Late luteal rescue in the baboon
(Papio cynocephalus)

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Numerous studies have used human chorionic gonadotrophin (HCG) administration to study the response of the primate ovary to gonadotrophin stimulation. These studies are generally performed in the luteal phase with very few studies of the follicular phase. We have studied the effect of both HCG and gonadotrophin releasing hormone (GnRH) agonist administered at the early follicular phase in normally cycling baboons (Papio cynocephalus). Five baboons were treated with increasing doses of HCG for 5 consecutive days starting on day 1 of the cycle and three untreated baboons served as controls. Follicular and luteal phase lengths were determined and serum samples were assayed for progesterone, oestradiol and 17α-OH progesterone. In a separate study, six baboons were treated with GnRH agonist (WY-40972) on days 2–6 of the cycle and saline-treated baboons served as controls (n = 5). Mean peak progesterone concentrations (± SE) during the treatment interval were 3.88 ± 0.56 ng/ml in HCG-treated baboons compared to 0.19 ± 0.07 ng/ml in controls (P < 0.001). A similar significant increase (P < 0.001) in serum 17α-OH progesterone concentrations was also observed (6.13 ± 1.12 ng/ml versus 1.13 ± 0.49 ng/ml). In association with the increase in luteal steroids there was also a significant prolongation of menstrual cycle length from 32.7 ± 1.2 days in controls to 46.8 ± 4.9 days in HCG-treated baboons (P < 0.05), which involved prolongation of the follicular phase (16.7 ± 1.2 days to 29.0 ± 4.6 days; P < 0.05) with no difference in luteal phase length or progesterone concentrations. In GnRH agonist-treated baboons, mean (± SE) cycle length was prolonged to 46.3 ± 1.6 days and in saline-treated controls was 32.8 ± 0.8 days (P < 0.001), again this was completely represented by the change in follicular phase length, from 13.4 ± 0.7 days in controls to 27.2 ± 2.1 days in agonist-treated baboons (P < 0.001). In contrast, there was no significant difference in luteal phase length between these two groups (19.4 ± 0.7 versus 19.2 ± 1.0 days). The prolongation of the follicular phase was accompanied by significant increases in both progesterone (P < 0.01) and oestradiol (P < 0.01) during GnRH agonist treatment above control concentrations. Luteal phase concentrations of these hormones were not different from controls. These results demonstrate the previously unreported finding that gonadotropin stimulation will rescue the corpus luteum in the next follicular phase.

Key words: baboon/corpus luteum/gonadotropin/menstrual cycle/progesterone

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Introduction

Human chorionic gonadotrophin (HCG) has been used extensively in non-human primates and women to study the ovarian response to gonadotrophic stimulation. These studies have generally been carried out in the luteal phase in order to mimic the luteal rescue which occurs as the implanting trophoblast initiates production of chorionic gonadotropin (Quagliarello et al., 1980; Ottbre and Stouffer, 1984). Very few studies have examined the ovarian response to HCG in the follicular phase and the emphasis of the present study is to examine early follicular phase stimulation with both HCG and the endogenous flare of pituitary gonadotrophins which occurs in response to a GnRH agonist administration (Gordon and Hodgen, 1991). These results present the unexpected finding that it is possible, in the normally cycling baboon, to rescue the corpus luteum following gonadotrophic stimulation in the early follicular phase. This is the first full report to demonstrate this physiological phenomenon.

Menstrual cycle in the baboon

Normally cycling mature female baboons (Papio cynocephalus) were housed in individual cages and observed daily for menses and changes in perineal sex skin. These indices allow us to determine the beginning and end of the menstrual cycle as well as providing a reliable estimate of the day of ovulation. Our own studies, as well as those of others, indicate that ovulation occurs 3 ± 1 days before sex skin deturgescence (Hendrickx and Kraemer, 1969; Wildt et al., 1977).

Follicular phase HCG administration

Experimental animals received 5 consecutive days of HCG administration on days 1–5 of the follicular phase in one of two different dose regimens, starting with a dose of either 250 IU of HCG source, working up to 500 IU of HCG (n = 2), or at the higher dose starting at 500 IU working up to 900 IU on the 5th day (n = 3). Since results were comparable in both dosage groups, these data have been combined as a single experimental group. Three control baboons were untreated. All animals were bled daily for the first week of the cycle during the treatment interval and then at 2–3 day intervals throughout the remainder of the menstrual cycle. A similar bleeding protocol was implemented in control animals. Animals had previously been trained for phlebotomy without anaesthesia. These protocols were approved by the Animal Care Committee of both The Ohio State University and the University of Oklahoma. In order to study these endocrine responses, the peak endocrine value (oestradiol, progesterone, 17α-OH progesterone) during the days of HCG administration was used to calculate the mean (± SE) and compared against similarly calculated means in the control group.

Serum was separated and remained frozen until assayed for progesterone, 17α-OH progesterone and oestradiol using solid phase commercially available radioimmunoassays (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA). The interassay coefficients of variation (CV) for progesterone were 6.7%, for 17α-OH progesterone 7.8% and for oestradiol 7.9% respectively. The intra-assay CV in all assays were <5%.

Follicular phase GnRH agonist treatment

Normally cycling baboons were treated with GnRH agonist (WY-40972; Wyeth Laboratories), 100 mg per day for cycle days 2–6 (n = 6) (Corbin and Bex, 1981). Blood samples were obtained prior to administration, for the next 2 days and every 3 days until the next menses. Serum was assayed for oestradiol and progesterone by radioimmunoassay. Normally cycling baboons, treated with saline on the same schedule, served as controls. This protocol was approved by the Animal Care Committee of the Department of Clinical Sciences and Reproductive Biology of the Southwest Foundation for Research and Education, San Antonio, Texas.

These serum samples were assayed for progesterone using antisera GDN 337 generously supplied by Dr G.D.Niswender, Colorado State University, CO, USA. The within-assay and between-assay CV were 7% and 15.9% respectively, with sensitivities of <0.1 ng/ml. Serum oestradiol was determined with a highly specific antisera, E2TGK, kindly supplied by Drs D.C.Collins and K.Wright, Emory University, Atlanta, GA, USA. The within-assay and between-assay CV were 6.4% and 17.5% respectively, with a sensitivity of <10 pg/ml.

For both HCG and GnRH agonist studies, statistical comparisons were made using a t-test with nonpool variances. Calculations were made using a Stat Graphics Plus statistical program (Manogistics, Rockville, MD, USA).

Consequences of follicular phase HCG administration

Mean (± SE) cycle length of HCG-treated baboons was 46.8 ± 4.9 days. In control animals, cycle length was 32.7 ± 1.2 days (P < 0.05). This prolongation of cycle length was completely represented by a prolongation of the follicular phase where mean follicular phase length in control animals was 16.7 ± 1.2 days, but in HCG-treated animals was prolonged to 29.0 ± 4.6 days (P < 0.5). Luteal phase length in these two groups was not significantly different at
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Figure 1. Mean ± SE follicular phase and luteal phase lengths in human chorionic gonadotrophin (HCG)-treated baboons and untreated controls. The significant prolongation in menstrual cycle length in HCG-treated baboons is completely represented by the significant prolongation (P < 0.001) of follicular phase length, while luteal phase length is unchanged.

16.0 ± 0 days for controls and 17.8 ± 1.2 days for HCG-treated animals (Figure 1).

There was a stimulation of progesterone concentrations within 2–3 days after the initiation of HCG administration and on the day of peak progesterone concentrations in HCG-treated animals the mean (± SE) of serum progesterone was 3.88 ± 0.56 ng/ml. In control animals, progesterone concentrations were never elevated in the follicular phase with a mean of 0.19 ± 0.07 ng/ml (P < 0.01). A similar relationship existed for oestradiol with peak concentrations in the HCG-treated group of 68.8 ± 13.4 pg/ml compared with 39.9 ± 13.4 pg/ml in control animals (difference not significant). The mean peak concentration of 17α-OH progesterone was significantly elevated with HCG treatment at 6.13 ± 1.12 ng/ml and 1.13 ± 0.49 in control animals (P < 0.05) (Figure 2). Not only were luteal phase lengths not significantly different, but peak concentrations of progesterone in the luteal phase were also not different, being 8.99 ± 1.41 ng/ml in the HCG-treated baboons and 8.12 ± 0.82 ng/ml in control animals.

Figure 3 presents serum progesterone, 17α-OH progesterone and oestradiol concentrations in three baboons. A is an untreated control to demonstrate the low serum concentrations of these steroids in the early follicular phase. B and C represent two HCG-treated baboons and show the increase in these steroids during HCG treatment and the prolongation of the follicular phase with this treatment.

Consequences of follicular phase GnRH agonist administration

Menstrual cycle length is prolonged from a mean (± SE) of 32.8 ± 0.8 menstrual cycle length in controls to 46.3 ± 1.6 days (P < 0.001) following treatment with GnRH agonist. As can be seen in Figure 4, the prolongation of the menstrual cycle is completely due to an effect on the follicular phase which is 13.4 ± 0.7 days in controls, but 27.2 ± 2.1 days in GnRH agonist-treated baboons (P < 0.001). The luteal phase length is 19.4 ± 0.7 days in controls and 19.2 ± 1.0 days in agonist-treated baboons, which is not significantly different and represents no change in luteal phase length. Figure 5 presents the mean (± SE) serum progesterone concentrations through the follicular phase and luteal phase. One can see the significant elevation (P < 0.01) in progesterone concentrations in the early follicular phase associated with GnRH agonist treatment. More importantly, the prolongation of follicular phase length compared to untreated controls is evident. The pattern and concentrations of progesterone in the luteal phase are not significantly different. Figure 6 presents mean (± SE) serum

Figure 2. Mean ± SE peak serum concentrations of progesterone, 17α-hydroxyprogesterone (17α-OHP) and oestradiol during the time of human chorionic gonadotrophin (HCG) treatment and the corresponding interval in untreated controls. Both progesterone and 17α-OHP are significantly (P < 0.001) elevated during the HCG treatment interval in comparison to controls. Concentrations of serum oestradiol are also elevated but do not attain statistical significance.
oestradiol concentrations in control and GnRH agonist-treated baboons in the follicular and luteal phase. Significant elevation ($P < 0.01$) is seen in GnRH agonist-treated baboons during the first few days of treatment, comparable to the changes for progesterone and, again, a slower increase in oestradiol related to the prolongation of the follicular phase. Concentrations of oestrogen in the luteal phase, while somewhat variable, are not different.

**Prolongation of the follicular phase and corpus luteum rescue**

Prolongation of luteal activity, both as increased progesterone and duration of progesterone production, when HCG is administered at the mid to late luteal phase has been demonstrated in both human and non-human primates (Quagliarello et al., 1980; Ottobre and Stouffer, 1984). In this study we report, for the first time, the ability to rescue luteal activity following administration of either HCG in the early follicular phase or GnRH agonist, which results in a flare of endogenous pituitary gonadotrophins. The stimulation of the corpus luteum hormones progesterone, oestradiol and 17α-OH progestrone indicates that this is a late luteal rescue since the magnitude of observed endocrine responses could not result from the small follicles present at the time of this early follicular phase treatment. In addition, both of these treatments result in a prolongation of the follicular phase and consequently of the menstrual cycle itself without any apparent effects on progesterone production or duration of the luteal phase. With both HCG and GnRH agonist treatment in the first few days of the menstrual cycle, the follicular phase length is doubled and results in a prolongation of the menstrual cycle. A similar arrest of cyclic ovarian function was reported with late follicular phase administration of HCG to the normally cycling monkey (Williams and Hodgen, 1980).

Treatment with HCG for the first 5 days of the menstrual cycle results in significant elevations of both progesterone and 17α-OH progesterone and non-significant increases in oestradiol. These hormones are all products of the corpus luteum and, at this stage of the cycle, would not be expected to arise from stimulation of small follicles. Similarly, treatment with GnRH agonist, which results in a flare of endogenous LH and FSH, results in the same late luteal rescue with corresponding elevations of progesterone and oestradiol.
Figure 5. Mean ± SE serum concentrations of progesterone in control and gonadotrophin releasing hormone (GnRH) agonist-treated baboons. (A) Follicular phase changes, (B) luteal phase. Note the significant increases in the follicular phase and the longer follicular phase. There is no difference in the luteal phase of control or agonist-treated baboons.

Figure 6. Mean ± SE serum concentrations of oestradiol in control and gonadotrophin releasing hormone (GnRH) agonist-treated baboons. (A) Follicular phase changes, (B) the luteal phase. There is a significant increase in the follicular phase during agonist treatment and the prolonged follicular phase exhibits a more gradual increase in oestradiol. Oestradiol concentrations in the luteal phase (B) are not different between agonist-treated and controls.

Prolongation of the menstrual cycle is similar with either treatment and accounted for by the prolongation of the follicular phase which is essentially doubled. Neither treatment has an effect on luteal phase activity. An earlier study in women (Sheehan et al., 1982) had reported that follicular phase treatment with GnRH agonist in normally cycling women would result in an inadequate luteal phase that might have some contraceptive utility. In the present study, this result was not observed since both luteal length and luteal progesterone concentrations were not significantly different between GnRH agonist-treated and saline-treated controls. In both HCG and GnRH agonist-treated groups, once ovulation had occurred, there was normal luteal activity based both on length and progesterone concentrations obtained in that luteal phase.

HCG administration, during the luteal phase, in both human and non-human primates has demonstrated that the luteal response varies with the stage of the luteal phase (Quagliarello et al., 1980; Ottobre and Stouffer, 1984) with little or no response in the early luteal phase but a dramatic response by the mid to late luteal phase. A mid-luteal model is frequently used to study ovarian responses to
HCG in an attempt to duplicate the endocrine effects of the concepive luteal phase. This age-related luteal response to gonadotrophin is also demonstrated by luteal cells in vitro (Stouffer et al., 1977). This is the first study to examine gonadotrophic stimulation in the early follicular phase, and specifically whether such a late luteal response occurs.

Preliminary studies in women have demonstrated similar responses and are the subject of continuing studies (Koulianos et al., 1990). In these human subjects treated with HCG in the early follicular phase, there is an immediate (within 24 h) increase in both progesterone and relaxin, as well as oestradiol. Relaxin, in particular, is taken to be a more definitive marker of the luteal response. In infertility patients, we have reported that with the short protocol of GnRH agonist and human menopausal gonadotropin the ovary responds to the endogenous flare of LH and FSH with a transient luteal stimulation as seen by progesterone and oestradiol increases (Castracane et al., 1996). These results demonstrate that the ability to rescue the corpus luteum, which has been reported in the late luteal phase, is also carried into the early follicular phase. The magnitude of the progesterational response of this rescue does not show the maximum concentrations of progesterone seen in the luteal phase and probably represents only a fraction of luteal tissue that remains responsive. This is undoubtedly due to luteolytic and apoptotic mechanisms that result in demise of the corpus luteum which are initiated in the luteal phase but not completed in the early follicular phase so that some portion of that corpus luteum is able to respond. The demonstration of LH receptors within the first few days of the menstrual cycle (Bramley et al., 1987), in what is referred to as the corpus albicans, is probably the basis for this response. It is important to remember that the original studies that defined the corpus albicans were histological and biochemical. It may be more appropriate, when we consider the definition of the corpus albicans, to consider not only its histology but also its biochemistry and ability to respond to gonadotrophins. Perhaps the term corpus albicans may best be applied at some point later than the first few days of the menstrual cycle. Further studies in this interesting area of luteal physiology are ongoing.

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