CONTROL OF OVULATION IN CATTLE WITH MELENGESTROL ACETATE

I. EFFECT OF DOSAGE AND ROUTE OF ADMINISTRATION

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Summary. Dairy heifers with normal oestrous cycles were treated with progestagen for 15 to 18 days beginning at the 15th day of the cycle. Daily oral doses of 0.25 to 8 mg of melengestrol acetate (6 α -methyl-6-dehydro-16-methylene-17-acetoxypregesterone: MGA) inhibited oestrus and ovulation in all heifers except one receiving 0.5 mg. Daily intravenous injections of 0.4 mg inhibited ovulation in eight out of eight heifers. Lower doses by either route suppressed oestrus but did not uniformly inhibit ovulation. Orally, MGA was about 300 to 900 times as potent as MAP (medroxyprogesterone acetate; Provera*) but only about ten to fifteen times as potent when both were compared by intravenous injection.

Groups of eight to ten heifers were fed doses of 0.2 to 2.0 mg daily beginning without regard to the stage of the oestrous cycle. Of seventy-two heifers, sixty-nine did not ovulate during treatment. The average interval from last feeding to oestrus or ovulation ranged from 2.7 days at 0.2 mg to 6.3 days at 2.0 mg. The conception rates for various groups varied from 25 to 88% at first service. Of the total, 42% conceived with one service and 82% with two services.

INTRODUCTION

The biological activities of melengestrol acetate (MGA) in laboratory animals have recently been described. The potency of MGA relative to medroxyprogesterone acetate (MAP) in various laboratory animal tests, characteristic of progestational agents, was two to four times greater (Duncan, Lyster, Hendrix, Clark & Webster, 1964). This paper reports that the potency of MGA in cattle as measured by ovulation inhibition is several hundred times that of MAP. A partial explanation for this marked difference in relative potency between species has been obtained.

METHODS

The studies reported were conducted on Holstein dairy heifers with normal oestrous cycles prior to treatment. The general procedures were similar to those used to determine the minimal effective dose of MAP for ovulation inhibition in cattle (Zimbelman, 1963a). The data in Table 1 were obtained between

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October 1961 and May 1962 with individual feeding for a 16-day period beginning on the 15th day of the oestrous cycle. Data on reproductive performance were collected from seventy-two heifers fed as groups of eight to ten each for 18 days beginning without regard to the stage of the oestrous cycle. Other groups of heifers received once daily administration of MGA by means of an intravenous injection. Two solutions were used for the injection. The first procedure involved using a solution of 0.4 mg MGA/ml of 95% ethanol. Immediately prior to injection, the 1 ml of ethanol was diluted with 9 ml of injectable saline in the syringe. The second procedure involved a solution of 0.1 mg MGA/ml in a solvent mixture of 25% dimethylacetamide (DMA) and 75% propylene glycol. The required 1, 2 or 4 ml of this solution were injected into the jugular vein. The controls received a DMA-propylene glycol solution and a sterile aqueous suspension of MAP injected intravenously.

Conclusions on the effectiveness of treatment were based on frequent palpations of the ovaries *per rectum* and twice daily observations for oestrus. All inseminations within a trial were performed with a single lot of frozen semen. Heifers were inseminated about 12 hr after being first observed in oestrus. During the time of maximal synchronization, heifers were also inseminated when first noted in oestrus. Pregnancies were diagnosed by rectal palpation between Days 30 and 45. Conception rates were based on these examinations. Pregnancies terminating between the time of diagnosis and calving will be discussed with the calving results.

RESULTS

The sequence of oral doses used to determine the minimal effective dose for MGA is shown in Table 1. Only one heifer ovulated during treatment with doses from 8.0 mg to 0.25 mg daily/heifer. Of the five heifers each receiving 0.125 and 0.0625 mg daily, ovulation occurred during treatment in four and five, respectively. However, only one and three, respectively, were observed in oestrus. Thus, as with MAP (Zimbelman, 1963a), it appeared that certain doses may suppress oestrus but allow ovulation to occur.

Three trials with group feeding were designed to confirm the dosage titration results. The first trial, conducted in January 1962, was a 2 × 2 factorial experiment involving two frequencies of feeding, once-daily (1 ×) and twice-daily (2 ×) and two doses per feeding, 0.5 and 1.0 mg. Of the ten heifers per group, only one receiving 0.5 mg daily showed oestrus and ovulated during treatment. The second trial, conducted in July 1962, involved three groups of eight heifers each group receiving either 0.2, 0.4 or 0.6 mg daily, with all feeding done twice daily. One heifer out of eight receiving 0.2 mg/day ovulated during treatment, but was not observed in oestrus. In October 1962, an additional eight heifers received 0.2 mg by once-daily feeding and again, one heifer out of eight ovulated during treatment and oestrus was observed in this animal.

The average interval from last feeding to first oestrus varied according to dose and whether or not ovulation occurred during treatment. Heifers were considered synchronized if they came in oestrus within 5 days after the first oestrus after-treatment for that group. The ranges for intervals from last feeding

to oestrus in synchronized heifers were 1.5 to 4.5 days at 0.2 mg and 4.0 to 9.0 days at 2.0 mg. The average intervals in the synchronized heifers from various dose groups were 2.7 days at 0.2 mg and 6.3 days at 2.0 mg. Seven animals falling out of the range and not considered synchronized had intervals from 7.0 to 21.0 days. Ovulation was found to have been inhibited in four of these seven heifers (intervals of 12.5, 12.0, 7.0 and 7.5 days), but had occurred during treatment in three others (9.5, 13.5 and 21.0 days intervals). The average intervals and ranges were similar for the groups receiving 0.2 to 0.6 mg daily to those reported for MAP at 150 to 500 mg daily (summarized in Zimbelman, 1963a). The longer return intervals encountered at daily dose levels of 1.0 to 2.0 mg apparently resulted from overdosing and are similar to those reported for high doses of CAP (Van Blake, Brunner & Hansel, 1963).

TABLE 1

Daily oral dose (mg)	No. of heifers*	During treatment†			Percentage inhibition	
		EO	O	I	Oestrus	Ovulation
0.0625	5	3	2	0	40	0
0.125	5	1	3	1	80	20
0.25	4	0	0	4	100	100
0.5	8†	0	1	7	100	88
1.0	4	0	0	4	100	100
2.0	4	0	0	4	100	100
4.0	4	0	0	4	100	100
8.0	4	0	0	4	100	100

* Total 38.

† Four heifers were fed one-half of dose twice daily, all others were fed only once daily. All feeding periods were for 16 days beginning 15 days after oestrus.

‡ The number of animals was categorized as follows: EO = oestrus was observed, ovulation occurred as expected; O = ovulation detected by rectal palpation, oestrus was not observed; and I = ovulation was not detected, considered as inhibited.

The average pre-treatment oestrous cycle length for the group-fed heifers was 21.1 days. The average intervals from the latest pre-treatment oestrus to the first post-treatment oestrus and from first to second post-treatment oestrus were 31.6 and 22.5 days respectively. Synchronization treatments would be expected to extend the interval by about one-half cycle length. The figure of 22.5 days (27.4 minus 4.9) indicates that those animals which failed to conceive had cycles of normal average length (Table 2).

The conception rate at first insemination ranged from 25% to 88% at the various dose levels, and was 42% for all groups. After two inseminations, 82% of the MGA-treated heifers had conceived. A similar range at first service has been reported for MAP at a narrower range of doses (Zimbelman, 1963a). Wagner, McAskill & Means (1963) reported that of forty CAP-treated heifers, 42% had conceived at first service and 75% had conceived by the second heat period. Of the forty heifers inseminated the second time in the present study, twenty-eight (70%) conceived. This conception rate would also indicate a return to normal or above normal fertility by the second oestrus.

TABLE 2
REPRODUCTIVE PERFORMANCE DURING AND AFTER GROUP FEEDING OF MELENGESTROL ACETATE TO DAIRY HEIFERS

Trial	Daily dose (mg) and times fed	No. animals	No. ovulated during treatment	No. sync.	Oestrous cycle length*		Days from last feeding to†		Post-treatment conception rates (%)‡	
					Pre	Post	First cycle	Second cycle	First service	Two services
A	2.0-2 x	10	0	10	20.3	31.8	6.3	27.0	40	90
	1.0-2 x	10	0	10	20.7	29.3	4.9	26.2	44	89
	1.0-1 x	10	0	9	20.9	32.9	6.1	27.0	40	90
	0.5-1 x	10	1	8	21.4	35.6	5.1	31.6	50	100
B	0.6-2 x	8	0	8	20.9	28.5	3.8	31.0	25	50
	0.4-2 x	8	0	8	22.0	31.0	2.6	22.8	25	75
	0.2-2 x	8	1	6	21.6	29.9	4.6	24.0	88	88
	0.2-1 x	8	1	6	21.3	32.9	5.5	28.0	25	63
Mean Total No. animals		72	3	65	21.1 72	31.6 72	4.9 72	27.4 40	42 30	82 58

* Oestrous cycle length: Pre = pre-treatment indicates the number of days of the pre-treatment oestrous cycle; Post = post-treatment indicates the number of days from the latest pre-treatment oestrus to first post-treatment oestrus or ovulation.

† Includes all heifers whether considered synchronized or not (see also Table 3).

‡ One heifer died from accidental cause prior to determining whether pregnant or not, one heifer bred only at first oestrus.

The data on intravenous injections (i.v.) were obtained in an attempt to explain the extremely high potency of MGA relative to MAP in heifers. The hypothesis was formed that the rumen microorganisms might be converting it into a more active compound. This seemed possible since MGA was found to be only about

TABLE 3
DEGREE OF SYNCHRONIZATION OBTAINED BY GROUP FEEDING MELENGESTROL ACETATE TO DAIRY HEIFERS

Trial	Daily dose (mg) and times fed	No. of heifers		Intervals from last feed to first oestrus		
		Treated	Sync.*	Synchronized* Average	Range	Not synchronized
A	2.0—2 ×	10	10	6.3	4.0—9.0	None
	1.0—2 ×	10	10	4.9	3.5—6.0	None
	1.0—1 ×	10	9	5.4	3.5—8.0	12.5
	0.5—1 ×	10	8	3.7	3.0—5.0	9.5†, 12.0
B	0.6—2 ×	8	8	3.8	2.5—7.5	None
	0.4—2 ×	8	8	2.6	1.5—3.5	None
	0.2—2 ×	8	6	2.7	1.5—3.5	7.5, 13.5†
C	0.2—1 ×	8	6	2.7	1.5—4.5	7.0, 21.0†
Total		72	65			

* See text for definition of synchronized.

† These intervals from heifers which ovulated during treatment.

TABLE 4
INHIBITION OF OVULATION BY ONCE DAILY INTRAVENOUS INJECTION OF PROGESTAGENS

Compound	Daily dose (mg)	Vehicle*	No. of heifers	During treatment†			Percentage inhibition	
				EO	O	I	Oestrus	Ovulation
MGA‡	0.1	PG	4	2	1	1	50	25
	0.2	PG	4	0	2	2	100	50
	0.4	PG	4	0	0	4	100	100
	0.4	EtOH	4	0	0	4	100	100
MAP‡	0.5	PG	4	4	0	0	0	0
	2.5	PG	4	0	3	1	100	25
	5.0	PG	4	0	0	4	100	100
	5.0	SAS	4	0	0	4	100	100

* Vehicles for administration were: PG = 75% propylene glycol, 25% dimethyl-acetamide with 0.1 mg MGA/ml or 1 mg MAP/ml; EtOH = 95% ethanol with 0.4 mg MGA/ml, diluted in the syringe with 9 ml of injectable saline; and SAS = sterile aqueous suspension of 1 mg MAP/ml.

† The number of animals was categorized as follows: EO = oestrus was observed, ovulation occurred as expected; O = ovulation detected by rectal palpation, oestrus was not observed; and I = ovulation was not detected, considered as inhibited.

‡ MGA = Melengestrol acetate; MAP = medroxyprogesterone acetate.

two to four times as potent as MAP in non-ruminants (Duncan *et al.*, 1964). It is evident from Tables 1 and 4 that the potency of MGA is essentially equal by intravenous injection or by oral administration. At 0.1 mg i.v. or 0.125 mg orally, ovulation was blocked in only one of four or five heifers. At 0.4 mg i.v.,

ovulation was blocked in all eight heifers with two different solutions for injection. That MGA was of equal or greater potency when administered orally than intravenously may be considered unusual. Intravenous injections of 5 mg MAP, either as a solution or as a suspension, blocked ovulation in eight heifers, thus indicating a greater potency for MAP when administered intravenously than when given orally (5 mg versus 180 mg).

Comparison of the mean gestation lengths and birth weights of twenty-four single calves conceived at the first (synchronized) service with those of eighteen single calves conceived at a later service indicated that all fell within the normal range. There was no apparent effect of synchronization on those traits. All term calves appeared normal upon gross examination.

DISCUSSION

Studies on laboratory animals (Duncan *et al.*, 1964) and pigs (unpublished) show that MGA administered orally has only two to four times the potency of MAP. In cattle, given orally it has 300 to 900 times the potency to inhibit ovulation and in sheep at least 150 times (Zimbelman, 1963b). It has been reported that MGA is also effective in the maintenance of pregnancy in bilaterally ovariectomized dairy heifers (Zimbelman & Smith, 1963). A daily oral dose of 4 mg was completely effective, while 1 mg was partially effective. The minimal effective dose of MGA for complete pregnancy maintenance was eight to twenty times that for inhibition of ovulation. Thus, the lack of complete pregnancy maintenance with daily oral doses of 240 mg of MAP can be explained and MGA again appears to be much more potent by this criterion of progestational activity in the dairy heifer.

The data on intravenous injections gives some insight into explaining the extreme potency of MGA in cattle. That MGA is equally or more potent when given orally may indicate that it is relatively well absorbed and not readily degraded. On the other hand, MAP is about thirty times more potent when given intravenously than when given orally. Whether the lowered oral effectiveness of MAP is due to poor absorption and/or rapid metabolism cannot be answered at this time. The relative potency of the two compounds at the active site is still unknown.

The chemical modifications which produce increased potency of progestagens have been discussed (Van Blake *et al.*, 1963; Zaffaroni, 1960), and the 6-dehydro (U-18,950) and 16-methylene (U-10,343) modifications following oral dosage of MAP have been studied in cattle in the manner described here for MGA (unpublished). Both modifications increased the oral potency several-fold, with the greatest increase due to the 16-methylene addition; MGA is structurally the 16-methylene addition on 6-dehydro-MAP. The antioviulatory properties of 6-dehydro-MAP in rabbits, cats and dogs were described by David, Edwards, Fellowes & Plummer (1963). The increased oral potency of CAP in heifers was ascribed to replacement of 6-methyl with 6-chloro (Van Blake *et al.*, 1963) but the 6-dehydro modification also is present in CAP. In addition, the 16-methylene addition on 6-dehydro-MAP (MGA) caused a greater increase in oral potency for cattle than did the 16-methyl addition (U-20,878) (unpublished). Formulae of the various compounds are given in Text-fig. 1.

The image displays three chemical structures of progestins, each with its name below it:

- Progesterone:** A steroid molecule with a ketone group at C3 and a methyl ketone group at C20.
- Medroxyprogesterone acetate (MAP):** A steroid molecule with a ketone group at C3, a methyl group at C13, and an acetate ester group at C20.
- Melengestrol acetate (MGA):** A steroid molecule with a ketone group at C3, a double bond between C4 and C5, a methyl group at C13, and an acetate ester group at C20.

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REFERENCES

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