DIFFERENTIAL EFFECT OF MELENGESTROL ACETATE OR PROGESTERONE-RELEASING INTRAVAGINAL DEVICES ON FOLLICULAR DEVELOPMENT, PROGESTERONE AND ESTRADIOL-17ß CONCENTRATIONS AND PATTERNS OF LUTEINIZING HORMONE RELEASE DURING THE BOVINE ESTROUS CYCLE

by

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DIFFERENTIAL EFFECT OF MELENGESTROL ACETATE (MGA) OR PROGESTERONE-RELEASING INTRAVAGINAL DEVICES (PRID) ON FOLLICULAR DEVELOPMENT, PROGESTERONE AND ESTRADIOL-17ß CONCENTRATIONS AND PATTERNS OF LUTEINIZING HORMONE RELEASE DURING THE BOVINE ESTROUS CYCLE

Edward E. Custer

(Abstract)

Two studies were conducted to determine if 7-d MGA or PRID treatment initiated on d 17 of the estrous cycle altered: 1) follicular development, 2) estradiol-17ß (E2) and progesterone (P4) concentrations, and 3) patterns of release of luteinizing hormone (LH). In both studies, Angus, Angus x Holstein or Holstein cows 2 to 6 yr of age were randomly assigned to receive either MGA (.5 $mg \cdot hd^{-1} \cdot d^{-1}$; n = 23) or PRID (n = 26) for 7 d or to serve as untreated controls (n = 14). Real time, B-mode ultrasound, equipped with a 7.5 mHz linear-array transrectal transducer, was used to conduct daily ovarian scans beginning 3 (Study 1) or 9 d (Study 2) after onset of estrus. Jugular venous blood samples (45 ml) were collected coincident with ovarian scans. In study 2, cows were fitted with indwelling jugular catheters 17 (Control, MGA and PRID), 20 and 23 d (MGA and PRID) after onset of estrus and blood samples were collected at 15-min intervals for 6 h for determination of LH. Interestrus interval was extended ($P<.05$) for 3 to 5 d in MGA-treated cows exhibiting two or three dominant follicles (classified as MGA-2 and MGA-3, respectively) or PRID-treated

cows compared to controls exhibiting two or three dominant follicles during the estrous cycle (control-2 and control-3, respectively). Forty-four percent of MGA-treated cows ovulated the dominant follicle present at the beginning of MGA treatment. In both studies, days from detection of the ovulatory follicle until ovulation were greater ($P<.01$) in MGA-2 and control-2 cows than control-3, MGA-3 and PRID cows. Diameter of the ovulatory follicle was greater ($P<.01$) 9 d before estrus and growth rate of the ovulatory follicle was less ($P<.02$) in MGA-2 and control-2 cows than control-3, MGA-3 and PRID cows. Serum P4 decreased 3 d earlier ($P<.02$) during the estrous cycle of MGA-2 and control-2 cows than control-3, MGA-3 and PRID cows. Serum E2 was greater ($P<.01$) 7 d before estrus in MGA-2 cows than all other treatment groups. Changes in mean and baseline LH concentrations and amplitude of LH pulses on d 17, 20 and 23 after onset of estrus did not differ ($P>.10$) among treatments. Luteinizing hormone pulse frequency was greater ($P<.03$) on d-20 after onset of estrus in MGA-2 cows than MGA-3 and PRID cows ($4.3 \pm .6$ vs $2.6 \pm .3$ and $3.2 \pm .4$, respectively). In addition, LH pulse frequency did not differ ($P>.10$) 17 or 23 d after onset of estrus among treatments. In conclusion, MGA treatment extended the dominance phase of development of ovulatory follicles, which resulted in the premature increase in serum E2 and frequency of LH release, whereas the dominant follicle present at the beginning of PRID treatment underwent atresia and another preovulatory follicle developed.

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Chapter I

Introduction

Controlling the estrous cycle in beef cows with progestogens has been the subject of ongoing research for the past five decades (Hansel and Beal, 1978; Patterson et al., 1989; Odde, 1990). In the United States < 5% of beef cows are bred artificially and less than 50% of beef producers realize the benefits of estrous synchronization programs (Corah and Kiracofe, 1989). Utilization of synthetic progestogens affords beef producers an opportunity to increase reproductive efficiency by inducing estrus in previously acyclic cattle (Beal and Goode, 1986; Patterson et al., 1989). However, most progestogen-synchronization programs have resulted in unacceptable conception rates, regardless of whether cattle are artificially inseminated or bred naturally at the synchronized estrus.

If the beef cattle industry is to realize the benefits of genetic progress, by expanding its utilization of artificial insemination, successful application of procedures designed to effectively synchronize estrus without adversely affecting reproductive efficiency must be implemented. To accomplish these goals, a thorough understanding of the effect of progestogens on physiological events (endocrine and follicular) which dominate the typical estrous cycle of beef cows is

essential. This review will describe endocrine and follicular events that occur during the bovine estrous cycle and examine the impact of progestogens on these variables to alter the reproductive capabilities of the mature beef cow.

Chapter II

Literature Review

The nonpregnant beef cow is a polyestrous, spontaneously ovulating, nonseasonal breeder. Average interestrus interval is usually 20 to 21 days for heifers and cows, with approximately 85% of all animals falling within the range of 17 to 25 days (Peters and Ball, 1987). Average length of estrus is 18 h with greater than 93% of all animals falling within the range of 10 to 27 h (Roberts, 1986). Ovulation occurs on average 10 to 16 h after the end of estrus with a range of 2 to 26 h (Roberts, 1986). The coordinated actions of hypothalamic, pituitary, ovarian and uterine secretory products perpetuates estrous cycles throughout the year.

Endocrine Patterns

The release of ovarian steroids, estradiol-17ß and progesterone, and anterior pituitary gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), is a dynamic process throughout the bovine estrous cycle.

Ovarian Steroids

Estrogen:

Throughout most of the bovine estrous cycle, systemic concentrations of estradiol-17ß remain basal, between 2 to 4 pg/mL (Hansel and Echternkamp, 1972; Glencross et al., 1973; Schallenberger et al., 1985), with no apparent pulsatile pattern of release (Schallenberger et al., 1985). Whereas, determination of estradiol-17ß concentrations in blood samples collected from the caudal vena cava established a well defined pattern of pulsatile release (Walters et al., 1984). Systemic and caudal vena caval basal concentrations of estradiol-17ß were greater during the early (d 4) than during the mid-luteal (d 11) phase of the estrous cycle (5.6 vs 4.0 and 23.6 vs 7.7 pg/mL, respectively; Walters et al., 1984). Both frequency and amplitude of estradiol-17ß pulses increased during the early compared to the mid-luteal phase of the estrous cycle (7.2 vs 3.6 pulses /12 h and 16.8 vs 6.4 pg/mL, respectively; Walters et al., 1984). During both the early and mid-luteal phase of the cycle, at least 90% of all estradiol-17ß pulses were preceded within a 60-min period by a pulse release of LH (Walters et al., 1984). Similar patterns of LH and estradiol-17ß release have been reported for ewes (Baird and McNeilly, 1981). However, pulsatile secretion of FSH may not be associated with the pulsatile secretion of estradiol-17ß during the bovine estrous cycle (Walters et al., 1984). In addition, systemic

concentrations of estradiol-17ß have been shown to increase above basal concentrations during three time periods during the estrous cycle. In naturally-occurring (Henricks et al., 1971; Shemesh et al., 1972; Glencross et al., 1973; Schallenberger et al., 1985) and prostaglandin-induced shortened estrous cycles (Schallenberger et al., 1984), mean systemic (Henricks et al., 1971; Shemesh et al., 1972; Glencross et al., 1973; Schallenberger et al., 1985) and caudal vena caval (Walters and Schallenberger, 1984) estradiol-17ß concentrations increased transiently to between 3 and 10 pg/mL during the first 3 d of proestrus. On the day before estrus, estradiol-17ß concentrations increased to 15 to 25 pg/mL as a result of an increase in both frequency and amplitude of estradiol-17ß pulses; however, an estradiol-17ß concentration greater than 10 pg/mL was not detectable until progesterone concentrations fell to less than 2 ng/mL (Henricks et al., 1971; Walters and Schallenberger, 1984). With the onset of estrus, estradiol-17ß concentration was equivalent to that on the day before estrus and within 2 to 5 h after the onset of estrus, estradiol-17ß concentrations returned to basal concentrations before the time of ovulation (Henricks et al., 1971; Shemesh et al., 1972; Glencross et al., 1973). Estradiol-17ß is lowest during the first 2 d after estrus (Glencross et al., 1973), then it begins to increase on about d 4 (Shemesh et al., 1972; Ireland and Roche, 1983a), with a second peak by d 6 to 7 after the proestrus estradiol-17ß peak (Glencross et al., 1973). A third

increase in estradiol-17ß concentrations occurs between d 10 to 16 of the cycle, with peak concentrations reported between d 11 and 13 (Hansel and Echternkamp, 1972; Shemesh et al., 1972). Similar estradiol-17ß concentrations have been reported during the first 30 d of pregnancy in the cow, when progesterone concentrations are at luteal phase concentrations (Glencross et al., 1973).

Progesterone:

The primary source of progesterone secretion during the bovine estrous cycle is the corpus luteum. Plasma progesterone concentrations increase rapidly during the first five days after ovulation (Glencross et al., 1973; Walters et al., 1984) and peak at 2 to 10 ng/mL between d 8 and 18 of the estrous cycle (Wetteman et al., 1972; Hansel and Echternkamp, 1972; Glencross et al., 1973; Schallenberger et al., 1985). These concentrations are maintained until the onset of luteolysis (Schallenberger et al., 1985). During the luteal phase, 45% of pulsatile progesterone releases are coincident with each LH/FSH pulse, and 44% of pulsatile progesterone releases occurred after FSH pulses that were not associated with LH pulses (Walters et al., 1984; Schallenberger et al., 1985). Prostaglandin-induced (Schallenberger et al., 1985) and natural luteolysis (Henricks et al., 1971) result in a steady decline in progesterone concentrations until basal concentrations of ≤ 1 ng/mL are

reached within 36 h after prostaglandin injection (Schallenberger et al., 1985).

Anterior Pituitary Gonadotropic Hormones

Luteinizing Hormone and Follicle-Stimulating Hormone:

Concentrations of LH and FSH are not maintained at a constant concentration throughout the estrous cycle in cows. Luteinizing hormone and FSH fluctuate in an episodic pattern, and this fluctuation is dependent upon the stage of the estrous cycle (Rahe et al., 1980; Walters et al., 1984; Schallenberger et al., 1985; Parfet et al., 1989). Basal concentrations of both LH and FSH remain relatively constant during the early and late luteal phases of the cycle (.6 to .8 and 42 to 48 ng/mL, respectively; Schallenberger et al., 1985). With the onset of luteolysis, basal concentrations of LH and FSH begin to increase (1.3 and 56 ng/mL, respectively; Schallenberger et al., 1985). During the early luteal phase of the cycle, d 1 to 4, LH release is characterized by low-amplitude (.3 to 1.8 ng/mL) high-frequency pulses (14 to 30 pulses/24 h; Rahe et al., 1980; Walters et al., 1984; Schallenberger et al., 1985; Parfet et al., 1989), and FSH pulse frequency parallels that observed for LH (Walters et al., 1984; Schallenberger et al., 1985). During the mid- (d 5 to 12) and late- (d 13 to 16) luteal phase of the cycle, LH release is typically classified as having high

amplitude (.8 to 7.0 ng/mL) low frequency pulses (3 to 8 pulses/ 24 h; Rahe et al., 1980; Walters et al., 1984; Schallenberger et al., 1985); FSH pulse frequency is increased relative to LH pulse frequency (6.9 and 3.4 pulses/24 h, respectively), with an average of one additional pulse of FSH between synchronous FSH/LH pulses (Walters et al., 1984; Schallenberger et al., 1985). Frequency of LH and FSH pulses increases during and after luteolysis (14 and 10 pulses/12 h, respectively), and the increase persists through proestrus and estrus (Schallenberger et al., 1984; Walters and Schallenberger, 1984; Schallenberger et al., 1985). Following the FSH/LH preovulatory surge, which results from an increase in frequency and amplitude of both FSH and LH pulses, LH pulse frequency decreases. In contrast, a second peak of FSH, due to an increase in amplitude with no alteration in frequency, begins 4 to 18 h after the onset of the preovulatory surge FSH/LH (Walters and Schallenberger, 1984; Findlay and Clarke, 1987).

To summarize, Walters et al. (1984) and Schallenberger et al. (1984) reported that estradiol-17ß, progesterone, LH and FSH are secreted in a pulsatile pattern during the estrous cycle. Parallel pulses of LH and FSH are secreted during the cycle as well as individual pulses of FSH. Progesterone pulses result from the stimulation of FSH and/or LH. Whereas, estradiol-17ß pulses are caused by the pulse release of LH. Frequency and amplitude of LH increase during proestrus, which stimulates development of a large estrogen-active, preovulatory follicle.

Follicular Development During the Estrous Cycle

Ovarian follicular development in the bovine is a process that involves recruitment, selection, growth, dominance atresia and ovulation during the estrous cycle (Spicer and Echternkamp, 1986; Ireland , 1987; Ireland and Roche, 1987; Fortune et al., 1988; Fortune et al., 1991; Roche and Boland, 1991). In 1960, based upon histological examinations, Rajakoski reported that two waves of growth of follicles ≥ 5 mm in diameter occur during the bovine estrous cycle. The first wave begins on d 3 to 4 and ends on d 12, and the second wave is initiated between d 12 to 14 and ends with a dominant follicle that is destined to ovulate. In a more recent study, Ireland et al. (1979) found that only 30% of heifers have large follicles between d 1 to 4 of the cycle, and, 88 and 73% have large follicles between d 5 to 10 and d 18 to 20 of the cycle, respectively, which supports the concept of follicular waves during the bovine estrous cycle that was put forth by Rajakoski (1960). These studies were not supported by the work of several researchers (Choudary et al., 1968; Donaldson and Hansel, 1968; Marion et al., 1968) who reported that follicles larger than 5 mm in diameter were not present on either ovary during the luteal phase of the cycle, but, atretic follicles larger than 5 mm in diameter were found on both ovaries throughout the entire cycle. In addition, Marion and Gier, (1968) observed that as many as 11 follicles grow to a diameter larger than 8 mm during the bovine estrous

cycle. These observations led to the conclusion that growth of follicles from one class size to another was a continuous process and was not dependent on stage of the estrous cycle.

In an attempt to more clearly define the pattern of follicular growth during the estrous cycle, Matton et al. (1981) "marked" (microinjection of India ink into the follicular stroma) the largest and second largest follicle on each ovary on d 3, 8, 13 and 18 of the estrous cycle. They found that the largest follicle on d 3 was slightly larger on d 8 and that 80% were still the largest follicle. The largest follicles on d 8 were smaller on d 13, and only 50% were still the largest follicle. By d 18, the largest follicles on d 13 were smaller, and only 10% were still the largest follicle. There was a rapid turnover of large follicles after d 18 of the cycle, which resulted from the largest follicles present on d 18 ovulating only identified within 3 d of ovulation. The results of this study did not support the two wave "hypothesis" of Rajakoski, but, it supports the idea of waves of follicular growth throughout the estrous cycle.

Ireland and Roche, (1983a; 1983b) collected follicles ≥ 6 mm in diameter from 6 to 7 heifers on d 3, 5, 7, 9, 11 and 13 of the estrous cycle and separated the follicles into two classes, either estrogen-active or estrogen-inactive. Fluid from estrogen-active follicles contained higher concentrations of estradiol-17ß than progesterone and androgens and had a low incidence of atresia. During d 3 to 7 of the cycle, a single

estrogen-active follicle > 6 mm in diameter developed, all estrogen-inactive follicles regressed, and no other estrogen-active follicles were identified until d 13 of the cycle. In support of the follicular wave hypothesis, Ireland and Roche (1987) reported that at least three periods of increased estradiol-17ß production by a single estrogen-active follicle, which was several millimeters larger than the largest subordinate follicle, were evident during estrus, early diestrus and mid-diestrus. This observation was similar to reports of Hansel and Echternkamp, (1972) and Shemesh et al., (1972) on the pattern of serum concentrations of estradiol-17ß during the bovine estrous cycle. These data, along with the observations of Staigmiller and England, (1982) and Ireland et al. (1984) that a single, large follicle on one ovary is responsible for increases in estradiol-17ß concentrations during early diestrus and proestrus and that cows have at least one large follicle capable of producing estradiol-17ß during most of the estrous cycle, support the hypothesis that follicular development in cattle occurs in waves over the course of a 21 d estrous cycle.

Before the development of diagnostic ultrasound, researchers were unable to monitor daily growth and regression of individual ovarian follicles during the bovine estrous cycle. The use of ultrasonography has overcome these limitations and enabled researchers to define more clearly the daily growth and regression of individual follicles (Pierson and Ginther, 1984; Reeves et al., 1984; Pierson and Ginther, 1986; Pierson and

Ginther, 1988). Accuracy of ultrasonography as a diagnostic tool for assessing ovarian structures (follicle and corpus luteum) was examined by Pierson and Ginther (1987). They compared *in vivo* ultrasonographic results with data collected on slices of excised ovaries. Ultrasonography tended to over estimate the number of 2 to 3 mm follicles, but it was an accurate estimator of the diameter of the largest follicle. In addition, there was 100% agreement between ultrasound imaging and ovarian slices for identification of the ovary bearing the corpus luteum (Pierson and Ginther, 1987).

The first report utilizing ultrasonography to evaluate ovarian follicular dynamics in cattle was published in 1984 (Pierson and Ginther, 1984). In that and subsequent studies (Pierson and Ginther, 1986; Pierson and Ginther, 1987), daily counts of follicles 2 to 3, 4 to 6, 7 to 10, 11 to 13 and > 13 mm in diameter were recorded for Holstein heifers over a period extending from 3 d before ovulation until 3 d after the subsequent ovulation. The profiles of all follicular size categories, except 4 to 6 mm, were bimodal throughout the estrous cycle. There was selective growth of a follicle to preovulatory size by d 6 of the cycle; the follicle became static for 5 to 6 d then regressed. Follicles that attained preovulatory diameter around d 18 to 20 of the cycle ultimately became ovulatory follicles.

Quirk et al. (1986) demonstrated that ultrasonagraphy could be used to monitor daily changes in individual follicles

with antral diameters of ≥ 5 mm. Studies utilizing daily ultrasonic monitoring indicate that heifers and cows can have either two, three or four waves of follicular growth during the estrous cycle (Pierson and Ginther, 1988; Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a; Knoph et al., 1989; Driancourt, 1991; Driancourt et al., 1991; Taylor and Rajamahendran, 1991a). In 33 two- and three-wave interestrus intervals several characteristics of follicular development have been identified. The dominant follicle of the first wave of a two- or three-wave cycle becomes detectable as part of a cohort of 4 to 6 mm follicles as early as the d after ovulation (Pierson et al., 1989) and can be identified on average by d 2 to 5 of the cycle (Savio et al., 1988). The dominant follicle of the first wave grows (1.2 to 2.0 mm/d) to a maximum diameter (12 to 16 mm) by d 6 to 7, then it is relatively static between d 6 to 10 of the cycle (Sirois and fortune, 1988; Savio et al., 1988; Ginther et al., 1989a; Ginther et al., 1989d). Following this static phase, the dominant follicle of the first wave decreases (-1.0 mm/d) in size and becomes undetectable between d 15 and 25 of the cycle (Savio et al., 1988; Ginther et al., 1989a; Ginther et al., 1989d; Knoph et al., 1989). Once the dominant follicle of the first wave becomes identifiable, growth of subordinate or secondary follicles (follicles that grow during periods when dominant follicles are evident) ceased (Savio et al., 1988; Ginther et al., 1989a; Ginther et al., 1989d). When the dominant follicle is in the growing phase,

emergence of the next follicular wave is suppressed (Ko et al., 1991). In cows exhibiting either two or three follicular waves, no differences were found with respect to characteristics of the dominant or subordinate follicles of the first wave (Sirois and Fortune, 1988; Savio et al., 1988; Ginther et al., 1989a; Ginther et al., 1989d).

The dominant follicle of the second wave was the ovulatory follicle in cows with two waves, but it becomes atretic in cows with three follicular waves. The dominant follicle of the second wave of a three-wave cycle becomes identifiable between d 9 to 12 of the estrous cycle, grows (1 to 1.6 mm/d) to a maximum diameter (8 to 11 mm) between d 14 to 16 (Fortune et al., 1988; Savio et al., 1988), enters the static phase between d 16 to 19, and then becomes non-detectable by d 19 to 25 after the previous ovulation (Fortune et al., 1988; Savio et al., 1988). The dominant follicle of the second wave of a two-wave cycle is first detected on d 9 to 10 of the cycle and grows (1.2 mm/d) to a maximum diameter (16 to 17 mm) on the d before ovulation (d 20 to 21; Ginther et al., 1989a; Ginther et al., 1989d). Diameter of the ovulatory follicle of a two-wave cycle on the d before ovulation is not different from the diameter of the dominant follicle of the first wave during the static phase (Ginther et al., 1989a; Ginther et al., 1989d). The ovulatory follicle of a three-wave cycle is identifiable by d 16 of the cycle and reaches maximum diameter (12 to 19 mm) on the d before ovulation (Fortune et al., 1988; Savio et al., 1988). Persistence

(detected ≥ 5 mm) of the ovulatory follicle for a three-wave cycle was shorter than that for nonovulatory dominant follicles of the first or second wave of a three-wave cycle (6 versus 17 and 13 d, respectively) and ovulatory and nonovulatory dominant follicles of a two-wave cycle (6 versus 10 and 17 d respectively; Fortune et al., 1988; Ginther et al., 1989a; Ginther et al., 1989b; Ginther et al., 1989c).

The timing and mechanism for selection of the ovulatory follicle has received extensive attention. The ovulatory follicle of two- and three-wave cycles was not consistently the largest follicle on either ovary until the d of estrus. However, the ovulatory follicle was always one of the two largest follicles during the 3 d before estrus (Scaramuzzi et al., 1980; Quirk et al., 1986; Sirois and Fortune, 1988; Pierson and Ginther, 1988). Diameter of estrogen-active and estrogen-inactive follicles were not different 12 to 72 h after prostaglandin-induced luteolysis on d 8 or 9 of the cycle (Ireland and Roche, 1982). During the LH surge one dominant, estrogen-active follicle is present per pair of ovaries (Ireland and Roche, 1982a; Staigmiller et al., 1982). These observations expand and support the earlier work of Dufour et al. (1972) and Staigmiller and England (1982), who reported that the ovulatory follicle was not identifiable by size alone until not more than three days before estrus.

Ginther et al., (1989b) examined temporal associations for two versus three waves of follicular activity during the bovine

estrous cycle. For 18 two-wave and 4 three-wave interovulatory intervals in heifers, events that occurred during the first half of the interovulatory interval were not associated with the emergence of a third follicular wave. Day of emergence of the ovulatory follicle was earlier for two- versus three-wave intervals (d 9.6 and 16, respectively). Interval from detection of the ovulatory follicle until ovulation was longer in two- versus three-wave intervals (11 and 7 d, respectively). Diameter of the ovulatory follicle on the d before ovulation was not different in two- versus three-wave intervals (17 and 18 mm, respectively). Interovulatory interval was shorter for two- versus three-wave intervals (20 and 23 d, respectively), and the average day of luteal regression (progesterone < 1 ng/mL) was earlier for two- versus three-wave intervals (d 16 and 19, respectively). For all heifers, luteal regression occurred after emergence of the dominant follicle of the ovulatory wave.

To further investigate the role of the corpus luteum in regulating the number of follicular waves during the estrous cycle, several researchers examined the capability of the dominant follicle of the first wave to ovulate after premature luteal regression. Savio et al. (1990a) induced luteolysis (prostaglandin analog) on d 7 of the cycle, a time when the dominant follicle of the first wave was in the late growing or early static phase of development. The dominant follicle of the first wave ovulated in 37 of 43 treated heifers. Within 24 h after treatment with prostaglandin, plasma progesterone

concentrations were < 1 ng/mL in all heifers, indicating complete luteolysis had occurred. Similar results have been reported for heifers treated on d 5 (late growing phase) or 8 (static phase) of the cycle; however, treatment on d 12 (late static or regressing phase) of the cycle resulted in selection of the ovulatory follicle from the second wave (Kastelic et al., 1990a; Lavoir and Fortune, 1990). Therefore, the dominant follicle of the first wave is capable of ovulating when luteolysis occurs before detection of the second follicular wave. These studies expand and support the hypothesis that inhibition of final maturation of the dominant follicle results from an increase in progesterone concentration which acts to inhibit LH secretion (Abeyawardene and Pope, 1987). Turnover of dominant follicles has also been reported in prepubertal heifers (Roche and Boland, 1991), postpartum anestrous cows (Savio et al., 1990b; Savio et al 1990c; Dimmick et al., 1991; Lucy et al., 1990; Murphy et al., 1991; Perry, 1990) and during pregnancy in cattle (Bellin et al., 1984; Guilbault et al., 1986; Ginther et al., 1989c; Driancourt et al., 1991; Taylor and Rajamahendran, 1991b; Thatcher et al., 1991).

The results from these studies indicate that follicular development in cyclic cattle is not a continuous process. Rather it occurs in waves of follicular growth (normally two or three per estrous cycle). The life span of the corpus luteum is an important factor in determining whether an animal will

exhibit either two or three waves of follicular growth during an estrous cycle.

Synchronization With Progestogens

Christian and Casida (1948) were among the first researchers to demonstrate that daily injections of progesterone completely suppressed estrus and ovulation in cattle. Long term progesterone treatment (18 to 21 d) resulted in 80 to 90% estrous synchronization, and, conception rate at the synchronized estrus was 15 to 20% lower than that for untreated controls (Hansel et al., 1966; Roche, 1974a). Short-term progesterone treatment (< 10 d) overcame the reduced conception rate at the synchronized estrus, but, it resulted in reduced estrous response compared to long term progesterone treatment (Roche, 1974a). Wiltbank and Kasson (1968) combined a short-term progestogen treatment (9 d) with the luteolytic effects of estradiol valerate early in the cycle (Wiltbank et al., 1961). This treatment alleviated the poor estrous response reported by Roche (1974b), while maintaining conception rates comparable to those of untreated controls. These studies were the basis for the development of SynchroMate-B (SMB) which combines a 9-d, 6-mg norgestomet implant with an injection of 5 mg estradiol valerate and 3 mg norgestomet at the time of implant insertion. Odde (1990) reviewed several studies utilizing SMB and found a range of 77 - 100% estrous response after implant removal and

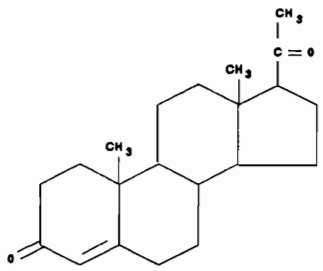
conception rates at the synchronized estrus ranging from 33 to 68%.

Melengestrol acetate (MGA; 6-methyl-17-alpha-acetoxy-16 methylene-pregn-4,6-diene-3,20-dione), is an orally active progestational steroid developed in 1962 which is capable of maintaining pregnancy and suppressing estrus and ovulation in cyclic cows and heifers (Zimbelman and Smith, 1966). Melengestrol acetate is approved by the Food and Drug Administration for use within the United States in feedlot heifers to increase feed efficiency and average daily gain by inhibiting estrus and ovulation without inhibition of follicular development. Estrus and ovulation are suppressed when MGA is given at a dose of .5 $mg \cdot animal^{-1} \cdot day^{-1}$, with the minimal effective dose .42 mg/day (Zimbelman, 1966; Zimbelman and Smith, 1966). Structurally, MGA is closely related to naturally-occurring progesterone and is biologically characterized as an analog of medroxyprogesterone acetate (MAP; Figure 1). Biological differences are evident among the three compounds when administered orally. Progesterone is essentially orally inactive, whereas, MGA is 300- to 900- times more potent than MAP. Commercially, MGA is available in premixes containing 100 or 200 mg/.45kg and designated as MGA-100 and MGA-200.

Synchronization programs utilizing MGA initially met with limited success. Long term feeding (14 to 21 d) of MGA resulted in a high percentage (60 to 100%) of cows exhibiting estrus after MGA withdraw, however, conception rates at the

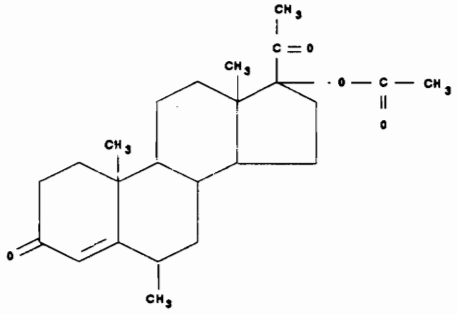
synchronized estrus ranged from 40 to 50% lower than those of untreated controls (Hill et al., 1971; Henricks et al., 1973; Roche and Crowley, 1973). Short-term MGA feeding, 5 d (Moody et al., 1978) or 7 d (Patterson et al., 1986; Beal et al., 1988;) with an injection of prostaglandin$F_2\alpha$ on the last day of MGA feeding effectively synchronized estrus in a large percentage of animals treated, however, conception rates at the synchronized estrus were lower than those of untreated controls. Even though conception rates are reduced at the synchronized estrus after MGA treatment, pregnancy rates at the end of the breeding season are equivalent to untreated controls (Zimbelman et al., 1970).

More recently, researchers have attempted to circumvent reduced fertility associated with long- and short-term MGA feeding programs by utilizing a 14-d MGA treatment period followed by an injection of a luteolytic agent 17 d after the last day of MGA feeding (Brown et al., 1988). This method effectively places all animals between d 11 to 14 of the cycle at the time of injection of the luteolysin. This results in a reduction in the variability of responsiveness to the prostaglandin injection (Stevenson et al., 1984) and alleviates unacceptable conception rates associated with long- and short-term MGA treatment (Patterson et al., 1989a; Patterson et al., 1989b; Patterson et al., 1989c). Estrous response ranged from 68 to 96% with a mean conception rate of 72% at the estrus after prostaglandin injection. During the first 30 d of the breeding



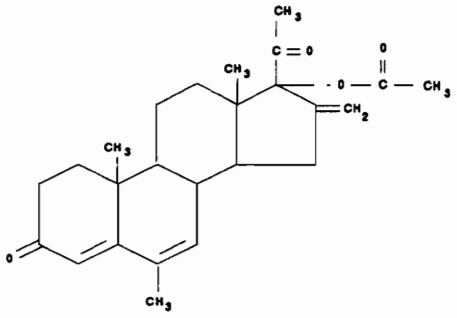
PROGESTERONE

Pregn-4-ene-3,20-dione



MAP

6-alpha-methyl-17-alpha-acetoxy-pregn-4-ene-3,20-dione



MGA

6-methyl-17-alpha-acetoxy-16-metylene-pregn-4, 6-diene-3,20-dione

Figure 1. Structural formulas for progesterone, Melengestrol acetate (MGA) and medroxyprogesterone acetate (MAP). Adapted from Patterson et al., (1989).

season, 83% of treated heifers conceived under natural-service conditions when bulls were joined with heifers 17 d after the 14 d MGA synchronization treatment. To maximize the effectiveness of the program under natural-service conditions, a cow to bull ratio of no more than 20:1 is recommended (Patterson, 1990; Patterson et al., 1990).

Progesterone-releasing intravaginal devices (PRID) have also been utilized in research efforts to control the bovine estrous cycle. In heifers treated with a PRID for 7 d and given $PGF_2\alpha$ on d 6 of PRID treatment, estrus was synchronized, and conception rates (79%) were greater than those for untreated controls (Hansel and Beal, 1978). Beal (1983) reported similar results in cyclic and noncyclic cows, which indicates that PRID's were effective in inducing estrus in previously acyclic cows. Presently, PRID's and MGA are not approved by the FDA as a means of estrous synchronization in breeding cattle. Furthermore, reduced conception rates which are associated with synchronization programs utilizing progestogens have limited their use by the cattle industry.

Physiological Alterations Associated with Progestogen Synchronization

The physiological mechanism which results in reduced conception rates at the synchronized estrus after progestogen

treatment remains unclear. The after variables have been identified relative to the adverse progestogen effects.

Stage of the Cycle:

Stage of the estrous cycle at the beginning of progestogen treatment influences conception rates after either MGA or SMB. Hill et al. (1971) were among the first to report that heifers administered MGA for 14 d beginning on d 15 of the cycle had lower pregnancy rates than heifers beginning treatment on d 4 of the cycle. In agreement with this study, Henricks et al. (1973) reported a 23% reduction in pregnancy rate in heifers treated with MGA for 14 d beginning on d 15 of the cycle compared to untreated controls. Furthermore, at d 40 after breeding, heifers treated with MGA had a 31% reduction in the number of embryos compared to untreated controls. More recently, Beal et al. (1988) and Patterson et al. (1989) reported reduced conception rates in heifers and cows treated with MGA for 7 d beginning late (≥ d 11) in the estrous cycle compared to those in which MGA treatment began early (≤ d 11) during the estrous cycle. Brink and Kiracofe (1988) reported similar results for heifers and cows that began SMB treatment early (≤ d 11) compared to late (≥ d 11) during the estrous cycle (Table 1). Hall (1991) compared the effects of MGA and PRID treatment either early or late during the cycle and reported a 23% increase in conception rate for animals treated with a PRID late

in the cycle compared to those treated with MGA. Melengestrol acetate and PRID treatment initiated during the early stage of the cycle resulted in comparable conception rates (78 and 73%, respectively). In a more recent study, Sanchez et al. (1992) reported a significant increase in conception rate for both heifers and cows at the estrus after 10 d of norgestomet treatment when treatment was imposed in the absence of a corpus luteum. This would be comparable to treatments initiated late during the estrous cycle in the studies outlined in Table 1.

Table 1. Conception rates of heifers and cows treated with SMB or MGA + prostaglandin (PGF) beginning either early (≤ d 11) or late (≥ d 11) during the estrous cycle.

Stage of the estrous cycle	Aggregate conception rates for stage of the estrous cycle treatment began		
	SMB[a]	MGA + PGF[b]	MGA + PGF[c]
Early	22/47 (47%)	27/35 (77%)	42/64 (66%)
Late	13/38 (37%)	8/26 (31%)	13/36 (36%)

[a]SMB (from Brink and Kiracofe, 1988).
[b]7 d MGA + Fenprostalene d 7 (from Patterson et al., 1989).
[c]7 d MGA + Lutalyse d 7 (from Beal et al., 1988).

Luteal Dysfunction:

Luteal dysfunction, characterized by a short luteal phase and(or) insufficient luteal progesterone production, has been reported after synchronization with synthetic progestogens. Favero et al. (1988) synchronized 183 beef heifers and cows with SMB and artificially inseminated 48 h after implant removal. Forty-one animals were bled on d 6 or 7 and d 10 or 14 after artificial insemination for determination of serum progesterone concentrations. Of the animals responding to the synchronization treatment, 11% exhibited estrus within 8 to 10 d after artificial insemination and(or) had elevated (> 1 ng/mL) progesterone concentrations on d 6 to 7 after artificial insemination and low progesterone concentrations (< 1 ng/mL) on d 10 to 14 after artificial insemination. These characteristics are indicative of luteal insufficiency. Removal of data collected from non-synchronized animals and those with luteal dysfunction resulted in an increase in pregnancy rate of 14%.

King et al. (1986) examined the relationship between synchronization with SMB and serum progesterone concentration on the day of embryo transfer on subsequent pregnancy rates. Of the recipients (heifers synchronized within ± 24 h of the donors and having palpable corpora lutea) receiving demi-embryos after synchronization with SMB, 25% had progesterone concentrations < 1 ng/mL on the d of transfer (d 6 to 7). Furthermore, SMB-synchronized heifers having sub-luteal phase progesterone

concentrations resulted in a 43% reduction in pregnancy rate compared to heifers with progesterone concentrations between 1 and 7 ng/mL. Heifers treated with SMB, which had < 1 ng/mL serum progesterone, had structurally-normal corpora lutea and estrous cycles of 17 to 24 d. Furthermore, when data from SMB-treated heifers with < 1 ng/mL were eliminated from the analysis, pregnancy rate for SMB-treated heifers was not different from that of heifers synchronized with Estrumate (King et al., 1986). The authors postulated that synchronization with SMB which results in sub-luteal phase concentrations of progesterone and low pregnancy rates either delayed corpora lutea function or resulted in a continuously reduced serum concentration of progesterone which may have altered the intrauterine environment.

Sperm Transport and Viability:

There is a void in the literature as to the effect of progestogens on sperm transport and sperm viability in cattle, whereas, the ewe has been utilized extensively to investigate this effect of exogenous progestogens on these events. Hawk and Conley (1972) group fed ewes either 60 mg medroxyprogesterone-acetate (MAP) or 300 µg MGA daily from d 8 to 25 of the estrous cycle or treated ewes with an intravaginal sponge containing 60 mg MAP from d 8 to 25 of the cycle. Ewes were inseminated following estrous detection either 2, 3 or 4 d after the last d

of progestogen treatment. Blank sponges did not decrease the number of sperm cells within all segments of the reproductive tract. Feeding or intravaginal treatment with progestogen decreased the number of sperm cells in the oviduct and uterus, whereas, only intravaginal progestogen treatment reduced the number of sperm cells within the cervix and vagina. This study is in agreement with the earlier work of Quinlivan and Robinson (1969) who reported significant decreases in the number of spermatozoa in the vagina, cervix and uterus by 24 h after insemination in intravaginal progestogen synchronized ewes. In addition, Pritchard et al. (1970) reported that rabbits treated daily with MGA up until 1 to 3 days before the time of artificial insemination had a reduction in the number of fertilized ova if semen deposition was in the vagina compared to the upper uterine horn.

Sperm destruction, defined as the proportion of tailless sperm relative to the number of sperm recovered from the vagina, increased by 66 and 56% in ewes treated with MAP-impregnated sponges between d 8 to 22 and 14 to 22 of the cycle, respectively, compared to untreated and blank-sponge-treated ewes (Hawk et al., 1970). Furthermore, following an 18-d MGA synchronization treatment, sperm recovered at the synchronized estrus from heifers and cows removed a higher percentage of tetracycline hydrochloride (indicative of physiological alterations to sperm, such as loss of sperm head coat, which results in damaged acrosomes or damaged membranes and leads to

increased phagocytosis) than sperm recovered from heifers and cows after spontaneous estrus (Lauderdale and Ericsson, 1970). The authors postulated that progestogen treatment may result in sperm which have a shorter fertile life and could be more susceptible to leukocytic phagocytosis.

Oviductal-Uterine Physiology and Ova Viability and Transport:

Wordinger et al. (1976) examined the effect of 14-d MGA treatment beginning on d 15 of the cycle on histochemical and histological features of the ampullary portion of the oviduct. Within the ampulla, folded epithelia consist of ciliated and nonciliated columnar cells (Wordinger et al., 1976). Ampullary epithelia of cyclic cows 3 d after mating are characterized by numerous cytoplasmic and nuclear extrusions protruding from nonciliated cells. Secretory granules are found within the cytoplasmic extrusions (Wordinger et al., 1976). Heifers treated with MGA for 14 d beginning on d 15 of the cycle have fewer cytoplasmic and nuclear extrusions than untreated controls (Wordinger et al., 1976). These features characterize a heifer during estrus and early proestrus or ovariectomized heifers treated with estradiol. Ampullary epithelial cell heights were not adversely affected by MGA treatment (Wordinger et al., 1976). The authors hypothesized that a decreased amount of cytoplasmic and nuclear extrusions are indicative of a reduction in the secretory capability of the ampulla.

Endometrial surface and glandular epithelial cell height increases in cows treated with MGA for 15 d (Wordinger et al., 1971). Melengestrol acetate treatment beginning on d 15 of the cycle resulted in a decrease in glycogen content in glandular and surface epithelial cells. Heifers treated with MGA beginning on d 4 of the cycle reflected a tissue glycogen content similar to that of untreated controls (Wordinger et al., 1971). The authors indicated that this could act to decrease energy reservoirs which are essential for the developing blastocyst.

Rate of ova recovery is decreased by 50% (Reed and Rich, 1971) and fertilization rate of recovered ova is reduced by 50 to 75% in cows treated with MGA for 15 d compared to untreated controls (Reed and Rich, 1971; Wordinger et al., 1976). Treatment with MGA did not adversely affect ovulation since an ovum was recovered from each experimental heifer. In contrast, Wishart and Young, (1974) found no difference 4 d after estrus in the number of ova recovered or fertilized between 9- or 21-d norgestomet-treated heifers compared with untreated controls. Furthermore, 21-d norgestomet treatment resulted in 26% of recovered embryos in the 2 or 4 cell stage of cleavage, whereas, only eight-cell embryos, indicative of normal cleavage progression, were recovered from 9-d norgestomet-treated heifers and untreated controls.

To evaluate ova transport, Reed and Rich (1971) ligated oviducts into four equal segments then flushed each segment with

saline to recover ova. Ova transport was increased in cows fed .5 mg/d MGA for 15 d. Ova from MGA-treated cows entered segment 2 of the oviduct, near the ampullary-infundibular junction, 15 h after ovulation compared to controls at 30 h after ovulation. Segment four of the oviduct, near the ampullary-isthmus junction, contained ova from MGA-treated cows several hours earlier than was detected in untreated controls. Alterations in ova transport have been implicated as a cause for reduced implantation rate after ovulatory delay in rats. Banik and Pincus (1964) observed an increase in ova transport in estrogen-treated rats which resulted in a reduction in implantation rate.

Follicular Development and Estradiol-17ß Production:

Over the past six decades numerous researchers have reported the adverse effects of progestogens on follicular development. One of the first reports by Ulberg et al. (1951) showed that daily injections of 12.5 mg of progesterone starting at d 15 of the cycle and continuing until d 28 resulted in development of one large follicle (18 to 28 mm) in 100% of the animals treated. In each case the large follicle ovulated after the post-treatment estrus. Trimberger and Hansel (1955) reported similar results in which 47% of heifers treated with progesterone had one large follicle that persisted throughout the treatment period. In addition, Ray et al. (1961) reported that animals receiving a single injection of progesterone (.76

mg/.45kg) on d 16 of the cycle had significantly larger follicles than those treated on d 8 of the cycle (19 and 8 mm, respectively). More recently, Lee et al. (1988) found that an i.v. injection of 2.5 mg progesterone administered to multiparous cows at the onset of standing estrus (period when cows would not stand to be mounted) blocked the preovulatory surge of LH and generally resulted in persistent follicles 10 d after estrus.

Synthetic progestogens, such as MGA and norgestomet, which are utilized in synchronization programs, have also been examined to determine their effect on follicular development. Long-term feeding, either 14 (Zimbelman, 1966; Zimbelman and Smith, 1966b) or 21 d (Guthrie et al., 1970), of MGA to heifers at a dose (.44 to .85 mg/d) which inhibited ovulation in almost all animals resulted in increased follicular fluid volume to three times that of controls. This increase in follicular fluid was primarily from the largest follicle present rather than from a general stimulation of follicular activity. Based on cervical mucous smears, these large follicles were determined to be estrogenic despite inhibition of estrus and ovulation by MGA treatment (Zimbelman, 1966; Zimbelman and Smith, 1966b). When SMB was administered without regard to day of the estrous cycle, 77% of treated heifers had a dominant follicle (> 10mm) persist while the implant was in place and all heifers ovulated the persistent follicle upon implant removal (Jones et al., 1989). Similar results have been reported when norgestomet implants

were given during proestrus (d 18 to 20; Rajamahendran and Taylor, 1991; Taylor and Rajamahendran, 1991c). However, if 9-d norgestomet implants are administered during early diestrus (d 6 to 7) or mid-diestrus (d 11 to 12) the dominant follicle present on either ovary at the time of implant insertion regressed and a newly-selected follicle developed to become the ovulatory follicle (Taylor and Rajamahendran, 1990; Rajamahendran and Taylor, 1991).

In other studies, when cows were treated with 9-d norgestomet implants after $PGF_2\alpha$-induced luteolysis on d 8 of the cycle, dominance of the first follicular wave was extended beyond d 18 of the cycle in 15 of 16 cows (Savio et al., 1990d). However, the original dominant follicle ovulated after implant removal on d 23 only in cows in which implants had been replaced on d 18. These results do not support the suggestion of Mikeska and Williams (1988) that decreased conception rates after norgestomet-synchronization may result from retarded selection or maturation of the ovulatory follicle. Even though norgestomet treatment resulted in premature development of the ovulatory follicle compared to heifers with 3 follicular waves, prolonged maintenance of the ovulatory follicle was not detrimental to pregnancy rates (60%; Rajamahendran and Taylor, 1991).

Beal et al. (1990) also observed a stage of cycle effect of MGA treatment on follicular development. Cows fed MGA beginning on d 17 of the cycle arrested development of the

dominant follicle present at the beginning of treatment. Feeding MGA beginning on d 7 of the cycle had little effect on follicular development (Table 2). It was noted that estradiol-17ß concentrations > 2 pg/mL persisted for 1 wk before ovulation when MGA treatment began on d 17 compared to heifers treated on d 7 of the estrous cycle. Chow et al. (1972) also reported elevated estradiol-17ß concentrations before estrus when MGA treatment began without regard to stage of the cycle. In that study, estradiol-17ß concentrations ranged from 3 pg/mL 4 d before estrus to 9 pg/mL on the day before estrus in treated cows compared to 1 and 6 pg/mL in control cows. Henricks et al. (1973) reported no increase in estrogen concentrations during a 14-d MGA feeding period initiated on d

Table 2. Effect of MGA treatment beginning on d 7 or 17 of the estrous cycle on subsequent follicular development.

	Days Before Ovulation[a]			
Treatment	**7**	**5**	**3**	**1**
MGA d 7	**5.4**	**9.4**	**12.5**	**15.7**
MGA d 17	**10.8**	**12.5**	**13.6**	**15.0**

[a]Follicular diameter (mm; from Beal et al., 1990).

15 of the cycle and no alterations during proestrus compared to untreated controls. However, in animals that became pregnant

after MGA treatment, elevated concentrations of plasma estrogen were confined to fewer days before estrus than in animals which failed to become pregnant (Henrick et al., 1973). In a more recent study, Coleman et al. (1990) reported elevated concentrations of estradiol-17ß before the synchronized estrus in beef cows treated with MGA for 21 d. Elevation of estradiol-17ß during proestrus did not carry over to the second estrous period after MGA treatment (Coleman et al., 1990). This is not surprising since reduced conception rates after progestogen treatment are also confined to the synchronized estrus. These elevated concentrations of estradiol-17ß before first estrus subsequent to MGA treatment may play an important role in understanding reduced fertility after synchronization with progestogens.

Sirois and Fortune (1990) reported a prolonged persistence of the developing ovulatory follicle in heifers treated with one controlled internal drug-releasing device (CIDR) for 14 d beginning on d 14 of the estrous cycle compared to heifers receiving two CIDR's. In addition, their was complete absence of follicular recruitment in the heifers receiving one CIDR. Plasma progesterone concentrations were maintained between one and two ng/mL after regression of the corpus luteum in one-CIDR-treated heifers, whereas, progesterone concentrations ranged between 4 and 5 ng/mL in heifers treated with two CIDR's. Treatment with one CIDR resulted in increased estradiol-17ß concentrations before estrus compared to heifers treated with

two CIDR's. The authors postulate that the higher concentrations of progesterone in heifers treated with two CIDR's acted to inhibit LH pulse frequency and consequently lowered androgen synthesis which resulted in lower concentrations of estradiol-17ß and inhibition of follicular maintenance during the treatment period. In addition, low progesterone concentrations associated with treatment with one CIDR are attained coincident with decreasing endogenous progesterone after natural luteolysis and may be the impetus for increased LH support necessary for continued development of a dominant follicle. The results from this study are unclear, because not all heifers treated with one CIDR arrested development of a dominant follicle and three of six heifers in the two CIDR treatment group failed to arrest development of the dominant follicle present at CIDR insertion. The confusing results may be due to an inappropriate timing of CIDR insertion. If CIDR insertion had occurred around the time of natural luteolysis there would have been an asynchronous decline in progesterone between the CIDR and the corpus luteum which may have inhibited the increase in LH support that seems essential for maintenance of a dominant follicle. Swanson et al. (1989) reported an effect of the corpus luteum on alteration of follicular development after treatment with a CIDR. In the absence of a corpus luteum, CIDR insertion increased the mean number of large follicles (> 9mm), whereas, in the presence of a corpus luteum CIDR insertion decreased the mean number of large

follicles. Furthermore, CIDR insertion after induced luteolysis suspended follicular waves and prolonged the persistence of dominant follicles present at the time of CIDR insertion. Importantly, LH concentrations were lower in heifers treated with a CIDR after induced luteolysis (Swanson et al., 1989).

In a more recent study, Sawyer et al. (1992) examined the effect of progesterone-releasing intravaginal devices (PRID) during the early and midluteal phase of the estrous cycle on subsequent follicular development. During the mid-luteal phase, the dominant follicle present at PRID insertion remained static or grew slowly and became the ovulatory follicle. During the early phase, the dominant follicle regressed slowly over 9 d and a second wave follicle grew rapidly and became the ovulatory follicle. If the estradiol benzoate tablet was not removed from the PRID, regression of the dominant follicle occurred regardless of stage of the cycle at the time of PRID insertion. These results do not agree with the work of Rajamahendran and Walton (1990) who reported that administration of estradiol valerate during the late luteal phase of the cycle disrupted follicular development and resulted in maintenance of dominant follicles. In addition, maintaining progesterone at luteal phase concentrations either naturally during pregnancy (Ginther et al., 1989c) or by daily treatment with 150 mg of progesterone for 90 d (Bergfelt et al., 1991) resulted in continued periodic appearance of follicular waves throughout the entire treatment period. Furthermore, induced persistent dominant follicles,

resulting from proestrus norgestomet treatment, resume follicular turnover after PRID insertion on d 3 of norgestomet treatment (Taylor and Rajamahendran, 1991c) which coincides with a reduction in LH pulse frequency.

Data from these studies support the hypothesis that progesterone production by the corpus luteum allows for continued emergence of follicular waves throughout the estrous cycle. The emergence of follicular waves is dependent on expression of low-frequency, high-amplitude LH pulses normally exhibited during the luteal phase of the cycle (Sirois and Fortune, 1990). Synthetic progestogens appear to be effective in inhibiting estrus and ovulation, but when administered coincident with or subsequent to luteal regression, they are ineffective in maintaining luteal phase LH pulse frequency, which results in premature development of the ovulatory follicle.

Ovulatory Delay:

A great deal of work has been accomplished utilizing the rat as a model to study the effects of delayed ovulation on subsequent reproductive fitness and alterations in hormonal secretion. Mature female rats (4 to 5 months) normally exhibit estrous cycles of 4 to 5 days, whereas, aged female rats (10 to 12 months) display irregularities in cyclicity which can result in estrous cycles extending to 6 days or longer (Lu et al.,

1979a). An induced delay of ovulation in female rats, exhibiting 4- or 5-day estrous cycles, with sodium pentobarbital administered on the afternoon of proestrus, results in an early and prolonged rise in plasma estradiol-17ß concentrations in relation to the time of ovulation (Butcher et al., 1975; Butcher and Pope, 1979; Page and Butcher, 1982). A similar premature and prolonged elevation in plasma estradiol-17ß concentration occurs during spontaneously-prolonged estrous cycles in older rats (Lu et al., 1979a; Lu et al., 1979b; Page and Butcher, 1982) and is paralleled by an increase in estradiol-17ß concentration in follicular fluid (Page and Butcher, 1982; Lerner et al., 1990). These increases in plasma estradiol-17ß concentrations are similar to those reported in cattle when synchronization with synthetic progestogens begins during late diestrus, which results in delaying estrus and ovulation (Beal et al., 1990; Coleman et al., 1990).

The elevated estradiol-17ß concentrations noted in spontaneously-prolonged estrous cycles of older rats is associated with alterations in follicular development. The number of antral follicles is reduced (Peluso et al., 1974) and diameter of the preovulatory follicle is increased in older rats exhibiting ovulatory delay compared to young mature rats exhibiting normal estrous cycles (Peluso et al., 1974; Lerner et al., 1990).

Several studies have attempted to more clearly define the underlying mechanism responsible for decreased fecundity that is

accompanied by fertilization of intrafollicularly aged oocytes resulting from delayed ovulation. Butcher et al. (1974) have shown that oocytes remain in meiotic arrest during the 48-hr ovulatory delay and resume maturation at the normal time on the afternoon of the preovulatory surge of gonadotropins. Increases in ova degeneration as well as abnormal and retarded development of ova has been observed in mature rats after induced ovulatory delay (Butcher et al., 1975). Peluso and Butcher (1974a) have also noted that follicularly-aged oocytes collected after induced delay of ovulation exhibit a 50% reduction in the number of cortical granules compared to untreated controls (99.4 and 46.4% per 100 µg of plasma membrane, respectively). Also, the follicularly-aged oocytes contained empty vesicles of the Golgi complexes and no indication of cortical granule synthesis, as well as cortical granule forming bodies which were larger, more diffuse and often exhibited broken limiting membranes. The authors postulated that this reduced concentration of and/or reduced ability to synthesize cortical granules in follicularly-aged oocytes may cause the zona pellucida to be less effective in blocking polyspermy, which account for the increased incidence of polyspermy in rats after 2-d ovulatory delay (Fugo et al., 1966). In addition, the metabolic state of the aged oocyte may have been increased prematurely as indicated by the presence of elongated mitochondria with shelf-like cristae which are indicative of eight-cell rat embryos and not unfertilized oocytes (Fugo et al., 1966).

Chromosomal abnormalities have been shown to be associated with ovulatory delay in the rat. Butcher and Fugo (1967) obtained 390 embryos after ovulatory delay and 410 control embryos and observed a greater occurrence of abnormal chromosomal counts in ovulatory-delayed animals than in untreated mature rats (18 and 6, respectively). Furthermore, Peluso and Butcher, (1974b) reported a reduction in RNA synthesis and no alteration in protein synthesis in oocytes after ovulatory delay compared to untreated mature rats. It was also reported that the follicular aging process did not alter RNA or protein synthesis by the cumulus cell mass, however, follicular aging did result in an increased dispersement of the cumulus cell mass (Peluso and Butcher, 1974).

In an elegant study, Butcher et al. (1969) examined the relationship between intrauterine environment and physiological alterations associated with induced ovulatory delay. Blastocysts recovered from untreated or induced-ovulatory-delay rats were transferred into untreated or induced-ovulatory-delay recipients. Delayed ovulation in the donor increased the incidence of embryonic death and of small abnormal embryos by 11 and 12% respectively, whereas, implantation rate was unaffected when ovulation was delayed in the donor. Implantation rate was decreased by 23% when the blastocysts from control donors were transferred to induced-ovulatory-delayed, pseudopregnant rats. Embryonic development and/or death were not significantly increased. The authors concluded that alterations in

intrauterine environment acted to reduce implantation rate, whereas, increased embryonic death and abnormal development were the result of changes within the ovum.

Butcher and Pope (1979) examined the effects of the early preovulatory rise of estradiol-17ß on subsequent embryonic development at d 4 (blastocyst stage) and d 11 (mid gestation) of gestation. A 48-h delay of ovulation with sodium pentobarbital resulted in abnormal development and retarded growth of embryos at both stages of development. Implantation rate was decreased and embryonic death was increased. The detrimental effects of delayed ovulation were reversed after treatment with antiserum against estradiol-17ß (ASE), whereas, diethylstilbestrol treatment reinitiated the detrimental effects in ASE-treated rats. The results from these studies support the hypothesis of Butcher et al. (1979) and Peluso and Butcher (1974) that the early rise or prolonged elevation of preovulatory concentrations of estradiol-17ß in relation to the time of ovulation may be responsible either directly, by acting on the oocyte to alter the ultrastructural characteristics, or indirectly, by altering the intrauterine environment, for abnormal development and embryonic death after delayed ovulation.

Progestogens and LH Secretion

An understanding of the impact of either synthetic progestogens (MGA or SMB) or exogenous progesterone (PRID), on the secretion of gonadotropins, especially LH, appears to be essential in the unraveling of the mechanism(s) responsible for the reduced fertility associated with synchronization programs utilizing progestogens. Melengestrol acetate (either .5 or 1.0 mg/d) group fed to heifers for 14 d without regard to stage of the cycle had no significant effect on mean serum LH concentrations (Randel et al., 1972). In contrast, Hill et al. (1971) found that mean serum LH concentrations were elevated in beef heifers fed .5 mg MGA per day for 14 d when treatment began on day 15 compared to d 4 of the estrous cycle (1.05 ± .49 and .38 ± .12 ng/mL, respectively). In a more recent study, Smith and Day, (1990) treated prepubertal beef heifers with .5 mg MGA per day for 16 d and reported an increase in the episodic release and mean concentration of LH on d 14 and 17 after the initiation of treatment, Table 3. Amplitude of LH pulses were unchanged throughout the experimental period.

Savio et al. (1990d) treated cows with norgestomet implants on d 8 of the cycle, then on d 18 of the cycle implants were replaced with a new implant or retained the d 8 implant until implant removal on d 23. Pulse frequency of LH increased on d 10 in norgestomet-treated cows compared to untreated controls (5.4 and .7 pulses/8 h, respectively). Furthermore,

implant replacement on d 18 resulted in an increase in LH pulse frequency compared to cows which retain implants from d 8 (7 and 3 pulses/ 8 h, respectively). In contrast, Taylor and Rajamahendran, (1991c) reported that norgestomet treatment had no effect on LH pulse frequency or amplitude on the d of implant insertion (d 8 of the cycle) or d 3 of norgestomet treatment.

The use of natural progesterone, in the form of PRID, has also been shown to alter the secretory pattern of LH. Roche and Ireland (1981) reported an increase in mean serum concentrations of LH in heifers which received a PRID (2% progesterone) for 7 d beginning on d 17 to 18 compared to those treated between d 8 and 10 of the estrous cycle (1.51 and 1.24 ng/mL, respectively). In a subsequent study, Ireland and Roche

Table 3. Comparison of mean LH concentration and frequency of LH pulses before, during and after 16 d MGA treatment.[a]

Item	Day of Treatment		
	0	14	17
LH Pulses/8 h			
MGA	2.70	5.20	5.90
Control	2.90	2.50	4.20
Mean LH ng/mL			
MGA	.73	1.15	1.00
Control	.70	.72	.77

[a]From Smith and Day, 1990.

(1982) treated heifers with PRID (4% progesterone) for 12 or 14 d beginning between d 6 and 10 of the estrous cycle and found that as progesterone concentrations declined below 2 ng/mL while PRID's were in the vagina, episodic release of LH increased from 2.2 ± .4 pulses per 4.67 h to 3.6 ± .3 pulses per 4.67 h. PRID (6.75 and 20% progesterone) treatment which maintained progesterone concentrations above 2 ng/mL inhibited increases in mean concentration and pulse frequency of LH (Roche and Ireland, 1981; Ireland and Roche, 1982). More recently, Roberson et al., (1989) reported an increased LH pulse frequency in cows receiving subnormal luteal progesterone concentrations (.5 PRID, 2.14 ng/mL, after induced luteolysis) compared to cows receiving normal luteal progesterone concentrations (two PRID's, 6.19 ng/mL, after induced luteolysis) or untreated controls (6.73 ng/mL) (21.36, 14.64 and 6.72 pulses/ 24 h, respectively). While PRID's were in the vagina estradiol-17ß concentrations were higher in cows receiving subnormal luteal phase concentrations of progesterone. In agreement with these studies, Taylor and Rajamahendran, (1991c) reported a decrease in LH pulse frequency, amplitude and basal concentrations after insertion of a PRID on d 3 after the beginning of norgestomet treatment.

It appears from these studies, that in the absence of a corpus luteum, synthetic progestogens, such as MGA or norgestomet, and subluteal phase concentrations of exogenous progesterone released from a PRID, are capable of inhibiting

estrus and ovulation, but not pulse frequency of LH. Conversely, when luteal phase concentrations of exogenous progesterone are maintained by the combination of two PRID's, estrus and ovulation, as well as LH pulse frequency are suppressed.

Chapter III

Statement of the Problem

One of the major disadvantages of utilizing progestational compounds in estrous synchronization programs is the reduced fertility that is associated with breeding at the first estrus subsequent to treatment. At the present time, there is a void in the literature as to the mechanism(s) which acts to reduce fertility. Unusual follicular development, alterations in gonadotropin (LH) secretory patterns, elevated estradiol-17ß concentrations during the preovulatory period and stage of the estrous cycle at the time progestogen treatment is initiated have each been implicated. If we are to alleviate or reduce the adverse effect of exogenous progestogens on the reproductive capabilities of beef cattle, we must understand the basic physiological alterations that result from progestogen treatment. If we can accomplish this goal, we will have at our disposal the tools with which to increase the reproductive efficiency of the beef cow.

The following studies were conducted at the Catawba Research Station, Virginia Polytechnic Institute and State University, Catawba, Virginia.

Chapter IV

Differential Effect of Melengestrol Acetate or Progesterone-Releasing Intravaginal Devices on Follicular Development, Progesterone and Estradiol-17ß Concentrations and Patterns of Luteinizing Hormone Release During the Bovine Estrous Cycle

Introduction

One of the major disadvantages of utilizing progestational compounds in estrous synchronization programs is the reduced fertility that is associated with breeding at the synchronized estrus (Patterson et al., 1989; Odde, 1990). Stage of the cycle in which exogenous progestogens are administered influences conception rates at the synchronized estrus. Cows treated with Synchro Mate-B (SMB; Brink and Kiracofe, 1988) or melengestrol acetate (MGA; Beal et al., 1988) beginning before d 13 of the estrous cycle had a higher conception rate than did cows given SMB or MGA after d 13 of the estrous cycle.

Follicular development in cyclic cattle is not a continuous process; it occurs in either two or three waves per cycle (Rajakoski, 1960; Pierson and Ginther, 1988; Savio et al., 1988; Sirois and Fortune, 1988). The lifespan of the corpus luteum is an important factor in determining whether an animal exhibits two or three waves of follicular growth throughout an estrous cycle (Ginther et al., 1989c). Administration of exogenous progestogens alters follicular development in cyclic cattle. Sirois and Fortune (1990) reported a prolonged

persistence of the developing ovulatory follicle in heifers given one controlled internal drug-releasing device (CIDR) beginning on d 14 of the estrous cycle. One CIDR left in place for 14 d maintained progesterone concentrations in plasma at concentrations lower than those recorded during the luteal phase of the cycle. Conversely, two CIDR's, maintained progesterone concentrations at luteal phase concentrations and did not arrest development of the dominant follicle present at the beginning of treatment. In similar experiments, MGA fed from d 17 of the cycle arrested development of the dominant follicle present at the beginning of treatment, however, feeding MGA to cows from d 7 of the cycle had little effect on follicular development (Beal et al., 1990). Similar findings have been reported when Norgestomet is administered during the proestrus stage of the estrous cycle (Jones et al., 1989; Rajamahendran and Taylor, 1991).

Persistence of the dominant follicle in cattle treated late in the estrous cycle with MGA (Beal et al., 1990) or one CIDR (Sirois and Fortune, 1990) is accompanied by a precocious increase in estradiol-17ß before ovulation. Furthermore, LH pulse frequency increases when PRID or CIDR treatment results in sub-luteal phase concentrations of progesterone (Ireland and Roche, 1981; Roberson et al., 1989; Sirois and Fortune, 1990). This coincides with alterations in follicular development normally associated with the proestrus stage of the bovine estrous cycle (Sirois and Fortune, 1990; Cupp et al., 1992). At

the present time there are no direct comparisons in the literature of the effects of MGA and progesterone-releasing intravaginal devices (PRID) given around the time (d 17) of natural luteolysis on subsequent follicular development, estradiol-17ß concentrations or patterns of luteinizing hormone release. Therefore, the objectives of this series of studies were to determine the effects of a 7-d administration of MGA or PRID beginning on d 17 of the estrous cycle on: 1) follicular development, 2) serum concentrations of estradiol-17ß and progesterone, and 3) patterns of luteinizing hormone release before and during progestogen treatment.

Experiment 1

Materials and Methods

This experiment was conducted in three replicates with mature, (2 to 14 yr of age) non-lactating Angus, Angus x Holstein or Holstein cows. Within each replicate, treatments were assigned to cows at the time they were observed in estrus. All breeds were represented within each treatment. For each replicate, each cow received an i.m. injection of prostaglandin $F_2\alpha$ ($PGF_2\alpha$, 25 mg; Lutalyse[1]) to synchronize estrus. Following $PGF_2\alpha$, cows were observed for signs of estrus twice daily (am:pm) for at least 30-min during each period. Following estrus, (day of estrus = d 0) cows were assigned to one of three treatments: 1) MGA (oral, .5 $mg \cdot hd^{-1} \cdot d^{-1}$; n = 12), 2) PRID (silastic coil impregnated with 1.55 g progesterone[2]; n = 15) or 3) untreated controls (n = 8). MGA and PRID treatments were initiated on d 17 of the estrous cycle and continued for 7 d. Cows in the MGA group received a daily bolus containing MGA, and the last bolus was administered on d 23. Removal of the PRID from cows in the PRID group occurred on d-24 after onset of estrus.

Beginning on d-3 after onset of estrus, ovaries were examined daily using a real-time, B-mode ultrasound instrument equipped with a 7.5 MHz linear array transrectal transducer

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[1]The Upjohn Co., Kalamazoo, MI.
[2]Sanofi Animal Health, Paris, France.

(Equisonics LS-300A[3]) to monitor development of follicles ≥ 5 mm in diameter. Fecal material was removed before examination of the ovaries. The transducer was inserted into the rectum and moved along the ventral surface of the rectum adjacent to the dorsal surface of the uterine horns and then moved laterally to examine each of the ovaries (Pierson and Ginther, 1984). The reproductive tract was not manipulated before or during the ultrasound examination. Images of the ovaries were viewed on a 48-cm monitor and recorded on video cassette to be reviewed later to record daily growth or regression of dominant follicles and corpora lutea. Daily ultrasound imaging of the ovaries continued until ovulation, as determined by the acute disappearance of the dominant follicle (Pierson and Ginther, 1984).

Jugular venous blood samples (45 mL) were collected daily, coincident with ultrasound examinations, beginning at d 3 post estrus and continued until ovulation had been recorded. Blood samples were allowed to clot at 22 C for approximately 16 h and then centrifuged at 1000 x g at 4 C for 30 min. Serum was harvested and frozen at -25 C until radioimmunoassayed for progesterone and estradiol-17ß.

Assays for Estradiol-17ß and Progesterone:

Progesterone concentration for samples collected during the three replicates were quantified by solid-phase

[3]Tokyo Keiki LS-300A, Products Group International, Boulder, CO.

radioimmunoassay[4]. Estradiol-17ß concentrations in daily samples for the three replicates were quantified by radioimmunoassay as described by Guthrie and Bolt (1983) and Lewis et al. (1989). Sensitivity of the progesterone assay was .1 ng/mL and for estradiol-17ß was 1.5 pg/mL. Inter- and intra-assay coefficients of variation (CV) were as follows: progesterone, 8.4 and 6.8% ; estradiol-17ß, 19.5 and 8.5%, respectively.

Statistical Analyses:

Data for interovulatory interval, day after onset of estrus when progesterone concentration declined below 1 ng/mL, day of emergence of the dominant follicle of the second wave, day of emergence of the ovulatory follicle, diameter of the dominant follicle present on d 17, days from detection of the ovulatory follicle until ovulation and diameter of the ovulatory follicle on the day before ovulation were analyzed by one-way analyses of variance for a completely randomized design using the GLM procedure of SAS (SAS, 1987).

Growth rate (mm/d) of the dominant non-ovulatory and ovulatory follicle of each follicular wave was calculated by linear regression (slope = growth rate) of follicular diameter on days of the estrous cycle. Data for regression analyses included observations from the first day follicular diameter was ≥ 5mm and continued until maximum diameter was reached (non-

[4]Diagnostics Products Corp., Los Angeles, CA.

ovulatory) or until ovulation occurred. Comparison of growth rates for follicular waves was analyzed by a one-way analysis of variance using the GLM procedure of SAS (SAS, 1987). Data for diameter of the ovulatory follicle and estradiol-17ß concentrations for the 9-d period before ovulation were analyzed using analysis of variance procedures with time as an independent variable (SAS, 1987). Shape of the curves for ovulatory follicular diameter and estradiol-17ß concentrations were determined by polynomial regression analysis (Allen et al., 1983). Test for homogeneity of residual variances were used to determine the effect of treatment on changes in follicular diameter and estradiol-17ß concentrations over time between control, MGA- and PRID-treated cows. Rate of increase in estradiol-17ß concentration relative to follicular diameter during the 9 d before estrus was calculated by linear regression (slope = increased serum estradiol-17ß concentrations) of estradiol-17ß concentration on diameter of the dominant follicle per pair of ovaries. Comparison of rate of increase of estradiol-17ß concentration relative to follicular diameter was analyzed by one-way analysis of variance using the GLM procedure of SAS (SAS, 1987).

Least-squares mean differences for interovulatory interval, days from detection of the ovulatory follicle until ovulation and growth rate of dominant follicles within follicular waves were determined using Tukey's honestly significant difference test (Ott, 1988). Least-squares mean

differences for day of detection of the dominant follicle of the second wave, day of detection of the ovulatory follicle, diameter of the dominant follicle present on d 17 after onset of estrus, diameter of the ovulatory follicle on the day before ovulation and day that progesterone concentration declined below 1 ng/mL were determined by T-test (SAS, 1987).

RESULTS

There were no treatment X replicate interactions (P>.10) for the variables examined in this study, therefore, data for each replicate within treatment were pooled for subsequent analysis.

Interestrus interval was lengthened by approximately 5 d in MGA- or PRID-treated cows compared to untreated control cows (P<.01, Table 4).

Table 4: Least-squares means and standard errors for interestrus interval for cows treated with MGA, PRID or no treatment (control) for 7 d beginning on d 17 of the estrous cycle.

Treatment	n	Interestrus Interval (d)
Control	8	$22.3 \pm .19^{a}$
MGA	12	$27.1 \pm .22^{b}$
PRID	15	$27.1 \pm .27^{b}$

^{a,b}Least-squares means within the same column without common superscripts differ P<.01.

Average progesterone concentrations for control, MGA- and PRID-treated cows through d 17 of the estrous cycle were similar to previously reported values (Hansel and Echternkamp, 1972; Glencross et al., 1972). PRID-treated cows exhibited an acute increase in progesterone concentration on the day after PRID

insertion (d 18). Progesterone concentrations remained elevated (>1 ng/mL) until PRID removal (d 24) then decreased to concentrations similar to those in MGA-treated cows (<.25 ng/mL; Figure 2). Diameter of the corpus luteum throughout the experimental period declined coincident with progesterone concentration in MGA-treated and untreated control cows.

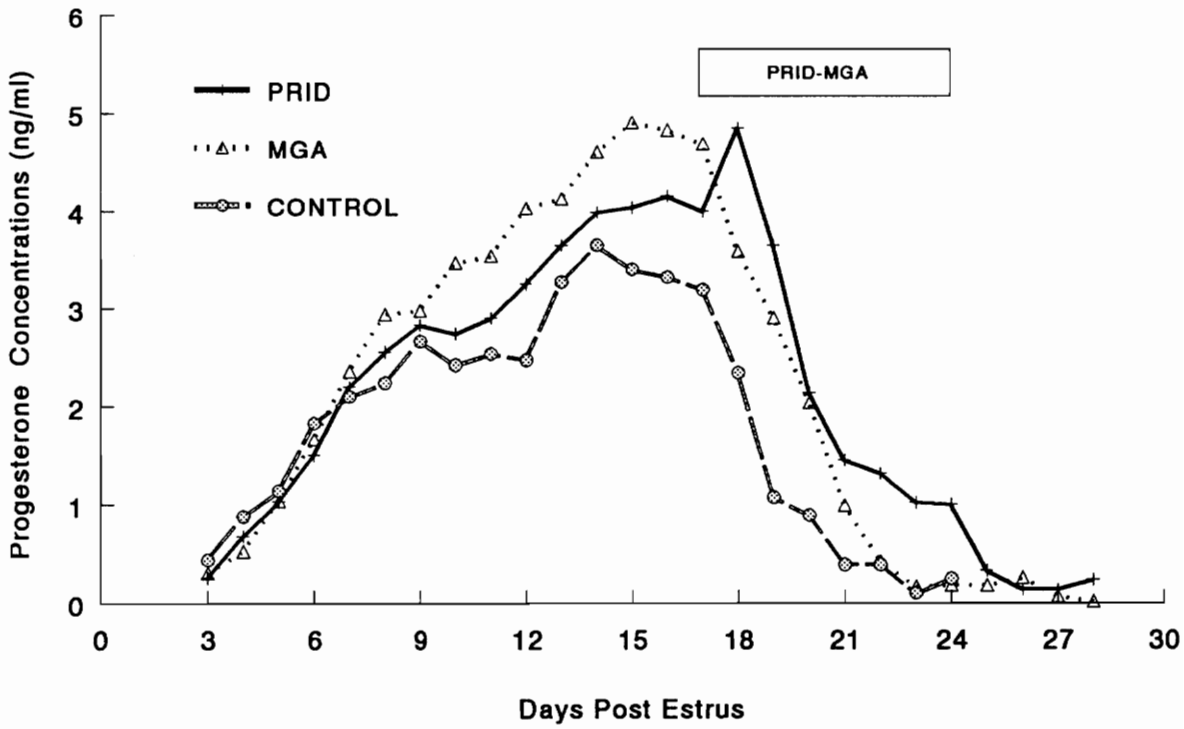

Figure 2. Concentrations of progesterone throughout the estrous cycle for cows administered MGA, PRID or no treatment (control) for 7 d beginning on d 17 of the estrous cycle.

Follicular development in all treatment groups was characterized by the growth and regression of dominant follicles throughout the interestrus interval. PRID-treated and control cows primarily exhibited three follicular waves, 14 of 15 and 7 of 8, respectively, whereas, 7 of 12 MGA-treated cows exhibited two follicular waves during the interestrus interval. Day of detection of the dominant follicle of the second follicular wave and diameter of the dominant follicle on d 17 of the estrous cycle did not differ (P>.10) among treatments (Table 5).

Table 5: Least-squares means and standard errors for day of detection of the dominant follicle of the second follicular wave and diameter of the dominant follicle on d 17 for cows treated with MGA, PRID or no treatment (control) for 7 d beginning on d 17 of the estrous cycle.

Treatment	n	Detection of Second Follicular Wave (d)	Dominant Follicle Diameter on d 17
Control	8	10.21 ± .75	10.60 ± .91
PRID	15	11.03 ± .72	11.56 ± .89
MGA	12	11.47 ± .49	10.00 ± .72

The dominant follicle present at the beginning of MGA or PRID treatment ovulated in 7 of 12 and 1 of 15 cows, respectively. Pattern of follicular growth and regression of dominant follicles throughout the interestrous interval for representative untreated control cows and those treated with MGA

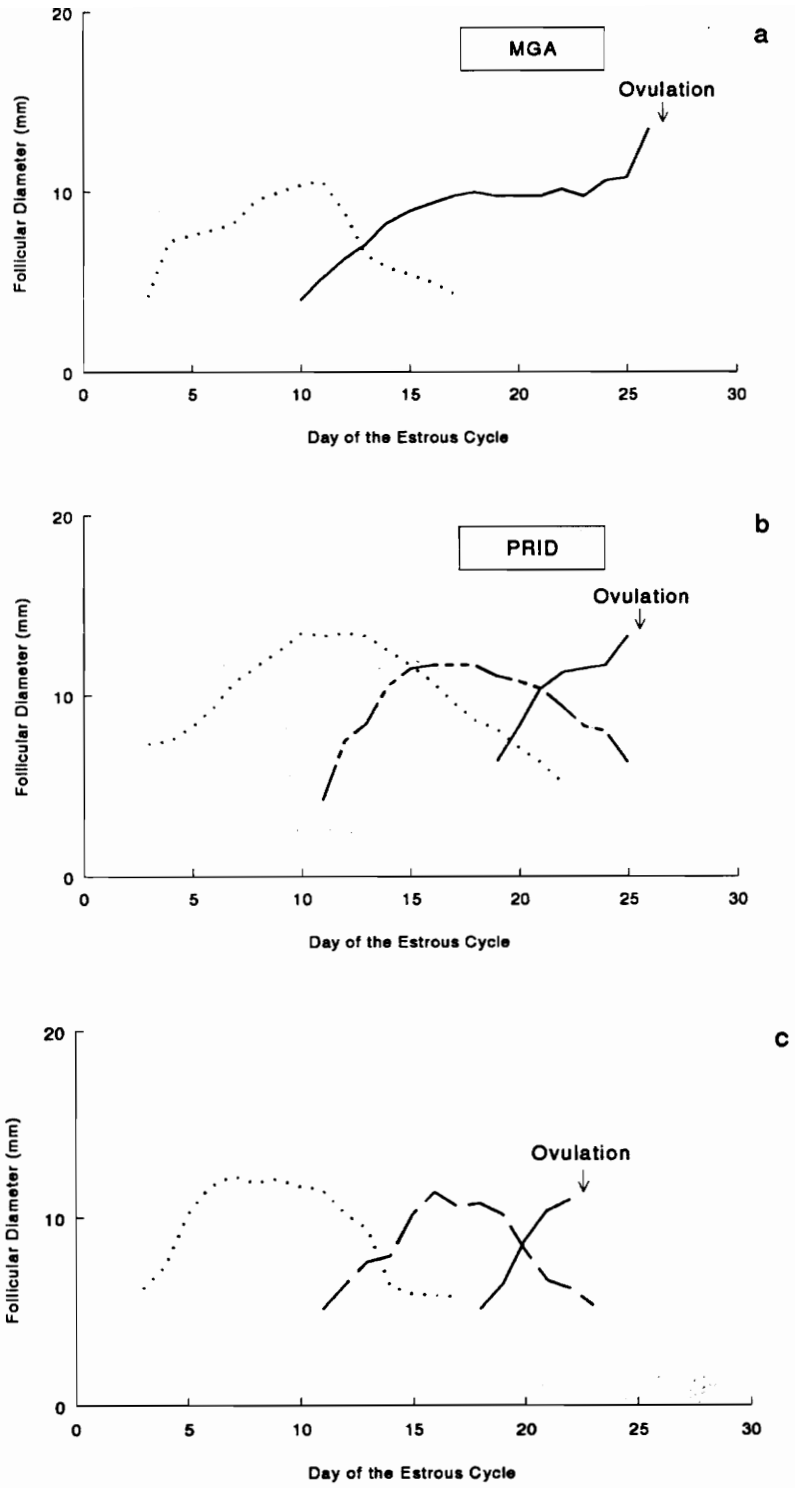

Figure 3. Growth and regression of dominant non-ovulatory and ovulatory follicles throughout the estrous cycle for a representative cow treated with MGA (a), PRID (b) or no treatment (c) for 7 d beginning on d 17 of the estrous cycle.

or PRID for 7 d beginning on d 17 of the estrous cycle are depicted in Figure 3.

Day of detection of the ovulatory follicle (≥ 5mm) occurred earlier (P<.001) during the estrous cycle and the interval from detection of the ovulatory follicle until ovulation was extended (P<.01) in MGA-treated cows that exhibited two follicular waves during the interestrus interval (Table 6). Similar trends were observed in control and PRID-treated cows characterized by two follicular waves (Table 6). Diameter of the ovulatory follicle

Table 6: Least-squares means and standard errors for days from detection (≥ 5mm) of the ovulatory follicle until ovulation (DTO), day of detection of the ovulatory follicle (DDOF) and diameter of the ovulatory follicle on the d before ovulation (DOF) for control, MGA-and PRID-treated cows exhibiting two or three follicular waves during the estrous cycle.

Treat	n	W[a]	DTO	DDOF	DOF
Control	1	2	11.0	11.0	13.5
PRID	1	2	17.0	11.0	17.0
MGA	7	2	16.7 ± .51[b]	11.6 ± .52[b]	15.1 ± .64[d]
Control	7	3	6.8 ± .51[c]	17.0 ± .53[c]	12.2 ± .64[e]
PRID	14	3	8.9 ± .36[c]	19.5 ± .37[c]	13.6 ± .45[e]
MGA	5	3	8.8 ± .59[c]	20.2 ± .62[c]	13.6 ± .76[e]

[a]Number of follicular waves throughout the interestrus interval.

[b,c]Least-squares means within the same column without common superscripts differ P<.01.

[d,e]Least-squares means within the same column without common superscripts differ P<.07.

on the day before ovulation tended to be greater (P<.07) in MGA-treated cows characterized by two follicular waves than control, PRID and MGA-treated cows exhibiting three follicular waves (Table 6). A similar trend was observed in the PRID-treated cow that exhibited two follicular waves (Table 6).

The luteal phase of the estrous cycle, characterized by serum concentrations of progesterone ≥ 1 ng/mL, was approximately 2 to 3 days shorter (P<.01) in MGA-treated cows that exhibited two versus three waves of follicular growth throughout the interestrus interval (Table 7). A similar trend was observed for control and PRID-treated cows exhibiting two follicular waves (Table 7).

Table 7. Least-squares means and standard errors for day of the estrous cycle when progesterone concentration was first detected below 1 ng/mL in cows treated with MGA, PRID or no treatment (control) for 7 d beginning on d 17 of the estrous cycle.

	Number of Follicular Waves Per Cycle	
Treatment	**2 (n)**	**3 (n)**
MGA[c]	19.83 ± .62[a] (7)	21.80 ± .68[b] (5)
PRID	20.00 (1)	23.42 ± .41 (14)
Control	17.00 (1)	19.71 ± .58 (7)

[a,b]Least-squares means within the same row without common superscripts differ at P<.01.
[c]Two waves during the interestrus interval resulted in persistence of the dominant follicle of the second wave.

Growth rate of dominant non-ovulatory follicles of waves 1 and 2 did not differ among treatment groups (P>.05, Table 8). However, growth rate of the dominant follicle of the ovulatory wave was greater (P<.02, Table 8) for control and PRID-treated cows than for MGA-treated cows.

Table 8. Least-squares means and standard errors for growth rate (mm/d) of dominant non-ovulatory and ovulatory follicles for cows treated with MGA, PRID or no treatment (control) for 7 d beginning on d 17 of the estrous cycle

Wave of Follicular Growth	PRID	MGA	Control
Wave 1	1.03 ± .08	1.13 ± .09	1.00 ± .11
Wave 2	1.18 ± .11	.76 ± .18	1.02 ± .16
Ovulatory wave	1.12 ± .09[a]	.77 ± .10[b]	1.38 ± .12[a]

[a,b]Least-squares means within the same row without common superscripts differ P<.05.

Changes in follicular diameter for the 9-d period before ovulation were linear for each of the three treatment groups. There was no difference (P>.10) in the linear nature of the curves for control and PRID-treated cows, therefore, data from these groups were pooled for further analysis. The change in follicular diameter of the ovulatory follicle during the 9-d period before ovulation for MGA-treated cows was less rapid (P<.001, Figure 4) than that recorded for cows in the combined control and PRID-treated groups. This is best explained by the

observation that most of the cows fed MGA ovulated the dominant follicle present on d 17. Hence, the diameter of the ovulatory follicle was larger 9 d before ovulation in MGA-treated cows than in control or PRID-treated cows. Because of the persistence of the dominant follicle in MGA-treated cows, the ovulatory follicle grew less in the days before ovulation.

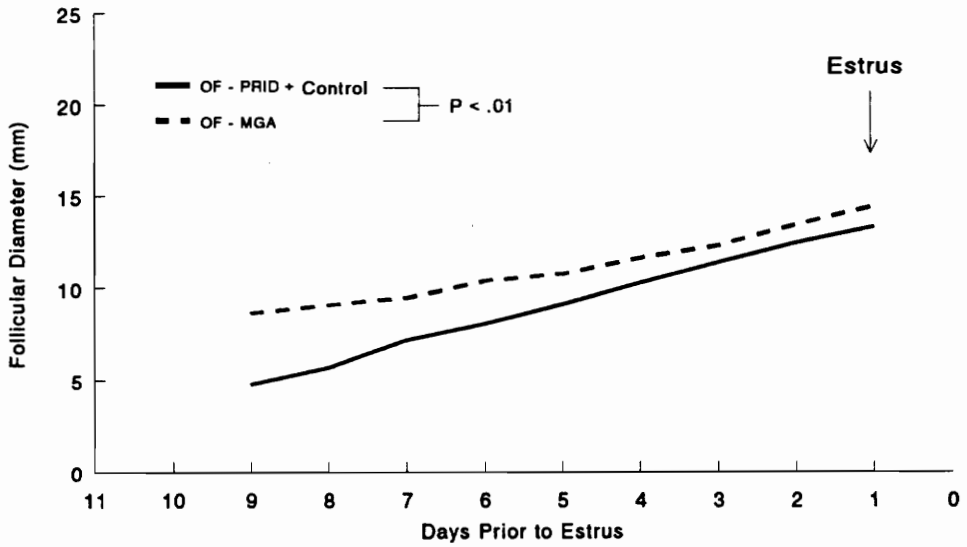

Figure 4. Diameter of the ovulatory follicle (OF) 9 d before ovulation in cows treated with MGA or PRID (controls inclusive) for 7 d beginning on d 17 of the estrous cycle.

Figures 5, 6 and 7 represent individual cows that received MGA then PRID treatments during two consecutive replicates of this study. In each case, MGA treatment resulted in persistence and ovulation of the dominant follicle of the second wave that was present at the initiation of treatment. However, PRID treatment resulted in regression of the dominant follicle of the second wave that was present at the beginning of treatment and the subsequent selection of a "new" ovulatory follicle from a third follicular wave.

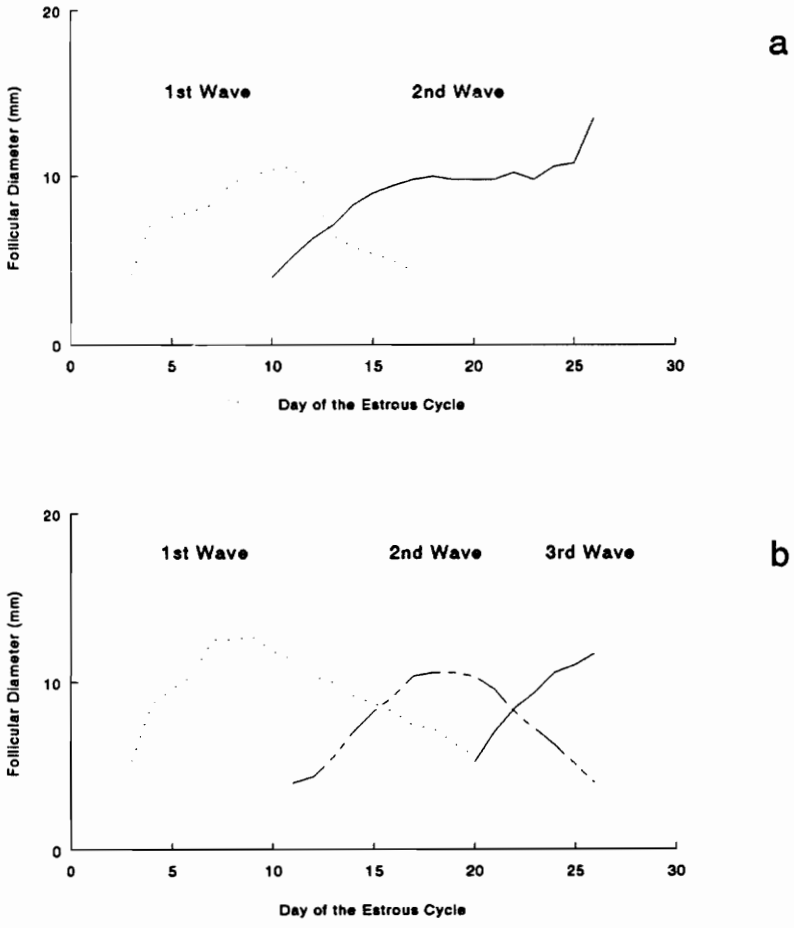

Figure 5. Follicular development throughout the interestrus interval for cow #761 which received MGA (a) and PRID (b) treatment during replicate 1 and 2 of this study, respectively.

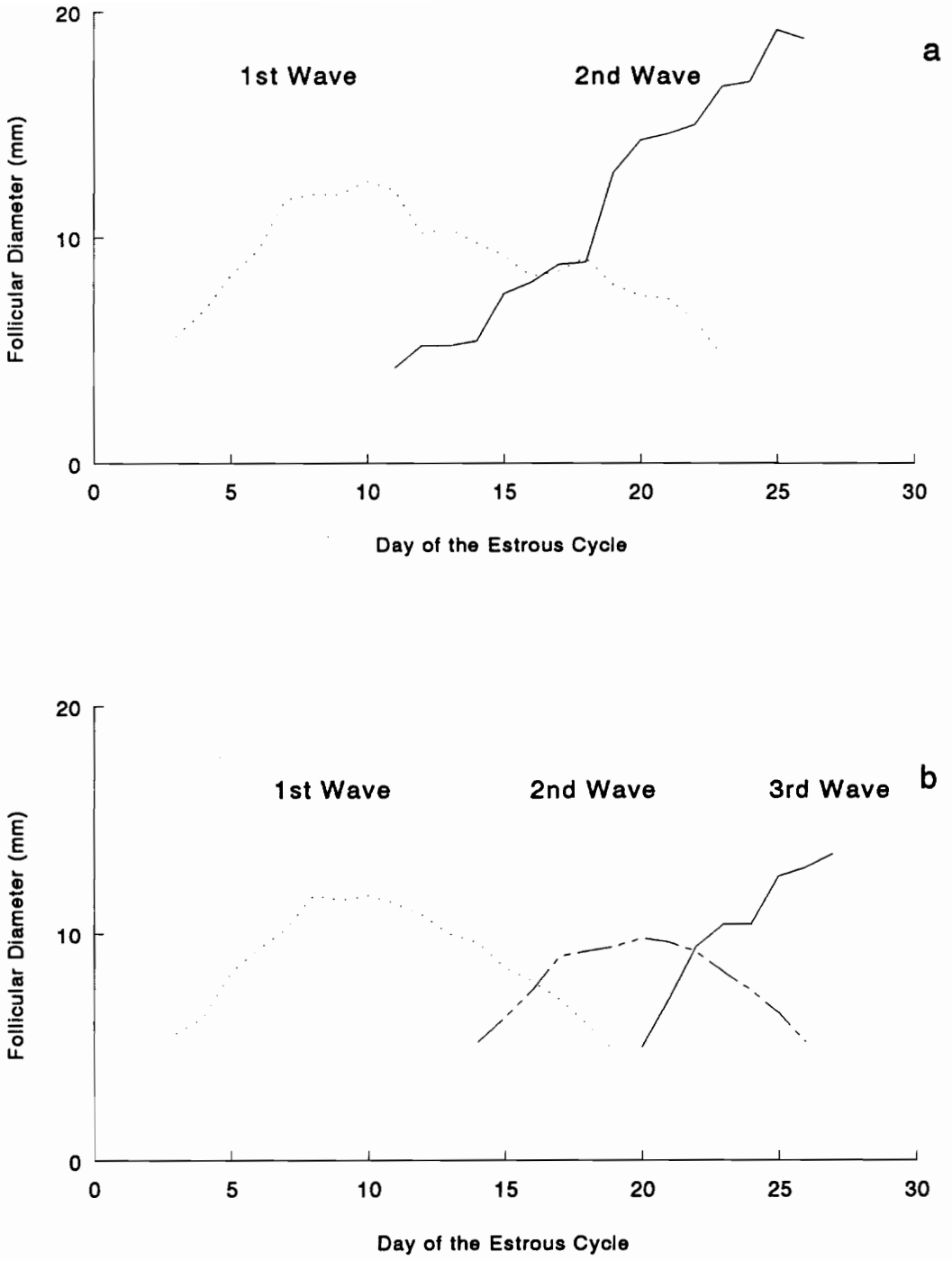

Figure 6. Follicular development throughout the interestrus interval for cow #E164 which received MGA (a) and PRID (b) treatment during replicate 1 and 2 of this study, respectively.

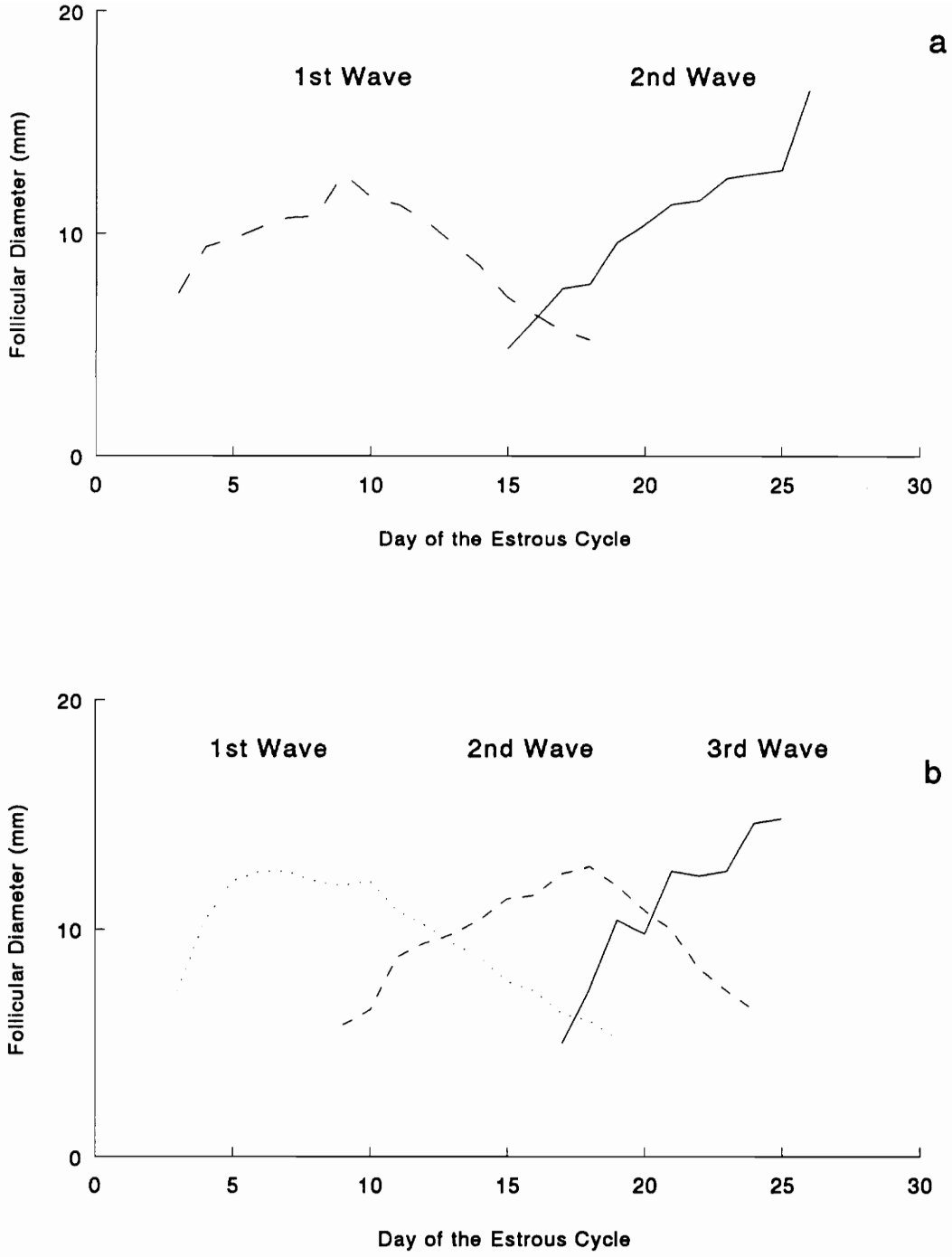

Figure 7. Follicular development throughout the interestrus interval for cow #K166 which received PRID (b) and MGA (a) treatment during replicate 1 and 2 of this study, respectively.

Estradiol-17ß concentrations were within the normal range of previously reported values through d 17 of the estrous cycle (Shemesh et al., 1972; Glencross et al., 1973) and before d 17 did not differ (P>.10, Figure 8) among treatments.

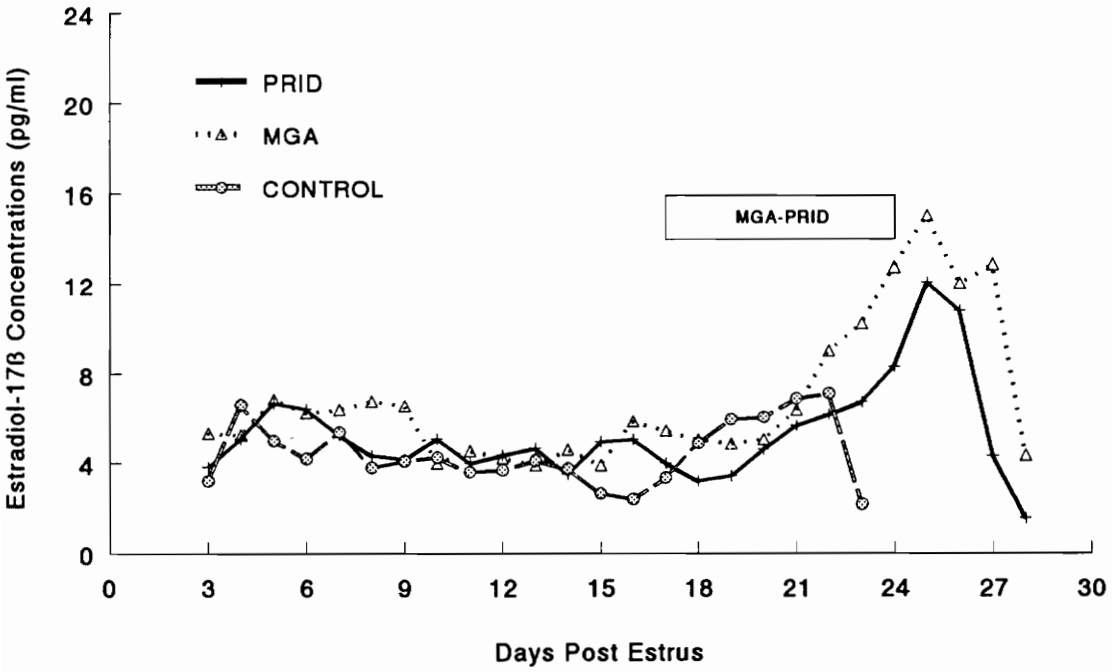

Figure 8. Mean concentration of estradiol-17ß throughout the estrous cycle of cows treated with MGA, PRID or no treatment (control) for 7 d beginning on d 17 of the estrous cycle.

To examine the effect of treatment on estradiol-17ß concentrations during the 9 d period before estrus, data were normalized to the day of estrus for each treatment. Changes in

estradiol-17ß concentrations during the 9-d period before estrus were quadratic for control, MGA- and PRID-treated cows. There was no difference (P>.10) in the quadratic trend of the estradiol-17ß curves for control or PRID-treated cows, therefore, data for these two groups were pooled for subsequent analysis. Estradiol-17ß concentrations were sustained at higher concentrations (P<.01, Figure 9) during the 9-d period before ovulation in MGA-treated cows than the combined group of PRID- or untreated-control cows.

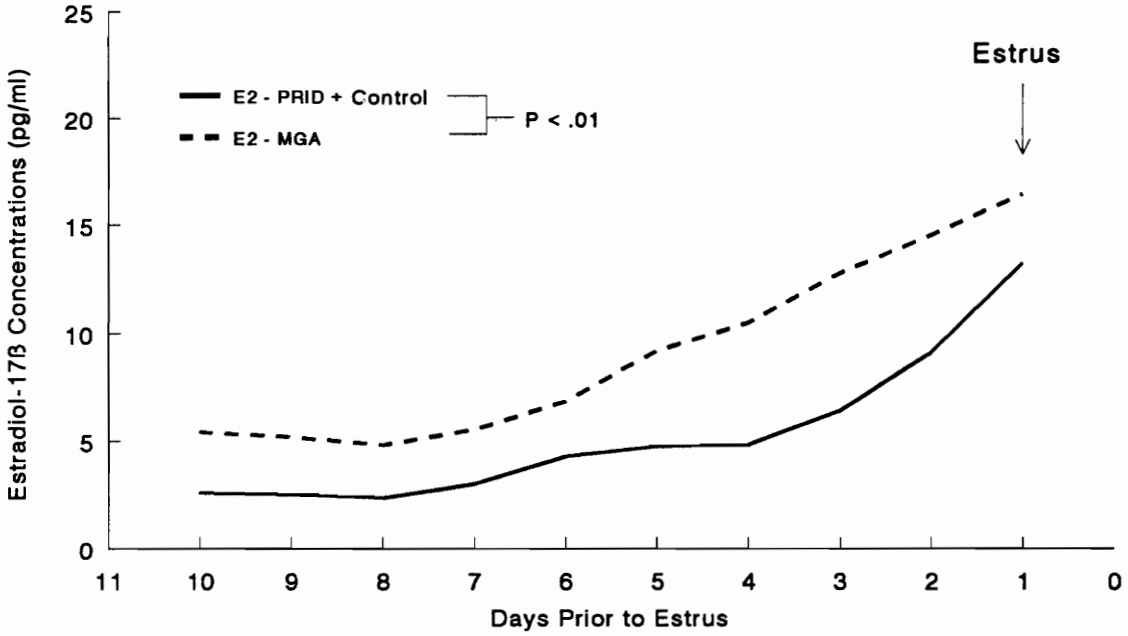

Figure 9. Concentration of estradiol-17ß for 9 d before ovulation in cows treated with MGA or PRID (controls inclusive) for 7 d beginning on d 17 of the estrous cycle.

Furthermore, there was a linear relationship between serum estradiol-17ß concentrations and diameter of the ovulatory follicle during the 9 d period before ovulation in all treatment groups (Figure 10). Regression coefficients representing the slope of the regression line was larger ($P<.05$) for MGA-treated than the combined group of control- and PRID-treated cows for rate of change in estradiol-17ß concentration relative to follicular diameter during the 9 d before estrus (Figure 10).

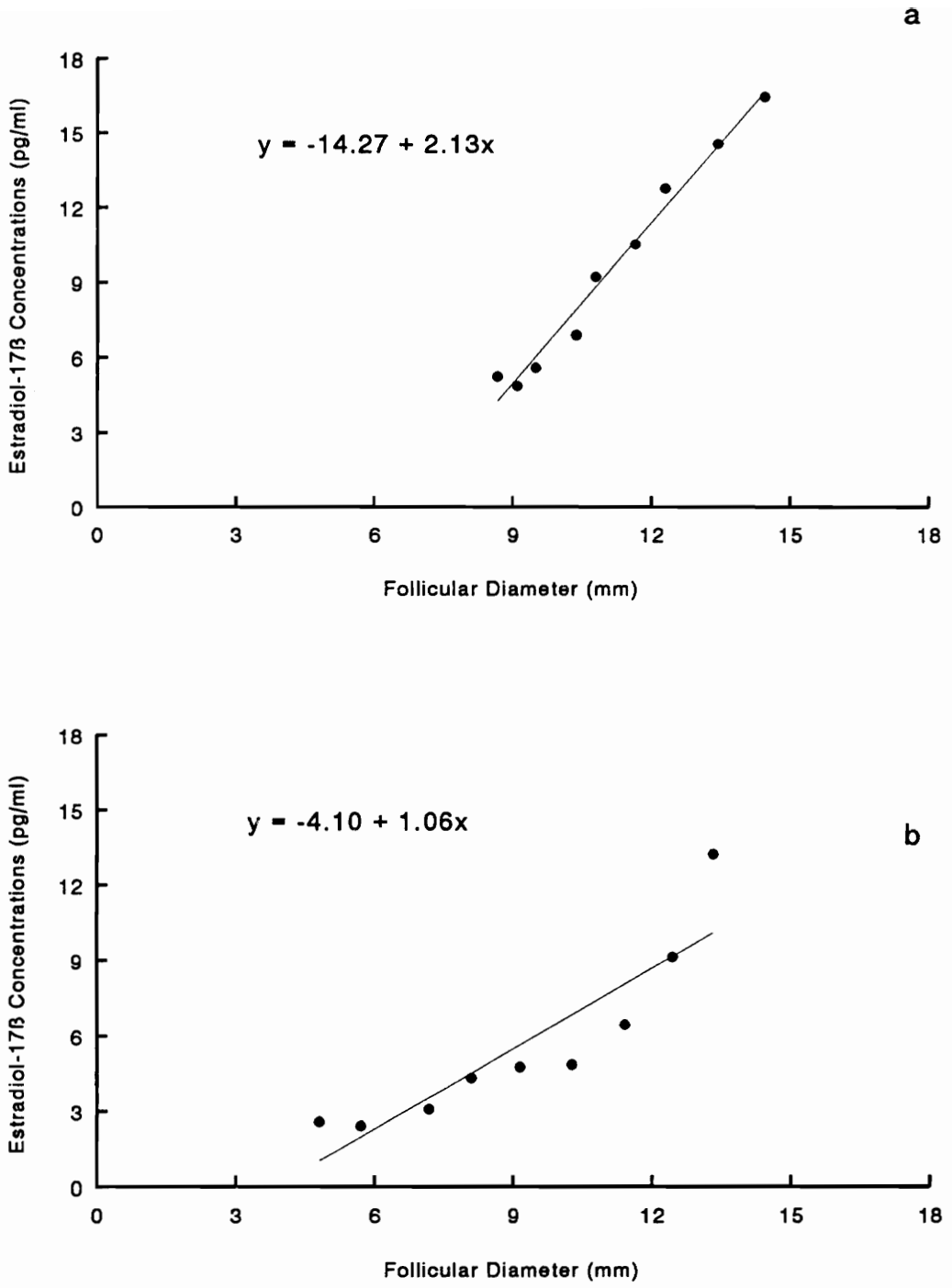

Figure 10. Relationship between estradiol-17ß concentration and diameter of the ovulatory follicle for the 9 d period before ovulation in cows treated with MGA (a) or PRID + untreated controls (b) for 7 d beginning on d 17 of the estrous cycle.

Experiment 2

Materials and Methods

This experiment was conducted in two replicates with mature (2 to 14 yr of age), non-lactating, Angus or Angus x Holstein cows. Within each replicate, treatments were assigned to cows at the time they were observed in estrus. All breeds were represented within each treatment. For each replicate, each cow received an i.m. injection of prostaglandin $F_2\alpha$ ($PGF_2\alpha$, 25 mg; Lutalyse[5]) to synchronize estrus. Following $PGF_2\alpha$, cows were observed for behavioral signs of estrus twice daily (am:pm) for at least 30 min during each period. Following estrus, (day of estrus = d 0) cows were assigned to one of three treatments: 1) MGA (oral, .5 $mg \cdot hd^{-1} \cdot d^{-1}$; n = 11), 2) PRID (silastic coil impregnated with 1.55 g progesterone[6]; n = 11) or 3) untreated controls (n = 6). MGA and PRID treatments were initiated on d 17 of the estrous cycle and continued for 7 d. Cows in the MGA-treatment group received a daily gelatin-encapsulated bolus[7] containing .5 mg MGA and the last bolus was administered on d 23 post estrus. Removal of the PRID from cows in the PRID-treatment group occurred on d 24 post estrus.

Based on the observations of Fortune et al. (1988) and Kastelic et al. (1990) that the dominant follicle of the second follicular wave was identifiable (≥ 5mm in diameter) on d 9 to

[5] The Upjohn Co., Kalamazoo, MI.
[6] Sanofi Animal Health, Paris, France.
[7] Jorgensen Laboratories, Loveland CO.

10 of the estrous cycle, ultrasonic imaging of the ovaries was initiated on d 9 post estrus. Ovaries were observed daily using a real-time, B-mode ultrasound instrument equipped with a 7.5 MHz linear array transrectal transducer (Equisonics LS-300A[8]) to monitor the growth and regression of follicles ≥ 5mm in diameter. Fecal material was removed before examination of the ovaries. The transducer was inserted into the rectum and moved along the ventral surface of the rectum adjacent to the dorsal surface of the uterine horns and then moved laterally to examine each ovary (Pierson and Ginther, 1984). The reproductive tract was not manipulated before or during the ultrasonic examination. Images of ovaries were viewed on a 48-cm monitor and recorded on video cassette to be reviewed later to record development and regression of dominant follicles and the corpus luteum. Daily ultrasound imaging of the ovaries continued until ovulation, as determined by the acute disappearance of the ovulatory follicle (Pierson and Ginther, 1984). Jugular venous blood samples (45 mL) were collected daily, coincident with ultrasound examinations, beginning at d 9 post estrus and continuing until ovulation had been recorded.

Intensive Blood Sampling for LH:

Cows assigned to each treatment within each replicate were bled intensively on d 17, 20 and 23 after onset of estrus. The d-17 bleeding served as the pre-treatment sampling period for

[8] Tokyo, Keiki LS-300A, Products Group International, Boulder, CO.

MGA- and PRID-treated cows. Cows assigned to the control group were intensively bled on d 17 only. On d 17 after onset of estrus, all cows were fitted with an indwelling jugular catheter (14-Ga x 14 cm). Once a silastic extension tube (61 cm) had been attached, the catheter and the tubing were flushed with 10 mL of 3% sodium citrate and a "test" blood sample was drawn and discarded to insure that the catheter was functional. Cows were allowed to recover after the catheterization for at least 1 h before the beginning of the intensive sampling period. Indwelling jugular catheters were again inserted on d 20 and 23 after onset of estrus in all MGA- and PRID-treated cows.

Blood samples were collected at 15-min intervals for 6 h beginning at approximately 1100 h on d 17, 20 and 23 after onset of estrus. Blood samples were allowed to clot at 22 C for approximately 16 h, then were centrifuged at 1000 x g at 4 C for 20 min. Serum was decanted and stored at -25 C until assayed for luteinizing hormone. Blood samples collected daily were allowed to clot at room temperature for approximately 4 h, then were centrifuged at 1000 x g for 30 min. Serum was harvested and stored at -25 C until radioimmunoassayed for estradiol-17ß and progesterone.

Assays for Estradiol-17ß, Luteinizing Hormone and Progesterone:

Estradiol-17ß concentrations in daily samples for the two replicates were quantified by a double antibody radioimmunoassay as described by Sirois and Fortune. (1990). Progesterone

concentration in daily samples for the two replicates were quantified with solid-phase radioimmunoassay kits[9] . Luteinizing hormone was quantified by a double antibody radioimmunoassay. Validation and procedures for this assay are as described by Custer (1988). Assay sensitivity for estradiol-17ß, luteinizing hormone and progesterone were 1.5 pg/mL, .55 ng/mL and .10 ng/mL, respectively. Inter- and intra-assay coefficients of variation were as follows: estradiol-17ß, 14.8 and 9.7%; luteinizing hormone, 8.46 and 1.66%; and progesterone, 7.9 and 6.2%, respectively.

Statistical Analyses:

Data for interovulatory interval, day after onset of estrus progesterone concentration declined below 1 ng/mL, day of detection of the dominant follicle of the second follicular wave, day of detection of the ovulatory follicle, diameter of the dominant follicle present on d 17 after onset of estrus, days from detection of the ovulatory follicle until ovulation and diameter of the ovulatory follicle on the day before ovulation were analyzed by one-way analyses of variance for a completely randomized design using the GLM procedure of SAS (SAS, 1987).

Growth rate (mm/d) of the dominant non-ovulatory and ovulatory follicle of each follicular wave was calculated by linear regression (slope = growth rate) of follicular diameter

[9] Diagnostic Products Corp., Los Angeles, CA.

on day of the estrous cycle. Data for regression analysis included observations from the first day follicular diameter was ≥ 5mm and continued until maximum diameter was reached (non-ovulatory wave) or until ovulation (ovulatory wave) occurred. Comparison of slopes for growth rates for follicular waves was analyzed by one-way analysis of variance using the GLM procedure of SAS (SAS, 1987).

Data for diameter of the ovulatory follicle for the 9-d period before ovulation was analyzed using analysis of variance procedures with time as an independent variable (SAS, 1987). Shape of the curve for ovulatory follicular diameter was determined by polynomial regression analysis (Allen et al., 1983). Test for homogeneity of residual variances were used to determine the effect of treatment on changes in follicular diameter over time between control, MGA- and PRID-treated cows. Comparison of estradiol-17ß concentration on the d diameter of the dominant follicle of the second wave was significantly for MGA-2 cows than control-3, MGA-3 and PRID-treated cows was analyzed by one-way analysis of variance using GLM procedures of SAS (SAS, 1987).

Least-squares mean differences among treatments for the following variables were determined by T-test (SAS, 1987).

(a) interovulatory interval

(b) days from detection of the ovulatory follicle until ovulation

(c) growth rate of dominant follicles within follicular waves

(d) day of detection of the dominant follicle of the second wave

(e) day of detection of the ovulatory follicle

(f) diameter of the dominant follicle present on d 17 after onset of estrus

(g) diameter of the ovulatory follicle on the day before ovulation

(h) day in which progesterone concentration declined below 1 ng/mL

(i) estradiol-17ß concentration on the day diameter of the dominant follicle of the second wave diverged significantly between treatments

Patterns of LH release for each cow were characterized by the mean (ng/mL), baseline (ng/mL), pulse frequency (pulses/6 h) and pulse amplitude (ng/mL) for frequent samples collected on d 17, 20 and 23 after the onset of estrus. Baseline and amplitude were determined using algorithms (PC Pulsar) as described by Merriam and Wachter (1982). A peak was defined as a value that was at least two standard deviations above the baseline with at least one value increasing to or decreasing from the peak. Amplitude was calculated by subtracting the highest concentration of LH within a peak from the baseline. To test the hypothesis that progestogen treatment did not contribute to the variation in LH patterns on d 17, 20 and 23 after onset of estrus; mean, baseline, pulse frequency and amplitude of LH pulses were analyzed by analysis of variance for a completely random design using GLM procedure of SAS (SAS, 1987). Two additional analyses of LH characteristics were performed. The first differentiated between MGA-treated cows which arrested

development of the dominant follicle present at the beginning of treatment versus MGA-treated cows which continued follicular turnover throughout treatment while the second considered changes in LH characteristics on d 17, 20 and 23 within treatment groups.

Results

There was no treatment X replicate interaction for the variables examined in this study, hence, data for each replicate within treatment were pooled for further analysis. Interestrus interval was extended by approximately 3 to 5 d in PRID- and MGA-treated cows which exhibited two (MGA-2) or three (MGA-3) follicular waves during the interestrus interval compared to untreated controls (P<.01; Table 9). Control cows exhibiting two follicular waves (Control-2) had 2 to 3 d shorter interestrus intervals than controls exhibiting three or four follicular waves (Control-3; P<.01; Table 9).

Table 9: Least-squares means and standard errors for interestrus interval for MGA-, PRID- or untreated control cows exhibiting two, three or four follicular waves throughout the interestrus interval.

Treatment	n	Follicular Waves	Interestrus Interval (d)
Control	3	2	22.0 ± .48[a]
Control	3	3,4	24.7 ± .48[b]
MGA	3	2	26.7 ± .48[c]
MGA	8	3	26.9 ± .30[c]
PRID	11	3	26.3 ± .25[c]

[a,b,c]Least-squares means within the same column without common superscripts differ P<.01.

Progesterone concentration and corpus luteum diameter from d 9 through 17 of the cycle did not differ (P>.10; Figure 11) among treatments. PRID-treated cows had an acute increase in progesterone concentration after PRID insertion (d 17). Progesterone concentrations remained elevated (> 1 ng/mL) until PRID removal (d 24) then decreased to concentrations similar to cows in the MGA-2 and MGA-3 treatment groups (Figure 11). Progesterone concentration declined below 1 ng/mL approximately 3 d earlier (P<.01; Table 10) during the interestrus interval in MGA-2 and control-2 cows compared to MGA-3 and control-3 cows (Figure 11).

Table 10. Least-squares means and standard errors for day of the estrous cycle progesterone concentration declined below 1 ng/mL in control, MGA- and PRID-treated cows.

	Waves During the Estrous Cycle	
Treatment	**2 (n)**	**3 (n)**
MGA	**19.00 ± .88[a] (3)**	**22.43 ± .57[b] (8)**
PRID	**--------**	**24.18 ± .46 (11)**
Control	**20.33 ± .87[a] (3)**	**23.00 ± .88[b] (3)***

[a,b]Least-squares means within the same row without common superscripts differ at P<.01.
***Includes 3 and 4 wave cows.**

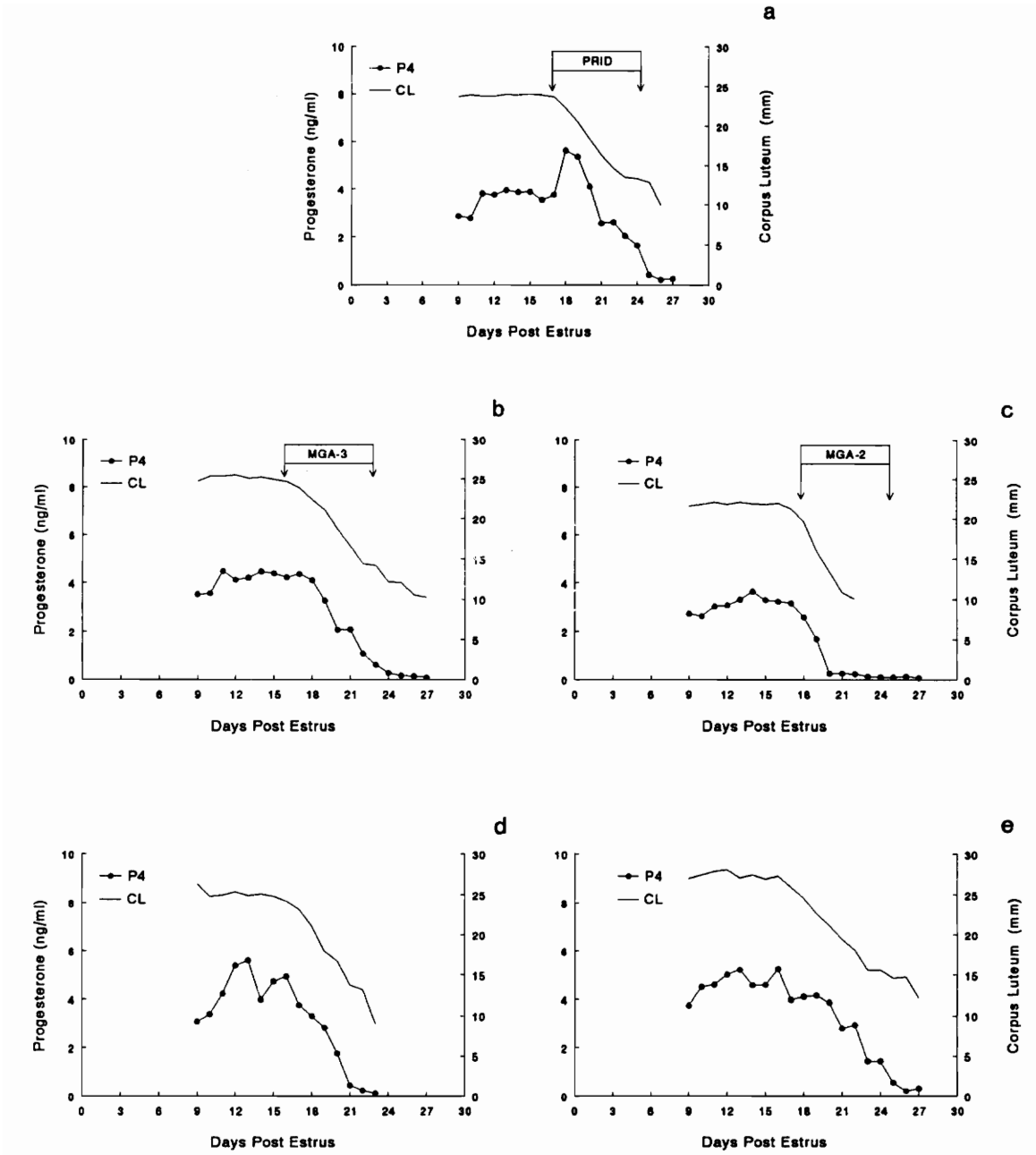

Figure 11. Progesterone concentration and corpus luteum diameter for PRID- (a), MGA-3 (b), MGA-2 (c), control-2 (d) and control-3 (e) cows throughout the interestrus interval.

Cows within each treatment group exhibited waves of follicular growth throughout the interestrus interval. All 11 PRID-treated cows exhibited three follicular waves, whereas, 3 of 11 MGA-treated cows exhibited two follicular waves during the experimental period. The dominant follicle present on d 17 of the interestrus interval ovulated in 3 of 11 MGA-treated cows and 0 of 11 PRID-treated cows. Figure 12 depicts representative animals from each treatment group which were characterized by either two, three or four waves of follicular growth. There was no treatment or treatment X waves per cycle interaction ($P>.10$; Table 11) with respect to day of detection of the dominant follicle of the second wave, growth rate of the dominant follicle of the second wave or diameter of the dominant follicle present on either ovary at the initiation of progestogen treatment (d 17).

Day of detection of the ovulatory follicle occurred approximately 7 d later during the interestrus interval for control-3, MGA-3 and PRID-treated cows than for control-2 and MGA-2 cows ($P<.01$; Table 12). Days from detection (≥ 5mm) of the ovulatory follicle until ovulation was less ($P<.03$; Table 11) and growth rate of the ovulatory follicle was greater ($P<.02$; Table 11) for control-3, MGA-3 and PRID-treated cows than for control-2 and MGA-2 cows. Diameter of the ovulatory follicle on the day before ovulation was larger in MGA-2 cows than all other treatment groups ($P<.01$; Table 12).

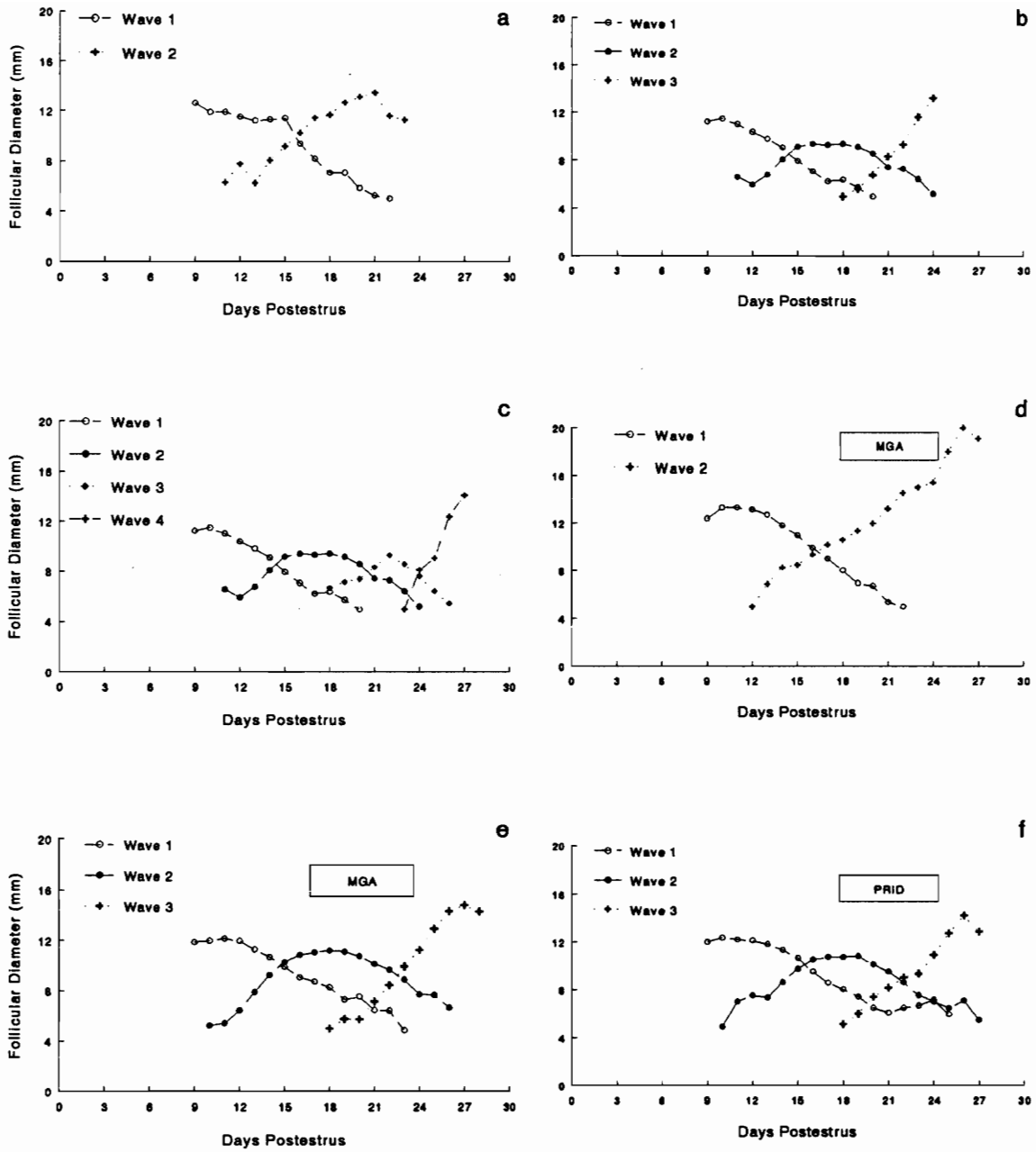

Figure 12. Growth and regression of dominant non-ovulatory and ovulatory follicles throughout the interestrus interval for control (a,b,c), MGA- (d,e) and PRID-treated (f) cows exhibiting two, three or four follicular waves.

Table 11. Least-squares means and standard errors for day of detection of the dominant follicle of the second follicular wave (DDSW), growth rate of the dominant follicle of the second wave (GRSW) and diameter of the dominant follicle present on day 17 postestrus (DD17) in control, MGA- and PRID-treated cows.

Treatment	n	Waves	DDSW	GRSW	DD17
Control	3	2	12.33 ± .98	------	11.41 ± .57
Control	3	3,4	12.00 ± .70	.86 ± .14	9.33 ± .57[a]
MGA	3	2	12.33 ± .70	------	11.41 ± .57
MGA	8	3	11.25 ± .43	1.01 ± .09	11.01 ± .35
PRID	11	3	11.82 ± .37	1.09 ± .07	10.71 ± .30

[a]Least-squares means within the same column without common superscripts differ P<.05.

Table 12. Least-squares means and standard errors for day of detection of the ovulatory follicle (DDOF), days from detection until ovulation (DDTO), growth of the ovulatory follicle (GROF), and diameter of the ovulatory follicle on the day before ovulation (DOF) in control, MGA- and PRID-treated cows.

Treatment	n	Waves	DDOF	DDTO	GROF	DOF
Control	3	2	12.33 ± .98[a]	10.66 ± .77[e]	.82 ± .16[g]	13.96 ± 1.35[a]
Control	3	3,4	20.00 ± .98[b]	5.66 ± .77[c]	1.75 ± .16[f]	13.51 ± 1.35[a]
MGA	3	2	13.00 ± .98[a]	14.66 ± .77[d]	.98 ± .16[g]	20.39 ± 1.35[b]
MGA	8	3	20.25 ± .60[b]	7.75 ± .47[c]	1.47 ± .10[f]	15.53 ± .83[a]
PRID	11	3	20.36 ± .51[b]	6.91 ± .40[c]	1.49 ± .08[f]	14.48 ± .71[a]

[a,b]Least-squares means within the same column without common superscripts differ P<.01.
[c,d,e]Least-squares means within the same column without common superscripts differ P<.03.
[f,g]Least-squares means within the same column without common superscripts differ P<.02.

Changes in diameter of the ovulatory follicle for the 9-d period before ovulation were linear for cows exhibiting two, three or four follicular waves within each treatment. The increase in follicular diameter of the ovulatory follicle during the 9-d period before ovulation was less rapid for MGA-2 cows than all other treatments ($P<.01$; Figure 13). This is best explained by the observation that diameter of the ovulatory follicle was larger 9 d before ovulation in MGA-treated cows characterized by two follicular waves.

Estradiol-17ß concentrations were similar to previously reported values from d 9 through d 17 of the estrous cycle (Shemesh et al., 1972; Glencross et al., 1973). There was no treatment or treatment X waves per cycle interaction for estradiol-17ß concentrations before d 18 of the estrous cycle ($P>.10$; Figure 14).

Changes in estradiol-17ß concentrations during the 9-d period before ovulation were cubic for control, MGA- or PRID-treated cows characterized by either two or three follicular waves. There was no difference ($P>.10$) in the cubic trend of the estradiol-17ß curves during the 9 d before ovulation for control, MGA- or PRID-treated cows characterized by two or three follicular waves.

Estradiol-17ß concentration was lower 7 d before ovulation in control-3, PRID and MGA-3 cows than MGA-2 cows ($P<.05$; Figure 15). This coincided with the day diameter of the dominant

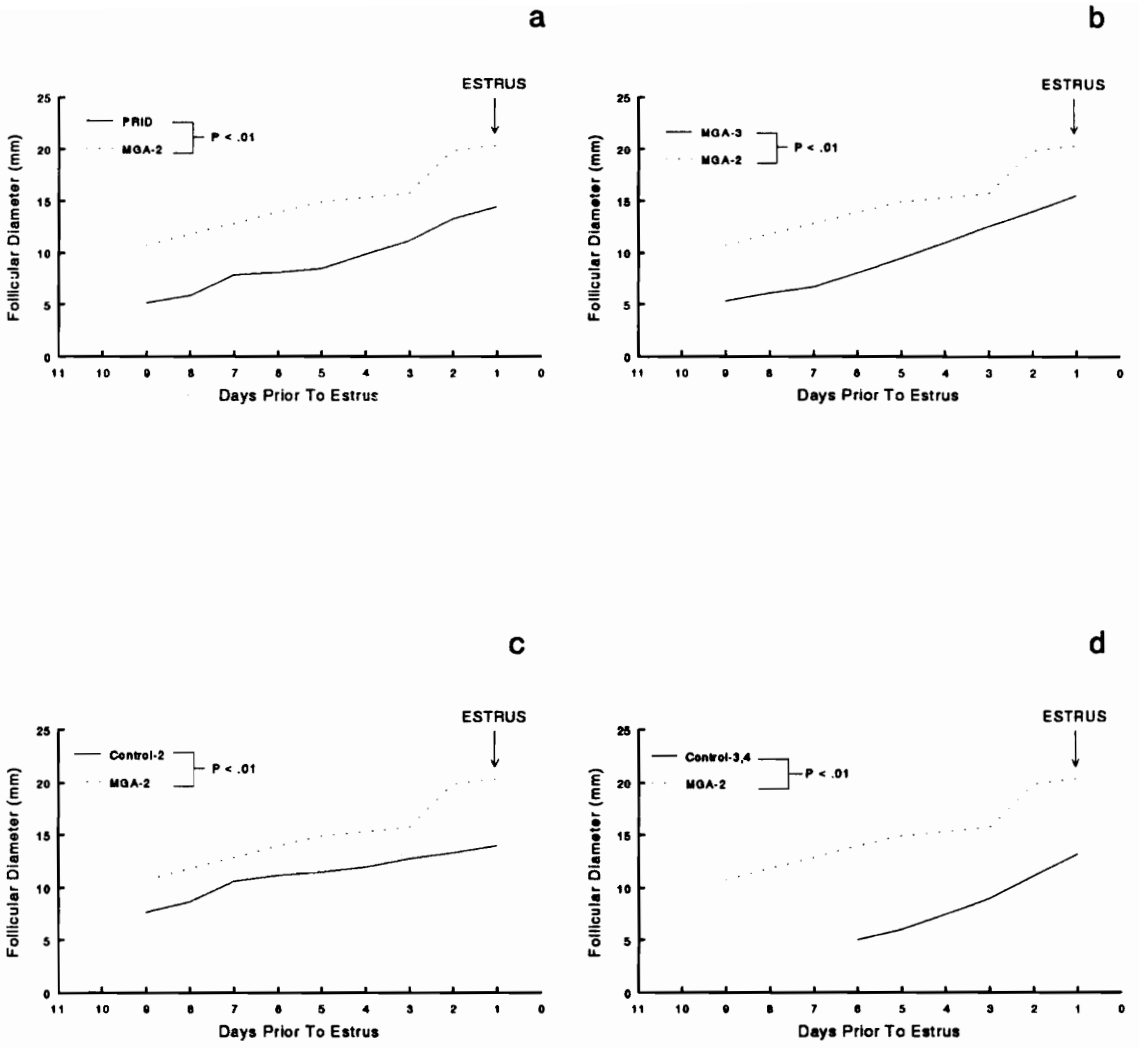

Figure 13. Diameter of the ovulatory follicle for the 9-d period before ovulation in MGA-treated cows exhibiting two follicular waves compared with PRID (a), MGA-treated cows exhibiting three follicular waves (b) and untreated controls exhibiting two or three follicular waves (c,d).

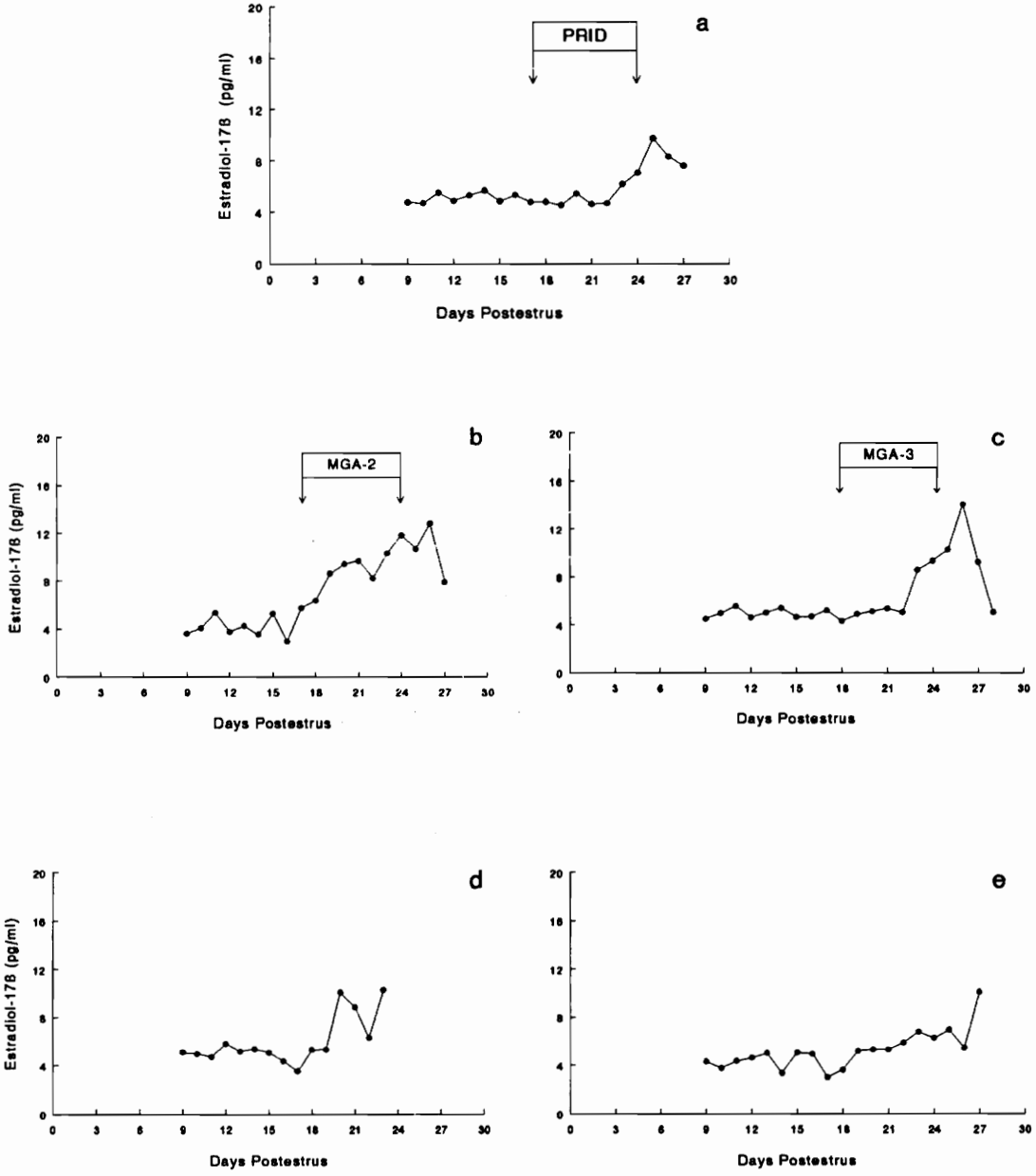

Figure 14. Mean concentration of estradiol-17ß throughout the interestrus interval for PRID- (a), MGA- (2-waves,b; 3-waves,c) and untreated control cows (2-waves,d; 3+4 waves,e).

follicle of the second wave was larger (P<.05; Figure 15) in MGA-2 cows than control-3, MGA-3 and PRID-treated cows. Diameter of the dominant follicle of the second follicular wave and estradiol-17ß concentrations 7 d before ovulation did not differ between control-2 and MGA-2 cows (P>.10; Figure 16).

Mean and baseline concentrations of luteinizing hormone, pulse frequency and amplitude of episodic LH pulses on d 17 of the interestrus interval did not differ among treatments (P>.10; Table 13). There was no treatment or treatment X waves per cycle interaction on mean, baseline concentration or amplitude of LH pulses on d 20 or 23 of the interestrus interval (P>.10; Table 13). Frequency of LH pulses was greater in MGA-treated than PRID-treated cows on d 20 of the interestrus interval (P<.075; Table 13). This was a direct result of an increase in LH pulse frequency on d 20 of the interestrus interval in MGA-2 cows (P<.03; Table 14). Figure 17 depicts characteristics of pulsatile LH release on d 17 of the interestrus interval for control, MGA- and PRID-treated cows. Figures 18 and 19 illustrate patterns of episodic LH release on d 20 and 23 of the interestrus interval for MGA-2, MGA-3 and PRID-treated cows.

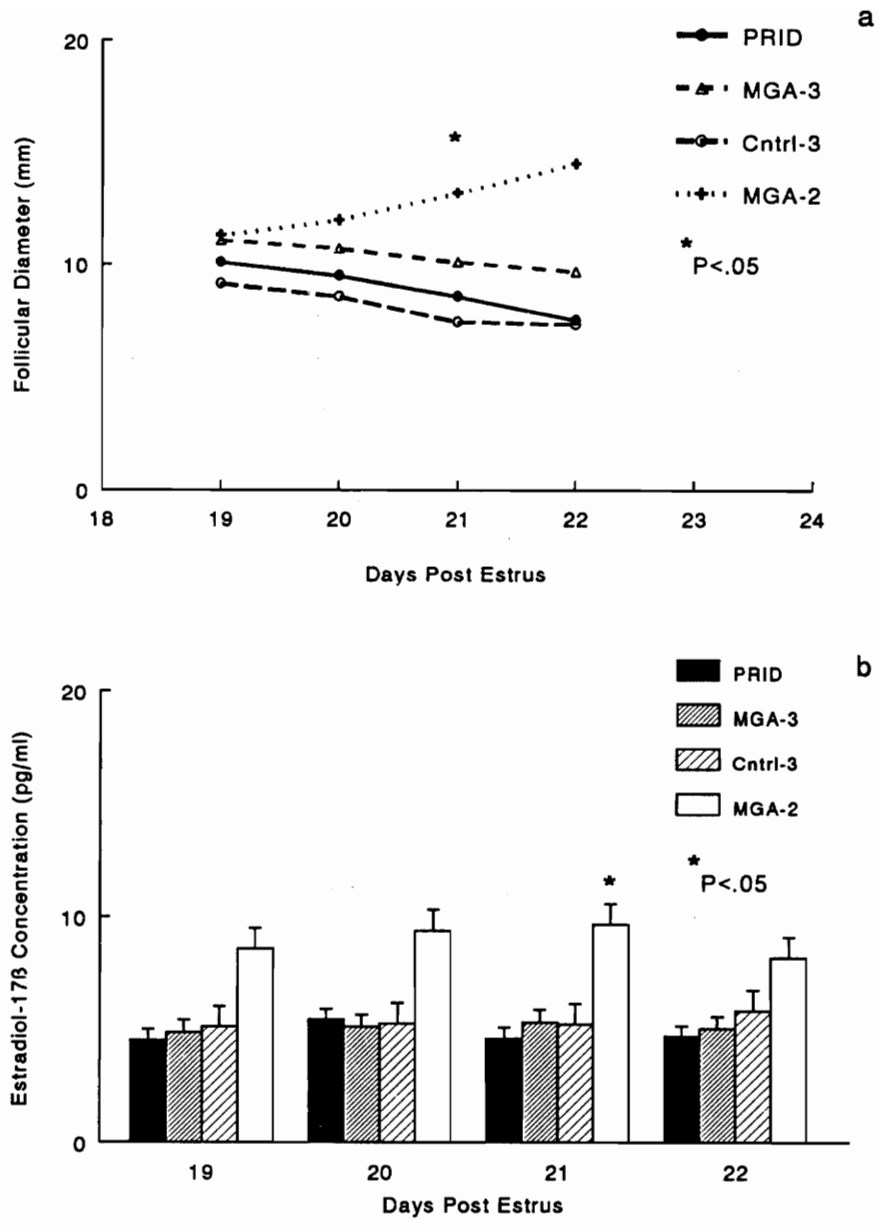

Figure 15. Diameter of the dominant follicle of the second follicular wave (a) and estradiol-17ß concentrations (b) on d 19, 20, 21 and 22 after the onset of estrus for control, PRID and MGA-treated cows characterized by three follicular waves and MGA-treated cows characterized by two follicular waves.

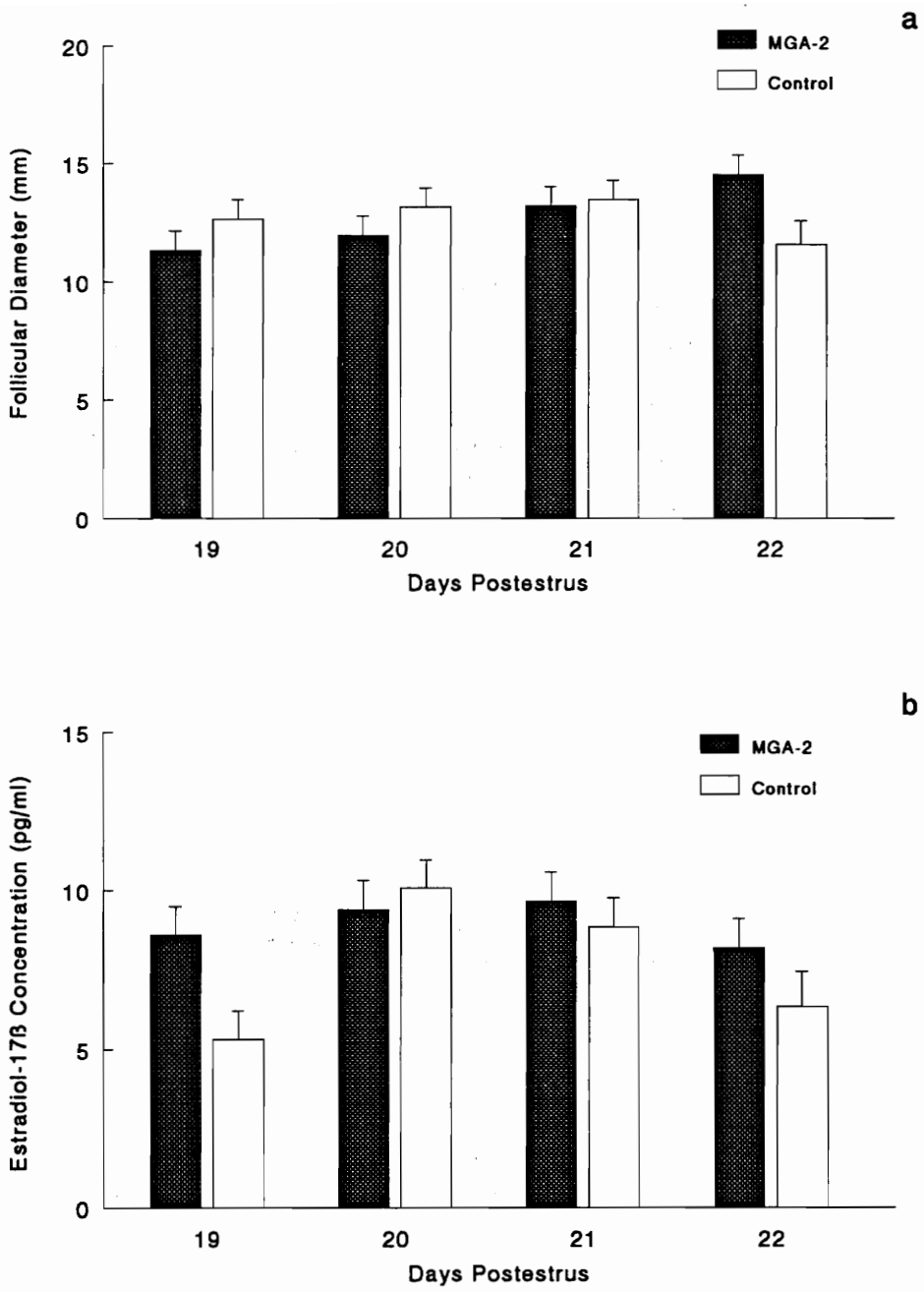

Figure 16. Diameter of the dominant follicle of the second follicular wave (a) and estradiol-17ß concentrations (b) on d 19, 20 21 and 22 after the onset of estrus for control and MGA-treated cows characterized by two follicular waves.

Table 13. Least-squares means and standard errors for characteristics of luteinizing hormone patterns on day 17, 20 and 23 postestrus for control, MGA- and PRID-treated cows.

Characteristic	Treatment		
	Control	PRID	MGA
No. of cows	6	11	11
Day 17			
Mean[a]	1.75 ± .17	1.74 ± .13	1.64 ± .13
Baseline[a]	1.60 ± .16	1.57 ± .12	1.42 ± .16
Frequency of LH pulses[b]	2.83 ± .52	2.55 ± .38	2.83 ± .52
Amplitude of LH pulses[a]	.53 ± .16	.60 ± .12	.83 ± .12
Day 20			
Mean		1.81 ± .13	1.91 ± .13
Baseline		1.61 ± .13	1.52 ± .13
Frequency of LH pulses		2.64 ± .34[c]	3.54 ± .34[d]
Amplitude of LH pulses		.73 ± .10	.81 ± .10
Day 23			
Mean		1.90 ± .13	1.90 ± .13
Baseline		1.70 ± .14	1.77 ± .17
Frequency of LH pulses		3.36 ± .22*	3.27 ± .22
Amplitude of LH pulses		.64 ± .06	.61 ± .06

[a] $(ng \cdot ml^{-1}) \cdot 6\ h^{-1}$.

[b] (pulses/6 h).

[c,d] Least-squares means within the same row without common superscripts differ at $P<.08$.

* PRID d20 vs PRID d23 ($P<.075$).

Table 14. Least-squares means and standard errors for luteinizing hormone pulse frequency on day 20 after onset of estrus for PRID- and MGA-treated cows exhibiting two or three waves of follicular growth throughout the interestrus interval.

Treatment	n	Frequency of LH Pulses[a]
PRID	11	2.63 ± .33[b]
MGA-2	3	4.33 ± .63[c]
MGA-3	8	3.25 ± .39[b]

[a](pulses/6 h)

[b,c]Least-squares means within the same column without common superscripts differ at P<.03.

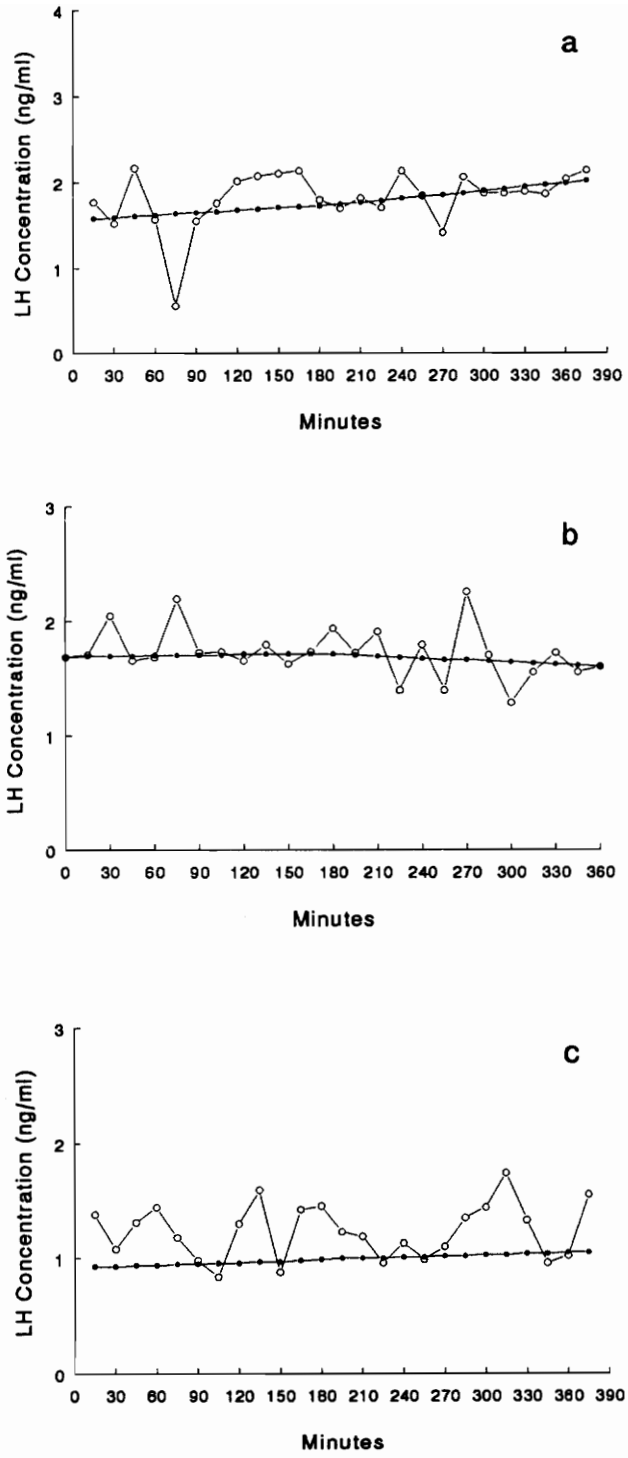

Figure 17. Patterns of luteinizing hormone release on d 17 of the interestrus interval for representative control (a; n=1), PRID- (b; n=1) and MGA-treated cows (c; n=1). Solid line represents the smoothed baseline determined by PC Pulsar.

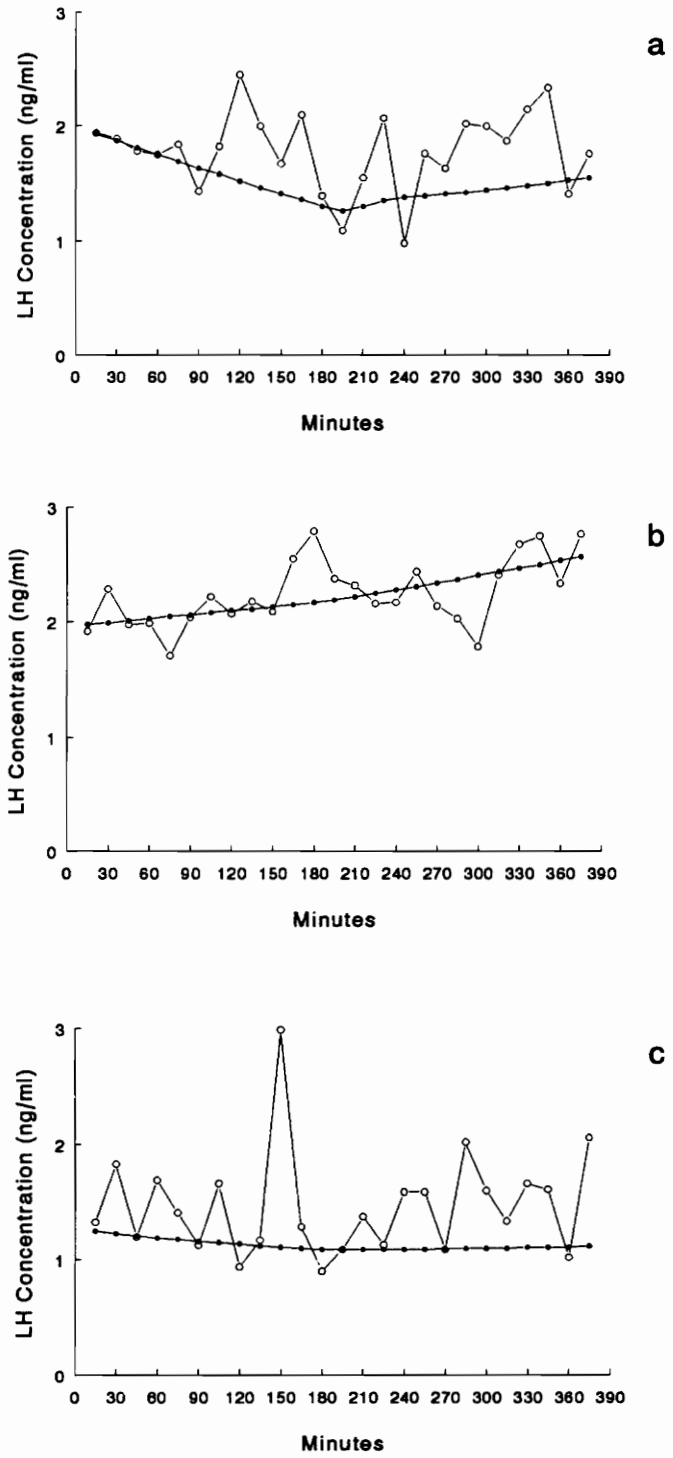

Figure 18. Patterns of luteinizing hormone release on d 20 of the interestrus interval for representative PRID (a; n=1), MGA-3 (b; n=1) and MGA-2 cows (c; n=1). Solid line represents the smoothed baseline determined by PC Pulsar.

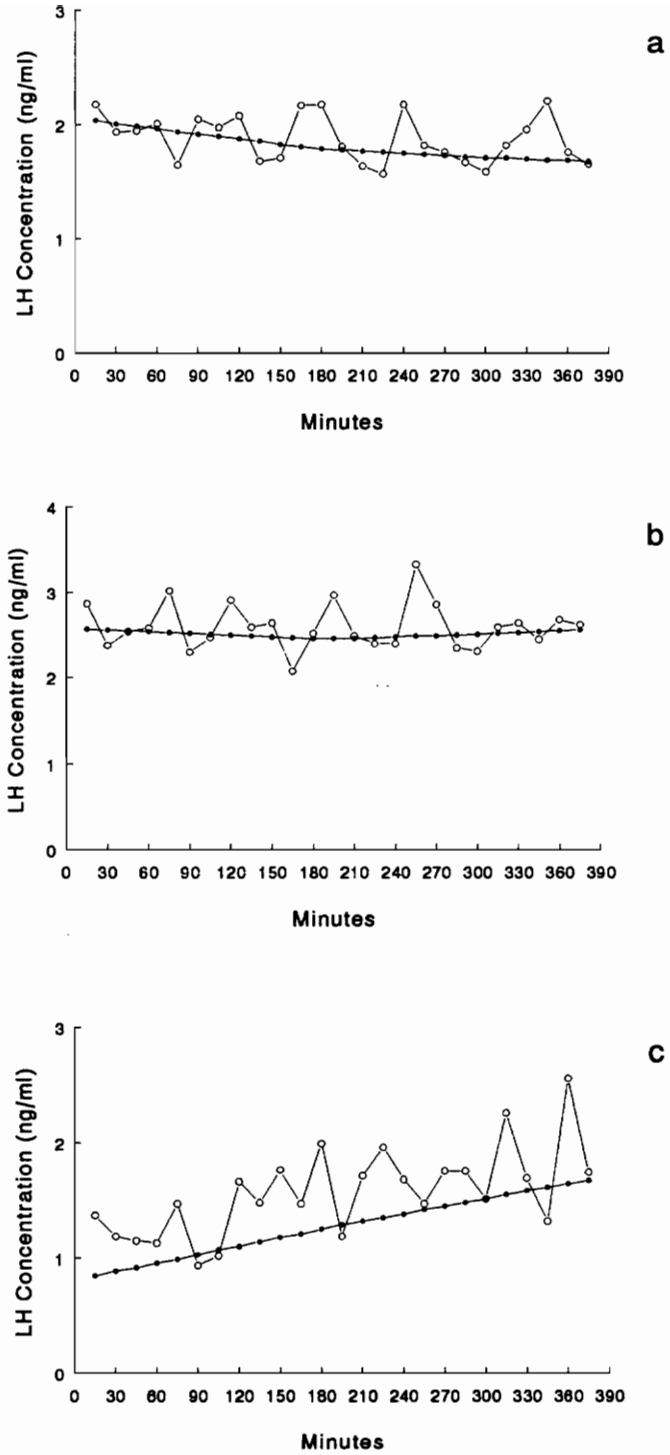

Figure 19. Patterns of luteinizing hormone release on d 23 of the interestrus interval for representative PRID (a; n=1), MGA-3 (b; n=1) and MGA-2 cows (c; n=1). Solid line represents the smoothed baseline determined by PC Pulsar.

Discussion

Artificially lengthening the interestrus interval with exogenous progestogens, such as CIDR's, MGA or norgestomet, alters the hormonal environment, as well as the normal pattern of follicular development associated with the proestrus stage of the bovine estrous cycle (Beal et al., 1990; Sirois and Fortune, 1990; Taylor and Rajamahendran, 1991). In this series of studies, 7-d of MGA or PRID treatment initiated on d 17 of the estrous cycle delayed estrus and ovulation by approximately 3 to 5 d, regardless of the number of dominant follicles characterizing the interestrus interval. These data are consistent with the results of Beal et al. (1990) and Sirois and Fortune (1990) in which progestogen treatment artificially lengthened the bovine estrous cycle. Life span of the corpus luteum did not appear to be influenced by MGA or PRID treatment, since corpus luteum diameter declined coincident with that of cows in the control group. In Experiment 1 and 2, decline in serum progesterone concentration in MGA-treated cows paralleled that of untreated control cows. In contrast, progesterone concentrations increased after PRID insertion on d 17, then declined progressively, to subluteal (< 2 ng/mL) phase concentrations within 4 d after PRID insertion. These concentrations were maintained until PRID removal on d 24 of the interestrus interval. This pattern of progesterone release from a PRID agrees with the results of Ireland and Roche (1981) in

which heifers received vaginal coils containing 2% progesterone during the follicular phase of the estrous cycle.

In both studies, control, MGA- and PRID-treated cows exhibited waves of follicular growth throughout the interestrus interval, which agrees with the observations of Pierson and Ginther (1988), Beal et al. (1990), Sirois and Fortune (1990) and Taylor and Rajamahendran (1990). In control, MGA- and PRID-treated cows, observable characteristics of follicular development before d 17 were not a determining factor in the emergence of either two or three follicular waves during the interestrus interval. In agreement, Ginther et al. (1989) reported that characteristics of follicular development occurring during the first half of the interovulatory interval were not associated with the later emergence of a third follicular wave.

Control cows in Experiment 1 were predominantly (7 of 8) characterized by 3 follicular waves, whereas in Experiment 2, there was an equal distribution of two and three wave intervals among the control cows. In both studies, the average length of the interestrus interval was 2 to 3 d shorter for control cows characterized by two-wave compared to three-wave intervals. This was not unexpected, since Ginther et al. (1989b) reported a significant increase (2 to 4 d) in the mean length of the interovulatory interval for heifers with three- versus two-wave intervals. In Experiment 1, prolongation of the interestrus interval was accompanied by a predominance (7 of 12) of cycles

characterized by two waves of follicular growth in MGA-treated cows, whereas only 3 of 11 MGA-treated cows exhibited two waves of follicular growth in Experiment 2. In contrast, in both studies, PRID-treated cows characteristically exhibited (26 of 27) three follicular waves during the interestrus interval. This difference resulted primarily due to a prolonged persistence and ovulation of the dominant follicle of the second wave present at the beginning of treatment in 10 of 24 MGA-treated cows. This finding confirms and supports the results reported by Beal et. (1990) that MGA treatment initiated during late diestrus arrested development of the dominant follicle present at the beginning of MGA treatment. The reduced rate of follicular growth of the ovulatory follicle noted in MGA-treated cows characterized by two follicular waves reflects the persistence and ovulation of the dominant follicle present at the beginning of MGA treatment. Further support for this notion comes from the equivalent growth rate of the ovulatory follicle in control, MGA- and PRID-treated cows characterized by three follicular waves.

In both studies, the dominant follicle present at the beginning of PRID treatment underwent atresia (26 of 27 cows) with the subsequent selection of the ovulatory follicle occurring after PRID insertion on d 17 of the estrous cycle. This was unexpected, because Sirois and Fortune (1990) reported that heifers treated with 1 CIDR for 14 d beginning on d 14 of the estrous cycle arrested development and ovulated the dominant

follicle present at CIDR insertion. An explanation for the divergent results may lie in the timing of initiation of PRID and CIDR treatments and resultant serum progesterone concentrations.

Sirois and Fortune (1990) indicated that progesterone concentrations must be maintained at normal luteal phase concentrations for continuation of follicular waves during an artificially-lengthened estrous cycle. In support of this hypothesis, Bergfelt et al. (1991) reported that non-pregnant heifers treated daily with 150 mg of progesterone for 100 d continue periodic emergence of follicular waves. Similar findings have been reported during the first 60 d of pregnancy in which serum progesterone concentrations are maintained at luteal phase concentrations for extended periods of time (Ginther et al., 1989c). In a more recent study, Cupp et al. (1992) reported that in the absence of a corpus luteum, heifers receiving two PRID's, which maintained serum progesterone concentrations between 5 to 6 ng/mL, continued development of dominant follicles, whereas heifers receiving .5 PRID, which maintained serum progesterone concentrations between 1 to 2 ng/mL, experienced arrested development of the dominant follicle which grew approximately 1 mm/d throughout the experimental period. Sub-luteal phase serum progesterone concentrations are apparent at the time of natural luteolysis (d 18 to 20) in heifers treated with 1 CIDR on d 14 of the estrous cycle (Sirois and Fortune, 1990). However, in the present studies, PRID

insertion on d 17 of the estrous cycle, nearer the time of natural luteolysis, maintained serum progesterone concentrations at luteal phase concentrations (>2 ng/mL) until at least d 21 of the artificially-lengthened interestrus interval.

Sub-luteal phase concentrations of progesterone (<2 ng/mL) increase the frequency of luteinizing hormone (LH) pulses in cattle and the pattern was characteristic of LH pulsatile release during the follicular phase of the estrous cycle (Roberson et al., 1989; Cupp et al., 1992). Furthermore, serum progesterone from exogenous sources, such as a PRID, or from the corpus luteum influence LH release in a similar manner (Bergfeld et al., 1992). Sirois and Fortune (1990) postulated that the decrease in progesterone associated with treatment with 1 CIDR beginning on d 14 of the estrous cycle may have induced an increase in LH pulse frequency, that resulted in maintenance and ovulation of the dominant follicle present at CIDR insertion. Initiation of PRID treatment on d 17 of the estrous cycle in Experiment 2 did not result in any alteration in the pattern of LH release on d 20 of the interestrous interval relative to the release pattern of LH during the pre-treatment sampling period on d 17 of the interestrous interval. By d 23 of the interestrus interval, when serum progesterone concentrations were evident at sub-luteal phase concentrations, LH pulse frequency increased to values observed during the follicular phase of the bovine estrous cycle (Rahe et al., 1980; Roberson et al., 1989; Cupp et al., 1992). Therefore, it is plausible to

postulate that the dominant follicle present at the time of PRID insertion on d 17 of the estrous cycle, had become static or had entered the regressing stage of follicular development before the initiation in the decline in serum progesterone to sub-luteal phase concentrations. This may have resulted in a follicle unable to respond to an increase in LH pulse frequency which resulted due to the decreasing progesterone to sub-luteal phase concentrations during the later stages of PRID treatment (Roberson et al., 1989; Cupp et al., 1992).

In both studies, serum progesterone concentrations declined below 1 ng/mL at approximately d 19 of the interestrous interval in MGA-treated cows which arrested development of the dominant follicle present at the beginning of MGA treatment. This was an average of 3 d earlier than in MGA-treated cows which failed to arrest development of the dominant follicle present at the beginning of MGA treatment. MGA-treated cows that arrested development of dominant follicles may have been predisposed to having two waves of follicular growth during the estrous cycle. This idea seems plausible because control cows exhibiting two follicular waves during the estrous cycle had progesterone concentrations which dropped below 1 ng/mL on d 18 to 19 of the interestrous interval, at a time comparable to the decline in MGA-treated cows that arrested development of dominant follicles. In addition, in control cows that exhibited three follicular waves during the interestrous interval progesterone concentrations declined below 1 ng/mL around d 21

which was similar to the time of decline in MGA-treated cows that continued follicular turnover throughout the interestrous interval.

The early decline in progesterone to proestrus phase concentrations in MGA-treated cows that arrested follicular development may have resulted in an increased LH pulse frequency at the time of natural luteolysis. This would allow for the necessary gonadotropic support for the continued growth and subsequent ovulation of the dominant follicle present at the beginning of MGA treatment. This idea is supported by the results of Experiment 2 in which LH pulse frequency increased 35% from d 17 to d 20 of the interestrus interval in MGA-treated cows that arrested development of the dominant follicle present at the beginning of MGA treatment. Smith and Day (1990) also reported an increase in LH pulse frequency on d 14 of a 16 d MGA treatment schedule designed to induce estrus in previously pre-pubertal heifers. Therefore, it is possible that in the present study, MGA treatment acted to enhance or failed to inhibit LH pulse frequency in the absence of luteal phase concentrations of progesterone. Furthermore, maintenance of luteal phase concentrations of progesterone through d 21 of the interestrous interval maintained LH pulse frequency at luteal phase concentrations through d 20 of the interestrus interval in MGA-treated cows failing to arrest development of the dominant follicle present at the beginning of MGA treatment. Finally, neither PRID or MGA treatment had any effect on mean, baseline

or amplitude of LH pulses on d 17, 20 or 23 of the interestrus interval, regardless of the number of dominant follicles characterizing the interestrus interval. This was not unexpected, since Roberson et al. (1989) reported an increase in LH pulse frequency in cows given sub-luteal phase concentrations of progesterone without any apparent increase in baseline or mean concentrations of LH.

In both studies, persistence of the dominant ovulatory follicle in MGA-treated cows was accompanied by a precocious increase in estradiol-17ß 7 d before ovulation. Similarly, Beal et al. (1990) and Coleman et al. (1990) reported a premature preovulatory rise in estradiol-17ß when MGA treatment was initiated during the late diestrus phase of the estrous cycle. Furthermore, Sirois and Fortune (1990) observed an increase in plasma estradiol-17ß concentrations in heifers administered 1 CIDR for 14 d beginning on d 14 of the estrous cycle compared to heifers receiving 2 CIDR's during the 14 d treatment period. Cupp et al. (1992) reported similar results when heifers received .5 PRID in the absence of a corpus luteum. Sirois and Fortune (1990) hypothesized that maintenance of higher concentrations of progesterone in heifers receiving two CIDR's was indirectly responsible, as a result of negative feedback on LH release (pulse frequency), for the significantly lower concentrations of estradiol-17ß observed during the CIDR treatment. Results from the current studies support and expand on the hypothesis put forth by Sirois and Fortune (1990).

MGA- and PRID-treated cows which maintained progesterone concentrations at luteal phase concentrations, and continued follicular turnover throughout treatment, did not have an increase in LH pulse frequency on d 4 of treatment and did not exhibit an increase in estradiol-17ß 7 d before ovulation. In addition, the rate with which estradiol-17ß increased relative to follicular diameter during the 9 d before ovulation was more rapid in MGA-treated cows that arrested development of the dominant follicle present at the beginning of MGA treatment compared to PRID and MGA-treated cows which continued follicular turnover during the interestrous interval. This was a direct result of MGA-treated cows arresting development of a follicle destined to become the ovulatory follicle.

The premature increase in estradiol-17ß concentration before ovulation in MGA-treated cows which arrested development of dominant follicles has also been reported after induced delay of ovulation in sodium pentobarbital treated rats (Butcher et al., 1975) and in older rats undergoing spontaneously prolonged estrous cycles (Page and Butcher, 1982). In the rat, numerous physiological alterations have been reported to result from the premature preovulatory increases in estradiol-17ß noted previously. Retarded growth of embryos at d 4 and 11 of gestation, as well as a reduction in implantation rate and an increase in embryonic death have been reported after an induced premature rise in estradiol-17ß (Butcher and Pope, 1979). Furthermore, oocytes exposed to prolonged elevations of

estradiol-17ß after induced delay of ovulation, exhibited a 50% reduction in the number of cortical granules and lacked any indication of cortical granule synthesis, which resulted in an increased incidence of polyspermy (Fugo and Butcher, 1966). The results from these studies support the hypothesis that a premature elevation of preovulatory concentrations of estradiol-17ß before ovulation may be acting directly on the oocyte to alter ultrastructural characteristics or acting indirectly by altering the intrauterine environment, which leads to embryonic death after delayed ovulation (Peluso and Butcher, 1974; Butcher et al., 1979). Since both rats and cattle have been shown to have premature increases in estradiol-17ß when undergoing ovulatory delay, it is possible that alterations in oocyte ultrastructure, as well as intrauterine environment noted in rats may also be present in cattle. Expanding our current, limited understanding of the role of premature increases in estradiol-17ß on subsequent fertility in MGA-treated cows will require research efforts directed at the oocyte and intrauterine environment as described previously for the rat.

In conclusion, the results from this series of studies demonstrate that 7-d of MGA, but not PRID treatment, initiated around the time of natural luteolysis altered the normal course of follicular development, as well as disrupting the hormonal milieu normally associated with the follicular phase of the bovine estrous cycle.

Implications

Seven-day MGA and PRID treatment inhibited estrus and ovulation in all cows. Results of this study indicate that MGA treatment altered the normal course of follicular development by arresting development of a dominant follicle present at the beginning of MGA treatment. Furthermore, LH pulse frequency was increased in MGA-treated cows which maintained dominant follicles throughout the interestrous interval. In addition, MGA treatment resulted in a premature increase in serum concentrations of estradiol-17ß before ovulation. The differential effects of MGA and PRID treatment on follicular development and secretory patterns of estradiol-17ß and LH offers an excellent model which will enable future research efforts to better define the physiological mechanism underlying subnormal fertility associated with progestogen treatment.

Chapter V

Summary

These experiments were conducted to determine the effect of 7-d MGA or PRID treatments, initiated around the time of natural luteolysis (d 17), on follicular development, ovarian steroid production and the pattern of release of LH. Both MGA and PRID treatment extended the interestrus interval. In MGA or PRID treatments, follicular characteristics before d 17 were not associated with subsequent treatment effects. PRID treatment did not alter follicular development, estradiol-17ß concentrations or the release pattern of luteinizing hormone.

Melengestrol acetate treatment arrested development of dominant follicles in 42% of animals treated. Extending the dominance phase of follicular development resulted in a premature increase in estradiol-17ß concentrations, as well as an increase in luteinizing hormone pulse frequency. Luteolysis occurred earlier in MGA-treated cows in which development of dominant follicles was arrested and was comparable to control cows defined as having two follicular waves. Therefore, the after model best describes the course of events associated with MGA extension of follicular dominance. First, cows appear to be predisposed to having two follicular waves during the normal estrous cycle, with the second wave producing the ovulatory follicle. Predisposition to two follicular waves results in

hastening the onset of luteolysis, defined by a reduction in serum progesterone concentrations below 1 ng/mL. The decline in serum progesterone to sub-luteal phase concentrations allows for an increase in luteinizing hormone pulse frequency, which has been shown to be a prerequisite for continued maintenance of a dominant follicle. Increased luteinizing hormone support results in continued production of estradiol-17ß by the arrested dominant follicle. Therefore, arresting follicular development with MGA offers researchers an experimental model with which to more clearly define the underlying mechanism(s) of follicular dominance in cattle.

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