SYNCHRONIZATION OF ESTRUS, CONCEPTION RATE, AND EMBRYONIC MORTALITY IN BEEF CATTLE FOLLOWING TREATMENT WITH PROGESTERONE-RELEASING INTRAVAGINAL DEVICES OR MELENGESTROL ACETATE IN CONJUNCTION WITH PGF$_{2\alpha}$

by

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(ABSTRACT)

The objective of these experiments was to determine the effects of and/or interactions among estrous synchronization treatments, reproductive status, and stage of the estrous cycle on estrous response (ER), first service conception rates (CR), pregnancy rates (PR), and embryonic mortality (EM) after 25 d of gestation. Angus or Angus crossbred cattle (n=391) at two locations were assigned to receive either melengestrol acetate for 7 d (MGA-PGF; .5 mg/hd/d, n=136) or progesterone releasing intravaginal device for 7 d (PRID-PGF; n=139) or to serve as untreated controls (n=116). All animals in MGA and PRID treated groups coincidentally received 25 mg prostaglandin F2α (PGF) on the final day of treatment. Real time, B-mode, ultrasound with a 7.5 MHz linear-array transducer was used to conduct three ovarian scans at 7-d intervals beginning 7 d prior to initiation of treatment. Jugular blood samples were collected at each scanning period. Serum was harvested and stored at 4°C until radioimmunoassayed for progesterone (P₄). Serum P₄ levels in conjunction with ovarian scans were used to
determine cycling status and stage of the estrous cycle at
initiation of treatment. Cattle treated with PRID-PGF
exhibited a greater synchronized ER (P < .06) than MGA-
treated cattle. Cycling animals had a greater ER than non-
cycling animals, regardless of treatment (P < .01).
Anestrous postpartum cows and prepubertal heifers treated
with PRID-PGF exhibited a greater ER (P < .05) within 7 d
than either MGA-treated or untreated control animals.
Conception rates of cattle treated with PRID-PGF beginning
late (> Day 16) in the estrous cycle were improved over
those of MGA-treated cattle (P < .13) at the same stage.
Pregnancy rate at 21 d was higher in PRID-treated cattle
than untreated controls (P < .01). Ultrasound scans
for embryonic viability were conducted at 25, 45, and 65 d
of gestation. Calving data was collected to characterize EM
between 65 d and term. The majority of embryonic loss
occurring after 25 d of pregnancy occurred before 45 d.
Synchronization treatment had no effect on the extent of EM
occurring after 25 d of gestation. Embryonic mortality
occurring between d 45 and 65 (2%) and between d 65 and term
(3%) when combined were similar in magnitude to EM occurring
between 25 and 45 d of gestation (4.8%). In conclusion,
PRID for 7 d combined with PGF was a superior
synchronization treatment for the mixed group of cyclic and
anestrous cattle.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Review of the Literature</td>
<td>1</td>
</tr>
<tr>
<td>Estrous Synchronization</td>
<td>1</td>
</tr>
<tr>
<td>Methods of Estrous Synchronization</td>
<td>3</td>
</tr>
<tr>
<td>Progestogens</td>
<td>4</td>
</tr>
<tr>
<td>Progestogen-Estrogen Combinations</td>
<td>7</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>10</td>
</tr>
<tr>
<td>Progestogen-Prostaglandin Combinations</td>
<td>12</td>
</tr>
<tr>
<td>Ultrasound and Reproduction</td>
<td>16</td>
</tr>
<tr>
<td>Postpartum Anestrous Cows</td>
<td>21</td>
</tr>
<tr>
<td>Prepubertal Heifers</td>
<td>29</td>
</tr>
<tr>
<td>Embryonic Mortality</td>
<td>33</td>
</tr>
<tr>
<td>Objectives</td>
<td>42</td>
</tr>
<tr>
<td>ANALYSIS OF ESTROUS SYNCHRONIZATION, CONCEPTION RATES, AND PREGNANCY</td>
<td>43</td>
</tr>
<tr>
<td>RATES IN CATTLE TREATED WITH EXOGENOUS PROGESTOGENS AND PGF$_{2}$a</td>
<td></td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>43</td>
</tr>
<tr>
<td>Animals</td>
<td>43</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>44</td>
</tr>
<tr>
<td>Blood Handling and Hormone Assay</td>
<td>46</td>
</tr>
<tr>
<td>Progesterone Profile Interpretation</td>
<td>48</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>48</td>
</tr>
<tr>
<td>Results</td>
<td>50</td>
</tr>
<tr>
<td>Discussion</td>
<td>57</td>
</tr>
<tr>
<td>ANALYSIS OF THE OCCURRENCE AND TIMING OF EMBRYONIC MORTALITY OCCURRING</td>
<td>63</td>
</tr>
<tr>
<td>AFTER DAY 25 GESTATION IN CATTLE</td>
<td></td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>63</td>
</tr>
<tr>
<td>Animals</td>
<td>63</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>64</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>65</td>
</tr>
<tr>
<td>Results</td>
<td>67</td>
</tr>
<tr>
<td>Discussion</td>
<td>70</td>
</tr>
<tr>
<td>Conclusions</td>
<td>75</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>76</td>
</tr>
<tr>
<td>Vita</td>
<td>90</td>
</tr>
</tbody>
</table>
LIST OF TABLES

1. Estrous response and pregnancy rates of untreated cows and heifers or animals treated to synchronize estrus..........................51

2. Estrous synchronization, conception rates, and pregnancy rates in cycling vs non-cycling (NC) cattle..........................................................52

3. Estrous synchronization, conception rates, and pregnancy rates in anestrous postpartum cows and prepubertal heifers.................................55

4. Estrous synchronization, conception rates, and pregnancy rates in suckled vs non-lactating postpartum cattle..............................................56

5. Influence of stage of the estrous cycle at the initiation of treatment on conception rates of cyclic cows and heifers treated with MGA-PGF or PRID-PGF.......................................................56

6. Embryonic mortality rates in cattle treated to synchronize estrus.................................68

7. Embryonic mortality rates by location.................69
LIST OF FIGURES

1. Distribution of estrus in MGA-PGF and PRID-PGF treated cattle......................53
REVIEW OF THE LITERATURE

ESTROUS SYNCHRONIZATION

A high pregnancy rate achieved within a short breeding season is highly desirable for the reproductive management of beef replacement heifers and lactating cows. The optimum length of the breeding season should be 60 d for mature cows and 45 d for virgin heifers. Successful synchronization of estrus enables manipulation of the estrous cycle and ovulation to bring a large percentage of treated females into a tightly synchronized, fertile estrus at a predetermined time. A synchronized group of cattle is expected to cycle within a 3 to 4 d period. If the breeding season begins with a synchronization program, a beef producer is afforded more opportunities to achieve a high pregnancy rate within a shorter period of time. Since the bulk of the herd is bred over an abbreviated interval, the breeding season is shorter and more intensive, while the calving period is more confined and predictable. Calves born earlier have heavier weaning weights, and the dams have increased postpartum intervals to the beginning of the next breeding season (Odde, 1990).

Synchronization of estrus also allows the producer greater opportunity to take advantage of artificial insemination (AI). Artificial insemination promotes the use
of genetically superior breeding stock. It also provides access to more reliable predictions of calving ease, maternal characteristics and growth traits of performance tested animals. An AI program reduces the cost of maintaining herd bulls and the possibility of poor conception rates due to subfertility. Furthermore, herd replacements sired by genetically superior sires are of a higher quality allowing more effective culling practices (Eller and Whittier, 1988).

Despite the advantages, only a small percentage of beef cattle are treated to synchronize estrus and artificially inseminated. Proper facilities and labor for intensive handling of animals are required to initiate a synchronization and AI program. Most beef producers are unwilling or unable to expend the time, effort or money to initiate an AI program. Conversely, synchronization of estrus reduces the time spent heat checking in herds that are already using AI breeding programs.

In general, estrous synchronization programs are more successful if cows are cycling at the beginning of the breeding season. The most common reasons cows and heifers are anestrous at the start of the breeding season include:

1) the interval from calving to the start of breeding season is too short;
(2) cows received inadequate energy either before or after calving; 
(3) cows are two or three years of age; or 
(4) heifers have not yet reached puberty (Wiltbank, 1984).

To enhance the likelihood of cyclicity, heifers must be at least 13 months of age and weigh two-thirds of their mature body weight prior to breeding. Similarly, after calving mature cows must be fed to gain weight and be in moderate to good body condition.

METHODS OF ESTROUS SYNCHRONIZATION

Since a mixture of cows and heifers with either active and inactive ovaries (anestrous) are likely to be present in a herd at the beginning of the breeding season, a successful synchronization program must be capable of inducing and controlling estrus and ovulation in both circumstances.

Treatments must synchronize regression of the corpus luteum (CL, luteolytic treatments), delay periovulatory endocrine events (progestogen treatments) until the CL has regressed in cycling cows (Roche et al., 1981b) or induce estrus and ovulation in anestrous cows and prepubertal heifers.
PROGESTOGENS

Progestogens are used in estrous synchronization programs to suppress heat and ovulation. The first attempt at control of the estrous cycle in cattle implemented daily injections of progesterone (Christian and Casida, 1948). Later, it was determined that progestogens could be administered orally (Zimbelman and Smith, 1966). One of the more commonly used progestogens today is oral 17α-acetoxy-6-methyl-16-methylene-4, 6-prenadiene-3, 20-dione (melengestrol acetate). Melengestrol acetate (MGA) is a progestational steroid with unique oral activity when fed to the ruminant at doses of 0.5 to 1.0 mg daily (Zimbelman et al., 1970; Hill et al., 1971; Randel et al., 1972). Melengestrol acetate successfully synchronizes estrus in cows and heifers by allowing follicular development but inhibiting estrus. In 14 of 15 trials using cattle fed MGA at 0.5 to 1.0 mg for 10 to 18 d, Zimbelman et al. (1970) reported as many MGA-fed cattle were in estrus during a 6-d synchronized interval as were untreated herdmate controls during a 20-d period.

Stage of the estrous cycle prior to initiation of treatment appears to be important in the success of using progesterone to synchronize cycling cattle. Synchronization of estrus is better in cattle which begin treatment on d 4 of the cycle than in cattle which begin treatment on day 15
(Hill et al., 1971). Postpubertal heifers in the follicular phase of the cycle treated with exogenous progesterone via Controlled Internal Drug Releasing (CIDR) devices inserted into the vagina appeared (via ultrasound examination) to suspend follicular waves. In particular, large follicles persisted longer than in untreated control cattle or cattle that had a CL at the beginning of treatment. Cattle in which treatment is begun with an exogenous progestogen during the follicular phase of the cycle may suffer from an alteration in the follicular wave pattern when compared to cattle that had a CL at the initiation of treatment (Swanson et al., 1990).

Medroxyprogesterone acetate (MAP), dihydroxyprogesterone acetophenide (DHPA) and 6-chloro-6-dehydro-17-acetoxy progesterone (CAP) are other orally-active, progestational compounds that have been used to successfully inhibit estrus in cattle (Zimbelman, 1963; Hansel et al., 1966; Wiltbank et al., 1967). Inhibition of ovulation and estrus was accomplished using doses of MAP of 0.18 to 0.36 mg per kg of body weight (Zimbelman, 1963) and of up to 500 mg per cow using DHPA (Wiltbank et al., 1967). Satisfactory synchronization of estrus was obtained in a trial comparing MAP and CAP in which CAP was fed at levels of 10 mg/head/d for 18 d (Hansel et al., 1966).
Conception rates are often reduced in cattle bred at the estrus immediately following administration of a progestogen for >10 d. After summarizing 24 studies using MGA, Zimbelman et al. (1970) concluded that conception rates from breeding at first synchronized estrus post-treatment were about 70% of the rate of untreated control animals bred at a spontaneous estrus. They noted that this effect seemed to be limited to breeding at estrus occurring within 10 d of progestogen withdrawal.

The cause for reduced fertility after treatment with progestogens remains elusive. Hill et al. (1971) reported altered follicle populations when progestogen-treated cows were compared to untreated controls. A shift in cervical mucus indices (CMI, ratio of chloride to protein) occurred such that the peak did not coincide with estrus. Normally, CMI peaks for 24 to 48 h at or around the time of estrus. Melengestrol acetate did not appear to influence the life-span or progesterone secretion profile of the CL that was present at the initiation of treatment, but plasma concentrations of luteinizing hormone (LH) were elevated. Furthermore, more uncleaved ova were obtained from heifers treated with MGA (Hill et al., 1971), and of those that were viable, a higher incidence of retarded cleavage rates were observed (Wishart and Young, 1974).
Guthrie et al. (1970) concluded that MGA caused an unusual form of follicular atresia in the cow characterized by degeneration of the granulosa cells in most follicles, intact membrana propria, vascularization of the theca interna, and proliferation of the thecal glandular cells. In the absence of a CL, the ovaries had few normal follicles, some hyperplastic follicles, many showing obliterative atresia, and a number of atretic follicles with thickened theca interna. It has also been suggested that infertility is associated with excessive amounts of progesterone preceding estrus observed in cattle treated with progestogens (Lamond et al., 1971). Finally, Hawk (1971) reported that sperm transport is reduced following insemination following MAP treatment in the ewe possibly due to sperm breakage, specifically, tailless sperm. A combination of the above mentioned factors, as well as those influences as yet unnamed, may contribute to reduced conception rates at first postpartum breeding in cattle treated with progestogens.

PROGESTOGEN-ESTROGEN COMBINATIONS

Progesterone delays periovulatory events and has been shown to successfully synchronize estrus in cycling (Beal et al., 1988) and non-cycling cattle (Beal and Good, 1986), but low fertility is often associated with prolonged (18 to 24d)
administration (Wiltbank et al., 1965; Roche et al., 1981b). Limiting the duration of treatment to 9 to 12 d has been used in attempts to overcome the problem of reduced conception rates (Wiltbank et al., 1971; Gonzalez-Padilla et al., 1975a; Williams and Ray, 1980). However, luteolytic agents are necessary to give precise control of estrus and ovulation when progestogens are administered for less than 14 d (Roche et al., 1981b). When administered during the early part of the estrous cycle, estrogens are luteolytic (Wiltbank et al., 1961), and are, therefore, often used in conjunction with progestogen treatments in synchronization regimens.

A 9-d, 6-mg norgestomet implant plus an injection of 5 mg estradiol valerate (EV) and 3 mg of norgestomet given at the time of implantation were able to synchronize estrus in cycling animals and to induce a fertile estrus in many prepubertal heifers (Gonzalez-Padilla et al., 1975a). Wiltbank et al. (1971) reported that a regimen which used 60 or 200 mg norethandrolone (17-ethyl-19-noresterone) implants for 16 d was less effective than similar implants for 9 d with an injection of EV on the day of implantation. Not only was synchrony of estrus better, but pregnancy rates were also higher.

The high biological activity of norgestomet (17α-acetoxy-11β-methyl-19-nor-preg-4-ene 20, dione) enables it
to be released in effective levels from a small polymer implant. Treatment with a norgestomet implant and an injection of EV and norgestomet at the beginning of the implant insertion is now commercially available as Syncro-Mate B (SMB) and is approved by the Food and Drug Administration (FDA) for use in beef cattle and dairy heifers for estrous synchronization regimens (Odde, 1990).

Syncro-Mate B elicits a tight synchrony of estrus and ovulation which permits artificial insemination at a fixed time after the end of treatment. This procedure is referred to as timed or mass mating (Odde, 1990) and eliminates the necessity of heat detection. Timed breeding also gives non-estrus but synchronously ovulating animals the opportunity to become pregnant. Wishart and Young (1974) reported that approximately 50% of non-estrus heifers inseminated at fixed times after SMB implant removal became pregnant. Insemination at 48 and 60 hr after implant removal resulted in a greater proportion of pregnant animals than a single insemination at 48 hr.

The literature documenting the success of SMB is extensive (Miksch et al., 1978, Spitzer et al., 1978a,b), but conception rates have been variable. Wishart and Young (1974) reported better success with shorter (9 d) implantation periods and explained that delayed embryonic
cleavage occurred when a longer (18 to 21 d) implantation period was used.

Stage of the cycle can be an important factor mediating the success of SMB. The number of animals showing estrus within a 5 to 7 d period is lower in animals treated in either the early or late stages of the cycle (Spitzer et al., 1978a). Brink and Kiracofe (1988) reported an effect of stage of the cycle at the time of treatment on conception rates as well. Heifers treated late (> d 11) in the cycle had reduced conception and pregnancy rates when compared to those treated early in the cycle (≤ d 11), possibly due to prolonged progestogen exposure resulting in a relatively long period of progesterone dominance.

PROSTAGLANDINS

Prostaglandin F\textsubscript{2α} (PGF) and analogues of PGF are effective as luteolytic agents in cycling cattle from the 5th to the 16th day of the estrous cycle (Moody, 1979). Because approximately 25% of the cyclic cattle presented for synchronization treatment could be expected to be within 5 d of their last estrus, this limitation reduces the practical value of PGF as a method of estrous synchronization for cycling cattle. Furthermore, often only 30 to 50% of the postpartum cows and limited numbers of heifers in a herd are cycling at the beginning of the breeding season, eliminating
another group of cattle for which this treatment would be effective.

However, there is an important advantage to the use of prostaglandins versus progestogens for synchronization of estrus in cattle. Although synchrony of estrus is often less than maximal (Lauderdale et al., 1974; Beal, 1983), conception rates after treatment are similar to those of untreated controls and higher than those recorded after estrous synchronization with progestogens (Lauderdale et al., 1974; Moody, 1979; Lauderdale et al., 1980, DeSilva et al., 1984; Chenault et al., 1990).

Cycling animals that are more than 16 d into the estrous cycle are not of great concern in a PGF synchronization program. They would be fairly well synchronized with cattle responding to the PGF treatment. Most systems that utilize PGF are designed to manage the cycling cattle that are less than 5 d of the estrous cycle. These regimens may be classified into one of the four following categories:

1. Two PGF injections 11 d apart and inseminate after the second injection according to estrus or at a preset time (Lauderdale et al., 1980, DeSilva et al., 1984).

2. Cattle are detected for estrus and inseminated for 5 d. Those not detected in estrus by d 5 are
injected with PGF. Breeding continues until 80 hr post PGF injection at which time all cattle not previously inseminated are bred (Lauderdale et al., 1980).

3. Inject PGF and inseminate for 4 d according to estrus. Then, 11 d later, inject a second time with PGF and breed at a preset time all animals that were not previously inseminated (Burfening et al., 1978).

4. Inject PGF and inseminate for 5 d according to detection of estrus (Lauderdale et al., 1980).

The fourth method is not recommended because it is not effective for animals that are in the first 5 d of the cycle. Otherwise, the above mentioned programs are quite appropriate for use in pubertal heifers and cycling postpartum cows.

PROGESTOGEN–PROSTAGLANDIN COMBINATIONS

Because administering progestogens for a long period of time (>14 d) is deleterious to conception rates for cattle bred at a synchronized estrus, means by which the length of progestogen treatment can be shortened are highly desirable. Rather than administer EV to cause luteal regression at the beginning of treatment, PGF may be administered at the end to cause luteolysis. The success of this regimen has been
well documented (Wishart, 1974; Deletang, 1975; Beal and Good, 1986).

In one of the first attempts to utilize progestogens and PGF for estrous synchronization, Wishart (1974) implanted cycling heifers with norgestamet for 5 d and placed PGF transcervically on the last day of treatment. This regimen resulted in a higher estrous response than administration of PGF alone because none of the animals were in the early stage of the cycle when PGF is not luteolytic. Furthermore, fertility was not adversely affected when compared to that of untreated controls bred after a spontaneous estrus. Later trials also reported favorable conception rates (Deletang, 1975; Roche, 1976).

Concerning anestrous cattle, MGA fed for 7 d with a PGF injection on the last day of MGA feeding was able to induce estrus in the majority of cows that were diagnosed as anestrous prior to treatment. When 5-, 7-, or 9-d treatment regimens were compared, it was concluded that a 7 d progestogen treatment was the most appropriate to induce a favorable estrus response (Beal and Good, 1986). Treatment with SMB was also able to induce estrus in many anestrous cows and prepubertal heifers (Beal et al., 1984).

Progesterone releasing intravaginal devices (PRIDs) inserted intravaginally for 7 to 9 d with PGF administered at the end of treatment or EV injected at the beginning of
treatment have been used to synchronize estrus. This method is not yet approved for use in the United States. The PRID-PGF combination has been successful in synchronizing estrus in both cycling and non-cycling cows with no impairment of conception rates compared to those of untreated controls (Beal, 1983).

The claim that short-term treatment with progestogens elicits normal fertility is not unrefuted. Conception rates for cattle treated for 5 to 9 d with MGA followed by a luteolysin at the end of treatment have been reported to be significantly reduced (Beal et al., 1988, Chenault et al., 1990). Cattle fed progestogens beginning late in the cycle exhibit depressed conception rates, an effect which may be related to extending the estrous cycle and delaying ovulation (Beal et al., 1988).

A practical utilization of progestogens for estrous synchronization in cattle should allow initiation of treatment irrespective of stage of the cycle and provide acceptable fertility following a synchronized estrus. Realizing that a producer must know or control the stage of the estrous cycle to optimize success with short term progestogen regimens, Coleman et al. (1989) attempted to design a treatment that would take advantage of the estrous synchronizing characteristics of MGA without compromising fertility. Cattle were fed MGA for 21 d followed 14 d later
by a single injection of luteolysin, but conception rates were intermediate to those of untreated controls. Further investigations designed to group cattle that are in an optimum stage of the estrous cycle is warranted.
ULTRASOUND AND REPRODUCTION

Through the use of a real-time, B-mode ultrasound scanning device, structures in the reproductive tract of the cow that could previously only be palpated per rectum can be viewed by a trained ultrasound operator. Recently, ultrasound scanning has been used in vivo in the bovine to monitor ovarian structures (Reeves et al., 1984; Pierson and Ginther, 1984a), to examine the uterus (Pierson and Ginther, 1987), to aid in pregnancy diagnosis (Archibong and Diehl, 1982; Pierson and Ginther, 1984b; Curran et al., 1986), to evaluate the technique of artificial insemination (Beal et al., 1989), and to determine the sex of fetuses in utero (Muller and Wittowski, 1986).

Presently, there are three different forms of ultrasound devices in use: A-, B-, and M-mode. A-mode is a one-dimensional image of echo amplitude versus distance. B-mode produces a two-dimensional display of soft-tissue cross sections. M-mode is an adaptation of B-mode which generates a tracing of the scan on light-sensitive paper. Real-time ultrasound scanning creates a moving two-dimensional image on a video monitor (Reeves et al., 1984).

Ultrasonography employs high frequency sound to produce cross-sectional images of soft tissues. These waves are produced by crystals within the head of the transducer which have piezoelectric properties. The waves are propagated
through the soft tissues as a sound beam (Reeves et al., 1984). Depending on what tissues the beam interfaces with, the image will appear very bright (solid tissue) or very dark (fluid) on a cathode ray tube screen (Edmondson et al., 1986). Solid tissues are very reflective or echogenic to ultrasound waves, whereas, fluids are non-echogenic and absorb ultrasound waves (Edmondson et al., 1986).

Before the advent of ultrasound scanning, studies on the changing morphology of the ovaries in cattle were limited to data collected from slaughterhouse specimens, data collected through rectal palpation or by marking structures with India ink (Pierson and Ginther, 1984a). Ultrasonography of the bovine ovary is a rapid and accurate method of visualizing ovarian structures. The time required for the examination of the ovaries in cattle is reported to be 20 to 150 sec (Reeves et al., 1984).

Because follicular fluid is nonechogenic, follicles appear as black, roughly circumscribed areas from 2 to 20 mm in diameter. Ovulation is readily detected by the acute disappearance of the large follicle (>13 mm) on an ovary. The corpus luteum or ovulation site is visible by 3 d after ovulation. The CL is more dense and, therefore, presents a gray echogenic pattern with an easily defined border. Often, cavities within corpora lutea are observed, but are distinguishable from the cavity of a follicle by their less-
spherical lumen and surrounding luteal tissue. These lumens are a temporary morphology which eventually condenses into a dense core (Pierson and Ginther, 1984a).

Follicular cysts are generally differentiated from follicles by their size (25 to 55 mm). Furthermore, because luteinized tissue is distinguishable with ultrasound, luteinized cysts can be differentiated from follicular cysts (Edmondson et al., 1986; Omran et al., 1988).

Regarding ultrasound and pregnancy detection, it is important to note that early pregnancy detection in cows has become a key aspect of monitoring reproductive efficiency. There are three methods widely available to the producer for pregnancy diagnosis: transrectal palpation, assay for progesterone, and return to estrus. Transrectal palpation of the uterus is most often used and can be performed by a skilled palpator, usually a veterinarian, 42 to 70 d after insemination. Recently, radioimmunoassay or enzyme immunoassay of progesterone in the blood or milk approximately three weeks after last service have been used as an alternative method because it allows earlier detection of pregnancy (Hanzen and Delsaux, 1987). Less accurate is the use of failure to show estrus following mating or AI, and even less reliable, some cattlemen still rely on external indications, such as udder development or an increase in abdominal size (White et al., 1985). The use of
Doppler ultrasound for detection of fetal pulse has been investigated but is limited to the later stages of pregnancy (Pierson and Ginther, 1984b).

Ultrasound confirmation of pregnancy is possible by d 20 based on the appearance of the CL and uterine echotexture. Caution must be practiced when using the presence of fluid in the lumen as an indication of pregnancy, because small amounts of intrauterine fluid are commonly observed near the time of estrus as well as during the first 3 wk of pregnancy (Kastelic et al., 1989). The embryonic heartbeat is easily visible by approximately d 23, allowing confirmation of a viable pregnancy. Extremely high accuracy of pregnancy diagnosis using ultrasonography has been reported (White et al., 1985; Hanzen and Delsaux, 1987). The assay for progesterone can be performed as early as ultrasound diagnosis, but milk or blood samples are more difficult for many beef producers to collect and have analyzed. Ultrasonography is a tool that can be used without prior knowledge of breeding date and can be performed earlier than rectal palpation.

The ultrasonic appearance of the embryo is a small echogenic line on d 20 which develops a "C" shape between d 22 and 30. From d 30 to approximately d 39 the embryo adopts an L shape, after which time the head, trunk, and
limbs become distinguishable. The amnion is visible by approximately d 30 (Curran et al., 1986).

It is recognized that pregnancy diagnosis through ultrasonography is no more accurate than guessing until d 18 (Kastelic et al., 1989) after which time accuracy increases rapidly. This device could prove of great value to the producer and, especially, to the researcher investigating events occurring in utero.
POSTPARTUM ANESTRUS

The calving interval is a major factor used to assess the reproductive efficiency of cattle. The economic optimum for a beef cattle producer is to have each cow produce one calf yearly. Unfortunately, individual cows will exceed the average economic optimum of 365 d. As years go by, this tends to deteriorate progressively, decreasing the rate of calf production and increasing the culling of otherwise good cows (Peters, 1984).

To successfully maintain a 365-d calving interval, the calving to conception interval should be no more than 82 d. Successfully achieving conception within this interval is dependent upon three factors:

(1) the reestablishment of normal ovarian cycles;
(2) the observation of estrous behavior; and
(3) pregnancy rate following a natural or AI service (Peters, 1984).

Regarding pregnancy rates, 90% fertilization rates are possible, but embryonic survival is much lower (refer to embryonic mortality section). It is the first factor, the reestablishment of ovarian cycles, that will be discussed in detail in this section.

Short et al. (1990) characterized the four general reasons that cows are infertile for a variable period of time following parturition:
(1) general infertility;
(2) anestrus;
(3) short estrous cycles; and
(4) uterine involution.

Of these, the last three are specifically related to the postpartum period. General infertility is an independent and, often, negotiable factor.

Kiracofe (1980) observed that uterine involution had no relationship to the length of the anestrous period but, rather, is a barrier to fertility early (< 30 d) after calving. In agreement, Short et al. (1974) found that by inseminating into the tip of the uterine horn of postpartum cows, more fertilized ova were recovered from early postpartum cows than if semen was inseminated in the uterine body. They concluded that the involuting uterus of a cow is a barrier to sperm transport. Although uterine involution is a permanent factor soon after calving that must be accepted, this is somewhat inconsequential since most cows are not in heat early enough after calving to allow uterine involution to play a significant role in determining the interval from calving to conception.

Short estrous cycles (7 to 12 d) during the first 30 to 40 d after calving are commonly observed in beef and dairy cattle herds. It has been established that the ova released during a short cycle are normal and capable of being
fertilized. However, the CL regresses too early for the ovary to receive the luteotropic signal from the gravid uterus to maintain pregnancy (Graves et al., 1968; Ramirez-Godinez et al., 1982). Recent evidence indicates that the uterus is the indirect cause of the short cycle observed in many postpartum cattle. High levels of exogenous PGF released from the uterus during the period of involution are believed to cause the CL to regress prematurely. In support of this theory, elevated levels of PGF metabolite [13, 14 dihydro-15-keto-prostaglandin F$_{2\alpha}$ (PGFM)] have been recorded in postpartum cattle exhibiting short estrous cycles (Copelin et al., 1987). A normal CL lifespan has been observed in cows pretreated with a progestogen which acts, presumably, by decreasing levels of PGF released from the uterus (Gonzalez-Padilla, 1975b; Ramirez-Godinez et al., 1982). This would indicate a possible advantage for the producer using an estrous synchronization regimen which includes the use of a progestogen.

Postpartum anestrus is the most serious problem facing cattlemen attempting to manage postpartum cows to maintain a \( \leq 365\)-d calving interval. Minor factors affecting postpartum intervals (PPI) are season, breed, age or parity, dystocia, presence of a bull, uterine palpation, and carryover effects of the previous pregnancy. The primary factors involved in lengthening this period are the suckling
of a calf and nutrition (Tervit et al., 1977; Peters, 1984; Short et al., 1990). The factors affecting the length of postpartum anestrus will be reviewed recognizing that individual examination of these factors is quite difficult as they all interact.

It is well established that the postpartum interval to first ovulation observed in fall-calving cows is shorter than that observed in spring-calving cows. A higher proportion of fall-calving cows also become pregnant by d 100 postpartum (King and Macleod, 1984; Hansen, 1985; Montgomery et al., 1985). These seasonal effects may be related to changes in photoperiod. The effect on ovulation is mediated via the pineal gland, and its release of melatonin (Sharpe, 1986). It has been suggested that commercial beef producers consider fall breeding rather than summer breeding to insure mating during the most fertile season and to minimize neonatal calf losses. This is a practical approach for producers who have the resources to provide adequate nutrition throughout all seasons of the year (King and Macleod, 1984).

Concerning breed and genotype, breed has been considered a significant factor determining the interval to first ovulation. In one study using Angus, Simmental, and Holstein heifers selected for high and low milk production, 50, 88, and 92% of the Angus, Simmental, and Holstein
heifers, respectively, had initiated their first estrous cycle by 30 d postpartum. Breed was the factor that most affected interval to first estrus regardless of level of milk yield, breeding value, or changes in body weight or body condition (Masilo et al., 1991). Holsteins tended to have a longer interval to first estrus and to conception than Herefords, and diet influenced reproduction more in Holsteins than in Herefords (Hansen et al., 1982). It is difficult to conclude to what degree the differences attributed to genotype are due to true physiological differences or created by factors such as the average amount of milk produced by that particular breed.

Regarding age and parity, younger, primiparous cows have longer PPI and lower reproductive potential than multiparous cows. Dystocia is more prevalent in younger cattle and can increase the PPI. Calving difficulty also affects subsequent reproductive performance. Cows experiencing dystocia weaned fewer calves, had calves that were born later than those of herdmates and delivered calves that weighed less (Tervit et al., 1977). The negative effect of a prolonged and difficult labor on postpartum reproductive performance can be somewhat mediated by early obstetrical assistance (Doornbos et al., 1984; Bellows et al., 1988).
The presence of a bull has been used in an attempt to decrease the PPI, but results have been equivocal. Stumpf et al. (1987) reported few stimulatory effects of bull exposure, but others (Zalesky et al., 1984; Alberio et al., 1987; Gifford et al., 1989) have found the duration of postpartum anestrus in multiparous and primiparous cows was decreased when cows were exposed to bulls. This would seem to suggest that the use of a teaser bull in breeding programs could be advantageous.

One of the primary factors affecting PPI is nutrition. This includes the quantity and quality of feed, reserves stored by the animal, and competition for nutrients. The competition for nutrients consumed in the diet is referred to as nutrient partitioning and proceeds as follows: (1) basal metabolism, (2) activity, (3) growth, (4) basic energy reserves, (5) pregnancy, (6) lactation, (7) additional energy reserves, (8) estrous cycles and initiation of pregnancy, and (9) excess reserves (Short et al., 1990). It is apparent from the above energy hierarchy how easy it is to upset the reproductive efficiency of postpartum cattle by improper dietary management. Adequate energy is more important than adequate protein, and precalving nutrition has a greater effect on the first 60 d after calving than postcalving nutrition (Tervit et al., 1977). Body condition scores (BCS) are a frequently used and relatively simple way
to monitor nutrient reserves in cattle. Cows calving with BCS ≥ 5 on a scale of 1 to 9 (1 = emaciated; 9 = extremely fat) can withstand postpartum periods of weight loss common to a lactating cow without sacrificing reproductive performance (Warren et al., 1988).

Suckling is the second major factor affecting postpartum anestrus. PPI can be decreased by complete, short-term, or partial weaning (Williams, 1990). The interval to first estrus is affected by the intensity and, especially, the duration of the suckling period (Tervit et al., 1977). The suckling stimulus acts to increase the anestrus period by depressing LH secretion from the pituitary (Dunlap et al., 1981) and by preventing the release of LH in response to estradiol (Radford et al., 1978). Hence, suckling prevents the mechanism normally responsible for the establishment and maintenance of cyclic ovarian function. Although decreasing the suckling stimulus helps to initiate LH pulsatility, it could cause increases in the potential for calf diseases and reduced weaning weights (Short et al., 1990).

The temporal relationship between the hypothalamus, pituitary and ovary that initiates cyclicity after calving was summarized by Short et al. (1990): (1) functional competence of the hypothalamus and pituitary, (2) pulsatility of gonadotropin releasing hormone (GnRH) at 1 to
2 pulses per h, (3) pulsatile release of LH, (4) follicular growth/maturation and estrogen production, (5) preovulatory LH release and estrus, (6) ovulation, CL formation and progesterone production, (7) regression of the CL under the influence of PGF or fertilization, pregnancy, and viable offspring.

During the early postpartum period, the GnRH pulse generator is inhibited by low levels of estrogen (Acosta et al., 1983) which causes low serum concentrations of LH. Postpartum cows treated with pulsatile or continuous infusions of GnRH exhibited a shorter PPI, but not all cows responded to treatment indicating that other factors may be involved. The ovary is involved in that as the follicle pool grows, the pituitary and hypothalamus are hypersensitive to the negative feedback of estrogen which is eventually overcome, resulting in the preovulatory surge of LH and ovulation. The adrenal gland, prolactin, oxytocin and direct neural influence of the udder have been investigated and ruled out as control mechanisms which block or retard the steps leading to the preovulatory surge of LH (Short et al., 1990).
PREPUBERTAL HEIFERS

Each day that a heifer is maintained before giving birth to a calf decreases the efficiency of lifetime production. The goal of the beef producer is to select and manage heifers that will exhibit estrus and conceive early in the breeding season as yearlings. Cows that calve early in the first calving season are more likely to conceive during the breeding season throughout their lifetime, thereby maximizing their efficiency (Greer et al., 1983). Reducing the age at puberty increases economic efficiency and is especially critical under current management systems in which heifers are bred at 14 to 15 months of age to calve at approximately 24 months of age (Short and Bellows, 1970).

Factors similar to those which delay estrus in postpartum cows affect the age at which heifers reach puberty (defined as first ovulation in heifers) including age, weight/nutrition, season, and breed (Grass et al., 1982; Greer et al., 1983). The possibility of an added effect through the presence of a bull (Berardinelli et al., 1978; Roberson et al., 1987), the presence of puberal heifers, or the presence of mature cows (Nelsen et al., 1985) were investigated and ruled out as contributing factors.

Age and weight are the primary factors controlling the timing of puberty in cattle (Wiltbank et al., 1966).
Heifers should be 13 to 15 months of old at the start of the breeding season and should weigh approximately two-thirds of what they will weigh as mature cows in average condition. Nutrition should be a priority in prepuberal heifers since they must be fed from weaning to the start of the breeding season to meet the target weight. In many beef herds, this means gaining between 1.5 and 2 pounds per day (Eller and Whittier, 1988). Feeding diets low in energy can delay onset of estrus in heifers (Short and Bellows, 1970; Grass et al., 1982).

Season affects the onset of puberty in heifers. Winter conditions during the peripuberal period delay puberty (Grass et al., 1982; Hansen, 1985). Schillo et al. (1983) observed that regardless of season of birth, heifers exposed after six months of age to temperatures and photoperiods of spring and summer were younger at puberty than heifers exposed to autumn and winter conditions. In the same study, environment affected sexual maturation differently in the second six months compared to the first six months raising the question: although certain seasons may hasten puberty if they occur during the first six months of a heifer's life, do they delay it later in life? There is no one season of birth that predisposes an animal to early puberty, but photoperiod and temperature can exert an effect on this process (Hansen, 1985).
Certain breeds and breed-crosses will reach puberty at earlier ages (Laster et al., 1972). Furthermore, weight at puberty varies among breeds with the faster growing breed-crosses reaching puberty at a heavier weight. Similarly, Wiltbank et al. (1966) reported that Herefords were older and heavier at first estrus when compared to Angus and Shorthorn breeds. Breed-of-sire, breed-of-dam, and preweaning average daily gain all had a significant effect on age at puberty.

There are two primary endocrine events which occur just prior to puberty in heifers. The first involves the marked fluctuation of LH which rises and falls randomly in the months preceding first estrus. In addition to the preovulatory peak of LH (day 0), there are peaks of similar magnitude and duration approximately 11 and 9 d before estrus. Concentrations of LH between d-9 and d 0 do not fluctuate like those prior to d-11 and are similar to those observed during the luteal phase of a normal estrous cycle (Gonzalez-Padilla et al., 1975b).

The second event which occurs just prior to puberty in heifers is the rise in plasma progesterone concentration. Most often, there are two periods, between d -20 and d 0, during which progesterone increases significantly (Gonzalez-Padilla et al., 1975b). The source of progesterone associated with these prepubertal rises is the ovary
(Berardinelli et al., 1979). There is some debate, however, whether the source within the ovary is a short-lived CL or luteinized follicle.

The significance of the increase in progesterone is probably two-fold. First, progesterone may establish a phasic pattern of release of LH. Secondly, progesterone may act to sensitize the ovaries to LH (Berardinelli et al., 1979).

Puberty may be induced in young (< 9 mos) heifers with little or no ovarian activity. Treatment with an estrogen alone was able to induce LH release but not ovulation. Pretreatment with progesterone, called progesterone priming, was necessary for estradiol to induce estrus and ovulation (Short et al., 1976).
EMBRYONIC MORTALITY

Reproductive failure is a significant source of production losses for a cattle producer. Fertilization rates of 85 to 90% have been reported in cows (Hill et al., 1970) while calving rates to a single service average 55% (Sreenan and Diskin, 1986). Using these estimates, embryonic mortality, defined as losses that occur after fertilization, can contribute up to a 35% loss in the net calf crop.

An increase in the interval between service and return to estrus beyond the usual range of 17 to 25 d is often considered to reflect embryonic mortality. However, when losses occur prior to maternal recognition of pregnancy (d 17), the embryo dies too early to prevent secretion of the uterine luteolysin, prostaglandin, and the cycle of the dam is normal in length (Ayalon, 1978). Hence, increased intervals from insemination to the return to estrus are an inadequate method of determining embryonic mortality.

A well-trained technician can detect pregnancy in cows by inserting the hand into the rectum and palpating through the rectal and uterine walls for fetal membranes or the amniotic vesicle within the uterus as early as 35 to 40 d after insemination, but accuracy is greatly improved by waiting until 40 to 50 d (Sasser and Ruder, 1987).
Several tests for the detection of pregnancy early after insemination have been developed, such as those for pregnancy specific proteins, but these are not yet widely used. The only test commonly in practice is an assay for progesterone in milk or serum which can be used to confirm the absence of a CL and, therefore, non-pregnancy near the time of next expected estrus (Sasser and Ruder, 1987). Other tests such as early pregnancy factor (EPF) in serum, bovine trophoblast protein 1 (btp-1), and pregnancy-specific protein B (PSPB) may develop into useful early tests for pregnancy, but are liable to be expensive methods of monitoring embryonic viability.

Traditional studies of embryonic development and mortality involve slaughterhouse or necropsy specimens or early conceptuses flushed from the uterus through a transcervical cannula. Recently, the use of transrectal real-time, B-mode ultrasonography has improved methods of detecting and monitoring bovine embryos. The embryonic heartbeat can be detected as early as 21 d after insemination, and can be applied as a useful and relatively easy method of confirming embryonic viability (Curran et al., 1986). Kastelic et al. (1989) evaluated the accuracy of ultrasonography for pregnancy diagnosis 10 to 22 d after insemination in heifers. Pregnancy diagnosis was no more accurate than guessing until 18 d when diagnosis was based
primarily on the appearance of the CL and uterine echotexture. The conceptus itself was not visible as a small echogenic line until after 20 d.

The extent and timing of embryonic mortality in the cow varies considerably according to different sources. Diskin and Sreenan (1980) demonstrated that embryonic survival rate was reduced between d 8 (87%) and 16 (66%). Roche et al. (1981a) observed pregnancy rates in Hereford-cross heifers of 84% and 60% at d 8 and 18, respectively, and concluded that the greater part of embryonic mortality occurred during this time. Subsequently, Smith et al. (1982) observed that the primary loss of potential embryos in heifers occurs before d 3, and that additional losses were negligible. Alternatively, Chaffaux et al. (1986) reported a high incidence (23%) of embryonic death in dairy cattle occurring between d 30 and 60 after insemination. Finally, Maurer and Chenault (1983) reported that in both parous and nonparous beef females, the preponderance of embryonic mortality occurred by d 8 of gestation. Although results of these studies are inconsistent, the available data seems to indicate that the greatest proportion of embryonic loss occurs sometime before d 15 in the cow.

Because of the complexity of reproductive processes, the causes of embryonic mortality are numerous and, in many cases, interrelated. Ayalon (1978) classified nonpathogenic
factors for embryonic death into two main categories: (1) genetic factors such as breed, family, inbreeding, and blood groups, and (2) environmental factors such as nutrition, age, climate, hormonal imbalance, and uterine environment. Of these, genetic factors have been characterized to a greater extent by early researchers. Casida (1950) found no significant differences in the extent of embryonic mortality in two breeds of dairy cattle. Exploring family and inbreeding as a cause of embryonic loss, Hawk et al. (1955) found no significant difference in losses between the embryos of inbred and outbred dams or inbred or outbred embryos. However, there did exist a trend for both inbred embryos, and especially inbred embryos in inbred dams, to have higher embryonic mortality at 150 d of gestation than the corresponding outbreds. Furthermore, Echternkamp et al. (1990) suggested that low embryonic survival was associated with multiple ovulations, and they observed that livability of twins tended to be greater in the left uterine horn than in the right uterine horn. This phenomena is interesting from the evolutionary standpoint since there is greater ovulatory activity on the right ovary.

Inherited genetic deficiencies that cause embryonic death are not uncommon in cattle. Rausch et al. (1963) examined the association between bovine blood transferrin types, as determined by disc electrophoresis, and fertility.
Using several hundred matings, he detected no significant effect of globulins. Conversely, Ashton and Fallon (1962) determined that matings between cattle homozygous for beta-globulin exhibited an average fertilization rate, but suffered extensive (11%) embryonic death. Structural chromosomal abnormalities that contribute to early embryonic mortality have also been identified (Moraes et al., 1987). When these abnormalities are identified, carrier animals should be culled. Recently, an inherited deficiency of uridine monophosphatase synthase, a condition originally identified in humans, has been discovered in dairy cattle (Shanks and Robinson, 1989). The enzyme catalyzes the conversion of orotic acid to UMP, the precursor of all other pyrimidine nucleotides, and is lethal embryonically in cattle homozygous for the trait.

Regarding nutrition and embryonic mortality, heifers subjected to short-term undernutrition suffer increased embryonic loss compared to heifers fed a balanced diet (Hill et al., 1970). Pregnant cows fed 70% of their calculated optimum energy requirements during the third trimester when the fetus is gaining approximately .45 kg per d exhibited an increased incidence of calf morbidity and mortality (Corah et al., 1975). Limiting protein in the diet resulted in reduced fetal weight and resultant increases in mortality. Furthermore, deficiencies in copper, vitamin A and D have
also been related to fetal resorption, abortions and stillbirths, respectively (Maas, 1987).

The relationships between age of the dam and embryonic death have been explained by Erb and Holtz (1958). Based upon intervals of return to service, they concluded that heifers had a higher rate of embryonic death (15%) than cows of fourth or fifth parity (.8%). Alternatively, Echternkamp et al. (1990) reported that embryonic survival decreased as age of the dam increased beyond 5 yr.

The quality of semen is also a factor in embryonic loss. Kidder et al. (1954) examined the fertilization rates of 64 bulls of two different breeds with variable fertility. Fertility was based on 60- to 90- d nonreturn rates. There were no significant breed differences in fertilization rates or embryonic death. After pooling the breeds for further comparisons, the embryonic death rate, estimated as the percent of fertilized ova that died, was 25.5 and 14.9% for the high- and low-fertility bulls, respectively. Bearden et al. (1956) observed fewer normal embryos (57.7%) in heifers bred to low-fertility bulls than heifers bred to bulls of high fertility (86.1%) at 33 d post-estrus. More recently, lower fertility and higher proportions of low quality embryos have been reported from the use of bulls having high proportions of cratered sperm (Miller et al., 1982). Following heat insult, one bull produced a high number of
cratered and diadem defective sperm which were defective in fertilization and maintaining the embryo (Saacke et al., 1991).

In the dam, hormonal imbalance is frequently blamed for embryonic mortality. Erb et al. (1976) concluded that nonfertile cows exhibited an increased incidence of hormonal asynchrony prior to ovulation. Cows that were nonfertile due to either fertilization failure or loss subsequent to fertilization exhibited a delayed increase in luteinizing hormone. Half of the cows that ovulated after insemination had low plasma progesterone 6 or more days after ovulation. Variations in plasma estrogen in nonfertile cases could have resulted in failure of the ova to enter the uterus at the proper time. Lamming et al. (1989) observed a small but measurable depression in progesterone output in animals which failed to maintain early embryos, but concluded that the differences were not sufficient to explain the losses.

Uterine environment is critical to embryonic survival and development as demonstrated by embryo transfer trials. Rowson et al. (1972) confirmed that the requirements for synchronization of the estrous cycle with embryo transfer are acute and should not vary more than 1 d. Especially convincing were the extremely high number of successful pregnancies (91.1%) when synchronization was exact. In agreement, Sreenan and Beehan (1974) obtained pregnancy
rates of 91.7% and 71.4% for synchronous and asynchronous transfers, respectively.

The early bovine embryo is extremely sensitive to maternal heat stress. Climatic thermal stress has been shown to be a cause of hormonal depression and low fertility in the bovine (Stott and Wiersma, 1973). Cows exposed to cooler temperatures during the summer had markedly higher breeding efficiency (58%) than those having access to only conventional shade (35%). Lewis et al. (1984) observed that prepartum heat stress increased 13,14-dihydo-15-keto-prostaglandin F2α (PGFM) concentrations in Holstein cows suggesting an increase in the production of PGF by the uterine endometrium. Intermittent thermal stress from 30 h after the onset of estrus until d 7 of pregnancy increased the incidence of abnormal or retarded embryos recovered from dairy heifers (Putney et al., 1989) and maternal heat stress between d 8 and 16 after insemination reduced conceptus weight and caused an increased trend towards pregnancy failure in beef cattle (Biggers et al., 1987). Finally, temperature elevation of cultured d 17 bovine conceptuses and uterine endometrium induced a large reduction in conceptus protein synthesis and secretion and secretion and stimulated release of PGF (Putney et al., 1988a,b). This suggests that high temperatures may alter conceptus metabolic activity and lead to reduced growth rates and
failure of conceptuses to produce biochemical signals in adequate amounts required for preventing CL regression (Putney et al., 1989).
OBJECTIVES

These experiments were designed to determine the effects of and/or interactions among estrous synchronization treatments, reproductive status (cyclic or non-cyclic; suckled or non-lactating) and stage of the estrous cycle (cyclic only) on:

(1) estrous response within 7 d after end of treatment or over 21 d in untreated controls,
(2) first service conception rate,
(4) pregnancy rates at 7 and 21 d post-insemination, and
(3) the extent and timing of embryonic mortality occurring after d 25 of gestation.
ANALYSIS OF ESTROUS SYNCHRONIZATION, CONCEPTION RATES, AND PREGNANCY RATES IN CATTLE TREATED WITH EXOGENOUS PROGESTOGENS AND PGF\textsubscript{2}\textalpha

MATERIALS AND METHODS

Animals.

Two hundred sixty-two Angus cows or heifers ranging in age from 14 mo to 11 yr and averaging 2.9 yr of age (SD = 2.1 yr) were used at Wehrmann Angus, Newmarket, Virginia. Animals (78 virgin heifers [H], 47 non-lactating cows [NL], 137 suckled cows [S]) were housed on pasture and fed corn silage based rations. Within S, 44 were fall calving cows (September to November), and the remainder were spring calving (January to March). Days postpartum (DPP) ranged for S from 20 to 88 d averaging 58.1 d (SD = 21.3 d).

One hundred twenty-nine Angus-crossbred cattle ranging in age from approximately 15 mo to 14 yr and averaging 4.3 yr (SD = 2.9 yr) were used at Catawba Research Station, Catawba, Virginia. Animals (19 H, 29 NL, 81 S) were housed on pasture and fed corn silage based rations. All cattle had calved in the fall (September to November). Days postpartum for S ranged from 28 to 87 d and averaged 64.2 d (SD = 15.0 d).
Experimental Design.

Cattle at the two locations were stratified by age and DPP within 5 and randomly assigned to receive one of three treatments:

1) Control - No estrous synchronization treatment. These cattle received the same rations and were managed similarly to treated cattle;

2) Melengestrol Acetate (MGA-PGF) - Each animal was fed .5 mg of 17α-acetoxy-6-methyl-16-methylene-4, 6-prenadiene-3, 20-dione per d (MGA; Upjohn Co., Kalamazoo, MI) for 7 d and received a 25 mg injection of prostaglandin F2α, i.m. (Lutalyse; Upjohn Co., Kalamazoo, MI) on the last d of MGA feeding;

3) Progesterone-Releasing Intravaginal Device (PRID-PGF) - Each animal had one PRID, a silastic coil impregnated with 1.55 g progesterone (Sanofi Animal Health, Paris, France), inserted within the vagina and left in place for 7 d. An injection of prostaglandin F2α (PGF, 25 mg, i.m.) was administered at the time the PRID was removed.

The time of PGF administration in Groups 2 and 3 occurred on the same day. Animals in all three groups were observed for signs of behavioral estrus at least twice daily, early morning and late afternoon, for 30 d beginning at the time of PGF administration. Animals in the treated groups were considered to have exhibited a synchronized
estrus if estrus was observed within 7 d after the administration of PGF. Each animal observed in estrus was artificially inseminated by one inseminator (Wehrmann Angus) or one of two inseminators (Catawba) approximately 12 h after detection of estrus. Cattle were bred to the frozen semen of 1 of 14 Angus sires (Wehrmann Angus) or 1 of 5 Angus sires (Catawba). Sires were of proven fertility.

Cattle were determined to be cycling or non-cycling prior to treatment based on ultrasonic (Equisonics LS-300A with 5 MHz transducer; Tokyo Keiki Co., Ltd.) evaluation of ovarian status 7 d prior to treatment and on the day of initiation of treatment. Cycling status was based on the presence or absence of a corpus luteum at either ovarian evaluation.

Blood samples were collected via 10-ml vacutainer tubes 7 d prior to treatment, on the day of initiation of treatment, and on the 7th and final day of treatment. Stage of the estrous cycle was classified in treated animals as early, mid, or late based on analysis of serum progesterone concentrations of blood samples (see below).

Conception rates (cows pregnant/cows inseminated) were determined by ultrasonic examination for pregnancy at approximately 25 d of gestation. Pregnancy rates (cows pregnant/cows per group X 100) were also based on this examination.
Blood Handling and Hormone Assay.

Immediately upon collection of blood, tubes were stored at room temperature and out of direct sunlight. The blood was allowed to clot for approximately 1 h before centrifugation at 3000 RPM for 20 min. Serum was collected into 12 x 75 mm plastic tubes, immediately frozen in liquid nitrogen vapor, and stored at -20° C.

After all serum samples from one location were collected, tubes from both treated groups were assayed for progesterone (P₄) content using radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA). Tubes were randomized within each assay. Crossreactivity of the P₄ antibody with MGA was < .01%.

Progesterone Profile Interpretation.

Frequency distribution curves were fitted to the progesterone values to determine a lower limit of serum P₄ for cycling animals. For both the Wehrmann and Catawba samples, .6 ng/ml was used as the indicator of for cyclicity.

For cows at both locations, the concentration of P₄ in each of the three blood samples taken 7 d before treatment, at the initiation of treatment and at PGF injection was used to estimate the stage of the estrous cycle. A concentration of ≥ .6 ng/ml was used to identify beef females with a
corpus luteum (Days 5 through 17 of the estrous cycle [estrus = Day 0]). This period of time within the 21 d estrous cycle was identified as the middle of the cycle, with < Day 5 being early and > Day 17 being late. Progesterone values at each of the three sampling times were identified as ≥ .6 ng/ml (H = high) or < .6 ng/ml (L = low). Blood samples were collected 7 d apart, hence, a high percentage of the cattle had high P₄ on two sampling days. Individuals with low P₄ at all three sampling periods were considered to be anestrus. Cattle in Groups 2 and 3 were determined to have been at the following stages of the estrous cycle at the time of initiation of the treatments:

<table>
<thead>
<tr>
<th>Stage of cycle</th>
<th>Sample 1 (-7 d)</th>
<th>Sample 2 (0 d)</th>
<th>Sample 3 (+7 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Early</td>
<td>Low</td>
<td>High</td>
<td>High</td>
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<tr>
<td>Middle</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>

Cattle in the PRID-PGF group were assigned a H or L at the third sampling time in accordance with the ultrasound scan as the RIA results were always above .6 ng/ml due to progesterone in the serum from the PRID. This assignment is believed to be accurate because of all cases in which both ultrasound evaluation of the ovaries for CL and P₄ assays were conducted (1173 observations), the ultrasound scan and
RIAs results were in agreement with the exception of three individual cows which evidently had small CLs, as evidenced by P4 concentration > .6 ng/ml, that was not detected by the ultrasound operator.

**Statistical Analysis.**

In a preliminary step to identify independent variables of interest, estrous response, conception rate, and pregnancy rate, were analyzed using SAS (1985) General Linear Models procedure (GLM) with location (1 df), treatment (2 df), parity (2 df; 1=virgin heifer, 2=first parity, 3=second or greater parity), cyclic status (1 df), non-lactating vs suckled (1 df) and stage of the estrous cycle (2 df) as main effects. All two way interactions were included. Location and parity were eliminated from the model as they were not significant with the exception of an interaction between location and cyclicity within tests for the dependent variable estrous response. This effect is inherent to the experimental design as more of the cattle were cycling (98 vs 69%; location x cyclicity, P < .02) at Catawba than at Wehrmann Angus. Least-squares means differences for interval to estrus in treated groups were determined using Tukey's honestly significant difference test.
Because the effect of stage x treatment on conception rates was insignificant, an apparent difference between conception rates for cattle that began treatment late in the estrous cycle was analyzed directly using a chi-square procedure (SAS, 1985).

Sire and inseminator were not included as main effects in the statistical analysis of conception rate because they were confounded with location. No single sire or inseminator was utilized at both locations. Inseminators (Catawba) and sires (both locations) were randomly distributed among treatment groups within location, hence, neither variable should affect the ability to validly measure treatment effects.

Estrous response, conception rate and synchronized pregnancies were analyzed using Biological Medical Data Processing (BMDP) 4f two-way and multi-way frequency tables for categorical data (BMDP Statistical Software, 1983) with treatment (2 df), cycling status (1 df), non-lactating vs suckled (1 df) and stage of the estrous cycle (2 df) as main effects. When main effects with more than 1 df were significant, a separate analysis of each pair was conducted. BMDP values reported are from chi-square contingency tables.
RESULTS

Across both locations, only one cow was found to have lost a PRID at the end of the 7 d treatment period (99.3 % retention rate). The estrous response for that animal occurred within the synchronized period, and those data were included in the data set.

The proportion of cows in estrus within 168 h after treatment with either MGA-PGF or PRID-PGF was different (71 vs 81%, respectively, ; P < .06; Table 1). Estrous response observed in untreated controls over 21 d (74%) did not differ from that of PRID-treated cattle (P < .17) or MGA-treated cattle (P < .6).

The mean interval from PGF to the onset of estrus was shorter (P < .05) in the PRID-PGF group (2.9 ± .11 d) at both locations and for both cows and heifers, than the interval from PGF to estrus in the MGA-PGF group (4.2 ± .13 d; Figure 1).

The percentage of cows in estrus was greater (P < .01) for cows that were cyclic prior to treatment than for cows that were previously anestrous (Table 2). Treatment with PRID-PGF induced estrus in a greater proportion of cows and heifers that were diagnosed as anestrous prior to treatment than MGA-PGF (P < .02) and the proportion that were induced was greater than the percentage of the anestrous control
TABLE 1. ESTROUS RESPONSE AND PREGNANCY RATES OF UNTREATED COWS AND HEIFERS OR ANIMALS TREATED TO SYNCHRONIZE ESTRUS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>MGA-PGF$_2$α</th>
<th>PRID-PGF$_2$α</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. animals</td>
<td>116</td>
<td>136</td>
<td>139</td>
</tr>
<tr>
<td>Animals in estrus, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First 7 d$^b$</td>
<td>23.3</td>
<td>71.3</td>
<td>81.3</td>
</tr>
<tr>
<td>First 21 d</td>
<td>74.1</td>
<td>78.7</td>
<td>82.7</td>
</tr>
<tr>
<td>Animals pregnant, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 7 d$^c$</td>
<td>17.2</td>
<td>44.9</td>
<td>56.8</td>
</tr>
<tr>
<td>After 21 d$^d$</td>
<td>40.0</td>
<td>45.6</td>
<td>56.8</td>
</tr>
</tbody>
</table>

$^a$Treatment effects analyzed by pair-wise comparisons.
$^b$All comparisons differed (P < .06).
$^c$Controls differed from both treated groups (P < .01) and MGA-PGF treated cattle differed from those treated with PRID-PGF (P < .09).
$^d$Controls differed from PRID-PGF treated cattle (P < .01).
TABLE 2. ESTROUS SYNCHRONIZATION, CONCEPTION RATES, AND PREGNANCY RATES IN CYCLING VS NON-CYCLING (NC) CATTLE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Status</th>
<th>No.</th>
<th>Sync %&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CR %</th>
<th>PR %&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Cycling</td>
<td>88</td>
<td>78.4</td>
<td>60.9</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>NC</td>
<td>28</td>
<td>60.7</td>
<td>64.7</td>
<td>32.0</td>
</tr>
<tr>
<td>MGA-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>Cycling</td>
<td>103</td>
<td>82.5</td>
<td>65.9</td>
<td>54.4</td>
</tr>
<tr>
<td></td>
<td>NC</td>
<td>33</td>
<td>36.4</td>
<td>41.7</td>
<td>15.2</td>
</tr>
<tr>
<td>PRID-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>Cycling</td>
<td>115</td>
<td>84.4</td>
<td>75.0</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>NC</td>
<td>24</td>
<td>66.7</td>
<td>69.1</td>
<td>50.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent in estrus within 7 d of the end of treatment or within 21 d in untreated controls. Estrous response was greater in cycling than in anestrous cattle (P < .01).

<sup>b</sup>Pregnancy rate within 7 d of the end of treatment or within 21 d in untreated controls. Pregnancy rates were higher in cattle which were previously cyclic (P < .02).
Figure 1. Distribution of estrus in MGA-PGF and PRID-PGF treated cattle.
animals (P < .01) that spontaneously exhibited estrus and ovulated within 7 d (Table 3).

The mean conception rate (cows pregnant/cows inseminated x 100) of cows exhibiting estrus during the synchronized breeding period was 65.2%. The conception rate of cows that were cyclic (65.7%) or anestrus (62.2%) prior to treatment did not differ (P < .2). The effects of cyclic status on conception rate also did not differ among treatments (P < .2).

The conception rates of cows that were treated with MGA-PGF or PRID-PGF did not differ (P < .2), nor did they differ between treated cows and untreated control cows bred at spontaneous estrus (P < .3).

Because a greater number of cyclic cows exhibited a synchronized estrus and were inseminated, pregnancy rates (cows pregnant/cows per group x 100) 7 d after treatment were greater among animals that were cycling (P < .02) than for those that were anestrus prior to treatment (Table 2). Pregnancy rate at 7 d (Table 1) differed by 12% (P < .09) between MGA-PGF and PRID-PGF groups as well as by 27% or more between untreated control cattle and either treated group (P < .01).

A greater percentage of non-lactating cows responded to the estrous synchronization treatments (P < .08) and became pregnant in the first 7 d of the breeding season (P < .03)
TABLE 3. ESTROUS SYNCHRONIZATION, CONCEPTION RATES, AND PREGNANCY RATES IN ANESTROUS POSTPARTUM COWS AND PREPUBERTAL HEIFERS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Sync %&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CR %&lt;sup&gt;c&lt;/sup&gt;</th>
<th>PR %&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
<td>25.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MGA-PGF&lt;sub&gt;2&lt;/sub&gt;α</td>
<td>31</td>
<td>32.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PRID-PGF&lt;sub&gt;2&lt;/sub&gt;α</td>
<td>23</td>
<td>65.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>73.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.8&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent in estrus within 7 d.  
<sup>b</sup>Pregnancy rate within 7 d.  
<sup>c,d,e</sup>Numbers within the same column with different superscripts are different (P < .05).

than did suckled, postpartum cows (Table 4). Conception rates, however, did not differ between suckled and non-lactating cows (P < .12).

Conception rates of cows beginning treatment late in the estrous cycle were lower than those treated either early or mid- in the estrous cycle (P < .05). An apparent interaction between the effects of the treatments and stages of the estrous cycle (stage x treat; P < .19) on conception rates (Table 5), was not significant at the .05 probability level. However, cows assigned to receive MGA-PGF beginning late in the estrous cycle exhibited a lower (P < .13) conception rate compared to that of animals assigned to receive PRID-PGF beginning late in the estrous cycle.
TABLE 4. ESTROUS SYNCHRONIZATION, CONCEPTION RATES AND PREGNANCY RATES IN SUCKLED VS NON-LACTATING POSTPARTUM COWS

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Sync %(^a)</th>
<th>CR %</th>
<th>PR %(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suckled</td>
<td>194</td>
<td>71.7(^c)</td>
<td>66.9</td>
<td>47.9(^e)</td>
</tr>
<tr>
<td>Non-lactating</td>
<td>87</td>
<td>85.7(^d)</td>
<td>72.6</td>
<td>62.2(^f)</td>
</tr>
</tbody>
</table>

\(^a\)Percent in estrus within 7 d of the end of treatment or within 21 d in untreated controls.  
\(^b\)Pregnancy rate within 7 d of the end of treatment or within 21 d in untreated controls.  
\(^c,d\)Numbers with different superscript within a column are different (P < .08).  
\(^e,f\)Numbers with different superscript within a column are different (P < .03).

TABLE 5. INFLUENCE OF STAGE OF THE ESTROUS CYCLE AT THE INITIATION OF TREATMENT ON CONCEPTION RATES OF CYCLIC COWS AND HEIFERS TREATED WITH MGA-PGF OR PRID-PGF

<table>
<thead>
<tr>
<th>Stage of the estrous cycle</th>
<th>Early</th>
<th>Mid</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>MGA-PGF(_2\alpha)</td>
<td>13</td>
<td>84.6</td>
<td>41</td>
</tr>
<tr>
<td>PRID-PGF(_2\alpha)</td>
<td>16</td>
<td>68.8</td>
<td>43</td>
</tr>
</tbody>
</table>

\(^a,b\)Numbers in the same column with different superscripts are different (P < .13).
DISCUSSION

The debate as to the success of synchronization regimens lies within their ability to induce a fertile estrus in all cattle and, especially, to induce and control a fertile estrus in previously anestrous cattle. It is well documented that synchronization schemes involving progestogens combined with prostaglandins are more successful, and, in fact, have quite comparable success rates when cattle are cycling prior to treatment. It is also established that suckled postpartum cattle are often non-cycling. In the present study, suckled cows exhibited a lower synchronized estrous response and subsequent pregnancy rate compared to cattle which were non-lactating (Table 4). This is presumably due to the inhibition of LH pulsatility caused by the suckling of the calf (Williams, 1990).

Combining PRID with PGF resulted in greater estrous synchronization and a more consistent level of fertility when compared to that resulting from a combined treatment with MGA and PGF. The key to the success of the PRID treatment in causing a higher rate of estrous synchronization lies almost solely in the results obtained from anestrous postpartum cattle and prepubertal heifers. A better estrous response within 7 d of PGF injection was observed in cattle treated with PRID-PGF because it was more effective than MGA-PGF at inducing and/or controlling estrus.
in cattle which were previously anestrous (Table 3). The PRID-PGF combination also induced more anestrous animals to exhibit estrus than occurred spontaneously during the same period in the control group. Furthermore, cattle induced to estrus with PRID-PGF had a significantly higher conception rate than those induced with MGA-PGF. It followed that 7- and 21-d pregnancy rates were improved not only over that of MGA-PGF, but also over that of untreated controls (Table 1).

The results of this experiment did not confirm earlier reports (Zimbelman et al., 1970; Beal et al., 1988; Chenault et al., 1990) of reduced conception rates among animals exposed to exogenous progestogens. In the present study, cycling and non-cycling cattle treated with PRID-PGF had conception rates comparable to untreated controls which exhibited estrus and ovulated spontaneously. Similar results have been reported by other authors (Beal, 1983).

Greater estrous synchronization was achieved by the PRID-PGF treatment in the present study in part because of a greater ability to induce estrus in anestrous cattle (Table 3). This ability of PRID to induce estrus in previously acyclic cattle has been reported by other authors (Beal, 1983). The reason for this improved response is unclear, but it may be related to recruitment of the ovulatory follicle from the existing population. This concept is discussed in greater depth below.
Once the PRID-PGF treatment initiated cyclicity in anestrous cows, the first estrus was more fertile than that following MGA-PGF treatment. To explain this finding, it is necessary to first examine the different conception rates obtained from cattle that were previously cyclic. Lower conception rates were obtained from animals treated with MGA-PGF beginning late in the estrous cycle than those treated with PRID-PGF (Table 5). The subfertility of this first estrus after treatment may be related to the difference in development of ovulatory follicles in cows treated with MGA or PRID. Cattle normally exhibit two or three waves of follicular growth during a 21 d cycle. Individuals treated with MGA beginning late (> Day 14) in the estrous cycle demonstrated persistence and ovulation of the dominant follicle that was present at the beginning of treatment (Patterson et al., 1989; Beal et al., 1990; Custer et al., 1991). Conversely, most dominant follicles present in PRID-treated cattle late in the cycle undergo atresia and another preovulatory follicle develops from a different follicular wave (Custer et al., 1991). Furthermore, estradiol 17-β levels greater than 2 pg/ml persist for one week prior to ovulation when the growth of the dominant follicle is arrested during 7 d treatment with MGA (Beal et al., 1990). Similar findings have been reported in cattle treated for 21 d with MGA (Coleman et al., 1990). This
alteration in the preovulatory follicle and disruption of systemic hormonal dynamics may be related to reduced conception rates following treatment with MGA.

Prepubertal heifers and postpartum anestrous cows also undergo follicular wave patterns although no CL is present. To date, no study has examined the follicular dynamics of cattle induced to estrus with MGA through the use of ultrasonography. It is possible that similar alterations as described above may be occurring. If true, these deviations from the normal sequence of events preceding the first puberal or postpartum ovulation may be related to the reduction in conception rates observed in this study in non-cyclic cattle treated with MGA.

An interaction between the effect of stage of the estrous cycle and progestogen treatment on follicular dynamics is just one of many ideas that have been presented in the literature as a possible cause for depressed conception rates after treatment with exogenous progestogens, particularly MGA. Melengestrol acetate is a more potent progestogen than the native progesterone released from the PRID, and infertility has been associated with excessive progesterone exposure before estrus (Lamond et al., 1971). Rate of clearance of MGA from the blood is unknown, but estrus has been reported to be delayed in anestrous cows fed MGA (Beal and Good, 1986). Levels of
luteinizing hormone (LH) are reported to be elevated in cattle treated with MGA (Hill et al., 1971), and sperm transport through the uterus is altered in sheep treated with MGA (Hawk, 1971). Finally, collection of more uncleaved ova (Hill et al., 1971) and identification of retarded cleavage rates (Wishart and Young, 1974) in fertilized ova collected after MGA treatment have been reported. Results of these studies indicate that reduced conception rates following treatment with an exogenous progestogen may not be related solely to altering follicular development. Furthermore, this study and others suggest that the length of progestogen exposure (Wishart and Young, 1974) and the nature of the progestogen that is used (MGA vs PRID; Custer et al., 1991) may be critical to determining post-treatment fertility.

While either treatment used in this study appeared to be an effective method of synchronizing estrus in cyclic cows, the PRID-PGF combination was more effective at inducing and controlling a fertile estrus in cattle that were diagnosed as anestrus prior to treatment. This fact, along with superiority of pregnancy rates at 7 and 21 d after insemination in PRID-PGF versus MGA-PGF treated animals may make PRID-PGF the synchronization regimen of choice. The use of either of the synchronization schemes is
currently not approved by the U.S. Food and Drug Administration.
ANALYSIS OF THE OCCURRENCE AND TIMING OF EMBRYONIC MORTALITY IN CATTLE AFTER DAY 25 OF GESTATION

MATERIALS AND METHODS

Animals.

One hundred thirty-four Angus-crossbred cattle at Catawba Research Station, Catawba, Virginia were used in 1989 to determine the extent of embryonic mortality after d 25 of gestation. Twenty-seven heifers (H), 57 nonlacting (NL), and 50 suckled, postpartum cows (S) at one location were bred according to spontaneous estrus. Animals ranged in age from 15 mo to 13 yr and averaged 4.2 yr (SD = 2.8 yr). Cattle were maintained on barley-silage based rations (Location 1). All cattle were fall calving (September to November). Days postpartum (DPP) ranged from 53 to 130 d and averaged 87.0 d (SD = 15.8).

Two hundred sixty-two Angus cattle (78 H, 47 NL, 137 S) at Wehrmann Angus, Newmarket, Virginia ranging in age from 14 mo to 11 yr and averaging 2.9 yr (SD = 2.1 yr) were housed on pasture and fed corn silage based rations (Location 2). Within S, 44 were fall calving (September to November) cows, and the remainder were spring calving (January to March). Days postpartum ranged from 20 to 88 d and averaged 58.1 d (SD = 21.3 d).

One hundred twenty-nine Angus-crossbred cattle (19 H, 29 NL, 81 S) at Catawba Research Station, Catawba, Virginia ranging in age from approximately 15 mo to 14 yr and
averaging 4.3 yr (SD = 2.9 yr) were housed on pasture and fed barley silage based rations (Location 3). All cattle had calved in the fall (September to November). Days postpartum ranged from 28 to 87 d and averaged 64.2 d (SD = 15.0 d).

**Experimental Design.**

One hundred thirty-four cattle at the first location were used to assess the degree of embryonic mortality occurring between 25 to 45 d and 45 to 65 d of gestation. Cattle were bred by artificial insemination to 1 of 12 sires following detection of spontaneous estrus. Cattle were diagnosed pregnant according to transrectal examination of the uterus by one of two operators using a B-mode, real-time, linear scanning ultrasound diagnostic system (Equisonics LS-300A with 5 mHz transducer; Tokyo Keiki Co., Ltd.). The observation of a fetal heartbeat was used to indicate fetal viability.

Animals at Locations 2 and 3 were used in the previously described experiment to synchronize estrus. Those animals not returning to estrus were examined by ultrasound for pregnancy at 20 to 25 d post breeding from which conception rates were determined. Ultrasound examinations of fetal viability were performed again at 40 to 45 d at both locations and at 60 to 65 d of gestation at
Location 3 only. Calving data were also collected on cattle at Location 1 and 2 to determine which animals carried their calves to term. From these observations, information on embryonic mortality was gathered.

Statistical Analysis.

In a preliminary step to identify independent variables of interest, data from cattle that were treated to synchronize estrus (Locations 2 and 3) were analyzed using the General Linear Models (GLM) procedure (SAS, 1985) for effects of location (1 df), parity (2 df), suckling status (1 df), and pretreatment cyclicity (1 df) on embryonic mortality at 45 d (EM45), 65 d (EM65), and at term (EM280). All two-way interactions were included. None of these effects were statistically significant with the exception of pretreatment cyclicity.

Embryonic mortality at 45 d, EM65, and EM280 were further analyzed using Biological Medical Data Processing (BMDP) 4f two-way and multiway frequency tables for categorical data (BMDP Statistical Software, 1983) with treatment (2 df), and pretreatment cyclicity (1 df) as main effects. These two variables were included with no expectation that treatment would affect embryonic mortality. Pretreatment cyclicity was included in the analysis again,
due to the small sample size and possible biases, using Forward Stepwise Regression in BMDP 4f (BMDP, 1983).

Using data from all three locations combined, EM45, EM65, and EM280 were analyzed using BMDP for effects of parity (2 df) and location (2 df). No significant effects were found.

Sire and inseminator were not included as main effects in the statistical analysis of the combined data because these variables were confounded with location. No single sire or inseminator was utilized at both locations. Inseminators (Location 3) and sires (both locations) were randomly distributed among treatment groups within location, hence, neither variable should affect the ability to validly measure main effects.
RESULTS

The use of ultrasound technology proved to be a valuable asset in the assessment of embryonic loss from 25 d through 65 d of gestation. Fetal viability was determined through the observation of a heartbeat, maintenance of placental integrity, and general appearance/echogenicity of the embryo. The presence of a viable fetus at 25 d but not at 45 d, 65 d or term was considered a fetal loss. In two instances, the placenta appeared to be pulling away from the wall of the uterus although a heartbeat was still detected. Both calves were non-viable at the next pregnancy check. In one occurrence, a non-viable twin was found in one uterine horn with a viable twin in the second uterine horn which was carried to term. Lastly, one deformed calf was observed through 65 d of gestation and was carried to term at which time it was stillborn.

There was no effect of synchronization treatment on the occurrence of embryonic mortality between 25 d of gestation and term (Table 6) although there was a trend for control cows to lose more embryos (10.7%; 3/28) than cycling cows (2.3%; 4/171; P < .05). These three individual animals were all untreated, control postpartum cows.

With knowledge of the above results, data from the cattle at all three locations were pooled for analysis. The
total embryonic loss from d 25 to term for this data set was 3.5%. A measurable amount of embryonic loss (4.8%) occurred between 25 and 45 d of gestation (Table 7). More embryos were lost from 25 to 45 d of gestation than at any other time measured. Embryonic loss between d 45 and 65 (2%) and between d 65 and term (3%) when combined were similar in magnitude to the loss occurring between 25 and 45 d of gestation.

**TABLE 6. EMBRYONIC MORTALITY RATES IN CATTLE TREATED TO SYNCHRONIZE ESTRUS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EM45&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>EM65&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>EM280&lt;sup&gt;c&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4/56 (7.1)</td>
<td>0/18 (0.0)</td>
<td>0/20 (0.0)</td>
</tr>
<tr>
<td>MGA-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>2/63 (3.2)</td>
<td>0/27 (0.0)</td>
<td>1/34 (2.9)</td>
</tr>
<tr>
<td>PRID-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>1/80 (1.2)</td>
<td>2/35 (5.7)</td>
<td>0/44 (0.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>EM45 = embryonic loss between 25 and 45 d of gestation. Data gathered at Location 1, 2, and 3.
<sup>b</sup>EM65 = embryonic loss between 45 and 65 d gestation. Data gathered at Location 1 and 3.
<sup>c</sup>EM280 = embryonic loss between 65 d gestation and term. Data gathered at Location 1 and 2.
### TABLE 7. EMBRYONIC MORTALITY RATES BY LOCATION

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EM45&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>EM65&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>EM280&lt;sup&gt;c&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location 1</td>
<td>9/133 (6.8)</td>
<td>2/124 (1.6)</td>
<td>6/122 (4.9)</td>
</tr>
<tr>
<td>Location 2</td>
<td>6/118 (5.1)</td>
<td>NA&lt;sup&gt;d&lt;/sup&gt; (0)</td>
<td>1/112 (0.9)</td>
</tr>
<tr>
<td>Location 3</td>
<td>1/81 (1.2)</td>
<td>2/80 (2.5)</td>
<td>NA&lt;sup&gt;e&lt;/sup&gt; (0)</td>
</tr>
<tr>
<td>TOTALS</td>
<td>16/332 (4.8)</td>
<td>4/204 (2.0)</td>
<td>7/234 (3.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>EM45 = embryonic loss between 25 and 45 d gestation.  
<sup>b</sup>EM65 = embryonic loss between 45 and 65 d gestation.  
<sup>c</sup>EM280 = embryonic loss between 65 d gestation and term.  
<sup>d</sup>Data on EM65 not gathered at this location.  
<sup>e</sup>Calving data at this location not available at this time.
DISCUSSION

The classical approach to studying embryonic mortality in cattle has been through the determination of interestrous intervals or recovery of the conceptus. Recovery of the conceptus is a costly and time-consuming process, but it is one of the most accurate methods available to assess early embryonic mortality. Some animals with fertilized eggs cycle normally because the embryo dies too early to prolong the estrous cycle. However, when death of the conceptus is sufficiently early that it neither extends the cycle nor causes subsequent infertility, the economic consequences are the same as fertilization failure.

Interestrous intervals are often used to determine embryonic loss when the embryo is viable long enough to prolong the cycle and make it abnormal in length (> 21 d). There are several obvious disadvantages to this method including failure to detect heat and an inability to verify the presence of the embryo (e.g. a postpartum cow displaying a 24 d cycle has not necessarily conceived and lost an embryo).

Using transrectal ultrasonography, a viable embryo may be identified as early as 21 d of gestation. Once a heartbeat is detected, the potential for determining the precise time of embryonic death is limited only by the frequency of observations (Kastelic et al., 1991).
Previous studies (Diskin and Sreenan, 1980; Roche et al., 1981a; Maurer and Chenault, 1983) have indicated that the bulk of embryonic mortality occurs prior to 17 d of gestation. In the present study, embryonic mortality occurring prior to 25 d of gestation is inestimable due to the limits of ultrasonography. This study is, therefore, only a good characterization of the rate of embryonic loss after approximately d 25 of gestation.

Boyd et al. (1969) reported losses of 8% between Day 21 and 60. Similarly, Kummerfeld et al. (1978) estimated a 7.2% loss of embryos during a 28- to 75-d observation interval in which milk progesterone samples were taken three times weekly. In this same study, a delayed return to estrus estimate of embryonic mortality yielded 22.7%, clearly demonstrating the bias of this method. Other authors (Beghelli et al., 1986) have reported rates as high as 35% for the same period of gestation. In the latter study, embryonic loss was based on a high progesterone level at 21 to 23 d after insemination, but the absence of a fetus at rectal palpation at 60 d. Such a method of estimating embryonic mortality rates is deficient and may lead to overestimation of embryonic loss because the presence of an embryo at 21 d is not verifiable.

In the present study, an appreciable amount (4.8%) of embryonic mortality occurred between 25 and 45 d of
gestation with smaller amounts (2.0% between 45 and 65 d and 3.0% from 65 d to term) occurring thereafter. Furthermore, the results of these two experiments illustrate that although conception rates after estrous synchronization with a progestogen may be depressed compared to untreated controls, there is, apparently, no effect of treatment on embryonic viability after 25 d of gestation (Table 6).

The finding that non-cycling untreated controls lost more embryos than either MGA-PGF or PRID-PGF induced animals is interesting. One might speculate that these postpartum cows lacked the progesterone "priming" of the uterus received by progestogen treated animals (Ramirez-Godinez et al., 1981), and that this might have affected the ability of the dam to maintain the embryo.

Whether palpation and manipulation of the reproductive tract during ultrasound examination could contribute to embryonic loss has been investigated. An earlier study examining the effects of manual palpation on embryonic viability concluded that uterine manipulation can cause a release of PGF$_{2\alpha}$ as measured by PGFM levels in the blood (Wann and Randel, 1990). Ultrasound examination of the uterus by a skilled operator involves considerably less manipulation of the reproductive tract than manual palpation. It is assumed that the exam performed as a part
of this experiment would not lead to embryonic loss due to elevated levels of PGF$_{2\alpha}$.

Once an embryo is lost, the fate of the conceptus and of the corpus luteum (CL) are important in determining the interval from embryonic death to ovulation. In one study (Kastelic and Ginther, 1989), embryonic loss was induced using PGF on d 28 and 42 of pregnancy. Ultrasound examinations revealed that luteolysis preceded cessation of a heartbeat and was characterized by rapid loss of a fairly intact conceptus. On the average, embryonic death and ovulation occurred 2.4 and 2.6 d after treatment, respectively. When cochloline was injected into the uterus on d 42, average time to embryonic death and ovulation were 1.2 and 20.8 d, respectively. Similarly, embryonic loss occurred immediately when the amnion was ruptured at d 42, but mean interval to ovulation was 35 d. The latter two treatments were associated with maintenance of the CL and prolonged retention of the conceptus, which degenerated excessively.

From these findings, it is apparent that lysing of the CL and expulsion of the conceptus are important to shortening the interval from embryonic death to ovulation. Through the use of ultrasonography, embryonic losses can be accurately assessed as early as 21 d of gestation, providing the opportunity for the animal which has lost an embryo to
return to estrus during a 60-d breeding season, thereby improving the net calf crop and reducing the impact of losses due to embryonic mortality.

In conclusion, these results indicate that the greatest proportion of embryonic mortality after 25 d of gestation occurs between 25 and 45 d, but that this amount is quite small (<5%) compared to the rate of embryonic mortality reported in previous studies using other methods. It is reasonable to conclude from these results that a greater loss in the net calf crop occurs due to fertilization failure or embryonic loss prior to maternal recognition of pregnancy, with a minimal loss occurring due to embryonic death during gestation.
CONCLUSION

The effectiveness of two progestin-based synchronization treatments was compared. The first progestogen, MGA, is synthetically manufactured, and the other, PRID, utilizes progesterone native to the cow. MGA and PRID were comparable at controlling estrus in cyclic cattle, but the PRID was the treatment of choice for inducing a fertile estrus in previously acyclic cattle.

MGA has received considerable attention in the literature focusing on reduced fertility occurring at the first estrus after feeding. PRID treatment, apparently, does not elicit this same depression in conception rates. An understanding of the mechanisms behind this difference might provide a clearer understanding of manipulation of the estrous cycle in both cyclic and acyclic cattle to elicit a fertile estrus.

The advent of linear array ultrasound technology has made the characterization of embryonic death after d 25 more reliable and accurate. In regards to embryonic mortality rates occurring after 25 d of gestation, estrous synchronization with a progestogen and PGF had no effect on embryonic viability. No appreciable loss of embryos was observed with the greatest loss (< 5%) occurring between 24 and 45 d of gestation.
LITERATURE CITED


VITA

Stacey Jane Hall, daughter of Kathryn and William Michael Hall, was born May 5, 1967 in Lompoc, California. She graduated from Homer L. Ferguson High School in June, 1985. She received a Bachelor of Science degree in Animal Science and Dairy Science from Virginia Polytechnic Institute and State University in December, 1989. She began work on her Master of Science degree in Animal Science (Physiology) under the direction of Dr. W. E. Beal at Virginia Polytechnic Institute and State University in August, 1989. She is a member of the American Society of Animal Science, and hopes to enter the teaching profession someday.

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