Chorionic Gonadotropin Secretion During Pregnancy in the Chimpanzee (*Pan troglodyte*)[1] (36323)

M. T. Clegg and M. Weaver

Delta Regional Primate Research Center, Covington, Louisiana 70433

The excretion of high levels of gonadotropins during pregnancy has been described for a number of sub-human primate species, the rhesus monkey (*Macaca mulatta*) (1–3), the gorilla (*Gorilla gorilla*) (4), the Marmoset (*Callithrix jacchus*) (5), the Baboon (*Papio cynocephalus*) (6), and the Chimpanzee *Pan troglodyte*) (7). Since Elder and Bruhn (7) used the Friedman pregnancy test (8), they could only record positive or negative responses. The present report extends their observations to include an analysis of the temporal changes in urinary gonadotropin titers taking place throughout pregnancy in two chimpanzees (*P. troglodyte*). Also included are some observations on the influence of the nursing infant on the maternal reproductive cycle.

Methods. The two adult female chimpanzees (Loni and Molly) were proven breeders both having produced one or more infants prior to this study. The animals were caged individually and fed purina monkey chow supplemented with fruit daily. A number of menstrual cycles were observed for each female based on changes in the swelling and regression of the genital area and the finding of blood spots. They were paired at the time of maximal genital swelling with known fertile males. After mating, when ejaculation was visually determined, they were again separated. This procedure was repeated daily for the following 3 or 4 days. Urine samples were collected at weekly intervals thereafter until 1 week postpartum. Urine samples were either refrigerated at 3° within 2 hr of collection and subsequently transferred to a deep freezer or placed directly into the freezer.

Bioassays carried out on 21–23-day-old immature female rats consisted of twice daily injections of either straight urine or urine extracts over 4 days. Necropsy was performed approximately 24 hr after the last injection at which time ovarian and uterine weights were determined. Concomitantly, with each assay, dose–response curves for a standard HCG (human chorionic gonadotropin, Ayerst Lab.) preparation were carried out. Extracts of urine prepared by the alcohol precipitation method (9) were used during the first analysis of changes in gonadotropic titers during pregnancy on Loni. Because of poor recovery rates with this extraction procedure, straight urine was used during the second analysis on Molly. This procedure was feasible since the material appeared nontoxic in that body weights of recipient rats were not affected.

Pooled pregnant chimpanzee urine was extracted by a reverse dialysis procedure which involved dialyzing the urine against saturated $(NH_4)_2SO_4$. The extract obtained had an activity of approximately 13.5 IU HCG equivalents/mg of protein. Biological and immunological comparisons between this extract and HCG were performed. Tests included parallelism in dose–response curves based on the increase in ovarian weight response of immature rats, and cross-reaction on gel diffusion plates according to the procedure of Ouchterlony (10).

Observations on the length and frequency of menstrual cycles during nonpregnancy, pregnancy, and lactation were also made.

Results. Figure 1 gives the linear relationship between immature female rat ovarian weight response and graded doses of HCG. The sensitivity of this assay, as given by $\lambda = 0.022$, makes it a useful bioassay to serve as a standard for the estimation of equivalent

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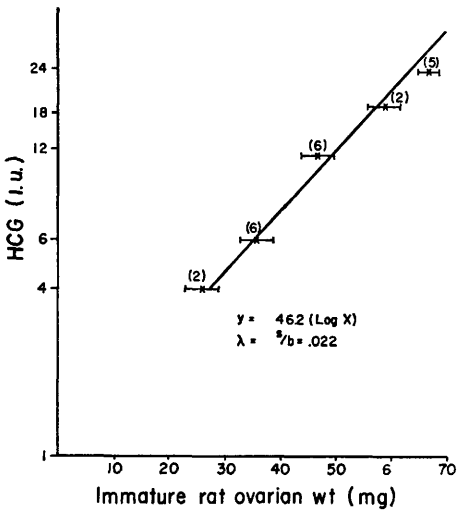

FIG. 1. Graph illustrating the highly significant relation between log of HCG dose (IU) and increase in ovarian weight of immature rats: (horizontal bars) range or standard error of mean.

activity in pregnant chimpanzee urine. Figure 2 shows that, when graded doses of pregnancy chimpanzee urinary extracts are compared with the HCG standard, a highly significant parallelism exists between the two curves and indicates similarity of biological activity.

Figure 3 is a photograph of an agar gel diffusion plate showing the precipitin lines formed by interaction between anti-HCG (center well) and either HCG (3 bottom wells) or pregnant chimpanzee urine extract (2 upper wells). The uniformity of the lines and lack of spurring indicate the immunological similarity between the two antigens.

In Fig. 4, each point represents the urinary titer as determined for each bioassay. The recorded date of conceptual mating for Loni was 3-24-69 and for Molly was 10-7-69. Both demonstrate that significant levels of gonadotropin associated with pregnancy appeared during the first and second weeks postmating and reached peak levels by days 30 and 40. In the case of Loni, the gonadotropic peak was followed by a rapid decline after day 44. Low but significant activity as indicated by stimulated recipient rat uterine weight persisted up to the week preceding parturition (which occurred on 10-7-69), 199 days postmating. In Molly, peak levels of urinary gonadotropic activity continued until after day 66 postmating, began to decline after day 80, and persisted at low levels until day 190. No activity was detectable thereafter; and parturition occurred on 5-21-70, 221 days postmating.

During the course of this study, data were obtained on the length of the menstrual cycle in 3 nonpregnant animals. Table I is an

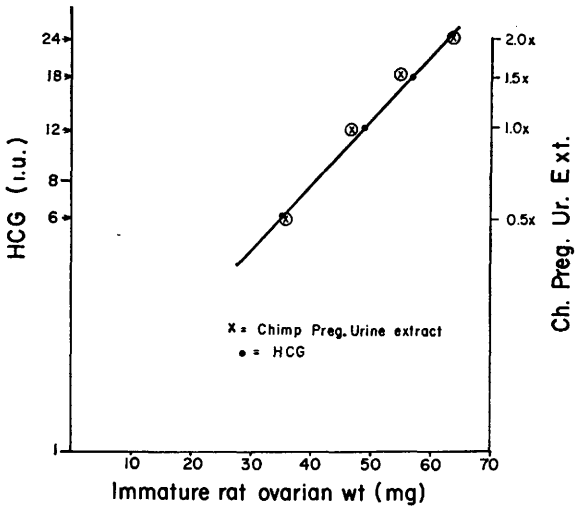

FIG. 2. Graph illustrating the highly significant parallelism in log dose–response curves between HCG and chimpanzee pregnant urine extract: (X) ml of extract (1.0 ml of extract contains 8.91 mg of protein).

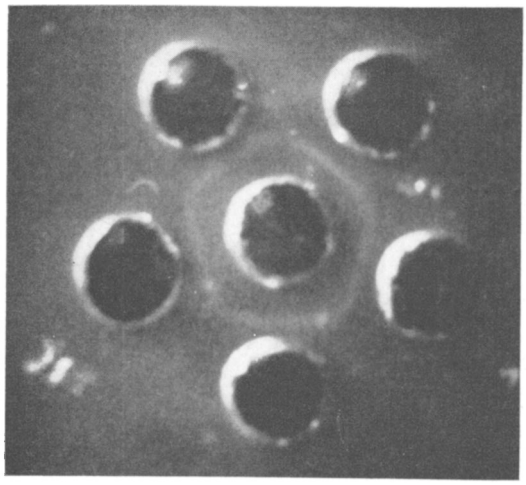

FIG. 3. Photograph of agar gel diffusion plate showing precipitin lines formed by interaction between anti-HCG (center well); and either HCG (3 bottom wells); or pregnant chimpanzee urine extract (2 upper wells).

analysis of these observations. The mean cycle length for 21 cycles was 35.5 days. Cycle lengths varied by as much ±7 days from the mean both within and between individuals. As a general rule cycles were abolished during pregnancy; although, in the case of Loni, one cycle was observed immediately subsequent to mating. Lactation and nursing also abolished reproductive cycles. Loni gave birth on Oct. 7, 1969; and was allowed to retain the infant. Throughout the period of suckling, the mother's vulvar and perineal region remained detumesced, indicating a failure of reproductive cyclicity. Although the infant was apparently healthy and grew normally, it died of a cardiac abnormality on Aug. 24, 1970 (or approx 11 months of age). Vulvar swelling in Loni was initiated almost immediately after loss of the infant and reached peak tumescence by Sept. 20, 1970 (27 days); again on Nov. 4, 1970 (45 days) and again on Dec. 1, 1970 (26 days) at which time she was mated. Another swelling peak occurred by Feb. 1, 1971 (60 days) followed by detumescence and no further cycles. On Jan. 6, 1971 urinary gonadotropic activity was first detected as indicated by a stimulation of recipient rat uterine weight. Thereafter, urinary gonadtropin titers reached levels of 850 and 1300 by 1-18-71 and 1-27-71, respectively. By Mar. 1, titers had dropped to approximately 200 IU HCG equiv/100 ml of urine. On Apr. 19, Loni aborted a fully developed fetus of approximately 4 months age.

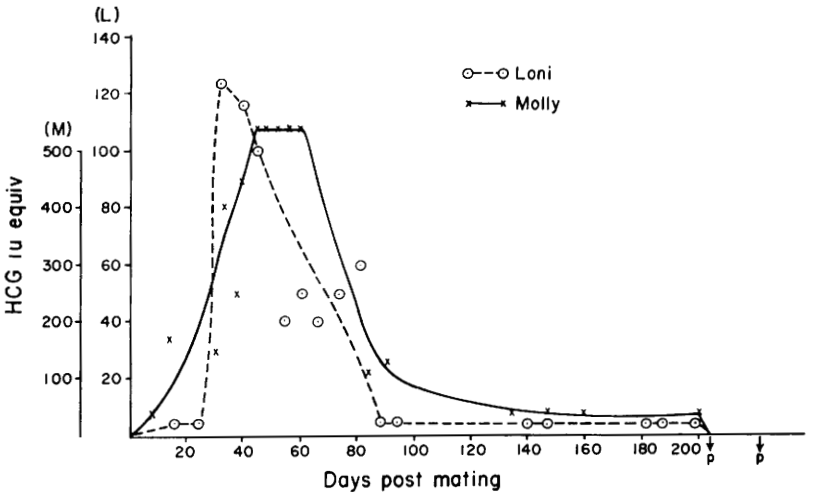

FIG. 4. Temporal changes in urinary gonadotropic titers in two chimpanzees occurring throughout pregnancy: (each point on the curve) the activity (HCG IU equivalents/100 ml of urine) for one bioassay. Bioassays were made on unextracted urine from Molly (M); or on urinary extracts prepared by alcohol precipitation (9) from Loni (L). Points below 10 IU HCG equivalents indicate activity based on a stimulation of immature recipient rat uterine weight.

TABLE I. An Analysis of Cycle Lengths for Three Mature Female Chimpanzees.

	No. of cycles (days duration)				Mean cycle length (days)
	26–31	32–37	38–43	44–49	
Katie	3	4	2		35
Dea	3	2	2	1	35
Molly	1		3		39
Composite	7	6	7	1	$\bar{x} = 35.5$

Mollie gave birth on 5-21-70. The infant was removed immediately postpartum. She first showed signs of vulvar swelling on Jul. 1, 1970; and reached peak tumescence by Jul. 18, 1970 (58 days), again on Aug. 30, 1970 (43 days), and on Oct. 1, 1970 (32 days). On this last date she mated, detumesced, and showed no further cycles. Based on recipient rat uterine weight response, low, but positive, gonadotropic titers could be detected in the urine on weeks 4, 8, and 10 postmating. By week 11, urinary gonadotropic titers had reached levels equivalent to 125 IU HCG/100 ml. During this week, X-rays showed the presence of a fetus, positively confirming pregnancy. Tests of urine from the week 14 postmating showed no evidence of gonadotropic activity; and X-rays taken during week 21 failed to show the presence of a fetus or its remains.

Discussion. It is interesting to compare the temporal changes in urinary gonadotropic levels taking place in the pregnant chimpanzee, as described in this study, with those found in the pregnant human. Peak levels of activity in the chimpanzee were found between days 30 and 50, postconception. In the human, highest amounts, reported by Evans *et al.* (11) and Browne and Venning (12), were present from weeks 6 to 10. After this time, they drop rapidly to reach a low, but fairly constant, level up to a time just preceding parturition. This similarity between the two of times of appearance and disappearance during gestation; of the remarkable parallelism in dose–response curves and of the cross reaction with anti-HCG, suggest that the gonadotropin produced during pregnancy by these two closely related primates share common biological properties. In one other study involving higher apes, Tullner and Gray (4) reported on the changes in gonadotropic levels occurring throughout pregnancy in one gorilla. Their data show that gonadotropic peak activity was not found until weeks 16 to 23, much later than those found in our study or those reported for the human.

The mean menstrual cycle length of 37 days and the interval from conception to parturition as reported in this study agrees reasonably well with other previously reported data (13). Graham (13) reports that the mean interval from conception to delivery is about 228 days. The gestation lengths for the two chimpanzees in this study were 199 and 227 days. Reproductive cycles, as a rule, do not occur during gestation. Douglas and Butler (14) state that a female chimpanzee will not begin cycling following the birth of her baby until approximately 3 months after the baby discontinues nursing. We likewise observed that genital swelling was abolished during lactation; but, in contrast, was initiated almost immediately when lactation stopped. These observations, together with the general knowledge that the sex skin of the chimpanzee is quiescent during lactation, suggest a lactational amenorrhea.

Summary. Quantitative alterations in urinary gonadotropin excretion were studied throughout pregnancy in two chimpanzees (*Pan troglodyte*). Weekly urine samples were collected following mating and gonadotropic activity determined by the increase in ovarian weights of immature female rats. Gonadotropic activity was first detected during weeks 1 and 2 postmating, and had reached peak titers by days 30 to 40. These levels persisted for 2 to 3 weeks and declined rapidly, thereafter, to low, but detectable, levels which were maintained until 1 to 3 weeks

prepartum. Reproductive cyclicity as determined by periodic swelling and detumescence of the genital and anal region disappeared during lactation. Biological and immunological tests of extracts from pregnant chimpanzee urine indicate a close relationship to human chorionic gonadotropin and the temporal changes in gonadotropic titers occurring throughout gestation are comparable to those reported for the human.

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