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Effects of Gonadotropin-Releasing Hormone Treatment on Ovarian Steroid Production during Midpregnancy[1,2] (42319)

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Abstract. During the second half of pregnancy, ovarian testosterone (T) through its conversion to estradiol (E) promotes progesterone (P) synthesis by the ovary which maintains the pregnancy. To determine if the administration of gonadotropin-releasing hormone (GnRH) disrupts pregnancy by suppressing ovarian production of T or its conversion to E, rats were treated from Day 11 through Day 18 of pregnancy with 50 or 100 μg/day of GnRH or 1, 5, or 10 μg/day of a GnRH agonist (GnRH-Ag; WY-40972) using an osmotic minipump. Rats were bled daily from the jugular vein under light ether anesthesia and on Days 14 or 18 of pregnancy both jugular and ovarian blood samples were obtained. While the GnRH-Ag treatment at the dose of 5 or 10 μg/day terminated pregnancy within 48 hr as indicated by vaginal bleeding, 1 μg/day terminated pregnancy more slowly. Neither dose of GnRH was effective in terminating pregnancy through Day 18. By Day 14, peripheral levels of plasma P in rats treated with 0, 1, 5, or 10 μg of GnRH-Ag were 97 ± 9, 24 ± 1, 13 ± 3, and 8 ± 1, respectively. In the same groups, levels in the ovarian vein were 3205 ± 633, 1317 ± 273, 360 ± 113, and 228 ± 73 ng/ml. By Day 18, serum P levels in the peripheral circulation and in the ovarian vein were declining even more dramatically. Daily administration of P (4 mg) and E (0.5 μg) simultaneously with GnRH-Ag at the dose of 5 μg/day from Days 11 through 14 reversed the abortifacient effect of GnRH-Ag and maintained pregnancy indicating that the GnRH-Ag effect is not directly on the uterus. Ovarian vein levels of T on Days 14 or 18 of pregnancy were either not different from controls at 1407 ± 163 or 1476 ± 122 pg/ml, respectively, or increased dramatically in certain groups. Ovarian vein levels of E were either not different from controls at 292 ± 13 pg/ml on Day 14 or increased significantly in rats treated at the dose of 1 μg/day of GnRH-Ag. However by Day 18, treatment with GnRH-Ag at all doses suppressed ovarian secretion of E. These results suggest that while the GnRH-Ag induces abortion in rats by suppressing ovarian production of P, this abortifacient effect is not due to a fall in ovarian T levels nor to its aromatization to E in the ovary.

Although endogenous gonadotropin-releasing hormone (GnRH) is indispensable in maintaining normal reproductive function through its control of gonadotropin secretion, chronic administration of GnRH or its highly active agonistic analogs induces a "paradoxical" antifertility effect in a variety of laboratory species of both sexes. GnRH agonists have multiple sites of action including the pituitary, the gonads, and the reproductive tract. GnRH agonists initially stimulate the release of large amounts of gonadotropins; subsequently occurring desensitization of the pituitary and the gonads is believed to be responsible for the inhibition of gonadal functions (1). However, luteal suppression with resultant antifertility has been noted for pregnant or pseudopregnant rodents (2, 3) and rabbits (4). Several studies have indicated that this abortifacient effect of GnRH is due to its ability to suppress plasma progesterone levels (5, 6). This effect of GnRH is reversible upon progesterone replacement (7). It has been demonstrated that GnRH disrupts pregnancy by its direct action on the ovary (8).

Results from previous investigations revealed that during early pregnancy luteinizing hormone (LH) stimulates luteal synthesis of testosterone (9) which is aromatized to estradiol by the corpus luteum (10, 11). The locally formed estradiol then promotes progesterone synthesis by the ovary (12). Therefore, it was of interest to determine if the administration of GnRH disrupts pregnancy by suppressing ovarian steroid production during pregnancy.

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We have chosen the model wherein administration of GnRH to intact pregnant rats (prior to Day 12 pregnancy) results in 100% termination of pregnancy (13).

Materials and Methods. *General.* Pregnant Sprague–Dawley rats were obtained from Holtzman Company (Madison, Wis.). The first day sperm were found was designated as Day 1 of pregnancy. They were kept in a room at 26°C under a controlled photoperiod of 14 hr of light (lights on from 0400 to 1800 hr EST) and 10 hr of darkness. Purina rat chow and water were provided *ad libitum.* A clean, but not aseptic, technique was used during all surgical procedures.

Treatment. On Day 11 of pregnancy laparotomy was performed under ether anesthesia in rats to observe the implantational sites. Then, an osmotic minipump (Alza; Model 2002) was implanted sc on the dorsal surface of each rat. The pumps delivered GnRH or GnRH-agonist (GnRH-Ag; Wyeth-40972) at a dose of 50 or 100 μg/day or 1, 5, 10 μg/day, respectively. In one experiment rats treated with GnRH-Ag (5 μg/day) also received progesterone and estradiol daily sc at a dose of 4 mg and 0.5 μg, respectively, in 0.25 ml of corn oil. Rats were observed daily for vaginal bleeding. Minipumps with GnRH or GnRH-Ag were incubated in saline at room temperature overnight prior to implantation in the rats.

Bleeding. Rats were bled from the jugular vein daily from Day 11 through Day 18 of pregnancy under ether anesthesia. The jugular vein was tapped through the unbroken skin using a heparinized syringe. On Days 14 or 18 of pregnancy ovarian blood was collected over a period of 15 min by techniques developed by Shaikh and Shaikh (14) and used by us (15) previously. Blood volume was measured and centrifuged at 4°C. Plasma was stored frozen at −20°C.

Autopsy. Rats were sacrificed with an overdose of ether following the ovarian vein bleeding. At autopsy, the uteri were removed and weighed for the measurement of fetal weight. The presence of at least one normal fetus at autopsy, along with the presence of a heart beat, was the criterion of pregnancy.

RIAs. Progesterone. Plasma concentrations of progesterone were measured after hexane extraction using a highly specific antiserum provided by Dr. G. D. Niswender (GDN 337). The specificity, validity, and reliability of this RIA have been reported earlier (16).

17β-Estradiol. After extraction with diethyl ether, plasma levels of 17β-estradiol were measured using the procedure described by Korenman *et al.* (17). The assay employs a highly specific antiserum prepared against 17β-estradiol-6-BSA (GDN 224) supplied by Dr. G. D. Niswender. The sensitivity of the assay is 10 pg.

Testosterone. Plasma testosterone concentrations were measured after an ethyl acetate–hexane extraction using a highly specific antisera (obtained from Radioassay Systems Laboratories, Calif.). The antisera cross-reacts less than 15% with dihydrotestosterone, less than 2% with other androgens, and not at all with progesterone. The sensitivity, specificity, and reliability of this assay have been reported previously (15).

Statistics. The data were analyzed for differences by one-way analysis of variance followed by Neuman–Kauls test when differences were significant. Values were considered significantly different when $P < 0.05$.

Results. *Effects of GnRH or GnRH-Ag treatment on fetal weight.* GnRH-Ag at a dose of 5 or 10 μg/day terminated pregnancy within 48 hr as indicated by vaginal bleeding. By Day 14 the abortion was complete in these rats. The effect of GnRH-Ag at the dose of 1 μg/day was abortifacient but slow and therefore abortion was complete only by Day 18. GnRH at 50 or 100 μg/day had no effect on fetuses as they looked normal as late as Day 18. Fetal weights (Fig. 1) were significantly lower on Day 14 in rats treated with 5 or 10 μg/day of GnRH-Ag compared to those of controls. By Day 18 in rats treated with GnRH-Ag at all doses the abortion was complete. Therefore no weight gain was observed for fetuses in these rats in contrast to the apparent weight gain in controls and in rats treated with GnRH.

Effects of GnRH or GnRH-Ag treatment on jugular or ovarian vein plasma concentrations of progesterone. The treatment of GnRH at a dose of 100 μg/day and GnRH-Ag at a dose of 5 or 10 μg/day suppressed plasma concentrations of progesterone in peripheral circulation (Table I) within 24 hr and remained low for the duration of the experiment. However, the suppressive effect of GnRH-Ag at the

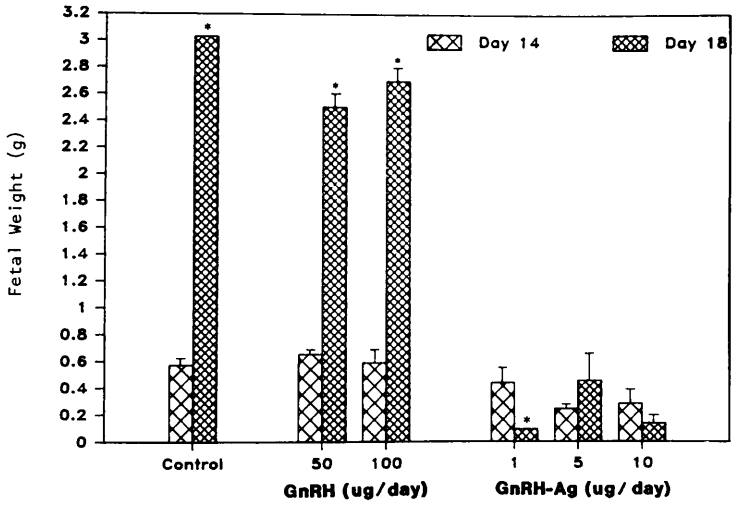

FIG. 1. Effects of GnRH or GnRH-Ag treatment on fetal weight. Each rat was implanted sc on the dorsal surface with an osmotic minipump on Day 11 of pregnancy at a dose of 50 or 100 μg/day of GnRH or at a dose of 1, 5, or 10 μg/day of GnRH-Ag. Sham surgery was performed in controls but they received no treatment. On Days 14 or 18 fetuses were weighed at autopsy. The average weight of a single fetus was calculated for each rat. Results are expressed as the means ± SEM (n = 4–6 rats). *$P < 0.05$ when compared to Day 14 weights within each group.

dose of 1 μg/day on plasma progesterone levels was evident only after 48 hr and remained low thereafter. Even though the average plasma progesterone level was 53 ± 5 on Day 18 in rats treated with GnRH at a dose of 100 μg/day compared to 118 ± 5 in controls, this level was sufficient to sustain the pregnancy. Throughout pregnancy, the primary source of progesterone is the ovary. Progesterone concentrations in the ovarian vein plasma on Day 14 and on Day 18 (Fig. 2) indicated that the fall in progesterone concentration in peripheral circulation is due to a dramatic decline in progesterone levels in the ovarian vein plasma.

Effects of daily injections of progesterone and estradiol on fetal weight (Fig. 3). When rats treated with GnRH-Ag at a dose of 5 μg/day were supplemented daily with progesterone (4 mg) and estradiol (0.5 μg), pregnancy and normal fetal weights were maintained. Exogenous injections of progesterone and estradiol failed to bring the plasma levels of progesterone to those observed in controls; nevertheless, they were physiologically sufficient to sustain pregnancy. These data suggest that the abortifacient effect of GnRH-Ag is not due to its direct effect on the uterus or on the fetus.

Effects of GnRH or GnRH-Ag treatment on

TABLE I. PLASMA PROGESTERONE CONCENTRATIONS (ng/ml) IN JUGULAR VEIN

Day of pregnancy	Treatment					
		GnRH (μg/day)		GnRH-Ag (μg/day)		
	Control	50	100	1	5	10
11	82 ± 8	89 ± 6	76 ± 4	85 ± 7	73 ± 4	87 ± 6
12	84 ± 7	91 ± 9	49 ± 7*	74 ± 19	18 ± 3*	15 ± 1*
14	97 ± 9	100 ± 15	54 ± 6*	24 ± 1*	13 ± 3*	8 ± 1*
18	118 ± 5	91 ± 4	53 ± 5*	6 ± 1*	8 ± 1*	5 ± 1*

Note. Results represent the Means ± SE (n = 3–9 rats). Rats were treated from Days 11 to 18 with 50 or 100 μg/day of GnRH or 1, 5, or 10 μg/day of GnRH agonist (WY-40972) using an osmotic minipump.
* Significantly different from controls.

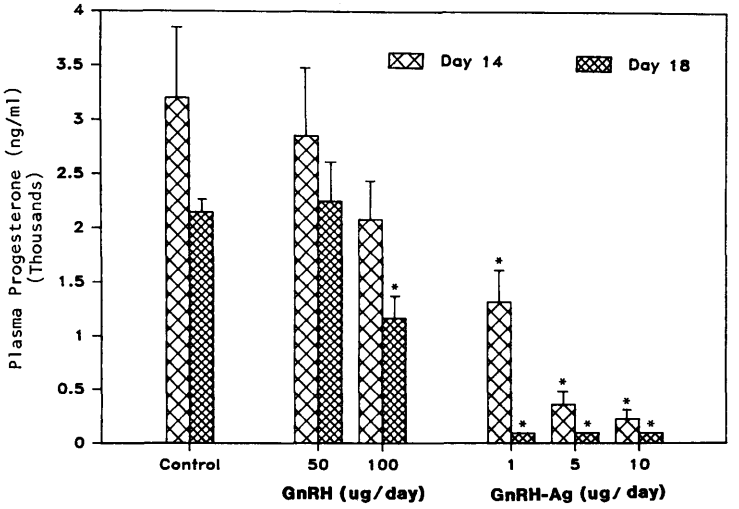

FIG. 2. Progesterone levels in the ovarian vein plasma on Days 14 or 18 of pregnancy. Rats were treated as described in the legend to Fig. 1. Results are expressed as the means ± SEM (n = 3–8 rats). *$P < 0.05$ when compared to control values on respective days.

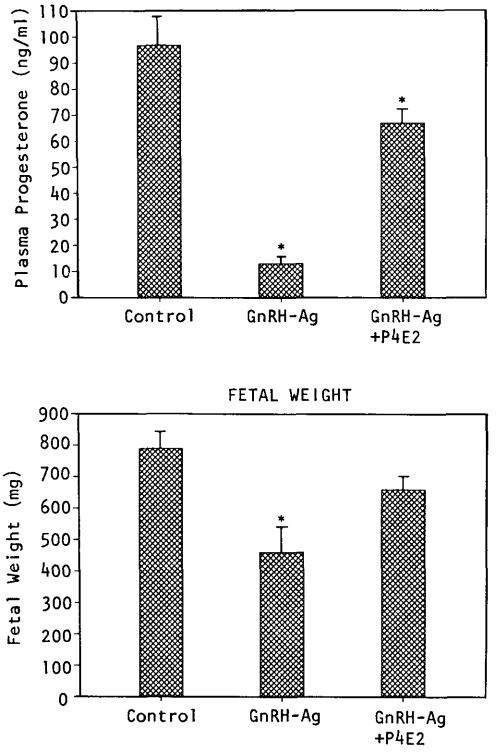

FIG. 3. Effects of daily treatment with progesterone (4 mg) and estradiol (0.5 μg) (P4E2) on fetal weight in GnRH-Ag (5 μg/day) treated rats. The treatment started on Day 11 and continued through Day 14 until rats were sacrificed. Results are expressed as the means ± SEM (n = 4–6 rats). *$P < 0.05$ when compared to control values.

plasma levels of testosterone in the ovarian vein (Fig. 4). Treatment with GnRH or GnRH-Ag had no suppressive effects on testosterone levels in the ovarian vein plasma on either Days 14 or 18 compared to controls. In fact, at some doses GnRH or GnRH-Ag significantly elevated the levels of testosterone when compared to controls.

Effects of GnRH or GnRH-Ag treatment on plasma levels of estradiol in the ovarian vein (Fig. 5). Treatment with GnRH had no suppressive effects on estradiol levels in the ovarian vein plasma on either Day 14 or Day 18. The levels were either comparable to controls or elevated in rats treated at the dose of 50 μg/day on Day 14. Similarly, by Day 14 GnRH-Ag treatment either had no suppressive effects on estradiol levels in the ovarian plasma at all doses or elevated estradiol levels in rats treated at the dose of 1 μg/day. In contrast, all doses of GnRH-Ag treatment suppressed the estradiol levels in the ovarian vein plasma by Day 18 when compared to controls.

Discussion. The present studies indicate that administration of GnRH induces abortion in rats due to its suppressive effects on ovarian progesterone secretion; furthermore, there was no evidence to suggest that a GnRH-induced fall in ovarian secretion of testosterone or estradiol was involved in this process.

Our results also reveal that GnRH induces

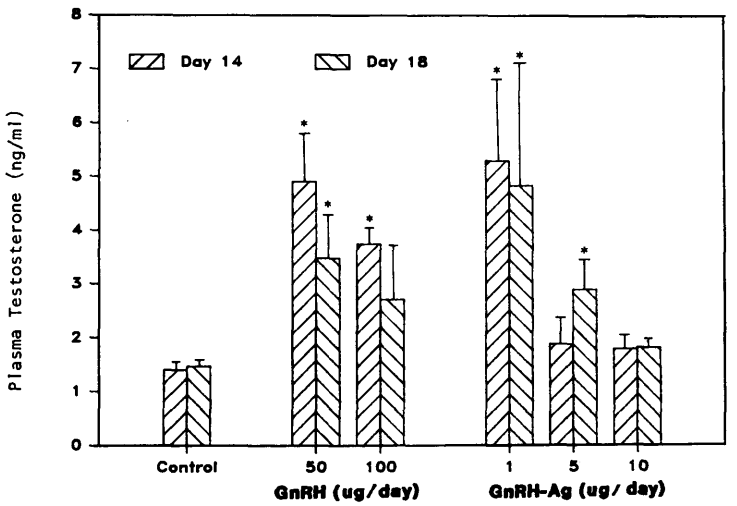

FIG. 4. Testosterone levels in the ovarian vein plasma on Days 14 and 18 of pregnancy. Rats were treated as described in the legend to Fig. 1. Results are expressed as the means ± SEM (n = 3–5 rats). *$P < 0.05$ when compared to control values on respective days.

abortion in rats at the level of the ovary and not due to its direct effect on the uterus (Fig. 3). This is consistent with the results of Macdonald *et al.* (8) who have demonstrated the direct suppressive effect of GnRH on ovarian steroidogenesis *in vivo* using pseudopregnant, hysterectomized, hypophysectomized, prolactin-injected rats.

During the second half of pregnancy the luteotropic activities of rat placenta are well established (18). After Day 12 of pregnancy the luteotropic function of the pituitary shifts to the placenta. The placenta secretes rat placental lactogen (rPL), a prolactin-like hormone (19) as well as a putative rat chorionic gonadotropin (rCG), a LH-like hormone (20). At this time, plasma LH and prolactin levels are greatly diminished (21). Although rCG has been detected in the rat placentas by radioreceptor assays (22) and found to possess struc-

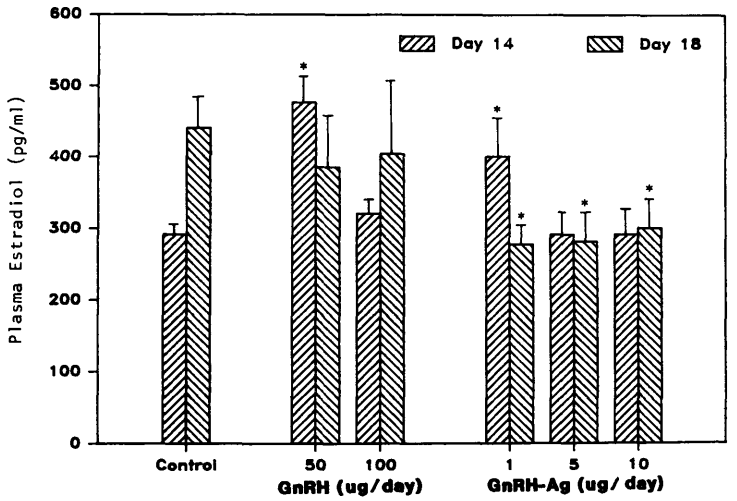

FIG. 5. Estradiol levels in the ovarian vein plasma on Days 14 and 18 of pregnancy. Rats were treated as described in the legend to Fig. 1. The results are expressed as the means ± SEM (n = 3–4 rats). *$P < 0.05$ when compared to control values on respective days.

tural similarities with human chorionic gonadotropin (hCG) (23), recent investigations do not detect any mRNA encoding for α or β subunit of LH or CG-like species at any stage of pregnancy (24–26). Since high levels of LH alone (27) during early pregnancy in the rat or chorionic gonadotropin in baboon (28) do not cause suppression of progesterone, high levels of the speculated rCG may not be the likely candidate responsible for suppressing plasma progesterone in this study. Since there are no methods available for the quantification of rCG, ovarian secretion of testosterone was measured in this study because hCG has been shown to stimulate ovarian testosterone production *in vivo* (9).

Normal or higher than normal levels of testosterone on Days 14 or 18 of pregnancy or normal or higher than normal levels of estradiol on Day 14 of pregnancy in the ovarian plasma occurred at a time when ovarian production of progesterone was completely suppressed (Fig. 2). In those rats where GnRH-Ag treatment was effective in suppressing progesterone production, neither the testosterone production by the ovary nor its conversion to estradiol was impaired due to the treatment. The lesion must be at a level beyond estradiol production in the ovary. GnRH-Ag treatment suppresses estradiol levels in the ovarian plasma at all doses on Day 18 of pregnancy. Since these levels were normal or above normal on Day 14 in rats that were aborting, the low levels on Day 18 may be an expression of postabortive events as opposed to the cause of inducing abortion.

Long term treatment with GnRH agonists, in addition to suppressing ovarian receptors for LH, also suppresses ovarian receptor content for prolactin and prolactin production (29) and suppresses plasma concentration of rat placental lactogen (rPL) (7). rPL has been shown to maintain receptors for estradiol (30) after Day 12 of pregnancy. It has been demonstrated that GnRH binds to its high-affinity receptor sites in luteal cell membranes (27) which are similar to pituitary receptor sites (31). Currently studies are in progress to determine if GnRH has any suppressive effects on rPL and/or luteal receptors for estradiol, and if so, whether there is any change observed in the number of GnRH binding sites in luteal cell membranes?

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