FOLLICULAR DYNAMICS, ESTRADIOL-17\beta CONCENTRATIONS, AND LUTEINIZING HORMONE RELEASE FOLLOWING NORGESTOMET IMPLANT INSERTION DURING ESTRUS SYNCHRONIZATION WITH MELENGESTROL ACETATE

by

Eric G. Faber

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Animal and Poultry Sciences
(Physiology of Reproduction)

APPROVED:

W.E. Beal, Chairman

G.S. Lewis

R.G. Saacke

W.D. Whittier

May, 1995

Blacksburg, Virginia
FOLLICULAR DYNAMICS, ESTRADIOL-17ß CONCENTRATIONS, AND LUTEINIZING HORMONE RELEASE FOLLOWING NORGESTOMET IMPLANT INSERTION DURING ESTRUS SYNCHRONIZATION WITH MELENGESTROL ACETATE

Eric G. Faber

(Abstract)

The objective of this experiment was to determine whether norgestomet implant insertion following melengestrol acetate (MGA) administration altered LH pulse frequency and follicular dynamics. Multiparous Angus cows were randomly assigned to receive MGA (.5 mg•cow⁻¹•d⁻¹; MGA; n = 14) for 18 d or to receive MGA (.5 mg•cow⁻¹•d⁻¹; MGA-N; n = 11) for 15 d and a norgestomet implant for 4 d beginning on d 15. Ultrasound was used to record images of each ovary in cows beginning on d 8 of MGA administration. On d 16, serial blood samples were collected from all cows in replicate one (MGA, n=6; MGA-N, n=6) for quantification of LH pulse frequency. A persistent, dominant follicle was identified in all cows on d 8 of MGA administration. Forty-three percent and 64% (P > .10) of MGA and MGA-N cows, respectively, initiated a new wave of follicular development during treatment that was the source of the ovulatory follicle. Pulse frequency of LH did not differ between MGA and MGA-N cows or between cows that ovulated a persistent (PERSIST) follicle and those that ovulated a follicle from a new follicular wave (NEW). Growth rate of the ovulatory follicle for the 7 d preceding ovulation was greater in PERSIST than in NEW cows (P < .01). Diameter of the ovulatory follicle on the day preceding ovulation was greater in PERSIST cows than in NEW cows (P < .01). In conclusion, MGA administration caused a persistent follicle to develop, but that follicle was unable to be regressed consistently by supplemental norgestomet administration.
ACKNOWLEDGEMENTS

I thank my graduate committee, Drs. Bill Beal, Greg Lewis, Richard Saacke, and Dee Whittier, for ensuring that meetings were quick and efficient (they usually had places to go and people to see besides me) and for making their queries pertinent and thought-provoking. I recognize my committee chairman, Dr. Bill Beal for quick manuscript revisions (He knew I wasn’t cut out for a long-term, professional career as a Masters student). At a time when pragmatic research is often deemed unworthy to receive funding, Bill Beal accomplishes applied research that results in solid answers for cow-calf producers. If relating more about the applied meaning of several statistical procedures in three hours of one-on-one conversation than could be taught in an entire year of Biometry means someone is an excellent teacher, then Dr. Greg Lewis is an esteemed pedagogue.

I thank Lee Johnson (Physiology group lab tech) for answering questions and repairing equipment that other people could not (this would cover about 90% of my queries and breakdowns). If fixing most American products ever becomes less expensive than buying them new, Lee could become CEO of a multi-million dollar general repair shop. Lee, however, is most appreciated for his interest in and fascination with general life occurrences. It is not unusual, in the course of 15 minutes of conversation with Lee, to learn about topics ranging from the exact temperature of hickory burning in a smokehouse used for curing hams to the repayment policies of the World Bank. Lee packages his expertise in an ego that is smaller than the Intel central processing chips he knows so much about.

I thank the farm crew (Henry Dickerson and Alan Lee) at Catawba for subtly suggesting which cows needed to be culled (because of their dispositions, not because their patterns of follicular dynamics were aberrant) from my study. Their suggestions resulted not only in greater animal welfare, but also kept anyone from realizing that I am capable of becoming angry, and, in some rare instances, swearing.
I thank the graduate students in Dairy Science for accepting me as one of their own (even though I couldn’t participate in any group pictures or be seen publicly with them). Their conversations (at least the ones that occurred near the instigation of social gatherings and, thus, before inebriation) with me were not only intellectually stimulating, but sometimes even bordered on having practical application. During my data analysis, when I issued an SOS they answered questions about SAS, and thereby kept me from being SOL.

One Dairy Science student, Amin Ahmadzadeh, deserves my accolades for providing invaluable assistance for my LH assay. I appreciated not only Amin’s helpful attitude but also his amazing work velocity (watching Amin’s hands while he pipettes is as dizzying as trying to count the number of wing flaps a hummingbird makes in one minute).

Many other people contributed to this thesis and to my development at Virginia Tech that will not be mentioned here. I hope that I showed my gratitude (I usually attempted to) for your help after it was provided.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter I:</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Chapter II:</td>
<td>3</td>
</tr>
<tr>
<td>Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>Progestogens and Estrus Response</td>
<td>3</td>
</tr>
<tr>
<td>Melengestrol Acetate</td>
<td>3</td>
</tr>
<tr>
<td>Progestogen-Estrogen Combinations</td>
<td>4</td>
</tr>
<tr>
<td>Progesterone Releasing Intravaginal Devices</td>
<td>5</td>
</tr>
<tr>
<td>Progestogen-Prostaglandin Combinations</td>
<td>6</td>
</tr>
<tr>
<td>Fertility Following Progestogen Synchronization Systems</td>
<td>8</td>
</tr>
<tr>
<td>Stage of the Estrous Cycle When Progestogen Treatment Begins</td>
<td>9</td>
</tr>
<tr>
<td>Physiological Changes Following Progestogen Treatment</td>
<td>10</td>
</tr>
<tr>
<td>Persistent Follicles and Fertility</td>
<td>12</td>
</tr>
<tr>
<td>Progestogens and Luteinizing Hormone Secretion</td>
<td>15</td>
</tr>
<tr>
<td>Chapter III:</td>
<td>20</td>
</tr>
<tr>
<td>Statement of the Problem</td>
<td>20</td>
</tr>
<tr>
<td>Chapter IV:</td>
<td>21</td>
</tr>
<tr>
<td>Follicular Dynamics, Estradiol-17β Concentrations, and Luteinizing Hormone Release Following Norgestomet Implant Insertion During Estrus Synchronization With Melengestrol Acetate</td>
<td>21</td>
</tr>
<tr>
<td>Introduction</td>
<td>21</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>23</td>
</tr>
<tr>
<td>Real-Time Ultrasound Examination</td>
<td>24</td>
</tr>
<tr>
<td>Daily Blood Sampling</td>
<td>24</td>
</tr>
<tr>
<td>Serial Blood Sampling for Luteinizing Hormone</td>
<td>24</td>
</tr>
<tr>
<td>Assays for Progesterone, Estradiol-17β, and Luteinizing Hormone</td>
<td>25</td>
</tr>
<tr>
<td>Statistical Analyses</td>
<td>26</td>
</tr>
<tr>
<td>Results</td>
<td>28</td>
</tr>
<tr>
<td>Discussion</td>
<td>42</td>
</tr>
<tr>
<td>Implications and Summary</td>
<td>53</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Least squares means for interval from treatment to estrus for cows treated with MGA-N or MGA</td>
<td>29</td>
</tr>
<tr>
<td>2. Number (percentage) of cows treated with MGA-N or MGA that ovulated a new (NEW) or persistent (PERSIST) follicle following treatment</td>
<td>29</td>
</tr>
<tr>
<td>3. Least squares means for interval from treatment to estrus for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle</td>
<td>29</td>
</tr>
<tr>
<td>4. Number (percentage) of cows that ovulated a persistent (PERSIST) or new (NEW) follicle with an ovulatory follicle was identifiable on d 8 of the experiment</td>
<td>33</td>
</tr>
<tr>
<td>5. Number (percentage) of cows treated with MGA-N or MGA with an ovulatory follicle that was identifiable on d 8 of the experiment</td>
<td>33</td>
</tr>
<tr>
<td>6. Least squares means for day of regression of the persistent follicle and day of emergence of the ovulatory follicle from the new follicular wave for NEW cows treated with MGA-N or MGA</td>
<td>34</td>
</tr>
<tr>
<td>7. Least squares means across MGA-N and MGA treatments for serum estradiol-17β concentrations (pg/mL) on d 8 and d 16 for cows that ovulated a new (NEW) follicle, or a follicle that persisted from d 8 to ovulation (PERSIST)</td>
<td>34</td>
</tr>
<tr>
<td>8. Least squares means for growth rate of the ovulatory follicle for the 6 d preceding ovulation for cows treated with MGA-N or MGA</td>
<td>37</td>
</tr>
<tr>
<td>9. Least squares means for growth rate of the ovulatory follicle for the 6 d preceding ovulation for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle</td>
<td>37</td>
</tr>
<tr>
<td>10. Least squares means for diameter of the ovulatory follicle on the day preceding ovulation for cows treated with MGA-N or MGA</td>
<td>39</td>
</tr>
<tr>
<td>11. Least squares means for diameter of the ovulatory follicle on the day preceding ovulation for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle</td>
<td>39</td>
</tr>
</tbody>
</table>
12. Least squares means for number of LH pulses in 6 h on d 16 for cows treated with MGA-N or MGA. ................................................................. 39

13. Least squares means for number of LH pulses in 6 h on d 16 for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle................................................................. 39
LIST OF FIGURES

Figure page

1. The experimental design for Replicates 1 and 2. Procedures for the two replicates were identical, except for blood collection, which only occurred in Replicate 1. .................................................. 25

2. Follicular dynamics relative to the experimental design for cows treated with MGA-N (a) or MGA (b) that ovulated a follicle that emerged from a new follicular wave (NEW; a) or ovulated a follicle that persisted from d 8 to ovulation (PERSIST; b). Ovulation occurred within 24 h of the last day the new (a) or persistent (b) follicles are depicted ................................................. 31

3. Follicular dynamics relative to the experimental design for cows treated with MGA-N (a) or MGA (b) that ovulated a follicle that persisted from d 8 to ovulation (PERSIST; a) or ovulated a follicle that emerged from a new follicular wave (NEW; b). Ovulation occurred within 24 h of the last day the persistent (a) or new (b) follicles are depicted ................................................. 32

4. Follicular dynamics in cows treated with MGA-N (a) or MGA (b) that regressed their persistent follicle and had their ovulatory follicle emerge from a new follicular wave (NEW) before day 15, the day of implant insertion in cows treated with MGA-N. Ovulation occurred within 24 h of the last day the new follicles are depicted .................................................................................. 36

5. Relationship between diameter of the ovulatory follicle and days preceding ovulation for cows that ovulated a follicle that persisted from d 8 to ovulation (PERSIST; a) or that ovulated a follicle from a new follicular wave (NEW; b). The change in diameter of the ovulatory follicle was greater for NEW cows than for PERSIST cows (P < .01) ........................................................................... 38

6. Patterns of luteinizing hormone release for cows treated with MGA-N (a) or MGA (b) that ovulated a new (NEW; a) or persistent (PERSIST; b) follicle. Pulses are indicated by asterisks. Sampling occurred on d 16, 1 d after the insertion of a norgestomet implant into cows treated with MGA-N ................. 40
7. Patterns of luteinizing hormone release for cows from MGA-N (a) or MGA (b) treatments that ovulated a persistent (PERSIST; a) or new (NEW; b) follicle. Pulses are indicated by asterisks. Sampling occurred on d 16, 1 d after the insertion of a norgestomet implant into cows treated with MGA-N .......................... 41

8. Concentrations of progesterone relative to the experimental design for cows in Replicate 1 treated with MGA-N or MGA................................................................. 43
Chapter I

Introduction

The goal of most cow-calf operations is to produce calf weaning weight at the least possible cost per pound. The use of genetically superior, proven sires not only allows producers to realize a lower cost of production but also adds value to the calves. Sires that are genetically superior for economically important traits are available to all producers through several companies that market semen to be used in artificial insemination (AI).

Artificial insemination is the most practical way to use genetically superior, proven sires in a cost-effective manner. However, less than 5% of the beef cows in the United States are bred artificially each year, primarily because labor, time, and facilities are inadequate to implement AI (Odde 1990).

Synchronization of estrus facilitates the use of genetically superior sires; their semen is available from semen companies. Because females are in estrus at a predetermined time, labor and time requirements to implement AI are greatly reduced. Estrus synchronization involves altering the estrous cycle so that a high percentage of females will exhibit estrus at a fixed time. If genetic improvement from AI is to be facilitated by estrus synchronization regimens, those regimens should elicit a highly synchronized estrus that results in excellent fertility.

Synchronization of estrus also provides females more opportunities to be bred in a breeding season, which can increase pregnancy rate and, thus, reduce costs that result from maintaining nonpregnant or late-pregnant cows. For example, if a breeding season is 63 days, cows in estrus on day 1 would have four opportunities to become pregnant during the breeding season. If an estrus synchronization regimen was not used, cows not
in estrus by d 1 of the breeding season (approximately 95%) would only have 3 opportunities to become pregnant.

In general, estrus is synchronized by using prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) or progestogens. Prostaglandin F$_{2\alpha}$ is effective only in cows that are cycling and that are between d 5 and 16 of the estrous cycle at the time of injection. The only way to synchronize all cows is to inject PGF$_{2\alpha}$ twice at a 10- to 13-d interval so that those cows that were less than d 5 or greater than d 16 of the estrous cycle at the first injection will respond to the second injection.

Progestogens inhibit cows from exhibiting estrus and ovulating and thereby, extend the estrous cycle in cows that begin treatment with fewer days left in their estrous cycle than the length of the progestogen treatment. Progestogens are often used for approximately 14 d, and most cows exhibit estrus within 6 d after cessation of treatment. Additionally, progestogens may induce estrus in prepuberal heifers (Gonzalez-Padilla et al., 1975; Short et al., 1976) or in anestrous postpartum cows (Britt et al., 1974; Hall, 1991). If some of these acyclic cows exhibit estrus and become pregnant, overall pregnancy rates will be increased, compared with estrus synchronization programs that only use PGF$_{2\alpha}$.

This review focuses on the limitations of estrus synchronization regimens that use progestogens and the physiological changes associated with extended estrous cycles. The review will describe some protocols that have been designed to circumvent problems associated with estrus synchronization with progestogens.
Chapter II

Literature Review

Progestogens and Estrus Response

*Melengestrol Acetate*:

Melengestrol acetate (MGA; 6-methyl-17-α-acetoxymethylene-pregna-4,6-diene-3,20-dione), is an orally-active progestogen developed in 1962 that is approved for suppression of estrus in feedlot heifers. Numerous references from the early 1970's are available detailing the use of MGA to synchronize estrus (Zimbelman and Smith 1966; Zimbelman et al., 1970; Hill et al., 1971). In general, .5 to 1 mg·cow⁻¹·d⁻¹ MGA was fed for approximately two-thirds of the length of the estrous cycle and cows were observed for estrus and inseminated following MGA removal. In one study, cows were fed .5 or 1 mg·cow⁻¹·d⁻¹ for 14 d beginning on d 4 or 15 of the estrous cycle (Hill et al., 1971). When treatment began on d 4, 80% of the cows exhibited estrus within 10 d following the removal of MGA. Conversely, only 53% of those cows that began treatment on d 15 exhibited estrus during the same period. Of all cows that exhibited a synchronized estrus, those that received .5 and 1 mg·cow⁻¹·d⁻¹, respectively, were in estrus an average of 4.8 (range = 3-7 d) and 7.3 d (range = 5-10 d) after the last day of MGA administration (Hill et al., 1971).

Because higher levels of MGA caused a longer interval from the end of treatment to estrus and because the minimal effective daily dose to inhibit ovulation was .42 mg (Zimbelman and Smith, 1966), most subsequent research involved feeding .5 mg·cow⁻¹·d⁻¹ of MGA. In the Hill et al. (1971) study, estrus response was lower if MGA feeding began on d 15 of the estrous cycle compared to d 4. Henricks et al. (1973) reported that only 56% of the heifers fed MGA beginning on d 15 of the estrous cycle exhibited a synchronized estrus. Guthrie et al. (1970) suggested that the cause of this reduced estrus
response was that one large follicle became partially luteinized and possibly cystic when MGA feeding began coincident with or after natural luteolysis. Additionally, Coleman et al. (1990) noted that fewer cows were in estrus in a 7 d period after 21 d of MGA feeding compared with control cows that received one injection of PGF$_{2\alpha}$. Conversely, Zimbelman et al. (1970) reviewed 15 studies in which MGA was administered and concluded that the number of cows in estrus following MGA feeding was at least equal to untreated controls in 14 of 15 studies. However, Zimbelman et al. (1970) used a liberal definition of a synchronized estrus (within 20 d following the last MGA feeding).

_Progestogen-Estrogen Combinations:_

Estrogens are luteolytic when administered < d 9 of the estrous cycle (Wiltbank et al., 1961). Estrogen administration can be combined with short term (<14 d) progestogen treatments to effectively synchronize estrus. The knowledge that estrogen was luteolytic, combined with the discovery that long-term progestogen treatments reduced estrus response, led to the development of the synchronization system now marketed as SYNCRO-MATE-B (SMB). In this system 5 mg of estradiol valerate and 3 mg of norgestomet are injected concomitant with the insertion of a 6 mg norgestomet implant, which is removed 9 d later. When cows are treated with estrogen at the beginning of progestogen exposure, those cows that are < d 9 of the cycle should regress their corpora lutea while the implant is in place and exhibit estrus after short-term progestogen exposure is terminated. If cows are > d 9 of the cycle, progestogen exposure for 9 d should allow them to regress their corpora lutea naturally and exhibit estrus within 3 d after norgestomet implant removal. In trials conducted by Wiltbank and Gonzalez-Padilla (1975), 5 d estrus response rates ranged from 77 to 100% in treated heifers. Similarly, high estrus rates have been reported in cyclic postpartum cows that had the same treatment (see Odde, 1990).

Brink and Kiracofe (1988) reported that the interval to estrus and the percentage of cows that exhibited a synchronized estrus did not differ when cows began SMB
treatment early (< d 12) or late (> d 12) in the estrous cycle. However, other researchers have reported that estrus response was reduced when SMB treatment began prior to d 5 of the estrous cycle. Fanning et al., (1992) inserted norgestomet implants and injected 5 mg of estradiol valerate to cows on d 2 of the estrous cycle and reported that 58 and 63%, respectively, of cows injected with 3.0 and 4.5 mg of norgestomet exhibited estrus within 5 d after implant removal. The percentage of cows in estrus in this study is numerically lower than in most other reports. In a similar experiment, cows received norgestomet implants concomitant with an injection of estradiol valerate on d 3 or 9 of the estrous cycle. Cows treated on d 9 exhibited a higher estrus response at 48 (82 vs 46%) and 96 h (91 vs 55%) after implant removal (Pratt et al., 1991). In both of these studies, more cows that did not exhibit estrus had progesterone values > 1 ng/mL than cows that exhibited estrus. The results of Fanning et al., (1992) and Pratt et al., (1991) are the only reports of reduced estrus response after SMB treatment, and both of these groups reported that estrus response was reduced in cows that began treatment early in the cycle.

*Progesterone Releasing Intravaginal Devices:*

Progestogens or progesterone can also be applied to a sponge or pessary that is placed in the vagina to release the progestogen slowly. One such device, a Progesterone Releasing Intravaginal Device (PRID) has been used to synchronize estrus (Roche 1976; Wehrman et al., 1993). In the study by Wehrman et al. (1993), cows were injected with PGF$_{2\alpha}$ on the day of PRID insertion to remove the effects of the corpus luteum on follicular dynamics. Most cows or heifers that received 1 or 2 PRID for 10 d exhibited estrus within 7 d after PRID removal (Wehrman et al., 1993). Estrus response was 98 and 94% for cows that had 1 or 2 PRID, respectively, and was 88 and 85% for heifers that had 1 or 2 PRID, respectively. Cows and heifers with only one PRID exhibited estrus earlier than cows and heifers with two PRID (45 vs 60 h; Wehrman et al., 1993). Roche (1976) performed a trial in which cows and heifers were fitted with an intravaginal
coil containing either 3.1 g or 2.1 g of progesterone. At the time of coil insertion all cows were administered 5 mg estradiol benzoate and 50 mg of progesterone. Estrus response rate was 90 and 93% for cows and heifers, respectively. The majority of cows exhibited estrus within 2 d after coil removal (Roche 1976). Nearly all studies with PRID or SMB have reported shorter and more highly synchronized intervals to estrus than have been reported from studies utilizing long-term administration of MGA.

Progestogen-Prostaglandin Combinations:

Combining short-term (< 9 d) progestogen treatment with PGF$_{2a}$ to synchronize estrus has been used by administering PGF$_{2a}$ injections on either the last day or next to last day of progestogen exposure (Whittier et al., 1986; Hall, 1991). These protocols reduce the time of progestogen required to synchronize estrus in a high proportion of cows. Prostaglandin F$_{2a}$ is not effective prior to d 5 of the estrous cycle (see Odde 1990). If progestogens are administered for 7 d to block ovulation, no cows will be early in the cycle when PGF$_{2a}$ is injected at the end of progestogen exposure. Hence, the estrus response to a single PGF$_{2a}$ injection should be high. Additionally, those problems that result from feeding MGA for 14 d to cows that are late in the cycle should be reduced.

Chenault et al. (1990) fed MGA for 7 d and injected PGF$_{2a}$ on the last d of MGA feeding. In the 6 d following the PGF$_{2a}$ injection, 72% or 52% of heifers exhibited estrus that were fed MGA with a PGF$_{2a}$ injection on the last day or only given a PGF$_{2a}$ injection, respectively. Hall (1991) administered PRID to cyclic cows and heifers for 7 d and injected PGF$_{2a}$ concomitant with PRID removal. In the 7 d following PRID removal, 81% of cows exhibited estrus vs 23% of untreated controls. Similarly, Beal (1983) fitted lactating cows with PRID for 7 d and administered PGF$_{2a}$ 1 d before PRID removal. Sixty-three percent of PRID-treated cows exhibited estrus within 7 d following PRID removal vs only 28% of controls treated with two PGF$_{2a}$ injections 11 d apart or 10% of untreated controls in the same time period (Beal, 1983).
Norgestomet implants have also been used in conjunction with PGF$_{2\alpha}$ to synchronize estrus. Whittier et al. (1986) treated heifers with norgestomet implants for 7 d and injected Alfaprostol®, an analogue of PGF$_{2\alpha}$, concomitant with implant removal. They compared this treatment to the standard SMB treatment. Estrus rates for the 5 d following implant removal were similar between groups (94 vs 98%), but synchrony of estrus was higher in the SMB group. The lower synchrony of estrus in the norgestomet-Alfaprostol® group may have been because the Alfaprostol® was not injected until the time of implant removal. Some heifers had corpora lutea present at the time of Alfaprostol® injection. In these heifers, it is likely that progesterone from their waning corpus luteum inhibited estrus even after norgestomet implant removal. Thus, in heifers with a corpus luteum present at implant removal, the interval from treatment to estrus may have been longer than for heifers without a corpus luteum at the end of treatment. Heersche et al. (1979) inserted norgestomet implants for 7 d and injected PGF$_{2\alpha}$ on the day of or 24 h prior to implant removal in 281 heifers. Thirty-six hours after implant removal 55 and 36% of heifers that were injected with PGF$_{2\alpha}$ 24 h before or at the time of implant removal were in estrus, respectively. To further demonstrate that presence of a corpus luteum was the limiting factor in the timing of estrus, they reported that the estrus response within 26 h after implant removal was 5.7 and 51.7%, respectively, for heifers that had a corpus luteum and those that did not at the time of implant removal (Heersche et al., 1979).

Synchronization of estrus following long term (>14 d) MGA or PRID treatment has been high, except in cases in which > 0.5 mg·cow$^{-1}$·d$^{-1}$ MGA was fed or in some studies in which MGA feeding began late in the estrous cycle. SYNCRO-MATE-B and other progestogen treatments that are coupled with estradiol-17β as a luteolysin have allowed synchronization rates to remain high, but with shorter times of progestogen exposure. Additionally, short-term (7 to 9 d) MGA, norgestomet, or PRID treatments have all resulted in high estrus response rates when used in conjunction with a PGF$_{2\alpha}$ injection at or near the cessation of progestogen exposure.
Fertility Following Progestogen Synchronization Systems

Despite the high rate of synchronization of estrus with progestogens, fertility at the estrus following progestogen treatment has been reduced compared with control cows. Zimbelman et al. (1970) summarized 24 studies in which MGA was fed for 10 to 18 d and reported that fertility in the cows synchronized with MGA was reduced by 30%. The reduction in fertility was confined to cows that were in estrus within 10 d following treatment cessation. In fact, 28-d pregnancy rates were greater for MGA-treated cows than for untreated controls (Zimbelman et al., 1970). Long-term MGA feeding periods (> 14 d) caused a greater reduction in fertility than shorter MGA feeding periods. Coleman et al. (1990) fed MGA for 21 d and reported conception rates to be reduced by 47%, compared with control cows injected with PGF$_{2\alpha}$. However, when MGA feeding periods were shortened, the reduction in fertility compared with controls was less severe than with long-term MGA feeding periods. Chenault et al. (1990) demonstrated that MGA feeding for 7 d followed by PGF$_{2\alpha}$ injected on the last day of MGA feeding caused only an 11% reduction in fertility. Patterson et al. (1989) reported a 14% reduction in fertility in heifers that were fed MGA and injected with fenprostalene (a PGF$_{2\alpha}$ analogue) on the last day of MGA feeding compared with control heifers. Synchronization trials with other progestogens have yielded similar results when treatments of long and short duration were compared. Roche (1974) noted that fertility following 18 or 21 d of a progesterone ear implant was 20% lower than that of heifers that received a progesterone ear implant for only 9 or 12 d.
Stage of the Estrous Cycle When Progestogen Treatment Begins:

The antifertility effect associated with progestogen seems to be related to the stage of the estrous cycle when treatment begins. Specifically, short-term progestogen treatment caused a reduction in fertility when the treatment was initiated late, rather than early in the estrous cycle. Brink and Kiracofe (1988) divided SMB-treated heifers into groups that began SMB treatment either early (< d 12) or late (> d 12) in the estrous cycle and reported that conception rates were 46 and 36% for cows that began treatment early or late in the cycle, respectively. Patterson et al. (1989) fed MGA for 7 d, administered fenprostalene on the last day of MGA feeding, and recorded a 51% reduction in fertility for cows that began treatment later than d 12 of the estrous cycle compared with heifers that began treatment before d 12. In another study, conception rates were reduced by 30% for heifers that began a 7 d MGA treatment on or after d 14 of the estrous cycle compared with those that began treatment before d 14 (Beal et al., 1988).

Research trials that evaluated the interaction between stage of the estrous cycle and fertility following progestogen treatment help to explain the reduction in fertility after short-term progestogen treatments. That fertility is intermediate between that of untreated control cows and that of long-term progestogen-treated cows. Cows that began treatment early in their estrous cycle so that their estrous cycle was not extended did not have a reduction in fertility compared to untreated control cows. Conversely, cows that begin short-term progestogen treatments late in their estrous cycle exhibited estrus later than they would have without progestogen treatment (their estrous cycles were extended). The fertility of these cows with extended estrous cycles was lower than fertility of cows that did not have their cycle extended. The reduced fertility observed when short-term progestogen treatment is begun late in the cycle is comparable to the fertility of long-term progestogen-treated cows.
Physiological Changes Following Progestogen Treatment:

Numerous studies have reported a reduction in fertility of cows that began progestogen treatment late in the estrous cycle and had their estrous cycles extended, but the reason for this reduction in fertility is not understood entirely. Hill et al. (1971) fed MGA to heifers beginning on d 4 or 15 of the estrous cycle and noted that proestrus was prolonged by 3 d. This unusually long proestrus was accompanied by cervical mucus secretion similar to that which occurs at estrus. All control heifers had cervical mucus secretion that reached its highest level coincident with standing estrus. However, only 39% of MGA-treated heifers exhibited standing estrus coincident with maximal cervical mucus secretion. Of those cows that began MGA on d 15 of the cycle, only 23% exhibited standing estrus coincident with maximal cervical mucus secretion (Hill et al., 1971). They speculated that the reason for the extended proestrus period and premature mucus secretion was related to their discovery of one large follicle present in a higher proportion on the ovaries of cows fed MGA for 14 d compared with untreated control cows (Hill et al., 1971). Zimbelman and Smith (1966) noted that there was an increased incidence of one large, estrogen-active follicle in heifers fed MGA. Other studies from the 1970's verified that the large follicle produced more estradiol-17β than would be produced by a normal preovulatory follicle during a normal estrous cycle and that the preovulatory increase in estradiol-17β was precocious (Henricks et al., 1973; Randel et al., 1973).

Sequentially monitoring ovaries with transrectal real-time ultrasound has allowed researchers to determine the daily pattern of growth and regression of individual follicles (Pierson and Ginther, 1988). Ultrasound has been used to determine that follicles develop and regress in waves, with each wave lasting approximately 10 d. Each wave has a follicle that becomes dominant and suppresses the development of other subordinate follicles during that wave (Pierson and Ginther, 1988). At the end of one wave and the beginning of the next, the dominant follicle of the first wave regresses and a new follicle from the next cohort is “selected” as the next dominant follicle (Ireland,
1987). Usually, two to four waves occur during an estrous cycle. In cows that ovulate a single follicle (i.e., those that do not have multiple ovulations), the last wave of follicular development within each estrous cycle culminates with the ovulation of the dominant follicle from that wave.

The use of ultrasound allowed researchers to conclusively determine that one large follicle remained dominant when cows without a corpus luteum were treated with progestogens. The dominant follicle remaining during progestogen treatment has been termed persistent, because it does not regress and continues to suppress the development of subordinate follicles.

Jones et al. (1989) treated heifers with SMB at random stages of the estrous cycle and noted that 77% of heifers developed one persistent follicle (>10 mm) during SMB treatment. That persistent follicle ovulated after SMB treatment. Savio et al. (1993) inserted norgestomet implants into cows on d 8 of the estrous cycle, concomitant with administration of a luteolytic dose of PGF<sub>2α</sub>, and monitored daily follicular development with ultrasound. Implants were removed on d 23 of the extended estrous cycle. In all eight cows, the dominant follicle present when the implant was inserted remained dominant until d 18. In five of eight cows, the dominant follicle remained dominant after implant removal (d 23) and went on to ovulate (Savio et al., 1993).

Sequentially monitoring follicular dynamics with ultrasound, coupled with serum estradiol-17β measurements, has enabled researchers to determine that one follicle became dominant, estrogen-active, and persisted during MGA feeding. Beal et al. (1990) fed MGA for 7 d beginning on d 17 of the estrous cycle. The MGA feeding did not end until cows were on the equivalent of d 24 of the estrous cycle and the average day of ovulation did not occur until the equivalent of d 28 of the estrous cycle. When the estrous cycle was extended, dominant follicle size was 4 mm larger 4 d prior to ovulation than was dominant follicle size for the same cows 4 d prior to ovulation during a normal estrous cycle (Beal et al., 1990). It is likely that the follicle was larger because there were more days from when it reached antral size to its ovulation than in an estrous cycle of normal length. This large, persistent follicle present during MGA feeding also
produced more estradiol-17β than the preovulatory follicle of the cows during the subsequent, normal estrous cycle (Beal et al., 1990). Custer (1992) demonstrated that follicular diameter and estradiol-17β concentration in the 7 d preceding ovulation were greater in cows fed MGA for 7 d beginning on d 17 of their estrous cycle than in untreated control cows. Savio et al. (1993) noted that estradiol-17β was higher in the days preceding ovulation and reached preovulatory levels earlier in norgestomet-treated cows that ovulated a persistent follicle than in another group of cows that ovulated a follicle that was not persistent.

Progestogen treatments that begin late in the estrous cycle result in reduced fertility at the estrus following treatment cessation. When treatments begin late in the cycle, the estrous cycle is extended, and one follicle becomes dominant and persistent until its ovulation following treatment cessation. The persistent follicle results in a precocious and sustained increase in estradiol-17β prior to ovulation that, in one study, was related to a lengthened period of proestrus.

**Persistent Follicles and Fertility:**

A dominant follicle becomes persistent in cows, without a corpus luteum, treated with progestogens for ≥ 14 d. Using a model with rats that are ovulatory delayed, the persistent dominant follicle and its increased secretion of estrogen may partially explain the reduction in fertility in progestogen-treated, ovulatory delayed cattle. Butcher et al. (1975) induced ovulatory delay in rats by giving sodium pentobarbital on the day of proestrus and reported that preovulatory concentrations of estradiol-17β were produced earlier relative to ovulation than in control rats. This phenomenon also occurs in aged rats, which naturally exhibit longer estrous cycles than young rats. Peluso et al. (1974) noted that, on the day of ovulation, estradiol-17β was higher, the number of antral follicles lower, and the diameter of the preovulatory follicle larger in aged rats, which exhibited longer estrous cycles.
Oocyte fertilization rates in cows receiving long-term progestogen treatments are low. Henricks et al. (1973) fed MGA for 14 d beginning on d 15 of the estrous cycle and reported that more uncleaved or unfertilized ova were recovered from MGA-treated cows than from untreated control cows. These results are supported by those of Hill et al. (1971), who demonstrated that heifers fed MGA for 14 d had fewer fertilized ova and more unrecovered ova than untreated controls. Wordinger et al. (1976) recovered 50% fewer fertilized ova from heifers fed MGA than from untreated control heifers. In this study, ovulation was not affected by MGA treatment because ova were recovered from all treated and control cows. Conversely, Hill et al. (1971) reported that heifers fed MGA sometimes did not ovulate. They indicated that heifers fed MGA for 14 d did not yield ova 100% of the time. The results of Hill et al. (1971) may be explained by Guthrie et al. (1970), who reported that heifers fed MGA for 14 d formed a partially luteinized follicle that did not ovulate following MGA removal.

Wishart and Young (1974) treated heifers with daily injections of norgestomet and collected ova 4 d after mating. They detected no difference in ova recovery or ova fertilization rate among cows treated with norgestomet for nine consecutive days, 21 consecutive days, or untreated control heifers. However, there were more embryos at the 2-cell or 4-cell stage in the 21-d group than in the 9-d or control group. In the 9-d and control groups, all embryos were at the 8-cell stage (Wishart and Young, 1974). In a subsequent study, more embryos that had delayed cleavage rates were recovered 3 and 4 d after mating from 21-d norgestomet-treated than from untreated control heifers (Wishart, 1977).

The detection of delayed embryo cleavage rates in progestogen-treated cows are corroborated by studies in the rat that suggest a link between increased levels of estradiol-17β and reduced embryo development rate. Butcher and Pope (1979) reported that embryo development was reduced and abnormalities increased at d 4 and 11 of gestation in rats that had ovulation delayed by 48 h with sodium pentobarbital. When ovulatory-delayed rats were treated with estradiol-17β antiserum, embryo development at d 4 and 11 was equal to controls and there was not an increased level of abnormalities.
(Butcher and Pope, 1979). However, when ovulatory-delayed rats were treated with estradiol-17β antiserum and diethylstilbestrol (synthetic estrogen), the same detrimental effects seen in ovulatory-delayed rats not treated with estradiol-17β antiserum were observed (Butcher and Pope, 1979). These findings indicate that increased concentrations of estradiol-17β, for an extended time before ovulation, either directly or indirectly cause embryo development rates to be reduced.

Reduced embryo cleavage rates in progestogen-treated cows, while related to a prolonged increase in estradiol-17β, may be further explained by Wordinger et al. (1971). They demonstrated that surface and glandular epithelial height in the uterus was increased in cows fed MGA for 15 d beginning on d 15 of the estrous cycle. Glycogen was reduced in these cells, which they speculated could decrease energy for the embryo. Additional explanation is offered by Wordinger et al. (1976), who fed MGA for 14 d beginning on d 15 of the estrous cycle to determine whether the delayed embryo cleavage (reflected as reduced fertility) in cattle was caused by an interaction between MGA, ovarian changes, and histologic changes of the oviduct. They reported that there were more cytoplasmic and nuclear extrusions in nonciliated oviductal epithelial cells in control heifers than in MGA-treated heifers examined 3 d after mating (Wordinger et al., 1976). Because secretory granules were found within the cytoplasmic extrusions, they speculated that the lack of extrusions may reflect a reduced amount of secretion in the ampullary region of the oviduct in MGA-treated cows and, thus, may be evidence of an abnormal environment for the dividing embryo.

Research with rats has yielded information about abnormalities in the oocyte that result from ovulatory delay, which may serve as a model for understanding progestogen-induced ovulatory delay in cattle. Peluso and Butcher, (1974) delayed ovulation in rats for 48 h with sodium pentobarbital and collected oocytes 5 to 8 h after ovulation and compared ultrastructural characteristics of oocytes from delayed and non-ovulatory delayed young rats. Control oocytes were characterized as having evenly distributed cortical granules (synthesized from golgi complexes) beneath the plasma membrane while ovulatory-delayed oocytes had empty golgi vesicles with no evidence of cortical granule
synthesis (Peluso and Butcher, 1974). Furthermore, there were fewer cortical granules attached to the plasma membrane in oocytes of ovulatory-delayed rats, which would lead to a greater chance of polyspermy. Another abnormality associated with ovulatory-delayed oocytes was the occurrence of shelf-like cristae on the mitochondria. This type of mitochondria was not observed in control oocytes, but is commonly observed in 8-cell rat embryos (Peluso and Butcher, 1974). Their results lend evidence to the belief that the metabolic signals in ovulatory-delayed oocytes are premature relative to ovulation. Embryos that develop from oocytes that are ovulatory-delayed have an asynchronous rate of chromosomal division and extranuclear development.

**Progestogens and Luteinizing Hormone Secretion**

The mechanisms that allow a dominant follicle to become persistent during progestogen exposure are related to the presence or absence of a corpus luteum. During an estrous cycle of normal length, follicular growth occurs in two to four waves, with each wave having a dominant follicle that develops, suppresses the growth of subordinate follicles, and then regresses or ovulates. The fate of the dominant follicle is related to the status of the corpus luteum. During corpus luteum presence and high progesterone, the dominant follicle regresses and a new follicular wave is recruited. Following corpus luteum regression, instead of regressing, the dominant follicle continues to grow and eventually ovulates.

The mechanisms by which progesterone indirectly causes regression of a dominant follicle and development of a new wave of follicular growth are related to the frequency of luteinizing hormone (LH) pulses from the anterior pituitary. During the luteal phase of the estrous cycle, LH release is characterized as high amplitude and low frequency (Rahe et al., 1980), while during proestrus the frequency of LH pulsatility increases (Schallenberg et al., 1985). When LH pulsatility is reduced, as in the luteal phase, the dominant follicle is likely to regress and a new cohort of follicles will develop from the next follicular wave. To test the hypothesis that progesterone indirectly causes
the regression of a dominant follicle, Custer (1992) inserted PRID in cows for 7 d beginning on d 17 of the estrous cycle. The dominant follicle present at treatment initiation regressed and a newly-selected follicle from the subsequent wave developed and ovulated in each of 11 cows (Custer 1992). Pulse frequency of LH in PRID-treated cows on d 20 was comparable to untreated control cows sampled on d 17 and was lower than the same PRID cows on d 23, one day prior to PRID removal (Custer 1992). Progesterone concentrations released by the PRID were initially high and then gradually declined. Hence, decreased pulsatility of LH on d 20 was caused by the inhibition of LH release by progesterone.

Results reported by Wehrman et al. (1993) support Custer’s (1992) finding that an increased concentration of progesterone was more likely to cause regression of a dominant follicle. Estradiol-17β was reduced in cows without a corpus luteum fitted with two PRID compared to those fitted with only one PRID for 10 d immediately prior to the breeding season (Wehrman et al., 1993). Cows fitted with two PRID had higher levels of progesterone in the plasma and regressed their dominant follicles while cows fitted with one PRID had lower levels of plasma progesterone and maintained a persistent follicle that secreted increased amounts of estradiol-17β. Kojima et al. (1992a) reported that cows without a corpus luteum fitted with two PRID secreted less estradiol-17β than cows fitted with 1 PRID. These results help to indicate that the reason two-PRID cows secreted less estradiol-17β than one-PRID cows was because progesterone was higher and LH pulse frequency was too low in cows treated with two PRID to allow the dominant follicle to become persistent and to secrete abnormally high amounts of estradiol-17β (Kojima et al., 1992a). This suggests a clear link between sub-luteal levels of progesterone released from one PRID, high LH pulsatility, and the development of a persistent, estrogen-active follicle.

Other progestogens do not seem to have the same effect on LH release and follicular turnover as progesterone supplied by a PRID or the corpus luteum. In the absence of a corpus luteum, MGA does not suppress LH pulsatility and therefore allows the dominant follicle to become persistent. Custer (1992) administered MGA for 7 d
beginning on d 17 of the estrous cycle and all cows that had two follicular waves during
the estrous cycle developed a persistent follicle that ovulated after MGA removal (Custer
1992). In cows that had three follicular waves, the prolonged presence of the corpus
luteum likely was the reason that the dominant follicle of the second wave regressed and
the third wave subsequently developed. Indeed, on d 20 MGA-treated cows with two
follicular waves (shorter corpus luteum lifespan), had higher LH pulse frequency than
MGA-treated cows with three follicular waves (longer corpus luteum lifespan; Custer,

Kojima et al. (1992b) investigated the effect of MGA on LH secretion in cows,
without a corpus luteum. Cows were fed .5, 1.0, or 1.5 mg·cow⁻¹·d⁻¹ of MGA in the
absence of a corpus luteum, and LH pulse frequency was compared to that of untreated
control cows with a corpus luteum. Luteinizing hormone pulse frequency was higher in
MGA-treated cows than in controls (Kojima et al., 1992b). Despite administration of
levels of MGA three times that required to inhibit ovulation, LH pulsatility was not
inhibited in the group that received 1.5 mg·cow⁻¹·d⁻¹ of MGA.

Norgestomet implants administered to cows on d 8 of the estrous cycle
concomitant with a luteolytic dose of PGF₂α caused a persistent follicle to develop that
went on to ovulate after implant removal in five of eight cows (Savio et al., 1993). The
persistent follicle developed as a result of increased LH pulsatility, as norgestomet-
treated cows had higher pulsatility on d 10 than cows with a corpus luteum present (5.2
vs .8 pulses / 8h; Savio et al., 1993). When Savio et al. (1993) replaced the original
implants that were inserted on d 8 with a new norgestomet implant after d 18, all eight
cows regressed their persistent follicle and developed a new follicular wave that produced
the eventual ovulatory follicle (Savio et al., 1993). One day after the insertion of the new
implant, LH pulsatility was higher in the group that retained their original implant than in
the group that had their original implant replaced (7 vs 3 pulses / 8 h, respectively; Savio
et al., 1993).

It has been inferred from the Savio et al. (1993) study that supplemental
norgestomet provided by replacing a previously inserted implant quickly causes a
reduction in LH pulsatility. In turn, decreased LH pulsatility causes regression of a dominant persistent follicle. Two days after the insertion of an original implant, LH pulsatility was higher in norgestomet-treated cows than in untreated controls (Savio et al., 1993). However, just 24 h after the insertion of a new implant all cows regressed their persistent follicle as a result of the decrease in LH pulsatility. Surprisingly, three cows in the group that retained their original implant had their dominant follicles regress and had a new follicle develop after d 18. It is not known whether this was caused by a reduction in LH pulsatility, or whether the persistent follicle was predestined to regress and be replaced by a follicle from the subsequent follicular wave.

Anderson and Day (1994) performed an experiment to determine if acute progesterone administration could cause regression of a persistent dominant follicle, similar to the effect of norgestomet reported by Savio et al. (1993). Melengestrol acetate was fed to cyclic heifers for 11 d, and PGF$_{2\alpha}$ was administered on d 2. All heifers developed a dominant, persistent, estrogen-active, follicle by the time progesterone (200 mg) or the sesame oil vehicle (control treatment) was injected on d 9 of MGA feeding. In heifers injected with progesterone, the persistent follicle regressed, and a new follicular wave appeared within three days (Anderson and Day, 1994). Additionally, the diameter of the persistent follicle and estradiol-17β concentrations decreased immediately in the group injected with progesterone. In contrast, the dominant persistent follicle continued to increase in diameter until its ovulation in heifers fed MGA with no progesterone injection (Anderson and Day, 1994).

The overall picture relating the reduced fertility, development of a persistent follicle, and increased LH pulse frequency of cattle treated with progestogens in the absence of a corpus luteum seems to be clearer. In the absence of a corpus luteum, cows treated with progestogens exhibit high LH pulse frequency and develop a persistent, estrogen-active follicle that usually ovulates following progestogen removal. Progesterone from a corpus luteum or from administration of two or more PRID can block the development of a persistent follicle. Alternatively, after a persistent follicle has developed during progestogen administration, supplemental progestogen (norgestomet or
progesterone) can be added to the original treatment to cause reduced LH pulsatility, regression of the persistent follicle, and the development of a new follicular wave that is the source of the ovulatory follicle.
Chapter III

Statement of the Problem

Estrus synchronization regimens that use progestogens and that begin late in the estrous cycle cause extended estrous cycles, but usually result in a high estrus response and a high synchrony of estrus. However, fertility to insemination at the synchronized estrus is reduced. High LH pulsatility results when progestogens are administered in the absence of a corpus luteum. This high LH pulse frequency causes one dominant follicle to become persistent and secrete an increased amount of estradiol-17β, which is thought to be either the direct or indirect cause of the reduced fertility. One way to avoid the reduced fertility that results after cows ovulate an oocyte from a persistent follicle is to cause cows to regress their persistent follicle and develop a new follicular wave before the synchronized estrus. If the persistent follicle that develops during progestogen treatment can be regressed, the negative effects associated with its persistence will be removed and fertility following progestogen treatment should be improved.
Chapter IV

Follicular Dynamics, Estradiol-17β Concentrations, and Luteinizing Hormone Release Following Norgestomet Implant Insertion During Estrus Synchronization With Melengestrol Acetate

Introduction

The use of progestogens for synchronization of estrus results in reduced fertility to insemination at the estrus following progestogen removal (see Odde, 1990). Specifically, cows that begin short-term progestogen treatment late in the estrous cycle have reduced conception rates to the insemination at the synchronized estrus compared with cows that begin treatment early in the estrous cycle (Brink and Kiracofe, 1988; Patterson et al., 1989). Administration of norgestomet (Savio et al., 1993), melengestrol acetate (MGA; Custer, 1992) or low levels of progesterone from a progesterone releasing intravaginal device (PRID; Kojima et al., 1992a) to cattle without a corpus luteum causes the development of a dominant, persistent, estrogen-active follicle that ovulates following treatment. The presence and ovulation of a persistent follicle has been directly related to lower fertility of cattle treated with progestogens in the absence of a corpus luteum (Stock and Fortune, 1993).

Persistence of a dominant follicle in cattle treated with progestogens, in the absence of a corpus luteum, is related to the luteinizing hormone (LH) pulse frequency. Compared with cows with a corpus luteum, pulse frequency of LH increases when low levels of progesterone (Roberson et al., 1989), norgestomet (Savio et al., 1993) or MGA (Kojima et al., 1992b) are administered to cows without a corpus luteum. This increased LH pulse frequency allows a persistent follicle to be maintained from the beginning of progestogen exposure to ovulation following progestogen removal.

If the pulse frequency of LH is reduced during progestogen treatment, the persistent follicle that had developed will regress and be replaced by an ovulatory follicle
from a new wave of follicular development. Savio et al. (1993) inserted norgestomet implants into cows without a corpus luteum and replaced the implants 10 d following their insertion. The pulse frequency of LH was lower 24 h after the insertion of a new implant compared to cows that did not have their original implant replaced (Savio et al., 1993). Additionally, all cows in the group that had their implant replaced regressed the persistent follicle that had developed while the original implant was in place and developed an ovulatory follicle from a new follicular wave. Similarly, cows regressed their persistent follicle that had developed during MGA feeding and had the ovulatory follicle emerge from a new follicular wave after progesterone was injected during MGA feeding (Anderson and Day, 1994). Therefore, the objective of this experiment was to determine the effects of a norgestomet implant inserted after 15 d of MGA administration on 1) the ovulation of a persistent follicle or the emergence of an ovulatory follicle from a new follicular wave, 2) the pattern of LH release, and 3) serum concentrations of estradiol-17β.
Materials and Methods

The experiment was conducted in two replicates with nonlactating (Replicate 1; n=12) or lactating (Replicate 2; n=15) multiparous, Angus or Angus-crossbred cows. In Replicate 1, pretreatment estrous cycles were synchronized by inserting a PRID (Sanofi Animal Health, Paris, France) for 7 d and administering two i.m. injections of PGF$_{2\alpha}$ (12.5 mg and 12.5 mg; Lutalaye; Upjohn Co., Kalamazoo, MI) 12 h apart on the day before PRID removal. In Replicate 2, pretreatment estrus was synchronized with one injection of PGF$_{2\alpha}$ (25 mg). Beginning on d 9 or 10 of the estrous cycle, cows were administered a gelatin capsule bolus daily containing MGA (17α-acetoxy-6-methyl-16-methylene-4, 6-prenadiene-3, 20-dione; Upjohn, 5 mg•cow$^{-1}$•d$^{-1}$; Figure 1). On d 15 of MGA administration, 11 cows from Replicates 1 and 2 were randomly assigned to receive a norgestomet implant (6 mg; Sanofi) for 4 d (MGA-N). The implant was inserted on the day (d 15) that the last MGA bolus was administered to ensure that cows would not exhibit estrus and ovulate between MGA treatment and implant insertion. Fourteen cows from Replicates 1 and 2 were administered MGA daily for 18 d (MGA) but did not receive a norgestomet implant. Following the cessation of MGA administration (d 18) in MGA cows and the removal of the norgestomet implants (d 19) in MGA-N cows, four times daily observations for estrus were begun. The interval to estrus was defined as the time from the removal of the norgestomet implant in group MGA-N or 24 h following the last MGA administration in group MGA. Two cows were not detected in estrus within 12 d following treatment and their data were not included in the analysis.
Real-Time Ultrasound Examination:

On d 8 of MGA administration, ovaries were monitored with a real-time, B-mode ultrasound machine (Aloka 500-V; Corometrics Medical Supply, Inc., Wallingford, CT) equipped with a 5.0 MHz linear-array transrectal transducer. Beginning 12 d after the initiation of MGA treatment, ovaries were monitored daily with ultrasound until each cow ovulated (Figure 1). Images were recorded and ovarian maps were drawn characterizing the location and diameter of all follicles >5 mm present on both ovaries each day. For all cows, the ovulatory follicle was determined to be the follicle identified on d 8, the first ultrasound evaluation (PERSIST) or from a new wave of follicular development that emerged after the first ultrasound evaluation (NEW).

Daily Blood Sampling:

Blood samples (15 mL) were collected from the jugular vein daily from cows in Replicate 1 (6 MGA-N, n=6; MGA, n=6) beginning on d 1 of MGA administration and concluding when the last cow was observed in estrus (Figure 1). Samples were allowed to clot at 22°C for approximately 4 h and were centrifuged at 1,000x g for 15 min. Serum was harvested and frozen at -25°C until radioimmunoassayed for progesterone and estradiol-17β.

Serial Blood Sampling for Luteinizing Hormone:

In Replicate 1, frequent blood samples were collected from all cows (MGA, n = 6; MGA-N, n = 6) for determination of LH pulse frequency. Serial blood sampling for later LH quantification began on d 16, 24 h after the norgestomet implant had been inserted into the MGA-N cows. Cows were fitted with an indwelling jugular catheter (14-Ga x 14 cm). A silastic extension tube (61 cm) was attached to the catheter and immediately flushed with 3% sodium citrate. Blood samples were collected at 15-min. intervals for 6 h and were allowed to clot at 22°C for approximately 4 h, then they were stored on ice until being centrifuged at 1,000x g at 4°C for 15 min. Serum was stored at -25°C until radioimmunoassayed for LH.
Figure 1. The experimental design for Replicates 1 and 2. Procedures for the two replicates were identical, except for blood collection, which only occurred in Replicate 1.

**Assays for Progesterone, Estradiol 17β, and Luteinizing Hormone:**

For determination of progesterone concentrations for d 1 to 28 of the experiment, duplicate samples were quantified using a radioimmunoassay described by Gengenbach et al. (1977). For determination of estradiol-17β concentrations on d 8 and 16 of the experiment, quadruplicate samples were quantified using a radioimmunoassay as described by Sirois and Fortune (1990). The sensitivity of the assays were .1 ng/mL for progesterone and 1.5 pg/mL for estradiol-17β. For progesterone, twelve assays were performed and resulted in inter- and intraassay coefficients of variation (CV) of 21.9 and 12%, respectively. Two estradiol-17β assays were performed and resulted in intra- and interassay CV of 18 and 14%, respectively.

Luteinizing hormone was determined as described by Niswender et al. (1969), with some modifications. Purified LH (USDA-bLH-B-6) and serum in assay buffer were incubated with rabbit antiovine serum (USDA-309-684p; 1:35,000 dilution) for 24 h at
22°C. Radiolabeled LH (¹²⁵I; USDA-bLH-B-6) was added and the samples were incubated for an additional 48 h at 4°C. A second antibody (sheep antirabbit gamma globulin serum) was added at a 1:8 dilution to the samples and they were incubated an additional 48 h at 4°C. The sample tubes were then centrifuged for 45 min. at 4°C and the supernatant was decanted to separate bound from free radiolabeled LH prior to counting with a gamma counter. All samples were assayed in duplicate in three assays. The inter- and intraassay CV were 25 and 18%, respectively.

The pulse frequency of LH was defined as described by Walters et al. (1984). An LH pulse was defined as an increased LH concentration that exceeded the previous sample by least four times the CV of the assay and was followed by a decreased LH concentration (Walters et al., 1984).

Statistical Analyses:

Data for number of LH pulses detected in 6 h, estradiol-17β concentrations on d 8 and 16 of the experiment, the interval from removal of implants or from 24 h after cessation of MGA administration to estrus, and the diameter of the ovulatory follicle on the day prior to ovulation, were analyzed with analyses of variance for a completely randomized design using the GLM procedures of SAS (1987). Replicate (1 or 2), treatment (MGA or MGA-N), and source of the ovulatory follicle (NEW or PERSIST) were included as main effects, and all possible interactions were included. Data for estradiol-17β concentrations came only from Replicate 1, therefore replicate was not included in the analysis for estradiol-17β concentrations. Data for the proportion of cows that ovulated a follicle that persisted from d 8 to ovulation (PERSIST) or that ovulated a follicle from a new follicular wave (NEW) by treatment group (MGA-N or MGA) were analyzed with a Z statistic for testing differences between two proportions (Zar, 1984). Data for the proportions of cows with an ovulatory follicle identified on or after d 8 were analyzed with a Z statistic for testing differences between two proportions (Zar, 1984).

The overall shape of the curve (linear, quadratic, cubic, quartic, or pentic) for diameter of the ovulatory follicle was determined for each treatment (MGA-N or MGA)
or each source of the ovulatory follicle (NEW or PERSIST) by using a polynomial regression analysis in which time was included as a covariate with diameter (Allen et al., 1983). Replicate (1 or 2), treatment (MGA-N or MGA) and source of the ovulatory follicle (NEW or PERSIST) were evaluated as main effects and all possible interactions were included. Using the previously determined shapes of the curve for the diameter of the ovulatory follicle for the 6 d preceding ovulation, the profiles were compared by treatment (MGA-N or MGA) or by source of the ovulatory follicle (NEW or PERSIST).
Results

In this experiment, conducted in two replicates, estrus was synchronized by administering MGA to cows for 18 d (MGA) or by administering MGA for 15 d and inserting a norgestomet implant for 4 d beginning on the last day of MGA administration (MGA-N). Eighty-seven percent (25/27) of cows in both treatment groups exhibited estrus and ovulated within 12 d following treatment. Two cows were not detected in estrus within 12 d following treatment and their data were not included in the analysis. The data were analyzed to determine the effect of the two treatments on follicular dynamics, hormonal profiles, and estrus response. In addition, follicular dynamics, the hormonal profiles, and estrus responses of cows that ovulated a follicle that persisted from d 8 to ovulation (PERSIST) or cows that developed an ovulatory follicle from a new follicular wave during treatment (NEW), were analyzed.

There were no interactions (P > .10) between the effects of the treatments (MGA-N or MGA) or ovulatory follicle source (NEW or PERSIST) and replicates for all variables analyzed. Therefore, data were combined across replicates for subsequent analysis. Similarly, there was no interaction (P > .10) between the effect of ovulatory follicle source (NEW or PERSIST) and treatment (MGA-N or MGA) for all variables analyzed. Therefore, data were combined across treatments to determine the effects of ovulatory follicle source.

The interval from the end of treatment to estrus averaged 93.9 ± 6.9 h and did not differ between cows treated with MGA or MGA-N (Table 1). Some cows in each treatment group ovulated a follicle that persisted from the time of the first ultrasound evaluation (d 8) until ovulation. The percentage of cows that ovulated a persistent follicle was not decreased by the MGA-N treatment. In other words, insertion of a norgestomet implant did not consistently initiate a new wave of follicular development during treatment (Table 2). The interval to estrus was shorter (P < .05) in cows that ovulated a persistent follicle (PERSIST) than in cows that ovulated a follicle from a new follicular wave (NEW), regardless of treatment (Table 3).
Table 1. Least squares means for interval from treatment to estrus for cows treated with MGA-N\(^a\) or MGA\(^b\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. cows</th>
<th>Interval to estrus (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-N</td>
<td>11</td>
<td>95.8 ± 11.4</td>
</tr>
<tr>
<td>MGA</td>
<td>14</td>
<td>92.9 ± 9.8</td>
</tr>
</tbody>
</table>

\(^a\) Interval to estrus was defined as the time from norgestomet implant removal to estrus.  
\(^b\) Interval to estrus was defined as the time from 24 h following the last MGA administration to estrus.

Table 2. Number (percentage) of cows treated with MGA-N or MGA that ovulated a new (NEW)\(^a\) or persistent (PERSIST)\(^b\) follicle following treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. NEW cows</th>
<th>No. PERSIST cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-N</td>
<td>7/11 (63)</td>
<td>4/11 (37)</td>
</tr>
<tr>
<td>MGA</td>
<td>6/14 (43)</td>
<td>8/14 (57)</td>
</tr>
</tbody>
</table>

\(^a\) Number of cows that had their ovulatory follicle emerge from a new follicular wave that developed during treatment.  
\(^b\) Number of cows that ovulated a follicle that persisted from d 8 to ovulation.

Table 3. Least squares means for interval from treatment to estrus for cows across MGA-N\(^a\) and MGA\(^b\) treatments that ovulated a persistent (PERSIST) or new (NEW) follicle.

<table>
<thead>
<tr>
<th>Ovulatory follicle source</th>
<th>No. cows</th>
<th>Interval to estrus (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERSIST</td>
<td>12</td>
<td>77.1 ± 11.1(^e)</td>
</tr>
<tr>
<td>NEW</td>
<td>13</td>
<td>111.6 ± 9.9(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Interval to estrus was defined as the time from norgestomet implant removal to estrus.  
\(^b\) Interval to estrus was defined as the time from 24 h following the last MGA administration to estrus.  
\(^c,d\) Least squares means within a column with different superscripts differ (P < .05).

Patterns of follicular development depicting the initiation of a new follicular wave or follicular persistence during treatment with MGA-N or MGA are shown in Figure 2. These patterns represent the \textit{a priori} expectations of cows treated with MGA-N or MGA. Cows treated with MGA-N were expected to initiate a new wave of follicular development, whereas cows treated with MGA were expected to ovulate a persistent follicle that developed during treatment. Of the cows treated with MGA-N,
seven of 11 responded with a new wave of follicular growth during treatment, as depicted for cow K193 in Figure 2a. Figure 2b depicts the follicular dynamics of cow K174, one of eight cows fed MGA that ovulated a persistent follicle.

Patterns of follicular development that were unexpected for cows in the MGA-N or MGA treatment groups are depicted in Figures 3a and 3b, respectively. Cows treated with MGA-N were not expected to ovulate the dominant, persistent follicle initially identified on d 8. However, four of 11 cows treated with MGA-N ovulated a persistent follicle (PERSIST), as depicted for cow M33 in Figure 3a. Conversely, cows treated with MGA were not expected to initiate a new follicular wave during treatment. Six of 14 cows treated with MGA ovulated a follicle that emerged from a new wave of follicular development (NEW), as is depicted for cow M63 in Figure 3b.

Analysis of the follicular dynamics depicted for the cows that ovulated a persistent follicle (PERSIST; Figures 2b and 3a) indicated that the ovulatory follicle was identifiable earlier than in cows that developed a new follicular wave (NEW; Figures 2a and 3b). The ovulatory follicle was detected on d 8, the first day of ultrasound examination, for all cows that ovulated a persistent follicle (PERSIST; Figures 2b and 3a). Therefore, there were more days between its detection and ovulation than for the ovulatory follicle from NEW cows (Figures 2a and 3b).

For all cows that ovulated a persistent follicle (PERSIST), the ovulatory follicle could be identified on d 8, the first ultrasound evalution (Table 4). For those cows that ovulated a follicle from a new follicular wave (NEW), the ovulatory follicle could not be identified on d 8 for any cows (Table 4). The percentage of cows that ovulated a persistent follicle did not differ between MGA-N or MGA treatments (Table 2). Therefore, there were no differences in the proportion of cows treated with MGA-N or MGA that had their ovulatory follicle identified on d 8 (Table 5).
Figure 2. Follicular dynamics relative to the experimental design for cows treated with MGA-N (a) or MGA (b) that ovulated a follicle that emerged from a new follicular wave (NEW; a) or ovulated a follicle that persisted from d 8 to ovulation (PERSIST; b). Ovulation occurred within 24 h of the last day the new (a) or persistent (b) follicles are depicted.
Figure 3. Follicular dynamics relative to the experimental design for cows treated with MGA-N (a) or MGA (b) that ovulated a follicle that persisted from d 8 to ovulation (PERSIST; a) or ovulated a follicle that emerged from a new follicular wave (NEW; b). Ovulation occurred within 24 h of the last day the persistent (a) or new (b) follicles are depicted.
Table 4. Number (percentage) of cows that ovulated a persistent (PERSIST) or new (NEW) follicle with an ovulatory follicle that was identifiable on d 8 of the experiment.

<table>
<thead>
<tr>
<th>Ovulatory follicle source</th>
<th>No. cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERSIST</td>
<td>12 (100)(^a)</td>
</tr>
<tr>
<td>NEW</td>
<td>13 (0)(^b)</td>
</tr>
</tbody>
</table>

\(^a,b\) Proportions within a column with different superscripts differ (P < .01).

Table 5. Number (percentage) of cows treated with MGA-N or MGA with an ovulatory follicle that was identifiable on d 8 of the experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-N</td>
<td>4 (36)</td>
</tr>
<tr>
<td>MGA</td>
<td>8 (57)</td>
</tr>
</tbody>
</table>

For cows that regressed their persistent follicle and ovulated a follicle from a new follicular wave (NEW), the day of regression of the original persistent follicle was defined as the day it began to continuously decrease in diameter. The timing of regression of the persistent follicle and the emergence of the new ovulatory follicle varied among cows. Across MGA-N and MGA treatments, the average day of regression of the persistent follicle was d 15 and the average day of emergence of the ovulatory follicle from the new follicular wave was d 16 (Table 6). Treatment with a norgestomet implant, however, did not seem to cause cows that ovulated a follicle from a new follicular wave (NEW) to regress their persistent follicle earlier or have their new ovulatory follicle emerge earlier than cows treated with MGA. The NEW cows treated with MGA-N or MGA had a similar temporal pattern of dominant follicle regression and follicular emergence (Table 6).

All cows had a dominant, persistent follicle on d 8 of the experiment. However, on average, NEW cows developed a new follicular wave on d 16 of the experiment. Therefore, NEW cows were expected to have lower serum concentrations of estradiol-17\(^\beta\) on d 16 compared to cows that had a dominant follicle persist from d 8 to ovulation (PERSIST). However, estradiol-17\(^\beta\) concentrations on d 8 did not differ between PERSIST and NEW cows (Table 7) but estradiol-17\(^\beta\) concentrations on d 16 were less for NEW than for PERSIST cows (P < .05).
Table 6. Least squares means for day of regression of the persistent follicle$^a$ and day of emergence of the ovulatory follicle$^b$ from the new follicular wave for NEW cows treated with MGA-N or MGA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. cows</th>
<th>Day of regression</th>
<th>Day of emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-N</td>
<td>7</td>
<td>15.5 ± .5</td>
<td>16.1 ± .7</td>
</tr>
<tr>
<td>MGA</td>
<td>6</td>
<td>15.4 ± .6</td>
<td>16.6 ± .8</td>
</tr>
</tbody>
</table>

$^a$ Day relative to the start of MGA administration that the persistent follicle began to continuously decrease in diameter.

$^b$ Day relative to the start of MGA administration that the ovulatory follicle from the new follicular wave could first be identified.

Table 7. Least squares means across MGA-N and MGA treatments for serum estradiol-17β concentrations (pg/mL) on d 8 and d 16 for cows that ovulated a new (NEW) follicle, or a follicle that persisted from d 8 to ovulation (PERSIST).

<table>
<thead>
<tr>
<th>Ovulatory follicle source</th>
<th>No. cows</th>
<th>Day 8</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEW</td>
<td>5</td>
<td>4.7 ± .6</td>
<td>2.5 ± .4$^a$</td>
</tr>
<tr>
<td>PERSIST</td>
<td>7</td>
<td>4.5 ± .8</td>
<td>4.1 ± .4$^b$</td>
</tr>
</tbody>
</table>

$^{a,b}$ Least squares means within a column with different superscripts differ (P < .05).

From Table 6, it has been inferred that some cows from both treatment groups had their ovulatory follicle emerge from a new follicular wave before the time that norgestomet implants were inserted into cows treated with MGA-N. Thirteen cows treated with MGA-N or MGA developed new ovulatory follicles and five of these 13 had a new follicular wave begin prior to the time of implant insertion for cows treated with MGA-N. The follicular dynamics relative to the experimental design are depicted in Figure 4 for cow O2 (MGA-N; Figure 4a) or cow O21 (MGA; Figure 4b).

The growth rate of the ovulatory follicle for the 6 d preceding ovulation did not differ between cows treated with MGA-N or MGA (Table 8). However, the growth rate of the ovulatory follicle for the 6 d preceding ovulation was greater for cows that had their ovulatory follicle emerge from a new follicular wave (NEW) than for cows that
ovulated a follicle that persisted from d 8 to ovulation (PERSIST; P < .01; Table 9). For PERSIST cows the ovulatory follicle was identified on d 8, the first day of ultrasound examination. Therefore, there were more days between the first identification of the ovulatory follicle and ovulation in PERSIST cows than in NEW cows. For PERSIST cows, the ovulatory follicle reached a higher percentage of its ovulatory diameter 6 d preceding ovulation than the ovulatory follicle for NEW cows and, thus, did not grow as rapidly in the 6 d preceding ovulation.

The shape of the curve for ovulatory follicular diameter for the 6 d preceding ovulation for cows that had their ovulatory follicle emerge from a new follicular wave (NEW) and for cows that ovulated a follicle that persisted from d 8 until ovulation (PERSIST) was linear. Figure 5 depicts the regression line for daily change in diameter of the ovulatory follicle for the 6 d preceding ovulation for PERSIST (Figure 5a) or NEW (Figure 5b) cows. The change in diameter was greater for NEW cows than for PERSIST cows (P < .01; Figure 5).

The average diameter of the ovulatory follicle on the day prior to ovulation did not differ between cows treated with MGA-N or MGA (Table 10). The average diameter of the ovulatory follicle on the day preceding ovulation was larger for cows treated with MGA-N or MGA that ovulated a persistent follicle (PERSIST) than for cows that had a new follicular wave emerge (NEW; P < .01; Table 11).

Not all cows that had their ovulatory follicle persist from d 8 until ovulation (PERSIST) or cows that ovulated a follicle from a new follicular wave (NEW) exhibited high or low LH pulsatility, respectively. Figure 7 depicts cow N19 (Figure 7a; MGA-N), who ovulated a persistent follicle and had low LH pulsatility or cow O12 (Figure 7b; MGA), who ovulated a new follicle and had high LH pulsatility.
Figure 4. Follicular dynamics for cows treated with MGA-N (a) or MGA (b) that regressed their persistent follicle and had their ovulatory follicle emerge from a new follicular wave (NEW) before day 15, the day of implant insertion for cows treated with MGA-N. Ovulation occurred within 24 h of the last day the new follicles are depicted.
Table 8. Least squares means for growth rate of ovulatory follicle for the 6 d preceding ovulation for cows treated with MGA-N or MGA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. cows</th>
<th>Ovulatory follicle growth rate (mm•d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-N</td>
<td>11</td>
<td>.85 ± .3</td>
</tr>
<tr>
<td>MGA</td>
<td>14</td>
<td>.75 ± .3</td>
</tr>
</tbody>
</table>

Table 9. Least squares means for growth rate of the ovulatory follicle for the 6 d preceding ovulation for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle.

<table>
<thead>
<tr>
<th>Ovulatory follicle source</th>
<th>No. cows</th>
<th>Ovulatory follicle growth rate (mm•d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERSIST</td>
<td>12</td>
<td>.40 ± .2 *</td>
</tr>
<tr>
<td>NEW</td>
<td>13</td>
<td>1.21 ± .1 b</td>
</tr>
</tbody>
</table>

* *b Least squares means with different superscripts within a column differ (P < .01).

Cows treated with MGA-N or MGA did not differ in number of LH pulses recorded per 6 h on d 16, 1 d after the insertion of norgestomet implants into cows treated with MGA-N (Table 12). Additionally, there were no differences in number of LH pulses per 6 h for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle (Table 13). The LH pulsatility patterns for cow K193 (Figure 6a; MGA-N) or cow K174 (Figure 6b; MGA) are depicted in Figure 6. Cow K193 (Figure 6a) had a low number of LH pulses per 6 h, as was expected for cows treated with MGA-N. Cow K174 (Figure 6b) had a high number of LH pulses per 6 h, as was expected for cows treated with MGA.
a (PERSIST)

![Graph a](image)

b (NEW)

![Graph b](image)

Figure 5. Relationship between diameter of the ovulatory follicle and days preceding ovulation for cows that ovulated a follicle that persisted from d 8 to ovulation (PERSIST; a) or that ovulated a follicle from a new follicular wave (NEW; b). The change in diameter of the ovulatory follicle was greater for NEW cows than for PERSIST cows (P < .01).
Table 10. Least squares means for diameter of the ovulatory follicle on the day preceding ovulation for cows treated with MGA-N or MGA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. cows</th>
<th>Ovulatory follicle diameter (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-N</td>
<td>11</td>
<td>17.2 ± 1.1</td>
</tr>
<tr>
<td>MGA</td>
<td>14</td>
<td>16.1 ± .9</td>
</tr>
</tbody>
</table>

Table 11. Least squares means for diameter of the ovulatory follicle on the day preceding ovulation for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle.

<table>
<thead>
<tr>
<th>Ovulatory follicle source</th>
<th>No. cows</th>
<th>Ovulatory follicle diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERSIST</td>
<td>12</td>
<td>19.2 ± .7*</td>
</tr>
<tr>
<td>NEW</td>
<td>13</td>
<td>14.3 ± .4b</td>
</tr>
</tbody>
</table>

* b Least squares means within a column with different superscripts differ (P < .01).

Table 12. Least squares means for number of LH pulses in 6 h on d 16 for cows treated with MGA-N or MGA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. cows</th>
<th>No. LH pulses in six hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-N</td>
<td>6</td>
<td>2.4 ± .7</td>
</tr>
<tr>
<td>MGA</td>
<td>6</td>
<td>1.8 ± .7</td>
</tr>
</tbody>
</table>

Table 13. Least squares means for number of LH pulses in 6 h on d 16 for cows across MGA-N and MGA treatments that ovulated a persistent (PERSIST) or new (NEW) follicle.

<table>
<thead>
<tr>
<th>Ovulatory follicle source</th>
<th>No. cows</th>
<th>No. LH pulses in six hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERSIST</td>
<td>7</td>
<td>1.8 ± .7</td>
</tr>
<tr>
<td>NEW</td>
<td>5</td>
<td>2.4 ± .7</td>
</tr>
</tbody>
</table>
Figure 6. Patterns of luteinizing hormone release for cows treated with MGA-N (a) or MGA (b) that ovulated a new (NEW; a) or persistent (PERSIST; b) follicle. Pulses are indicated by asterisks. Sampling occurred on d16, one d after the insertion of a norgestomet implant into cows treated with MGA-N.
Figure 7. Patterns of luteinizing hormone release for cows from MGA-N (a) or MGA (b) treatments that ovulated a persistent (PERSIST; a) or new (NEW; b) follicle. Pulses are indicated by asterisks. Sampling occurred on d 16, one d after the insertion of a norgestomet implant into cows treated with MGA-N.
Discussion

Twenty-five of 27 (92.5%) cows exhibited estrus within 12 d following MGA-N and MGA synchronization treatments. Two cows from the MGA-N treatment were not detected in estrus with 12 d following norgestomet implant removal. The estrus response rate in this experiment is comparable with most other reported estrus response rates after long-term MGA feeding (see Zimbelman et al., 1970; see Odde et al., 1990).

The interval from implant removal or from 24 h following the last administration of MGA to estrus did not differ between cows treated with MGA-N or MGA, respectively. The reported value for hours from treatment to estrus for cows treated with MGA is slightly lower than that reported in other studies (Hill et al., 1971; Henricks et al., 1973); however, those studies measured the interval from the last MGA feeding to estrus, not from 24 h after the last MGA feeding to estrus. If compared on an equal basis, the reported values in this study for interval to estrus would have been similar to those reported by Hill et al. (1971) and Henricks et al. (1973).

The interval from norgestomet implant removal to estrus for cows treated with MGA-N in this study was longer than that reported for cows treated with SMB (Whittier et al., 1986; Brink and Kiracofe, 1988) or with a norgestomet implant and PGF$_{2\alpha}$ at or near the time of implant removal (Whittier et al., 1986; Heersche et al., 1979). It should be noted that the time of progestogen exposure was shorter in those studies. In this study, cows without a corpus luteum were administered MGA for 15 d prior to norgestomet implant insertion. Many studies have demonstrated that long-term MGA feeding, especially when initiated late in the estrous cycle, lengthens the interval from the cessation of MGA feeding to estrus (Hill et al., 1971; Henricks et al., 1973; Coleman et al., 1990). The longer interval from treatment to estrus may have been related to the administration of MGA prior to the insertion of the norgestomet implant.

Eight days after the initiation of MGA administration, all cows had a large, dominant follicle. This finding is in agreement with the observations of Hill et al. (1971) and Beal et al. (1990), who demonstrated that when cattle without a corpus luteum are
fed MGA, they develop one large follicle. Figure 8 depicts the progesterone profiles for cows treated with MGA-N or MGA. These profiles demonstrate that progesterone concentrations were low enough to allow MGA administration to cause a follicle to persist.

There were no differences between cows treated with MGA-N or MGA in the proportion of cows that ovulated a follicle from a new wave of follicular development (NEW). This observation is in contrast with the results of Savio et al. (1993), who reported that while cows developed a persistent follicle when treated with a norgestomet implant, all then regressed the persistent follicle and developed a new follicular wave when fitted with a second norgestomet implant. Two differences were apparent between this study and that of Savio et al. (1993). In the experiment conducted by Savio et al. (1993), the first progestogen administered was norgestomet, not MGA. Second, the supplemental norgestomet was administered on d 11 of treatment, which is 4 d earlier than the supplemental norgestomet was administered in this study.

Figure 8. Concentrations of progesterone relative to the experimental design for cows from Replicate 1 treated with MGA-N or MGA.
The inability of supplemental progestogen to consistently cause regression of a persistent follicle in this study also contrasts with the results of Anderson and Day (1994). They reported that all heifers developed a persistent follicle during 11-d MGA feeding, but the persistent follicle then regressed and a new follicular wave emerged after exogenous progesterone was administered. Although Anderson and Day (1994), administered MGA, as was done in this study, they injected progesterone rather than insert a norgestomet implant to cause regression of the persistent follicle. Additionally, they injected progesterone on d 9 of MGA feeding, which is 7 d earlier than the norgestomet implant was administered to MGA-N cows in this study.

The interval from treatment to estrus was shorter for cows that ovulated a follicle that ovulated a follicle that persisted from d 8 until ovulation (PERSIST) than for those that ovulated a follicle from a new wave of follicular development (NEW). These differences can be partially explained from the results of Anderson and Day (1994) who reported that the interval from the last MGA feeding to estrus was longer for heifers that ovulated a follicle from a new wave that developed following the progesterone injection than for heifers that ovulated a persistent follicle. They demonstrated that for heifers only fed MGA, the persistent, ovulatory follicle was larger and secreted more estradiol-17β at treatment cessation than the ovulatory follicle that developed from a new wave in heifers treated with progesterone (Anderson and Day, 1994). They reasoned that, compared with heifers that ovulated a persistent follicle, more days were required for a new follicle to secrete enough estradiol-17β to initiate estrus for heifers that ovulated a follicle from a wave that emerged close to treatment cessation.

A variable interval from treatment to estrus, similar to that observed by Anderson and Day (1994) may have occurred in this study, especially for those cows that had their new follicular wave emerge late in the MGA-N or MGA treatments. Estradiol-17β concentrations were not determined on the day of treatment cessation in this experiment. However, concentrations of estradiol-17β on d 16, 2 or 3 d prior to treatment cessation for cows treated with MGA or MGA-N, respectively, were determined. On d 16 of the experiment, cows that ovulated a follicle from a new wave of follicular development
(NEW) had lower concentrations of estradiol-17β than cows that ovulated a follicle that persisted from d 8 to ovulation (PERSIST). If these differences were maintained to treatment cessation, the higher concentrations of estradiol-17β on d 16 for PERSIST cows were likely the reason they exhibited estrus earlier than NEW cows.

The ovulatory follicle for those cows that ovulated a persistent follicle was identified on d 8, the first day ultrasound was used. The ovulatory follicle in NEW cows was identified on d 16. One reason that persistent follicles are implicated as a cause of reduced fertility is because of their increased lifespan relative to the ovulatory follicle from a cow with an estrous cycle that is not extended. Butcher and Pope (1979) reported that embryo development rates were reduced for rats that were ovulatory-delayed with sodium pentobarbital compared to untreated controls. Peluso and Butcher (1974) demonstrated that cortical granules and mitochondria in oocytes from rats that were ovulatory-delayed resembled those of 8-cell embryos, not oocytes.

For those cows across MGA-N and MGA treatments that had a new follicular wave emerge during treatment that was the source of the ovulatory follicle (NEW), the timing of regression of the persistent follicle and the emergence of the new follicular wave varied among cows. For some cows, these events occurred prior to d 15, which was unexpected. According to previous results (Savio et al., 1993), the insertion of a norgestomet implant after initial progestogen exposure was expected to cause regression of a persistent follicle and the emergence of a new follicular wave. Indeed, in the experiments performed by Savio et al. (1993), a new follicular wave emerged an average of 3 d after the insertion of the norgestomet implant. Furthermore, no cows in that study regressed their persistent follicles and developed a new follicular wave prior to implant insertion. The expected timing of follicular wave emergence following exogenous progestogen is corroborated by the report of Anderson and Day (1994), who demonstrated that in all heifers injected with progesterone on d 9 of MGA feeding, a new follicular wave emerged 3 d after the injection.

Based on the results of the experiments of Savio et al. (1993) and Anderson and Day (1994), cows in this study treated with MGA were expected to develop and maintain
a persistent follicle until its ovulation. Conversely, cows treated with MGA-N were expected to develop a new follicular wave after insertion of the norgestomet implant. For NEW cows across MGA-N and MGA treatments, five of 13 developed a new follicular wave prior to the time of implant insertion for cows treated with MGA-N. It is not known why some cows treated with MGA-N developed a new follicular wave prior to implant insertion or why any cows treated with MGA developed a new follicular wave. One factor may be the increased number of days that MGA was administered prior to implant insertion in this study compared to that of Savio et al. (1993). It is possible that if the norgestomet implant had been inserted after 11 d, instead of after 15 d of MGA administration, there would have been a greater incidence of follicular turnover for cows treated with MGA-N. In the Savio et al. (1993) study, three of eight cows that were treated with one norgestomet implant for 16 d developed a new follicular wave that was the source of the ovulatory follicle. The average day of emergence for the new follicle in those cows was d 16, the day of implant removal. This reported day of emergence is similar to the timing of emergence of a new wave in the present study for cows treated with MGA-N or MGA. It is possible that some persistent follicles are predestined to regress after a certain time, even in cows without a corpus luteum and under the influence of low levels of progestogen. No studies have used ultrasound to determine how long a dominant follicle remains persistent during long-term progestogen administration to cows without a corpus luteum.

Atretic, dominant follicles (i.e., those that are in a regressing phase of development) exhibit characteristics that are not observed in growing dominant follicles. A persistent follicle, even under the influence of low levels of a progestogen and, thus, high LH pulsatility, may eventually become atretic. This possibility would partially explain how some cows treated with MGA in the present study regressed their persistent follicles and developed a new follicular wave. The same events that are observed in atretic follicles during a normal estrous cycle may have occurred in persistent follicles that regressed.
One factor that is related to whether a persistent follicle becomes atretic and regresses is the number of granulosa cell divisions that have occurred. Hirshfield (1991) noted that nearly all follicles became atretic after the eighth or ninth generation of granulosa cell divisions and that a follicle needed to receive specific metabolic or endocrine signals to continue to develop after that time. Two of those signals are thought to be increased LH and FSH support relative to what subordinate follicles receive (Fortune, 1994), but many other factors may be involved. The increased LH pulsatility that occurs after corpus luteum regression is thought to allow a dominant follicle to continue development until it ovulates (Fortune, 1994). Similarly, when an estrous cycle is extended with low levels of a progestogen, one dominant follicle becomes persistent until it ovulates after the cessation of progestogen treatment (Stock and Fortune, 1993). Eventually, this persistent follicle may regress because the increased number of granulosa cell layers have decreased access to oxygen and other nutrients (Hirshfield, 1991). The threshold number of granulosa cell layers that can obtain enough nutrients to survive may be less during a time of low LH pulse frequency (i.e., d 5 to 16 of the estrous cycle) than during a time of high LH pulse frequency. Therefore, during an estrous cycle that is extended by administering progestogens, persistent follicles develop that exhibit an increased lifespan (i.e., more granulosa cell generations) compared to dominant follicles observed during a normal estrous cycle. However, even in an environment of high LH pulse frequency, a persistent follicle does not seem to have an infinite lifespan.

Badinga et al. (1991) removed the dominant follicle of the first follicular wave from cows that were on either d 5 or 12 of the estrous cycle. They added radiolabeled testosterone to media containing the follicular walls and measured tritiated water released to determine aromatase enzyme activity. Aromatase enzyme activity was greater for dominant follicles collected on d 5 than on d 12, indicating that atretic follicles (d 12) had a diminished ability to convert androgen to estradiol-17β compared with growing, healthy follicles (d 5; Badinga et al., 1991).
The findings of Badinga et al. (1991) corroborate those of Ireland and Roche (1983), who demonstrated that atretic or healthy follicles have a low or high estradiol-17β to androgen ratio, respectively. Ireland and Roche (1983) speculated that the reason for this characteristic may be related to their observations that the capacities of granulosa cells from atretic follicles to bind hCG and FSH were reduced compared with those of granulosa cells from healthy, estrogen-active follicles. Even in an environment of high LH pulsatility, as would occur under the influence of low levels of a progestogen, if the number of LH receptors was decreasing on the granulosa and theca cells the persistent follicle would lose the ability to respond to the high LH pulsatility.

Another method of differentiating atretic follicles from healthy follicles is to determine whether pycnotic granulosa cells are present; if a follicle contains granulosa cells that are pycnotic the follicle is assumed to be atretic. Ireland and Roche (1983) and Guilbault et al. (1993) classified follicles as histologically atretic based upon the appearance of pycnotic granulosa cells. Ireland and Roche (1983), however, indicated that a portion of the estrogen-active follicles classified as healthy had some pycnotic granulosa cells. They determined that classifying follicles as atretic or healthy was more accurate if classification was based upon the ratio of estradiol-17β to androgen than if classification was based upon the appearance of pycnotic granulosa cells. Additionally, they speculated that a precipitous decline in estradiol-17β production by a follicle may occur before histological indications of atresia.

Tilly et al. (1991) speculated that internucleosomal cleavage of cellular DNA, a general indication of apoptosis caused by calcium/magesium dependent endonuclease activity, may also exist in atretic follicles. Because the endonuclease separates DNA every 185-bp, only fragmented DNA is observable following endonuclease activity. They separated healthy or atretic porcine follicles and noted that fragmented DNA (i.e., DNA that exhibited internucleosomal cleavage) was observed in atretic, but not healthy follicles (Tilley et al., 1991).

Another hormone that may regulate how long a dominant follicle remains persistent is inhibin. Inhibin suppresses FSH release and may alter aromatase enzyme
activity (Beard et al., 1990). Guibault et al. (1993) removed dominant follicles that were either early or late in the first wave of follicular development and measured the α subunit of inhibin and the dimeric inhibin concentrations in the follicular fluid. Follicles collected during the early part of the first follicular wave had greater concentrations of the α subunit of inhibin than follicles from the later part of the first follicular wave. Concentration of the dimeric subunit of inhibin was greater in follicles from the later than from those collected during the early part of the first follicular wave (Guibault et al., 1993). The amount of α subunit of inhibin present was positively correlated with the estradiol-17β to androstenedione ratio, demonstrating that follicles from the early part of the first follicular wave were estrogen-active. Conversely, dimeric inhibin content was negatively correlated with the estradiol-17β to androstenedione ratio (Guilbault et al., 1993).

One problem related to linking inhibin concentrations to a suppressive effect on either the dominant follicle or subordinate follicles is that not all of the α subunit forms appear to be biologically active. However, Guilbault et al. (1993) observed that concentrations of dimeric inhibin were greater in atretic than in growing follicles. As dominant follicles become atretic, they may synthesize increasing amounts of dimeric inhibin and this may be a functional index of whether a follicle is atretic. Based on the findings of Guilbault et al. (1993), one could speculate that increasing contents of dimeric inhibin may, even in an environment of high LH pulsatility, have a local inhibitory effect on a dominant, persistent follicle.

It is likely that subtle changes in LH pulsatility, LH and FSH receptor populations, inhibin concentrations, aromatase enzyme activity, and many other factors interact to cause a follicle to remain estrogen-active and persistent or to become atretic. Once the follicle regresses, there are several characteristics that can be used to classify it as atretic. It is unknown whether those characteristics are causes of follicular atresia or are simply associated with atretic follicles.

The growth rate of the ovulatory follicle for the 6 d preceding ovulation was greater for cows that ovulated a follicle from a new follicular wave (NEW) than for cows
that ovulated a follicle that persisted from d 8 until ovulation (PERSIST). This is in agreement with the findings of Beal et al. (1990) and Custer (1992). Beal et al. (1990) also reported greater ovulatory follicle growth rates in the 7 d preceding ovulation for untreated control cows than for cows treated with MGA for 7 d beginning on d 17 of the estrous cycle. Custer (1992) demonstrated that the growth rate of the ovulatory follicle was greater for cows treated with a PRID for 7 d beginning on d 17 of the estrous cycle than for cows treated with MGA for the same time period. In Custer’s study (1992), all cows treated with a PRID continued to exhibit follicular turnover during treatment while most cows treated with MGA maintained a persistent follicle from the beginning of treatment until ovulation. Based on the responses to treatment in Custer’s (1992) study, cows treated with a PRID or MGA are analogous to NEW or PERSIST cows in the present study, respectively.

While growth rate of the ovulatory follicle was greater for cows that ovulated a follicle from a new wave of follicular development (NEW), ovulatory follicle diameter on the day preceding ovulation was greater for cows that ovulated a follicle that persisted from d 8 until ovulation (PERSIST). This is in agreement with earlier research involving the use of MGA; Zimbelman and Smith (1966) demonstrated that one large follicle was present on the ovaries of cattle fed MGA. Similarly, Anderson and Day (1994) noted that the diameter of the ovulatory follicle was greater on the day preceding ovulation for heifers only fed MGA than for heifers that received a progesterone injection near the end of MGA feeding. In Anderson and Day’s (1994) study, all cows that were only fed MGA ovulated a follicle that persisted throughout treatment whereas all cows that were injected with progesterone near the end of MGA feeding ovulated a follicle from a new follicular wave. Based on the responses to treatment in the Anderson and Day (1994) study, cows only fed MGA or injected with progesterone near the end of MGA feeding are analogous to PERSIST or NEW cows in the present study.

The patterns of LH release were expected to differ between cows treated with MGA-N or MGA in this study. Savio et al. (1993) inserted a norgestomet implant into cows without a corpus luteum for 16 d and noted that pulse frequency of LH was higher
on d 11 of the experiment than on d 2. This finding indicates that LH pulsatility can be expected to increase as the number of days cows are treated with progestogen increases. Kojima et al. (1992a) administered a PRID, a norgestomet implant, or MGA to cattle without a corpus luteum. In all three treatments, LH pulsatility was higher on d 8 of treatment than for control cows that were on d 13 of the estrous cycle. In both of the above investigations, the number of LH pulses / h for norgestomet-, PRID-, or MGA-treated cows was numerically higher than for cows treated with MGA in the present study. It is unknown why the pulse frequency of LH was lower in the present study compared to the reported values from Savio et al. (1993) and Kojima et al. (1992a). One possible explanation is that blood collection for determination of LH occurred 5 d later relative to the beginning of progestogen treatment than in the Savio et al. (1993) study and 8 d later relative to the beginning of progestogen treatment than in the Kojima et al. (1992a) study.

In this study, a norgestomet implant was inserted into cows after treatment with MGA. Cows were expected to have high LH pulsatility during administration of MGA, then exhibit an immediate decline in pulse frequency of LH after insertion of a norgestomet implant. Savio et al. (1993) inserted a new norgestomet implant into heifers after their original implant had been in place for 10 d. These heifers were compared to the group in which the original implant was left in place, as described above. Twenty-four h after insertion of the new implant, LH pulse frequency was lower for the group that received a new implant than for the group that retained their original implant. Similarly, Custer (1992) administered a PRID or MGA for 7 d to cows on d 17 on the estrous cycle and reported a reduction in LH pulse frequency on d 20 of the cycle for cows treated with a PRID compared to those treated with MGA. In Custer’s (1992) study, the PRID had a similar effect on LH pulse frequency that a fresh norgestomet implant had in the study from Savio et al. (1993)

The reduction in LH pulse frequency reported by Savio et al. (1993) and Custer (1992) seems to be closely related to whether cows regressed their persistent follicles that had developed during administration of the original implant. Savio et al. (1993) reported
that in the group that had their implant replaced, the reduction in LH pulsatility preceded the regression of a persistent follicle and emergence of a new wave that was the source of the ovulatory follicle for all of the cows treated. Custer (1992) reported that incidence of regression of a dominant follicle that was present at the beginning of PRID treatment was 100% for cows treated with a PRID but was lower for cows fed MGA. The failure of a norgestomet implant treatment to consistently cause regression of a persistent follicle and subsequent emergence of a new follicular wave in this study may be related to the failure of the implant to cause a sufficient reduction in pulse frequency of LH.

In conclusion, the results of this study indicate that a norgestomet implant after MGA administration does not cause a reduction in LH pulsatility compared to cows only treated with MGA. Additionally, cattle treated with a norgestomet implant, compared to those only treated with MGA, did not exhibit a greater incidence of regression of a persistent follicle and emergence of a new follicular wave that was the source of the ovulatory follicle.
Implications and Summary

For the use of progestogen-based estrus synchronization systems to be popular, they should result in high percentages of cows that exhibit a tightly synchronized estrus. Additionally, the fertility to the insemination at the synchronized estrus should be comparable to that obtained after a spontaneous estrus. Progestogens induce estrus in some anestrous cows or prepuberal heifers, which is not accomplished with PGF$_{2\alpha}$, and this may be an added advantage of using progestogens. Melengestrol acetate is a progestogen used to synchronize estrus that is inexpensive and easy to administer. Feeding of melengestrol acetate is more convenient compared with SYNCRO-MATE-B, which involves inserting a progestogen (norgestomet) implant.

Melengestrol acetate administration followed by insertion of a norgestomet implant into cows was studied in this experiment. The treatment was expected to result in a more tightly synchronized estrus than treatment with melengestrol acetate alone or treatment with a single PGF$_{2\alpha}$ injection. The insertion of a norgestomet implant was expected to cause regression of a persistent follicle that developed during melengestrol acetate treatment. After regression of this persistent follicle, a follicle from a new follicular wave was expected to ovulate after implant removal, which may result in improved fertility to insemination at the synchronized estrus.

The results of this experiment were not encouraging. Cows did not have a tightly synchronized estrus following norgestomet implant removal. Furthermore, insertion of the implant did not cause cows to consistently develop a new wave of follicular development that was the source of the ovulatory follicle. Therefore, it is likely that a 15-d treatment with melengestrol acetate followed by insertion of a norgestomet implant would result in a variable interval from treatment to estrus and low fertility compared with cows bred at a spontaneous estrus.

A norgestomet implant inserted after 15 d of melengestrol acetate administration did not consistently cause regression of a persistent follicle. That length of melengestrol acetate administration prior to implant insertion may have altered the response to the
implant. One method which may improve the treatment investigated in this experiment might be to reduce the number of days cows were fed melengestrol acetate prior to norgestomet implant insertion. A shortened period of melengestrol acetate administration may allow the implant to consistently cause regression of a persistent follicle and lead to exhibition of a more controlled estrus with a better chance of high fertility.
Chapter V

Literature Cited


VITA

Eric Glen Faber, son of Robert and Clarice Faber, was born November 28, 1969, in Seoul, South Korea. He graduated from Iowa Mennonite High School in May, 1988. He received a Bachelor of Science degree in Animal Science from Iowa State University in December, 1992. He began work towards his Master of Science degree in Animal and Poultry Sciences (Reproductive Physiology) at Virginia Polytechnic Institute and State University in January, 1993, under the direction of Dr. W.E. Beal.

Eric G. Faber