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Effects of 17-Hydroxyprogesterone Caproate (17-OHPC) Administration to Pregnant Squirrel Monkeys *(Saimiri boliviensis boliviensis)*

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Poor reproductive performance of the squirrel monkey *(Saimiri boliviensis boliviensis)* in captivity and a relative progesterone (P) deficiency in pregnancy have been reported. Since premature births may contribute to pregnancy wastage, we attempted to measure the effectiveness of 17-hydroxyprogesterone caproate (17-OHPC) treatment of pregnant squirrel monkeys to prevent early deliveries. Based on clearance studies of nonpregnant animals, 25 mg of 17-OHPC was administered at 6-day intervals to a test group of 31 pregnant monkeys while the control group of 29 received saline. Venous blood was obtained at 6- to 12-day intervals for measurement of 17-hydroxyprogesterone (17-OHP), P, 17-B estradiol (E), and androstenedione (A), and dihydroepiandrosterone (DHEA) levels by radioimmunoassays. The treated group had a significant increase in serum 17-OHP ($P < 0.001$), P ($P < 0.01$), and DHEA ($P < 0.05$) levels compared to controls. The numbers of live births, stillbirths, or neonatal deaths did not differ significantly between groups. Although 17-OHPC administration appeared to increase P and 17-OHP levels, this did not alter the duration of pregnancy nor delay the onset of labor. A significant fall in 17-OHP, P, and E levels was observed 6–12 days before delivery.

Key words: pregnancy, 17-OH progesterone, progesterone, 17-B estradiol

INTRODUCTION

Pregnancies in squirrel monkeys *(Saimiri boliviensis boliviensis)* are characterized by a high incidence of prenatal mortality [Abee et al., 1990] and early abortions [Diamond et al., 1985]. A variety of causes may be responsible for pregnancy wastage, such as rapid, strong labor, narrow pelvic outlets [Aksel & Abee, 1983], or prematurity. Additionally, neonatal loss of infants is high and the cause of this poor reproductive performance is not clearly understood.

Bars on importation of squirrel monkeys have made efforts to optimize reproduction in the breeding colonies very important. Our laboratory had addressed this issue by exploring some therapeutic modalities to reduce pregnancy loss and pre-

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vent prematurity. In a previous longitudinal study by our group, an apparent progesterone (P) deficiency throughout pregnancy was observed [Diamond et al., 1987]. Since P is an important steroid in maintenance of pregnancy in many species, increasing tenfold between early and late human pregnancy [Tulchinsky & Okada, 1975], we hypothesized that the lack of increase in P concentrations during pregnancy in laboratory-housed squirrel monkeys contributes to low birth weights and premature deliveries. Therefore, this steroid was selected to test our hypothesis. Treatment with 17-hydroxyprogesterone caproate (17-OHPC) has been shown to improve the outcome of human pregnancies [Johnson et al., 1979; Kauppila et al., 1980; and Yemini et al., 1985]. We attempted to improve pregnancy outcome by increasing the bioavailability of P during the second half of gestation. We treated pregnant squirrel monkeys with either long-acting 17-OHPC or sham-treated with saline, starting 60–70 days prior to the expected date of delivery, and evaluated and compared the progress of pregnancy, the length of gestation, and the delivery outcome of each group.

METHODS

Animals

Sixty mature female squirrel monkeys *(Saimiri boliviensis boliviensis)* were housed in breeding groups with sexually mature males. Environmental conditions and care of the animals have been described previously [Diamond et al., 1984]. Pregnancy was diagnosed by palpation on a bi-weekly basis, after the commencement of the breeding season. Animals were randomly assigned to treatment or control groups.

Treatment was started at mid-gestation, 60–70 days prior to estimated time of delivery. Thirty-one pregnant squirrel monkeys were given 25 mg of 17-OHPC intramuscularly every six days and the 29 control animals received normal saline at similar intervals. The treatment dose of 17-OHPC was selected as follows: the usual dose used to treat pregnant women to prevent premature labor, 250 mg per week, [Johnson et al., 1979; Yemini et al., 1985] was reduced tenfold based on the body weight, surface area, and the metabolic rate of pregnant female squirrel monkeys. Isolated values of 17-hydroxyprogesterone (17-OHP) in male squirrel monkeys, cyclic females with high circulating estrogen levels, and pregnant females were 4, 24, and 81 ng/ml, respectively. Based on this additional information, the actual amount and the frequency of injections of 17-OHPC were determined as follows: in two non-cyclic females with baseline 17-OHP levels less than 10 ng/ml, serum values of 17-OHP were measured daily after administration of 25 or 50 mg of 17-OHPC (Figure 1). A sixfold increase over baseline was achieved between days three to five when animals received 50 mg of 17-OHPC intramuscularly. With the 25 mg dose, the values were elevated fourfold over baseline by day 3 and maintained the same concentration through day 6. On treatment days 7 and 8, 17-OHP levels fell to 12 and 10 ng/ml, respectively. Therefore, this dose which elevated 17-OHP concentrations by 15–20 ng/ml above the baseline was selected, and a six-day administration interval was chosen to maintain 17-OHP levels at the desired therapeutic concentrations.

Blood Sampling

Blood sampling was performed without anesthesia at 6–12 day intervals before the injection of 17-OHPC or placebo. The treatment was continued until the expected time of delivery. Almost all of the animals went into labor within 4 to 13 days of the anticipated date of delivery. If an animal aborted prior to reaching 70 days of gestation, treatment was stopped and it was removed from the protocol.

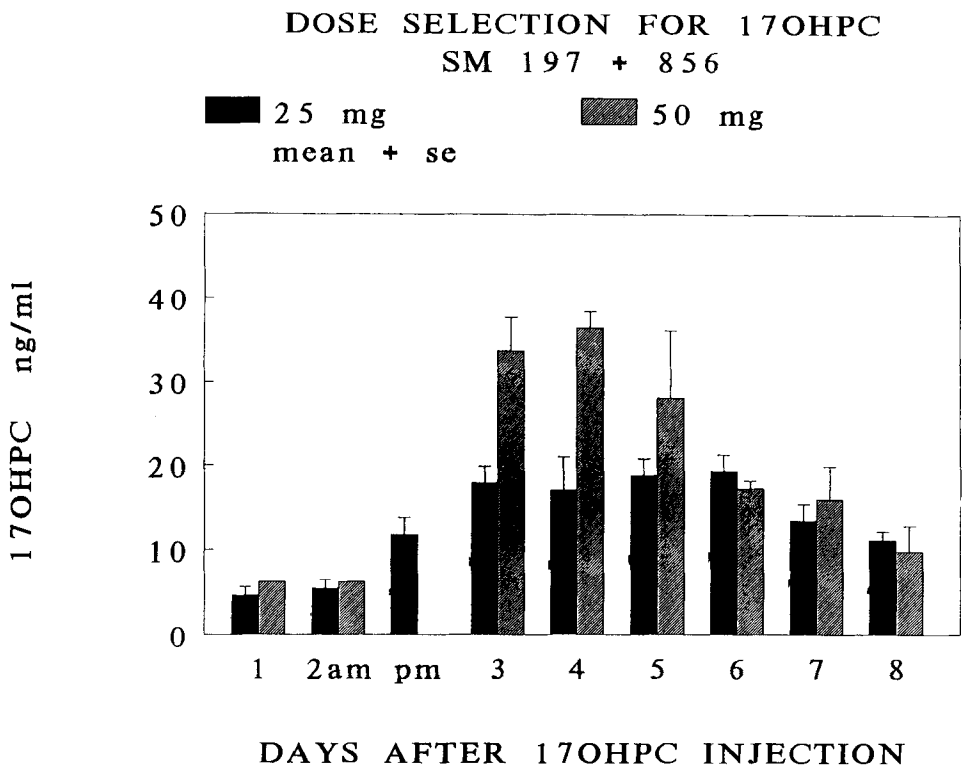

Fig. 1. The mean serum values after administration of 25 mg or 50 mg of 17-OH progesterone caproate (17-OHPC) to 2 non-cycling animals.

During the study, blood was collected every 6 days throughout the second half of pregnancy. Blood (1.5 ml) was obtained from a femoral vein between 8 and 9 AM and 17-OHPC or saline was administered subsequently. The procedure took no more than 3 minutes and the animals habituated well to this procedure. This brief period of stress has been shown not to affect circulating levels of gonadal hormones [Yeoman et al., 1988].

Hormone Measurements

17-OHP was assayed with an extraction procedure using the ED-64 antibody kindly provided by Dr. G. P. Chrousos, National Institutes of Health. The assay required 200 ul of a 1:100 serum dilution which was extracted with 3 ml of a 1:1 mixture of anesthesia (diethyl ether with 1.5% ethanol) and petroleum ether. Recoveries were later calculated from addition of small amounts of tritiated tracer. The dried organic phase was suspended in 0.5 ml of absolute ethanol of which 100 ul was used for recoveries and the remaining 400 ul was dried. The residue was resuspended in 100 ul of 0.1 N phosphate-buffered saline 0.1% gel (pH 7.8) for assay. Antibody (100 ul of 1:6700 working dilution) was added and incubated for 30 minutes at 30°C. Tritiated tracer hormone (100 ul, 5–7000 cpm) was then added for a second incubation of 1 hour at 30°C. Dextran-coated charcoal was used to separate the bound from free fraction before scintillation counting. The standard curve extended from 10 to 1000 pg/tube with coefficients of variation of 6.9% within and 9.6% between assays. The sensitivity of the assay was 5 pg per tube. Prior to

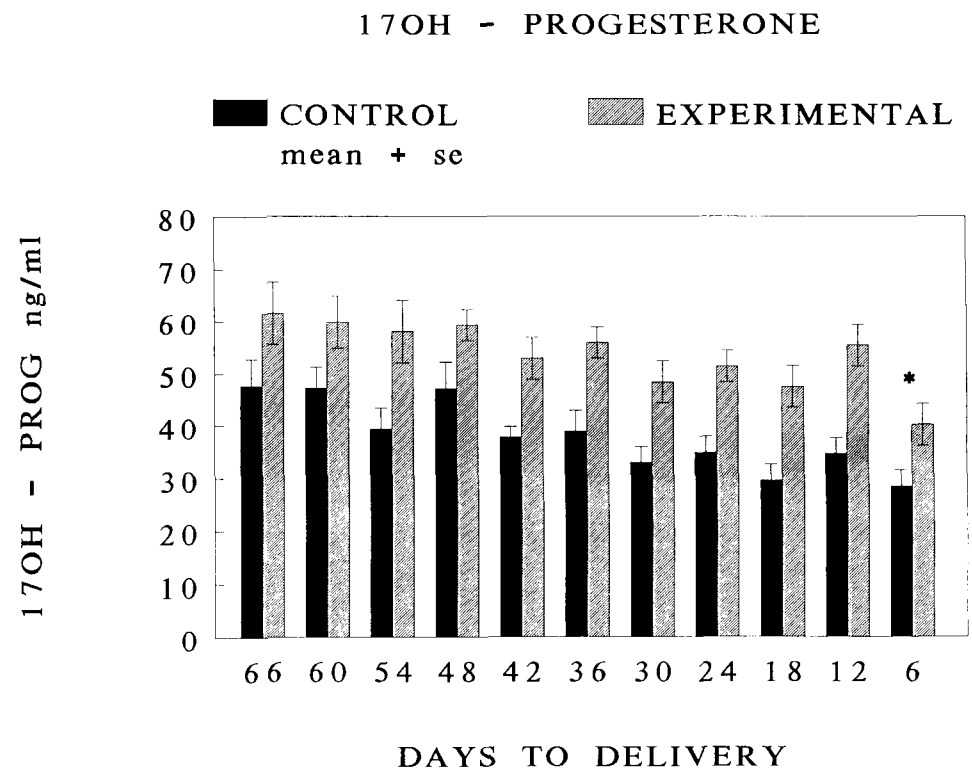

Fig. 2. Mean plasma 17-OH progesterone (17-OHP) levels at each sampling in experimental and control animals. *$P < 0.005$.

running all the samples, chromatography of the serum was performed to evaluate 17-OHP values; however, since chromatographed versus non-chromatographed serum values were still within the same range, it was decided that there were no benefits of chromatography in this assay procedure.

Progesterone, 17-B estradiol, (E) [Diamond et al., 1984], dehydroepiandrosterone (DHEA), and androstenedione (A) [Wiebe et al., 1984] were measured by previously published methods.

Statistical Analysis

A two-way repeated measure, analysis of variance (ANOVA) was used to test for differences between the 17-OHPC treated and control groups. Each group was sampled repeatedly over the days of the experiment. Significant main effects were further tested by a Duncan multiple range, post hoc analysis to determine which means differed significantly at the 0.05 probability level.

RESULTS

As shown in Figure 2, significantly higher serum concentrations of 17-OHP were achieved when pregnant monkeys were treated with 25 mg of 17-OHPC every 6 days ($P < 0.001$). As expected from the dose selection study (Figure 1), there was at least 15–20 ng/ml difference between treatment and control groups for each sampling day. In general 17-OHP concentrations at mid-gestation gradually de-

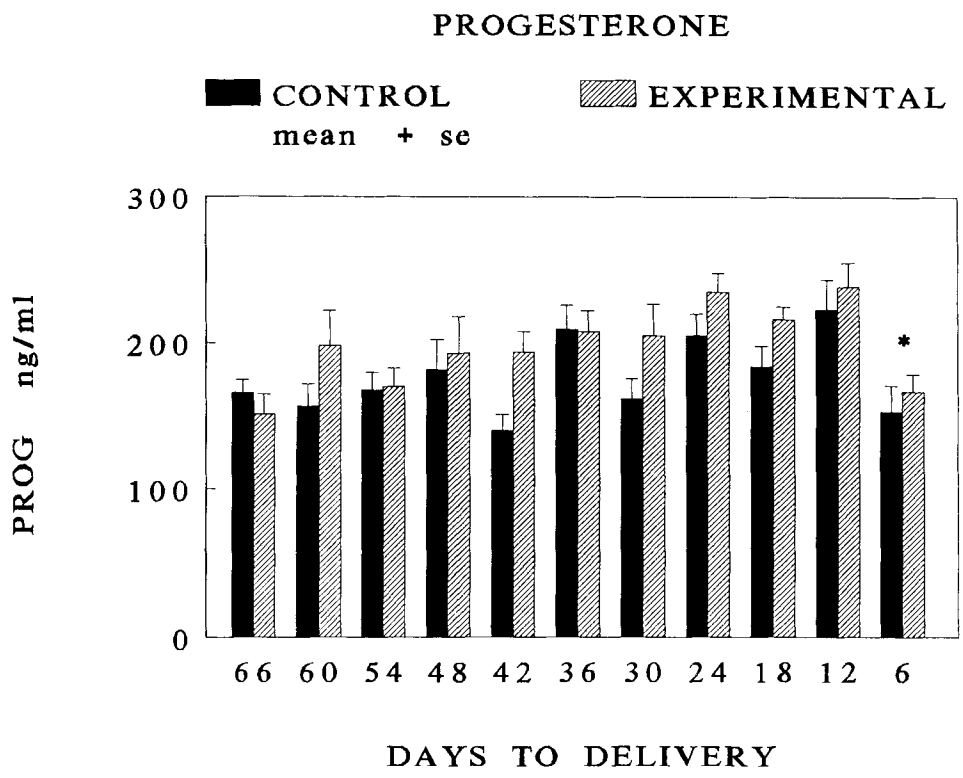

Fig. 3. Treatment with 17-OH progesterone caproate (17-OHPC) increased progesterone (P) concentrations in the experimental group. *$P < 0.005$.

clined from 45–50 ng/ml to 30–35 ng/ml in the control group, as the pregnancies progressed. This decline was not clearly evident in the experimental group. Mean 17-OHP levels (52.7 ± 1.3 ng/ml) in the experimental group were significantly higher than those (36.7 ± 0.1 ng/ml) of the control group ($P < 0.001$). The lowest level of 17-OHP was measured in the control group 6 days prior to delivery ($P < 0.005$).

As reported previously, mean P levels of pregnancy were comparable to luteal phase concentrations in this species [Diamond et al., 1984]. Treatment with 17-OHPC increased P concentrations to a mean of 193.9 ± 5 ng/ml in the experimental group, while a mean P level of 177.4 ± 5 ng/ml was observed in the control group ($P < 0.01$; Figure 3). A drop in P levels from day 12 to day 6 occurred in both groups prior to onset of labor ($P < 0.005$).

Total DHEA levels were relatively low and significant fluctuations in the treatment group registered a trend toward higher concentration of DHEA, 1.27 ± 0.1 ng/ml versus 0.9 ± 0.05 ng/ml in the control group ($P = 0.05$, Figure 4).

Androstenedione (A) levels, however, appeared to be totally unaffected by 17-OHPC treatment. Androstenedione levels both in control and treatment groups remained stable throughout pregnancy (Figure 5).

The 17-B estradiol levels during the second half of the gestation resembled 17-OHP levels in control animals, appeared to plateau at mid-pregnancy, and declined slowly throughout gestation. Although a tendency toward higher E con-

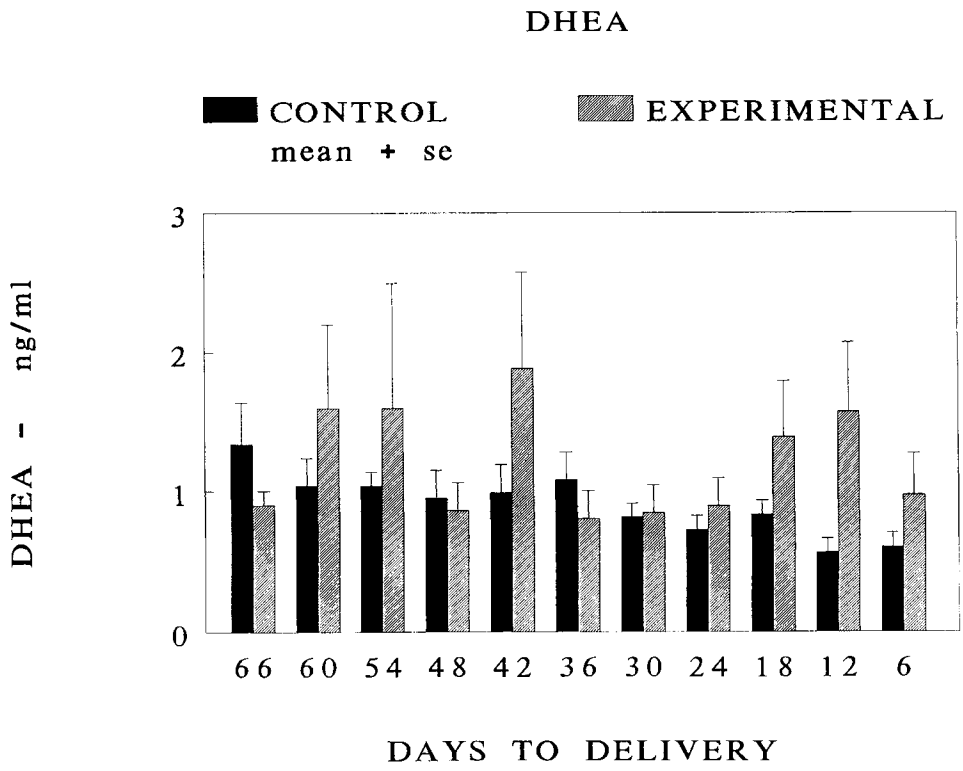

Fig. 4. Dihydroepiandosterone (DHEA) levels showed significant fluctuations in the treatment group.

centrations was noted in the treatment group, with wide fluctuations from controls on days 36, 24, and 12 to delivery, this observation did not quite reach statistical significance ($P = 0.0565$; Figure 6). A significant drop in E from day 12 to day 6 was observed in both groups before onset of labor at $P < 0.001$.

The duration of pregnancy in experimental and control groups ranged between 137 and 158 and between 140 and 154 days, respectively. There were 13 livebirths, 6 stillbirths, 10 neonatal deaths, and 2 late abortions (< 120 days) in the experimental group whereas 16 livebirths, 3 stillbirths, and 10 neonatal deaths were observed in the control group. These findings were not statistically different.

DISCUSSION

The duration of pregnancy in the squirrel monkey ranges from 141 to 162 days in length [Stolzenberg et al., 1979; Diamond et al., 1987], and a sizeable portion of pregnancy losses occur during the first half of gestation. Clewe [1969] was able to detect early pregnancy losses within 50 days of gestation by the mouse uterine assay, and in our group such losses were determined by serum chorionic gonadotropin measurements [Diamond et al., 1985]. In a group of 27 females of reproductive age followed between February and June there were 48 to 71% positive pregnancy tests, whereas the live birth rate was only 30% [Diamond et al., 1985]. Pregnancy wastage continued into the second half of gestation due to premature deliveries, stillbirths, and neonatal deaths.

Progesterone in human gestation is produced initially by the corpus luteum

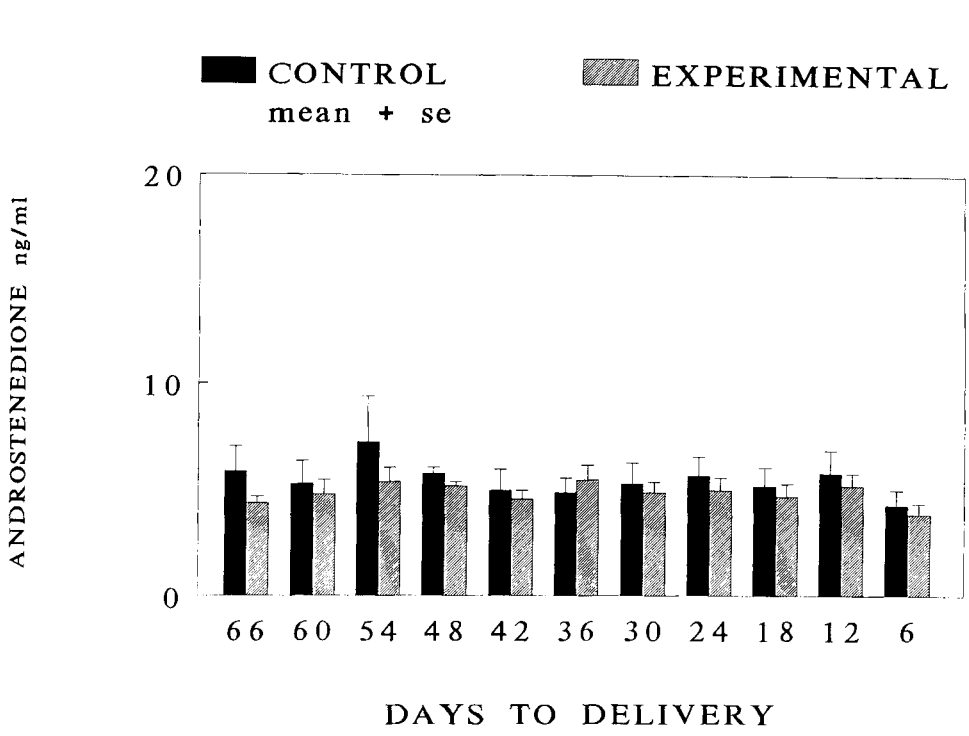

Fig. 5. Androstenedione (A) appeared to be totally unaffected by 17-OHPC treatment.

and later by the placenta, and is considered an important steroid for maintenance of human pregnancy [Tulchinsky & Okada, 1975]. In pregnant squirrel monkeys, P concentrations decline following an initial rise during the luteal phase despite rising chorionic gonadotropins, and once again reach luteal phase levels during the second half of the pregnancy [Diamond et al., 1987], demonstrating a possible P deficiency. As P has significant relaxing effects on myometrial function in human pregnancies [Kauppila et al., 1980], any treatment geared toward a P dominance in the system may suppress premature labor [Johnson et al., 1979; Yemini et al., 1985]. In the present study, we attempted to increase the P effects on pregnancy by administering 17-OHPC at mid-gestation. Although 17-OHPC injections increased maternal concentrations of 17-OHP, P, and DHEA levels significantly, pregnancy outcomes did not change.

In pregnant squirrel monkeys, serum E levels also rise after implantation, reach a peak level at mid-gestation (70 days), and decline slightly during the rest of the pregnancy [Diamond et al., 1987]. We observed the same type of an E curve in this study which involved more frequent sampling of the dams than our 1987 study. Also, in this study 17-OHP levels appeared to follow the P curve, as well as A and DHEA concentrations. There was no evidence of a rise in androgenic steroids during the pregnancy. Therefore, the data gave no clear support to the theory that increased androgens provide the substrate, through aromatization, for the rising E levels during the second half of pregnancy in this species as reported in other primates [Hearn, 1983]. Androstenedione and DHEA levels were not signif-

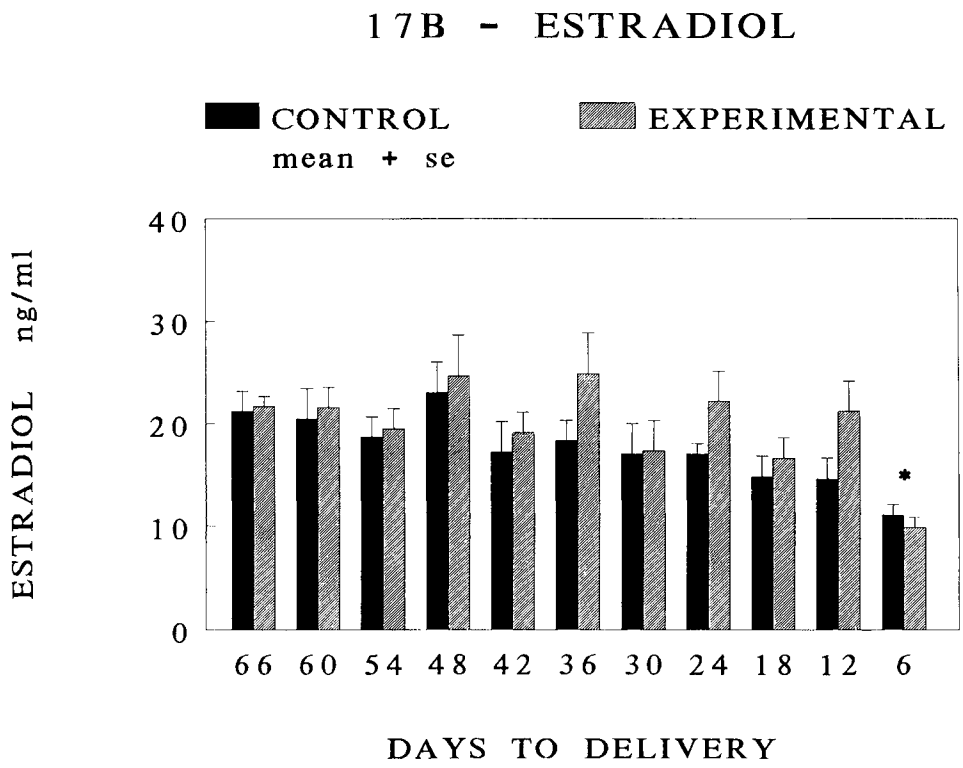

Fig. 6. 17-B estradiol (E) appeared to reach a plateau at mid-pregnancy in both groups and declined slowly. *$P < 0.001$.

icantly elevated during pregnancy compared to values obtained in cyclic animals during the breeding season. Although the possibility exists that testosterone, through aromatization, is the intermediate androgen, leading to E formation, this androgen could not be measured in this study due to limitations in the amount of serum available.

An interesting and unexpected finding was the consistent drop in 17-OHP, P, and E concentrations, a week to 10 days before onset of labor, in both the experimental and control groups, suggesting that this drop in E, P, and 17-OHP is causally related to onset of labor (Figs. 2, 3, and 6). It now appears possible to predict, rather precisely, the onset of labor by observing a 25 to 40% fall in P or E levels within a week of the expected date of delivery.

CONCLUSIONS

1. Treatment of pregnant squirrel monkeys with 17-OHPC did not alter the numbers of livebirths, stillbirths, or neonatal deaths, although it increased P and 17-OHP concentrations in serum.

2. The steroid data revealed a significant fall in 17-OHP, P, and E levels a week to 12 days before delivery.

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