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# Endocrinologic Comparison of Activin A Secretion During Pregnancy and Early Lactation in Japanese Monkeys, Chimpanzees, and Humans

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**Secretion of activin A in Japanese monkeys and chimpanzees during pregnancy and early postpartum periods was investigated, and the results were compared with those in humans. Plasma activin A increased throughout pregnancy in Japanese monkeys, and the level was significantly higher in the third and fourth quarters than in the first quarter. After parturition in the Japanese monkey, circulating activin A decreased to levels seen during the normal menstrual cycle. In chimpanzees, plasma activin A remained low until the third quarter of pregnancy and abruptly increased in the fourth quarter. After parturition in chimpanzees, however, circulating activin A concentrations still remained as high as levels in the fourth quarter until 42 h after parturition, then decreased to nadir levels at 1 mo after parturition, suggesting that activin A in chimpanzees may be secreted from other organs such as uterus in addition to placenta. Positive staining with inhibin/activin α-, βA-, and βB-subunit antisera was observed in the cytoplasm of the syncytiotrophoblast of term placenta in the Japanese monkey, chimpanzee, and human. These results demonstrated that circulating activin A gradually increased from early pregnancy in Japanese monkey, whereas an abrupt increase occurred at late pregnancy in chimpanzees. These results also demonstrated that the syncytiotrophoblast of placenta is the source of activin A in Japanese monkeys and chimpanzees as well as humans.**

**Key Words:** Nonhuman primates; activin A; inhibin subunits; pregnancy; lactation.

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## Introduction

Activin is a homodimeric or heterodimeric glycoprotein consisting of βA- and βB-subunits, classified into three types: activin A (βA-βA), activin B (βB-βB), and activin AB (βA-βB). Activin A was initially found as a stimulator of follicle-stimulating hormone secretion *(1–3)* and also as an erythroid differentiation factor *(4)*, a member of the transforming growth factor-β superfamily that is a large group of intracellular proteins involved in many aspects of development. Activins have been identified in many other tissues including the pituitary gland, bone marrow, pancreas, and adrenal glands, showing that activin has numerous biologic activities. On the other hand, inhibin is a heterodimeric glycoprotein consisting of an α-subunit and either a βA-subunit (inhibin A) or a βB-subunit (inhibin B), and it has been reported that inhibin is secreted from ovary and placenta in female primates *(5–11)*. It has been shown that inhibin and activin play important autocrine and paracrine roles in folliculogenesis, oocyte maturation, and corpus luteum function *(12,13)*.

During pregnancy in humans, both the inhibin/activin βA- and βB-subunit mRNAs and associated proteins have been identified in the placenta, deciduomata, and fetal membranes *(14–17)*. It was reported in humans that maternal serum levels of both activin A and inhibin A during pregnancy increased toward term and activin A and inhibin A were detected in placenta *(18–26)*. These reports suggested that both activin A and inhibin A are mainly secreted from placenta during pregnancy in humans *(27–30)*.

In pregnant nonhuman primates, only a few hormonal profiles of circulating inhibin were reported in marmosets *(31,32)*, baboons *(33,34)*, Japanese monkeys *(5,8,9)*, and chimpanzees *(7)*, but none have been reported for circulating activin. In Japanese monkeys, circulating inhibin A showed an abrupt rise at the second quarter and maintained its level until term, whereas circulating inhibin B gradually increased until the fourth quarter *(5)*. On the other hand, in chimpanzees, circulating inhibin A and B concentrations

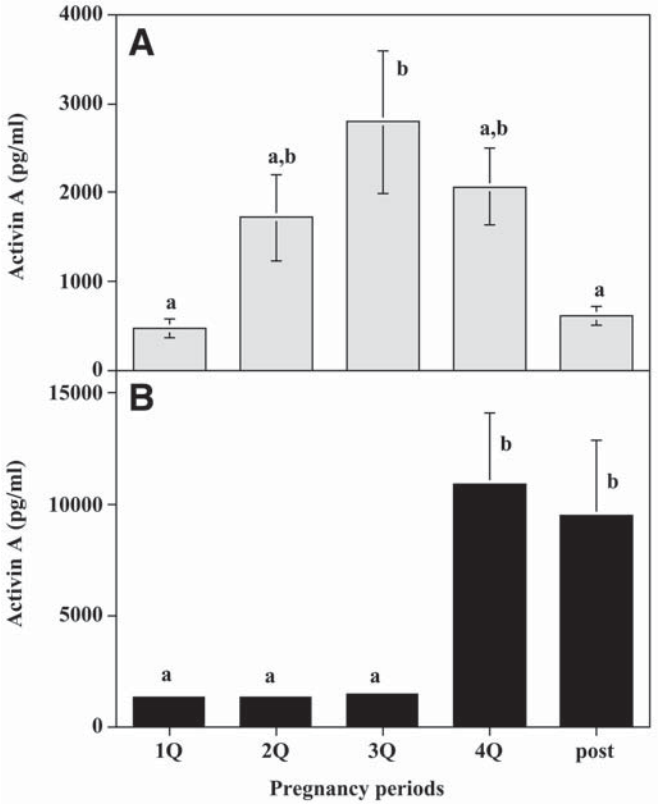


**Fig. 1.** Changes in maternal circulating concentrations of Activin A during pregnancy and early postpartum period (post) in Japanese monkeys (**A**) and chimpanzees (**B**). Pregnancy periods: 1Q: 0–39 d, 6–9 wk; 2Q: 40–79 d, 10 wk; 3Q: 80–119 d, 20 wk; 4Q: 120 d, 25 wk, post within a week after parturition, 18–48 h after parturition in the Japanese monkeys and chimpanzees, respectively. Each value represents the mean ± SEM (Japanese monkeys: $n = 4$; chimpanzees: $n = 5$). Different letters in each graph indicate a significant difference ($p < 0.05$) between each group (Tukey-Kramers test).

remained low throughout pregnancy *(7)*. Since activin shows close interactions with inhibin, it is assumed that activin maintains a different endocrine profile and has a different source of secretion during pregnancy in different primates. Therefore, in the present study, to clarify the profile and localization of activin in different primates, we measured circulating activin A during pregnancy in chimpanzees and Japanese monkeys by enzyme-linked immunosorbent assay (ELISA) and investigated the localizations of inhibin/activin subunits in placenta of those animals by immunohistochemistry.

## Results

### *Concentrations of Activin A During Normal Menstrual Cycle and Pregnancy*

As shown in Fig. 1A, plasma concentrations of activin A in Japanese monkeys were significantly ($p < 0.05$) higher in the third quarter than in the first quarter. After parturition in Japanese monkeys, concentrations of circulating activin A decreased to nonpregnant menstrual cycle levels (640.5 pg/mL at follicular phase; 549.5 pg/mL at luteal phase).

In chimpanzees (Fig. 1B), plasma concentrations of activin A were significantly higher ($p < 0.05$) in the fourth quarter than in the first to third quarters. Within 42 h after parturition in chimpanzees, concentrations of circulating activin A still remained as high as levels in the fourth quarter, and then decreased to nadir levels 1 mo after parturition.

Although only one sample of each animal was used, concentrations of activin A in homogenates of placentas were much higher in a Japanese monkey and a chimpanzee than in a human (360 ng/g in Japanese monkey; 108.3 ng/g in chimpanzee; 4.2 ng/g in human).

### *Immunohistochemichal Localization of Inhibin/Activin Subunits in Placenta*

As shown in Fig. 2, positive staining with inhibin/activin α-, βA-, βB-subunit antisera was observed in the cytoplasm of syncytiotrophoblast of term placenta in a Japanese monkey, a chimpanzee, and a human. Normal rabbit serum did not show any immunohistochemical staining within either trophoblasts or interstitial cells (Fig. 2A,E,I).

## Discussion

The present study provided patterns of circulating levels of activin A during pregnancy and early lactation in Japanese monkeys and chimpanzees. In Japanese monkeys, the steady rise in circulating levels of activin A was observed during gestation. By contrast, in chimpanzees, activin A remained low until the third quarter, then abruptly increased at the fourth quarter.

In addition, activin A was detected in homogenates of placenta of chimpanzees and Japanese monkeys as well as humans at high concentrations; furthermore, inhibin/activin α-, βA-, and βB-subunits were localized to syncytiotrophobrast in the placenta. These findings suggest that in chimpanzees and Japanese monkeys, the major sources of activin A as well as of inhibin A and inhibin B *(5,7)* are syncytiotrophoblast, as in humans *(27,28,35)*. The different profile of activin during pregnancy may be owing to the different placental morphologies among these three primate taxa. Placentas of macaques are characterized as bidiscoid and implanted in superficial, straight connections between maternal and fetal tissues, whereas human and chimpanzee placentas are characterized as single disk and implanted in interstitial, irregular connections between maternal and fetal tissues *(36)*. In chimpanzees, however, the circulating concentrations of activin A remained high even 42 h after parturition, although placentas were delivered about 1 h after parturition. In human, circulating activin A declined immediately following delivery, becoming undetectable within 6 h postpartum *(37)*, suggesting that there

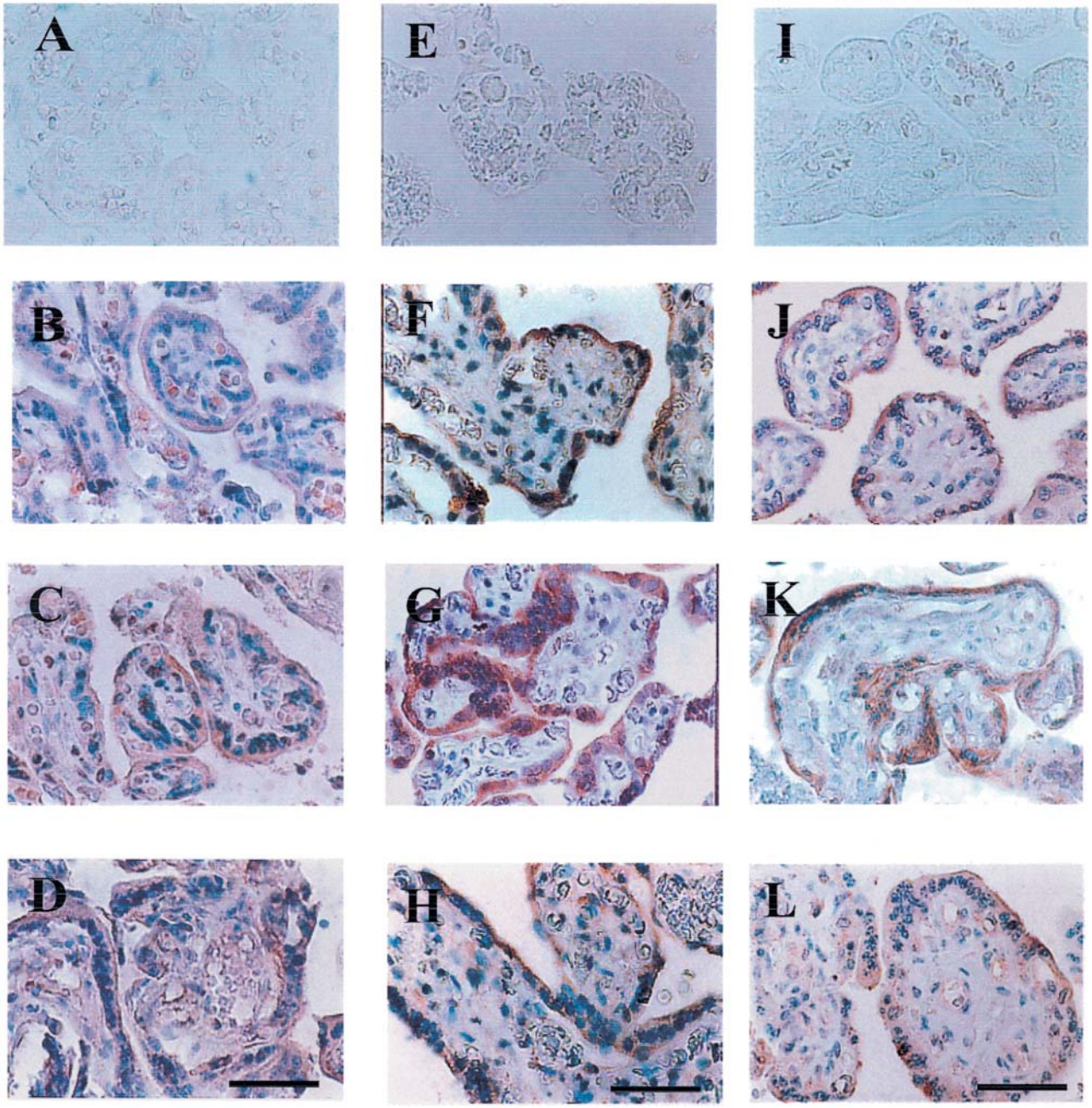


**Fig. 2.** Immunohistochemical staining for α-subunit (B, F, J), βA-subunit (C, G, K), and βB-subunit (D, H, L) in the placenta of Japanese monkeys (**A–D**), humans (**E–H**) and chimpanzees (**I–L**) obtained just after parturition. The localization of all three subunits was observed in the syncytiotrophoblast in all animals. Controls were stained with normal rabbit serum (A, E, I), and showed no immunohistochemical staining in all animals. Bar = 30 μm.

might be other sources of activin A, such as the uterus, in addition to placenta in chimpanzees.

There are close interactions between activin and inhibin secretion during pregnancy in humans *(18)*. Regarding activin A in the present study, in Japanese monkeys and humans, circulating activin A showed a steady rise during pregnancy, reached peak levels at late pregnancy, followed by an abrupt decline after delivery. This was also true for inhibin A in Japanese monkeys and humans. On the other hand, the patterns of inhibin B are completely different from inhibin A and activin A between Japanese monkeys and humans. In Japanese monkeys, plasma levels of inhibin B were already elevated at the early stage of pregnancy and continued to rise to peak levels at the fourth quarter, followed by an abrupt decline after delivery. By humans, however, levels of inhibin B in maternal circulation remained

low at all stages of pregnancy. By contrast, in chimpanzees, circulating inhibin A and inhibin B levels remained low throughout pregnancy, whereas activin A levels dramatically rose at the late stage of pregnancy.

The roles of maternal activins are not well established, though activins have been shown to regulate placental hormone secretion in vitro *(16)*. During pregnancy in humans, placental secretion of activin A, as well as inhibin A and inhibin B, is altered in some gestational diseases. It has been reported that activin A shows abnormally high levels at the third trimester in patients with preeclampsia, gestational hypertension, and preterm delivery *(27,38)*. This suggests that activin A may be an important marker of gestational diseases and fetal risk surveillance. As a model of humans for studying these diseases and risks, these comparative data on humans and nonhuman primates were confirmed to be important for identifying the applicability of different primate species for these human problems.

Collectively, the data of the present study provide evidence that placental syncytiotrophoblasts are a source of activin A increased in the late stages of pregnancy in Japanese monkeys, chimpanzees, and humans.

## Materials and Methods

### *Animals and Samples*

Five pregnant female chimpanzees (*Pan trogrodytes*) were used. The mean pregnancy period of the five pregnancies was 229.0 ± 5.0 d (range: 213–243 d). Blood samples were collected at 6–9, 10, 20, and 25 wk of pregnancy and 18–42 h postpartum; blood was drawn from the cubital vein under general anesthesia induced by dropelidol (Sankyo, Tokyo, Japan) and ketamine (Sankyo). Eight pregnant Japanese monkeys (*Macaca fuscata fuscata*) were also used. The mean pregnancy period of the eight pregnancies was 165.0 ± 3.2 d (range: 157–180 d). Animals were housed individually in an air-conditioned room with controlled temperature (20–25°C) and lighting (lights on: 6:00 AM to 6:00 PM). The day of ovulation was designated d 0 of the pregnancy, and the pregnancy was divided into five stages as follows: first quarter (0–39 d), second quarter (40–79 d), third quarter (80–119 d), fourth quarter (120 d until parturition), and postpartum (1 wk after parturition). All the blood samples were drawn from the cubital vein without anesthesia once in a quarter and 1 wk after parturition. Japanese monkeys were well acclimated to blood collections. The serum samples were stored at –20°C until the assay. Animal care and the experimental protocol were approved by "Guidelines for the Care and Use of Laboratory Primates" prepared by the Primate Research Institute, Kyoto University, Japan (1986).

### *Samples of Placentas and Treatments*

After vaginal delivery, placenta samples from a chimpanzee, a Japanese monkey, and a woman were immediately fixed in 4% paraformaldehyde (Sigma, St Louis, MO) in 0.05 *M* PBS, pH 7.4, and embedded in paraffin for immunohistochemistry.

For the ELISA, after the delivery, the placentas from a chimpanzee, a Japanese monkey, and a woman were homogenized with 0.85% (w/v) NaCl solution (1 g of placenta/mL) using a homogenizer (Phiscotoron, Nichion, Tokyo, Japan) and centrifuged at 25,000*g* for 30 min at 4°C. The supernatants were used for the ELISA for activin A.

### *ELISA for Activin A in Blood and Placenta*

Concentrations of activin A in peripheral blood and placental homogenates were measured using a commercially available ELISA kit (Serotec, Oxford, UK) developed for human activin A *(39)*. Recombinant human activin A was used as an assay standard. This ELISA kit allows for the specific measurement of total activin A concentrations. As a pilot study, dose-response curves of serially diluted placental homogenates (0.195–3.125 ng for Japanese monkey and 0.75–1.56 ng for chimpanzee) and plasma samples (31.2–62.5 µL for Japanese monkey and chimpanzee) were parallel to the standard curves of activin A, indicating that it was possible to measure the concentrations of activin A in peripheral plasma and placental homogenate of the Japanese monkey and the chimpanzee.

### *Immunohistochemical Localizations of Inhibin/Activin α, βA, and βB in Placentas*

Paraffin-embedded placentas were cut serially at 6 µm and sections were placed on glass slides coated with poly-L-lysine (Dako Japan, Kyoto, Japan). Tissue sections were deparaffinized with xylenes and treated in an autoclave in 0.01 *M* sodium citrate buffer (pH 6.0) at 121°C for 15 min to retrieve the antigen. The sections were then incubated with 3.0% $H_2O_2$ in methanol at room temperature for 1 h, and 0.5% casein–phosphate-buffered saline (PBS) at 37°C for 80 min to protect from nonspecific staining. Following treatment, the sections were incubated with primary antiserum against each inhibin/activin subunit, diluted with 1:10 block ace (Dainippon, Osaka, Japan), for 16 h at 37°C. The primary antisera against inhibin/activin α-, βA-, βB-subunits were used at dilutions of 1:6000, 1:10,000, and 1:4000, respectively. The primary antiserum against each inhibin/activin subunit was anti-[Tyr30]-porcine inhibin α-chain (1-30)-$NH_2$ conjugated to rabbit serum albumin ([Tyr30]-porcine inhibin α-chain [1-30]-$NH_2$ was kindly provided by Dr. N. Ling, Neuroendocrine Inc., San Diego, CA; anticyclic inhibin/activin βA [81-113]-$NH_2$ [#305-24-D] and anticyclic inhibin/activin βB [80-112]-$NH_2$ [#305-25-D] were kindly provided by Dr. W. Vale, The Salk Institute for Biological Studies, La Jolla, CA). For negative controls, normal rabbit serum was used instead of primary antiserum. After incubation, the sections were treated with 0.25% (v/v) biotinylated goat antirabbit secondary antiserum (Vectastain ABC kit; Vector, Burlingame, CA)

in 1:10 blocking ace for 1 h at 37°C, and then were incubated with 2% avidin-biotin complex (Vectastain ABC kit) in 1:10 block ace for 30 min at 37°C. Peroxidase activity was shown by incubation in 0.025% 3,3-diaminobenzidine tetrachloride (Sigma) in 0.01 *M* PBS, pH 7.4, containing 0.01% $H_2O_2$ for 1–30 min. The sections were counterstained with Lily-Mayers hematoxylin and observed under a light microscope.

### *Statistical Analyses*

All data are presented as the mean ± SEM. When a significant effect was obtained with one-way analysis of variance, the significance of the difference between two means was analyzed using the Tukey-Kramers test. A value of $p < 0.05$ was considered to be statistically significant.

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