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Suppression of Extravillous Trophoblast Invasion of Uterine Spiral Arteries by Estrogen During Early Baboon Pregnancy

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The present study determined whether estrogen plays a role in regulating invasion and remodeling of the uterine spiral arteries by extravillous trophoblasts during early baboon pregnancy. The level of trophoblast invasion of spiral arteries was assessed on day 60 of gestation (term is 184 days) in baboons untreated or treated on days 25–59 with estradiol or aromatizable androstenedione. The administration of estradiol or androstenedione increased ($P < 0.01$) maternal serum estradiol levels approximately 3-fold above normal. The mean ± SE percentage of spiral arteries/arterioles invaded by extravillous cytotrophoblasts in estradiol-treated baboons for vessels with diameters of 26–50 μm (0.0 ± 0.0), 51–100 μm (1.2 ± 0.7) and >100 μm (13.2 ± 5.5) was 100%, 90%, and 75% lower ($P < 0.001$), respectively, than in untreated baboons (2.4 ± 1.2%; 11.0 ± 5.5%, and 54.5 ± 8.5%, respectively). Similar results were obtained with androstenedione treatment. However, the distribution of uterine spiral arteries grouped by diameter or number of arteries per basal plate area, i.e. microvessel density, were similar in untreated and estrogen-treated baboons. We suggest, therefore, that the low levels of estrogen exhibited during early primate pregnancy are required to permit normal progression of trophoblast vascular invasion and that the surge in estrogen which occurs during the second-third of normal pregnancy has a physiological role in suppressing further arterial trophoblast invasion. Consequently, we propose that the estrogen-dependent restraint of spiral artery invasion/remodeling ensures optimal blood flow dynamics across the uteroplacental vascular bed to promote normal fetal growth and development.
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Keywords: Trophoblast; Invasion; Estrogen; Baboon; Spiral arteries

INTRODUCTION

During the first one-third of human and several nonhuman primate pregnancies a select population of extravillous placental cytotrophoblasts lose their proliferative phenotype, migrate and invade the spiral arteries of the uterus. Histological studies of the human, baboon and macaque [1–8] show that cytotrophoblasts enter and colonize the walls of spiral arterioles/arteries by displacing endothelial cells from the basal lamina and replacing vascular smooth muscle cells within the tunica media. Consequently, the structure of and blood flow dynamics within these arteries become modified, apparently to promote implantation and blood flow to the developing conceptus [9,10]. The role of intravascular and interstitial cytotrophoblast cells in vessel modification has not been resolved. However, signals between cytotrophoblasts and the extracellular matrix involving integrins, cell adhesion molecules, matrix metalloproteinases, gap junctions, as well as growth factors, appear to play an important role in anchoring cytotrophoblasts to the extracellular matrix, and cytotrophoblast differentiation, migration and vascular invasion [11–19]. A reduction in trophoblast vascular invasion and spiral artery remodeling may represent an underlying defect in human placental development that results in complications of pregnancy, including fetal growth restriction and preeclampsia [20–23]. Relatively little is known, however, about the in vivo regulation of the process of uterine artery invasion by trophoblasts.

Our laboratories have shown that estrogen has a major role in regulating morphological and functional differentiation of the villous trophoblast and signals between the placenta and fetus important to fetal growth and development in the baboon

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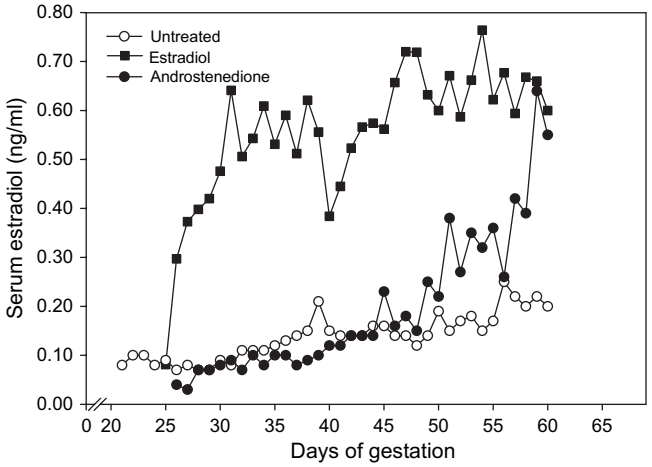

Figure 1. Maternal peripheral serum estradiol levels in baboons untreated or treated daily on days 25–59 of gestation with estradiol or androstenedione as detailed in the footnote of Table 1.

[24–27]. Moreover, estrogen regulates the villous cytotrophoblast expression of growth factors, i.e. vascular endothelial growth factor (VEGF) [28], that appear to have a role in extravillous cytotrophoblast vascular invasion [29,30]. In the present study, therefore, we utilized the baboon to test the hypothesis that estrogen also plays an important role in regulating the extravillous cytotrophoblast pathway involving invasion of the uterine spiral arteries by trophoblasts during early primate pregnancy.

MATERIALS AND METHODS

Animals

Female baboons (*Papio anubis*), originally obtained from the Southwest Foundation for Biomedical Research (San Antonio, TX) and weighing 13–15 kg, were employed in this study. Baboons were housed individually in large primate cages and received 20% protein pellets (Harlan Primate Diet, Madison, WI) and fresh fruit twice daily, multiple vitamins daily, and water ad libitum. Females were paired with male baboons for 5 days at the anticipated time of ovulation as estimated by menstrual cycle history and turgescence of external sex skin and day 1 of pregnancy was designated as the day preceding perineal deturgescence. Baboons were cared for and used strictly in accordance with USDA regulations and the NIH Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996). The present experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine.

At 1- to 2-day intervals throughout the study period, baboons were sedated briefly with ketamine HCl (10 mg/kg body weight, i.m.) and a 2–4 ml blood sample was obtained from a maternal peripheral saphenous vein. Serum estradiol levels were determined by RIA using an automated chemiluminescent immunoassay system (Immulite, Diagnostic Products Corp., Los Angeles, CA) as described previously [31].

To examine the effect of elevating estrogen early in pregnancy on vascular invasion, placentas were removed by cesarean section on day 60 of gestation (length of gestation is 184 days) from halothane-anesthetized baboons untreated ($n = 5$) or treated daily on days 25–59 with estradiol benzoate (0.35 mg/day s.c. in 1.0 ml sesame oil, $n = 5$) or androstenedione (30 mg/day s.c. in 1.0 ml sesame oil, $n = 6$). Androstenedione is readily taken up by and converted to estrogen within the primate placenta [32,33], resulting in a physiological distribution of estrogen locally within the placental trophoblast.

Placental sampling and vascular invasion

At least eight randomly-selected blocks (each approximately 5 mm^3) of placental basal plate, i.e. maternal–fetal tissue that adheres to the placenta at delivery, were obtained from each baboon. Because trophoblast invasion of uterine spiral arteries does not extend beyond the decidua in the baboon [34], quantification of the level of vascular invasion was confined to the decidual basalis. Tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned at 4 μm, and processed for hematoxylin/eosin histology and cytotrophoblast/epithelial cell-specific cytokeratin immunocytochemistry. Light microscopy (Nikon Eclipse E 1000 M, Tokyo, Japan) and an image analysis system (IP Lab, version 3.63, Scanalytics, Inc., Fairfax, VA) were used to analyze all arteries in each tissue sample and categorize them into groups with diameters of <25, 26–50, 51–100, and >100 μm. Arterial diameter was measured via an eyepiece micrometer as the smallest distance across the center of the vessel lumen from the inside edge of the surrounding smooth muscle (not invaded) or

Table 1. Maternal serum estradiol levels and placental and fetal body weights in baboons

Treatment	*N*	Estradiol (ng/ml)		Placental weight (g)	Fetal body weight (g)
		Saphenous	Uterine		
Untreated	5	0.18 ± 0.02	0.55 ± 0.08	30.8 ± 1.8	12.1 ± 0.6
Estradiol	5	0.59 ± 0.09*	1.12 ± 0.26*	26.9 ± 1.4	11.7 ± 1.2
Androstenedione	6	0.60 ± 0.15*	1.51 ± 0.34*	25.1 ± 2.8	11.0 ± 1.3

Mean ± SE maternal saphenous and uterine vein serum estradiol levels and placental and fetal body weights on day 60 of gestation in baboons untreated or treated daily on days 25–59 of gestation (term = 184 days) with estradiol benzoate (0.35 mg/day, s.c.) or androstenedione (30 mg/day, sc).
*Values different ($P < 0.01$) than in untreated baboons (ANOVA and Newman–Keuls multiple comparison test).