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Blastokinin Secretion, Ovarian Activity, and Embryo Survival after Melengestrol Acetate in Rabbits[1] (40025)

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Blastokinin (uteroglobin) comprises approximately 40-50% of the total protein in rabbit uterine fluid during the days around the time of implantation (1-3). In ovariectomized rabbits, the concentration of blastokinin in uterine flushings increases dramatically after progesterone treatment (4, 5), but only slightly if estrogen is given (6). Metabolites of progesterone also stimulate blastokinin secretion (7), as do some oral contraceptive steroids (8).

However, the physiological significance of this protein has yet to be determined. Administration of melengestrol acetate (MGA), a synthetic progestogen, lowered fertility in rabbits, allegedly through altering the time course of uterine protein secretions (9). In an attempt to elucidate further the possible functions of blastokinin in embryo survival in rabbits, we studied the effects of melengestrol acetate (MGA) administration on blastokinin secretion and ovarian activity. The ability of supplemental blastokinin to increase implantation rate of transferred rabbit embryos was examined under conditions known to reduce fertility.

Materials and methods. General procedures. A series of four experiments was conducted. In the first experiment, progesterone and estradiol were measured in blood samples collected prior to and during MGA or corn oil treatment and during a subsequent period of pseudopregnancy induced by treatment with human chorionic gonadotropin (HCG). In the second and third experiments, blastokinin was assayed in uterine flushings collected from rabbits sacrificed during MGA or corn oil treatment or during the period of induced pseudopregnancy which followed. In the final experiment, embryos collected from superovulated donors were transferred in medium containing blastokinin or rabbit blood serum albumin to pseudopregnant recipients which had been treated with MGA or corn oil. Significance of differences between treatments was analyzed by paired t test.

Treatment procedures. In all four experiments, treatments consisted of daily injections (10 days) of either 10 μg MGA/kg body weight dissolved in corn oil or corn oil alone. Pseudopregnancy was induced by intravenous administration of 100 IU of human chorionic gonadotropin[3] on the third day following MGA or corn oil treatment (9).

Serum and uterine fluid samples. In the first experiment, blood samples were collected on alternate days from a marginal ear vein of six corn oil- and six MGA-injected mature New Zealand white female rabbits. Samples were collected from 12 days pretreatment until Day 7 of pseudopregnancy. For three rabbits in each treatment group, sampling began on the first day and continued on odd-numbered days, whereas the remaining rabbits were sampled on even-numbered days. Serum was obtained by centrifugation of whole blood after it had been allowed to clot at room temperature. Samples were stored frozen until assayed for progesterone and estradiol.

In the second experiment, uterine flushings were obtained from mature does on Days 1 to 9 of pseudopregnancy (three does/treatment/day) following 10 days of MGA or corn oil injections. In the third experiment, uterine flushings were recovered (three does/treatment/day) from

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does given MGA or corn oil and sacrificed on Days 3, 6, and 9 of treatment or on the second day following the last injection. Does were sacrificed by cervical dislocation, and uteri were excised immediately, trimmed of fat, rinsed with tap water, and blotted to remove any traces of blood. Each uterine cornus was flushed with 3 ml of 0.33 *M* sodium chloride. Flushings were centrifuged at 700*g* to remove cellular debris and those obviously contaminated with blood were discarded. Samples were stored frozen until assayed for blastokinin.

Radioimmunoassays. Progesterone was assayed by specific radioimmunoassay as previously described in our laboratory (10). Briefly a highly specific antibody[4] (diluted 1:4500 in 1:400 normal rabbit serum in 0.01 *M* phosphate-buffered saline, pH 7.1) was added to tubes containing dried benzene–hexane (1:2) extracts of serum or standard quantities of progesterone. Then [^{3}H]progesterone was added to each tube and the tubes were incubated at 4° for approximately 18 hr. Dextran-coated charcoal was used to separate free from antibody-bound progesterone. Melengestrol acetate (MGA) was tested for its ability to inhibit binding of [^{3}H]progesterone to the antibody. At a concentration of 1 μg per tube, the cross-reactivity was less than 0.01%; thus it was not detectable in blood samples from MGA-treated rabbits.

Estradiol was assayed by specific radioimmunoassay using an antibody prepared against estradiol-6-oxime conjugaed to human serum albumin. Validation data for the assay have been reported (11). The procedure was similar to that for progesterone, except free and antibody-bound estradiol were separated by addition of sheep anti-rabbit γ-globulin.

Blastokinin was assayed according to the procedure of Mayol and Longenecker (12). Purified blastokinin was iodinated at room temperature by the chloramine T method and was incubated with a blastokinin antibody in the presence of unlabeled standard concentrations of reference blastokinin[5] or with uterine flushing from treated rabbits. A parallel dose–response curve of Day 6 pseudopregnant rabbit uterine extracts with the standard reference preparation was obtained. There was no cross-reactivity of the antibody with rabbit serum samples. To avoid interassay variation, all uterine fluid samples from an experiment were included within a single assay.

Embryo Transfers. Embryos, recovered 72 hr after induction of pregnancy in superovulated donors (9), were transferred to recipients pretreated with MGA or corn oil as described above. Transfers were made at an equivalent stage of pseudopregnancy. Donor oviducts and uteri were flushed with Krebs–Ringer bicarbonate and ova were collected into watch glasses (9). After examination for evidence of cleavage, ova were placed in medium supplemented (1.5 mg of protein/400-μl transfer volume) with either purified blastokinin as prepared by the method of Mayol and Longenecker (12) or rabbit blood serum albumin. The transfer procedure was essentially that described by Staples (13). Each animal served as its own control by receiving a minimum of four embryos per horn; one horn supplemented with blastokinin, the other with serum albumin. Results were recorded as the number of implantation sites on Day 11 of pregnancy per number of ova transferred.

Results. No differences in serum progesterone or estradiol concentrations were detected between rabbits given MGA or corn oil ($P > 0.10$; Figs. 1 and 2). Estradiol fluctuated between 4 and 18 pg/ml prior to and during MGA or corn oil treatment and peaked around 16 pg/ml on the second day of pseudopregnancy. Progesterone was low (<1 ng/ml) prior to pseudopregnancy and peaked on the sixth and seventh days of pseudopregnancy (the last days samples were taken). Similar numbers of large (>2-mm) follicles, hemorragic follicles, and corpora lutea were found on the ovaries of does from each treatment group (Table I).

The effect of corn oil or MGA administra-

[4] The rabbit antiprogesterone prepared against 6β-succinylprogesterone conjugated to bovine serum albumin was supplied by Dr. G. D. Niswender, Colorado State University.

[5] Purified blastokinin, guinea-pig anti-rabbit blastokinin, and blastokinin reference preparation were supplied by Dr. R. F. Mayol.

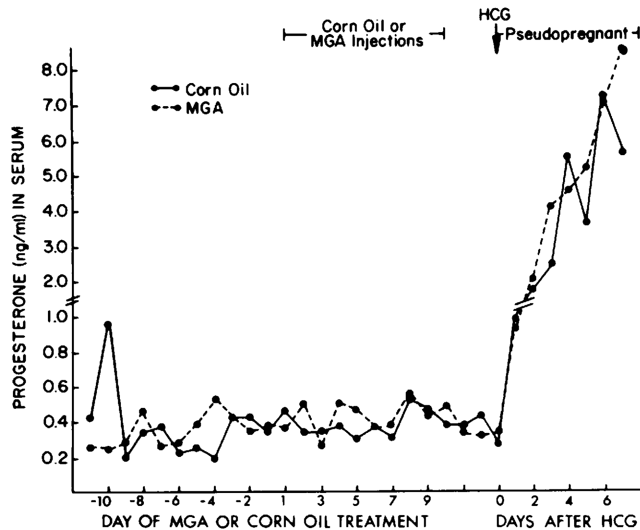

FIG. 1. Progesterone in blood samples collected prior to and during corn oil or MGA treatment and during the subsequent period of induced pseudopregnancy.

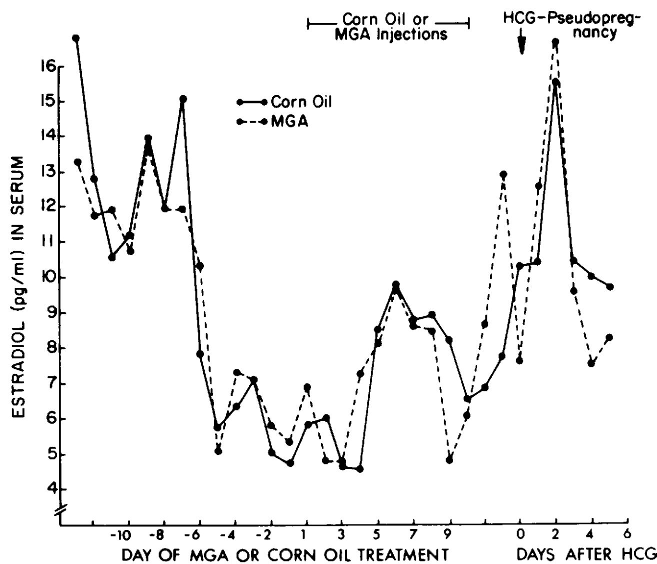

FIG. 2. Estradiol in blood samples collected prior to and during corn oil or MGA treatment and during the subsequent period of induced pseudopregnancy.

tion on blastokinin production during treatment and subsequent pseudopregnancy is shown in Fig. 3. Does given corn oil averaged 0.0004 mg of blastokinin/ml of uterine flushing prior to pseudopregnancy. On Day 3 of pseudopregnancy following corn oil treatment, blastokinin concentrations increased to 1.5 mg/ml, continued to rise until Day 5, plateaued until Day 8 (1.94–2.39 mg/ml), and then fell to 0.29 mg/ml on Day 9. Rabbits given MGA had blastokinin concentrations of 0.006, 0.202, and 0.108 mg/ml on Days 3, 6, and 9 of MGA treatment, respectively ($P < 0.01$). By Day 3 of pseudopregnancy, MGA-treated animals had only 0.1 mg/ml ($P < 0.01$) of blastokinin in the uterine fluid. This concentration increased to 1.6 mg/ml on Day 4, plateaued by Day 7 (1.75–2.23 mg/ml), and then decreased to 0.75 and 0.31 mg/ml

TABLE I. NUMBER OF OVARIAN STRUCTURES ON DAY 7 OF PSEUDOPREGNANCY IN RABBITS PRETREATED WITH CORN OIL OR MELENGESTROL ACETATE (MGA).

Ovarian structure	Treatment	
	Corn oil	MGA
Corpora lutea	10.3 ± 1.4[a]	11.2 ± 1.5
Hemmoragic follicles	2.5 ± 0.3	3.3 ± 0.4
Large follicles[b]	8.3 ± 2.0	10.7 ± 1.9

[a] Mean ± standard error of mean.
[b] Follicles >2 mm in diameter.

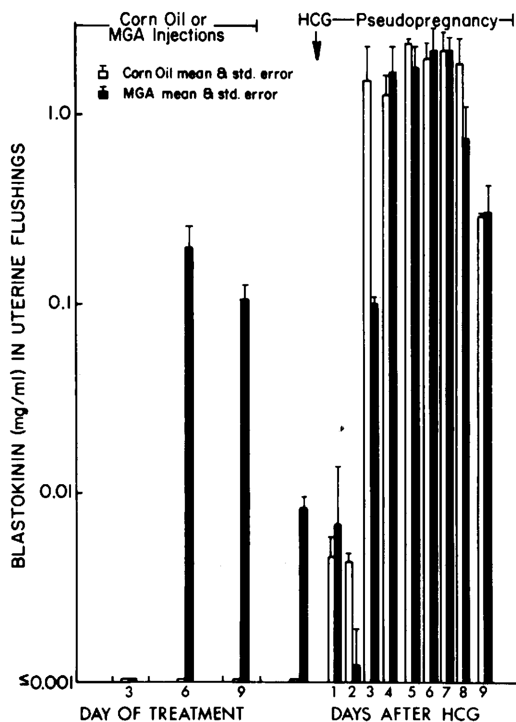

FIG. 3. Blastokinin in uterine flushings collected during MGA or corn oil treatment and during the subsequent period of induced pseudopregnancy.

by Days 8 and 9, respectively. The pattern of blastokinin secretion during MGA treatment, although of lesser magnitude, was highly correlated with secretion during pseudopregnancy ($r = 0.89$). Again follicle numbers did not differ between groups.

Blastokinin-supplemented medium was not able to improve implantation rates in does pretreated with MGA when compared with medium containing rabbit serum albumin (4.3 vs 0%). However, when protein-supplemented medium was used to transfer embryos to corn oil-treated controls, addition of blastokinin enhanced ($P \simeq 0.1$) survival compared with serum albumin; implantation rates were 32.6 and 23.1%, respectively (Table II). Six of eight does receiving each treatment were pregnant at autopsy on Day 11.

Discussion. These studies support contentions that progestin administration reduces embryo survival by altering the uterine environment (9). The concentration of blastokinin in uterine fluid was reduced on Day 3 of pseudopregnancy in MGA-treated rabbits at a time when fertilized ova first reach the uterus. Lower blastokinin concentrations on Day 3 of pseudopregnancy following MGA were probably due to refractoriness to progesterone secreted by the rabbits' ovaries. This concept is supported by the fact that blastokinin secretion declined in the face of continued progresterone administration to ovariectomized rabbits (4). Since blastokinin concentration was elevated during MGA treatment and began to decline before MGA treatment ended (Fig. 3), the refractory period was evident even before pseudopregnancy was induced.

Because our radioimmunoassay for progesterone could not detect MGA in serum, we could not determine the amount of progestational activity to which MGA-treated animals were exposed during and subsequent to treatment. Runic *et al.* (14) suggest that progestational compounds require longer to be cleared from the system than the natural hormone, and this could account for the delayed rise in blastokinin during the period of pseudopregnancy following MGA treatment.

TABLE II. IMPLANTATION RATE ON DAY 11 OF PREGNANCY OF 72-hr EMBRYOS TRANSFERRED TO MGA- OR CORN OIL-TREATED RECIPIENTS IN MEDIA SUPPLEMENTED WITH RABBIT BLOOD SERUM ALBUMIN OR BLASTOKININ.

Recipient pretreatment	Media supplement[a]	
	Rabbit serum albumin	Blastokinin
Corn oil	9/39[b] (23.1%)	14/43 (32.6%)
MGA	0/43 (0%)	2/47 (4.3%)

[a] 1.5 mg of protein/400-μl transfer volume.
[b] Number of embryos present on Day 11/number of 72-hr embryos transferred.

These results also lend credence to reports (15, 16) that blastokinin stimulates growth and development of early blastocysts *in vitro*. Blastokinin was able to enhance implantation in control animals, but was unable to overcome the infertility effects of progestin administration.

Summary. Progesterone and estradiol were measured in blood and blastokinin was assayed in uterine flushings collected from rabbits prior to and during treatment with melengestrol acetate (MGA) or corn oil and during the subsequent period of induced pseudopregnancy. Blastokinin was elevated during MGA treatment, but was transiently suppressed on Day 3 of pseudopregnancy after MGA when levels in corn oil-treated rabbits were 10-fold higher than those in rabbits given MGA. Estradiol and progesterone were not different between rabbits given the two treatments. Addition of purified blastokinin to embryo transfer media increased implantation rates in corn oil-treated recipients, but not in recipients pretreated with MGA.

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