CONTROL OF OVULATION IN CATTLE WITH MELENGESTROL ACETATE

V. EFFECT OF OVULATION INDUCTION ON CORPUS LUTEUM DEVELOPMENT DURING MGA TREATMENT

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Summary. Sixty-four normally cycling Holstein heifers were randomly assigned into four equal groups (three treatment and one control). Each of the three treatment groups received 0.5 mg of melengestrol acetate (MGA*) daily and a single injection of either oestradiol cypionate (ECP*), human chorionic gonadotrophin (HCG) or pituitary luteinizing hormone (PLH) between Days 22 and 26 following oestrus or Days 8 to 12 of MGA feeding. The control animals received neither MGA nor an injection. Heifers from each treatment group were spayed (ovariectomized) on Days 2, 5, 8 and 11 from the injection and control heifers were spayed on Days 1, 4, 7 and 10 from oestrus. The corpora lutea (CL) and the largest follicles on the ovaries of each heifer were observed for size and colour.

Oestrus was observed in only the ECP-treated group within 24 hr following the injections (nine of sixteen animals). Ovulation occurred following the injection in all of the sixteen animals in the HCG group and in fifteen of the sixteen animals in each of the ECP and PLH groups. All the control heifers ovulated following oestrus.

Normal CL were present in all the treated heifers spayed by Day 5. On subsequent days, increasing numbers of heifers had CL which were regressing.

The percentages of heifers spayed on Day 8 and Day 11 after injection with regressing CL were: 0% and 50% for the ECP group; 25% and 100% for the HCG group; and 75% and 50% for the PLH group. All the heifers in the control group had normally developing CL regardless of the day of spaying.

The follicles from those animals which had regressing CL were generally larger than those from control heifers.

INTRODUCTION

Previous studies in this series have shown that the use of ECP, HCG and PLH to induce ovulation in MGA-treated heifers may have been associated with

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improper development of the induced CL. Loy, Zimbelman & Casida (1960) and Wiltbank, Ingalls & Rowden (1961) have reported that administration of oestrogen to cycling cows caused early regression of the CL. In addition, the former investigators found that injections of progesterone beginning early in the cycle had a detrimental effect on the formation and function of the CL.

This experiment was designed to examine the interval from ovulation until abnormal development of the CL was evident.

MATERIALS AND METHODS

The design of the study is shown in Table 1. Sixty-four normally cycling Holstein dairy heifers were selected. They were placed at random into four groups (sixteen animals/group). Treated animals received 0.5 mg MGA daily and a single injection of one of three ovulation-inducing agents; controls received neither MGA nor an injection.

TABLE 1

DESIGN OF STUDY—EFFECT OF OVULATION INDUCTION ON CL DEVELOPMENT DURING MGA TREATMENT

Control			Treated					
MGA dosage	Inducing agent None No. heifers	Spayed* days from oestrus	MGA dosage	Inducing agents† ECP HCG PLH No. heifers			Spayed* days from injection	Total heifers
None	4	1	0.5 mg/day	4	4	4	2‡	16
	4	4		4	4	4	5‡	16
	4	7		4	4	4	8§	16
	4	10		4	4	4	11§	16
Total	16			16	16	16		64

* Bilateral ovariectomy—supravaginal incision.

† Single i.v. injection between Days 22 and 26 from previous oestrus (8 to 12 of MGA feeding).

‡ MGA began on Day 15 of the oestrous cycle and continued to spaying.

§ MGA began on Day 15 of the oestrous cycle and continued for 7 days from injection.

MGA treatment began on Day 15 of the oestrous cycle and was continued up to 7 days past the time of injection. The ovulation-inducing agents and the dosages used were: oestradiol cypionate (ECP: Upjohn Oestradiol Cypionate, cottonseed oil vehicle), 10 mg; human chorionic gonadotrophin (HCG; Upjohn Chorionic Gonadotrophin, 5000 i.u./vial), 5000 i.u.; pituitary luteinizing hormone (PLH; Armour PLH, 25 mg/vial), 50 mg. These compounds were administered as single intravenous injections between Days 22 and 26 of the cycle (Days 8 to 12 of MGA feeding); at this time the CL of the current cycle had regressed (<12 mm) and a palpable follicle of 12 mm or larger was present.

All heifers were bilaterally spayed by supra-vaginal incision on either Day 1, 4, 7 or 10 from oestrus for the control animals or on Day 2, 5, 8 or 11 from the injection for the treated animals (day of oestrus or injection = Day 0). This was done in order that the spaying of both the control and treated heifers would be at approximately equivalent days from ovulation.

The development of the CL was determined by gross inspection of the ovaries immediately following spaying. The CL and the largest follicle of each animal were measured in millimetres. In addition, colour comparisons between CL of control and treated animals were made to determine whether or not CL were developing normally.

All animals were observed for oestrus twice daily for the entire period. They were palpated rectally at regular intervals before and after the time of injection. All heifers which received the ovulation-inducing agents were palpated every 12 to 24 hr for 2 to 3 days following the injections, until the follicles had disappeared or until they were spayed. Generally, the animals spayed on Day 2 were spayed too early to determine ovulation by palpation so the ovaries of these heifers were examined closely for ovulation rupture points following spaying. Ovulation was determined similarly for the control heifers spayed on Day 1 from oestrus. The presence or absence of CL on the ovaries of any of the other heifers was a check on the palpation results.

TABLE 2
EFFECT OF OVULATION-INDUCING AGENTS ON CORPUS
LUTEUM DEVELOPMENT DURING MGA TREATMENT

MGA dose	No. of heifers	Ovulation inducing agent	Day spayed	Normal CL development	
				% by stage	No. and % of total group
None	4	None	1	NA*	12:12
	4		4	100	100%
	4		7	100	
	4		10	100	
0.5 mg	4	ECP 10 mg i.v.	2	NA	10:12
	4		5	100	83%
	4		8	100	
	4		11	50	
0.5 mg	4	HCG 5000 i.u. i.v.	2	NA	7:12
	4		5	100	58%
	4		8	75	
	4		11	0	
0.5 mg	4	PLH 50 mg i.v.	2	NA	6:12
	4		5	100	50%
	4		8	25	
	4		11	50	

* NA = Not applicable since development of CL could not be determined at this stage.

RESULTS

The effects of the ovulation-inducing agents on ovulation and subsequent CL formation are shown in Table 2. All animals in the HCG group ovulated by Day 2 to 3 following injection, as did all but one each in the ECP and PLH groups. All of the heifers ovulated following oestrus in the control group. Normal CL were present at spaying subsequent to ovulation in the injected groups as follows: ten of twelve ECP (83%); seven of twelve HCG (58%); and six of twelve PLH (50%). All the heifers spayed on Day 5 from injection that ovulated

TABLE 3
SIZE OF CORPUS LUTEUM AND LARGEST FOLLICLE SUBSEQUENT TO OVULATION

Group	No. heifers	% of group		Size (mm)									
		Normal CL		Individual CL					Largest follicle*				
		Day 2	Day 5	Day 8	Day 11	Day 2	Day 5	Day 8	Day 11	Day 2	Day 5	Day 8	Day 11
Control	16	NA†	100	100	100	NA†	10	18	18	8	10	14	16
						NA	12	20	22	8	10	16	15
						NA	14	20	22	8	10	16	18
ECP	16	NA	100	100	50	NA	18	22	26	12	10	15	16
						NA	10	18	10†	<6	10	14	15†
						NA	10	18	20†	6	6	16	20†
HCG	16	NA	100	75	0	NA	14	20	20	6	8	10	10
						NA	18	22	22	12	10	12	10
						§							
PLH	16	NA	100	25	50	NA	12	16	10†	8	10	15	16†
						NA	14	18†	12†	8	12	20†	20†
						NA	16	18	14†	8	8	18	24†
						NA	16	20	16	8	10	18	<6
PLH	16	NA	100	25	50	NA	14	14†	10†	6	15	16†	20†
						NA	16	14†	10†	8	12	18†	22†
						NA	20	14†	20	8	10	20†	15
						NA	§	20	22	10	18	14	18

* Corresponds to the same animals with CL on same day of spaying.

† NA = Not applicable since CL were merely ruptured points.

‡ CL appear to be regressing.

§ Failed to ovulate.

|| New CL from very recent ovulation. CL from induced ovulation almost completely regressed (< 10 mm).

had a normally forming CL. By Day 8, 100%, 75% and 25% and by Day 11 only 50%, 0% and 50% of the heifers in the ECP, HCG and PLH groups, respectively, had normal CL.

One heifer in the HCG group spayed on Day 11 had a very recent ovulation (16 mm) as indicated by the general appearance of the CL. A very small regressing CL (<10 mm) was noted where the induced CL had been palpated previously; thus it was concluded that a CL had been induced by HCG but did not form normally. The colour of the CL classified as regressing was a bright yellowish-orange compared to a brown or brownish-yellow for the newly developing CL.

The sizes of the CL and the largest follicle from each animal spayed on each of the 4 days are shown in Table 3. Comparisons between size and general appearance of the CL of the control and injected groups were the criteria by which normal CL development was determined. In most cases, there were marked differences between the size of the CL classified as normal and those not considered normal. Further, where the CL were classified as regressing, regardless of size, the colour confirmed regression.

The follicle size of the heifers that had regressing CL was generally larger than the follicle size of the comparable control heifers and also of heifers that had normally developing CL within injected groups.

Oestrus was induced only in the heifers injected with ECP. Of the sixteen ECP-treated heifers, nine (56%) were observed in oestrus within 24 hr from the injection. No HCG or PLH heifers were observed in oestrus.

DISCUSSION

The results show that ovulation can be effectively induced by either ECP, HCG or PLH when injected during MGA treatment. However, oestrus was induced only with ECP. These results agree with those in the previous reports in this series where it was shown that following ECP or gonadotrophin injection, ovulation occurred in almost all heifers, but oestrus was observed only in heifers receiving oestrogen injection. In these previous studies using oestrogens and gonadotrophins, there was some indication that CL did not develop normally following the induced ovulation. The present study confirms these earlier findings and indicates that abnormal development is evident after about 5 days. We suggested that this was due to a deficiency of gonadotrophin, probably LH, brought about by the inhibitory action on the pituitary gland of the oestrogen or of the progestagen treatment continued after the oestrogen injection. This would agree with the interpretations of Loy *et al.* (1960) and Wiltbank *et al.* (1961). These investigators showed that oestrogen injections beginning early in the cycle caused early regression of the CL. Loy *et al.* (1960) also showed that progesterone injections beginning early in the oestrous cycle exerted a detrimental effect on the formation and function of the CL. The injection of gonadotrophin may induce ovulation by direct action on the ovary, but continued MGA treatment may prevent additional release of pituitary LH in quantities or at times as needed for normal CL development. It is likely that enough endogenous or exogenous gonadotrophin was available for the initial stages of CL formation in each of the injected groups since all induced CL

appeared normal from heifers spayed 5 days later. However, the increased incidence of regressing CL from heifers spayed at the later stages supports the concept of a deficiency of a CL maintaining factor, probably LH.

The average size of follicles in this study corresponds quite closely to the average size of the largest follicles described by Hansel (1959) for normally cycling Holstein heifers. He described the sizes as follows: 1 to 4 days post-oestrus, 13 mm; 5 to 8 days post-oestrus, 11 mm; 9 to 13 days post-oestrus, 14 mm; and 18 to 21 days post-oestrus, 20 mm. Asdell (1964) indicated that the size of follicles at ovulation is 16 to 19 mm. Whether or not the largest follicle on the ovaries of heifers which had regressing CL in this study would have ovulated soon cannot be determined. Several follicles were of ovulatory size. One animal had recently ovulated, and an impending ovulation appeared near in others. The formation and early regression of CL induced during MGA treatment appears similar to the pattern of those subjected to oxytocin treatment (Armstrong & Hansel, 1959) during their early formative period.

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