CONTROL OF OVULATION IN CATTLE WITH MELENGESTROL ACETATE

III. INDUCING OVULATION DURING MGA TREATMENT

L. W. SMITH AND R. G. ZIMBELMAN

Agricultural Division, The Upjohn Company, Kalamazoo, Michigan, U.S.A.

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Summary. The following compounds were used in an effort to induce oestrus and ovulation in Holstein dairy heifers during melengestrol acetate (MGA®) treatment: oestradiol cypionate (ECP®), oestradiol-17 β , Carbestrol®, human chorionic gonadotrophin (HCG), pituitary luteinizing hormone (PLH), pregnant mare serum gonadotrophin (PMSG), oxytocin, neostigmine, amphetamine, nafoxidine hydrochloride and clomiphene citrate. MGA feeding began on Day 15 of the oestrous cycle and continued for 15 to 20 days. All the above compounds were administered between Day 8 and Day 13 of MGA feeding. The compounds were given by various routes of administration. Only the oestrogens (ECP, oestradiol-17 β and Carbestrol) effectively induced oestrus and ovulation, their effectiveness seeming to depend on the method of administration and the type of vehicle in which they were administered. ECP was effective at 5, 10 and 20 mg when injected intravenously (i.v.) in an oil vehicle. Oestradiol-17 β was effective at 10 mg when injected intramuscularly (i.m.) in an oil vehicle. Both were less effective when the vehicle was propylene glycol. Carbestrol was effective only when given orally at 40 mg and 60 mg levels.

The gonadotrophins (HCG, PLH and PMSG) did not produce consistent results. When the follicles disappeared, a subsequent corpus luteum did not always appear to develop.

The other compounds tested had no observable effect on oestrus or ovulation.

INTRODUCTION

The effectiveness of hormones which control the oestrous cycle is measured by the number of animals which come into oestrus and ovulate during a short post-treatment interval. The effectiveness of melengestrol acetate (MGA) in inhibiting oestrus and ovulation in heifers has been described previously (Zimbelman & Smith, 1966). Although the controlled breeding period of several days achieved through the use of progestagen offers many advantages over conventional breeding methods, it is not precise enough to relieve the rancher of the task of detecting oestrus.

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In addition, some reduced conception rates have been noted following control of oestrus by oral progestagens. It has been suggested that these may be due to the failure of ovulation or improper timing of insemination. Since the precise induction of ovulation would provide control over these factors, a search for ovulation-inducing agents was undertaken.

MATERIALS AND METHODS

Two hundred and four Holstein dairy heifers were given oral MGA at dosages of 0.4 and 0.5 mg daily. All heifers were individually fed and received the MGA treatment for 15 to 20 days beginning on the 15th day of the oestrous cycle. The heifers were divided into the treatment groups (control and injected) at random as they became eligible for treatment. A heifer was eligible for treatment if the corpus luteum (CL) of the present cycle had regressed (12 mm or less) and a follicle, 12 mm or greater, was present. The size of each was determined by rectal palpation. All treated heifers received a single administration of the possible ovulation-inducing compounds between Day 8 and Day 13 of MGA treatment. The compounds and the dosages used were:

Oestradiol cypionate (ECP), 1, 5, 10 and 20 mg

Oestradiol-17 β , 10 mg

Carbestrol (3-ethyl-4-(p-methoxy-phenyl)-2-methyl-3-cyclohexene-1-carboxylic acid), 20, 40, 50 and 60 mg

Human chorionic gonadotrophin (HCG—Upjohn), 2500 and 5000 i.u.

Pituitary luteinizing hormone (PLH—Armour), 50 and 100 mg

Pregnant mare serum gonadotrophin (PMS—Upjohn), 200, 500 and 2000 i.u.

Oxytocin (POP—Armour), 200 i.u.

Neostigmine (Pitman-Moore), 50 mg

Amphetamine (Pitman-Moore), 300 mg

Nafoxidine hydrochloride (1-{2-[p-3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl phenoxy]ethyl}-pyrrolidine hydrochloride), 900 and 1000 mg

Clomiphene citrate (2-[p-(β -chloro- α -phenylstyryl)phenoxy]-triethylamine citrate), 1000 mg

The route of administration and vehicle used is mentioned in the results when pertinent. Following the injection, the animal remained on MGA treatment for 7 days. All control animals received either an injection of the vehicle alone or no injection during the MGA treatment period.

All animals were palpated through the rectum at various intervals before and after the injections to determine the status of CL regression, follicular development or ovulation. In the first part of the study, the heifers were palpated only once after the injection (on the last day of MGA treatment), to determine CL presence, but later it became apparent that ovulations could not be determined accurately by this method. Thus, in the latter part of the study, palpations were made each morning and afternoon for 48 hr following the injections to determine follicle disappearance, and on the last day of MGA treatment to determine CL formation subsequent to the follicle disappearance.

All the animals were observed twice daily for oestrus. None was inseminated.

RESULTS

MGA effectively inhibited oestrus and ovulation in these heifers. Only 2% of the heifers ovulated before the time that the injections were to be given and none of the control (MGA only) heifers ovulated before the last day of MGA feeding. All the control heifers cycled within 2 to 7 days after last feeding.

Effectiveness of ECP and oestradiol-17 β

These compounds were administered either i.v. or i.m. in cottonseed oil or propylene glycol. Intravenous injections of ECP in oil effectively induced both oestrus and ovulation at 5, 10 and 20 mg. The percentage of heifers showing oestrus within 24 hr after the injection ranged from 50% to 100% and the percentage which had a palpable CL by 7 days after the injection ranged from 75% to 100%.

TABLE 1

OVULATION INDUCTION BY OESTRADIOL-17 β OR ECP DURING CONTINUOUS TREATMENT WITH ORAL MELENGESTROL ACETATE (MGA)

MGA* dose (mg)	No. of heifers	Compound injected				During MGA treatment			First post- treatment cycle
						% in oestrus	% ovulated		
		Name	Amount (mg)	Route	Vehicle†		Follicle disappearance	Corpus luteum	% 2 to 7 days§
0.5	4	ECP	1	i.m.	Oil	0	ND‡	0	75
0.4	14	None	—	i.v.	PG	0	ND	0	100
	8	ECP	5	i.v.	Oil	100	ND	75	25
	8		10	i.v.	Oil	75	ND	100	0
	4		10	i.m.	Oil	0	ND	0	75
	4		20	i.v.	Oil	50	ND	100	0
0.5	8	ECP	10	i.v.	Oil	63	88	75	37
	4		10	i.v.	PG	0	75	0	100
	4		10	i.m.	PG	0	100	25	50
	8	None	—	—	—	0	0	0	100
0.5	4	Oestradiol (U-3143)	10	i.v.	Oil	0	100	25	75
	4		10	i.m.	Oil	75	100	75	25
	4		10	i.v.	PG	25	25	25	75
	4		10	i.m.	PG	25	100	50	50

* MGA treatment continued for 1 week after injection.

† Oil = cottonseed oil vehicle; PG = propylene glycol.

‡ ND = Not determined.

§ Days after last feeding.

Intramuscular injections of ECP in oil at 1 and 10 mg levels were ineffective in inducing oestrus or ovulation. Intramuscular and intravenous injections of ECP in propylene glycol at 10 mg were ineffective in inducing oestrus, but were effective in inducing ovulation as indicated by follicle disappearance (75% to 100%). However, CL were not detected and most animals cycled within 2 to 7 days after last feeding.

The dosage of oestradiol-17 β was always 10 mg; results varied with the route of administration and the vehicle used. Oestrus was effectively induced

in only the group injected i.m. in an oil vehicle. Ovulation, as indicated by follicle disappearance, was induced in all heifers except those injected i.v. with a propylene glycol vehicle. Only 25% of these heifers ovulated. Normal CL were not palpable in all heifers in which the follicles had disappeared. The failure of CL formation was associated with the occurrence of the first post-treatment cycle 2 to 7 days after last feeding.

Effectiveness of Carbestrol

Dosages of 20, 50 or 60 mg of Carbestrol in propylene glycol administered either i.v. or i.m. did not consistently induce oestrus or ovulation. All but one of these heifers cycled 2 to 7 days after last feeding.

TABLE 2

OVULATION INDUCTION BY CARBESTROL DURING CONTINUOUS TREATMENT WITH ORAL MELENGESTROL ACETATE (MGA)

MGA dose (mg)	No. of heifers	Carbestrol injected			During MGA treatment			First post-treatment cycle
					% in oestrus	% Ovulated		
		Amount (mg)	Route	Vehicle		Follicle disappearance	Corpus luteum	% 2 to 7 days§
0.4	10	—	i.v.	PG*	0	ND‡	0	100
	4	20	i.v.	PG*	0	ND	0	100
	3	50-60	i.v.	PG*	33	ND	0	100
	3	60	i.m.	PG*	0	ND	33	67
	4	40	Oral	Crystals†	75	ND	75	25
	4	60	Oral	Crystals†	75	ND	100	0

* Carbestrol solution prepared in propylene glycol.

† Crystalline carbestrol prepared in 20 mg capsules.

‡ ND = Not determined.

§ Days after last feeding.

When Carbestrol was administered orally at dosages of 40 and 60 mg, 75% of the heifers showed oestrus and all but one had a palpable CL. Only one animal cycled within 2 to 7 days after last feeding.

Effectiveness of gonadotrophins

Three gonadotrophins (HCG, PLH and PMSG) were administered i.v. except the high dosage level of HCG which was given both i.v. and i.m. The PLH- and PMSG-treated heifers were palpated for follicle disappearance.

When HCG was administered, CL were not uniformly detected. At the dosage levels used, 2500 i.u. (i.v.), 5000 i.u. (i.v.) and 5000 i.u. (i.v. and i.m.), 75%, 50% and 40% of the animals, respectively, had detectable CL.

When either 50 or 100 mg of PLH was injected, the follicle disappeared in all heifers. However, none of the heifers given 50 mg and only 50% of those given 100 mg had a detectable CL by 7 days after the injection.

PMSG was administered at dosages of 200, 500 and 2000 i.u. Ovulation was not induced in any of the heifers at the 200 i.u. dosage. At the 500 i.u. dosage, the follicles disappeared and the CL was palpable in one heifer. At the 2000 i.u.

dosage, follicles disappeared in both heifers but follicular development was so extensive a few days later that detection of a CL by Day 7 was impossible in both heifers. By the next oestrous cycle, determination of ovarian structures was again possible.

In general, the occurrence of a cycle at 2 to 7 days after last MGA feeding reflected the absence of a detectable CL on the last day of MGA.

TABLE 3

OVULATION INDUCTION WITH GONADOTROPHIN DURING CONTINUOUS TREATMENT WITH ORAL MELENGESTROL ACETATE (MGA)

MGA dose (mg)	No. of heifers	Compound injected			% ovulated		First cycle post-treatment % 2 to 7 days†
		Name	Amount	Route	Follicle disappearance	Corpus luteum	
0.4	4	HCG	2500 i.u.	i.v.	ND*	75	25
	4		5000 i.u.	i.v.	ND	50	25
	5		5000 i.u.	i.v. & i.m.	ND	40	60
0.5	4	PLH	50 mg	i.v.	100	0	75
	4		100 mg	i.v.	100	50	25
	2	PMS	200 i.u.	i.v.	0	0	100
	2		500 i.u.	i.v.	50	50	50
	2		2000 i.u.	i.v.	100	—	50

* ND = Not determined.

† Days after last feeding.

Effectiveness of miscellaneous compounds

Two hundred i.u. oxytocin, 50 mg neostigmine and 300 mg amphetamine had no effect on ovulation. Follicles did not disappear and detectable CL did not form in any of the four heifers injected with each compound. There was

TABLE 4

OVULATION INDUCTION WITH VARIOUS AGENTS DURING CONTINUOUS TREATMENT WITH ORAL MELENGESTROL ACETATE

MGA dose (mg)	No. of heifers	Compound injected			% ovulated		First cycle post-treatment % 2 to 7 days†
		Name	Amount	Route	Follicle disappearance	Corpus luteum	
0.5	4	Oxytocin	200 i.u.	i.v. and s.c.	0*	0	75
	4	Neostigmine	50 mg	i.v. or s.c.	0	0	100
	4	Amphetamine	300 mg	s.c.	0	0	100
	4	Nafoxidine hydrochloride	1000 mg	i.v.	ND	0	100
	3		900 mg	i.v.	0	0	100
	4	Clomiphene citrate	1000 mg	i.v.	0	0	50

* ND = Not determined.

† Days after last feeding.

increased salivation, production of watery faeces and lack of coordination with neostigmine, and a condition of hyperexcitability with amphetamine. The

majority of these heifers cycled within 2 to 7 days after last feeding. Two compounds, nafoxidine hydrochloride and clomiphene citrate, that are known to have anti-oestrogenic properties were injected i.v. Doses of 900 or 1000 mg of nafoxidine hydrochloride and 1000 mg of clomiphene citrate had no apparent effect on ovulation. Follicles did not disappear and/or detectable CL did not form in any of the heifers treated. There were no adverse side effects with either compound. Almost all heifers cycled within 2 to 7 days after last feeding.

DISCUSSION

It has been postulated that oestrogen injections may cause rapid release of LH in laboratory animals (Hellbaum & Greep, 1946; Byrnes & Meyer, 1951) and in the bovine (Loy, Zimbelman & Casida, 1960). The results in this study appear to substantiate this postulate providing that the dosages of oestrogen are large enough to overcome the inhibitory effect of MGA on the release of LH. The general ineffectiveness of the i.m. injections of the oestrogens may indicate that the release of oestrogens at any one time from the injection site was not large enough to overcome this inhibitory effect. The vehicle also appeared to influence the effectiveness of the oestrogens and may reflect the rapidity with which a critical blood level is reached.

It was interesting to note that some of the CL induced by ECP regressed earlier than normal. This response may have been expected when MGA was continued for 7 days after the injection. Loy *et al.* (1960) reported that progesterone injected on the day after oestrus exerted a detrimental effect on the weight, histology and progesterone content of CL. The early luteal regression in the present study appeared chronologically similar to that produced by Armstrong & Hansel (1959) with oxytocin administration.

Regardless of this early regression of some of the ECP-induced CL, ECP injected i.v. appeared to cause both follicle disappearance and CL formation more frequently than the gonadotrophins at the dosages used in this study. When adequate dosages of the gonadotrophins were given, follicles disappeared regularly but CL failed to form consistently. Thus, we conclude that exogenous LH may cause rupture of the follicles by acting directly on the ovary but may fail to induce complete CL formation. Since LH appears to be the luteotrophic hormone in the bovine (Simmons & Hansel, 1964), CL formation or maintenance may be incomplete when only enough LH is provided for rupture of the follicle. The fact that ECP induced both ovulation and CL formation more frequently than some other treatments may be due either to a prolonged persistence of oestrogen levels or to prolonged activation of the hypothalamic (or other) centre causing a more normal endogenous release of LH.

The miscellaneous agents were used in an attempt to induce oestrus and ovulation in heifers. Armstrong & Hansel (1959) have shown that oxytocin blocks CL formation in heifers when injected at a critical time during the oestrous cycle. This could be due to either inhibition of LH release or exhaustion of LH release from the pituitary. Thus it seemed desirable to determine whether or not oxytocin would stimulate endogenous LH release. The results were negative at the dosage used. Similar results were obtained with the other miscellaneous

agents. Cholinergic (neostigmine) and adrenergic (amphetamine) stimulators failed to induce ovulation as did the anti-oestrogens. It has been reported that clomiphene citrate may have a gonadotrophin-releasing activity in Stein-Leventhal syndrome in which the ovaries are anovulatory for prolonged periods (Greenblatt, 1961). This gonadotrophin-releasing effect was not demonstrated under the conditions of this study.

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