

Blood pressure and heart rate in the ovine fetus: ontogenic changes and effects of fetal adrenalectomy

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¹Laboratory for Pregnancy and Newborn Research, Department of Physiology, College of Veterinary Medicine, and ⁴Department of Human Development, Cornell University, Ithaca, New York 14853-6401; ³Laboratory of Perinatal Neuroethology, Department of Psychology, Binghamton University, Binghamton, New York 13902; and ²Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 113 Tokyo, Japan

Unno, Nobuya, Chi H. Wong, Susan L. Jenkins, Richard A. Wentworth, Xiu-Ying Ding, Cun Li, Steven S. Robertson, William P. Smotherman, and Peter W. Nathanielsz. Blood pressure and heart rate in the ovine fetus: ontogenic changes and effects of fetal adrenalectomy. *Am. J. Physiol.* 276 (*Heart Circ. Physiol.* 45): H248–H256, 1999.—Ontogenic changes in baseline and 24-h rhythms of fetal arterial blood pressure (FABP) and heart rate (FHR) and their regulation by the fetal adrenal were studied in 18 fetal sheep chronically instrumented at 109–114 days gestation (GA). In the long-term study, FABP and FHR were continuously recorded from 120 days GA to spontaneous term labor (>145 days GA) in five animals. Peak times (PT) and amplitudes (Amp) of cosinor analysis were compared at 120–126, 127–133, and 134–140 days GA. Consistent, significant linear increases in FABP and linear decreases in FHR were observed in all fetuses. Significant 24-h rhythms in FABP and FHR were observed during all the time windows. In the adrenalectomy study, to test the hypothesis that fetal cortisol plays a key role in cardiovascular maturation, fetal adrenals were removed in eight animals (ADX); sham fetal adrenalectomy was performed on five animals (Con). Cortisol (4 µg/min) was infused intravenously in four ADX fetuses from day 7 postsurgery for 7 days (ADX+F). No significant changes in PT and Amp in FABP and FHR were observed. Plasma cortisol levels remained low in Con and ADX fetuses (<4.9 ng/ml). Cortisol infusion increased fetal plasma cortisol to 22.3 ± 3.2 ng/ml (mean \pm SE) on day 13 in ADX+F fetuses. FABP increased in control and ADX+F but not ADX fetuses; FHR decreased in control and ADX but rose in ADX+F fetuses. These results suggest that, in chronically instrumented fetal sheep at late gestation, 1) increases in FABP and decreases in FHR are maintained consistently from 120 to 140 days GA, with distinct 24-h rhythms, the PT and Amp of which remain unchanged, and 2) the physiological increase in FABP is dependent on the fetal adrenal; bilateral removal of the fetal adrenals does not prevent the ability of cortisol to produce a sustained increase in FABP.

cardiovascular system; circadian rhythm; adrenal; cortisol

IN THE SHEEP FETUS during late gestation, arterial blood pressure (FABP) increases steadily (6, 10, 22) and fetal heart rate (FHR) declines steadily (22). It has been postulated that the FABP increases during the last few

weeks of gestation as a result of both an increase in cardiac output and a rise in peripheral vascular resistance (18), whereas the decrease in FHR has been ascribed to a baroreflex response to the increased FABP (19), resulting in increased parasympathetic influence via the vagus on basal FHR (48). However, the exact mechanisms responsible for these changes are unknown, partly because ontogenic changes in FABP and FHR have not been fully characterized.

It has been shown that influences of β -sympathetic and parasympathetic activity on baseline FHR increase with gestational age in the sheep fetus (47). In addition, plasma concentrations of hormones that have stimulatory effects on the fetal cardiovascular system increase with gestational age (25, 34, 41). However, relative roles of the fetal endocrine and autonomic nerve system in the ontogenic changes in fetal cardiovascular system have not been characterized. Because glucocorticoids have a pronounced stimulatory effect on blood pressure (BP) in adult (40) and fetal (11, 12, 45, 50) sheep, it is possible that the ontogenic changes in the fetal cardiovascular system are, at least in part, regulated by the fetal hypothalamic-pituitary-adrenal axis. In one previous study, bilateral adrenalectomy (ADX) in fetal sheep at 119 to 133 days of gestation (GA) produced no significant changes in FHR and FABP compared with intact fetuses (35). In a second study, short-term (5 h) intrafetal cortisol infusion at >132 days GA to intact fetuses produced an increase in plasma cortisol concentration to 6.3 ± 0.7 ng/ml and caused a transient increase in FABP and a decrease in FHR (50). Finally, continuous cortisol infusion at a rate of 4 µg/min to intact fetuses induced a sustained increase in FABP for up to 48 h when administered at 103–120 days GA, but had no effect at 130–137 days GA (12, 45). These studies demonstrated that glucocorticoids can act to increase FABP, although they are not required for the maintenance of basal FABP. In addition they showed that the effect of glucocorticoids on FABP is gestational age dependent. In adult sheep, extensive investigations have been conducted on the BP increases produced by both ACTH and cortisol. It has been shown that ACTH administration induces an increase in cardiac output with a consequent rise in BP within 24 h unaccompanied by changes in peripheral vascular resistance (40). In adult sheep it has also been reported that the ACTH-induced BP increase is not abolished by treat-

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ment with α - and β -adrenergic blockade, angiotensin-converting enzyme inhibitors, or ganglion blockades (43), suggesting that mechanisms in addition to the sympathoadrenomedullary and the renin-angiotensin system may be involved in mediating the hypertensive response to ACTH.

Short-term cortisol infusion in intact fetal sheep at >132 days GA decreases plasma norepinephrine and epinephrine concentrations (50). This suppression of the fetal sympathoadrenomedullary system suggests that catecholamines play a relatively unimportant role in the cortisol-induced BP increase. However, more direct experiments to explore the roles of catecholamines in the maintenance of the elevated BP have not been conducted. Inasmuch as a previous study demonstrated that cortisol stimulated epinephrine release from cultured fetal adrenal medulla cells (17), it is possible that the adrenal medulla plays a role in the maintenance of the cortisol-induced FABP increase. Furthermore, no information is available that addresses the effects of fetal ADX on the ontogenic changes in FHR or the effects of prolonged elevation of plasma cortisol levels on basal FHR.

Several studies have demonstrated the existence of 24-h rhythms in FABP (7) and FHR in sheep (7, 9, 24). However, no study to date has examined whether there are ontogenic changes in amplitudes and peak times of the 24-h rhythms in these fetal cardiovascular variables. It has been suggested that glucocorticoids play a significant role in the regulation of 24-h rhythms of FHR in the human fetus (1); however, there is no information on the changes in the 24-h rhythms of FABP and FHR after fetal ADX or sustained premature increases in plasma cortisol concentrations in the sheep fetus.

In the present study we studied the chronically instrumented sheep fetus to test the hypothesis that fetal cortisol plays a key role in cardiovascular maturation in late gestation. We characterized 1) the ontogenic changes in FABP and FHR and 2) the ontogenic changes in the amplitude and the peak time of 24-h rhythms in these fetal cardiovascular variables by measuring hourly FABP and FHR continuously between 120 and 140 days GA. In addition we also investigated 1) the effects of fetal ADX on the ontogenic changes in FABP and FHR and 2) the effects of fetal ADX on cortisol-induced increases in FABP between 117 and 123 days GA.

MATERIALS AND METHODS

Care of Animals

Eighteen Rambouillet-Columbia crossbred ewes bred on a single occasion only and carrying a fetus of known gestational age were used. All procedures were approved by the Cornell University Animal Care and Use Committee. All facilities were approved by the American Association for the Accreditation of Laboratory Animal Care. From 7 days before surgery, the ewe was housed in a metabolic stall with ad libitum alfalfa cubes and water in a room with controlled light-dark cycles (lights on at 0700 and off at 2100).

Surgical Instrumentation

Surgery was performed under halothane general anesthesia on five ewes between 113 and 114 days GA in the long-term study and on 13 ewes between 109 and 113 days GA in the ADX study using techniques that have been described in detail (30, 31, 36). Briefly, polyvinyl catheters were inserted into a maternal carotid artery and jugular vein and advanced into the arch of the aorta and superior vena cava, respectively. The uterus was then exposed through a midline abdominal incision.

Long-term study. Hysterotomy was performed, and fetuses were instrumented with polyvinyl vascular catheters inserted via the carotid artery and jugular vein. Multistranded stainless steel wire (Cooner Sales, Chastsworth, CA, catalog no. AS 632) electrodes were sewn to the myometrium.

ADX study. Hysterotomy was performed. In eight fetal sheep both fetal adrenal glands were exposed and isolated via a retroperitoneal approach and removed. In five fetuses the adrenals were exposed but not removed (Con; $n = 5$) (30). Fetuses were instrumented with polyvinyl catheters inserted via the femoral artery and tibial vein. An amniotic cavity catheter was placed in all ewes.

After surgical preparation of the ewe and fetus, all fetal catheters and leads were grouped to exit the lateral abdominal wall of the ewe at a single point. Surgical closure was accomplished in layers. The ewe returned to the laboratory. During the four days after surgery, the ewe received 1 g/day iv ampicillin sodium. The ewes were fed daily, and water was available ad libitum. All fetuses were allowed to recover for at least 5 days after surgery before being studied.

Maternal and fetal arterial blood samples (0.5 ml) were taken daily after the surgery for measurements of blood gases and pH on a blood gas analyzer (ABL500, Radiometer, Copenhagen, Denmark). Measurements were corrected to 39°C. A heparin solution (10 U/ml physiological saline) was continuously infused at a rate of 0.5 ml/h into each vascular catheter to ensure that catheters remained open.

Data Acquisition

From the sixth day after the surgery, FABP, FHR, and myometrial electrical activities were recorded continuously throughout the study with the use of a data acquisition system that collected data averaged every second (16). FABP and amniotic cavity pressure were measured continuously with the use of a calibrated pressure transducer (Cobe, Lakewood, CO) connected to the fetal artery and amniotic cavity catheters. Amniotic fluid pressure was taken as the zero pressure reference for FABP. FHR was calculated from the BP systolic peak to peak intervals. In each fetus, averaged values of FABP and FHR were calculated every 40 s and analyzed with an IBM compatible personal computer using Microsoft Excel. Inappropriate signals due to blood samplings, fetal movements, and catheter malfunctioning were excluded. Hourly and daily averaged values for FABP and FHR were calculated beginning at 0000 (Eastern Standard Time) on 120 days GA in the long-term study and at 1600 on the sixth day after surgery in the ADX study.

Experimental Protocols

Long-term study. After the onset of labor, confirmed by the presence of irreversible contraction-type myometrial electrical activities, the ewe and the fetus were killed with an overdose of pentobarbital sodium (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI) and the body weight was determined.

ADX study. From six days after surgery, 5 ml of maternal and fetal blood were collected daily between 0900 and 1000, and plasma was removed, frozen in liquid N₂, and stored at -20°C until assayed for ACTH and cortisol. ADX fetuses were divided into two groups. Four adrenalectomized fetuses (ADX+F) were continuously infused with cortisol (Solu-Cortef, Upjohn, Kalamazoo, MI) via the fetal venous catheter at a rate of 4 µg/min starting at 1600 on the seventh day after surgery until necropsy. The four other adrenalectomized fetuses (ADX) received vehicle alone. Fetuses were delivered by cesarean section and killed by exsanguination while under halothane general anesthesia at 123–125 days GA. Completeness of ADX was confirmed in all ADX and ADX+F fetuses by careful inspection of the surgical sites. Tissues were collected and weighed.

RIA for ACTH and Cortisol

Plasma ACTH concentrations were measured with a commercial RIA kit (INCStar, Stillwater, MN) validated for hormone measurements in sheep plasma (46). Assay sensitivity (90% B/B₀) was 9 pg/ml. The intra- and interassay coefficients of variation (CV) for quality control samples containing 34.7 (pool of the assay kit), 10.9 (fetal pool), and 53.9 (maternal pool) pg/ml were 6.8 and 12.5%, 12.8 and 19.0%, and 6.5 and 10.7%, respectively. Plasma cortisol concentrations were measured with a commercially available RIA kit (Diagnostic Products, Los Angeles, CA) validated for measurements in sheep plasma (46). Intra-assay CV was 8.8% for a quality control sample containing 36.1 ng/ml ($n = 20$). Interassay CV was 2.3% for a quality control sample containing 29.9 ng/ml ($n = 20$). Assay sensitivity (90% B/B₀) was 4.9 ng/ml ($n = 27$).

Statistical Analysis

All data are presented as means ± SE. Data were analyzed first by the summary of measures method (27) to focus the statistical comparisons.

Long-term study. Daily average values for FABP and FHR were determined in each fetus. Changes in FABP and FHR with gestational age were analyzed with one-way repeated-measures (RM) ANOVA followed by the Student-Newman-Keuls (SNK) test for post hoc comparisons. Cosinor analysis was performed on hourly average values in each animal to determine the presence of 24-h rhythms (33). Cosinor curves were fitted to the hourly FABP and FHR data in each animal over 7-day periods (120–126, 127–133, and 134–140 days GA) after the subtraction of the linear component based on the results of the linear regression analysis. Among animals that revealed a significant 24-h rhythm, peak times and amplitudes were compared among three groups using one-way RM ANOVA. For the statistical analysis of arterial blood gases and arterial pH (pHa), one-way RM ANOVA was applied on weekly average values, which was followed by the SNK test.

ADX study. Fetal body and organ weights were compared among the three treatment groups using one-way ANOVA. Changes in daily values of fetal blood gases and pH were analyzed among the three groups using one-way ANOVA and in each treatment group using one-way RM ANOVA. Data for fetal plasma ACTH and cortisol concentrations were compared among the three treatment groups by one-way ANOVA or by Kruskal-Wallis nonparametric ANOVA where appropriate. Changes in daily FABP and FHR values were compared by one-way RM ANOVA in Con and ADX fetuses. In ADX+F fetuses, daily FABP and FHR were analyzed between the values on the day before infusion (*day -1*), 1 day after the commencement of infusion (*day 1*), and the fifth day of infusion (*day 5*) with the use of one-way RM ANOVA. Post hoc analyses for multiple comparisons were performed with the SNK test. Cosinor analysis was carried out to determine the presence of 24-h rhythms and to evaluate any change produced by ADX and ADX+F. Cosinor curves were fitted to the hourly FABP and FHR data averaged in each animal from *day 1* to *day 6*. Peak times and amplitudes were compared over three treatment groups by one-way ANOVA.

For all statistical tests, differences were considered to be significant when $P < 0.05$.

RESULTS

Long-Term Study

Spontaneous labor was confirmed by the presence of contraction-type myometrial electrical activity at 146.7 ± 0.5 days GA. At necropsy, fetal body weight was 4.5 ± 0.4 kg.

Arterial blood gases and pH in ewes and fetuses. Mean values for arterial blood gases and pHa in ewes and fetuses are presented in Table 1. There were no significant changes in pHa and arterial blood gases.

Overall changes in FABP and FHR. After we deleted unusable, corrupt, or unavailable periods of recording signals, hourly values were obtained in $95 \pm 2\%$ of total period in each fetus. No cardiovascular data were analyzed within 2 days of labor. Daily average values for FABP and FHR from 120 to 143 days GA are presented in Fig. 1. FABP increased steadily with gestational age from 35.2 ± 1.7 mmHg on 120 days GA to 47.4 ± 2.4 mmHg on 143 days GA, whereas FHR decreased with gestational age from 178 ± 3 beats/min on 120 days GA to 143 ± 2 beats/min on 140 days GA. Between 140 and 143 days GA baseline FHR increased.

Ontogenic changes in 24-h rhythms in FABP and FHR. Hourly values in FABP and FHR beginning at 2400 on 120 days GA until 2300 on 143 days GA are illustrated in Fig. 2. Cosinor analysis on 24-h rhythms of cardiovascular variables revealed significant 24-h

Table 1. Arterial blood gases and pH in ewes and fetuses in the long-term study

Days GA	Ewes			Fetuses		
	pHa	Pa _{CO₂}	Pa _{O₂}	pHa	Pa _{CO₂}	Pa _{O₂}
120–126	7.46 ± 0.02	37.7 ± 1.7	115.1 ± 2.5	7.35 ± 0.01	52.7 ± 1.0	23.6 ± 0.6
127–133	7.44 ± 0.01	37.8 ± 0.8	116.3 ± 2.3	7.35 ± 0.00	53.2 ± 1.2	23.0 ± 0.3
134–140	7.44 ± 0.01	37.6 ± 0.8	116.5 ± 1.9	7.36 ± 0.00	52.4 ± 1.4	22.5 ± 0.8

Data are presented as means ± SE of 5 animals. Blood samples were taken at 0900–1000 every day beginning at 120 days gestation (GA). Data were averaged in each animal to obtain representative values for each period. pHa, Pa_{CO₂}, Pa_{O₂}, arterial pH, PCO₂, and PO₂, respectively. No significant differences were observed throughout the study period.

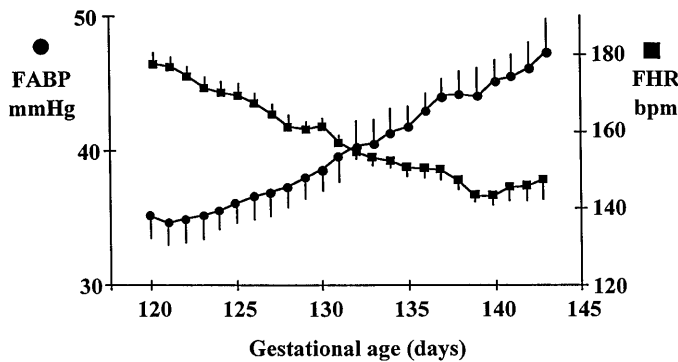


Fig. 1. Daily fetal arterial blood pressure (FABP) and daily fetal heart rate (FHR) (*n* = 5 ewes). Values were presented as means ± SE. FABP increased from 120 to 143 days gestation (GA), whereas FHR decreased steadily from 120 to 140 days GA. bpm, Beats/min.

rhythms in FABP in four of the five fetuses between 120–126 and 127–133 days GA and five fetuses during 134–140 days GA; FHR showed significant 24-h rhythms in all of the five fetuses during all three gestational age windows. Peak times and amplitudes of 24-h variations in FABP and FHR remained unchanged throughout the study period (Table 2).

ADX Study

Fetal body and organ weights. At necropsy ADX fetuses and ADX+F fetuses did not show any significant difference in body weight or in the weight of the fetal heart, lungs, kidneys, liver, spleen, or thymus compared with Con fetuses (Table 3).

Fetal blood gases and pH. pHa and arterial partial pressure of CO₂ values were well maintained throughout the experimental period, although pHa values in ADX fetuses were significantly lower than those in Con fetuses on the eighth and tenth day after surgery and

Table 2. Peak time amplitude of 24-h rhythms in FABP and FHR in the long-term study

Days GA	Peak Time		Amplitude	
	FABP, h	FHR, h	FABP, mmHg	FHR, beats/min
120–126	22.7 ± 0.5	23.9 ± 0.3	1.1 ± 0.2	5.2 ± 0.4
127–133	22.9 ± 0.5	23.1 ± 0.2	1.1 ± 0.2	5.5 ± 0.4
134–140	22.5 ± 1.2	23.6 ± 0.2	1.1 ± 0.2	6.0 ± 0.4

Values are means ± SE of 5 fetuses, except in fetal arterial blood pressure (FABP) at 120–126 days GA and 127–133 days GA, where *n* = 4. FHR, fetal heart rate. No significant differences were observed throughout the study period. Lights were on at 0700 and off at 2100.

pHa values in ADX+F fetuses increased significantly after cortisol infusion. Arterial partial pressure of O₂ (Pa_{O₂}) in ADX fetuses was significantly lower than Con fetuses between the sixth and ninth day after surgery. This difference in Pa_{O₂} was not observed during the rest of the experimental period. A serial analysis revealed a significant increase of Pa_{O₂} in ADX+F fetuses after cortisol infusion (Table 4).

Plasma ACTH and cortisol concentrations. In Con fetuses, fetal plasma ACTH levels remained stable throughout the observed period (22.2 ± 3.3, 20.6 ± 2.4, and 22.2 ± 3.3 pg/ml on day -1, day 3, and day 6, respectively). Plasma ACTH concentrations in ADX fetuses were significantly greater compared with those in Con fetuses (113 ± 36, 107 ± 31, and 128 ± 37 pg/ml on day -1, day 3, and day 6, respectively). In the ADX+F group, the plasma ACTH concentration on day -1 was 120 ± 93 pg/ml. After cortisol infusion, plasma ACTH concentrations decreased and reached a level of 14.6 ± 2.5 pg/ml on day 3. Fetal plasma ACTH remained low (15.1 ± 1.8 pg/ml on day 6) during the rest of the study period.

There was no significant difference in maternal plasma cortisol concentration among the three groups, except on day 1, when the ADX+F mothers showed significantly higher cortisol concentrations compared with Con and ADX mothers. No consistent interrelationship was found between maternal and fetal plasma cortisol concentrations. In Con and ADX fetuses, plasma

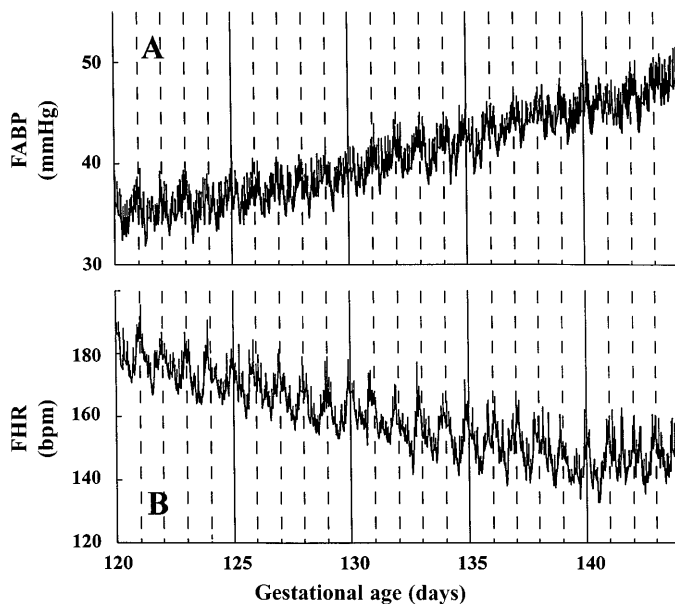


Fig. 2. Hourly FABP (A) and hourly FHR (B; *n* = 5) beginning at 0000 on 120 days GA until 2300 on 143 days GA. Values are presented as means ± SE.

Table 3. Fetal body and organ weights

	Con	ADX	ADX+F
Body wt	3,025 ± 180	3,050 ± 155	3,095 ± 111
Heart	22 ± 2	22 ± 3	24 ± 0
Lung, total	105 ± 7	89 ± 4	114 ± 8
Left	40 ± 4	34 ± 1	46 ± 3
Right	65 ± 4	54 ± 3	68 ± 5
Adrenal			
Left	0.16 ± 0.06		
Right	0.16 ± 0.06		
Kidney			
Left	10.5 ± 1.0	11.1 ± 1.1	10.5 ± 0.5
Right	10.4 ± 0.7	12.0 ± 0.7	10.4 ± 0.5
Liver	113.7 ± 5.8	108.8 ± 9.6	121.6 ± 13.0
Spleen	8.8 ± 1.4	7.6 ± 1.1	6.6 ± 0.8
Thymus	6.5 ± 1.9	4.9 ± 0.2	4.6 ± 0.2

Values are means ± SE in grams; *n* = 4 ewes each group. Con, control; ADX, adrenalectomized; ADX + F, ADX and cortisol-infused fetuses.

Table 4. Arterial blood gases and pH for fetuses of Con, ADX, and ADX + F in the ADX study

Days of Infusion		pHa	PaCO ₂ , mmHg	PaO ₂ , mmHg
-1	Con (5)	7.34 ± 0.01	52.3 ± 3.0	23.2 ± 1.2
	ADX (4)	7.31 ± 0.02	56.8 ± 1.6	17.8 ± 0.9*
	ADX + F (4)	7.34 ± 0.01	50.5 ± 2.8	20.0 ± 0.8
0	Con (5)	7.34 ± 0.01	52.3 ± 2.1	22.6 ± 0.8
	ADX (3)	7.30 ± 0.01†	57.4 ± 3.4	17.4 ± 1.2*†
	ADX + F (4)	7.34 ± 0.01	52.5 ± 2.0	21.0 ± 1.0
1	Con (5)	7.34 ± 0.01	52.9 ± 2.1	22.7 ± 1.0
	ADX (4)	7.30 ± 0.01*	58.2 ± 1.8	17.6 ± 0.9*†
	ADX + F (3)	7.32 ± 0.02	52.0 ± 1.1	21.6 ± 1.8
2	Con (5)	7.33 ± 0.01	54.9 ± 2.3	21.5 ± 0.8
	ADX (4)	7.30 ± 0.01	55.4 ± 2.1	18.1 ± 0.8*†
	ADX + F (4)	7.33 ± 0.01	49.5 ± 1.7	24.0 ± 1.2
3	Con (4)	7.34 ± 0.00	54.7 ± 2.2	20.8 ± 0.8
	ADX (4)	7.30 ± 0.00*†	55.0 ± 2.6	20.3 ± 1.8
	ADX + F (4)	7.34 ± 0.01	48.1 ± 2.3	23.8 ± 1.8
4	Con (4)	7.33 ± 0.01†	54.5 ± 2.2	21.2 ± 1.2
	ADX (4)	7.32 ± 0.01†	53.8 ± 2.6	19.4 ± 0.8
	ADX + F (4)	7.36 ± 0.01	49.4 ± 1.5	23.7 ± 1.3
5	Con (4)	7.33 ± 0.01†	53.1 ± 2.2	21.2 ± 1.2
	ADX (4)	7.32 ± 0.01†	53.3 ± 2.6	20.0 ± 1.5
	ADX + F (4)	7.38 ± 0.01	50.4 ± 1.8	23.4 ± 0.7
6	Con (4)	7.33 ± 0.01†	54.8 ± 2.5	21.0 ± 1.0
	ADX (4)	7.32 ± 0.00†	54.5 ± 2.8	17.5 ± 1.0†
	ADX + F (4)	7.37 ± 0.01	51.1 ± 1.4	23.9 ± 1.6

Values given are means ± SE; numbers in parentheses are number of fetuses. Significant differences: * ADX vs. Con; † ADX or Con vs. ADX + F (1-way ANOVA, $P < 0.05$).

cortisol concentrations remained below the assay sensitivity level for the volume of plasma extracted (4.9 ng/ml) throughout the observation period. In ADX+F fetuses, plasma cortisol concentrations increased significantly after the commencement of cortisol infusion and remained ~23 ng/ml (Fig. 3).

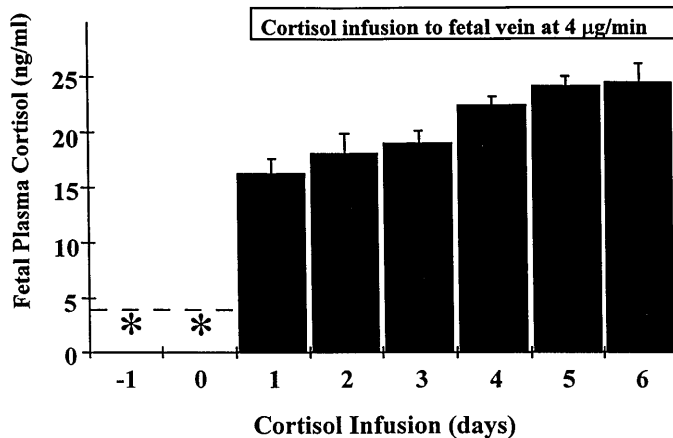


Fig. 3. Fetal plasma cortisol concentrations in adrenalectomized, cortisol-infused (ADX+F) fetuses. Values are means ± SE ($n = 4$). Dotted line indicates sensitivity of the assay. * On the sixth and seventh day after surgery, all values were below the sensitivity of the assay. Cortisol infusion to fetuses at a rate of 4 µg/min was commenced after the blood sampling on the seventh day after surgery and continued until necropsy. Values in control and ADX fetuses were below the sensitivity of the assay throughout.

Baselines of FABP and FHR. Daily baseline FABP and FHR values on the sixth day after surgery were 39.6 ± 1.4 and 190 ± 2 , 39.4 ± 1.6 and 185 ± 1 , and 44.9 ± 1.9 mmHg and 187 ± 3 beats/min in Con, ADX, and ADX+F fetuses, respectively. There were no differences among these groups. Hourly FABP and FHR values are shown for Con, ADX, and ADX+F fetuses in Fig. 4.

Changes in FABP and FHR in Con and ADX. During the study period there was a significant increase in FABP associated with a significant decrease in FHR in Con fetuses. In contrast there was no significant change in FABP in ADX fetuses, whereas the decrease in FHR was similar to those in Con fetuses.

Changes in FABP and FHR in ADX+F. FABP in ADX+F fetuses increased significantly on the first day of cortisol infusion compared with values of the preceding day (Fig. 4C). This increase was sustained throughout the study period. FHR also increased after cortisol infusion (Fig. 4F). This increase was gradual compared with the FABP increase and remained sustained throughout the infusion period, which reached a significant level on the fifth day after the commencement of infusion.

Changes in 24-h rhythms of FABP and FHR in ADX and ADX+F. Cosinor analysis revealed a significant 24-h rhythm in FHR in three of four Con fetuses, four of four ADX fetuses, and four of four ADX+F fetuses. No difference was found in the peak time or amplitude among the three treatment groups. The peak time and amplitude of each treatment group were not significantly different from those of the fetuses in the long-term study. Cosinor analysis was also applied to FABP, and a significant 24-h rhythm was found in two of four Con fetuses, four of four ADX fetuses, and three of four ADX+F fetuses.

DISCUSSION

Changes in Baseline FABP and FHR

Long-term study. This study is the first to report measurement of FABP and FHR continuously over the critical period of development from 120 days GA to delivery in the sheep fetus. This approach enabled detailed analysis on ontogenic changes in baseline FABP and FHR and in their 24-h rhythms. Kitanaka et al. (22) reported a steady increase in FABP and a simultaneous decrease in FHR from 110 to 120 days GA over 21 days in the sheep fetus. Because they measured FABP and FHR for only 1 h every day, it was difficult to detect small differences in the trajectory of developmental changes in these parameters. Brace and Moore (7) found that both FABP and FHR have 24-h rhythms in the late gestation sheep fetus, although they did not specify the gestational ages at which the study was conducted. In the present study, we used well-acclimated sheep and computer-based data acquisition using carefully calibrated transducers and amplifiers to achieve longitudinal continuous recording of FABP and FHR for 24 days. We demonstrated clear and consistent ontogenic changes in FABP and FHR from 120 to 143

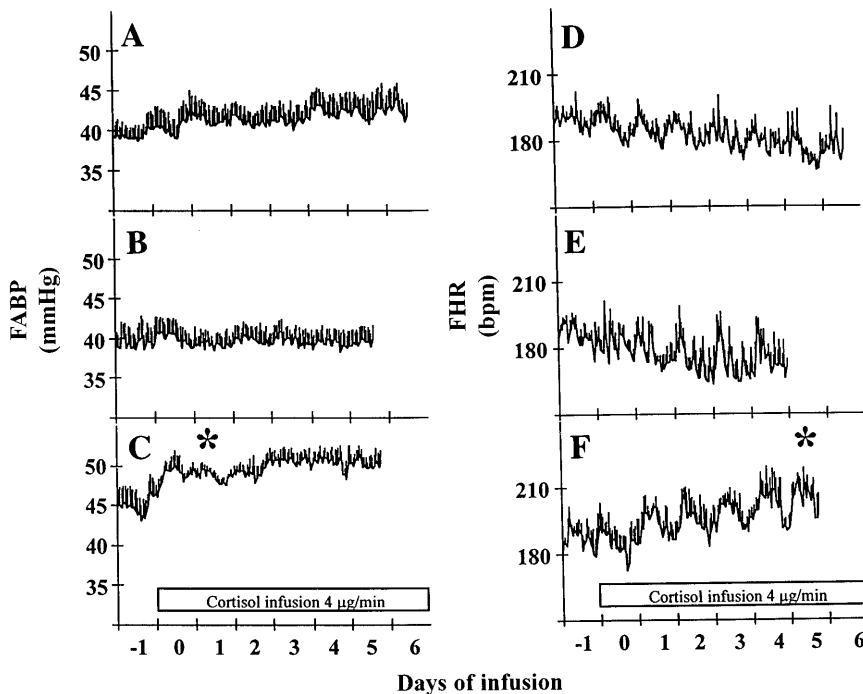


Fig. 4. Hourly FABP and hourly FHR. Values are means \pm SE beginning on the sixth day (i.e., 24-h before the beginning of cortisol and/or vehicle infusion) in control (Con), ADX, and ADX+F fetuses. Each day starts at 1600. *A*: hourly FABP in Con fetuses ($n = 5$). *B*: hourly FABP in ADX fetuses ($n = 4$). *C*: hourly FABP in ADX+F fetuses ($n = 4$); *D*: hourly FHR in Con fetuses ($n = 4$); *E*: hourly FHR in ADX fetuses ($n = 3$); *F*: hourly FHR in ADX+F fetuses ($n = 3$). Bar indicates period of cortisol infusion in *C* and *F*. *First day of significant sustained increase from day -1.

days GA with distinct 24-h rhythms. These findings strengthen previous studies and provide important information to understand the mechanisms of the ontogeny of the fetal cardiovascular system.

ADX study. To examine the possible roles of the fetal adrenals in the changes in FABP and FHR that have been characterized in the long-term study, we investigated the effects of fetal ADX on the normal gestational age-related changes in FABP and FHR and on the previously described increase in FABP produced by infusion of glucocorticoids to the fetus (12, 45, 50). A recent study reported a significant reduction in fetal body weight 4 wk after fetal ADX at 111–114 days GA (49). It is possible that long-term changes in fetal conditions after fetal ADX affect not only fetal growth but also ontogenic changes in the fetal cardiovascular system. Therefore, in the present study we evaluated the effect of ADX for 2 wk after fetal ADX at the critical period of adrenal development. At necropsy, no differences were observed in fetal body and organ weights among Con, ADX, and ADX+F fetuses, which supports the concept that overall fetal condition was substantially unchanged in all animals during the study period. In a previous study we demonstrated that the average fetal plasma cortisol over the 5 days before spontaneous vaginal delivery in control fetuses was 59 ± 10 ng/ml (28). Thus the levels of replacement we achieved (~ 23 ng/ml) were within the physiological range the fetus reaches in late gestation.

BLOOD PRESSURE. After ADX, the FABP increase that normally occurs with gestation was attenuated, suggesting a significant contribution of the fetal adrenals to the gestational age-related BP increase in fetal sheep. Although PaO_2 values in ADX fetuses were significantly lower than Con fetuses during the first one-half of the experimental period, it is not likely that this temporary

decrease in PaO_2 is related to the FABP profile in ADX fetuses, because the FABP profile did not change after the recovery of PaO_2 during the latter one-half of the experiment. Further studies are required to evaluate the precise causal mechanism and overall physiological relevance of the effect of ADX on the rise in FABP that occurs at this stage of gestation. Cortisol infusion to ADX fetuses beginning at 117 days GA resulted in a significant increase in FABP that was similar to previous findings in intact fetal sheep (12, 45). This increase in FABP was sustained for >6 days throughout the cortisol infusion period. These results clearly indicate that the fetal adrenal medulla does not play an indispensable role in mediating cortisol-induced FABP increases in late gestation fetal sheep. A previous study on adult sheep reported that total autonomic blockade does not attenuate ACTH-induced increases in FABP (43), supporting our conclusion that the adrenal medulla is not critically involved. However, these observations do not exclude the possibility of interaction of glucocorticoids at either the receptor or postreceptor level with locally released catecholamines. The sustained effects of cortisol on FABP for up to 6 days in the present study support and extend the results of a previous study of the effects of 48-h cortisol infusion to intact fetuses in which the FABP increase after cortisol infusion was evaluated for 48 h (12). Our findings also suggest that the cortisol-induced increase in FABP is not transient but may involve a fundamental change in the regulation of the fetal cardiovascular system.

The chronic hypertensive effect of glucocorticoids during development we and others have demonstrated may play a role in the more long-term effects on BP that follow prenatal glucocorticoid exposure demonstrated in rats (3). Because growth retardation has been linked

with development of high BP later in life (2) and an increase in fetal plasma cortisol concentrations in cordocentesis samples obtained from growth-retarded human fetuses has been reported (13), this stimulatory action of cortisol on the fetal cardiovascular system could be involved in the mechanism of adult hypertension and/or cardiovascular diseases of fetal origin. In addition, our findings of maintained effects on BP over 6 days also have relevance to possible consequences of repeated antenatal glucocorticoid therapy administered to women in threatened premature delivery over a lengthy period of gestation.

FETAL HEART RATE. Because the gestational age-related FHR changes are unaffected by ADX, our findings suggest an insignificant role of fetal adrenal maturation in this aspect of cardiac function. Changes in FHR in ADX+F fetuses indicate a stimulatory effect of sustained elevation of plasma cortisol on basal FHR despite the concurrent increase in FABP. Because baroreflexes are present and functional in the late gestation sheep fetus (5), this stimulatory effect could be a result of alteration of the setting of the baroreflex responses. However, this is unlikely because changes in FHR in ADX+F fetuses were completely opposite to FHR changes in Con and ADX fetuses. Alternatively, in rats, it has been demonstrated that glucocorticoids increase postsynaptic sensitivity of the cardiovascular system to norepinephrine (8). It has been also reported that glucocorticoids enhance the sensitivity of the pacemaker β -adrenergic receptors to catecholamines (29). In adult sheep, Spence et al. (43) reported that acute ganglion blockade increased FHR to a greater level in ACTH hypertensive sheep than in normotensive controls and suggested that ACTH treatment may have a direct chronotropic action on the heart. Glucocorticoids have also been suggested to play a key role in the developmental changes in the function of cardiac β -adrenergic receptors in the rat (32). In the sheep fetus, a recent study (44) demonstrated that intrafetal cortisol infusion at a rate of $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 60 h to fetal sheep at 128 days GA produced no changes in myocardial β -adrenergic receptor density and affinity; however a significant increase in adenylate cyclase activity in myocardial tissue was observed. Therefore it is likely that the increase in FHR after cortisol infusion to ADX fetuses results from a stimulatory effect of cortisol directly on the fetal heart. It is also likely that postreceptor events are involved in the changes in FHR after cortisol infusion. Additionally, the baroreceptors probably play a role in this cortisol-induced increase in FHR because FHR in ADX+F fetuses began to increase on the second day of cortisol infusion, contrasting with the FABP increase that occurred immediately on the first day (Fig. 4, C and F). Thus the early rise in FABP may dampen the mechanisms that lead to the increase in FHR. In the long-term study, we observed a steady decrease in baseline FHR from 120 to 140 days GA and an increase between 140 and 143 days GA (Fig. 1). It is possible that, in physiological conditions in sheep parturition, the chronotropic effect of cortisol is not strong enough to override mechanisms that cause a baseline

FHR decrease, such as baroreflexes, before circulating cortisol starts to increase exponentially at ~ 140 days GA.

Twenty-four-hour rhythms in fetal cardiovascular system. Synchronized diurnal variations in BP and heart rate exist in the adult in many species, including rats (42), rabbits (14, 38), marmosets (39), monkeys (15), and humans (26), peak times of which correspond to the active period for respective species. In the sheep fetus, similar diurnal rhythms in FABP and FHR have been described (7). However, ontogenic changes in the cardiovascular diurnal rhythms during fetal life have not been characterized. Results of the present study support previous findings and further indicate the absence of ontogenic changes in the 24-h rhythms in FABP and FHR between 120 and 140 days GA in the sheep fetus. The mechanisms responsible for the 24-h rhythms in the cardiovascular system have not been fully identified. It has been shown that the suprachiasmatic nucleus, which is known as a "biological clock" in mammals (23), plays a role in this phenomenon (20, 37). There is substantial evidence that suggests that diurnal rhythms in BP and heart rate are under sympathetic control (4, 21). A previous study in the human fetus reported that the 24-h FHR rhythm disappears after maternal and fetal adrenal gland suppression with triamcinolone (1), suggesting the adrenocortical regulation of fetal 24-h rhythms. The lack of changes in amplitudes of the 24-h rhythm in FHR observed in the present study may suggest that the 24-h rhythm in the fetal cardiovascular system is regulated by an independent factor from fetal development, such as maternal endocrine environment, and/or that the fetal mechanisms for this phenomenon are already established at 120 days GA. Furthermore, the lack of an effect of fetal ADX with or without subsequent continuous cortisol supplementation on the 24-h rhythm of FHR suggests the lack of involvement of both fetal adrenal cortical and medullary effects on the fetal 24-h rhythm. Both the human and sheep data would be compatible with a role for the maternal but not the fetal adrenal in regulating these rhythms. Further studies are required to elucidate the mechanisms regulating these cardiovascular 24-h rhythms.

In summary, we have demonstrated in fetal sheep that 1) there is a consistent increase in FABP baseline and a decrease in FHR baseline at 120–140 days GA; 2) the normal gestational age-dependent increase in FABP that occurs in late gestation is attenuated by ADX at 110 days GA in fetal sheep; 3) cortisol infusion beginning at 117 days GA to adrenalectomized fetal sheep produces a sustained increase in FABP and FHR, which is maintained up to 6 days; 4) 24-h rhythms in FABP and FHR exist from 120 to 140 days GA, and their peak times and amplitudes do not change throughout the study period; and 5) the 24-h rhythm in FHR remained unaffected by fetal ADX with or without subsequent cortisol supplementation. Taken together, these findings obtained in the present study indicate that glucocorticoids of fetal adrenal origin play an important role in regulating ontogenic changes in baseline FABP

during late gestation in the sheep fetus, whereas their role in baseline FHR regulation does not appear to be prominent until 140 days GA, and that the 24-h rhythms in FHR that exist during the last 3 wk of gestation are not regulated by the fetal adrenal.

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