Table 2. Transmission of the Z allele according to the sex of the heterozygous parent. N.S., not significant.

Number of families	Number of children					P
	Total	MM		MZ		
		Girls	Boys	Girls	Boys	
		Father MZ				
12	41	4	6	15	16	< .01
		Mother MZ				
11	27	8	8	2	9	N.S.

in the course of population studies of volunteer blood donors or during studies of the families of homozygous Z deficient individuals.

The Pi types were determined by thin-layer isoelectric focusing in a *p*H range of 4 to 6, as previously described (*6*), and phenotypes were confirmed by print immunofixation (*6*). Concentrations of α_1-antitrypsin in the serum were determined by single radial immunodiffusion. In all subjects with apparent M phenotypes, serum values did not suggest the presence of the (−) allele in the heterozygous state (M−). The data were analyzed by the chi-square test for goodness of fit (*7*).

Analysis of Pi phenotypes in all children (Table 1), irrespective of the sex of the heterozygous parent, indicated a higher than expected number of MZ phenotypes, but this increase did not reach statistical significance. In contrast, when the families were separated according to the sex of the MZ parent (Table 2), a statistically significant increase in the number of MZ children was found ($P < .01$) in families where the father carried the Z allele. No significant departure from the expected frequency was found when the mother possessed the Z allele.

In their original study of the familial transmission of Pi phenotypes in 77 Norwegian families, Fagerhol and Gedde-Dahl (*8*) found that their inheritance was compatible with a simple autosomal codominant mode; however, their study included only five families with MM × MZ matings. In later studies, Fagerhol (*9*) and Kueppers (*10*) proposed that the Pi polymorphism is due to positive selection in favor of the variant alleles. The present results suggest a significant increase in the number of MZ children among the progeny when the father carries the Z allele in the heterozygous state. Gedde-Dahl *et al.* (*11*) have previously described another genetic abnormality associated with the Z allele in males, in that males heterozygous for the Z allele have a significantly lower frequency of recombination between Pi and the *Gm* locus (coding for immunoglobulin G heavy-chain markers) than males with other phenotypes or females heterozygous for the *Z* allele.

Our observation suggests that sperm cells carrying the Z allele have selective advantage over those carrying the M allele. Further investigations will be required to determine whether this advantage is expressed during meiosis, migration of the sperm in the female reproductive tract, or fertilization.

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Induction of Luteolysis in the Human with a Long-Acting Analog of Luteinizing Hormone-Releasing Factor

Abstract. *Subcutaneous injection of 50 micrograms of a long-acting analog of luteinizing hormone-releasing factor on each of two successive days during midluteal phase in normally cycling women induced a short luteal phase and premature menstruation. These events were associated with luteolysis, as evidenced by the consistent and parallel premature decline of progesterone and estradiol levels compared with those in control cycles. This finding may prove to be useful in the prevention or interception of implantation.*

The hypothalamic luteinizing hormone-releasing factor (LRF) is indispensable in follicular maturation, ovulation, and the maintenance of corpus luteum function (*1*). However, recent studies in animals have disclosed an unexpected and paradoxical antifertility effect of large doses of the decapeptide LRF (*2*). Several potent LRF agonists have been shown to inhibit ovulation, prevent implantation, and terminate pregnancy in the rat (*3*). In one report (*4*), daily administration of an LRF agonist for 1 month also inhibited ovulation in women. However, the irregular bleeding consequent to the disturbance of normal follicular estrogen secretion represents a major complicating factor for practical use as a contraceptive.

The present study was designed to determine whether luteolysis can be induced by administration of [D-Trp^6, Pro^9 NEt]-LRF, a long-acting LRF agonist approximately 140 times as potent as LRF (*5*, *6*). Five regularly cycling women volunteered for this study, and written informed consents were obtained. The luteal phases of their menstrual cycles were monitored by basal body temperature recording and by determining daily serum levels of gonadotropin, estradiol, and progesterone (*7*) beginning on day 10 of the cycle. In the midluteal phase, a subcutaneous injection of 50 μg

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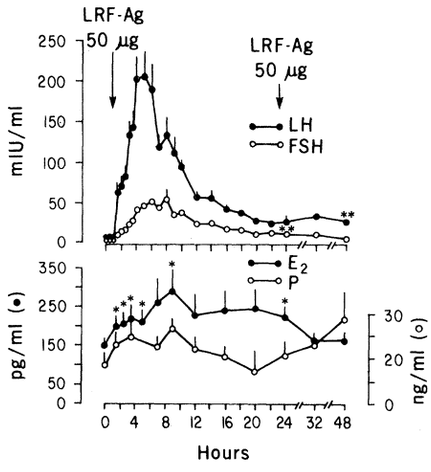


Fig. 1. Time course and quantitative changes in serum LH, FSH, estradiol (E_2), and progesterone (P) concentrations (mean ± standard error) in response to two injections of 50 μg of the LRF agonist (Ag) in the midluteal phase of normal cycling women ($N = 4$). Gonadotropin release after the first injection (at 1 hour) showed a marked and sustained increment, which remained significantly above basal level (** $P < .005$, by Student's t-test) at 24 hours. However, gonadotropin response was completely refractory to the second injection. Significant increases in circulating E_2 occurred at the times marked (*$P < .05$), with the peak level occurring 4 hours after peak gonadotropin release. There were no accompanying changes in serum progesterone levels.

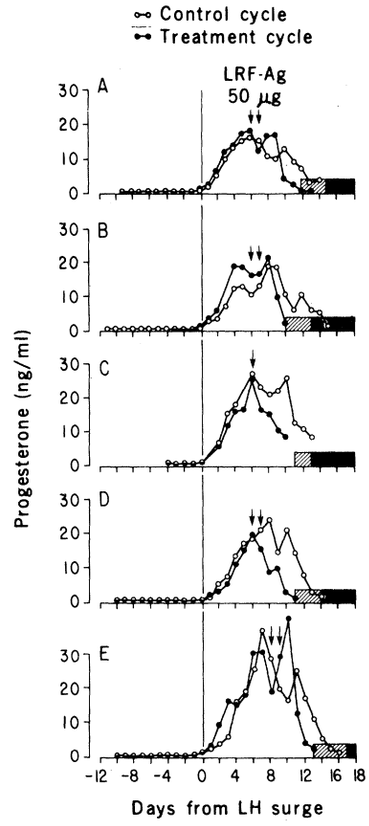
of the LRF agonist (*8*) was administered on each of two successive days to four subjects; the fifth subject received a single injection of 50 μg of the LRF agonist. The acute effects of this treatment on gonadotropin release and corpus luteum function were evaluated by measuring changes in circulating luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, and progesterone concentrations in the frequent and appropriately timed blood samples obtained through an indwelling venous catheter (*9*).

In response to the first dose of LRF agonist (Fig. 1) there was a prompt and expected increase in serum LH and FSH concentrations, which reached peaks 25 and 10 times higher than their respective basal levels 5 hours after administration of the agonist. At 24 hours, the LH level remained significantly above the pretreatment value (28.2 ± 5.1 compared to 8.2 ± 1.8 mI.U./ml; $P < .005$). No further increase in either gonadotropin was seen after the second injection of LRF agonist, and the normal luteal phase gonadotropin levels were maintained. This finding is consistent with other evidence for desensitization of pituitary gonadotropes due to prolonged and sustained stimulation (*10*). Serum estradiol, but not progesterone, concentrations showed a twofold increase at the ninth hour ($P < .05$), 4 hours after the peak gonadotropin increments.

In all treated cycles, including that of the subject receiving only one injection, a uniform decline in serum progesterone levels occurred within 2 days after the administration of LRF agonist (Fig. 2). Onset of menses occurred 4 to 5 days after the first injection and was thus advanced by 2 to 4 days when compared with control cycles. Luteolysis occurred as indicated by the parallel fall of serum estradiol as well as progesterone in all subjects treated with LRF agonist (Fig. 3). The mean (± standard error) duration of the luteal phase, calculated from LH peak to the initiation of menses, was significantly ($P < .0007$) shortened in treated cycles (11.4 ± 0.5 days) compared to control cycles (14.4 ± 0.7 days).

Koyama *et al.* (*11*) observed a suppressive effect on plasma progesterone levels when five daily injections (100 μg) of an LRF agonist, [des-Gly-NH_2^{10}, Pro ethylamide^9]-LRF, were administered to six women, beginning within the first 5 days of the luteal phase of their cycles. However, there was no change in estradiol levels or in the duration of the luteal phase, indicating that luteolysis had not occurred. On the other hand, multiple administrations (every 4 hours) of LRF over 24 to 48 hours to women in the midluteal phase has recently been shown to have a luteolytic effect (*12*). In the present study, one or two smaller doses (50 μg) of a more potent and longer-acting LRF agonist were found to effect an early and concomitant decline in circulating levels of estradiol and progesterone and a shortened luteal phase, thus indicating the occurrence of premature luteolysis.

The mechanism of the luteolytic effect of LRF agonist is unknown. The "down regulation" of pituitary gonadotropic activity observed in our study (Fig. 1) is probably not responsible for luteolysis, since LH and FSH levels remained in the range for the normal luteal phase. Down regulation of ovarian LH receptors secondary to the sustained increase in circulating LH, as suggested by experiments in the rat (*13*), is also not a satisfactory explanation for our finding. The fact that



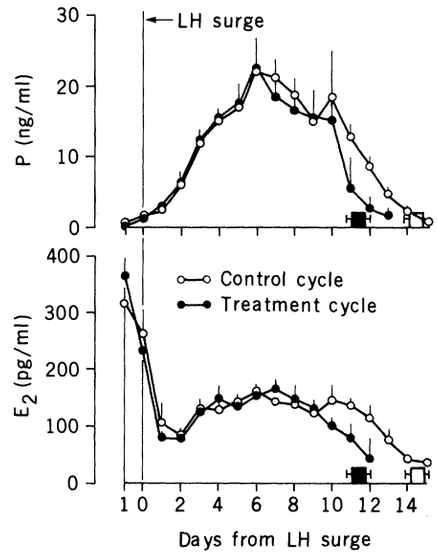


Fig. 2 (left). Pattern of premature decline in serum progesterone levels and early onset of menses in five women treated with 50 μg of LRF agonist compared with their respective control cycles. Data are centered around the midcycle LH peak (day 0). In all cases, LRF agonist treatment is associated with early decline of progesterone within 2 days after the first injection, resulting in menses 2 to 4 days earlier (indicated by horizontal boxes) than in control cycles. Fig. 3 (right). Parallel decline of serum estradiol (E_2) and progesterone (P) (mean ± standard error) in five women treated with LRF agonist during the midluteal phase compared with the succeeding control cycles. Data were centered around the midcycle LH peak (day 0). The mean duration of the luteal phase calculated from the LH peak to the onset of menses (indicated by boxes) is shortened by 3 days in the treated cycles ($P < .0007$).

massive serum elevation of endogenous (after implantation) or exogenous human chorionic gonadotropin promotes steroidogenesis and prolongs the life-span of the corpus luteum (*14*) appears to indicate that this type of down regulation may not occur in humans. Although estrogen itself may cause luteolysis (*15*), the twofold increase in estradiol is probably too small and too brief to exert a luteolytic effect (Fig. 1). Moreover, the finding by Gore *et al.* (*15*) that estrogen induces luteolysis in humans only when pharmacologic doses are administered in the period immediately after ovulation tends to exclude estrogen as an intermediary in the observed luteolytic action of LRF agonist. The recent demonstration by Hsueh and Erickson (*16*) that FSH-mediated estrogen and progesterone production by rat and human granulosa cells in culture is markedly inhibited by the addition of LRF as well as by LRF agonist indicates that this hypothalamic peptide may act directly on target cells in the ovary.

Although cellular mechanisms remain to be determined, the present study has demonstrated a luteolytic action of the LRF agonist, and a consequent reduction of corpus luteum steroidogenesis followed by early onset of menses. Since progesterone secreted by the corpus luteum is essential for implantation and the maintenance of early pregnancy (*17*) and since a short luteal phase is causally related to infertility in humans and in rhesus monkeys (*18*), the pattern of administration of LRF agonist used in this study may provide an effective means for the prevention or interception of implantation.

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Erythrosin B Inhibits Dopamine Transport in Rat Caudate Synaptosomes

Abstract. *Erythrosin B is a member of a class of fluorescein dyes that are suggested to elicit hyperkinesis when ingested by susceptible children. We found that erythrosin B inhibits dopamine uptake in rat caudate synaptosomes "uncompetitively" in the 10- to 800-micromolar range. Half maximal inhibition of uptake occurred at 45 micromolar. Uncompetitive inhibition denotes a decrease in efficacy of the dopamine membrane transport mechanism with an increase in affinity of dopamine to the carrier. Erythrosin B also decreased nonsaturable binding of dopamine to the synaptosome membrane. The inhibitory action of erythrosin B on dopamine uptake is consistent with the hypothesis that erythrosin B can act as a central excitatory agent able to induce hyperkinetic behavior.*

Food, drug, and cosmetic (FD&C) dyes have been suggested to play a major role in the etiology of a behavioral disturbance in children called hyperkinesis or minimal brain dysfunction (MBD) (*1*). Controlled behavioral experiments have not revealed a direct or a consistent relationship between FD&C dyes and hyperkinesis (*2*), although there are studies demonstrating that fluorescein-related FD&C dyes are biologically active. Apparently acting by their ability to dissolve in lipid membranes (*3*), FD&C dyes have been shown to affect a variety of biological systems. Eosin B (D&C Red No. 25), eosin Y (D&C Red No. 3), erythrosin B (FD&C Red No. 3), phyloxine B (D&C Red No. 8), and rose bengal inhibit fertilization in the sea urchin *Stronglyocentrotus purpuratus*, and erythrosin B prevents fertilization in members of four other phyla (*4*). Erythrosin B is also active pre- and postsynaptically in frog neuromuscular junction. Presynaptically, erythrosin B increases both the frequency and amplitude of miniature end-plate potentials, possibly by enhancing synaptic membrane and plasma membrane fusion. Postsynaptically, erythrosin B causes an increase in potassium conductance through muscle fiber membranes (*5*). Similarly, erythrosin B and related dyes increase potassium permeability of molluscan neuron membranes (*3*).

The capacity of fluorescein dyes to affect these systems suggests that the proposed relationship between hyperkinesis and food dyes may have a neurochemical basis. We have investigated this possibility by looking at the effects of erythrosin B (FD&C Red No. 3) on dopamine uptake in synaptosomes (*6*) prepared from rat caudate nucleus (*7*). Dopamine uptake is a presynaptic mechanism which terminates the action of dopamine after it is released into the synapse. The dopamine system was selected for study

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