomycin, 100 I. U./ml and 100 µg/ml) every day for maintenance. And in only one fetus (no. 12) out of six was recombinant tissue plasminogen activator (rt-PA, injected 1,200,000 I. U./2 ml/time) as a thrombolytic agent two times before parturition. Other catheters for fetal veins and amniotic and allantoic sacs were filled with sterile physiological saline with antibiotics added.

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Table 1. Details of catheters and animals

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Gestation length at surgery (day)</th>
<th>Type of catheter for fetal blood</th>
<th>Gravid uterus horn</th>
<th>Position of catheter</th>
<th>Duration of collection of fetal blood (day)</th>
<th>Duration of collection of fetal fluid (day)</th>
<th>Gestation length at parturition (days)</th>
<th>Sex of neonate</th>
<th>B.W. of neonate (kg)</th>
<th>Condition of neonate</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>258</td>
<td>A</td>
<td>Left</td>
<td>A hindlimb vein#</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
<td>–</td>
<td>–</td>
<td>Death for infection</td>
</tr>
<tr>
<td>2</td>
<td>258</td>
<td>A</td>
<td>Left</td>
<td>An artery on chorion</td>
<td>3</td>
<td>NT</td>
<td>283</td>
<td>M</td>
<td>19.0</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>239</td>
<td>A</td>
<td>Right</td>
<td>An artery on chorion</td>
<td>0</td>
<td>NT</td>
<td>286</td>
<td>F</td>
<td>46.0</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>255</td>
<td>A</td>
<td>Right</td>
<td>An artery on chorion</td>
<td>2</td>
<td>NT</td>
<td>269</td>
<td>F</td>
<td>30.5</td>
<td>Stillbirth</td>
</tr>
<tr>
<td>5</td>
<td>255</td>
<td>B</td>
<td>Left</td>
<td>An artery on chorion</td>
<td>1</td>
<td>NT</td>
<td>272</td>
<td>F</td>
<td>23.0</td>
<td>Normal</td>
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<tr>
<td>6</td>
<td>262</td>
<td>C</td>
<td>Left</td>
<td>An artery on chorion</td>
<td>3</td>
<td>NT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Death for accident</td>
</tr>
<tr>
<td>7</td>
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<td>Right</td>
<td>A hind limb vein#</td>
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<td>NT</td>
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<td>F</td>
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<td>8</td>
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<td>A left hindlimb vein</td>
<td>1</td>
<td>NT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Death for dehydrating</td>
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<td>9</td>
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<td>A left forelimb vein</td>
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<td>M</td>
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<tr>
<td>10</td>
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<td>D</td>
<td>Right</td>
<td>A right forelimb vein</td>
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<td>267</td>
<td>M</td>
<td>26.0</td>
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<tr>
<td>11</td>
<td>260</td>
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<td>A left hindlimb vein</td>
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<td>11</td>
<td>271</td>
<td>F</td>
<td>19.5</td>
<td>Normal</td>
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<tr>
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<td>262</td>
<td>D</td>
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<td>A right hindlimb vein</td>
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<td>A right hindlimb vein</td>
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<td>29</td>
<td>291</td>
<td>M</td>
<td>34.5</td>
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<tr>
<td>14</td>
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<td>A left forelimb vein</td>
<td>19</td>
<td>NT</td>
<td>273</td>
<td>M</td>
<td>33.0</td>
<td>Normal</td>
</tr>
<tr>
<td>15</td>
<td>262</td>
<td>D</td>
<td>Right</td>
<td>A right hindlimb vein</td>
<td>22</td>
<td>12</td>
<td>283</td>
<td>F</td>
<td>31.5</td>
<td>Normal</td>
</tr>
</tbody>
</table>

F: female, M: male. #: Right or left not known. NT: No tested. &: treated with rt-PA.

*: A: Polyvinyl tube for indwelling in humans, B: Medical tube made of polyethylene, C: Angiographic catheter without spring tube, D: Angiographic catheter covered with spring tube.
Sampling and assay

Fetal and maternal blood were collected (2–8 ml/day and 20 ml whole blood, respectively) once or twice a day until parturition and more than twice before and after parturition. The blood samples of mothers and newborn calves were collected from their jugular vein. The blood samples had EDTA added and were immediately placed in a refrigerated centrifuge at 4 degrees C, and the plasma was separated and then stored at –20 degrees C or less than until the assay. The plasma concentrations of cortisol were measured by radioimmunoassay (Ortho-Clinical Diagnostics Inc.). All data were statistically analyzed by t-test.

Results and Discussion

It has been reported that the continuous intravenous infusion of 3–5 µg/min/kg ritodrine hydrochloride adequately reduced uterine contraction in pregnant sheep [6–8]. Similarly, in this study, the uterine wall was so relaxed that we were able to grasp the fetal limb through the uterine wall and easily maintain its position. Parturition occurred on days 278 ± 7 (Mean ± SD) of gestation, which is considered to be a normal gestation period (Table 1).

In all cases, fetal blood samples were not obtained for more than three days following the operation via neither catheters A, B nor C. However, the samples were able to be collected for seven days or more after the operation via catheter D in six cases. Blood samples via catheter D were able to collect until 36 hours prior to parturition in one case and until 30 minutes or less prior to parturition in three cases. It is conceivable that these A, B and C type catheters were pulled out or
compressed and refracted by the organ within 2 or 3 days after operation, in view of the rumen volume recovered and motility from fasting before the operation. On the other hand, catheter D which was covered with a stainless spring tube was not refracted but bent by the organ. It is suggested, therefore, that catheter D enable to collect blood continuously more than 3 days.

Fetal fluid could be collected daily until parturition perfectly from three fetuses. In the other two fetuses, it was possible to obtain it daily except for one or two days.

Fetal and maternal cortisol levels were comparatively high after the operation, but both the cortisol levels decreased to the basal level, less than 10 ng/ml [3,9], within 24 hours (P<0.05). This suggests that the maternal and fetal blood from 2–3 days postoperation is suitable to use as samples for analyzing the changes in the cortisol concentration without the influence of surgical stress [4] (Fig. 5).

Fetal plasma cortisol began increasing about one week before parturition and the time was earlier and the level was higher than that of maternal cortisol (Fig. 6). The peak cortisol value obtained in our experiment was 85.3 ± 7.2 ng/ml (mean ± SE) on average in three fetuses/neonates. The concentration of cortisol became very low (29–56 ng/ml) immediately after the peak. This change in cortisol is the same as previously reported [3, 9]. In two out of seven cows in which catheter “D” was used, mild assistance was required at birth. The

Fig. 4. Catheter for fetal fluid with tool to prevent sucking chorion and with fixing tool to prevent catheter from slipping out of uterus.

Fig. 5. Change in plasma concentrations of maternal and fetal cortisol after operation. (mean ± S.E., n=6), *P<0.05 or **P<0.01 vs. respective operation time (0 h).
remaining five cows calved without assistance. Six of these seven neonates were seen to be fine and perfectly normal. Although parturition was normal, one fetus given rt-PA was stillborn. It is suggested that rt-PA may cause an unstable and higher cortisol level, remaining from abruption of the placenta [10–12]. We recommend that rt-PA should not be used for the maintenance of catheters in fetal veins.

From these results, this method can be recommended for fetal cannulation and the best catheter for long term collection was the 5 Fr. 1.5 m long angiographic catheter covered with spring tube. Samples collected by this method were also found to be useful for studying changes in bioactive substances in blood during the perinatal period in cattle.

**Acknowledgments**

We would like to thank Mr. N. Koike (Unitika Ltd.) for kindly supplying the fixing tools, and Gyokuhoudou Inc. for a supply of goods. Thanks are also due to Ms. F. Uozumi, Ms. K. Ishizawa and Ms. A. Kishimoto for their assistance.

**References**