Pregnancy Interception with a Combination of Prostaglandins: Studies in Monkeys

Abstract. *Treatment with combinations of synthetic prostaglandins, one with an ovarian site of action and one with a uterine site of action, terminated pregnancy in all rhesus monkeys given the injection on day 28 of fertile menstrual cycles. Single prostaglandins, even at higher doses, interrupted pregnancy in only one-third of the monkeys. The most effective treatment, 5-oxa-17-phenyl-18,19,20-trinor prostaglandin $F_{1\alpha}$ methyl ester plus 9-deoxo-16,16-dimethyl-9-methylene prostaglandin E_2, promptly intercepted early pregnancy after a single administration and without side effects.*

A long-standing objective in the search for new methods to control human fertility has been the development of a procedure to interrupt early pregnancy safely and effectively (*1*). Various chemical substances, including peptides, steroids, and the prostaglandins have been used in attempts to develop methods of pregnancy interception (*2*). The prostaglandins have been the most successful of the chemical methods used in a clinical setting. However, prostaglandins have not yet supplanted surgical procedures for the termination of first-trimester pregnancies because of frequent side effects, the need for relatively long durations of treatment to terminate pregnancy, and an incidence of incomplete abortion (*2*). I now report a series of preclinical studies in the rhesus monkey to show that two prostaglandins—one with an ovarian site of action (corpus luteum) and one with a uterine site of action—can be combined to provide an effective nonsurgical method for interception of early pregnancy. This approach offers significant advantages over the use of single prostaglandins, including higher efficacy rates, lower total drug doses, higher efficacy with a single administration, and no side effects.

Twenty-two nonpregnant female rhesus monkeys were studied during normal menstrual cycles, and 36 pregnant monkeys were studied during fertile cycles. Blood samples (5 ml) were collected from all monkeys beginning on day 20 of the menstrual cycle and continuing until menses in nonpregnant monkeys and until day 36 in pregnant animals. Serum was harvested from each blood sample and assayed by specific radioimmunoassays (*3*) for concentrations of progesterone (nonpregnant monkeys) or monkey chorionic gonadotropin (mCG), progesterone, and estradiol-17β (pregnant monkeys). Studies in nonpregnant monkeys were designed to determine the corpus luteum–inhibiting properties of prostaglandins by means of an assay described earlier (*4*). Briefly, the assay requires stimulating the monkey corpus luteum with human chorionic gonadotropin (hCG) on days 20, 21, and 22 of the menstrual cycle and administering the prostaglandin on day 22 at the time of the last hCG treatment. Changes in serum progesterone concentrations within 24 hours of prostaglandin administration are used as a measure of corpus luteum–inhibiting activity. Before the prostaglandin treatments were begun, pregnancy was confirmed in mated monkeys by the immunological detection of mCG in peripheral serum. Prostaglandins were given by intramuscular injection (*5*) beginning at 0700 hours on day 22 of the menstrual cycle in nonpregnant monkeys and on day 28 in pregnant animals; in some experiments, multiple injections were given at 8- or 12-hour intervals. Four synthetic prostaglandins were used in these studies: 5-oxa-17-phenyl-18,19,20-trinor prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) methyl ester (PGF-analog), 5-oxa-17-phenyl-18,19,20-trinor $PGF_{1\alpha}$ amide (PGF-analog amide), $PGF_{2\alpha}$-1,15 lactone, and 9-deoxo-16,16-dimethyl-9-methylene PGE_2 (PGE-analog). Six additional monkeys were studied in the third trimester at days 108 to 131 of pregnancy.

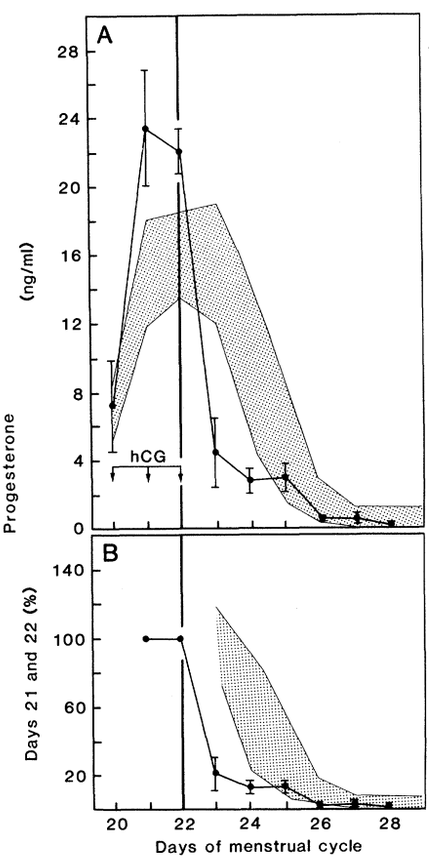

Fig. 1. Serum progesterone values (means ± standard errors) in monkeys ($N = 3$) treated with hcG on days 20 to 22 and PGF-analog on day 22 (7.5 mg three times at 8-hour intervals). The shaded areas depict the 95 percent confidence intervals for values in control monkeys ($N = 10$) receiving only hCG. (A) Serum progesterone concentrations in nanograms per milliliter. (B) Same data as a percentage of the mean value for days 21 and 22.

The initial experiments were conducted in nonpregnant monkeys to determine the corpus luteum–inhibiting properties of the prostaglandins. The results of an experiment with PGF-analog are shown in Fig. 1. Administration of hCG on days 20 to 22 increased serum progesterone values in both control and treated monkeys on days 21 and 22. Administration of PGF-analog on day 22 (7.5 mg three times at 8-hour intervals) resulted in a decline in serum progesterone concentrations from 22 ng/ml on day 22 to 4 ng/ml on day 23; this decline to 20 percent of the value before treatment differed significantly from the response seen in control monkeys ($P < 0.01$). Serum progesterone in treated monkeys also differed from control values ($P < 0.05$) on day 24 of the cycle but was similar thereafter. This result established an ovarian site of action for PGF-analog in the monkey because serum progesterone values declined in the presence of exogenous gonadotropin. Similar experiments were conducted with PGF-analog amide (7.5 mg three times at 8-hour intervals), $PGF_{2\alpha}$-1,15 lactone (5 mg twice, 12 hours apart), and PGE-analog (one 10-mg dose). The PGF-analog amide also significantly inhibited the monkey corpus luteum, although its potency was approximately one-third that observed for PGF-analog. $PGF_{2\alpha}$-1,15 lactone and PGE-analog did not inhibit the corpus luteum; mean progesterone values after treatment with these two prostaglandins fell within the 95 percent confidence limits for values seen in control monkeys. Earlier studies have documented in detail, however, that $PGF_{2\alpha}$-1,15 lactone and PGE-analog act on the monkey uterus to stimulate uterine myometrial contractions (*6*).

Experiments with single prostaglandins were conducted in pregnant monkeys; none of the prostaglandins was

effective in terminating early pregnancy when the treatments were given on day 28 of fertile menstrual cycles. Intramuscular injections of PGF-analog alone for 2 days (7.5 mg four times at 12-hour intervals), or for 3 days (7.5 mg nine times at 8-hour intervals) terminated pregnancy in only one of three, and one of two monkeys, respectively (*7*). When the PGE-analog or $PGF_{2\alpha}$-1,15 lactone was given alone, each terminated pregnancy in only one of three monkeys; the doses used were 0.5 mg three times at 8-hour intervals for PGE-analog, and 3 mg twice, 12 hours apart, for $PGF_{2\alpha}$-1,15 lactone.

Pregnancy in the rhesus monkey, as in all eutherian mammals, is maintained by progesterone. During very early pregnancy the corpus luteum is the essential source of this progesterone, but as the placenta develops it assumes primary responsibility for progesterone secretion. By day 35 from the previous menses, the presence of the ovary is no longer required in the monkey for the successful continuation of gestation. At day 28 of a fertile menstrual cycle, the monkey is in the midst of its luteal-placental transition in progesterone secretion (*8*). A similar luteal-placental shift in progesterone production occurs in the woman, but slightly later, at 6 to 8 weeks of pregnancy (*9*).

The low abortifacient efficacy of single prostaglandins in the monkey and the knowledge of the luteal-placental shift in progesterone production suggested that a combination treatment of prostaglandins—one with an ovarian site of action and one with a uterine site of action—would be effective for the interruption of early pregnancy. Administration of PGF-analog (7.5 mg three times at 8-hour intervals) with the PGE-analog (0.5 mg three times) promptly terminated pregnancy in all three treated monkeys. The interruption of pregnancy is illustrated in Fig. 2 by the prompt decline in serum hormone concentrations. Progesterone was nearly undetectable within 24 hours of the first prostaglandin injection; estradiol declined to 25 percent of the values preceding treatment, and mCG disappeared from the circulation within 3 days. The total doses of the prostaglandins were reduced in a second trial and administered as a single injection (7.5 mg of PGF-analog plus 0.5 mg of PGE-analog). Pregnancies were again interrupted in all treated monkeys, and serum concentrations of progesterone, estradiol, and mCG rapidly declined after the prostaglandin treatment (Fig. 3). Still lower doses of the two prostaglandins were only partially effective in terminating pregnancy (*10*). Side effects were observed in only one of the ten monkeys treated with this prostaglandin combination (*11*).

The concept that prostaglandins with different sites of action could be effectively combined to terminate pregnancy in the monkey was confirmed in additional studies. PGF-analog amide (7.5 mg three times at 8-hour intervals) plus PGE-analog (0.5 mg three times) terminated pregnancy in all three treated monkeys. Combination of PGF-analog [7.5 mg twice, 12 hours apart (N = 3) or 5 mg three times at 8-hour intervals (N = 3)] with $PGF_{2\alpha}$-1,15 lactone (3 mg twice or 1 mg three times) terminated pregnancy in all of the six treated monkeys. All of the experiments showed that adequate doses of two prostaglandins with different sites of action are highly effective for intercepting early pregnancy; pregnancy was terminated in 15 of 15 monkeys.

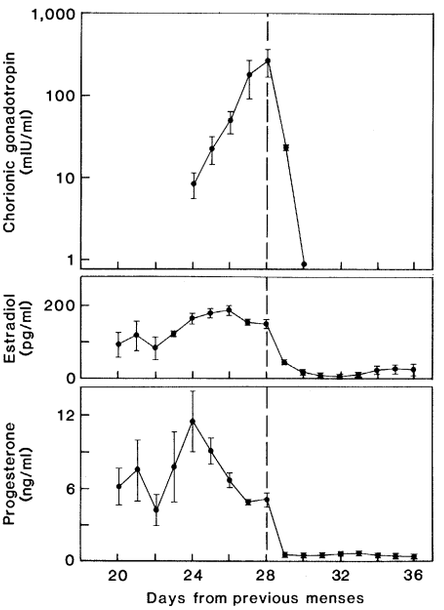

Fig. 2. Serum concentrations of mCG, estradiol-17β, and progesterone in rhesus monkeys treated intramuscularly with PGF-analog (7.5 mg three times at 8-hour intervals) plus PGE-analog (0.5 mg three times) on day 28 of fertile menstrual cycles. Points represent the means ± standard errors for values in three monkeys.

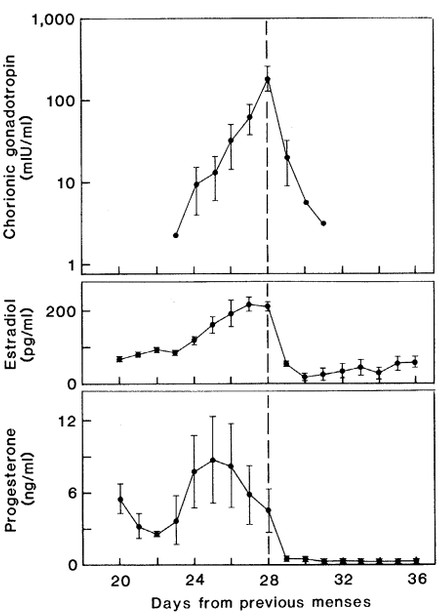

Fig. 3. Serum concentrations of mCG, estradiol, and progesterone in monkeys treated with a single intramuscular injection of PGF-analog (7.5 mg) plus PGE-analog (0.5 mg) on day 28 of fertile menstrual cycles. Points represent the means ± standard errors for values in three monkeys.

Administration of two of these prostaglandins to monkeys in the third trimester of pregnancy did not effectively interrupt gestation, suggesting that the combination was selective for early pregnancy. Pregnancy was interrupted in only one of three monkeys when PGF-analog was given alone (7.5 mg three times at 8-hour intervals) and in only one of three monkeys when PGF-analog (7.5 mg three times at 8-hour intervals) was given with PGE-analog (0.5 mg three times).

Once-a-month fertility control was recognized nearly two decades ago as a worthy objective in contraceptive research; however, such a method of controlling human fertility has never been successfully developed (*1*). Even though experiments in the rhesus monkey cannot be transferred to the woman with certainty, results in this species often have high predictability for the human. These studies in the rhesus monkey with a combination of prostaglandins suggest that nonsurgical, postconceptional human fertility control is attainable. Although the prostaglandins in these studies were administered by intramuscular injection, a preliminary trial with the prostaglandins given in a capsule (112.5 mg of PGF-analog plus 7.5 mg of PGE-analog) terminated pregnancy in one of three monkeys. A demonstration of partial oral activity offers the hope that modern pharmaceutical techniques could provide a convenient dosage form for prostaglandin combinations.

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5. Prostaglandins for injection were prepared as emulsions in 1 ml of 4 percent ethanol and 96 percent sterile aqueous vehicle (by volume); the aqueous vehicle contained (per milliliter): 10 mg of carboxymethylcellulose, 4 mg of polysorbate 80, and 0.42 mg propylparaben.
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7. Pregnancy maintenance was confirmed by continued normal secretion patterns of mCG, detection of uterine enlargement by bimanual palpation, and failure of the monkeys to show cycles of menstrual bleeding.
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10. Pregnancy was terminated in one of two monkeys treated with a single injection of 3.75 mg of PGF-analog plus 0.5 mg of PGE-analog and in one of two monkeys treated with a single injection of 3.75 mg of PGF-analog plus 0.25 mg of PGE-analog.
11. Mild anorexia lasting only one day was observed in one of the monkeys given the highest doses of the PGF-analog plus PGE-analog.
12. I thank A. D. Forbes, K. K. Forbes, and R. L. VanEyk for technical assistance.

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Anisotropies in the Perception of Three-Dimensional Surfaces

Abstract. *The appearance of certain three-dimensional surfaces was found to depend on the orientation of the depth contours forming the surface. This was true both when the depth was specified by motion parallax and when it was specified by binocular disparities. Slowly changing depth surfaces that generated a pattern of relative motions or disparities characterized by a one-dimensional expansion-compression were perceived differently from those that produced a shear transformation.*

The small differences between the two retinal images (binocular disparities) and the relative motion in each image when the observer moves (motion parallax) have both been shown to accurately specify the structure of three-dimensional surfaces (*1*, *2*). We report here that the perception of depth surfaces can be orientationally anisotropic, that is, the appearance of a surface can vary with the retinal orientation of the surface contours (*3*). Anisotropic effects were initially observed for surfaces in which the three-dimensional structure was specified stereoscopically, but we have since found similar effects for surfaces in which the structure was specified by motion parallax.

The most striking demonstration of these effects was found with the depth surfaces depicted in Fig. 1, a and b. The profile of the surface (Fig. 1c) consists of a sharp step or discontinuity in depth, flanked on either side by more gradual depth changes to areas that are equidistant from the observer. When this profile defines luminance (rather than depth) changes across a surface, observers typically report that the left-hand side appears lighter than the right. This effect is known as the Craik-O'Brien-Cornsweet illusion (*4*). In the luminance domain, the effect is usually attributed to the visual system's poor sensitivity to slow or low spatial frequency changes (which characterize the sloping areas of the profile) compared with its sensitivity to the sharp discontinuity at the center (*5*). We investigated the analogous effect in the depth domain. When the surface depicted in Fig. 1a was presented stereoscopically, the right flank of the surface appeared to be nearer the observer than the left (*6*). However, when the surface depicted in Fig. 1b was presented there was no illusion and the outer flanks appeared to be equidistant. Moreover, when the same depth surfaces of Fig. 1 were specified by parallax motions, rather than by binocular disparities, a similar anisotropy was observed.

Random dot techniques were used to study these anisotropies in both the stereoscopic and motion parallax domains. For stereopsis, two identical 256 by 256 pixel arrays were displayed on a pair of large-screen oscilloscopes (20 by 20 degrees of visual angle) and viewed independently by the two eyes at a distance of 57 cm. Disparities were introduced by feeding an additional (equal and opposite) signal to the x-inputs of the display scopes. The shape of the signal, in this case that of the Cornsweet profile, determined the shape of the simulated depth surface.

For the motion parallax display, the depth information was provided by the patterns of relative motion projected onto the retina during lateral movements of the observer's head. We demonstrated previously that the shape and relative depth of a three-dimensional surface can be perceived readily and unambiguously under these conditions (*2*). In this study, subjects monocularly viewed a single random dot array while making lateral head movements through a distance of 13 cm. The random dot array was systematically transformed during each head movement so as to produce a pattern of relative motions identical to that produced by a real three-dimensional surface. The relative motion between the rows of dots was produced by feeding an additional x-signal to the display oscilloscope and modulating the amplitude according to the lateral position of the observer's head. The shape of the signal again determined the shape of the simulated depth surface. In both displays the angular extent of the central, changing region of the depth surface was 10 degrees of visual angle. The depth change at the discontinuity corresponded to a disparity of 8 arc minutes.

The size of the illusion in both cases was measured with a nulling technique. The subject's task was to introduce a physical disparity between the outer flanks of the depth profile until any perceptual difference was canceled out and the flanks appeared to be equidistant. The curvature of the slowly changing

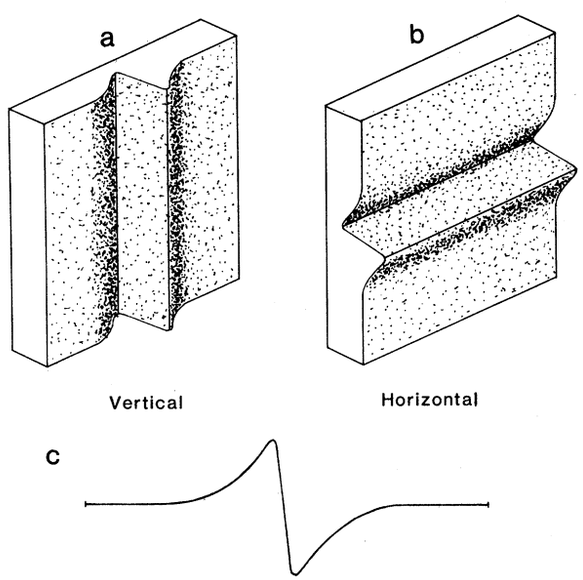

Fig. 1. (a and b) Perspective drawings of the depth surfaces used in the experiments. (c) Approximate shape of the Cornsweet profile, as constructed by adding a positive-going half-cycle sine wave and a negative-going half-cycle sine wave of one-eighth the spatial extent.