EFFECTS OF MELENGESTROL ACETATE (MGA) ON VESICULAR FOLLICLES IN THE BOVINE OVARY

by

Francisco Rogelio Cuevas-Correa

D.V.M., National Autonomous University of Mexico, 1963

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy and Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1969

Approved by:

H T Gier
Major Professor

G B Marion

TABLE OF CONTENTS

Page

INTRODUCTION 1

MATERIALS AND METHODS. 3

RESULTS AND DISCUSSION 5

SUMMARY AND CONCLUSIONS. 26

ACKNOWLEDGEMENTS 28

REFERENCES 29

INTRODUCTION

Estrus and ovulation can be successfully synchronized in dairy and beef cattle by use of natural or synthetic progesterone (Trimberger and Hansel, 1955; Wiltbank et al., 1965; Zimbelman, 1963). Synthetic progesterone which can be administered orally has been observed to be much more potent and economical than natural progesterone for synchronizing estrus in cattle. Furthermore, the potency of melengestrol acetate (MGA) for cattle as measured by ovulation inhibition is several hundred times that of medroxyprogesterone acetate (MAP) which is also several times more effective than progesterone (Zimbelman and Smith, 1966a).

Fertility attained at the synchronized estrus in cows, ewes and gilts, after progesteronic regulation of the estrous cycle, is variable but generally low (Trimberger and Hansel, 1955; Wiltbank, et al., 1965; Foote, 1962; Baker et al., 1954).

Possible causes of low fertility are not yet understood. The complexity of available data makes their interpretation difficult, since various immediate causes could result in reduced fertility (Zimbelman and Smith, 1966b).

Alteration in hormone balance produced by introduction of large quantities of exogenous progesterone appears to be the cause of low fertility at the synchronized estrus. The introduction of estrogen with progesterone might be less disruptive of the hormonal balance and thus improve fertility. Experimental evidence showed that injections of different levels of estrogen (10 to 80 mg) and progesterone (20 or 40 mg) increase synchronization of estrus but lowered fertility at the synchronized estrus (Wiltbank et al., 1965).

Brock and Rowson (1952) found that following injection of pregnant mare serum (P.M.S.) (300 I.U.) in the absence of a corpus luteum, the percentage of ovulations increased directly with the delay in estrus and many of the ova from cows in which estrus was delayed more than 5 days showed evidence of degeneration.

Ova released by injection of P.M.S. can be fertilized readily in the absence of a corpus luteum, but in presence of a corpus luteum or after daily injections of progesterone, no fertilization took place. In the presence of a corpus luteum or after injections of progesterone, ova passed through the oviduct at a greatly increased rate. In some cases ova movement was slowed by the injection of estrogens (Rowson, 1951).

Trimberger and Hansel (1955) inferred that either ovum degeneration or too rapid passage of the ova through the oviduct may be involved in the low conception rate following estrus control by progesterone injections in dairy cattle.

It has been suggested that progesterone has an inhibitory effect on the uterine muscular movement interfering with sperm transport in the uterus and decreasing the levels of fertility in gilts treated with progesterone (Baker et al., 1954).

Little attention has been given to the effects of administration of synthetic or natural progesterone on the follicular system of the bovine ovary. The importance of knowing the normal cyclic changes in the ovarian follicular system as a meaningful aid in assessing the influence of exogenous agents on the ovary has been supported strongly by Choudary et al., 1967 and Marion et al., 1968.

Stratum granulosum and theca interna of the ovarian follicle are extremely sensitive to changing levels of FSH, LH, estrogen and progesterone. Adverse hormonal combinations result in failure of the follicle and subsequent regression, commonly known as "follicular atresia" (Marion et al., 1968). Lawyners and Ferrin (1964) observed numerous involuting and atretic tertiary follicles in the ovaries of women treated with 5 mg of Lynestrol per day. The theca folliculi were poorly developed and some follicles showed congestion accompanied by hemorrhage. Numerous atretic follicles were also reported by Toth (1964) in rats treated with a variety of progestational substances.

A number of cows whose ovaries contained large follicles developed luteinized follicles during melengestrol acetate (MGA) treatment (Darwash, 1965).

Melengestrol acetate administered orally to cows was reported to produce estrogenic influence in the ovary; increasing follicular size, decreasing incidence of corpus luteum, increasing evidence of follicles and an estrogenic activity in the production of cervical mucus (Zimbelman and Smith, 1966b).

Due to the promising and increasing use of synthetic progesterone in the regulation of estrus in cows and to the low and variable fertility rates that have been obtained at the synchronized estrus, the following experiment was designed to investigate the effects of the synthetic progesterone (MGA) on the follicular system of the bovine ovary to elucidate the causes of low fertility after MGA treatment.

MATERIALS AND METHODS

Levels of 0.5 mg and 1 mg of MGA administered orally, reported to be effective in synchronization of estrus cycles in dairy cows by Darwash (1965) and Zimbelman and Smith (1966a), were used in this study.

One group of 3 and one of 33 clinically normal cycling dairy cows with known reproductive histories received orally 0.5 mg and 1 mg of melengestrol acetate (MGA) respectively per day for 14 days. Cows were in heat 4 or 5 days after treatment and were sacrificed on day after estrus. Another group of three cows was fed 1.0 mg of MGA per day for 14 days followed by no MGA treatment period of 11 days and then 1.0 mg was again fed for 10 days. Estrus was observed 4 days after termination of the first MGA treatment. No estrus was observed in the 4 day period between the end of the second MGA treatment period and the day of slaughter. A group of three normally cycling cows was used as control.

Ovaries from each group were recovered and fixed between 20 and 30 minutes after slaughter. Before fixation each pair of ovaries was weighed and measured for the maximum length, width and thickness. Ovaries were perfused with or immersed in Bouin's fixative. They were then cut into four or five slices each 8-10 mm thick to insure proper fixation. Slices ovaries were dehydrated in a series of isopropyl alcohol, infiltrated and embedded in paraffin. During this process except at embedding, ovary slices were kept in their original sequences by incomplete sectioning of the slices on the mesovarian edge.

Histological sections were made at 8, 10 or 12 u thick depending on the difficulties in sectioning presented by each embedded slice.

In order to obtain representative samples from each ovary, entire serial sections were made from each slice to fill two slides.

One slide was stained with periodic acid Schiff-Harris Haematoxylin and the other slide with Harris Haematoxylin and Mallory triple stain.

The stained sections were projected at a magnification of ten and the contours of the section and follicles were drawn on tracing paper. The maximum diameter including the theca interna of each follicle was measured from the drawings or with an ocular micrometer. Follicles above 0.4 mm diameter were classified as either normal or atretic in accordance with their size and histological conditions.

The morphological description of normality in an ovarian follicle as well as the classification of the stages of follicular atresia proposed by Marion et al. (1968) were adopted with some modifications. This system was used as a tool to evaluate histologically the effects caused by oral administration of MGA upon the bovine ovarian follicular system. The data of normal and atretic follicles reported by Choudary et al. (1968) based on cyclic changes observed in bovine vesicular follicles were compared with those found in the present work. The total count of all the normal and atretic follicles (stage 1 to 5) that were found in each pair of ovaries were classified as one of the following five classes; (1) from .4 mm to 1.0 mm, (2) from 1.1 mm to 2.0 mm, (3) from 2.1 mm to 4.0 mm, (4) from 4.1 to 8.0 mm, and (5) over 8.1 mm.

RESULTS AND DISCUSSION

The granulosa layer of the growing vesicular follicle is 8 to 10 cells thick and contains a basal layer of pseudo-columnar cells which are oriented perpendicularly against the membrana propria. Mitotic figures occur frequently in the stratum granulosum (Pl. I-A).

In the normal preovulatory follicle the basal cell layer of the stratum granulosum is no longer pseudo-columnar, and the entire stratum granulosum is transformed into a layer of polyhedral cells 5 or 6 cells thick, becoming plumped and hyperchromatic (Pl. I-E).

The membrana propria of the growing vesicular follicle and that of the preovulatory follicle is distinctly PAS positive and remains unbroken during normal growth (Pl. I-C).

Definite morphological changes from normality in the vesicular follicles indicate atresia, which may be of five different types and at least five recognizable stages. Follicular atresia, except in cystic atresia, is characterized by disintegration of granulosa cells and membrana propria and by dedifferentiation of glandular interna cells and shortening of the fibrocytes. During late stages, the fibrocytes of the theca interna resolve into a hyalinized substance intensively PAS positive (Pl. I, II).

Five stages of follicular atresia were categorized.

Stage 1 was characterized by (a) loosening of cells of the stratum granulosum leaving distinctly open spaces, (b) appearance of atretic bodies in the follicular lumen as consequence of nuclear disintegration, (c) broken membrana propria and deorientation of granulosa and theca interna, and (d) appearance of Call-Exner bodies in the granulosum or cumulus.

Atretic bodies, formed by clumps of chromatin material after disintegration of the cytoplasm, were heavily stained with Haematoxylin (Pl. I-5).

The order of occurrence of follicular degenerative processes seemed to be independent and no one seems to have priority over the others. One or all of these degenerative signs may occur in a follicle undergoing first stage of atresia.

Stage 2 is recognizable by the presence of numerous atretic bodies in the lumen as consequence of intensive disintegration of granulosa cells. In a few instances the membrana propria was still present but in most of the cases it had disappeared by this stage.

The theca interna glandular cells dedifferentiate into fibrocytes and fibrocytes become short and somewhat thickened. Blood vessels may constrict somewhat but remain a dominant feature of the interna (Pl. I-D).

From this point the degenerative follicles could follow any one of five types of degeneration depending on the structures involved in the atretic

EXPLANATION OF PLATE I

A. Growing vesicular follicle showing normal granulosa layer with a basal layer of pseudo columnar cells oriented perpendicularly against the membrana propria. Mitotic figures can be observed in the stratum granulosum.

B. Follicle in stage 1 atresia showing a Call-Exner body in the stratum granulosum.

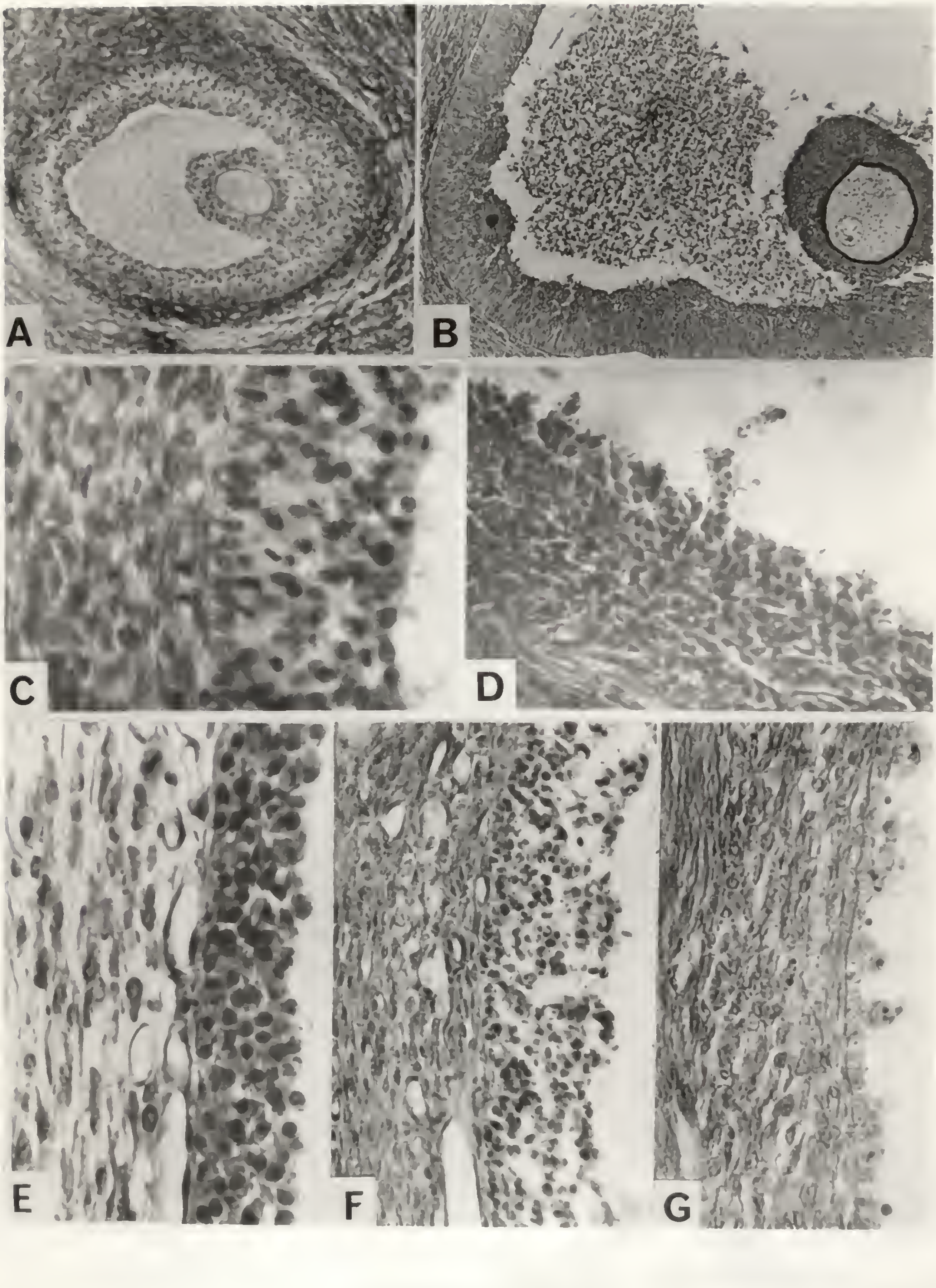
C. Normal vesicular follicle with an intact membrana propria distinctly PAS positive.

D. Numerous atretic bodies in a vesicular follicle in stage 2 atresia.

E. Normal pre-ovulatory follicle with stratum granulosum transformed into a layer of polyhedral cells.

F. Rapid process of atresia in a pre-ovulatory follicle is characterized by drastic disintegration of granulosa cells forming great numbers of atretic bodies.

G. Contracting follicle in stage 3 atresia. Granulosa layer has been reduced to a single cell layer and fibrocytes of theca interna have been shortened and rounded up.


A
B
C
D
E
F
G

processes; (A) contracting, (B) collapsing, (C) cystic, (D) luteinized, and (E) rapid atresia.

A. Contracting atresia. Most follicles undergo a simultaneous degeneration of granulosa and theca interna cells resulting in a contraction of the entire follicle rounding it up and preserving its spherical shape. Degeneration of granulosa cells continues progressively to the point that a single cell layer of granulosa remains. At the same time the fibrocytes of the theca interna round up (Pl. I-G).

B. Collapsing atresia. Follicles that start degeneration of theca interna and maintain stratum granulosum undergo a collapsing process characterized by drastic folding of the granulosa layer and eventual maintenance of a few granulosa cells as an interstitial gland (Pl. II-B).

C. Potential cystic follicles lose the granulosum down to a single cell layer, but maintain membrana propria and theca interna, then the follicle enlarges to approximately twice its size before atresia begins (Pl. II-C).

D. In some follicles, stratum granulosum cells and glandular interna cells hyperthrophy, enlarge like blossom cells and come in intimate proximity separated only by membrane propria. This was considered to be a process of follicular luteinization (Pl. II-D).

E. A rapid process of atresia occurs in preovulatory follicles and is characterized by a drastic disintegration of granulosa cells forming great numbers of atretic bodies (Pl. I-F).

Stage 3. The third stage of atresia is an advanced stage of degeneration in which the granulosa layer has been reduced to a single cell layer. During late phases of this stage there is an increase in the number of cell layers from one to 2 or 3 cells thick caused by contraction of the follicle approximately 1/3 of its maximum diameter. As mitotic figures could be observed during this late stage of degeneration, mitosis may contribute in part to increase the thickness of granulosum. The lumen was eventually filled with a spongy mass derived from the granulosa cells (Pl. I-G).

Stage 4 was assigned to those follicles in which theca interna cells started dissolution into a rim of hyalinized highly PAS positive material within the innermost border of the theca interna. This process of hyalinization extends progressively outwards involving 1/3 to 3/4 of the theca interna. The follicle by this time has decreased to less than 4 mm diameter. The terminal condition of collapsing atresia of a large follicle may reach this stage (Pl. II-A).

Stage 5. Finally the follicles that reach the late stage of degeneration contain no lumen and the theca interna is entirely hyalinized with only a few blood vessels and fibrocytes still remaining within the hyalinized substance. Most follicles at this stage vary in diameter between .4 mm and 2 mm and only a few range into the 4 mm class.

From here the remnants of the follicle are dissolved by or incorporated into the surrounding stroma.

Degeneration of the oocyte was observed to occur at any time during atresia of the follicle and it seems to be set independently of follicular degeneration. Normal appearing oocytes were observed rather frequently in follicles undergoing late stages of atresia (Pl. II-E, F). Degeneration of the oocyte was characterized by cytoplasmic vacuolization or granulation, degeneration of the nucleus and intensively PAS positive staining.

EXPLANATION OF PLATE II

A. Theca interna cells start dissolution forming a rim of hyalinized highly PAS positive material characteristic of vesicular follicles in stage 4 atresia.

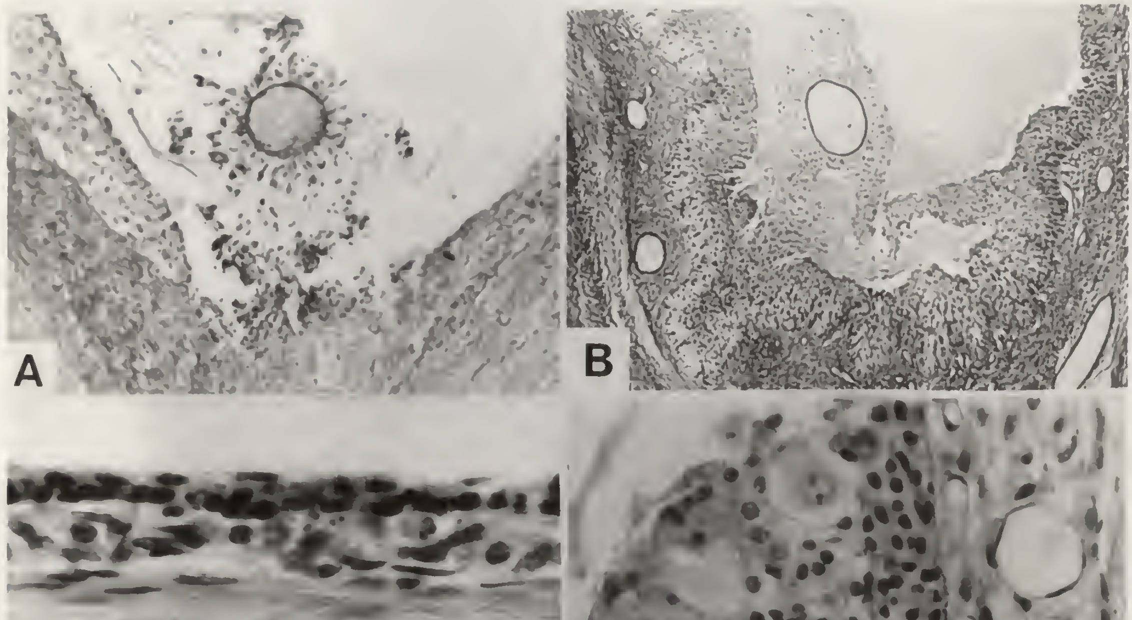
B. Vesicular follicle undergoing collapsing type of atresia. Granulosa layer drastically folds and granulosa cells are maintained as interstitial gland.

C. Cystic follicle with granulosa reduced to a single cell layer. Membrana propria and theca interna remain under functional condition.

D. Luteinizing follicle in which the hyperthrophy of granulosa and glandular interna cells causes the follicle to form a corpus luteum-like condition. Granulosa and interna cells remain separated by membrana propria.

E. Normal appearing oocyte retained in a vesicular follicle undergoing stage 3. This condition was observed rather frequently in all treated cows.

F. Normal appearing oocyte retained in a highly degenerated vesicular follicle undergoing stage 4 of atresia.


A
B
C
D
E
F

The average number of follicles by classes in the control and treated groups are presented in Table 1. Figures were calculated from the raw data by the formula:

$$\frac{\text{length of the ovary in mm X number of follicles in the category}}{\text{average size of follicular class X number of sections counted per ovary}}.$$

The follicles undergoing stage 5 of atresia were not included in these counts because their number was so great that the lesser numbers of active follicles became insignificant. Furthermore, they represent old atretic follicles that do not reflect the effect of MGA levels used during 14 days of treatment.

There was a progressive reduction in the number of follicles per pair of ovaries as the levels of MGA increased (Fig. 1).

Analysis of the average number of follicles shows that normal follicles in control cows (Fig. 1) were predominantly in the .4-1.0 mm class size and that there was a progressive decrease in the number of normal follicles of this size as the level of MGA was increased. Number of normal follicles in 1.1-2.0 and 2.1-4.0 mm in the treated cows are comparable to the number of normals in the control group.

Stage 1 of atresia predominantly in 0.4-1.0 mm follicles in each control and treated cows with exception of the 0.5 mg group (Fig. 1).

Stage 2 of atresia was predominantly seen in classes 1.1-2.0 and 2.1-4.0 mm follicles in controls and treated groups (Fig. 1).

Stage 3 of atresia was characteristically seen in follicles 2.1-4.0 mm in control and treated cows but was considerably reduced in number in treated cows (Fig. 1).

Table 1. Average number of normal and atretic follicles per cow slaughtered on the fourth day after terminal treatment.

Treatment MGA (mg)	Follicle size mm	Follicle class Stages of atresia Normal	1	2	3	4	Total	Total atretic
Control (3 cows)	.4 - 1	144	82	4	-	190	420	276
	1.1 - 2	47	24	12	7	63	153	106
	2.1 - 4	30	15	28	35	13	121	91
	4.1 - 8	3	2	4	6	-	15	12
	>8.1	1	1	1	2	-	5	4
	Total	225	124	49	50	266	714	489
.5 mg per day for 14 days (3 cows)	.4 - 1	86	-	-	-	3	89	3
	1.1 - 2	46	1	1	6	26	80	34
	2.1 - 4	35	5	14	14	7	75	40
	4.1 - 8	17	3	1	-	-	21	4
	>8.1	1	-	-	-	-	1	-
	Total	185	9	16	20	36	266	81
1 mg per day for 14 days (33 cows)	.4 - 1	39	59	1	1	18	118	79
	1.1 - 2	44	16	5	3	22	90	46
	2.1 - 4	17	16	11	12	9	65	48
	4.1 - 8	3	2	2	1	1	9	6
	>8.1	1	1	1	1	-	4	3
	Total	104	94	20	18	50	286	182
1 mg per day for 14 days; two periods (3 cows)	.4 - 1	10	34	-	-	5	49	39
	1.1 - 2	16	6	4	10	52	88	72
	2.1 - 4	22	4	6	13	3	48	26
	4.1 - 8	2	2	4	2	1	11	9
	>8.1	1	1	1	-	-	3	2
	Total	51	47	15	25	61	199	148

The number of follicles in stage 4 atresia in the control cows was much greater than the number of normals in the same group. Stage 4 was mainly observed in 0.4-1.0 mm class follicles and secondarily in 1.1-2.0 mm class follicles in the controls. Only a few follicles over 2.0 mm were observed in stage 4 of atresia (Fig. 1). Stage 4 of atresia in the treated cows was characteristic of 1.1-2.0 mm class follicles. Number of follicles 0.4-1.0 mm in diameter undergoing stage 4 was greatly reduced in all treated cows as compared with controls.

In the control cows the number of follicles decreased from the high number of normal follicles to a lower number in stages 2 and 3, increasing again in those follicles in stage 4 (Fig. 1).

Total number of follicles in atresia stages 2 and 3 in the treated cows were comparable to those in the controls (Fig. 1).

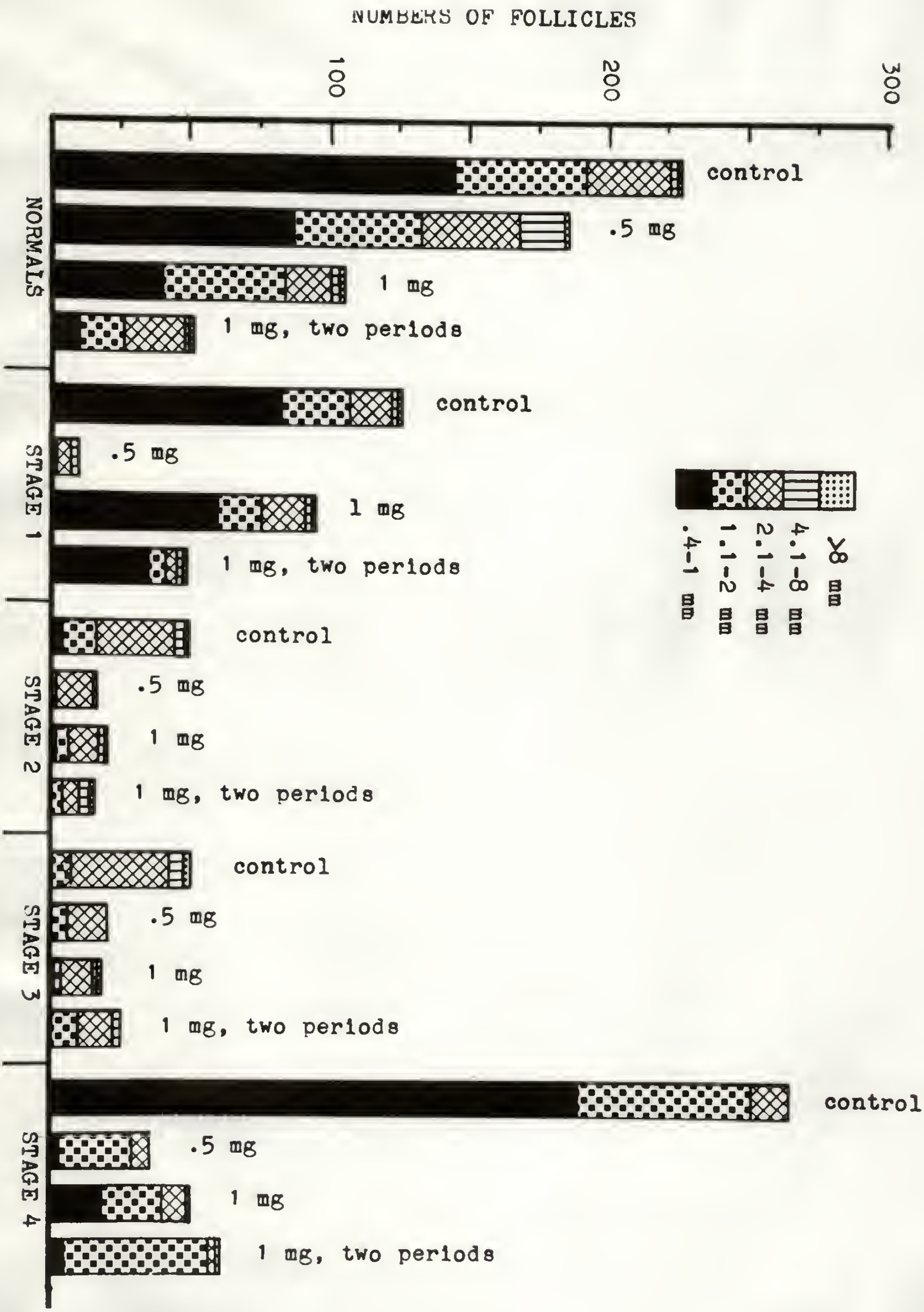
The low number of normal small tertiary follicles (0.4-1 mm) and presence of comparable numbers of normal and stage 1 atresia follicles from 1.1 to 4.0 mm diameter in treated cows suggests a decrease in the rate of development of secondary to early tertiary follicles as consequence of MGA treatment.

The great number of follicles undergoing stage 4 atresia in the control cows may be explained on the basis that this stage is a rather slow, long lasting process thus increasing the follicular counts in this stage while there is a normal rate of follicular regression. The decrease in number of follicles undergoing stage 4 of atresia in the treated cows as the levels of MGA increase can be interpreted as an accelerating effect of MGA on the rate of follicular dissolution of later stages of atresia.

Comparison of the average number of normal and atretic follicles is presented in Fig. 1. Vesicular follicles in ovaries of control cows is almost three times that of treated cows (Table 1). The number of normal follicles

EXPLANATION OF FIGURE 1

Average number of follicles by stage in control cows and those treated with MGA 0.5 mg 14 days; 1 mg 14 days; and 1 mg for 2 periods of 14 and 11 days. Patterns on the bars indicate the number of each size class.



NUMBERS OF FOLLICLES
100
200
300
NORMALS
control
.5 mg
1 mg
1 mg, two periods
STAGE 1
control
.5 mg
1 mg
1 mg, two periods
STAGE 2
control
.5 mg
1 mg
1 mg, two periods
STAGE 3
control
.5 mg
1 mg
1 mg, two periods
STAGE 4
control
.5 mg
1 mg
1 mg, two periods
.4-1 mm
1.1-2 mm
2.1-4 mm
4.1-8 mm
>8 mm

was considerably greater than the number of atretic follicles in cows receiving 0.5 mg of MGA. However, cows treated with 1.0 mg MGA for 14 days or 1.0 mg MGA for periods of 14 and 10 days had more atretic than normal follicles. These results indicate that low levels of MGA (0.5 mg) probably repress growth of small follicles, retard onset and early stage 1 of atresia, and accelerate stages 3 and 4 of atresia, consequently maintaining more follicles in normal conditions and reducing later stages.

The repressive effect of MGA on normal follicular growth was accentuated in the groups receiving higher levels of MGA (1.0 mg for 14 days or 1 mg for periods of 14 and 10 days) (Fig. 2), but the proportion of all size classes progressing to atresia approximated that in the control group.

Treatment with 1 mg MGA for periods of 14 and 10 days seemed to have a greater repressive effect on the rate of follicular development than did 1.0 mg for a single 14 day period.

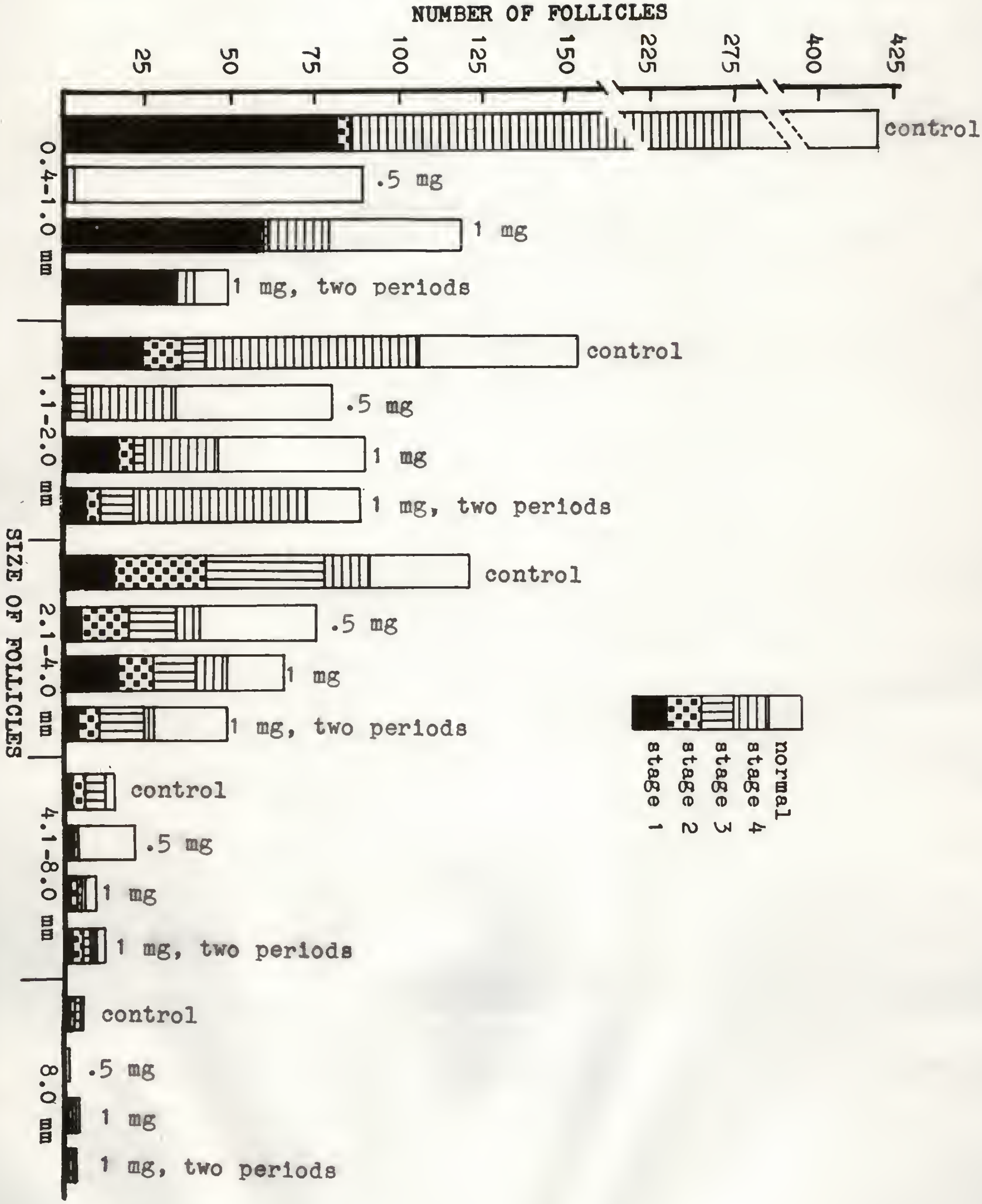
Figure 2 shows in detail the number of normal follicles and those in the different stages of atresia in the control and treated cows. In all treated groups, there was a reduction in the number of follicles in stage 1 of atresia, more drastic in the 0.5 mg MGA treated cows.

The number of follicles in stage 2, 3 and 4 was maintained nearly equally in the three treated groups and could be directly compared with control groups revealing the reduced number of follicles in the treated groups (Fig. 2). As follicles undergoing stage 1 of atresia were predominately in the .4-1 mm class, this again suggests a repressive effect of MGA on the development of early vesicular follicles.

Numbers of normal follicles in treated cows were reduced in comparison to control cows, but relative numbers of follicles were proportional to controls

EXPLANATION OF FIGURE 2

Numbers of follicles by size as determined for control cows and those treated with MGA, 0.5 mg 14 days, 1 mg 14 days, and 1 mg for two periods. Each bar indicates the average number of follicles within a treatment, as in Fig. 1. Number of follicles in each stage is designated by pattern within the bar.


NUMBER OF FOLLICLES
25
50
75
100
125
150
225
275
400
425
SIZE OF FOLLICLES
0.4-1.0 mm
1.1-2.0 mm
2.1-4.0 mm
4.1-8.0 mm
8.0 mm
control
.5 mg
1 mg
1 mg, two periods
normal
stage 4
stage 3
stage 2
stage 1

in each particular group (Fig. 2). Normal follicles were progressively fewer with increased dosage of MGA.

Ovaries of control cows contained follicles in 0.4-1.0 mm class undergoing stage 1 and 4 of atresia, but few follicles in stage 2. Cows receiving 0.5 mg MGA had no follicles in the 0.4-1.0 mm class undergoing stage 1 of atresia and few in stage 4.

In cows treated with 1 mg MGA for 14 days the number of normal 0.4-1.0 mm follicles was reduced without modification of the number in stage 1. Normal follicles in 0.4-1.0 mm class of cows treated with 1 mg MGA during two periods seemed to be highly repressed as were those in stage 1. Similar repression of the number of regressing follicles in stage 4 was observed.

The number of normal follicles of 1.1-2.0 mm in the treated groups were not greatly reduced. Stage 4 of atresia was diminished in all treated groups. The increase in number of follicles in stages 2 and 3 is indicative of an increased reaction of follicular size as degeneration advances in the follicular walls. The few follicles in the 2.1-4.0 mm class suggest that they reflect much better the variations in hormonal levels. By the time the follicles reach the 2.1-4.0 mm class fewer and fewer were observed beginning atresia. Few follicles (2.1-4.0 mm) in stage 4 atresia were found in any group. Number of follicles in stage 4 presented in figure 2 decreases as they increase in size. The remnants of follicles undergoing stage 4 atresia have been reduced to a small fraction of their original size by this stage. Follicles seemed to go through stage 4 at a rather slow rate and accumulate in large numbers in the 0.4-1.0 mm class as indicated in Fig. 2 for control groups.

The number of follicles in stage 1 atresia per treatment followed the same pattern as the normal follicles, decreasing in number as the follicular size increased. This might be a result of the decrease in normal follicles with increase in size, since normal follicles are the immediate source of atretic follicles in stage 1.

In the group receiving 0.5 mg MGA follicles undergoing stage 4 were disturbed in the 0.4-1.0 mm class decreasing in number where they were expected to increase. Follicles in classes 1.0-2.0 mm and 2.1-4.0 mm followed the expected pattern of distribution found in the controls. No follicles undergoing stage 3 were observed in the 4.1-8.0 mm class. This may indicate the repressive effect of MGA on the rate of growth of vesicular follicles and an accelerating effect on the rate of atresia during the late stages.

In cows treated with MGA the number of follicles undergoing stage 4 atresia was decreased as compared with controls (Fig. 2) again indicating stimulation to an increased rate of regression during the final stages.

Cows receiving 1 mg of MGA for 14 days followed by a 10 day treatment 11 days later showed the greatest repression on follicular counts.

Figure 3 shows the percent distribution by sizes of normal and atretic follicles in control and treated cows based on average numbers. Vesicular follicles in all treated groups showed deviations from the pattern of distribution of the control group.

A drastic decline was observed in frequency of follicles in class 0.4-1.0 mm to follicles in 2.1-4.0 mm in the control group (Table 2). A rather smooth decline in frequencies between all size classes was observed in treated cows. Follicles in the 8.1 mm class were increased in proportion but not in number in the groups treated with 1 mg MGA. This might suggest a relative increase on preovulatory follicles as a final output in the treated cows as a consequence of maintenance of a base level of normal, growing follicles in the larger sizes even with the repression caused by high levels of MGA (Fig. 3). It is obvious, then, that the interpretation of ovarian activity in terms of follicular development expressed as relative frequency should be critically analyzed since it may give a false indication of the true ovarian activity. Thus, Zimbelman and Smith (1966b) reported an increase in follicular size and increased incidence of detectable follicles (from 56% to 91%) as the number of days in treatment increased in a group of dairy and beef heifers treated with 0.4 mg of MGA for 24 days. Furthermore, they reported that lower dose of MGA (0.22 or 0.42 mg) was associated with a decreased incidence of follicles of a larger size and a higher dose (0.85 mg) was associated with high incidence but small follicle size. Again, this interpretation is in contrast with our findings since the results indicate a tremendous reduction in the number of follicles of treated cows and that this effect increases as the doses of MGA increase.

In the 0.5 mg of MGA group the relative proportion of normal follicles was greatly increased predominantly in the 0.4-1.0 mm class with comparable reduction in the proportion of follicles undergoing stage 1 and 4 of atresia in the 0.4-1.0 mm class. No atretic follicles were observed in the 8.1 mm class.

Cows receiving 1 mg MGA for one period showed a slight increase in the proportion of normal follicles over the controls. There was an apparent increase in proportion of follicles undergoing stage 1 (mainly in the 0.4-1 mm class) over the controls. Stages 2 and 3 of atresia kept comparable proportions to similar follicles in the control and 0.5 mg MGA treated groups.

The relative incidence of follicles in stage 4 of atresia was lower than that of the controls but above similar follicles in the .5 mg MGA group. The rate of atresia 4 seemed to increase in follicles at 1.1-2.0 mm class.

In cows receiving 1 mg MGA for two periods of 14 days, there was a decrease in frequency of normal follicles in all the size classes.

It was evident that low doses of MGA (0.5 mg/14 days) increased the percent of normal follicles, but normal follicles were repressed, increasing percent of atresia as the MGA doses and days in treatment increased (Fig. 4).

It is generally assumed that exogenous progesterone replaces the action of endogenous progesterone and prevents follicles from growing beyond the pre-ovulatory size. Follicular growth up to the preovulatory size is assumed to be due primarily to the action of FSH. The growth of follicles to preovulatory size, estrogen secretion and ovulation are caused by interaction between FSH and LH.

It has been suggested that exogenous progesterone prevents the release of LH from the pituitary rather than preventing action of LH after it is in the blood stream (Ulberg <u>et al</u>., 1951; Brock and Rowson, 1952). In contrast, Nellor and Cole (1957) suggested that exogenous progesterone inhibits the action of LH on extra-pituitary sites.

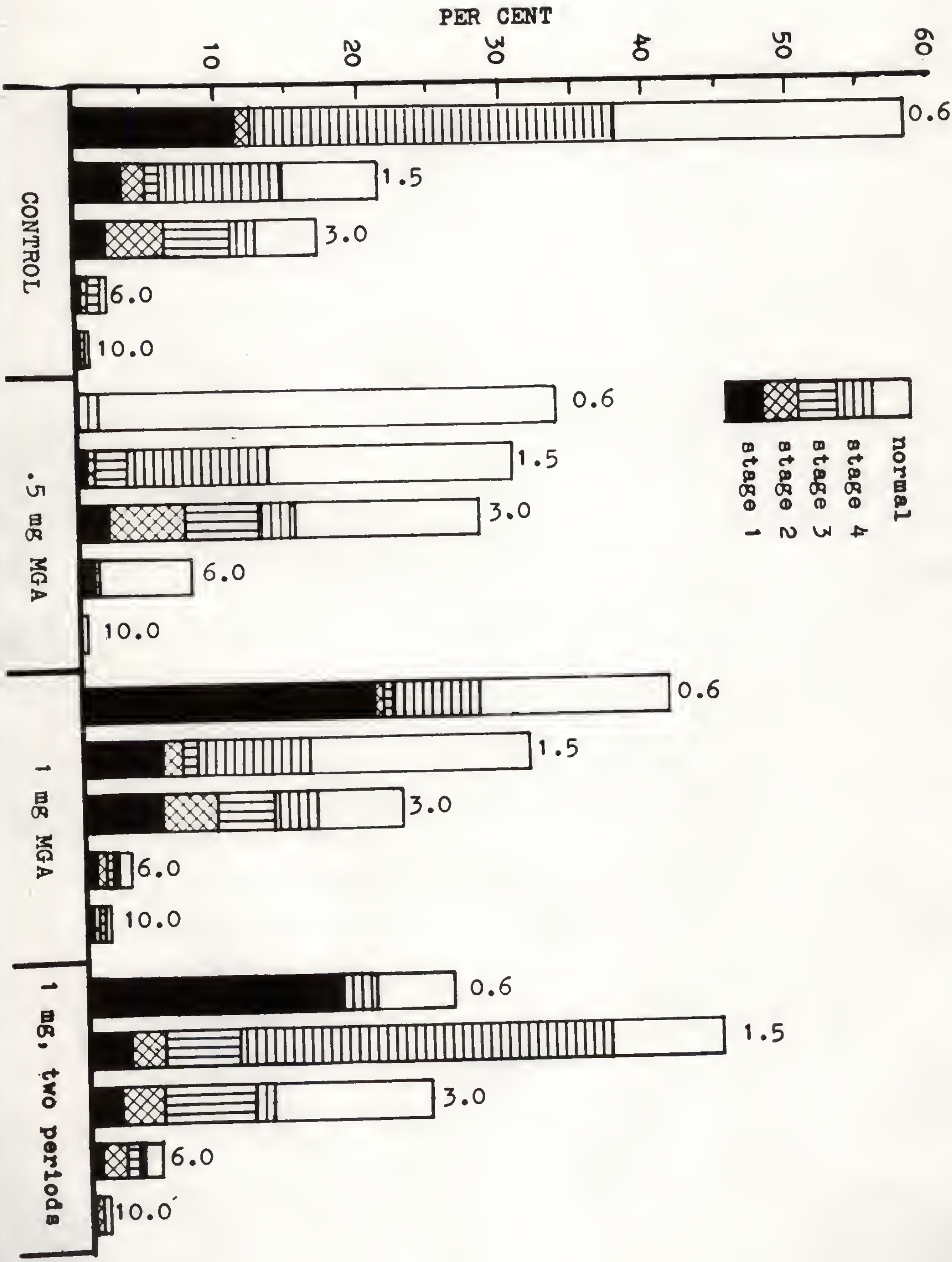
The drastic decrease in the number of early tertiary follicles and reduction in the number of large vesicular follicles indicate that MGA inhibits the levels of FSH as well as those of LH. Exogenous progesterone has considerable inhibition on ovarian function (Lauweryns and Ferrin, 1964) and on growth and size of bovine follicles (Nellor and Cole, 1957). However, Zimbelman and Smith (1966b) reported that MGA prevents the release of LH rather than the release of FSH from the pituitary and so causes inhibition of

Table 2. Relative frequency of normal and atretic follicles per cow.

	Follicular Size mm	Normal	Stages of Atresia 1	2	3	4	Total	Total Atretic
Control Group (3 cows) %	.4 - 1.0	20.11	11.44	.59	-	26.62	58.76	38.65
	1.1 - 2.0	6.60	3.32	1.69	.97	8.79	21.47	14.77
	2.1 - 4.0	4.29	2.13	3.99	4.84	1.87	17.12	12.83
	4.1 - 8.0	.43	.24	.59	.91	-	2.17	1.74
	8.1	.17	.06	.03	.32	-	.58	.41
	Total	31.60	17.19	6.89	7.04	37.28	100.00	68.40
MGA .5 mg (3 cows) %	.4 - 1.0	32.25	-	-	-	1.25	33.50	1.25
	1.1 - 2.0	17.37	.50	.50	2.25	9.88	30.50	13.13
	2.1 - 4.0	13.00	1.88	5.37	5.25	2.50	28.00	15.00
	4.1 - 8.0	6.50	1.00	.25	-	-	7.75	1.25
	8.1	.25	-	-	-	-	.25	-
	Total	69.37	3.38	6.12	7.50	13.63	100.00	30.63
MGA 1 mg/ 14 days (33 cows) %	.4 - 1.0	13.63	20.63	.35	.35	6.30	41.26	27.63
	1.1 - 2.0	15.39	5.59	1.74	1.05	7.69	31.46	16.07
	2.1 - 4.0	5.94	5.60	3.85	4.19	3.15	22.73	16.79
	4.1 - 8.0	1.05	.70	.70	.35	.35	3.15	2.10
	8.1	.35	.35	.35	.35	-	1.40	1.05
	Total	36.36	32.87	6.99	6.29	17.49	100.00	63.64
MGA 1 mg/ 14 days TWICE (3 cows) %	.4 - 1.0	5.38	17.51	-	-	2.52	25.41	20.03
	1.1 - 2.0	7.91	2.86	2.02	5.22	26.43	44.44	36.53
	2.1 - 4.0	11.12	2.02	2.86	6.40	1.69	24.09	12.97
	4.1 - 8.0	1.17	.84	1.85	.64	.17	4.87	3.70
	8.1	.34	.51	.34	-	-	1.19	.85
	Total	25.92	23.74	7.07	12.46	30.81	100.00	74.08

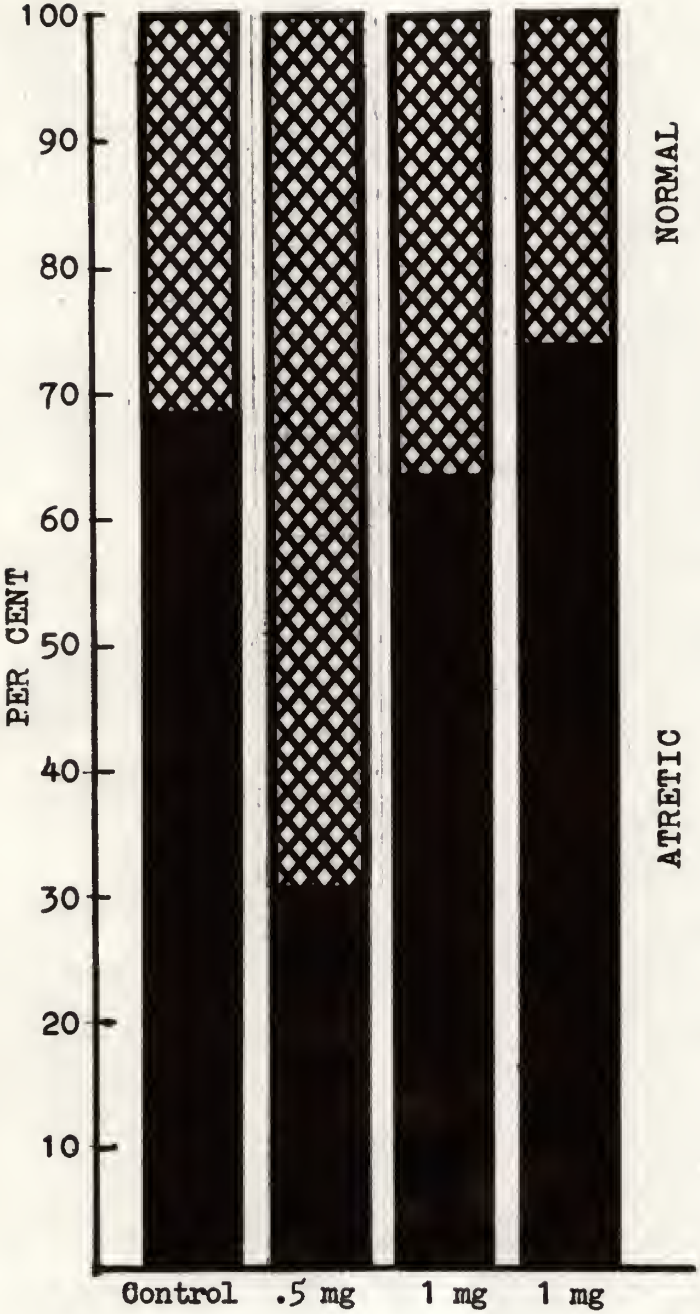
EXPLANATION OF FIGURE 3

Frequency of various size and condition of follicles as determined from different treatments. The follicular size average is used as description of the groups. Each bar indicates the number of follicles within a size category; condition of follicles is designated by pattern.


PER CENT
10
20
30
40
50
60
CONTROL
.5 mg MGA
1 mg MGA
1 mg, two periods
0.6
1.5
3.0
6.0
10.0
normal
stage 4
stage 3
stage 2
stage 1

EXPLANATION OF FIGURE 4

Relative frequency of normal and atretic follicles in the control cows; those treated with 0.5 mg 14 days; 1.0 mg 14 days; and 1.0 mg 14 days, no treatment 11 days, then 1.0 mg 10 days.



100
90
80
70
60
50
40
30
20
10
PER CENT
NORMAL
ATRETIC
Control
.5 mg
1 mg
1 mg

ovulation associated with an increase in both the incidence and size of palpable follicles.

Absence of estrus and prolongation of life span of the large follicles of cows under MGA treatment must be due to LH inhibition.

The increased rate of follicular dissolution during the late stages of atresia might be favored by an increase in vascular supply of the affected follicles, thus facilitating the removal of cellular debrids and accelerating the integration of the degenerated follicle into the ovarian stroma. Inhibition of levels of LH by MGA may be reflected by this increased vascular supply to the ovarian follicle indicating some sort of corticosteroid-like action.

SUMMARY AND CONCLUSIONS

A drastic depression of the rate of development and growth of early vesicular follicles (0.4-1.0 mm) in cow ovaries was evident as a consequence of the treatment with either 0.5 mg or 1.0 mg of melengestrol acetate during 14 days or with 1.0 mg of melengestrol acetate during two periods of 14 and 10 days separated by an 11-day period of no treatment.

The rate of depression on the transformation of secondary follicles to early vesicular follicles increased as the doses of melengestrol acetate and the days of treatment increased. Melengestrol acetate appeared to cause an acceleration in the rate of follicular atresia during the late stages resulting in a decrease in the number of follicles undergoing stage 4 of atresia in all treated groups. This effect was magnified as the doses of MGA increased and the time of treatment was prolonged.

Normal appearing oocytes were found rather frequently in vesicular follicles undergoing different stages of atresia. In a few instances the oocyte of vesicular follicles was atretic as manifested by nuclear destruction, cytoplasmic vacuolization and granulation, rupture and disintegration or collapsing of the zona pellucida, while the follicular walls were morphologically normal. It was concluded that degeneration of the oocyte is a separate event that may be set at any time during, before or after follicular atresia has begun.

The pattern of histological characteristics presented by normal and atretic follicles during their growth and degeneration seems to be a useful and efficient tool to evaluate the effects of exogenous hormones on the follicular system of the bovine ovary.

The use of this system to evaluate the effects of exogenous hormones on the histological structure of the ovary is recommended.

Melengestrol acetate seemed to prolong the life span of the few normal follicles found in the ovaries of treated cows as was shown by an increase in the relative number of normal follicles in each treated group.

There was a failure to recover any oocytes in the oviducts of treated cows from ovulated ovaries and no oocytes were found in any of serial sections of preovulatory follicles (15-20 mm) in the group treated with 1 mg of melengestrol acetate for two periods. Normal oocytes were found rather frequently in all smaller follicles but not in the big preovulatory follicles. It is concluded that melengestrol acetate depressed the ovarian function of cows under treatment. This high depression of the ovarian function might account for the low conception rates of cows inseminated at the synchronized estrus after treatment with melengestrol acetate.

ACKNOWLEDGEMENTS

The author wishes to express his indebtedness to Dr. G. B. Marion and Dr. H. T. Gier for their inestimable guidence and advice given to the author during the development of this research and during his entire program of studies. Thanks are expressed to the Department of Dairy Science and the Division of Biology for the facilities given in the use of appropriate equipment.

My gratitude is expressed to The Ford Foundation, to Dr. William K. Gamble (Representative of The Ford Foundation for Mexico, Central America and the Caribbean), for the aid received to improve my scientific education by supporting my studies and research.

I am specially grateful to my wife and my parents for their constant encouragement and cooperative attitude that helped me solve the problems presented during my studies.

REFERENCES

Baker, L. N., L. C. Ulberg, R. H. Grummer, and L. E. Casida. 1954. Inhibition of heat by progesterone and its effect on subsequent fertility in gilts. J. Animal Sci. 13: 648-657.

Brock, H., and L. E. Rowson. 1952. The production of viable bovine ova. J. Agric. Sci. 42: 479.

Choudary, J. B., H. T. Gier, and G. B. Marion. 1967. Cyclic changes in bovine follicles. J. Animal Sci. 27: 468-471.

Darwash, A. D. 1965. Effects of melengestrol acetate on postpartum reproductive activity of the dairy cow. Ph.D. dissertation. Kansas State University.

Foote, W. C. 1962. Use of progesterone to control reproduction in farm animals. Utah Farm and Home Sci. 23: 44-47.

Lauweryns, J., and J. Ferin. 1964. Effects on the ovary of prolonged administration of Lynestrenol: A histological study. Internat. J. Fertil. IX(1): 35-39.

Marion, G. B., H. T. Gier and J. B. Choudary. 1968. Micromorphology of the bovine ovarian follicular system. J. Animal Sci. 27: 451-465.

Nellor, J. E., and H. H. Cole. 1957. The influence of exogenous progesterone on follicular development and pituitary hormonal content of beef heifers. J. Animal Sci. 16: 151-157.

Rowson, L. E. 1951. Methods of inducing multiple ovulation in cattle. J. Endocrinol. 7: 260.

Toth, F. 1964. The effects of synthetic progestins on the ovaries. Internat. J. Fertil. 9: 151-153.

Trimberger, G. W., and W. Hansel. 1955. Conception rate and ovarian function following estrus control by progesterone injections in dairy cattle. J. Animal Sci. 14: 224.

Ulberg, L. C., R. E. Christian, and L. E. Casida. 1951. Ovarian response in heifers to progesterone injections. J. Animal Sci. 10: 752-759.

Wiltbank, J. N., D. R. Zimmerman, J. E. Ingalls, and W. W. Rowden. 1965. Use of progestational compounds alone or in combination with estrogen for synchronization of estrus. J. Animal Sci. 24: 990.

Zimbelman, R. G. 1963. Inhibition of estrus in ewes with oral progesterons. J. Animal Sci. 22: 868 (abstr.).

Zimbelman, R. G., and L. W. Smith. 1966a. Control of ovulation in cattle with melengestrol acetate. I. Effect of dosage and route of administration. J. Reprod. Fert. 11: 185-191.

Zimbelman, R. G., and L. W. Smith. 1966b. Control of ovulation in cattle with melengestrol acetate. II. Effects on follicular size and activity. J. Reprod. Fert. 11: 193-201.

EFFECTS OF MELENGESTROL ACETATE (MGA)

ON VESICULAR FOLLICLES IN THE BOVINE OVARY

by

Francisco Rogelio Cuevas-Correa

D.V.M., National Autonomous University of Mexico, 1963

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy and Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1969

One group of 3 and one of 33 normally cycling cows were treated respectively with melengestrol acetate .5, 1.0 mg during 14 days and one group of 3 was treated with 1.0 mg during periods of 14 and 10 days separated by an interval of 11 days.

Ovaries were recovered immediately after slaughter and perfused with or immersed in Bouin's fixative. Slides with serial sections of the ovaries were prepared and stained with Periodic Acid Schiff-Harris hematoxylin.

The morphological characteristics of normal and atretic follicles of normally cycling cows were used as control to evaluate the effects of MGA on the follicular system of treated cows. The histological pattern of normal and degenerating follicles was used in this study.

Follicles were classified as normals or in stages 1, 2, 3 or 4 of atresia in each one of the following size classes: 0.4-1 mm, 1.1-2 mm, 2.1-4.0 mm, 4.1-8 mm and 8 mm.

An enormous reduction in the number of normal follicles in the 0.4-1.0 mm class occurred in all treated cows. High reduction of normal follicles was observed as the levels of MGA and days in treatment increased. An accentuation of the rate and speed of atresia was observed in all treated groups.

A severe depression on ovarian function of all treated cows was manifested by the reduction of the number of follicles.

The percentage of normal (31%) and atretic follicles (69%) differed slightly from those reported in the literature.

There was a relative increase in the number of normal follicles above 2 mm in the treated cows. No oocytes were recovered from the oviducts of recently ovulated cows. No oocytes were found in serial sections prepared from big preovulatory follicles.

It was concluded that MGA has a highly repressive effect on ovarian function mainly on the formation of early vesicular follicles (0.4-1 mm). This effect was increased as the doses of MGA increased.

MGA accelerated the rate of follicular atresia but seemed to prolong the life span of normal follicles in the 2.1-4 mm and 4.1-8 mm classes.

The repression of the ovarian function and the apparent absence of oocytes in the pre-ovulatory follicles might account for the low fertility rates observed after insemination of cows at the synchronized estrus.

The histological pattern of normally cycling cows as the one used here to evaluate the effects of MGA seems to be useful and efficient to evaluate the effects of exogenous hormones in the follicular system of the bovine ovary.