

Fertility and Embryo Survival After Administration of Melengestrol Acetate in Rabbits¹ (37390)

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Altered sperm transport in the ewe (1) and altered uterine environment in the cow (2) have been implicated as causes of reduced fertility after estrous synchronization with progestogens. Pritchard, Wettemann, and Hafs (3) demonstrated reduced fertility in rabbits after 12 days of melengestrol acetate (MGA) administration. We conducted four experiments using the rabbit model (3) to test whether phenobarbital, given to enhance steroid clearance (4) or oxytocin, given to facilitate sperm transport (5) would improve fertility of insemination after MGA and to find whether altered uterine environment after MGA persisted to interfere with survival of fertilized ova.

Materials and Methods. Rabbits averaging about 3.5 kg and of mixed breeding were assigned at random to treatments. In the first experiment 16 control rabbits were given 12 daily subcutaneous (sc) injections of corn oil and 32 rabbits were given daily sc injections of MGA³ (10 µg/kg) dissolved in corn oil. The controls and 16 of the MGA-treated rabbits were inseminated 3 days after the last MGA or corn oil injection and the other 16 MGA rabbits were inseminated 7 days after MGA. Within each of these three treatment groups, phenobarbital was injected (50 mg/kg) sc daily into half (eight) of the rabbits for 3 days beginning on the final day of MGA or corn oil treatment. Within each of these six treatment subgroups, four rabbits were

inseminated vaginally and four were inseminated surgically in each uterine horn adjacent to the tubo-uterine junction. A single pool of semen from six males was extended in 0.1 M phosphate buffer (pH 7.1) to 24×10^6 sperm/ml, with 0.5 ml used for each vaginal insemination or 0.1 ml deposited surgically in each uterine horn. Superovulation was induced with injections of FSH⁴ (0.4 mg twice daily) for 3 days before insemination and HCG⁵ injected at the time of insemination, 12 hr after the final injection of FSH. At 30 hr after insemination, rabbits were killed by cervical dislocation, the number of ovulations was recorded, and oviducts and uterine horns were flushed with 0.85% sodium chloride. Cleaved ova were recorded as fertilized.

In the second experiment, 24 control rabbits were given corn oil sc and MGA was injected (10 µg/kg) sc daily into 24 rabbits for 12 days. The rabbits were superovulated, and at 3 days after the final MGA or oil injection; one-half (12) of the rabbits in each group was inseminated vaginally or in the uterus as in the first experiment. At about 1 hr after insemination, the rabbits were injected intravenously with 0.1 or 2 IU oxytocin per kg body weight. Fertilized ova were determined 30 hr after insemination as in the first experiment.

The third experiment was designed to determine whether the uterine factor(s) which affect sperm in MGA-treated rabbits also affect survival of fertilized ova transferred to the uterus or oviducts. Recipients were given corn oil (0.5 ml) or MGA (10 µg/kg) for 10 days, and were made pseudopregnant with an injection of HCG, 3 days after the final

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³ Supplied by Dr. R. G. Zimelman, The Upjohn Co., Kalamazoo, Mich.

⁴ Armour FSH-P.

⁵ Follutein, supplied by Dr. W. T. Davis, E. R. Squibb and Sons, New Brunswick, N.J.

MGA. Donor rabbits were superovulated and inseminated vaginally as in the first experiment, and killed at 48 or 72 hr after insemination. Ova were flushed from the uteri and oviducts with Krebs-Ringer phosphate buffer, and groups of eight eggs (four per uterine horn) were transferred to control and MGA-treated recipients. Ova were transferred to oviducts at 48 hr or to uteri at 72 hr after HCG, 5 or 6 days after the last MGA. The number of pregnant recipients and the percent implantations were recorded 11 days after HCG.

In the fourth experiment, we transferred groups of eight 4-day blastocysts from superovulated donors to the uteri (4 eggs to each uterine horn) of MGA-treated rabbits 2, 3, 4, 5, or 6 days after an ovulating dose of HCG, given 3 days after MGA. The MGA treatment procedures and transfer techniques were identical to those described for the third experiment. The number of pregnant recipients and percent implantations were recorded 11 days after HCG.

Results and Discussion. First experiment. We found an average of 40 ovulations per rabbit, and number of ovulations was not significantly influenced by pretreatment with MGA or phenobarbital, or by site of insemination (Table I). In the phenobarbital-treated rabbits, 73% of the ovulations were represented by ova flushed from oviducts or uteri, slightly ($p \cong 0.08$) higher than the 56% in rabbits which did not receive pheno-

barbital, but this effect of phenobarbital was restricted to rabbits which had been treated with MGA.

Fertility at 3 days after MGA withdrawal was significantly ($p < 0.01$) reduced relative to that of the controls (Table I) as Pritchard *et al.* (3) observed, but this restrictive effect of MGA on fertility largely disappeared ($p < 0.01$) by 7 days after MGA. Uterine inseminations were more fertile than vaginal inseminations ($p < 0.01$), but this difference was limited principally to rabbits inseminated 3 days after MGA when vaginal inseminations resulted in only 1% fertility. Phenobarbital significantly improved fertility among rabbits inseminated 3 or 7 days after MGA, but not among control rabbits.

Jones and Armstrong (4) demonstrated that phenobarbital induced hepatic microsomal enzymes related to sterol and steroid metabolism. Increased metabolism of a steroid presumably limits its biological effectiveness (6). If residual MGA is responsible for reduced fertility at 3 days after MGA, phenobarbital may improve fertility by enhancing clearance of MGA. On the other hand, Wette- mann and Hafs (7) suggested that cows have unusually high blood estradiol after withdrawal of MGA; phenobarbital may aid clearance of high estradiol concentration if this occurs in rabbits after MGA.

Second experiment. The average of 40 ovulations per rabbit was not significantly influ-

TABLE I. Average Number of Ovulations, Percent Ova Recovered and Fertility of Rabbits Treated with Phenobarbital After MGA.

MGA ($\mu\text{g}/\text{kg}$)	Period from MGA to in- semination (days)	Pheno- barbital (mg/kg)	Site of insemination					
			Vagina			Upper uterus		
			Ovulations (no.)	Ova recovered —————(%)—————	Ova fertilized	Ovulations (no.)	Ova recovered —————(%)—————	Ova fertilized
0	3	0	38 ^a	79	88	47	37	77
		50	30	71	52	38	49	89
10	3	0	41	31	0	53	42	50
		50	53	80	2	30	65	79
10	7	0	33	72	52	38	58	70
		50	34	93	86	46	63	95

^a Each entry represents the average of three or four rabbits.

TABLE II. Number of Ovulations, Ova Recovered, and Fertility of Rabbits Treated with Oxytocin at Insemination 3 Days After MGA.

MGA ($\mu\text{g}/\text{kg}$)	Oxytocin (IU/kg)	Site of insemination					
		Vagina			Upper uterus		
		Ovulations (no.)	Ova recovered ————(%)————	Ova fertilized ————	Ovulations (no.)	Ova recovered ————(%)————	Ova fertilized ————
0	0	46 ^a	92	71	16	62	83
	0.1	59	86	93	36	77	88
	2.0	53	69	87	38	46	95
10	0	52	78	7	22	34	82
	0.1	32	99	25	39	43	62
	2.0	43	42	48	38	73	47

^a Each entry represents the average of three or four rabbits.

enced by oxytocin or by MGA pretreatment (Table II). In contrast to the first experiment, however, rabbits inseminated into the uterus had fewer ($p < 0.01$) ovulations than rabbits inseminated intravaginally though the reason for this is not evident. Percent ovum recovery was not significantly influenced by MGA, oxytocin or site of insemination.

As in the first experiment, fertility at 3 days after MGA withdrawal was significantly reduced from the controls (Table II, $p < 0.01$). Also, upper uterine inseminations were more fertile than vaginal inseminations, but this effect was limited to rabbits treated with MGA. The data in Table II suggest that oxytocin improved fertility of vaginal inseminations, especially after MGA, but this influence of oxytocin only approached significance ($p \cong 0.20$). This result tends to support the contention (3) that reduced fertility after MGA may be related with altered sperm transport. Whether larger or repetitive doses of oxytocin may obviate reduced fertility after MGA remains to be tested.

Third experiment. When fertilized ova were transferred to oviducts or uteri, the percent pregnant recipients was significantly ($p < 0.05$) reduced in rabbits pretreated with MGA (Table III). In other words, most MGA-treated rabbits were unable to maintain pregnancy even though the fertilized ova were transferred 5 or 6 days after MGA withdrawal. Embryo survival was high (73%) in the two pregnant recipients to

which ova were transferred into the oviducts at 5 days after MGA, considerably lower (11%) in one rabbit given ova in the uterus on Day 6 after MGA. Nevertheless, the dominant conclusion from the data in Table III is that survival of transferred ova was reduced by the previous treatment with MGA. Altered uterine environment probably accounts for reduced survival of transferred ova at 5 or 6 days after MGA. Although fertility of inseminations was not reduced at 7 days after MGA (Table I), the ova in that case probably would not arrive in the uterus until about 10 days after MGA.

Fourth experiment. Altering the chronological relationship between the blastocyst and uterus resulted in normal pregnancy rates when Day-4 blastocysts were transferred to Day-5 MGA-treated recipients.

TABLE III. Pregnancy Rate and Embryo Survival in Pregnant Recipients at 11 Days After Transfer of Fertilized Ova to Oviducts or Uteri of MGA-Treated Rabbits.

MGA ($\mu\text{g}/\text{kg}$)	Ova trans- ferred to	Recipient rabbits		Embryo survival in pregnant recipients (%)
		Num- ber	No. pregnant	
0	Oviduct	10	7	43
	Uterus	8	6	42
10	Oviduct	11	2	73
	Uterus	9	1	11

TABLE IV. Pregnancy Rate and Embryo Survival at 11 Days After Transfer of Day-4 Blastocysts to the Uteri of MGA-Treated Rabbits at Various Intervals After MGA.

Day of transfer ^a	MGA ($\mu\text{g}/\text{kg}$)			
	0		10	
	Pregnant recipients Total recipients	Embryo survival (%)	Pregnant recipients Total recipients	Embryo survival (%)
2	0/4	—	0/4	—
3	4/5	42	1/5	25
4	2/3	60	1/5	75
5	1/4	13	3/5	54
6	2/5	13	0/3	—

^a Day of transfer is the days after HCG when Day-4 blastocysts were transferred to recipients. HCG was given 3 days after MGA.

^b Embryo survival is calculated for pregnant recipients only and is the percent of total blastocysts transferred represented by implants at autopsy.

Pregnancy rates were normal in Day-3 and -4 control recipients but were low in Day-5 control recipients. These results suggest that MGA delayed onset of the period when the uterus is most receptive to transferred ova; however, the limited number of recipients prohibit definite conclusions concerning fertility.

Recently, Beirer *et al.* (8) reported that treatment with estrogen 6 and 30 hr after mating in the rabbit altered the profile of proteins in uterine secretions; uteroglobin secretion was delayed for about 3 days and 4-day blastocysts developed normally only when transferred to the "delayed uterus." We suggest that reduced survival of transferred embryos in MGA-treated rabbits is possibly due to alteration in uterine secretions caused by altered steroid hormone environment (7, 9) resulting in asynchrony between the developing ova and the recipient uterus. However, we can not rule out the possibility that ovum transport may have been altered which would also cause asynchrony between the embryo and uterus.

Summary. Fertility of vaginal inseminations in rabbits treated with melengestrol acetate (MGA) was significantly improved when phenobarbital was administered at MGA withdrawal. Oxytocin administration

after vaginal insemination in MGA-treated rabbits resulted in partial restoration of normal fertility. Ova transferred from superovulated donors to synchronous MGA-treated recipients failed to survive, but ova transferred to MGA-treated recipients in which pseudopregnancy was one day advanced in relation to the donors resulted in normal pregnancy.

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