PLASMA GLUCOCORTICOID AND ADRENOCORTICOTROPIN CONCENTRATIONS

MEASURED SERIALLY IN GROWTH-RETARDED FETAL LAMBS

Anibal J. Llanos,* James C. Rose,**
Robert K. Creasy, James R. Green,
and María Serón-Ferré

From the Department of Obstetrics, Gynecology and Reproductive Sciences, Reproductive Endocrinology Center, and the Cardiovascular Research Institute, University of California, San Francisco, California 94143, and **the Department of Physiology and Pharmacology, the Bowman Gray Medical School, Winston-Salem, North Carolina.

*Recipient of a postdoctoral fellowship form the Bay Area Heart Research Association. Present address: Departamento de Medicina, Universidad de Chile, Casilla 16083, Santiago 9, Chile.

Summary

We studied the fetal pituitray-adrenal axis in 9 growth retarded fetal lambs by serially measuring plasma glucocorticoids and ACTH concentrations from 115 to 140 days of gestation and adrenal blood flow at 138 days of gestation. At each gestational age period studied, plasma glucocorticoid and ACTH concentrations were similar in both growth retarded and control fetuses (Figs. 1, 2). However, ACTH concentration tended to be higher in the last 20 days of gestation in the growth retarded fetuses. This resulted in a significantly lower glucocorticoid/ACTH ratio between 121 to 130 days of gestation in the growth retarded fetal lambs as compared to the control fetuses (212 ± 111 vs. 1042 ± 257). The glucocorticoid/ACTH ratio in the growth retarded fetuses became similar to the ratio of the control fetuses at 131 to 140 days (445 ± 145 vs. 554 ± 182). Adrenal blood flow was significantly increased at term in the growth retarded fetuses (535 ± 87 vs. 302 ± 40 ml/min/100g). These data indicate that the pattern of maturation of the fetal adrenal gland in the growth retarded fetal lamb is similar to that of the normal sized fetus. Because of the increased adrenal blood flow observed at 138 days of gestation, there is a suggestion of a decreased sensitivity to ACTH in these animals.

Speculation

Growth retarded fetal lambs have glucocorticoid and ACTH concentrations similar to those of control fetuses. Because adrenal blood flow is higher in growth retarded fetuses than in control fetuses, (indicating an increased ACTH flux to the adrenal in the growth retarded fetuses) there is possibly a decreased sensitivity to ACTH in the fetuses with growth retardation. This apparent insensitivity to ACTH may be due to a partial enzymatic blockade in the pathway of biosynthesis of glucocorticoids.

INTRODUCTION

In human pregnancies complicated by maternal cardiovascular disease, such as hypertension or pre-eclampsia, uteroplacental blood flow is probably chronically decreased (2,8,14) and the fetuses' growth is frequently retarded (18). The additional observation that the lecithin/sphingomyelin ratio in the amniotic fluid is prematurely increased in these pregnancies (7) suggests that in growth-retarded fetuses glucocorticoid production by the fetal adrenal gland increases prematurely. This hypothesis is supported by the findings that glucocorticoid administration to the mother reduces the incidence of respiratory distress in newborns from normal pregnancies (16). In opposition to this hypothesis are the finidings that human newborns with fetal growth redardation have low cortisol production (13) or normal urinary excretion of glucocorticoid metabolites (20).

In fetal lambs with growth retardation secondary to a reduction in uteroplacental blood flow the thymus weight is greatly reduced and the adrenal gland weight is normal in spite of a significant reduction of body weight (4). This finding also supports the hypothesis that adrenocortical function may be increased in growth-retarded fetuses. To determine whether this hypothesis is correct, we studied pituitary-adrenal funciton by serially measuring plasma glucocorticoid and adrenocorticotropin (ACTH) concentrations during the last fifth of gestation and adrenal blood flow at 138 days of gestation in growth-retarded fetal lambs.

MATERIALS AND METHODS

Preparation

Nineteen mixed breed Western ewes with known gestational ages were surgically prepared from 105 to 110 days of gestation. Polyvinyl catheters were inserted into fetal carotid and femoral arteries and jugular and femoral veins, the amniotic cavity, and a maternal femoral artery and vein. In nine ewes a catheter was also placed in the uterine artery; through this catheter we injected 15 μm diameter nonradioactive microspheres daily as described by Creasy et al. (4) in order to gradually embolize the maternal uteroplacental vascular bed. Ten ewes without a uterine catheter were used as controls. Five of the controls were studied for 25 to 30 days and the remaining five for 15 to 20 days. Arterial pH, blood gases, hematocrits, heart rate and arterial blood pressure were measured daily. Blood samples for glucocorticoid and ACTH measurements from the control fetuses were included in this study only if arterial pH, blood gases and hematocrit were normal. Beginning at least five days after surgery, blood samples (1.5 ml) were collected simultaneously from the fetal and maternal femoral artery between 0900 and 1200 hours, in chilled plastic syringes and tubes. The samples were immediately centrifuged at 4°C and the plasma was transferred to plastic tubes and stored at -20°C until the assays were performed. Each fetus and ewe was sampled once every five days for the glucocorticoid measurements and once every ten days for the ACTH measurements. (To perform cardiovascular studies that will be reported elsewhere, atropine (0.2 mg/kg) and propranolol (1 mg/kg) were injected into normal and embolization fetuses twice a week. In four pilot experiments the fetal glucocorticoid concentration did not change after the injection of either atropine or propranolol.)

Glucocorticoid Measurement

Cortisol was measured by the radioimmunoassay method of Abraham et al. (1) modified as follows: corticoids were extracted from 50 μl of plasma diluted with 100 μl of phosphate buffer by mixing with 4 ml of ethyl ehter in a 12 x 75 mm glass tube closed with a plastic cap. After placing the tube in an acetone-dry ice bath to freeze the aqueous phase, we decanted the organic phase into another tube. The ether extract was evaporated to dryness and then dissolved in 0.4 ml phosphate-saline-gelatin 0.1% buffer. ^{3}H-cortisol (400 cpm, New England Nuclear Corporation) in 100 μl of buffer, and 100 μl of antibody were diluted 1:5000 was then added to each tube. (Cortisol antibody S-6 #3 was obtained from Dr. G. Abraham, University of California, Los Angeles). Samples were incubated overnight at 4°C. Bound and free glucocorticoids were separated by absorption with dextran-coated charcoal as described for the original technique (1). Standards were extracted in a similar fashion. All samples were analyzed in duplicate. The coefficient of variation for duplicate samples was 8%. The inter-assay variation was 12%. Since the cortisol antibody cross-reacts significantly with several steroids other than cortisol (progesterone 5%, cortisone 5%, 11-deoxycortisol 28%, 21 deoxycortisol 72%, corticosterone 32%), we expressed our results as glucocorticoids because we did not separate cortisol by chromatography.

ACTH Measurements

ACTH was measured by radioimmunoassay as described elsewhere (23). We used an antiserum prepared by one of us (JCR,22). This antiserum cross-reacts with α^{1-39} ACTH (100%), N-terminal α^{1-24} ACTH (90%), and N-terminal α^{1-12} ACTH (< 30%). It does not cross-react with the α^{1-10}, α^{1-10} amide, α^{11-19}, α^{11-24} or α^{25-39} fragments of ACTH. We used sythetic human ACTH (140 U/mg) (provided by Drs. C.H. Li and J. Ramachandran (Hormone Research Laboratory, UCSF) for both standards and iodination. The limit of sensitivity of the assay was 1.5 pg. Parallellism was observed between purified ovine ACTH and ACTH extracted from plasma of rats, dogs, fetal and adult sheep, and monkeys. The interassay coefficients of variation were 9, 14, and 15% for pools of plasma with high, medium, and low ACTH concetrations. The intra-assay variation was 6% for one sample analyzed 10 times in a single assay.

Fetal Plasma Glucocorticoid/ACTH Ratio

This ratio was calculated by dividing the glucocorticoid concentration, expressed as pg/ml, by the ACTH concentration expressed as pg/ml; both hormones were measured in the same plasma sample.

Fetal Body Weight and Length, and Organ Weights

At the termination of the experiment the control and embolized ewes were sacrificed and the fetuses delivered in order to measure body weight, crown-to-rump length, and organ weights.

Adrenal Blood Flow

In five embolized fetuses we measured adrenal blood flow by the radiolabelled microspheres method (9). At 138 ± .9 days of gestation, we injected radiolabelled microspheres, 15 μm in diameter simultaneously into the fetal inferior and superior vena cava. Reference samples were taken from the carotid and femoral arteries. The fetuses were removed from the uterus and the individual organs were dissected, incinerated in an oven and counted for radioactivity in a 512-channel multichannel pulse-height analyzer (Searle Analytic, Des Plaines, Ill.) as described elsewhere (9). Blood flow to the various organs was calculated by a 370 IBM computer from the counts in the adrenal gland, the counts in the reference sample and the withdrawal rate of the reference sample. As a check of adequate mixing of microspheres in the blood, the right and left kidney and hemisections of the brain were counted separately. The number of microspheres in the adrenal gland was always more than 400.

Statistical Analysis

Student's unpaired t-test was used to compare the differences between the means of the embolization and control groups. Values in the text are given as a means ± SE.

RESULTS

Fetal Weight and Length, and Organ Weights

Body weight, and crown-to-rump length, were low in the embolized fetuses, but brain, and lung weights were similar to those in control fetuses (Table I). Liver and thymus weights were low in the embolization fetuses; so the brain-to-liver weight ratio was significantly higher in the embolization than in the control fetuses.

Fetal Arterial pH, Blood Gases, and Hematocrit

In the embolization fetuses, pO_2 decreased and hematocrit increased between 131 and 140 days of gestation (Table II). Blood pH and pCO_2 in embolization fetuses were similar to those in control fetuses througout the last 35 days of gestation. Heart rate and blood pressures were also similar in the two groups.

Fetal Adrenal Blood Flow

Adrenal blood flow at 138 days of gestation was significantly higher in the five embolization fetuses studied than in five control fetuses studied by Cohn et al. (3) in our laboratory using the same technique (535 ± 87 vs. 302 ± 40 ml/min/100 g respectively; $p < 0.05$).

Fetal Plasma Glucocorticoid and ACTH Concentrations and Ratios

Plasma glucocorticoid concentrations in embolization and control fetuses increased in similar fashion during the last 35 days of gestation (Fig. 1). In contrast, the mean ACTH concentration in the embolization fetuses was slightly but not significnatly higher ($0.10<p>0.005$) than in the control fetuses during this period (Fig. 2). The fetal glucocorticoid/ACTH ratio in the embolization fetuses was significantly lower than in the controls between 121 to 130 days of gestation but not after 130 days (Fig. 3).

Maternal Plasma Glucocorticoid Concentration

Glucocorticoid concentrations were similar in the embolization and the control mothers throughout the last 35 days of gestation (Fig. 4).

DISCUSSION

The gradual embolization of the sheep uterine-placental vascular bed with non-radioactive microspheres retarded the growth of the fetal lambs. The indications that fetal growth was retarded are the low fetal body weight and length, the low liver and thymus weights and the preservation of brain weight, findings similar to those we have reported before (4). The increase in brain-to-liver weight ratio observed, and the decrease in pO_2 and the increase in hematocrit found during the last 10 days of the study are also indicators of fetal growth retardation (4,5,6).

Our findings of a similar adrenal weight and of a similar concentration and pattern of increase of glucocorticoids with gestational age in the fetal growth retarded lamb and in the control lamb indicate that in spite of the chronic stress produced by placental embolization adrenocortical function is not increased nor is adrenocortical maturation accelerated in growth retarded fetal lambs. Plasma glucocorticoid concentration in growth retarded fetuses, produced by carunculectomy, agree with our findings (20). The sparing of the fetal adrenal gland may have been caused by the significant increase in the adrenal blood

flow that we observed in these fetuses at 138 days of gestation. The mild fetal hypoxemia (pO_2 = 18 torr) could be a stimulus for the increase in blood flow since acute severe hypoxemia (pO_2 = 12 torr) can stimulate fetal adrenal blood flow in normal sheep fetuses (3).

It is known that in normal fetal lambs the plasma glucocorticoid concentration does not change in response to an endogenous or exogenous ACTH challenge before 130 days of gestation (11,17,23) although ACTH is secreted in response to several fetal stresses from 110 days of gestation onwards (23). However, chronic infusion (5-7 days) of a large amount of ACTH (over 100 g/day) will accelerate adrenal maturation and cause an increase in glucocorticoid secretion and labor early in gestation (12,15). Our results show that ACTH tends to be higher in the fetal growth retarded lamb from 121 days of gestation to 140 days. However, there was no increase in glucocorticoid concentration in these fetuses from 121 to 130 days and therefore the glucocorticoid/ACTH ratio was lower than in the control fetuses. These results indicate that chronic exposure to slightly higher ACTH in concentrations in the growth retarded fetal sheep did not accelerate adrenocortical maturation. The apparent discrepancy between the responses to ACTH previously mentioned (11,12,15) and our data may be explained by the different amount of ACTH presented to the gland in both circumstances, since the daily production rate of ACTH in the growth retarded fetuses (estimated as the product of metabolic clearance rate of ACTH in the normal fetal sheep (10) and the mean plasma concentration of ACTH at 121-130 days that we measured) is approximately one tenth of the amount of ACTH that was given exogenously (12, 15).

The plasma glucocorticoid concentration of normal fetal lambs increases after 130-135 days of gestation when the fetal adrenal gland is stimulated with exogenous or endogenous ACTH (11,23,17). This indicates that the normal adrenal gland responsiveness to ACTH increases after 130 days of gestation. It has been shown in adult dogs (26) and rats (19) that glucocorticoid secretion rate by the adrenal gland correlates with the flux of ACTH to the gland rather than with the plasma ACTH concentration. The normal plasma glucocorticoid concentration found in the fetal growth retarded lamb in spite of an increased ACTH flux to the gland, suggest that the adrenal glands in these fetuses are less responsive to ACTH than the controls. It is possible that this apparent unresponsiveness may be due to a partial enzymatic blockade in the biosynthesis of glucocorticoid in fetal growth retarded lambs as a result of embolization of the utero-placental vascular bed.

Another possible explanation of the normal glucocorticoid concentration seen in the growth-retarded fetal lambs througout gestation is that glucocorticoid metabolic clearance rate may be increased. An increased metabolic clearance rate caused by increased fetal metabolism or by transfer to the maternal compartment would prevent an increase in the plasma glucorticoid concentration in the growth-retarded fetuses in spite of an increased glucocorticoid secretion rate of the fetal adrenal gland due to the high ACTH flux. But this possiblility seems unlikely because the umbilical blood flow in the growth-retarded fetuses is significantly reduced (5) as are the weights of both the liver and the placenta (4,5) organs where glucocorticoids are known to be metabolized.

The fetal growth retarded lambs maintain a normal plasma glucocorticoid concentration and a normal pattern of adrenocortical maturation. The mechanisms by which this normalcy is achieved remain to be elucidated. Our data suggests less responsiveness to ACTH of a normal sized fetal adrenal gland; this lesser responsiveness is compensated by an increased ACTH flux to the gland brought about by an increase in adrenal blood flow and a slight increase in plasma ACTH concentrations.

REFERENCES AND NOTES

1. Abraham, G.E., Buster, J.E., and Teller, R.C., Radioimmunoassay of plasma cortisol. Analyt. Letters, 5: 757 (1972).
2. Bieniarz, J., Julio, W. and Granier, L.: Perinatal factors affecting human development. Pan Americna Health Organization S.C. Publ. 185, Washington, D.C. 1969, p. 81.
3. Cohn, H.E., Sacks, E.J., Heymann, M.A., and Rudolph, A.M.: Cardiovascular responses to hypoxaemia and acidemia in fetal lambs. Am. J. Obstet. Gynecol., 120:817 (1974).
4. Creasy, R.K., Barrett, C.T., deSweit, M., Kahanapää, K.V., and Rudolph, A.M.: Experimental intrauterine growth retardation in the sheep. Am. J. Obstet. Gynecol. 112:566 (1972).
5. Creasy, R.K., deSweet, M., Kahanapää, K.V., Young, W.P., and Rudolph, A.M.: Pathophysiological changes in the fetal lamb with growth retardation. In: Foetal and Neonatal Physiology. Sir Joseph Barcroft Centenary Symposium. Comline, K.S., Cross, K.W., Dawes, G.S. and Nathanielsz, P.W. eds. p. 398 (Cambridge University Press, 1973).
6. Dawkins, M.J.R.: In discussion of Lloyd, J.K., Diabetic mellitus presenting as spontaneous hypoglycemia in childhood. April 24 meeting of the Royal Soc. of Med. Hypoglycemia in Childhood. Proc. Royal Soc. Med., 57:1063 (1964)
7. Gluck, L. and Kulovich, M.V.: Lecithin/sphingomyelin ratios in amniotic fluid in normal and abnormal pregnancy. Am. J. Obstet. Gynecol. 115:539 (1973).
8. Browne, J.C.M. and Veall, N.: The maternal placental blood flow in normotensive and hypertensive women. J. Obstet. Gynecol. Brit. Emp. 60:141 (1953).
9. Heymann, M.A., Payne, R.D., Hoffman, J.I., and Rudolph, A.M.: Blood flow measurements with radionucleide-labelled particles. Progr. Cardiovasc. Dis., 20:55 (1977).
10. Jones, C.T., Luther, E., Ritchie, J.W.K., and Worthington, D.: The clearance of ACTH from the plasma of adult and fetal sheep. Endocrinology 96:231 (1975).
11. Jones, C.T., Boddy, K., and Robinson, J.S., and Ratcliffe, J.G.: Developmental changes in the response of adrenal gland to hypoxemia of foetal sheep to endogenous adrenocorticotrophin as indicated by the responses of the adrenal gland of foetal sheep. J. Endocrinol. 72:279 (1977).
12. Kendall, J.Z., Challis, J.R.G., Hart, I.C., Jones, C.T., Mitchell, M.D., Ritchie, J.W.K., Robinson, J.S., and Thorburn, G.D.: Steroid and prostaglandin concentrations in the plasma of the pregnant ewes during infusion of adrenocorticotropin or dexamethapone to intact or hypophysectomized foetuses. J. Endocrinol., 75:49 (1977).
13. Kenny, F.M. and Preeyasombat, C.: Cortisol production rate, VI. Hypoglycemia in the neonatal and postantal period, and in association with dwarfism. J. Pediatr., 70:65 (1967).
14. Landesman, R. and Knapp, R.: ^{24}Na uterine muscle clearance in late prenancy. Am. J. Obstet. Gynecol. 80:90, (1960).
15. Liggins, G.C.: Premature parturition after infusion of corticotrophin or cortisol into foetal lambs. J. Endocrinol., 42:323 (1968).
16. Liggins, G.C. and Howie, R.N.: A controlled trial of antepartum glucocorticoid treatment for pevention of the respiratory distress syndrome in premature infants. Pediatrics, 50:515 (1972).
17. Llanos, A.J., Ramachandran, J., Creasy, R.K., Rudolph, A.M., and Serón-Ferré, M.: αMSH and ACTH in the regulation of glucocorticoid secretion during the perinatal period in sheep. Submitted for publication.
18. Mann, L.I., Tejani, N.A., and Weiss, R.R.: Antenatal diagnosis and management of the small-for-gestational age fetus. Am. J. Obstet. Gynecol. 120: 995, (1974).
19. Porter, J.C. and Klaiber, M.S.: Corticosterone secretion in rats as a funciton of ACTH input and adrenal blood flow. Am. J. Physiol., 209:811 (1965).
20. Reynolds, J.W. and Mirkin, B.L.: Urinary steroid levels in newborn infants with intra-uterine growth retardation. J. Clin. Endocrinol. Metab., 36: 576 (1973).
21. Robison, J.S., Jones, C.T., Challis, J.R.G., and Thorburn, G.D.: Observations on experimental growth retardation in sheep. Pediatr. Res., 10:891 (1976) Abstract No. 128.
22. Rose, J.C. and Newsone, H.H.: The rapid production of antisera to ACTH, angiotensin II and deoxycorticosterone with sufficient sensitivity to use in radioimmunoassay. J. Clin. Endocrinol. Metab. 35:469 (1972).
23. Rose, J.C., MacDonald, A.A., Heymann, M.A., and Rudolph, A.M.: Developmental aspects of the pituitary adrenal axis response to hemorrhagic stress in lamb fetuses in utero. J. Clin. Invest., 61:424 (1978).
24. Rudolph, A.M. and Heyman, M.A.: The circulation of the fetus in utero. Cir. Res., 21:163 (1967).
25. Stephenson, S.K.: Wool follicle development in the New Zealand Romney and N-type shee. IV - Prenatal growth and changes in body proportions, Aust. J. Agric. Res. 10:433 (1959).
26. Urquhart, J.: Adrenal blood flow and the adrenocortical response to corticotropin. Am. J. Physiol., 209:1162 (1965).

27. Acknowldegements: We wish to thank Ms. Francoise Mauray and Mr. Carl McWatters for their skillful technical assistance.
28. Supported by: U.S. Public Health Service grants HD 06619, HD11210-01, and HL 06285, CA-16428 and School of Medicine, Research and Evaluation Committee, Clough Foundation, University of California, San Francisco.

29. Send reprint requests to: R.K. Creasy, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA 94143.

30. Received for publication 2/6/79.
31. Accepted for publication 5/10/79.

TABLE I. Mean Body Weight and Length and Organ Weights In Growth-Retarded Fetal Lambs

	Controls n = 5	Growth-Retarded n = 9	% of Control
Gestational age (days)	138 ± 0.90	138 ± 0.60	100
Body weight (kg)	3.9 ± 0.08	3.2 ± 0.12**	82
Body length (cm)	50 ± 0.38	43 ± 1.00**	86
Adrenal (g)	0.51 ± 0.02	0.52 ± 0.01	102
Brain (g)	50 ± 2.00	47 ± 2.00	94
Lungs (g)	103 ± 8.00	80 ± 7.00	78
Liver (g)	132 ± 6.00	90 ± 3.00**	68
Thymus (g)	19 ± 2.00	6 ± 1.00**	32
Brain-to-liver weight ratio	0.38 ± 0.01	0.52 ± 0.02*	

Values are means SE; n = number of animals

*p <0.01

**p <0.001

TABLE II. Mean Femoral Arterial pH, Blood Gases and Hematocrit in Growth-Retarded Fetal Lambs from 131 to 140 Days of Gestation

Variable	Control n = 15	Growth-Retarded n = 27
pH	7.39 ± 0.007	7.39 ± 0.005
pCO_2 (torr)	44.7 ± 0.59	46.6 ± 0.71
pO_2 (torr)	23.1 ± 0.67	18.5 ± 0.45*
Hematocrit %	30.6 ± 0.98	35.0 ± 0.5**

Values are means ± SE

*p <0.02

**p <0.01

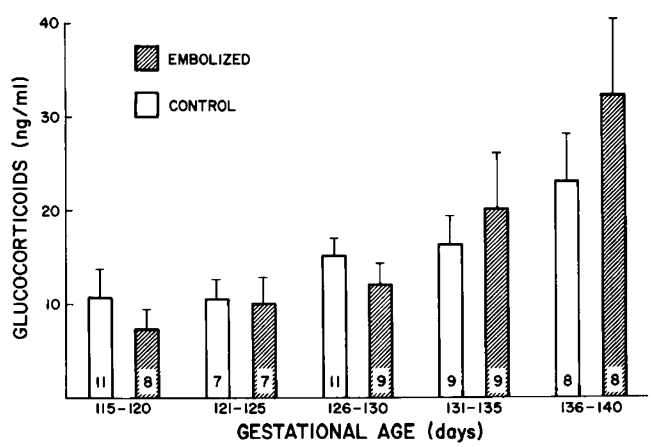

Fig. 1. Plasma glucocorticoid concentrations in control (open bars) and growth retarded (cross-hatched) fetal lambs from 115 to 140 days of gestation. Values are means ± SE. The number in each bar represents the number of fetuses studied during that time period.

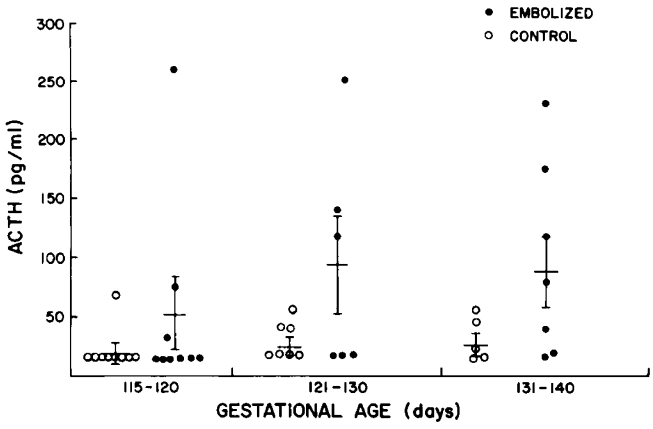

Fig. 2. Plasma ACTH concentrations in control (0) and growth retarded fetal lambs from 115 to 140 days of gestation. The horizontal line represents the mean and the vertical line the SE of each group.

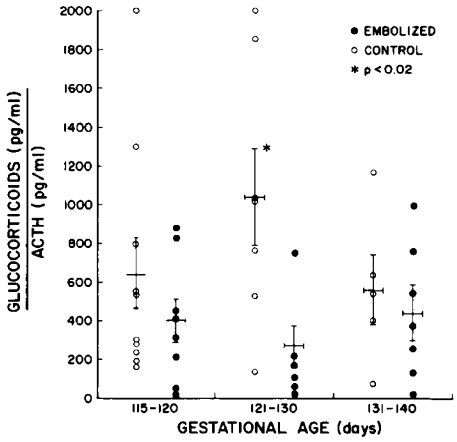

Fig. 3. Plasma glucocorticoid-to-ACTH concentration ratio in control (0) and embolized ewes from115 to 140 days of gestation. The horizontal line represents the mean and the vertical line the SE of each group.

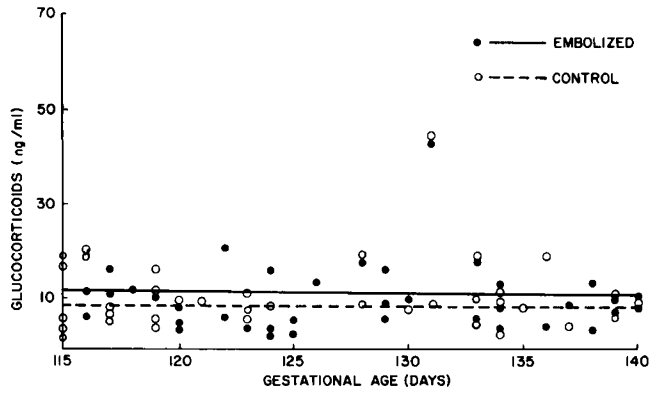

Fig. 4. Maternal arterial plasma glucocorticoid concentration in control (0) and embolized ewes from 115 to 140 days of gestation. The regression lines for each group are drawn in the picture.
----, ——, regression lines for control and embolized ewes, respectively.

0031-3998/79/1300-0000$02.00/0

Printed in U.S.A.