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CONTROL OF OVULATION IN CATTLE WITH MELENGESTROL ACETATE

II. EFFECTS ON FOLLICULAR SIZE AND ACTIVITY

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Summary. The effects of oral melengestrol acetate (MGA) on ovarian activity have been studied in 264 dairy and beef heifers. A total of ninety-eight heifers received 0·4 mg of MGA daily for short periods of time (18 to 32 days) and ovarian activity was determined by rectal palpation. As the incidence of a detectable corpus luteum decreased from 76% to 10%, the incidence of a detectable follicle increased from 56% to 91%. Follicular size also increased with time. Samples of cervical mucus were rated according to fern pattern, which was interpreted as due to an oestrogenic influence, and which occurred upon regression of the corpus luteum even though oestrus and ovulation were inhibited by MGA treatment.

A total of 166 beef heifers was used in long-term studies (105 to 116 days) of MGA treatment. Significant ($P<0·05$) increases were found in the weight of follicular fluid, primarily of the largest follicle from the pair of ovaries. Also indicative of increased oestrogenicity was an increased adrenal weight in puberal, intact MGA-treated heifers. The adrenal weights of spayed heifers tended to decrease with MGA treatment. The optimal dose for increased follicular activity appears to be near 0·4 mg MGA daily/heifer.

INTRODUCTION

A recent review of studies concerned with the synchronization of ovarian cycles in cattle and sheep indicated that there have been no reports on studies in ewes and cows on the mode of action of oral progestagens (Lamond, 1964). The present paper represents one of a planned series of publications describing studies of this nature with melengestrol acetate (MGA) in cattle. A preceding paper discussed the effects of dosage and route of administration (Zimbelman & Smith, 1966). The present paper describes follicular status and evidences of increased oestrogenic secretion during treatment.

METHODS

In the first phase of this study, four groups (ninety-eight head) of heifers, having normal ovarian and oestrous cycles, were used (Trial A—Table 1). A grain ration containing 0·4 mg MGA/heifer was fed daily for periods of time

ranging from 18 to 32 days. Heifers received their first treatment as a group on the same day, thus all stages of the oestrous cycle were represented. Ovarian status was determined at frequent intervals by rectal palpation of all heifers. Cervical mucus samples were taken from two groups with a plastic insemination pipette during predesignated palpations. The samples were collected from heifers beginning at 8 or more days after the start of MGA treatment and at intervals until ovulation had occurred. The mucus samples were rated according to a previously described procedure (Alliston, Patterson & Ulberg, 1958) based on the degree of the ferning pattern present in dried smears. Data on these four groups of heifers have been combined for brevity.

TABLE 1

DESIGN OF STUDIES TO DETERMINE EFFECT OF MGA TREATMENT ON FOLLICULAR ACTIVITY

Trial	*No. of heifers*	*Breed of heifers**	*Status of heifers†*	*Dose of* MGA		*Days fed*
				μg/lb of concentrate	*Daily‡ (mg)*	
A	16	D	P	–	0·4	18
	25§ (98)	D	P	–	0·4	24–32
	27§ (98)	D	P	–	0·4	23
	30 (98)	H	P	–	0·4	24–32
B	20 (40)	H	P	0	0	–
	20 (40)	H	P	20	0·50	105–116
C	18	A, H	P, O	0	0	–
	18	A, H	P, O	20	0·42	111
	18	A, H	P	0	0	–
	18 (126)	A, H	P	10	0·22	111
	18 (126)	A, H	P	20	0·44	111
	18 (126)	A, H	P	40	0·85	111
	18 (126)	A, H	I	20	0·42	111
Grand total	264					

* D = Dairy, primarily Holstein; A = Angus; H = Hereford.
† P = Puberal, not ovariectomized; P, O = Puberal, bilaterally ovariectomized; I = Immature or prepuberal, not ovariectomized.
‡ Average daily dose/heifer for entire period.
§ Cervical mucus smears were taken from these heifers.

Two trials were presented in the second phase of the study. In Trial B, there were two groups of twenty heifers each (Table 1). A control group received a mixed concentrate ration *ad libitum* in addition to a small amount of hay daily, while the treated group received the same concentrate ration containing 20 μg of MGA/pound. The average daily intake of MGA for this group of heifers was 0·50 mg/animal daily. Only heifers which were known to be puberal were used in this study. All heifers were weighed and the reproductive organs palpated *per rectum* at 2-week intervals from the start of treatment for 14 weeks. Five heifers from each group were slaughtered at 106, 110, 113 and 117 days from the start of treatment. Treatment continued until 24 hr before slaughter. At slaughter, the adrenal glands, ovaries and uterus were removed for study.

In Trial C of the second phase, there were seven groups of eighteen animals each with nine heifers of two breeds. Of these, six groups were considered

puberal, based on the palpation of a detectable corpus luteum before the start of treatment. The remaining group of heifers did not have a detectable corpus luteum at any of three consecutive weekly palpations immediately before treatment, thus they were considered as prepuberal. Two of the groups considered as puberal were spayed by supravaginal laparotomy at 11 to 13 days before the start of treatment.

One intact and one spayed group of puberal heifers were fed a mixed concentrate ration *ad libitum* plus a small amount of hay. The remaining groups received the same concentrate ration medicated with 10, 20 or 40μg of MGA/lb. The average daily intake was about 0·22, 0·43 and 0·85 mg of MGA/head daily for the three levels, respectively. Treatment continued for 111 days and six of eighteen heifers from each group were slaughtered at 112 days from the start of treatment. The twelve remaining heifers were switched to the unmedicated concentrate ration until slaughtered at 114 or 119 days. Thus one-third of each treated group was slaughtered at 1, 3 or 8 days after last feeding (ALF) of MGA. Rectal palpation and collection of tissues were similar to Trial B.

Statistical analyses were performed using χ^2 or analysis of variance where applicable. The analysis of variance of Trial C considered these main effects: breed, treatment and slaughter date. When mean squares due to interactions were not significantly ($P>0·10$) different from the error mean square, the sums of squares and degrees of freedom were pooled. The magnitude of difference required for significance between any two main effect classifications was determined by the method of Snedecor (1956).

Follicular fluid weights were determined by first weighing the intact, trimmed ovaries of each animal. In Trials B and C, the largest follicle was punctured and the ovary was re-weighed after removal of the follicular fluid. The ovary was then sliced with a special device holding razor blades at 5 mm intervals and the difference between the weight after slicing and blotting of fluid and the weight before puncturing the largest follicle was considered as total follicular fluid. In Trial C, the corpus luteum was also removed to allow comparisons of the non-luteal portion of the ovary.

RESULTS

A corpus luteum was detected by rectal palpation in about 75% of the treated heifers in Trial A at the beginning of treatment or at about the 1st week (Table 2). After 2 weeks of treatment, the incidence of a detectable corpus luteum decreased to 47% with a further decrease to 10% by 3 weeks. Of eight detectable corpora lutea at 22 to 24 days, five formed from an ovulation during treatment. These results confirm previous data indicating that 0·4 mg MGA daily effectively inhibited ovulation in most heifers. The percentage of animals with a follicle detected by rectal palpation increased from 55% at about the 1st week of treatment to 74% by the 2nd week and to 91% by 22 to 24 days of treatment. The average size of the largest palpable follicle increased gradually from 13·7 mm at the start of treatment to 16·3 mm at about 3 weeks.

Follicular size cannot always be assumed to be a reflection of a follicular activity, such as oestrogen production. Upon regression of the corpora lutea,

gross appearance of the heifers revealed signs usually associated with the period of oestrus, namely swelling of external genitalia and copious discharges of mucus from the vagina. Such heifers demonstrated interest in mounting other heifers, but would not stand to be mounted by other animals. Smears were made from

TABLE 2

OVARIAN STATUS OF HEIFERS RECEIVING 0·4 mg MGA DAILY

	Days of treatment when palpated			
	0 *to* 1	8 *to* 9	13 *to* 15	22 *to* 24
Number of heifers				
Palpated*	98	98	98	82*
With follicles	55	54	73	75
Corpus luteum				
Incidence (%)†	76	75	47§	10¶
Follicles				
Incidence (%)†	56	55	74	91
Size‡	13·7	14·3	15·0	16·3

* Only three of four groups fed for 22 days or greater—Trial A.
† Percentage of heifers palpated with a palpable corpus luteum or follicle.
‡ Estimated average size (mm) of largest follicle/heifer.
§ Of forty-six corpora lutea, two were from ovulation during treatment.
¶ Of eight corpora lutea, five were from ovulation during treatment.

TABLE 3

CERVICAL MUCUS SMEAR RATINGS OF HEIFERS AS INFLUENCED BY THE CORPUS LUTEUM AND ORAL MGA TREATMENT

Mucus smear ratings	*During* MGA *treatment*		*Following* MGA *treatment*		
	Corpus luteum present (%)	*Corpus* luteum regressed* (%)	*All smears* (%)	*Prior to*† *oestrus* (%)	*Prior to*‡ *ovulation* (%)
1 or 2	83	28	13	11	15
3 or 4	17	47	52	50	54
5 or 6	0	25	36	39	31
No. of smears	41	128	64	38	26
χ^2	39·78 ($P<0·005$)		0·60		
			6·52 ($P<0·05$)		

* Corpus luteum defined as regressed when size was 4 mm smaller than previous or no longer palpable.
† Cervical mucus was collected near the time of approaching oestrus, but prior to observed oestrus.
‡ Cervical mucus samples taken after oestrus was observed but prior to ovulation.

169 samples during MGA treatment and were classified as to whether the corpus luteum was present or had regressed. Regression for this purpose was defined as no longer palpable or more than 4 mm smaller than the estimated size at the previous palpation. Ratings of 1 or 2 indicate little if any fern pattern and represented 83% of the smears taken in the presence of the corpus luteum, but

only 28% of those with the corpus luteum regressed. Ratings of 3 or 4 indicate typical fern patterns over less than the entire area, and the incidence of this finding increased from 17% with the corpus luteum present to 47% after regression of the corpus luteum. Fern patterns over the entire area of the smear are rated as 5 or 6 and were found to make up 25% of the smears after corpus luteum regression. A χ^2 analysis of the data revealed that the distribution of smear ratings differed significantly. These data were interpreted to indicate an increased oestrogenic activity of the follicles which appeared during MGA treatment upon regression of the corpus luteum.

TABLE 4

OVARIAN STATUS OF HEIFERS DURING LONG-TERM ORAL MGA TREATMENT

Trial	*No. of heifers*	*Dose of* MGA	*Pre-treatment*	*Weeks of treatment* 2	4	6	8	10	12	14
			Incidence of detectable corpus luteum (%)							
B	20	0	68	80	85	85	65	60	85	80
	20	0·50	73	10	0	0	0	0	0	0
C	18	0	64	72	67	57	89	67	61	72
	18	0·22	66	57	22	22	28	28	28	33
	18	0·44	66	33	6	0	0	6	0	6
	18	0·85	67	11	0	0	0	0	0	0
	18*	0·42	0*	0	0	0	0	0	0	6
			Incidence of detectable follicle (%)							
B	20	0	18	55	45	20	30	50	25	25
	20	0·50	20	90	100	100	100	100	100	100
C	18	0	35	17	44	44	28	28	33	33
	18	0·22	43	67	67	72	67	61	78	57
	18	0·44	38	67	94	100	100	94	100	89
	18	0·85	35	61	94	94	100	100	100	94
	18*	0·42	31	72	78	100	94	94	100	100
			Size of largest detectable follicle (mm)†							
B	20	0	13	14	15	12	13	14	15	14
	20	0·50	14	15	18	17	17	16	18	18
C	18	0	15	12	14	13	12	14	14	14
	18	0·22	15	14	15	17	16	17	17	16
	18	0·44	14	13	16	17	18	17	17	16
	18	0·85	14	13	15	14	14	16	16	15
	18*	0·42	12	12	14	14	16	16	16	15

* Were not puberal at start of treatment.
† Mean of estimated follicle sizes to nearest whole number.

At the end of MGA treatment, additional mucus samples were obtained beginning near the time of expected oestrus. There was no significant difference between smears taken before oestrus and those taken after oestrus and ovulation as determined by rectal palpation. All sixty-four samples taken following MGA treatment were compared with those (128) taken during MGA treatment with the corpus luteum regressed. There was a further shift from 28% with little or no pattern to only 13% in this category (1 or 2), and an increase from 25% to 36% of the smears with typical fern patterns over the entire area (5 or 6). This additional increase in fern patterns is in agreement with the interpretation of a relationship of fern patterns to oestrogenic influence.

The heifers on long-term oral MGA treatment also confirmed that doses above 0·4 mg daily were effective in inhibiting ovulation. At 0·22 mg daily, the percentage of animals with a corpus luteum ranged from 22% to 33% during 4 to 14 weeks of treatment, compared with 57% to 89% for control animals. This indicates a partial inhibition of ovulation. The percentage of animals with a detectable follicle ranged from 17% to 55% at various times in control groups, from 57% to 78% with partial inhibition of ovulation (0·22 mg daily), and from 78% to 100% for groups (0·42 to 0·85 mg daily) with almost complete inhibition of ovulation. Follicular size of puberal heifers was greater at each 2-week period during which ovulation was inhibited with MGA. Follicular size for the immature heifers (0·42 mg) was less than the follicular size of comparable puberal heifers (0·44 mg) but the difference narrowed with time.

TABLE 5

OVARIAN WEIGHTS OF HEIFERS FOLLOWING LONG-TERM ORAL MGA TREATMENT

Trial†	MGA *dose*	*Ovarian weight (g)*			*Follicular fluid (g)*	
		Total intact	*Minus corpus luteum*	*After slicing*	*Largest follicle*	*Total*
B	0	13·30	–	11·34	1·08	1·96
	0·50	13·27	–	8·15*	3·93*	5·12*
	D§	2·63	–	2·17	0·97	1·30
C	0	13·82[a, b]	10·54[a]	8·68	0·95[a]	1·86[a]
	0·22	13·32[a, b]	11·97[a, b]	8·73	1·99[a, b]	3·24[a, b]
	0·44	15·96[b]	13·84[b]	8·72	3·36[b]	5·11[a]
	0·85	12·44[a]	12·04[a, b]	7·96	3·01[b]	4·08[a]
	0·42‡	12·09[a]	11·10[a, b]	6·74	2·87[b]	4·36[a]
	D§	3·34	3·31	2·07	1·63	1·93

* Significantly ($P<0·05$) different from untreated controls.
† See Table 4 for No. of animals/group.
‡ Were not puberal at start of treatment.
§ D = Magnitude of difference required for statistical significance ($P<0·05$) between two means.
(a and b) All means bearing the same superscript are not statistically different ($P<0·05$).

The mean follicular size at the lower ovulation-inhibiting doses (0·22 or 0·42 mg) was almost always greater than at the highest (0·85 mg) dose. The minimal effective dose for ovulation inhibition in most animals in a group (0·42 mg) produced the condition of greatest follicular development on a group basis with a maximal incidence and size of follicles. A lower dose was associated with a decreased incidence of follicles of a larger size, and a higher dose was associated with high incidence but smaller follicle size.

Measurements at slaughter confirmed that ovaries of all treated groups had an increase in follicular fluid weight. Only the group receiving 0·22 mg MGA daily failed to have a significant ($P<0·05$) increase. The maximum ovarian weight after removal of the corpus luteum was in the puberal group receiving 0·44 mg MGA daily. Differences between ovarian weights disappeared with removal of the corpus luteum and follicular fluid in Trial C. The differences in the weight of ovaries after slicing in Trial B would reflect the presence of a

corpus luteum in control animals. There was also an effect of slaughter date (or days ALF) on follicular fluid from the largest follicle and an interesting effect on uterine weight in Trial C. The increase in follicular fluid from Days 1 to 3 after the last feed (ALF), followed by a decline to Day 8, represented follicular enlargement followed by ovulation. While there was no significant overall effect of treatment on uterine weight, there was a significant ($P<0.05$) interaction of

TABLE 6

POST-TREATMENT REPRODUCTIVE PERFORMANCE OF HEIFERS FOLLOWING LONG-TERM TREATMENT WITH ORAL MGA

MGA dose	Days* ALF to slaughter	No. of heifers	From 2 to 7 days ALF		With regressed C.L. (%)	Weight of uterus‡	Follicular fluid weight (g)	
			In oestrus (%)	Ovulated (%)			Largest follicle§	Total¶
0·0	1	6	–	–	100	150	0·86	1·33
	3	6	0	0	100	143	0·97	2·19
	8	6	50	50	100	139	1·04	2·06
Total		18			100	144	0·95	1·86
0·22	1	6	–	–	83	150	2·10	3·21
	3	6	50	33	50	152	2·36	3·98
	8	6	33	33	100	143	1·51	2·54
Total		18			78	148	1·99	3·24
0·44	1	6	–	–	33	139	2·46	4·01
	3	6	0	0	17	150	4·64	6·38
	8	6	83	67	0	121	2·99	4·95
Total		18			17	137	3·36	5·11
0·85	1	6	–	–	0	176	3·48	4·59
	3	6	0	0	0	185	3·54	4·34
	8	6	50	50	0	120	2·02	3·31
Total		18			0	160	3·01	4·08
0·42†	1	6	–	–	17	146	2·69	4·20
	3	6	0	0	0	172	4·00	5·17
	8	6	50	67	33	119	1·93	3·70
Total		18			17	146	2·87	4·36

* ALF = after last feeding.
† These heifers were prepuberal at the beginning of the feeding period.
‡ Statistical analysis—slaughter date ($P<0.01$); treatment × slaughter ($P<0.05$).
§ Statistical analysis—treatment ($P<0.001$); slaughter date ($P<0.05$).
¶ Statistical analysis—treatment ($P<0.001$).

treatment with slaughter date. Gross observations made at slaughter are helpful in interpretation. The uteri of heifers treated with 0·42 to 0·85 mg tended to have accumulations of very tenacious mucus, both in the uterine horns and in the cervix, particularly at Days 1 and 3. This was not true at Day 8, indicating that the return to oestrus was associated with a loss of this mucus. Thus, the uterine weights in certain treated groups were, respectively, similar, higher and lower than those of control heifers at Days 1, 3 and 8 ALF. The uterine weights of the two groups of spayed heifers were not significantly different from each other. The average uterine weight for control spayed

heifers was 45 g compared with 61 g for spayed heifers receiving 0·42 mg MGA daily and with 144 g for intact control heifers. The weight of the uterus and cervix of treated heifers in Trial B (245 g) was not different from that of control heifers (255 g).

Adrenal weights of heifers following long-term treatment were also obtained. In Trial B, the left adrenal from treated heifers was significantly ($P<0·05$) larger than from control heifers. In Trial C, the tendency was for adrenal weights to be similar for spayed control heifers, intact control heifers and for the heifers which were not puberal when treatment began. However, all groups of heifers which were puberal and receiving MGA had greater adrenal weights. The weight

TABLE 7

ADRENAL WEIGHTS OF HEIFERS FOLLOWING LONG-TERM ORAL MGA TREATMENT

Trial	MGA *dose*	*Glands wt (g)*			
		Left	*Right*	*Total (g)*	*Per 100 lb carcass wt*
B	0†	9·20	8·62	–	–
	0·50†	10·58*	9·54	–	–
	D¶	0·92	1·00	–	–
C	0‡	8·82[a, b]	7·91[a]	16·73[a, b]	3·11
	0·42‡	8·27[a]	7·72[a]	15·99[a]	2·96
	0†	8·89[a, b]	8·37[a, b, c]	17·26[a, b, c]	3·05
	0·22†	10·16[b]	9·70[c]	19·86[c]	3·39
	0·44†	10·02[b]	9·39[b, c]	19·41[b, c]	3·30
	0·85†	10·16[b]	9·14[a, b, c]	19·30[b, c]	3·34
	0·42§	9·04[a, b]	8·13[a, b]	17·17[a, b, c]	3·15
	D¶	1·48	1·46	2·85	0·47

* Significantly ($P<0·05$) different from untreated controls.
† Puberal heifers with ovaries intact.
‡ Puberal heifers which were spayed prior to treatment.
§ Heifers were not puberal when treatment started.
¶ D = Magnitude of difference required for statistical significance ($P<0·05$).
(a to c) All means bearing the same superscript are not statistically different ($P<0·05$).

was significantly ($P<0·05$) greater only in the left adrenal gland when compared to intact puberal controls. It was found that an expression of adrenal weight in relation to carcass weight tended to reduce these differences. Comparison of the puberal spayed and puberal intact heifers receiving 0·42 to 0·44 mg MGA daily indicates that the increased adrenal weight was associated with the presence of ovaries. This level of MGA tended to decrease adrenal weight in spayed heifers and tended to increase adrenal weight in intact heifers. There was no effect of slaughter date on adrenal weight.

DISCUSSION

The inhibition of ovulation by MGA in heifers was associated with an increase in both the incidence and size of palpable follicles. This finding does not necessarily mean that MGA stimulates gonadotrophin (FSH) release but, rather, may simply reflect an opportunity for the same level of FSH to act on a given

follicle for a longer time than would ordinarily occur. MGA may prevent a release of LH in quantities needed for ovulation but may allow low levels of LH to be released for synergism with FSH. The increase in follicular fluid in the largest follicle accounted almost wholly for the increase in total follicular fluid from both ovaries. These enlarged follicles persisted on the ovaries for a time and some eventually might regress to be replaced with another follicle, while some ovulated at the first oestrous period following the end of MGA treatment.

That these enlarged follicles are functionally active may be deduced from several indications of oestrogen production, namely, swelling of external genitalia, frequent discharge of mucus through the vulva, an increase in ferning pattern of the cervical mucus and an increased adrenal weight. An increase in adrenal weights of heifers has been reported in association with diethylstilboestrol treatment (Clegg & Cole, 1954). These indications of increased follicular size and oestrogenic status during a period of MGA treatment suggests that MGA treatment did not simulate the corpus luteum in the means by which ovulation was inhibited. The data presented here do not eliminate the possibility that the level of progestagen may account for this difference. However, a level of MGA (0·85 mg daily) twice that which gave nearly complete inhibition of ovulation also allowed increased follicular development. Such findings appear to suggest that the influence of MGA was more on release of ovulating hormone (LH) from the pituitary gland than on that of follicle-stimulating hormone.

The increase in adrenal weight due to treatment with MGA contrasts with the decrease reported in rats (Duncan, Lyster, Hendrix, Clark & Webster, 1964) but the results in spayed heifers do tend to suggest that effects on adrenal size might occur in cattle under other circumstances.

The demonstration of the effects of MGA on follicle size, oestrogenic status and uterine weights might be related to the somewhat lower conception rate at the synchronized oestrus following administration of various progestagens (Lamond, 1964). These data would seem to indicate the potential complexity of determining the immediate cause of reduced fertility.

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