—**Original**—

# Fetal Plasma Prostaglandin $F_{2\alpha}$ and Cortisol Responses to High-Dose Endotoxin Administration in Fetal Goats

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**Abstract**

Intrauterine inflammation/infection has been associated with prenatal mortality and morbidity. However, few studies have been performed to investigate how the fetus responds to intrauterine inflammation/infection in utero. In the present study, fetal plasma prostaglandin (PG) $F_{2\alpha}$ and cortisol responses to high-dose fetal endotoxin administration were evaluated in late gestation goats (n＝8). After 160 μg/kg of fetal weight of endotoxin (*Escherichia coli*, O 111: B 4 lipopolysaccharide) administration via the fetal jugular vein over a 5-min period, fetal plasma $PGF_{2\alpha}$ and cortisol levels, fetal blood gases and pH were measured periodically.

After endotoxin administration, fetal plasma cortisol levels significantly increased to 9.5±0.8 ng/mL and 9.3±0.7 ng/mL after 1 and 3 h, respectively ($p<0.05$) and plasma $PGF_{2\alpha}$ levels did not change throughout the study. These results suggest that absent $PGF_{2\alpha}$ and attenuated cortisol responses to high-dose fetal endotoxin administration, relative to the adult, may be a self-protective mechanism that diminishes premature delivery and fetal asphyxia.
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**Key words**: fetus, endotoxin, prostaglandin $F_{2\alpha}$, cortisol

## Introduction

Prenatal exposure to inflammation/infection has been associated with numerous adverse outcomes that include abortion, premature delivery and increased risks of neonatal asphyxia and cerebral palsy[1,2]. It has now been established that endotoxins stimulate production of cytokines in many infections[3]. These in turn result in the secretion of prostaglandin (PG) $F_{2\alpha}$, which is responsible for uterine contraction[4] and cortisol, which is considered to determine the onset of labor pain in the fetal period[5].

The hypothalamo-hypophyseal-adrenal axis is almost established at 100 days gestation in fetal goats, which is slightly different from the time in sheep and cows. However, all of them accord with late gastation in human.

In previous studies, maternal endotoxin administration elevated fetal plasma $PGF_{2\alpha}$ and cortisol, which are, at least in part, associated with abortion, premature labor, fetal asphyxia or death[6–9]. However, the effects of fetal exposure to endotoxin in pregnancy outcome has not been clearly elucidated. Fetal plasma $PGF_{2\alpha}$ and cortisol responses to infusion of medium doses of endotoxin into goat fetuses (80 μg/kg of estimated fetal weight) have been examined only in our previous study[10]. We found that absent fetal $PGF_{2\alpha}$ and

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attenuated cortisol responses to fetal endotoxin administration, relative to the adult. However, the effects of high-dose endotoxin administration into the fetus on fetal plasma $PGF_{2\alpha}$ and cortisol levels have not been evaluated in late gestation goats.

The present study was designed to evaluate the extent of the effects of administration of high-dose endotoxin (160 μg/kg of estimated fetal weight) to the fetus on fetal plasma $PGF_{2\alpha}$ and cortisol release in late gestation goats.

## Materials and Methods

### 1. Surgical procedures

Eight pregnant mixed-breed goats mated on only a single occasion and of known gestational age (126～135 days, Term＝150 days) were used in the study. The animals were housed in rooms with controlled light cycles. They were fed each morning with alfalfa cubes and had free access to water at all times.

Surgery was performed on 8 pregnant mixed-breed goats. After a 24-h fast, the goats were anesthetized with a mixture of 1.5～2.5% halothane and oxygen. Under aseptic conditions the maternal skin and uterus were incised and the fetal head was delivered. Polyvinyl catheters (1.0 mm ID) were inserted into the fetal carotid artery and jugular vein and their tips advanced into the aortic arch and superior vena cava, respectively. An additional catheter was placed in the amniotic cavity for administration of antibiotics. Then, the fetal body was replaced into the uterus. All catheters were exteriorized through an incision in the maternal flank and protected in a nylon pouch. The goats were kept in metabolic carts and were allowed to recover for at least 4 days before experiments were begun.

Ampicillin was administered to the maternal goats on a daily basis (4 g/day, iv), and given to the fetus via instillation into the amniotic cavity (1 g/day).

### 2. Experimental protocol

This study was approved by the Ethical Committee of the Hobara Central Hospital, Fukushima, Japan. Experiments were started at 4 days after surgery. Prior to the start of each experiment, samples for measurement of fetal arterial $pO_2$, $pCO_2$ and pH were taken. Only fetuses with arterial blood gas and pH values in the normal ranges for our laboratory ($pO_2$＞17 mmHg and pH ＞7.28) were studied.

The protocol consisted of control and after endotoxin administration periods. After a 1-h control period, *Escherichia coli* endotoxin (160 μg/kg of estimated fetal weight) (O111:B4 lipopolysaccharide, Wako, Tokyo) was dissolved in 3 mL of sterile saline and infused into the fetal jugular vein over a 5-min period. Eight control fetal goats received an infusion of saline (3 mL) via the fetal jugular vein.

After endotoxin or saline administration, the condition of the fetal goats was monitored for the next 12 hours. Fetal arterial blood pressure was measured with a pressure transducer (Cobe laboratories, Lakewood, CO) and heart rate was determined with a cardiotachometer triggered by the arterial pulse pressure. Fetal arterial pressure and heart rate were recorded to a stripchart recorder (RM-6366, Nihon Koden, Tokyo, Japan), and values were sampled every 0.01 sec with an NEC computer, with minute averages stored on disk. Fetal plasma $PGF_{2\alpha}$ and cortisol levels, fetal blood gases and pH were measured periodically.

### 3. Blood sampling

Fetal blood samples were collected from the carotid artery at 30 min before the endotoxin or saline administration, and 0.5, 1, 3, 5, 9 and 12 h after endotoxin or saline administration. The first portion of each fetal blood sample (0.4 mL) was taken into an ice-cold heparinized syringe. Fetal blood gases and pH were measured (Model 148, CIBA-Corning, Medford, MA).

The next sample (2 mL) was taken into an ice-cold heparinized syringe, immediately transferred into an ice-cold centrifuge tube, and mixed with 100 μL of an aqueous solution containing EDTA (3 mM) and indomethacin (0.2 μg/mL) to prevent in vitro formation of prostaglandins. Samples were centrifuged at once (1,300 g for 5 min, 4℃). The plasma was collected and stored at −80℃ until analysis. Plasma $PGF_{2\alpha}$ level was determined by

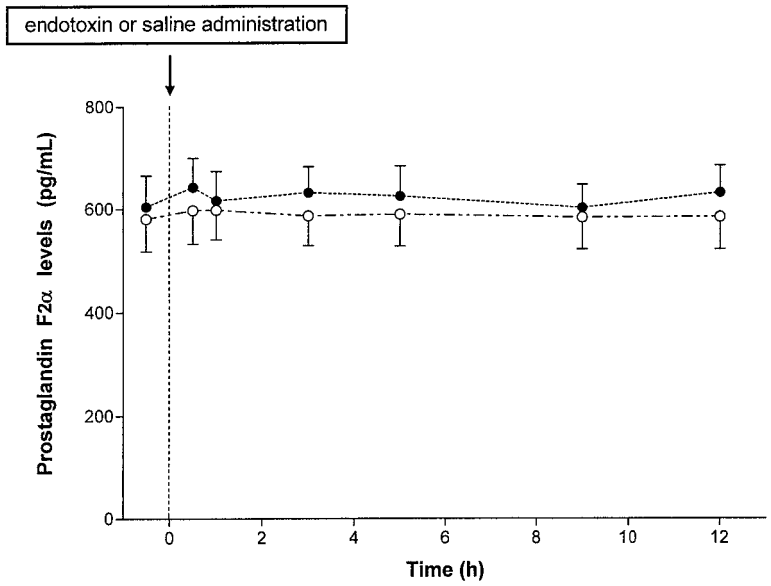


Fig. 1 Changes in fetal plasma prostaglandin $F_{2\alpha}$ levels after endotoxin or saline administration to late term fetal goats (n=8). Open circles, control fetuses; closed circles, endotoxin-administered fetuses.
Data are presented as mean±SEM.

a specific radioimmunoassay[11]. The detection limit of the assay was 2.5 pg/mL. There was no cross-reactivity between $PGE_2$ and $PGF_{2\alpha}$ The intra- and interassay coefficients of variation of $PGF_{2\alpha}$ assays were less than 5.2% and 6.8%, respectively.

Plasma cortisol level was measured by radioimmunoassay after methylene dichloride extraction, as described previously[4]. The intra- and interassay coefficients of variation were 6.3 and 7.4%, respectively. The assay sensitivity was 3.0 ng/mL.

The dose of endotoxin in this study (160 μg/kg of estimated fetal weight) was determined by a preliminary study (n=10). A higher dose of endotoxin (200 μg or more/kg of estimated fetal weight) killed the fetuses suddenly without affecting fetal cardiovascular conditions in the preliminary study.

### 4. Statistical analysis

Data are presented as mean±standard error of the mean (SEM). Student's t test was used to determine significant differences for single comparisons. Analysis of variance was used to determine the significant difference for repeated measures. If overall significance was observed, then individual group means were compared by the Bonferroni's post hoc multiple comparison test. Differences were considered significant at $p<0.05$.

## Results

### 1. Changes in fetal plasma $PGF_{2\alpha}$ levels

Changes in fetal plasma $PGF_{2\alpha}$ levels are shown in **Fig. 1**. Mean plasma $PGF_{2\alpha}$ levels after endotoxin and saline administration averaged 604±61 pg/mL and 581±63 pg/mL, respectively, during the control period, and did not change during the remainder part of study. Plasma $PGF_{2\alpha}$ levels in endotoxin-administered fetuses were not significantly different from those in control fetuses throughout the study.

### 2. Changes in fetal plasma cortisol levels

Changes in fetal plasma cortisol levels are shown in **Fig. 2**. In endotoxin-administered fetuses, plasma cortisol averaged 8.1±0.6 ng/mL during the control period, and then significantly increased to 9.5±0.8 ng/mL and 9.3±0.7 ng/mL after 1 and 3 h, respectively ($p<0.05$). During the remainder of the study, fetal plasma cortisol returned to basal levels. Plasma cortisol levels in endotoxin-administered

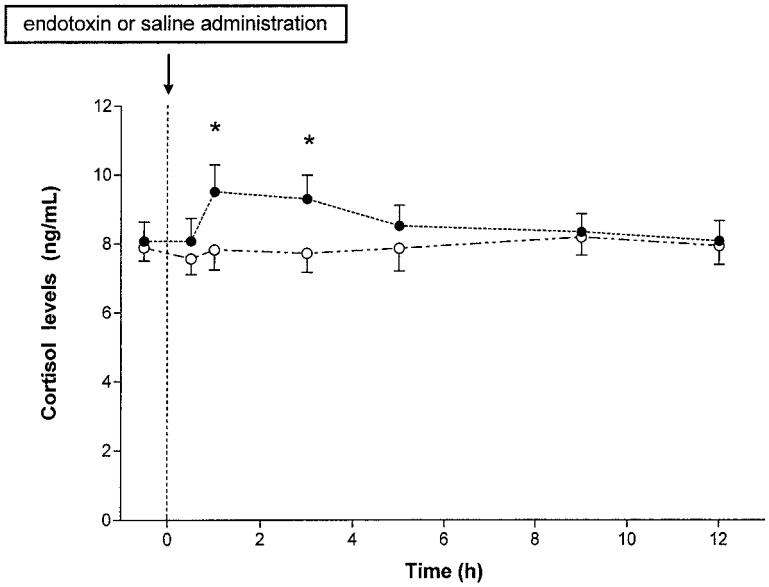


Fig. 2 Changes in fetal plasma cortisol levels after endotoxin or saline administration to late term fetal goats (n=8). Open circles, control fetuses; closed circles, endotoxin-administered fetuses.
Data are presented as mean±SEM.
*significantly different from the control period (−0.5 h) and control fetuses ($p<0.05$).

fetuses at 1 and 3 h were significantly different from those in control fetuses ($p<0.05$). Plasma cortisol levels in control fetuses did not change throughout the study.

### 3. Fetal blood gases and pH

**Table 1** shows the changes in fetal heart rate, mean arterial blood pressure, blood gases and pH. In endotoxin-administered fetuses, fetal blood $pO_2$ and pH significantly decreased after 3 and 5 h, and 5 h ($p<0.05$), respectively and $pCO_2$ significantly increased after 3 h ($p<0.05$). These values returned to initial levels after 12 h. However, the mean values of these variables remained within normal physiologic ranges. Fetal heart rate or mean arterial blood pressure did not change throughout the study. Control fetuses did not show changes in measured variables after saline administration.

## Discussion

In this study we have shown that fetal absent $PGF_{2\alpha}$ and attenuated cortisol responses to high-dose endotoxin administration to the fetus in late gestation goats. To date, the effects of high dose endotoxin on fetal $PGF_{2\alpha}$ and cortisol release have not been evaluated in late gestation goats.

In the present study, high-dose endotoxin administration to the fetus did not alter fetal plasma $PGF_{2\alpha}$ levels. To date, fetal goat plasma $PGF_{2\alpha}$ response to fetal endotoxin administration has been reported only in our previous study[10]. In that study, we infused medium doses of endotoxin to the fetuses (80 μg/kg of estimated fetal weight), which also demonstrated absent $PGF_{2\alpha}$ response in fetal goats. Since we administered two times higher dose of endotoxin in the present study, which is in the region of a lethal dose[12], the results observed here indeed reflect absent fetal $PGF_{2\alpha}$ response to endotoxin.

The exact mechanism of absent fetal $PGF_{2\alpha}$ response to endotoxin has not been elucidated. One possible explanation is that fetal plasma $PGF_{2\alpha}$ level is maximally elevated in late gestation[13], so it is no longer able to respond to endotoxin stimulation.

Further peripheral levels of $PGF_{2\alpha}$ do not necessarily reflect those in the action site, such as hypothalamo-hypophyseal axis. Further study is needed to clarify the mechanism of the absence of a response to endotoxin in the fetus.

Table 1 Changes in fetal heart rate, mean arterial blood pessure, blood gases and pH after endotoxin or saline administration

| | | –0.5h | 0.5h | 1h | 3h | 5h | 9h | 12h |
|---|---|---|---|---|---|---|---|---|
| Heart rate (bpm) | Control | 163 ± 11 | 164 ± 17 | 168 ± 19 | 160 ± 14 | 167 ± 21 | 162 ± 18 | 165 ± 15 |
| | Endotoxin | 167 ± 16 | 172 ± 12 | 170 ± 24 | 166 ± 13 | 162 ± 26 | 160 ± 10 | 163 ± 12 |
| MABP (mmHg) | Control | 48.2 ± 1.9 | 49.4 ± 2.4 | 48.8 ± 2.5 | 50.1 ± 2.7 | 50.6 ± 3.2 | 49.5 ± 2.6 | 49.0 ± 3.7 |
| | Endotoxin | 49.8 ± 2.2 | 49.5 ± 1.8 | 48.0 ± 3.1 | 48.6 ± 2.3 | 50.1 ± 2.0 | 48.8 ± 2.8 | 49.7 ± 3.9 |
| pO2 (mmHg) | Control | 19.4 ± 1.8 | 19.1 ± 2.2 | 19.9 ± 1.7 | 20.7 ± 1.9 | 20.5 ± 2.7 | 20.8 ± 2.3 | 19.7 ± 2.0 |
| | Endotoxin | 20.2 ± 1.3 | 19.7 ± 2.6 | 18.1 ± 3.5 | 17.0 ± 3.4* | 17.3 ± 1.8* | 18.0 ± 1.9 | 19.2 ± 2.1 |
| pCO2 (mmHg) | Control | 49.0 ± 1.4 | 49.5 ± 1.6 | 49.7 ± 0.8 | 49.2 ± 1.7 | 49.9 ± 1.6 | 50.8 ± 2.5 | 50.3 ± 1.3 |
| | Endotoxin | 50.5 ± 2.5 | 51.4 ± 2.0 | 51.3 ± 2.2 | 52.9 ± 1.1* | 51.8 ± 0.8 | 50.1 ± 2.6 | 51.2 ± 1.9 |
| pH | Control | 7.36 ± 0.02 | 7.35 ± 0.03 | 7.35 ± 0.02 | 7.33 ± 0.03 | 7.35 ± 0.01 | 7.34 ± 0.02 | 7.34 ± 0.03 |
| | Endotoxin | 7.33 ± 0.02 | 7.33 ± 0.04 | 7.32 ± 0.02 | 7.30 ± 0.04 | 7.29 ± 0.02* | 7.31 ± 0.03 | 7.30 ± 0.03 |

Date are presented as mean ± SEM. MABP, mean arterial blood pressure.
*$p < 0.05$ significant from the control period (–0.5h) and control fetuses.

In this study, fetal plasma cortisol levels slightly elevated after endotoxin administration. Fetal plasma cortisol response to maternal endotoxin administration has been reported in a few studies[6,8], which indicated that the extent of elevation of fetal plasma cortisol levels in previous and the present study was two to ten-fold less than that measured in their mothers.

The plasma cortisol response of fetal goats to high-dose endotoxin administration to the fetus has not been investigated to date. An attenuated cortisol response to medium doses of endotoxin administration to the fetus was observed in our previous study[10], and the same findings were observed after high-dose endotoxin administration in the present study, so the attenuated cortisol response observed in this study indeed reflects attenuated fetal cortisol response to endotoxin. The mechanisms of attenuated fetal plasma cortisol response are unknown. One possible explanation is that the fetus is generally less sensitive to endotoxin than is the adult[12]. Further study is needed.

In this study, the fetal cardiovascular system did not respond to endotoxin, and fetal blood gases after endotoxin remained within the normal range. These results are in accord with the previous study on pregnant sheep and goats[14,15]. These absent or attenuated responses in previous and the present study may be related to the immature adrenergic system, vascular shunt and poor responsiveness to endotoxin in the fetuses.

Maternal endotoxin administration increases fetal plasma $PGF_{2\alpha}$[9,16] and cortisol[8,17] levels which, at least partly, may be involved in the induction of abortion and onset of premature delivery. Further, endotoxin itself may be associated with fetal endotoxin shock under some conditions[18]. Therefore, the absent or attenuated sensitivity of the fetus to fetal endotoxin observed in this study may be beneficial for fetuses to maintain stable conditions in utero. However, it has been evident that infection in the pregnant uterine cavity may be chronic and remain without symptoms or signs for periods of weeks or even months[19,20]. Further study is needed to manage infection in the pregnant uterine cavity in the perinatal period.

In summary, the present study shows that absent $PGF_{2\alpha}$, attenuated cortisol and fetal cardiovascular responses to high-dose endotoxin administration to the fetus. These results suggest that fetal absent or attenuated responses, relative to the adult, may be a self-protective mechanism that diminishes premature delivery and fetal asphyxia.

Further study is needed to clarify the mechanism of the absence or attenuation of the response to endotoxin in the fetus.

## References

1. Fayez JA, Hasan A, Jonas HS, Miller GL: Management of premature rupture of membranes. Obstet Gynecol 1978; 52: 17–21.

2. Goldenberg RL, Hauth JC, Andrews WW: Intrauterune infection and preterm delivery. N Engl J Med 2000; 342: 1500–1507.
3. Romero R, King Wu Y, Brody DT, Oyarzun E, Duff GW, Durum SK: Human decidua: A source of interleulkin-1. Obstet Gynecol 1989; 73: 31–34.
4. O'Brien WF: The role of prostaglandins in labor and delivery. Clin Perinatol 1995; 22: 973–984.
5. Magyar DM, Fridhsal F, Elsner CW, Glaz T, Eliot J, Klein AH, Nathanielsz PW: Time-trend analysis of plasma cortisol concentration in the fetal sheep in relation to parturition. Endocrinology 1980; 107: 155–159.
6. Foley GL, Schlafer DH, Elsasser TH, Mitchell M: Endotoxemia in pregnant cows: Comparison of maternal and fetal effects utilizing the chronically catheterized fetus. Theriogenology 1993; 39: 739–762.
7. Tanaka K, Kawamura T, Asakura H, Araki T: Effects of maternal infection on prostaglandin production and uterine contraction in late-gestation pregnant goats. J Nippon Med Sch 1997; 64: 422–427.
8. Schlafer DH, Yuh B, Foley GL, Elsasser TH, Sadowsky D, Nathanieelsz PW: Effect of Salmonella endotoxin administered to the pregnant sheep at 133–142 days gestation on fetal oxygenation, maternal and fetal adrenocorticotropic hormone and cortisol, and maternal plasma tumor necrosis α concentrations. Biol Reprod 1994; 50: 1297–1302.
9. Harper MJK, Skarnes RC: Inhibition of abortion and fetal death produced by endotoxin and Prostaglandin $F_{2\alpha}$. Prostaglandins 1972; 2 : 295–309.
10. Kijima K, Yoneyama Y, Sawa R, Araki T: Effects of fetal endotoxin administration on plasma prostaglandin $F_{2\alpha}$ and cortisol levels in late-gestation fetal goats. Fetal Diagn Ther 1999; 14: 240–243.
11. Deayton JM, Young IR, Thorburn GD: Early hypophysectomy of sheep fetuses: effects on growth, placental steroidogenesis and prostaglandin production. J Reprod Fertil 1993; 97: 513–520.
12. O'brien WF, Cefalo RC, Lewis PE, Fletcher JR, Ramwell PW: The role of prostaglandins in endotoxemia and comparisons in response in the nonpregnant, maternal and fetal models. II Alterations in prostaglandins physiology in the nonpregnant, pregnant, and fetal experiments animals. Am J Obstet Gynecol 1981; 139: 535–539.
13. Challis JRG, Dilley JS, Robinson JS, Thorburn GD: Prostaglandins in the circulation of the fetal lamb. Prostaglandins 1976; 11: 1041–1042.
14. Harris WH, Pittman QJ, Veale WL, Cooper KE, Van Petten GR: Cardiovascular effects of fever in the ewe and fetal lamb. Am J Obstet Gynecol 1977; 128: 262–265.
15. Bech-Jansen P, Brinkmann III CR, Johnson GH, Assali NS: Circulatory shock in pregnant sheep. Am J Obstet Gynecol 1972; 113: 37–43.
16. Daels PF, Stabenfeldt GH, Hughes JP, Odensvik K, Kindahl H: Evaluation of progesterone deficiency as a cause of fetal death in mares. Am J Vet Res 1991; 52: 282–288.
17. Giri SN, Emau P, Cullor JS, Stabenfeldt GH, Bruss ML, Bondurant RH, Osburn BI: Effects of endotoxin infusion on circulation levels of eicosanoids, progesterone, cortisol, glucose and lactic acid, and abortion in pregnant cows. Vet Microbiol 1990; 21: 211–231. 16.
18. Morishima HO, Niemann WH, James LS: Effects of endotoxin on the pregnant baboon and fetus. Am J Obstet Gynecol 1978; 131: 899–902.
19. Gray DJ, Robinson HB, Malone J, Thomson RB Jr: Adverse outcome in pregnancy following amniotic fluid isolation of Urea plasma urealyticum. Prenat Diagn 1992; 12: 111–117.
20. Andrews WW, Hauth JC, Goldenberg RL, Gomez R, Romero R, Cassell GH: Amniotic fluid interleukin-6: correlation with upper genital tract microbial colonization and gestational age in women delivered after spontaneous labor versus indicated delivery. Am J Obstet Gynecol 1995; 173: 606–612.

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