CONTROL OF INTERVALS TO FIRST SERVICE AND ATTEMPTS TO IMPROVE FERTILITY IN DAIRY CATTLE USING PROSTAGLANDIN $F_{2\alpha}$ AND GONADOTROPIN-RELEASING HORMONE

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Matthew Christian Lucy

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Approved by:

Jeffrey A. Stevenson
Major Professor

BIOGRAPHY

Matthew Christian Lucy, son of Russell and Evelyn Lucy, was born June 4, 1960 in Syracuse, New York. He attended elementary and secondary schools at Baldwinsville, New York, graduating from C. W. Baker High School in 1978.

He attended Cornell University from September, 1978 to January, 1982 and received his Bachelor of Science degree in Animal Science from the College of Agriculture and Life Sciences. While a student at Cornell, he met Jacqueline S. Jamieson whom he married in August, 1984.

He enrolled in graduate school at Kansas State University in the Department of Animal Science and Industry in the fall of 1983. He served as a graduate research assistant while fulfilling the requirements for the Master of Science degree that he completed in August, 1985.

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LITERATURE REVIEW

Efficacy of Prostaglandin $F_{2\alpha}$ as a Luteolysin During the Estrous Cycle

Pharriss and Wyngarden (1969) first demonstrated the luteolytic effects of prostaglandin $F_{2\alpha}$ (PGF) in psuedopregnant rats. This discovery initiated studies with PGF and the bovine corpus luteum. The ability of PGF to induce estrus is not consistent throughout the estrous cycle. Rowson et al. (1972) showed that a single injection of PGF given during the first 5 days of the estrous cycle did not alter length of estrous cycles. In later work, Beal et al. (1980) demonstrated that twice daily injections given on days 3 and 4 could cause premature estrus in heifers. Similar injections given on days 2 and 3 or day 4 did not induce estrus, but inhibited subsequent secretion of luteal progesterone.

Prostaglandin $F_{2\alpha}$ is most effective for inducing luteolysis and subsequent estrus from day 6 through day 17 of the estrous cycle (Lauderdale, 1972; Liehr et al., 1972; Louis et al., 1972). Initially, 5 mg intrauterine doses of PGF were used to induce luteolysis (Lauderdale, 1972; Liehr et al., 1972; Louis et al., 1972). Later work indicated that greater doses (25 to 60 mg) given via intramuscular injection were equally effective (Stellflug et al., 1973; Tervit et al., 1973; Roche, 1974). Luteolysis following intrauterine (Louis et al., 1974) and intramuscular (Louis et al., 1973) PGF administration resulted in changes in serum luteinizing hormone (LH), progesterone, and estradiol that are similar to hormonal events following spontaneous regression of the corpus luteum (Chenault et al., 1974; Louis et al., 1973; Louis et al., 1974). Estrus, ovulation, and fertility following a PGF-induced luteolysis are normal (Lauderdale et al., 1974; Louis et al., 1975; Roche, 1974).

Intervals to Estrus after Prostaglandin $F_{2\alpha}$

Effect of Parity

The interval as well as variation in the interval to estrus and subsequent ovulation following PGF have been studied. Heifers, in general, show estrus between 2 and 3 days after PGF (Stellflug et al., 1973; Roche, 1974; Johnson, 1978). This interval is about 3 days for cows (Louis et al., 1972; Louis et al., 1974; Chenault et al., 1974; Hafs et al., 1975; Louis et al., 1975; Welch et al., 1975). Some evidence suggests that interval to estrus can be hastened by larger doses of PGF (Stellflug et al., 1973).

Effect of Stage of Estrous Cycle at Treatment

Interval to estrus is most affected by stage of the estrous cycle when PGF is administered. A luteolytic dose given early (days 5 to 9) in the cycle resulted in a shorter interval to estrus than did late (days 10 to 16) luteal phase administration in heifers and cows in one study (King et al., 1982). Others have examined more specific periods during the luteal phase. Macmillan (1983) found that cows injected on days 7 to 9 and days 14 to 17 show estrus sooner and with less variation than animals injected on days 10 to 13. A similar response has been seen in heifers (Jackson et al., 1979; Lauderdale, 1972). Conversely, Stevenson et al. (1984) showed shorter intervals to estrus in heifers given PGF during early luteal phase (days 5 to 8) compared with heifers treated in the late luteal phase (days 14 to 16). It is currently believed that effects of stage of cycle are the result of substantial waves of ovarian follicles that develop both early and late in the cycle and enable cattle treated at those times to reach estrus sooner after PGF (Macmillan and Henderson, 1984).

Estrous Synchronization Schemes

Administration of Prostaglandin $F_{2\alpha}$ Twice at 10 to 12 day Intervals

The luteolytic properties of PGF have led to development of various methods to synchronize estrus in a group of animals. One effective method is to inject PGF twice at a 10 to 12 day interval. This method allows for the

synchronization of estrus for all animals after the second injection regardless of stage of cycle when treatment was begun. At the first injection, cattle in the follicular phase of the cycle are unaffected, while those in the luteal phase undergo a PGF-induced luteal regression. Then, at the second injection, all cattle are in the luteal phase. Using this approach, Cooper (1974) was able to synchronize estrus in 171 out of 175 heifers in a 48-h period. In a group of cattle at random stages of the estrous cycle, as many as two-thirds of the treated animals may have luteolysis and subsequent estrus in response to PGF administration. Part of the success of the two injection regimen is due to the fact that those animals having luteolysis at the first injection are early in their estrous cycle at the time of the second injection and tighter synchrony of estrus results (Johnson, 1978). Studies of the two injection scheme for lactating dairy cows have been less convincing. Macmillan et al. (1977) found only 33 to 65% of cows showing estrus from 48 to 96 h after the second injection. In general, however, two injections give good synchrony of estrus in both heifers (Hafs et al., 1975; Leaver et al., 1976) and cows (Hafs et al., 1975; Christie et al., 1976; Hafs et al., 1978).

Another method is to inject PGF, inseminate at observed estrus, and then reinject all animals not yet inseminated 11 days later (Doornbos and Anderson, 1979; Roche and Prendville, 1979). Using this method, Roche and Prendville (1979) inseminated 138 out of 236 (58%) treated cows after the first PGF injection, while inseminating the remaining 98 cows after the second injection. This system conserves PGF, but does not synchronize the group at a single time period and is dependent on heat detection after the first injection.

Administration of a Single Dose of Prostaglandin $F_{2\alpha}$

Two other methods using a single injection of PGF also have been developed. The first involves a 5 to 7 day period of estrous detection before

PGF administration, during which all animals showing estrus are inseminated (Doornbos and Anderson, 1979; Han and Moody, 1979; Roche and Prendville, 1979). This ensures a majority of animals receiving PGF will be in the luteal phase. A second single PGF injection method involves ovarian palpation per rectum to determine if the cow has a functional corpus luteum (Lauderdale et al., 1974; Elmarimi et al., 1983; Plunkett et al., 1984). Only animals diagnosed to have a corpus luteum are treated with PGF. Both of these methods, although saving on hormone cost, suffer from the uncertainties associated with either heat detection, or rectal palpation for ovarian structures, respectively (Dawson, 1975). In addition, estrus following PGF is less synchronous because cattle are distributed randomly throughout the luteal phase at the time of PGF administration.

Methods of Insemination Following Prostaglandin $F_{2\alpha}$

Insemination at Estrus

Use of PGF allows for the artificial insemination (AI) of cattle at either observed estrus, or at a specific time interval following PGF. In general, insemination at estrus results in a higher conception rate than insemination by appointment because fixed time inseminations do not coincide always with naturally occurring estrus after PGF (Donaldson, 1977; Han and Moody, 1979; Stevenson et al., 1984). However, if animals are inseminated at estrus, conception rates (based on all animals treated) are sometimes lower because not all animals are observed in estrus following PGF injection (Seguin et al., 1978). Donaldson (1977) found that between 4 and 14% of treated cows were not detected in estrus following PGF. Therefore, success rate for AI at estrus is highly dependent on accuracy and frequency of heat detection after PGF.

Insemination by Appointment

In order to avoid problems associated with estrous detection after PGF,

timed AI regimens were developed. Insemination at a fixed time ensures that all PGF-treated animals, whether seen in estrus or not, will be inseminated. As would be expected, timing of the insemination is critical. Heifers are in estrus sooner and with greater synchrony than cows following PGF (Stellflug et al., 1973; Roche, 1974; Johnson, 1978; King et al., 1982). Acceptable conception for heifers was achieved with a single insemination at 72 to 80 h following the second of two PGF injections given at an 11-day interval (Hafs et al., 1978; Leaver et al., 1976; Jaster et al., 1982). Other intervals to timed AI following PGF (48 and 60 h) were shown to be less than optimal (Roche, 1977). Insemination of heifers on days 3 and 4 after PGF was also highly successful (Hafs et al., 1975; Roche, 1977; Roche and Prendville, 1979), but considering the success of a single timed AI, double insemination probably is not necessary (Hafs et al., 1978).

Conception rates for cows after one timed insemination following PGF have not been consistent across studies. While some investigators have demonstrated fertility equal to or greater than control animals inseminated at estrus (Chupin et al., 1977; Hafs et al., 1978; Young and Henderson, 1981), others have found one fixed time insemination after PGF to be inadequate (Han and Moody, 1979; Donaldson, 1977; Macmillan et al., 1980). The problem with a single insemination of cows after PGF is probably attributable to the variability in the interval to estrus. Although the average interval from PGF to estrus is about 72 h for cows, the variation in that interval may be quite large (Macmillan, 1983).

Type of synchronization regimen employed may affect the success of a timed AI. One PGF injection for estrous synchronization can result in lower conception after one timed AI due to more variable intervals to estrus resulting from treatments at all stages of the estrous cycle (Han and Moody, 1979;

Macmillan et al., 1980; Stevenson et al., 1984). Double injection methods reduce this problem because most cattle are grouped in their early to mid-luteal phase and their earlier and more synchronous estrus results in better synchrony between fixed timed AI and estrus (Hafs et al., 1978; Young and Henderson, 1981).

Due to the variable results in fertility after single timed inseminations, timed AI at 72 and 96 h after PGF maybe more reliable for cows. Results reported for double inseminations have been more consistent, and usually show conception equal to controls inseminated at estrus (Lauderdale, 1974; Christie et al., 1976; Hafs et al., 1978; Young and Henderson, 1981). Conception rates following double inseminations are less affected by stage of cycle because the variation in interval to estrus is less critical compared with a single timed AI. Both Lauderdale et al. (1974) and Plunkett et al. (1984) demonstrated that two inseminations following a single injection of PGF could yield conception comparable with controls inseminated at estrus. Some investigators are critical of the double insemination method, because it results in increased semen usage (Hafs et al., 1978) and does not always result in conception equal to one AI at an observed estrus (Macmillan, 1983).

Reports of timed AI experiments may be misleading due to the manner in which the results are calculated. Conception rates for an insemination by appointment following PGF may be based on all animals treated (Chupin et al., 1977), or only on those animals seen in estrus after PGF (Hafs et al., 1975; Macmillan, 1983). Therefore, the basis for conception rate calculations has a great effect on the apparent success of treatments.

Gonadotropin-Releasing Hormone and Insemination by Appointment

In an attempt to better synchronize ovulations of cattle following PGF administration, gonadotropin-releasing hormone (GnRH) has been used to induce

a preovulatory LH surge. When administered either 52 h (Cummings et al., 1977) or 64 h (Fernandez-lima, 1977) after a luteolytic dose of PGF, GnRH appeared to synchronize preovulatory LH surges and subsequent ovulation. In the first study, LH surges of 16 cows were synchronized to within 8 h when GnRH was given 52 h after PGF. Time of LH surges of control cows not given GnRH was less uniform with 93% occurring between 72 and 108 h. Graves et al. (1974) found that cows given GnRH at 60 h after PGF and inseminated 12 h later had conception equal to PGF-synchronized cows bred at estrus. Similar results were reported by Hansel and Fortune (1978) for heifers given GnRH at 60 h and bred at either 72 h or 72 and 96 h after PGF. Other research found lower fertility in PGF-synchronized cattle given GnRH and inseminated at a set time (Kinkie, 1976; Rodreguez, 1975; Roche, 1977; Burfening, 1978).

Problems of poor conception after PGF when GnRH and timed AI are both utilized may be related to the timing of GnRH. Administrating GnRH 48 h after PGF resulted in lower conception rates at a fixed time AI (72 h) compared with similarly treated controls not given GnRH in two studies (Rodreguez, 1975; Roche, 1977). More variable results in terms of conception were reported when GnRH was given at 60 h after PGF. Both Graves et al. (1974) working with cows, and Hansel and Fortune (1978) working with heifers, found that inseminations at 72 h after PGF (plus GnRH at 60 h) resulted in higher conception than PGF-treated animals inseminated at the same time without GnRH treatment. However, other studies employing similar regimens have produced opposite results (Kinkie, 1976; Burfening, 1978).

Timing of GnRH may affect signs of overt estrus after PGF in cattle. Rodriguez et al. (1975) found that GnRH given at 48 h after PGF decreased the percentage of cows seen in heat from 71% (controls) to 41% (GnRH-treated).

Insemination at Estrus or by Appointment

Due to the difficulties associated with both AI at estrus and a timed AI following PGF, methods of insemination combining the two methods have been investigated. Macmillan et al. (1977) found that one insemination at 72 h following PGF and the reinsemination of cows in estrus after 72 h resulted in acceptable conception based on all cows treated. This regimen allows for insemination of treated cattle with luteolysis without overt estrus, and reinsemination of those animals coming into heat too late to be fertile to the 72 h or 80 h timed AI. This technique is appealing since it results in optimal conception for PGF-treated cattle without the need for two inseminations of all animals.

Exogenous Hormonal Treatments and Fertility at Estrus

Progesterone

Concentration of progesterone (P) secreted by the corpus luteum rises in blood plasma or serum around day 5 of the estrous cycle and pregnant dairy cows have higher milk P than open cows as early as 1 wk after insemination (Lee and Ax, 1984). Thus, circulating P has been studied as one factor affecting fertility in cattle (Folman et al., 1973; Erb et al., 1976; Maurer and Echternkamp, 1982). Johnson et al. (1958) demonstrated that exogenous P administered on days 2, 3, 4, 6, and 9 after insemination increased fertility in dairy cattle. In other work, Kunkel et al. (1977) demonstrated higher embryonic survival of embryos transferred into recipient cows receiving repositol P at the time of surgery.

Human Chorionic Gonadotropin

The possibility that fertility can be enhanced by high progesterone levels during the luteal phase has led researchers to enhance corpus luteum (CL) function by exogenous luteotrophic treatments. Human chorionic gonadotrophin (hCG), due to its LH-like activity, augments CL function by increasing P and

subsequent fertility. However, results from these trials have been equivocal. Babler and Hoffman (1975) demonstrated an increase in conception of 15% points for dairy cattle given hCG at the time of AI. Conversely, Hansel et al. (1976) showed no difference for first-service conception in lactating dairy and beef cows given hCG at time of insemination when compared with controls. Although multiple doses of hCG given early in the estrous cycle can increase serum P concentration in heifers (Helmer and Britt, 1983), its exact effect on fertility has yet to be established.

Gonadotropin-Releasing Hormone

Gonadotropin-releasing hormone causes the release of LH and follicle-stimulating hormone (FSH) from the pituitary. A GnRH-induced LH release varies in magnitude and duration depending on the day of the estrous cycle when GnRH is administered (Kaltenbach, 1974). The greatest response of LH after GnRH occurs at and around the time of estrus prior to the spontaneous preovulatory LH surge (Coulson et al., 1980; Kaltenbach, 1974). Coulson et al. (1980) reported induced LH surges in four of five cows given GnRH at 66 h after PGF. These GnRH-induced LH surges were greater in magnitude, but shorter in duration than spontaneous preovulatory LH surges. The fifth cow in that study had a preovulatory LH surge prior to GnRH administration. In that case, additional GnRH-induced LH release was smaller in magnitude and shorter in duration than the GnRH-induced LH release of cows not having an earlier LH surge. Similarly, Kaltenbach et al. (1974) observed little or no increase in serum LH when GnRH was given immediately following a spontaneous LH surge.

The possibility that GnRH given around the time of estrus reduces pituitary LH secretion exists. Beck et al. (1983), using early postpartum (6 wk) dairy cows, investigated LH release associated with GnRH or saline administration at the onset of estrus and at insemination in a 2 x 2 factorial

experiment. They found the greatest amount of LH release in the group receiving GnRH at estrus and again at insemination. When looking at the magnitude of the second GnRH-induced LH peak, however, they found a decreased response in the GnRH-GnRH group compared to the saline-GnRH group. Perhaps GnRH given at the onset of estrus may hinder subsequent pituitary output of LH due to depletion of pituitary LH stores and(or) down regulation of pituitary GnRH receptors.

Efforts to determine the luteotrophic effects of GnRH have centered around measurement of serum P concentration during the estrous cycle following treatment but results have not been consistent. In one study, Lee and Ax (1984) found that GnRH given at AI to dairy cows that became pregnant resulted in higher milk P than those animals not receiving GnRH and becoming pregnant. Lee and Ax's (1984) data is not supported by data of Stevenson et al. (1985) since they found no difference in serum P concentration between GnRH and saline-treated cows that conceived, while GnRH-treated animals that failed to conceive had lower serum P than saline-treated cows that failed to conceive. Other research where GnRH was administered 5, 4, and 3 days prior to estrus (Helmer and Britt, 1983) demonstrated no effect on luteal function during the subsequent estrous cycle.

Although the main effect of GnRH on fertility in cattle is believed to be via pituitary LH release, the possibility of a direct effect of GnRH on the bovine ovary exists. Rippel and Johnson (1976) observed a decrease in ovarian weight for hCG-primed hypophysectomized rats given GnRH. Gonadotropin-releasing hormone also has been found to be inhibitory to granulosa and luteal cell function in the rat (Jones et al.,1980; Jones et al., 1981), human (Casper et al., 1979), and Rhesus monkey (Asch et al., 1981). Sheehan et al. (1982) found that administration of GnRH on days 1 to 3 of the

menstrual cycle caused luteal phase defects in women. Brown and Reeves (1984) assayed follicular and luteal tissue cells from cows, ewes, sows, and rats for GnRH receptors. They found no GnRH receptors in ovarian tissue of farm animals, while high affinity receptors were present in rat ovarian tissues. Therefore, it seems that unlike rat ovaries, farm animal ovaries may not be affected directly by GnRH because specific high affinity receptors for the hormone are not present in ovarian tissue.

In cattle, GnRH administration at the time of insemination has an effect on fertility which may not be consistent for all inseminations during the postpartum period. Whether or not GnRH administration at AI improves fertility at first postpartum service is not clear. Some investigators show a distinct advantage in conception when administering GnRH at first service (Schels and Mostafwi, 1978; Nakao et al., 1983). On the other hand, Stevenson et al. (1984) showed similar fertility in animals receiving GnRH or saline at the time of first service. In the same study, GnRH resulted in a 10% point increase in conception in repeat-breeder cows. This increased conception rate at repeat services with GnRH administration is consistent with other findings (Maurice et al., 1982; Lee et al., 1983). The reason for the improved conception for repeat-breeders is not clear, but may be related to the effect GnRH has on the time of ovulation, or subsequent luteal function (Stevenson et al., 1984).

Calving Intervals and Measures of Reproduction for Dairy Cows

Economics

Calving interval is an economically important measure of reproductive performance in dairy herds. Olds et al. (1979) found that for each day open between 40 and 140 days postpartum, 4.5 kg of annual milk for primiparous cows and 8.6 kg of annual milk for older cows were lost. These losses translated into $.71 and $1.18 less income over feed cost per day open for primiparous and

multiparous cows, respectively. This loss occurs because as days open increases, cows spend a greater proportion of their lactation in the less profitable part of their lactation curve (Call, 1978). Awareness of prolonged calving intervals is low among dairy farmers and 70% of the losses associated with elongated calving intervals were due to decreased potential income and not out-of-pocket expenses (Call, 1978). Thus, economic loss resulting from inefficient reproductive performance is subtle and not easily detected by the dairy producer.

Estrous Detection

Several factors affect calving interval in dairy herds. Among these, heat detection and interval to first service are most important (Pelisser, 1972). Barr (1975) investigated the influence of estrous detection on days open and found the correlation between conception failure and days open was .38, while the correlation between missed heats and days open was .92. These data suggest that failures in heat detection have greater effects on extending days open than failures in conception. In related work, Pelisser (1972) compared two herds with poor and good methods of heat detection and found a difference of 47% points in the number of cows detected in heat by 60 days postpartum. Thus, proportion of cows thought to be anestrus or "silent" ovulators is probably a function of heat detection. Zemjanis (1969) estimated that 90% of the anestrous cows examined were cycling, but heats went undetected due to poor heat detection.

Interval to First Service

Interval to first service and calving interval are interrelated (Bozworth et al., 1972; Britt, 1975; Pelisser, 1972; Slama et al., 1976). Slama et al. (1976) found that for Holstein cows, each day increase in interval to first service resulted in a .53 day increase in calving interval. Bozworth et al. (1972) compared herds with short and long calving intervals and found no difference in

conception or services per conception. However, the interval to first service in herds with short calving intervals was 28 days less than for herds with long calving intervals. Interval to first service also is related to income over feed costs and rolling herd average (RHA). Call and Stevenson (1985) found that as RHA and income over feed costs increased, calving interval as well as interval to first service decreased. These data illustrate the apparent profitable nature of shorter intervals to first service.

It is possible to manipulate interval to first service by breeding cows earlier postpartum (Britt, 1975). In general, the average interval to first service will be 3 wk longer than when the minimum postpartum insemination interval is decided by herd management (i.e., 40, 50, or 60 days). Therefore, inasmuch as most dairy producers do not first inseminate cows until 60 days postpartum, intervals to first service average around 80 days (Call, 1978). Breeding earlier postpartum can reduce interval to first service (Britt, 1975; Stevenson and Call, 1983). Stevenson and Call (1983) found that inseminating cows at the first detected estrus after 5 wk postpartum resulted in a 62-day interval to first service. Interval to first service could be controlled by PGF. This may ensure shorter interval to first service, however, at this time, this theory has not been tested.

Use of Prostaglandin $F_{2\alpha}$ for Cows with Unobserved Estrus

Prostaglandin $F_{2\alpha}$ can be used to reduce days to insemination for cows with unobserved estrus. Plunkett et al. (1984) showed a decreased interval from treatment to estrus, to first service, and to conception in lactating dairy cows with a palpable CL but unobserved estrus when treated with PGF. Similar results have been obtained in other studies (Seguin et al., 1978; Eddy, 1977; Elmarimi et al., 1983). Success of these regimens is dependent on the accuracy of rectal palpation for ovarian structures and heat detection. Rectal palpation

to determine a functional CL was found to be highly accurate (92 to 96%) in two studies (Seguin et al., 1978; Plunkett et al., 1984). Problems associated with heat detection after PGF administration can be eliminated by timed AI. Seguin et al. (1978) found that inseminating dairy cows by appointment at 72 and 96 h following PGF resulted in 59% of all treated cows pregnant within 5 days. The comparable figure for the control group inseminated at estrus following PGF was 24%. Plunkett et al. (1984) showed no difference in conception rates of treated cows inseminated at estrus or at 72 and 96 h after PGF. However, the importance of timed insemination for cows not observed in estrus was recognized by the fact that 33 cows (18% of the total) that were time inseminated became pregnant. These cows would not have been inseminated in the absence of estrus without the timed AI regimen.

Summary

Inefficient management of reproduction is not an option for the dairy producer if he or she plans to remain a viable part of the industry in the future. Efficient reproduction depends on the insemination of a majority of cows within 60 days postpartum so that a 12 to 13 mo calving interval can be achieved. In this regard, PGF may be a helpful tool for the dairy producer. If first services of all cows could be manipulated by PGF followed by inseminations by appointment, then no cow should have an interval to first service longer than desired. At present, the utility of PGF treatment in cows is not clear because some studies demonstrate reduced fertility at a timed AI due to the asynchrony of the estrous response and(or) lack of estrus after PGF. Acceptable fertility should be obtainable if the correct interval between PGF treatment and AI is used. Gonadotropin-releasing hormone also may be helpful because theoretically, it could time ovulations with inseminations. In addition, GnRH may have other fertility-promoting effects which at this time are not

known. Finally, the problem of no expression or detection of estrus after PGF needs to be addressed. The possibility that lack of estrous expression represents a source of failure for PGF treatment should be considered. An investigation into these aforementioned traits of PGF and GnRH may prove useful to the industry and aid the dairy producer to be more effective in reproductive management.

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Explanation for Luteolytic Failures after Prostaglandin $F_{2\alpha}$ in Early Postpartum Dairy Cows

ABSTRACT

Lactating Holstein cows (n=225) were used to study the effectiveness of two injections of prostaglandin $F_{2\alpha}$ at 11-day intervals to synchronize estrus for a timed insemination at first service from 54 to 60 days postpartum. Cows were assigned randomly at calving to one of four experimental groups. Control cows (n=59) were inseminated at spontaneous estrus beginning 6 wk postpartum. Remaining cows were given two 25-mg injections of prostaglandin $F_{2\alpha}$ (Lutalyse®) at 11-day intervals beginning 40 to 46 days postpartum. After the second of two injections of prostaglandin $F_{2\alpha}$ (treatment; 0 h) cows were either: 1) inseminated artificially at 80 h (n=55), 2) given gonadotropin-releasing hormone (100 µg Cystorelin®) at 72 h and inseminated artificially at 80 h (n=57), or 3) inseminated artificially at 72 and 96 h (n=54). Serum progesterone was measured in blood serum collected 0 and 48 h after each injection of prostaglandin $F_{2\alpha}$ and in 48 cows throughout the post-treatment luteal phase. Various progesterone profiles after luteolytic treatments were luteolysis (serum progesterone > 1 ng/ml at 0 h and < 1 ng/ml at 48 h), no luteolysis (serum progesterone > 1 ng/ml at 0 h and > 1 ng/ml at 48 h) and low progesterone (serum progesterone < 1 ng/ml at 0 h). Successful luteolysis at the second prostaglandin $F_{2\alpha}$ injection occurred in 119 of 166 (72%) treated cows. Failures of the two-injection regimen included no luteolysis and low progesterone preceding the second of two injections of prostaglandin $F_{2\alpha}$. No luteolysis occurred in 23 (14%) treated cows. Low progesterone occurred in 24 (14%) treated cows and was caused by no luteolysis after the first injection, lack of adequate serum progesterone by 11 days after luteolysis, and anestrus. Conception at first service was lower in treated cows (34.5%) than controls

(54.2%). Treated cows with luteolysis had higher conception (34.5%) than cows with treatment failures (2.1%). For treated cows not receiving gonadotropin-releasing hormone, conception tended to be greater in those cows having luteolysis after the first injection, while cows given gonadotropin-releasing hormone had similar fertility regardless of progesterone profile after the first injection. Successful use of prostaglandin $F_{2\alpha}$ to synchronize estrus in early postpartum dairy cows may be hindered by lack of its effectiveness, prolonged low progesterone after luteolysis, and anestrus. Employment of a two-injection method of estrous synchronization using prostaglandin $F_{2\alpha}$ may not be a practical method for handling of timed first inseminations early postpartum.

INTRODUCTION

Discovery and use of prostaglandin $F_{2\alpha}$ (PGF) as an estrous synchronization hormone in cattle has impacted the handling of heifers and cows ready for insemination (26). Unique synchronization regimens using PGF were developed so that inseminations could be accomplished without estrous detection (6, 11, 25). Administration of two injections of PGF at 10 to 12-day intervals is one method that was studied (6, 10, 13, 17). The first injection (PGF-1) regresses functional corpora lutea while otherwise having no effect. Two injections ensure that all cattle will have a corpus luteum (CL) susceptible to luteolysis at the time of the second PGF injection (PGF-2). Due to the nature of this system, any cow treated could conceive after a timed artificial insemination (timed AI) 2 to 3 days after PGF-2 (10, 12, 15). Furthermore, since all animals with luteal regression at PGF-1 (approximately 66%) will be early in the luteal phase at PGF-2, an earlier and less variable interval to estrus can be expected in response to PGF-2 (14, 30). Thus, the effectiveness of a double injection system is enhanced due to benefits of the stage of estrous cycle

realized by two-thirds of the treated cattle (13).

Success of a timed AI depends on the luteolytic response (% of treated cows induced into estrus), and the time and synchrony of estrus and ovulation in those cows responding to treatment (19). In this regard, current evidence suggests that large differences exist between cows and heifers. Heifers, in general, show a high response rate and precise estrus following PGF-2 (13, 14, 23, 27). In addition, single and double timed AI after PGF-2 have demonstrated conception rates consistently equal to untreated heifers bred at a detected estrus (10, 12, 24, 25). Data for cows, on the other hand, have produced equivocal results. Some investigations have shown promise for using the two PGF injection method, while other research has shown discouraging results in terms of response rate and conception rates after a timed AI (3, 4, 7, 9, 10, 11, 18, 19, 31).

Recognizing the inconsistencies in the success rate for the use of PGF in lactating cows, our objectives were to: 1) monitor serum progesterone in postpartum dairy cattle treated twice with PGF and time inseminated; 2) elucidate reasons for failure of the two injection system; and 3) determine the relationship of progesterone responses after PGF-1 and PGF-2 and conception rate at timed AI.

MATERIALS AND METHODS

Experimental Design

Lactating dairy cows (n=225) from the Kansas State University herd that calved between July 1, 1983 and February 1, 1985 were housed in an open air confinement system consisting of concrete lots with sheltered freestalls. At calving, cows were assigned randomly to one of four treatment groups. Cows in the control group (n=59) were inseminated at the first detected estrus after 6 wk postpartum. The remaining cows (PGF cows; n=166) received two injections

(i.m.) of PGF at an 11-day interval (25 mg dinoprost tromethamine; Lutalyse®) between 40 and 46 days postpartum. Following the second injection (PGF-2), cows were inseminated artificially by appointment (timed AI) according to one of the following schedules: 1) timed AI at 80 h (n=55); 2) gonadotropin-releasing hormone (GnRH; 100 µg Cystorelin®) at 72 h and timed AI at 80 h (n=57); 3) timed AI at 72 and 96 h (n=54). Only first services were synchronized as described while repeat services were performed at spontaneous estrus. Inseminations were by different inseminators during the first and second yr of the study. Pregnancy was determined by uterine palpation per rectum 40 to 60 d after AI.

Blood Collection

Blood was collected by coccygeal venipuncture at 0 and 48 h after PGF-1 and PGF-2. Additional blood was collected daily from 48 cows on days 0 to 10, and on alternate days from days 12 to 24 after PGF-2. Blood was stored at 5°C for 24 h until serum was harvested by centrifugation and held at -20°C until assayed for progesterone (P) using a validated radioimmunoassay (30).

Definitions

Luteolytic responses to PGF were defined as follows: 1) luteolysis (+) was indicated when serum P was greater than or equal to 1 ng/ml at the time of any PGF injection (0 h) and less than 1 ng/ml 48 h later; 2) no luteolysis (-) occurred when serum P was greater than or equal to 1 ng/ml at 0 h and greater than or equal to 1 ng/ml at 48 h, 3) low P (LP) was indicated when serum P was less than 1 ng/ml at 0 h. Possible responses were denoted by two symbols with the first symbol indicating the response to PGF-1 and the second representing the response to PGF-2. Nine possible results from treatment were: 1) luteolysis at both PGF-1 and PGF-2 (+,+); 2) low P at PGF-1 and luteolysis at PGF-2 (LP,+); 3) no luteolysis at PGF-1 and luteolysis at PGF-2 (-,+); 4) luteolysis at

PGF-1 and no luteolysis at PGF-2 (+,-); 5) low P at PGF-1 and no luteolysis at PGF-2 (LP,-); 6) no luteolysis at PGF-1 and PGF-2 (-,-); 7) luteolysis at PGF-1 and low P at PGF-2 (+,LP); 8) low P at both PGF-1 and PGF-2 (LP,LP); 9) no luteolysis at PGF-1 and low P at PGF-2 (-,LP). A successfully induced estrus (synchronized) was considered to have occurred in cows with luteolysis at PGF-2 (+ +, LP +, or - +). Remaining responses were considered to have yielded a PGF failure (nonsynchronized).

Statistical Analyses

Conception rate (CR) at first service was analyzed to determine effects of inseminator and treatment using procedure GLM from the Statistical Analysis System (1). Since no effect of inseminator was detected, conception data from the 2 yr were combined. Conception at first service and other enumeration data were analyzed by contingency Chi-square.

RESULTS

Luteolysis After PGF-1

Based on profiles of serum P, luteolysis occurred in 98 of 166 (59%) treated cows after PGF-1, and no luteolysis occurred in 18 cows (10.8%) when serum P was greater than 1 ng/ml at treatment. However, for cows with a functional CL (serum P > 1 ng/ml), 98 of 116 (84.5%) underwent luteolysis. Low P was detected in the remaining 50 cows at the time of PGF treatment (30.1%).

Luteolysis After PGF-2

The three progesterone responses for cows that had luteolysis after PGF-2 were + +, LP +, and - +. Serum progesterone profiles were similar for the three responses as illustrated in Figure 1. In these cases, luteolysis occurred after PGF-2 with a normal estrus and subsequent luteal phase. Frequency of progesterone responses for cows with luteolysis after PGF-2 is given in Table 1. Of the 166 treated cows, 119 (71.7%) had luteolysis after PGF-2. The + +, LP +,

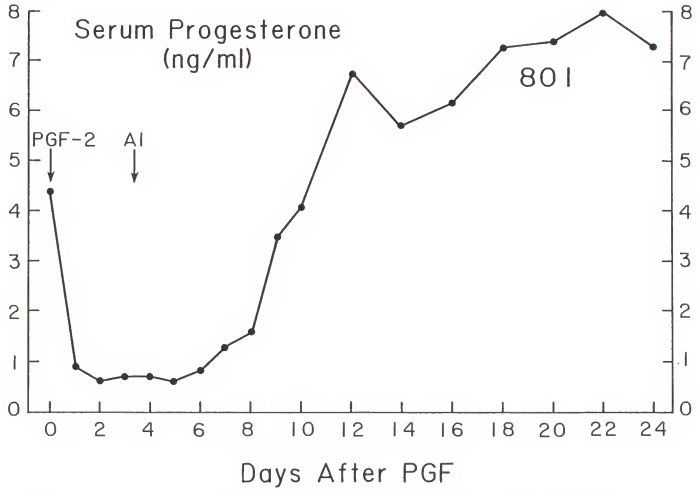

Figure 1. Serum progesterone after the second of two injections of prostaglandin $F_{2\alpha}$ in cow 801 having luteolysis resulting in estrus, and conception after timed AI (at 80 h).

TABLE 1. Frequency of serum progesterone (P) responses for cows with luteolysis after prostaglandin $F_{2\alpha}$ (PGF) and subsequent conception rate (CR) at first service[a].

Response[b]	Control	AI at 80 h	GnRH+AI at 80 h	AI at 72 and 96 h	Total CR	%CR	% Total[c]
+ +		8/27	9/22	13/25	30/74	40.5	62.2
LP +		1/13	5/14	1/11	7/38	18.4[d]	31.9
- +		0/0	3/5	1/2	4/7	57.1	5.9
Total	32/59	9/40	17/41	14/38	41/119		
%	54.2	22.5[d]	41.5	36.8	34.5[d]		

[a]The numerator denotes the number of first service conceptions. The denominator indicates the frequency of cows within each response and treatment.

[b]Symbols represent serum P response to the first (PGF-1) and second (PGF-2) prostaglandin $F_{2\alpha}$ injections, respectively. Luteolysis (+): serum P > 1 ng/ml at treatment and P < 1 ng/ml 48 h later. No luteolysis (-): serum P > 1 ng/ml at treatment and P > 1 ng/ml 48 h later. Low P (LP): serum P < 1 ng/ml at treatment.

[c]Percent of all cows with luteolysis.

[d]Different from control ($P<.05$).

and - + responses represented 44.6%, 22.9%, and 4.2% of all treated cows, and 62.2%, 31.9%, and 5.9% of cows having luteolysis at PGF-2, respectively.

No Luteolysis After PGF-2

No luteolysis after PGF-2 (+ -, LP -, - -) occurred in 23 cows. Serum progesterone after PGF-2 in cow K-49 (figure 2) illustrates that PGF apparently suppressed serum P in cows having no luteolysis, but estrus did not coinside with the timed AI. Frequency of progesterone responses for cows with no luteolysis after PGF-2 is given in Table 2. No luteolysis at PGF-2 accounted for 48.9% (23 or 47) of the PGF failures that led to a nonsynchronized estrus. The three nonluteolytic responses (+ -, LP -, and - -) represented 9.6%, 2.4%, and 1.8% of all treated cows and 34.0%, 8.5%, and 6.4% of cows with PGF failure, respectively.

Low Progesterone at PGF-2

Low progesterone at PGF-2 (+ LP, LP LP , - LP) was detected in 24 cows (51.1% of PGF failures) and the frequency of these responses is given in Table 2. A delayed rise in serum progesterone following luteolysis at PGF-1 caused the + LP response in 8 cows as is illustrated for cow 494 in Figure 3. This situation occurred in 4.8% and 17.0% of all treated cows and cows with PGF failures, respectively. Serum progesterone profiles after PGF that could account for the + LP response are illustrated in Figure 4 for cow 596 (delayed rise in serum progesterone after PGF-1) and Figure 5 for cow 804 (anestrus after PGF-1). In both cases, serum P concentrations were low at the time of the second PGF injection and accounted for the PGF failure.

No luteolysis at PGF-1 caused low P at PGF-2 in 8 cows as is illustrated for cow 685 in Figure 6 (- LP) and accounted for 17% of all cows with PGF failures, and 4.8% of all treated cows. Anestrus (LP LP) was evident in the remaining 8 cows and represented 4.8% of the treated cows.

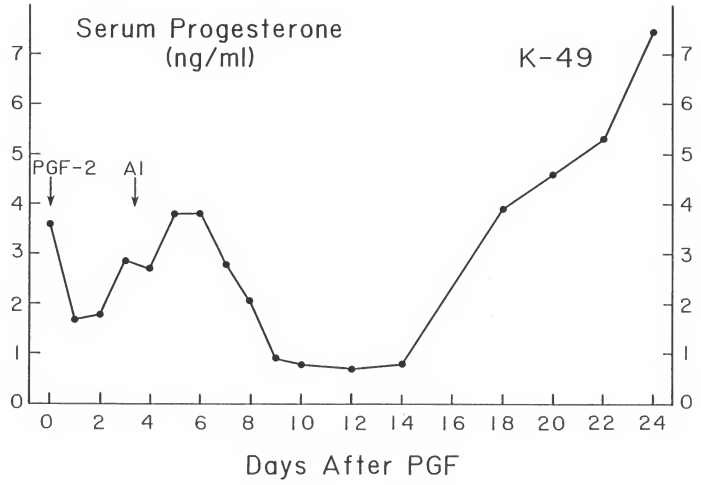

Figure 2. Serum progesterone after the second of two injections of prostaglandin $F_{2\alpha}$ in cow K-49 failing to undergo luteolysis and resulting in estrus 12 days after treatment.

TABLE 2. Frequency of serum progesterone (P) responses for cows with PGF failures after prostaglandin $F_{2\alpha}$ (PGF) and subsequent conception rate (CR) at first service[a].

Response[b]	Control	AI at 80 h	GnRH+AI at 80 h	AI at 72 and 96 h	Total CR	%CR	% Total[c]
+ -		0/6	0/6	0/4	0/16	0	34.0
LP -		0/1	0/2	0/1	0/4	0	8.5
- -		0/1	0/2	0/0	0/3	0	6.4
+ LP		0/3	0/1	0/3	0/8	0	17.0
LP LP		0/1	0/3	0/4	0/8	0	17.0
- LP		0/3	0/2	1/3	1/8	12.5	17.0
Total	32/59	0/15	0/16	1/15	1/47		
%	54.2	0	0	6.7	2.1		

[a]The numerator denotes the number of first service conceptions. The denominator indicates the frequency of cows within each response and treatment.

[b]Symbols represent serum P response to the first (PGF-1) and second (PGF-2) prostaglandin $F_{2\alpha}$ injections, respectively. Luteolysis (+): serum P > 1 ng/ml at treatment and P < 1 ng/ml 48 h later. No luteolysis (-): serum P > 1 ng/ml at treatment and P > 1 ng/ml 48 h later. Low P (LP): serum P < 1 ng/ml at treatment.

[c]Percent of all cows with PGF failures.

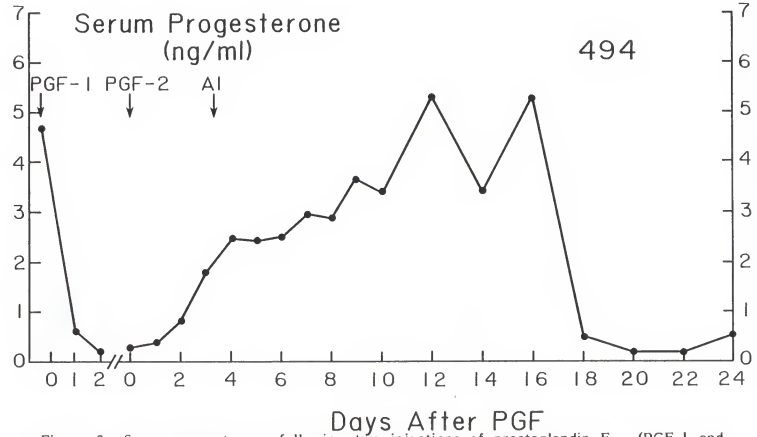

Figure 3. Serum progesterone following two injections of prostaglandin $F_{2\alpha}$ (PGF-1 and PGF-2) given at an 11-d interval. Cow 494 had luteolysis at PGF-1 and low progesterone at PGF-2 (+ LP).

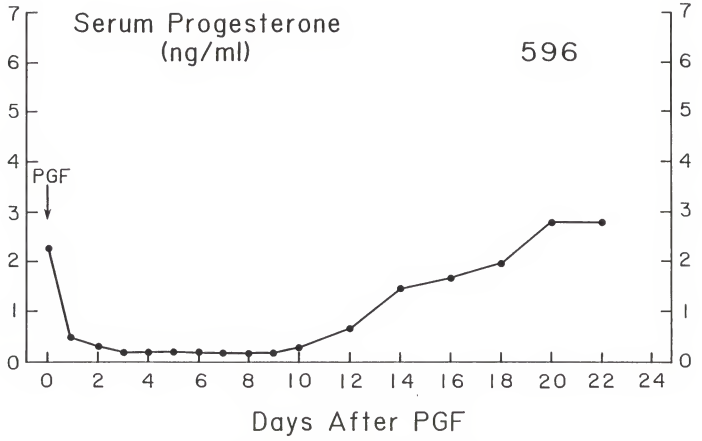

Figure 4. Serum progesterone after prostaglandin $F_{2\alpha}$ illustrating prolonged low progesterone following treatment in cow 596.

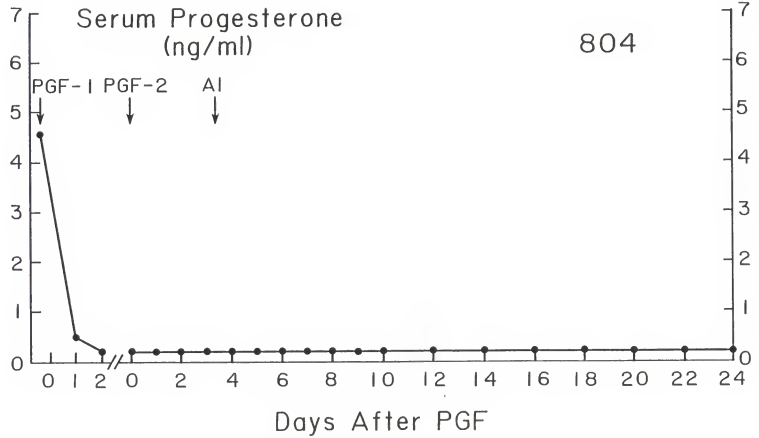

Figure 5. Serum progesterone following two injections of prostaglandin $F_{2\alpha}$ (PGF-1 and PGF-2) given at an 11-d interval. Cow 804 became anestrus after luteolysis at PGF-1 (+ LP).

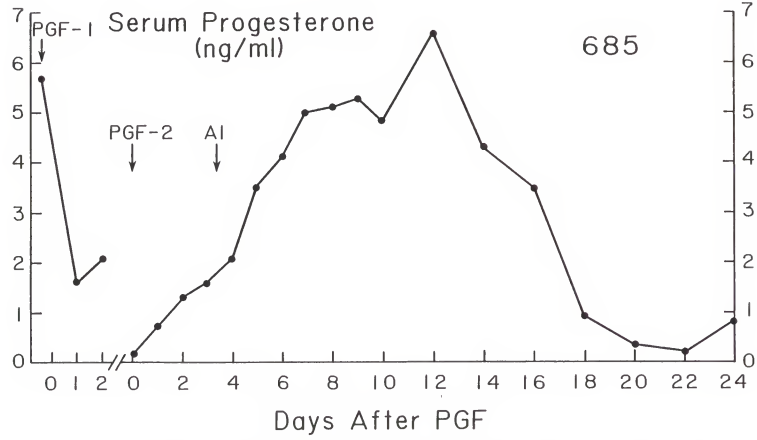

Figure 6. Serum progesterone following two injections of prostaglandin $F_{2\alpha}$(PGF-1 and PGF-2) given at an 11-d interval. Cow 685 had no luteolysis at PGF-1 and low progesterone at PGF-2 (- LP).

Combined Responses After PGF-1 and PGF-2

While the overall luteolytic response following PGF-1 occurred in 98 of 166 (59%) treated cows, the luteolytic response after PGF-2 increased ($P<.05$) to 119 of 166 (71.7%) cows. Although more cows had luteolysis after the second of two PGF treatments at 11-day intervals, it only accounted for a 21% increase in luteolysis. When combining responses to PGF-1 and PGF-2 for only cows with serum P greater than 1 ng/ml (functional CL), PGF successfully induced luteolysis in 217 of 258 cows (84.1%).

Conception Rate at First Service

Control cows (n=59) inseminated at estrus had a first service CR of 54.2%. Frequency and CR at timed AI for cows with luteolysis (Table 1) and failures (Table 2) are shown. As expected, CR of 119 cows with luteolysis at PGF-2 (34.5%) was greater ($P<.001$) than that of 47 cows with PGF failures (2.1%). Conception was lower ($P<.01$) in cows inseminated at 80 h (without GnRH) as well as for all cows categorized as LP + than for controls (Table 1).

Conception at first service was greater ($P<.05$) for those cows categorized as + + than LP + in the AI at 72 and 96 h group. A similar tendency ($P=.15$) existed for the AI at 80 h group. Cows receiving GnRH at 72 h and AI at 80 h did not differ ($P=.78$) in conception at first service in the + + and LP + groups. Within the LP + group, GnRH-treated cows inseminated at 80 h tended to have greater CR than cows not receiving GnRH and inseminated at 80 h ($P=.10$) or inseminated at 72 and 96 h ($P=.15$). One pregnancy resulted in a cow with PGF failure (- LP) inseminated at 72 and 96 h after PGF-2.

DISCUSSION

Successful synchronization of estrus for cows following two injections of PGF depends on several factors including the ability of PGF to regress the CL, and formation of new PGF-susceptible luteal tissue (CL) within 11 days after

the first injection of PGF. A third factor requires that all cows are no longer in a state of postpartum or lactational anestrus. Failure of a two injection regimen to induce synchronous estrus can be elucidated by examining these three factors.

We induced luteolysis in 84% of cows given PGF having serum P greater than 1 ng/ml (functional CL). However, those cows not having luteolysis (16%) at PGF-1 and (or) PGF-2 affected greatly the success of the treatment. In a two-injection scheme, the failure of luteolysis at PGF-1 is compounded by predisposing cows to potentially abnormal situations similar to the - LP response seen in Figure 6. In addition, no luteolysis at PGF-2 (Figure 2) resulted in no estrus during the timed AI period. Therefore, any deviations from the theoretically optimal 95 to 100% luteolytic response to PGF (observed by 14) may be unacceptible if two injections of PGF are to be used successfully to synchronize estrus in dairy cows.

Ability of PGF to induce luteolysis of a functional CL was low (84%) and may be related to the postpartum intervals when treatment began. Short estrous cycles with below normal serum P concentrations that occur frequently early postpartum (8, 20, 28) might indicate that luteal tissue at this time is not normal. Thus, PGF treatment which is luteolytic in heifers or later postpartum cows (7, 16) may not be equally effective in early postpartum cows. Additionally, the high metabolic rate associated with early lactation in dairy cattle (2, 5) may reduce the efficacy of PGF treatment through increased hormonal degradation or enhanced clearance rate. Another possiblity is that the 25-mg dose is not a sufficient dose for large Holstein cows. Earlier work suggested a trend towards increased luteolysis with 30 mg (22). Whether greater doses of PGF would circumvent these problems is unknown.

A second problem encountered in our study was the lack of elevated P 11

days after PGF-1. This situation denoted as + LP, occurred in nearly 5% of all treated animals (Figure 3). The lack of substantial serum P (indicative of a CL) by 11 days after PGF-1 caused PGF failure at PGF-2 and eliminated the possiblity of conception at the timed AI. Thus, the assumption that cows will have elevated serum P 11 days after luteolysis may be inaccurate for early postpartum dairy cattle. The extreme case of a + LP response was illustrated in Figure 5. Cow 804 appeared to become anestrus after PGF-1.

We encountered anestrus (LP LP) in nearly 5% of the treated cows. This is an additional hindrance to the use of PGF in early postpartum (days 40 to 60) dairy cows. Inasmuch as 5% anestrus at this time may not be abnormal (29), lack of estrous cyclicity is another stumbling block to synchronization of estrus with PGF.

While conception for + + cows was similar among PGF treatments, conception tended to be greater for GnRH-treated (35.7%) cows than for cows inseminated at 80 h (7.6%) and cows inseminated at 72 and 96 h (9.1%) in the LP + group (Table 1). The reason for this difference may be due to the stage of the estrous cycle when PGF-2 was administered. Cows in the + + group were likely to be in their early luteal phase at PGF-2, while the LP + cows would be expected to be distributed uniformly throughout the luteal phase. Investigations have shown that earlier and less variable estrus occurred after PGF when given to cattle in their early or very late luteal phase (14, 19, 30). Thus it seems likely that stage of the estrous cycle when PGF-2 was injected in the + + group yielded better CR at the timed AI than the LP + group except for GnRH-treated animals. The intention of the GnRH injection was to synchronize late ovulations with the timed AI. The theoretically less synchronous LP + group did not have lower conception than the + + group for cows receiving GnRH. Evidently, GnRH induced fertile ovulations in less synchronous cows that may

not have ovulated timely for possible conception to a timed AI.

The - + group numerically had the highest CR at a timed AI (57.1%), although only 7 cows were in this group. Stage of cycle could be responsible for this effect. Failure of luteolysis at PGF-1 could result in low serum P at the time of PGF-2 (Figure 6). However, if the cow is either in a very early or very late luteal phase at PGF-1, luteolytic failure at that time would not exclude the possiblity of the presence of a functional CL at PGF-2. Interestingly, the CL would either be early or late cycle but not midcycle (days 10 to 13). This could lead to the advantages of early and synchronous estrus after PGF due to a stage of the cycle, either early (d 7 to 9) or late (d 14 to 17) (19), when PGF-2 was administered.

Use of the two-PGF injection system for estrous synchronization in early postpartum dairy cows does not seem practical. We found 59% of the cows had luteolysis and were estrous synchronized after PGF-1 and following PGF-2, this figure increased to 72%. Given that only a 13 percentage point increase in synchronized estrus resulted from the second injection, it might be argued that a single injection yields optimal results when considering hormonal costs.

Unacceptable results for estrous synchronization shown for early postpartum dairy cattle should not discourage its use in other situations. Certainly PGF has applications for timed AI of replacement heifers as well as treatment of cows with unobserved estrus (21, 26). However, inducing estrus for timed AI at first service seems to be an impractical approach to handling first services in early postpartum dairy cattle.

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Controlling First Services and Days Open for Dairy Cows Using Prostaglandin $F_{2\alpha}$, Gonadotropin-Releasing Hormone, and Insemination by Appointment

ABSTRACT

Prolonged interval to first services may be one cause of lengthy and variable days open. Our objective was to determine the effect of controlling time of first services on days open and to evaluate three methods of artificial insemination by appointment after prostaglandin $F_{2\alpha}$. Holstein cows (n=283) were assigned randomly at calving to one of four treatment groups. Control cows (Group 1, n=77) were inseminated according to estrous detection beginning 6 wk postpartum. Estrous cycles of the remaining cows were synchronized using two 25-mg doses of prostaglandin $F_{2\alpha}$ at 11-day intervals beginning 40 to 46 days postpartum. Timed insemination regimens following the second of two prostaglandin $F_{2\alpha}$ injections (treatment; 0 h) were: 1) artificial insemination at 80 h (Group 2, n=74), 2) 100 µg gonadotropin-releasing hormone at 72 h and aritifical insemination at 80 h (Group 3, n=65), and 3) artificial insemination at 72 h and 96 h (Group 4, n=67). Only 72% of the cows had luteolysis (serum progesterone > 1 ng/ml at 0 h and < 1 ng/ml at 48 h). Average interval to first service was shorter in treated cows than controls (63, 57, 57, and 57 days for Groups 1 to 4). The variance associated with interval to first services for treated cows was 10% of controls. Conception rate at first service was greater in controls (51%) than treated cows (23, 29, and 31% for Groups 2 to 4). Among treated cows, conception was greater for cows with luteolysis after treatment (37.8%) than cows without luteolysis (2.6%). Treatment did not reduce days open compared with controls (96, 89, 111, and 104 days for Groups 1 to 4). However, variance associated with days open was less in Group 2 than controls. Services per conception were similar in Groups 1, 2, and 4 (1.8, 2.1, and 2.2,

respectively), but Group 3 (2.6) differed from controls and Group 2. These data demonstrate that although the variance in interval to first services for treated cows was reduced, days open were unaltered by treatments (except for Group 2). Failure of Groups 3 and 4 to have reduced variance in days open might be due to poor conception after timed inseminations and poor estrous synchronization-response to prostaglandin $F_{2\alpha}$. However, despite poorer fertility for treated cows, average days open did not vary from controls more than 16 days.

INTRODUCTION

Calving interval has a profound economic effect in the dairy herd (6). Numerous studies have demonstrated the beneficial nature of a 12 to 13 mo calving interval for a herd (1, 6, 8, 13) Intervals longer than optimum result in cows spending a greater proportion of their lactation in the less profitable part of their lactation curve (6). Thus, economic losses associated with prolonged calving interval often go undetected by dairy producers since they represent lost potential income and not out-of-pocket expenses (6).

Several factors affect calving interval in a dairy herd. Among these, heat detection and interval to first service (IFS) are most important (11). Poor heat detection has been linked to reproductive inefficiency in many studies and its effect on calving interval is well documented (2, 4, 11). Interval to first service has received less attention than heat detection as a measure of reproductive performance. However, calving interval and IFS are highly correlated (5, 9, 12), and optimal calving interval may not be attainable without decreasing IFS within a herd (6).

Reducing calving intervals to an optimal average on a herd basis does not optimize necessarily individual calving intervals for all cows. There remain cows with extremely short or extended intervals included in the herd average.

Reducing interval to first services will shorten calving intervals (9, 15), but postpartum inseminations earlier then 60 days may lower conception rates (5). One option available is to time all first services at approximately 60 days through the use of estrous synchronization. Therefore, the objectives of our study were two-fold: 1) to determine if reducing calving intervals or intervals from calving to conception (mean and variance) to more optimal length can be achieved by controlling the time of all first services with prostaglandin $F_{2\alpha}$ (PGF); and 2) to evaluate three different management systems using artificial insemination (AI) at first services following controlled periods of estrus.

MATERIALS AND METHODS

Experimental Design

Lactating Holstein cows (n=283) from the Kansas State University dairy herd that calved between July 1, 1983 and March 1, 1985 were used. Cows were housed in a free-stall confinement facility exposed to the environment. The diet consisted of a concentrate mix (16% protein) containing 50% corn and 50% milo grains, soybean meal, 1.5% Na bicarbonate, and minerals in a self-feeder and alfalfa hay fed ad libitum. Cows were milked twice daily at 0930 h and 2130 h. Two daily 30-min observation periods for estrous detection were conducted (0700-0900 h and 1600-1800 h). At calving, cows were assigned to one of four treatment groups (Groups 1 to 4). Control cows (Group 1; n=77) were inseminated artificially (AI) at first observed estrus after 40 days postpartum. Time of first services was controlled in the remaining cows (PGF cows; n=206) using two 25-mg injections (i.m.) of PGF (PGF-1 and PGF-2; Lutalyse®; dinoprost tromethamine) at an 11-d interval beginning 40 to 46 days postpartum. Following PGF-2 (0 h) cows were treated according to one of the following procedures: 1) timed AI at 80 h (Group 2; n=74); 2) 100 µg (i.m.) gonadotropin-releasing hormone (GnRH; Cystorelin®) at 72 h and timed AI at 80

h (Group 3; n=65); or 3) timed AI at 72 and 96 h (Group 4; n=67). Subsequent services were performed according to observed estrus. Cows were reinseminated until determined pregnant by uterine palpation per rectum at 40 to 46 d after AI or removed from the herd.

Blood Collection

Blood was collected by coccygeal venipuncture from a random group of PGF-treated cows (n=176) at 0 and 48 h after PGF-2. Samples were stored at 5°C for 24 h until serum was collected by centrifugation. Serum samples were held at -20°C until assayed for progesterone (P) by radioimmunoassay (14) to determine luteolytic response to PGF administration. A luteolytic response after PGF-2 was indicated when serum P exceeded 1 ng/ml at 0 h and was less than 1 ng/ml at 48 h. Cows with serum P > 1 ng/ml at 0 h and > 1 ng/ml at 48 h or having serum P < 1 ng/ml at 0 and 48 h at PGF-2 were indicated as having no luteolysis.

Statistical Analyses

A different inseminator was used during the first and second year of the study. Because no effect of inseminator was found, data were pooled. Interval to first service, conception rate at first service, days open (measure of calving interval), and services per conception (SPC) were analyzed by least-squares procedures from Statistical Analysis System (3). Treatment (n=4), lactation (primiparous or multiparous), season of calving (October 1 to January 31, February 1 to May 30, or June 1 to September 30), and luteolytic response to PGF (yes or no) were considered in various models. A measurement of the variation associated with the intervals to first service and to conception were calculated by determining a residual for each cow (absolute value of the difference between the observed value and treatment mean) and subjecting those residuals to analysis of variance using the preceding model. Orthogonal

contrasts were made between treatment means as well as comparisons with control (3). Enumeration data were tested for independence by chi-square.

RESULTS

Treatment Groups

Assignment of cows at calving to treatment resulted in 46, 45, 38, and 43 multiparous and 31, 29, 27, and 24 primiparous cows in Groups 1 to 4, respectively. Cows were distributed uniformly by season of calving and milk production (based on 305 day-2X ME) was similar among treatment groups. An unexplainable bias occurred in Group 2 in terms of cows having delayed postpartum conception (repeat-breeders). While all cows in Group 2 had conceived by 160 days postpartum, comparable numbers of cows for Groups 1, 3, and 4 were 6, 7, and 8, respectively. Days open (Mean $\pm$ SD) for these repeat-breeder cows were 221 $\pm$ 18, 212 $\pm$ 29, and 207 $\pm$ 30 for groups 1, 3, and 4 respectively.

Luteolytic Response to PGF

Three different progesterone responses were observed after the second of two PGF treatments. Cows either underwent luteolysis, failed to undergo luteolysis, or had low concentrations of serum P at the time of PGF treatment. Average serum P concentrations at 0 and 48 h for the three possible responses are given in Figure 1. Only 72% of treated cows were classified as having luteolysis after PGF-2. The remaining 28% had serum P levels that indicated a failure of the PGF treatment to initiate luteolysis. Thus, these cows were classified as having no luteolysis or low progesterone.

Interval to First Service

Interval to first service (IFS) was about 5 days shorter ($P<.01$) in PGF-treated cows than in controls (Table 1). By design, PGF cows had a less variable IFS than controls ($P<.01$). Beginning first services in control cows at

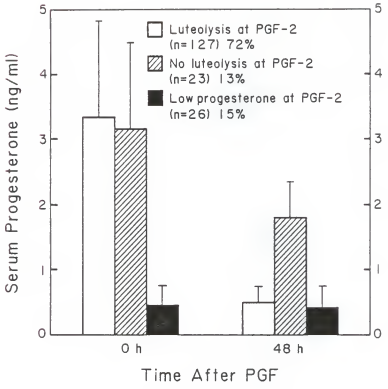

Figure 1. Mean (± SD) serum progesterone concentration for cows following the second of two injections of prostaglandin $F_{2\alpha}$ given at an 11-d interval.

TABLE 1. Average days and variance (residuals) of intervals to first service for controls and cows given prostaglandin $F_{2\alpha}$ (PGF) with or without luteolysis.

Treatment Groups:	1			2			3 GnRH at 72 h			4 AI at 72 h		
Luteolysis	Control			AI at 80 h			AI at 80 h			and 96 h		
	X	SE	n	X	SE	n	X	SE	n	X	SE	n
Yes				57.1	.4	40	57.6	.4	36	57.2	.4	35
Residuals[a]				1.8	.2	40	1.6	.2	36	1.7	.2	35
No				57.4	.6	13	56.0	.6	13	56.8	.7	13
Residuals				1.4	.3	13	1.3	.3	13	1.9	.4	13
Total[b]	62.6	1.4	77	57.1[c]	1.3	74	56.9[c]	1.4	65	57.1[c]	1.5	67
Residuals	16.6	.9	77	1.7[c]	.8	74	1.6[c]	.9	65	1.8[c]	.9	67

[a]Variation within treatment (residual) was expressed as the absolute value of (observed value - treatment mean).

[b]Total includes additional cows (n=56) from which no blood was collected after PGF treatment and luteolytic response based on serum progesterone concentrations could not be determined.

[c]Different from control ($P<.01$).

first detected heats after 40 days postpartum, resulted in a 3-wk delay to the average interval to first service for all control cows. The variance and mean IFS for cows given PGF were similar, without regard to luteolytic response.

Conception at First and Second Service

Conception rate (CR) at first service was greater ($P<.01$) for control cows than PGF cows (Table 2). The CR for the three AI treatments was similar. Considering all timed inseminations, cows with luteolysis had higher ($P<.01$) CR than cows (37.8% versus 2.6%) without luteolysis. When only cows with luteolysis were compared with controls, Group 2 had a lower first service CR ($P<.05$) while Group 3 and Group 4 were not different.

Second service CR (%) was 39.4 (n=33), 52.7 (n=55), 31.1 (n=45), and 50.0 (n=44) for Groups 1 to 4, respectively. The difference between Groups 2 and 3 (no GnRH versus GnRH prior to a single timed AI) for second service CR was significant ($P<.05$).

Services Per Conception

Of the 283 cows assigned to this experiment, 240 (85%) eventually conceived. The remaining 43 cows were either culled because of conception failure or for other management-related reasons (low milk production, illness, mastitis, etc.). Therefore, a similar percentage of cows were removed from the herd prior to conception between treatment groups (14.3, 17.6, 15.4, and 13.4 for Groups 1 to 4, respectively).

Services per conception (SPC) were similar among controls, Group 2, and Group 4 cows (Table 3). Group 3 cows required more ($P<.01$) SPC than all other groups. Within Groups 2, 3, and 4, SPC were higher ($P<.05$) in cows with no luteolysis than for the remaining cows as expected. When cows with luteolysis were considered, timing of AI or effect of GnRH did not alter SPC.

TABLE 2. Conception at first service (%) for controls and cows given prostaglandin $F_{2\alpha}$ (PGF) with or without luteolysis[a].

Treatment Groups:	1	2	3	4
Luteolysis	Control	AI at 80 h	GnRH at 72 h AI at 80 h	AI at 72 h and 96 h
Yes		10/40 (25.0)[b]	17/36 (47.2)[b]	15/35 (42.9)[b]
No		0/13 (0)	0/13 (0)	1/13 (7.7)
Total[c]	39/77 (50.6)	17/74 (23.0)[d]	19/65 (29.2)[d]	21/67 (31.3)[d]

[a]Conception after inseminations following the second of two injections of prostaglandin $F_{2\alpha}$ given at 11-day intervals except for controls that were inseminated at spontaneous estrus.

[b]Different from cows with no luteolysis ($P<.05$).

[c]Total includes additional cows (n=56) from which no blood was collected after PGF treatment and luteolytic response based on serum progesterone concentrations could not be determined.

[d]Different from control ($P<.01$).

TABLE 3. Services per conception for controls and cows given prostaglandin $F_{2\alpha}$ (PGF) with or without luteolysis.

Treatment Groups:	1			2			3 GnRH at 72 h			4[a] AI at 72 h		
Luteolysis	Control			AI at 80 h			AI at 80 h			and 96 h		
	X	SE	n	X	SE	n	X	SE	n	X	SE	n
Yes				1.9[b]	.2	32	2.3[b]	.3	35	1.9[b]	.2	32
No				2.9	.3	11	3.5	.4	9	3.0	.3	11
Total[c]	1.8	.2	66	2.1	.2	61	2.6[d]	.2	55	2.2	.2	58

[a]Double inseminations were counted as one service.

[b]Different from cows without luteolysis ($P<.05$).

[c]Total includes additional cows (n=56) from which no blood was collected after PGF treatment and luteolytic response based on serum progesterone concentrations could not be determined.

[d]Different from control ($P<.01$).

Days Open

Cows treated with PGF were similar to controls with respect to days from calving to conception (days open) (Table 4). Overall, Group 2 cows conceived earlier ($P<.10$) postpartum than cows in Groups 3 or 4. Cows with luteolysis had fewer ($P<.05$) days open than cows without luteolysis (97.6 versus 125.5 days). Variation in days open was reduced ($P<.01$) only in Group 2 compared with controls. Luteolytic success had no effect ($P=.19$) on variation in days open among PGF cows.

In an effort to account for an unexplainable absence of repeat-breeders in Group 2, days open were considered for only cows conceiving before 160 days postpartum. Days open (mean $\pm$ SD) for Groups 1 to 4 became 78 $\pm$ 28 (n=60), 89 $\pm$ 29 (n=61), 88 $\pm$ 31 (n=48), and 80 $\pm$ 26 (n=50), respectively.

DISCUSSION

Controlling the time of first service at approximately 60 days postpartum using PGF resulted in similar intervals to conception to control cows inseminated after 40 days postpartum. Variation in IFS in PGF-treated cows was reduced to 10% of that in control cows. However, the conciseness of IFS in PGF cows did not yield less variable days open compared with controls. Thus, our objective to reduce variation in days open was not realized. However, based on these results, beginning inseminations at 60 days by controlling the onset of estrus did not delay days open compared to beginning inseminations after 40 days for controls. Failure of this procedure to reduce the variability in days open may be related to the low CR at the timed AI. Greater than 70% of PGF cows did not conceive at first service and this fact probably decreased the effectiveness of the treatments to yield less variable days open.

Ability of the PGF treatment to synchronize estrus was low after two injections (72%). Insemination of cows with no luteolysis at first service

TABLE 4. Average days and variance (residuals) of intervals from calving to conception (days open) for controls and cows given prostaglandin $F_{2\alpha}$ (PGF) with or without luteolysis.

Treatment Groups:	1			2			3			4		
Luteolysis	Control			AI at 80 h			GnRH at 72 h AI at 80 h			AI at 72 h and 96 h		
	X	SE	n	X	SE	n	X	SE	n	X	SE	n
Yes				86.5	9.9	32	106.3	10.4	35	99.8	9.5	32
Residuals[a]				20.5	6.2	32	42.7	6.6	35	41.4	6.0	32
No				100.3	13.7	11	133.1	16.7	9	143.1	17.6	11
Residuals				25.0	8.7	11	45.9	10.5	9	64.7	11.1	11
Total[b]	95.9	6.1	66	89.1[c]	6.2	61	110.7	6.8	55	104.3	6.6	58
Residuals	40.8	3.8	66	25.5[d]	3.9	61	42.6	4.3	55	43.4	4.1	58

[a]Variation within treatment (residual) was expressed as the absolute value of (observed value - treatment mean).

[b]Total includes additional cows (n=56) from which no blood was collected after PGF treatment and luteolytic response based on serum progesterone concentrations could not be determined.

[c]Different from Groups 3 and 4 ($P<.10$).

[d]Different from control ($P<.01$).

increased the average and variance of days open because nonsynchronized cows had longer days open than those with luteolysis. It seems likely that if a greater proportion of cows had responded to PGF, this could have yielded shorter or less variable days open.

All timed AI treatments after PGF-2 resulted in similar conception that was inferior to controls. This was probably due to only 72% of the cows being in estrus that could have conceived to the timed AI. The timed AI method employed seemed to have an effect beyond first service fertility. Group 3 had a higher SPC than any other group. For unknown reasons GnRH (designed to synchronize late ovulations with AI) may have reduced fertility of subsequent services. Administration of GnRH at first service (Group 3) apparently decreased conception at second service. Some recent work suggested lower serum P concentrations in cows that failed to conceive after receiving GnRH at insemination (16). If this is true, and if serum P during the estrus cycle prior to insemination is important to subsequent fertility (as suggested by 7, 8) then lower conception in Group 3 at second service may have a physiological basis.

Cows without luteolysis before first service were peculiar in terms of SPC and days open. These cows required one more service on the average than those synchronized cows. The infertile first service received by cows with no luteolysis should have resulted in an increase in SPC consistent with first service CR for cows with luteolysis. It appears that the differences in SPC between the two groups of cows are caused by other factors in addition to the infertile first service. This suggests the possibility that animals having no luteolysis or low P at PGF-2 suffer from reproductive problems that may predispose poorer conception. A similar trend was seen in days open with cows failing to respond to PGF at first service eventually conceiving 28 days later than cows with luteolysis.

Perhaps a more effective approach to reducing the variation in days open would involve a simplified estrous synchronization regimen and only AI based on estrous detection. A single dose of PGF given to cows known to be in the luteal phase (based on heat-detection records or based on ovarian palpation for a corpus luteum) followed by an insemination at estrus and a timed AI for cows not observed in estrus might be another alternative. This procedure could make controlling IFS a more reasonable alternative to the dairy producer since it would reduce hormonal costs and probably increase conception at the controlled first service. Whether or not the additional benefit of reduced variation in days open is attainable by such a method, remains to be tested.

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Gonadotropin-Releasing Hormone at Estrus: Serum Concentrations of Luteinizing Hormone, Estradiol, and Progesterone

Summary

Administering gonadotropin-releasing hormone (GnRH) at the time of insemination improved fertility in dairy cows in previous studies. The objective of this study was to determine if the effect of GnRH is mediated through serum luteinizing hormone (LH) and(or) by altered secretion of serum progesterone (P) and estradiol-17ß (E) during the post-insemination period. Estrous cycles of 60 Holstein cattle were synchronized using two luteolytic doses of prostaglandin $F_{2\alpha}$ or PGF (Lutalyse® or PGF analog, Estrumate®) at an 11-d interval. Cattle were given either GnRH (100 µg Cystorelin®, n=31) or saline (n=29) at 72 h and inseminated artificially (AI) at 80 h after the second of two PGF injections (PGF-2). Serum P and E were measured in blood samples collected during 3 wk after AI (estrus). In 25 cows and heifers serum LH was measured in blood samples that were collected via indwelling jugular cannulae every 4 h on d 2 to 5 after PGF-2 and more frequent intervals during 240 min after GnRH (n=18) or saline (n=7). Ten females had a spontaneous preovulatory LH surge before GnRH (GnRH-spontaneous) while GnRH induced the preovulatory surge in six females. Two heifers appeared to initiate a spontaneous LH surge at or near the time of GnRH (spontaneous and(or) induced). The remaining seven cows had spontaneous LH surges with no subsequent change in LH after saline treatment. Duration of the GnRH-induced preovulatory LH surges was shorter ($P<.01$) than after saline, or after GnRH for cattle with spontaneous or spontaneous and(or) induced LH surges. Serum E before the LH surge was greater ($P<.05$) in saline, GnRH-spontaneous, and GnRH-spontaneous and(or) induced cattle than in GnRH-induced cattle. Among GnRH-treated animals, conception rate was lower ($P<.05$) for those with induced LH surges. Additionally, no GnRH-induced cattle

were observed in estrus. Serum P during 21 d after estrus was lower ($P<.05$) in both pregnant and open cattle previously treated with GnRH than after saline. Serum E was higher ($P<.01$) in nonpregnant cattle that received GnRH than in those that received saline. Serum P during the first wk after estrus was greater ($P<.01$) in saline controls, and GnRH-spontaneous cattle compared with GnRH-induced cattle. These data suggest that the use of GnRH after PGF for the purpose of inducing ovulations may not be worthwhile. Higher fertility after GnRH does not appear to be related to increased serum P, but may be a result of a delayed rise in serum P after ovulation.

Introduction

Recent evidence has suggested that conception rates were increased when gonadotropin-releasing hormone (GnRH) was administered at the time of a routine insemination after detected estrus (Schels and Mostafwi, 1978; Nakao et al., 1983). In addition, GnRH improved conception at a timed artificial insemination (timed AI) after estrous synchronization with prostaglandin $F_{2\alpha}$ (PGF) (Graves et al., 1974; Hansel and Fortune, 1978). Reasons for improved fertility after GnRH are not known. However, it is believed that use of GnRH after PGF may synchronize further ovulations of treated cows. Induction of ovulation has been demonstrated in several studies (Cummings et al., 1977; Fernandez-Lima, 1977). Furthermore, GnRH given after PGF may enhance fertility of all cattle regardless of its direct or indirect action on the ovulatory follicle and may act in a similar fashion at insemination following a spontaneous estrus (Schels and Mostafwi, 1978; Nakao et al., 1983).

Gonadotropin-releasing hormone improved fertility at first service in some (Schels and Mostafwi, 1978; Nakao et al., 1983), but not all (Stevenson et al., 1984) investigations. However, a more consistent effect of GnRH was seen for repeat services where administering GnRH generally enhanced fertility (Maurice

et al., 1982; Lee et al., 1983; Stevenson et al., 1984). The reason for consistent benefit of GnRH at repeat services, but not for all first services is unknown, but may be related to the ability of GnRH to induce ovulation in late ovulating repeat-breeding cattle (Dekruif, 1978)

Pregnant cattle have higher blood progesterone concentrations (P) during the first 3 wk following insemination than nonpregnant cattle (Lee and Ax, 1984; Stevenson et al., 1985). Thus, increasing P after insemination may be one way to improve fertility (Johnson et al.,1958; Kunkel et al., 1977). It is possible that LH released by GnRH could enhance fertility through its effects on luteal function. Helmer and Britt (1983) demonstrated increased P during the luteal phase of heifers given human chorionic gonadotropin (hCG) on d 2, 3, and 4 of the estrous cycle. Work using GnRH to augment serum P has produced mixed results. Lee and Ax (1984) demonstrated higher milk P during the luteal phase of cows given GnRH that became pregnant compared with similar saline-treated controls. Conversely, Stevenson et al. (1985) demonstrated no change in serum P for treated cows that became pregnant, while serum P in GnRH-treated cows that failed to conceive was less than nonpregnant saline controls.

This study was designed to elucidate the action of GnRH given to cows and heifers in estrus. Specifically, we wished to determine if the effect of GnRH is mediated through its effect on serum LH release and(or) through changes in serum P and estradiol during the post-insemination period.

Materials and Methods

Experimental Design

Holstein cows (n=37) and heifers (n=24) from the Kansas State University dairy herd were housed in an open-air confinement facility with concrete (cows) or dirt (heifers) lots having sheltered freestalls. Cows were estrous-synchronized using two 25-mg injections (i.m.) of prostaglandin $F_{2\alpha}$ (PGF or Lutalyse®;

dinoprost tromethamine) at an 11-d interval beginning 40 to 46 d postpartum. Following the second PGF injection (PGF-2; 0 h) cows were assigned randomly to receive either 100 µg GnRH (Cystorelin®; n=20) or saline (n=17) at 72 h and were timed inseminated (AI) at 80 h. Heifers (13 to 14 mo of age) were estrous-synchronized using two injections (i.m.) of cloprostenol (PGF analog or Estrumate®) at an 11-d interval. Following PGF-2, GnRH (n=12) or saline (n=12) and timed AI were handled as described for the cows. Animals were observed for signs of estrus during 30 min every 8 h.

Blood Collection

Blood was collected daily by coccygeal venipuncture from 0 to 10 d and alternately on d 12 to 24 after PGF-2. Fourteen cows and 12 GnRH-treated heifers were fitted with jugular cannulae and blood was collected once every 4 h for 3 d beginning at 32 h and 48 h after PGF-2 in heifers and cows, respectively. Additionally, blood was collected at 0, 20, 40, 60, 80, 100, 120, 150, 180, 210, and 240 min after either GnRH (7 cows, 12 heifers) or saline (7 cows). Blood was stored at 5° C for 24 h until serum was harvested by centrifugation. Blood sera were stored at -20° C until assayed.

Assay Procedures and Definitions

Serum hormone concentrations were measured using validated radioimmunoassays for progesterone (P; Stevenson et al., 1981), estradiol (E; Skaggs et al., 1985) and luteinizing hormone (LH; Skaggs et al., 1985). Serum P and E concentrations were determined in samples collected on d 0 to 24 and d 3 to 10 after PGF-2, respectively. Luteinizing hormone concentration was measured in samples collected every 4 h after PGF and during the 240 min frequent sampling period following GnRH or saline in animals fitted with jugular cannulae. Peak LH concentration and preovulatory LH surges after PGF-2 were determined from 4-h samples. In some cases, LH surges occurred during the 4 h after GnRH or

saline and the LH peak was determined from a 20 or 30 min sampling interval. Duration of the preovulatory LH surge was defined as the interval from when serum LH exceeded 1 ng/ml before the LH peak until serum LH declined to less than 1 ng/ml after peak LH. Since the point at which LH concentration went above or below 1 ng/ml was unlikely to be determined directly, it was derived from linear extrapolation of LH concentrations directly before and after serum LH reached 1 ng/ml.

Statistical Analyses

Hormonal data were analyzed by least-squares using procedure GLM from the Statistical Analysis System (SAS, 1979). Treatment (GnRH vs saline), lactational status (heifer vs cow), and pregnancy status were included in various pseudo split-plot models for repeated measurements or by factorial analysis of variance. Means were separated by selected LSD differences (SAS, 1979). Fertility data were analyzed by contingency Chi-square.

Results

Classification of LH Responses Associated with GnRH

There were three different LH responses associated with GnRH treatment and estrus. These LH responses are illustrated in figures 1 to 3. The first response (n=10, 5 cows and 5 heifers) was a normal spontaneous preovulatory LH surge followed by a variable GnRH-induced LH peak (figure 1). In this case, the endogenous LH surge occurred before GnRH treatment (GnRH-spontaneous). The second response (n=6, 2 cows and 4 heifers) was characterized as a GnRH-induced LH release because no endogenous LH surge occurred before GnRH treatment (figure 2). However, the preovulatory LH surge appeared to be induced by GnRH treatment 72 h after a luteolytic dose of PGF. The third response (n=2, heifers only) was characterized as spontaneous and(or) induced. The preovulatory LH surge appeared to begin just prior to 72 h and was then

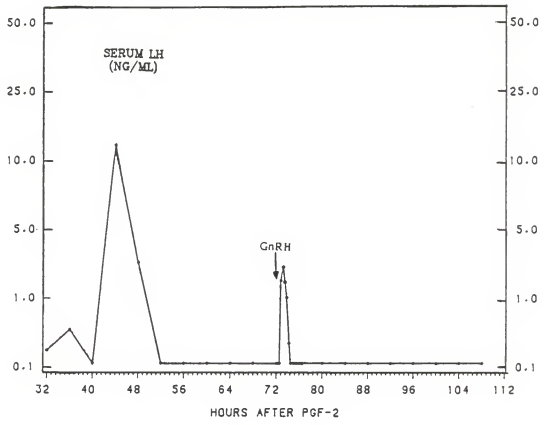

Figure 1. Serum luteinizing hormone (LH) following a luteolytic dose of prostaglandin $F_{2\alpha}$ (0 h) and gonadotropin-releasing hormone (GnRH) in a heifer having a spontaneous preovulatory LH surge at 44 h (GnRH-spontaneous) followed by a GnRH-induced LH release at 72h.

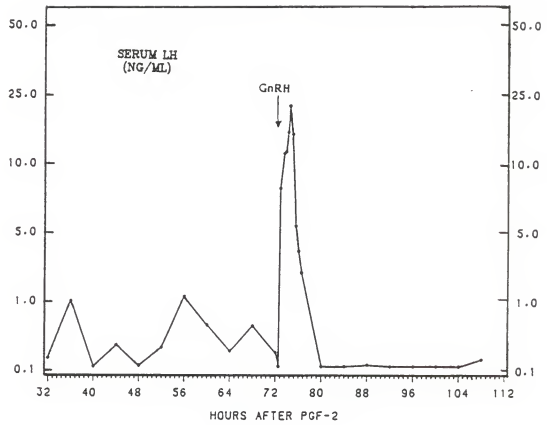

Figure 2. Serum luteinizing hormone (LH) following a luteolytic dose of prostaglandin $F_{2\alpha}$ (0 h) and gonadotropin-releasing hormone (GnRH) at 72 h in a cow having a GnRH-induced preovulatory LH surge.

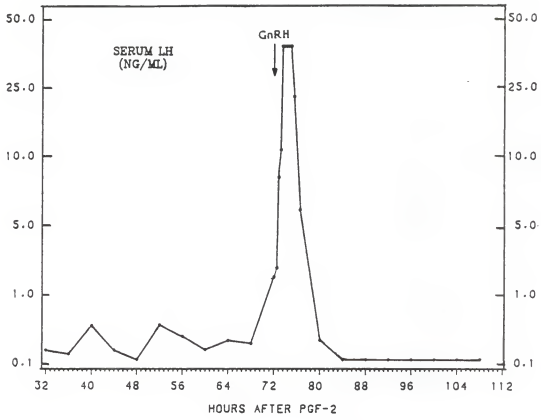

Figure 3. Serum luteinizing hormone (LH) following a luteolytic dose of prostaglandin $F_{2\alpha}$ (0 h) and gonadotropin-releasing hormone (GnRH) at 72 h in a heifer having a spontaneous and(or) induced preovulatory LH surge.

enhanced by GnRH treatment (figure 3). The LH response in these two heifers differed in magnitude and duration as described below, thus their response was dissimilar from the preceeding heifers and cows with GnRH-induced LH surges.

Characteristics of LH Surges

Individual LH surges and intervals to estrus after PGF-2 for lactating cows are given in table 1. Data are presented in an ordered sequence throughout table 1 by individual cow so information on each cow may be considered. Two cows with GnRH-induced LH surges were not detected in estrus but had LH surges that were about 7 h in duration with magnitudes exceeding 20 ng/ml. Serum estradiol at 72 h after PGF-2 ranged from 2.0 to 5.8 pg/ml in the two GnRH-induced cows. Five cows had spontaneous LH surges before GnRH that ranged from 7.8 to 14.2 h in duration and 6 to 20 ng/ml in magnitude. All five cows were observed in estrus either before or concomittant with the peak in their spontaneous LH surge. Serum estradiol concentration during 24 h before the spontaneous LH surge ranged from 4.9 to 12.1 pg/ml. Seven saline-treated control cows had spontaneous LH surges ranging from 8.5 to 16.8 h in duration and 5.3 to 18.2 ng/ml in magnitude. Six of seven control cows also were observed in estrus near the peak of their LH surge and serum estradiol concentration during the 24 h prior to the LH surge ranged from 4.1 to 10.2 pg/ml.

LH responses for individual heifers are presented in table 2. One of the original 12 heifers treated with GnRH was prepubertal based on her profile of serum progesterone after PGF and was omitted. Four heifers with LH surges induced by GnRH were not detected in estrus similar to the response of the cows with LH surges induced by GnRH. The LH response varied from 4 to 6.8 h in duration and from 8.5 to >40 ng/ml in magnitude. Serum estradiol concentration at 72 h after PGF-2 ranged from 2.7 to 4.9 pg/ml for

TABLE 1. CHARACTERISTICS OF ESTRUS AND INDIVIDUAL LUTEINIZING HORMONE SURGES IN SERUM OF LACTATING DAIRY COWS AFTER SALINE OR GONADOTROPIN-RELEASING HORMONE (GnRH)[a].

Treatment	n	Interval to estrus[b]	Estradiol[c]	Preovulatory LH surge[d]	Interval[e]	Duration[f]	Magnitude[g]
GnRH	2	NS[h],NS	5.8, 2.0	Induced	74.2, 74.5	7.1, 7.2	22.7, 22.2
GnRH	5	60, 60, 48, 56, 60	12.1, 4.9, 7.7, 5.0, 9.0	Spontaneous	64, 60, 52, 60, 64	7.8, 11.4, 11.2, 9.6, 14.2	12.7, 20.2, 6.0, 10.6, 8.8
Saline	7	60, 60, NS 60, 60, 68, 52	6.3, –, 4.5, 8.8, 6.1, 4.1, 10.2	Spontaneous	56, 64, 104, 68, 68, 68, 52	11.2, 9.8, 16.8, 9.2, 8.5, 10.8, 10.8	5.3, 7.3, 17.9, 8.9, 18.2, 42.4 6.6

[a]Traits described followed a luteolytic dose of prostaglandin $F_{2\alpha}$ (PGF). Data are presented in an ordered sequence by individual cow.

[b]Interval (h) to estrus after PGF.

[c]Serum estradiol during the 24 h before the LH surge or at 72 h after PGF if the LH surge was induced.

[d]Preovulatory surge occurred spontaneously or was induced by GnRH.

[e]Interval (h) to when peak LH occurred after PGF.

[f]Duration (h) of preovulatory LH surge.

[g]Peak concentration (ng/ml) of LH during the preovulatory LH surge.

[h]Cows were not observed in standing estrus during observation periods after PGF.

TABLE 2. CHARACTERISTICS OF ESTRUS AND INDIVIDUAL LUTEINIZING HORMONE SURGES IN SERUM OF HEIFERS AFTER SALINE OR GONADOTROPIN-RELEASING HORMONE (GnRH)[a].

Treatment	n	Interval to estrus[b]	Estradiol[c]	Preovulatory LH surge[d]	Interval[e]	Duration[f]	Magnitude[g]
GnRH	4	NS[h], NS, NS NS	2.7, 3.5, 2.0, 4.9	Induced	74.2, 74.5, 74.2, 73.8	5.1, 6.3, 4.0 6.8	10.4, 22.5, 8.5, >40
GnRH	2	84, 68	7.4, 9.7	Spontaneous and(or) induced	73.5, 73.5	10.1, 10.3	>40, >40
GnRH	5	51, 44, 44 44, 51		Spontaneous	68, 44, 48, 44, 48	11.1, 10.6, 11.1 8.2, 11.4	7.8, 13.4 ,8.7, 22.0, 25.4

[a]Traits described followed a luteolytic dose of prostaglandin $F_{2\alpha}$(PGF). Data are presented in an ordered sequence by individual cow.

[b]Interval (h) to estrus after PGF.

[c]Serum estradiol during the 24 h before the LH surge or at 72 h after PGF if the LH surge was induced.

[d]Preovulatory surge occurred spontaneously or was induced by GnRH.

[e]Interval (h) to when peak LH occurred after PGF.

[f]Duration (h) of preovulatory LH surge.

[g]Peak concentration (ng/ml) of LH during the preovulatory LH surge.

[h]Cows were not observed in standing estrus during observation periods after PGF.

GnRH-induced heifers. Two heifers had what appeared to be the beginning of a spontaneous LH surge immediately preceding GnRH treatment. Both heifers were detected in estrus and had serum estradiol concentration ranging from 7.2 to 9.6 pg/ml at 72 h after PGF-2. The duration of their LH response ranged from 10.1 to 10.3 h, while the magnitude of the LH peak exceeded 40 ng/ml in both heifers. The remaining five heifers were observed in estrus, and had spontaneous LH surges ranging from 8.2 to 11.1 h in duration and 7.8 to 25.4 ng/ml in magnitude. Adequate blood serum for estradiol was not collected from these heifers during the 24 h prior to the spontaneous LH surge, so no estradiol concentrations are available.

A summary of the spontaneous, spontaneous and(or) induced, or GnRH-induced LH surges are presented in table 3 for both heifers and cows. As expected, GnRH-induced and spontaneous and(or) induced animals had preovulatory LH surges that occurred later ($P<.01$) than the GnRH-spontaneous group. Time of LH surge in saline animals was not different from GnRH-treated animals. Duration of the LH surge was shorter ($P<.01$) in GnRH-induced animals than for GnRH-spontaneous, spontaneous and(or) induced, and saline-treated cows. Magnitude of the LH surge of spontaneous and(or) induced heifers was greater ($P<.05$) than all other types of preovulatory LH surges. Estradiol prior to the preovulatory LH surge was greater ($P<.05$) in spontaneous and(or) induced, GnRH-spontaneous and saline-treated cows than for GnRH-induced cattle. Conception rate to timed AI was greater ($P<.05$) in GnRH-spontaneous (7/10; 70%) compared with GnRH-induced animals (1/6; 17%). Both spontaneous and(or) induced animals became pregnant. Conception rate did not differ when GnRH-treated animals were compared with saline-treated females (56% vs 29%, respectively).

GnRH-Induced LH Release After Spontaneous LH Surges

TABLE 3. SUMMARY OF THE CHARACTERISTICS OF ESTRUS AND LUTEINIZING HORMONE SURGES IN SERUM AFTER SALINE OR GONADOTROPIN-RELEASING HORMONE (GnRH)[a].

Treatment	n		Interval to estrus[b]	Estradiol[c]	Preovulatory LH surge[d]	Interval[e]	Duration[f]	Magnitude[g]
GnRH	6	X	NS[h]	3.5	Induced	75.3[i]	6.2[j]	20.5
		SE		1		4.6	.9	4.3
GnRH	2	X	76	8.6	Spontaneous	76.7	10.6	>40
		SE	11.3	2	and(or) induced	8	1.5	
GnRH	10	X	51.8	7.7	Spontaneous	55.2	10.7	13.6
		SE	1.5	1		3.5	.6	3.3
Saline	7	X	55.0	6.7	Spontaneous	65.4	10.6	16.2
		SE	2.5	1		5	.9	4.7

[a]Traits described followed a luteolytic dose of prostaglandin $F_{2\alpha}$ (PGF). Data are presented in an ordered sequence by individual cow.

[b]Interval (h) to estrus after PGF.

[c]Serum estradiol during the 24 h before the LH surge or at 72 h after PGF if the LH surge was induced.

[d]Preovulatory surge occurred spontaneously or was induced by GnRH.

[e]Interval (h) to when peak LH occurred after PGF.

[f]Duration (h) of preovulatory LH surges

[g]Peak concentration (ng/ml) of LH during the preovulatory LH surge.

[h]Cows were not observed in standing estrus during observation periods after PGF.

[i]Different from GnRH-spontaneous ($P<.01$).

[j]Different from GnRH-spontaneous and saline-spontaneous ($P<.01$).

Time, duration, and magnitude of the GnRH-induced LH release for cows and heifers with previous preovulatory LH surges are presented in table 4. As expected, time of the LH release was precise and occurred at about 73.2 h in both heifers and cows. Duration of the LH release by GnRH was similar in both heifers and cows and ranged from 1.7 to 3.5 h and 0 to 3.5 h for heifers and cows, respectively. Average magnitude of LH release for heifers was about twice that of cows and the two groups were different ($P<.10$) in this respect.

Serum Progesterone and Estradiol After GnRH

Serum P and E concentrations after treatment with either GnRH or saline were analyzed considering heifers and cows separately and combined. Timed AI resulted in 5/11 (45.4%) pregnant heifers after GnRH treatment. Treatment employing saline resulted in 4/12 (33.3%) pregnancies. As expected, serum P during 21 d following insemination was greater ($P<.01$) in pregnant than open heifers. The saline-pregnant heifers had higher serum P ($P=.06$) than GnRH-pregnant heifers during the same period while open heifers given GnRH were similar ($P=.23$) in this respect. If only serum P during the first 7 d after estrus was considered, a tendency existed ($P=.16$) for saline-open heifers to have more P than GnRH-open heifers.

Serum E during the first week after AI was greater ($P<.01$) for GnRH-pregnant and GnRH-open heifers than for saline-pregnant and saline-open heifers, respectively. There was a tendency ($P=.13$) for pregnant animals to have higher E than open animals, however, the ratio of E/P was similar in pregnant and open heifers. Ratio of E/P tended to be greater ($P=.12$) for GnRH-pregnant heifers than for saline-pregnant heifers.

Gonadotropin-releasing hormone at 72 h and timed AI at 80 h following PGF-2 resulted in 9/20 (45%) pregnancies in lactating cows. Treatment employing saline resulted in 6/17 (35.5%) pregnant cows. Cows becoming

TABLE 4. CHARACTERISTICS OF THE LUTEINIZING HORMONE RESPONSE TO GONADOTROPIN-RELEASING HORMONE AFTER THE PREOVULATORY LUTEINIZING HORMONE SURGE[a].

Item	n		Interval[b]	Duration[c]	Magnitude[d]
Heifers	5		73.2, 73.2, 73.2, 73.2, 73.2	3.0, 1.7, 3.5, 2.0, 1.7	7.7, 2.8, 6.2, 5.9, 6.7
		X	73.2	2.4	5.9[e]
		SE	0	.5	1.0
Cows	5		73.2, 73.2, 73.2, 73.2, 73.5	0, 1.7, 1.3, 2.5, 3.5	.6, 1.6, 1.7, 4.9, 6.7
		X	73.3	1.8	3.1
		SE	.1	.5	1.0
Total	10	X	73.2	2.1	4.5
		SE	.2	.3	.7

[a]Traits described followed a luteolytic dose of prostaglandin $F_{2\alpha}$. Data are presented in an ordered sequence by individual cow.

[b]Interval (h) to when peak LH occurred.

[c]Duration (h) of LH release.

[d]Peak concentration (ng/ml) of LH during the LH release.

[e]Different from cows ($P<.10$).

pregnant following timed AI had higher P ($P<.05$) than cows failing to conceive when data for the 21 d after estrus were examined. A similar trend was observed during the first week after estrus ($P<.05$). A tendency existed for GnRH treatment to result in suppressed P compared with saline in both pregnant ($P=.11$) and open ($P=.14$) cows during 21 d following estrus. GnRH-pregnant cows had lower serum P ($P<.05$) than saline-pregnant cows if only the first week after estrus was considered. Within pregnant cows, ratio of E/P tended ($P=.15$) to be greater for GnRH-treated animals.

Pooled data for serum P and E for heifers and cows are illustrated in figures 4 and 5, respectively. Serum P during the 21 d after estrus was greater ($P<.001$) in pregnant than open females. GnRH decreased P in both pregnant ($P<.05$) and open ($P<.05$) animals compared with saline controls. Serum E during the 7 d after estrus was similar in pregnant and open animals. However, GnRH treatment induced higher E ($P<.01$) in open animals, but not in pregnant females.

The effect of the type of LH surge on subsequent P concentrations also was examined. Serum P concentration during the first week after estrus was analyzed (to eliminate effect of pregnancy on luteal function) considering only those cattle with available LH profiles. Cows and heifers were combined according to the type of their LH surge and serum P responses are presented in figure 6. Saline cows had higher ($P<.01$) serum P than GnRH-treated cattle. In addition, when GnRH induced the preovulatory LH surge, subsequent serum P was lower ($P<.01$) and slower ($P<.01$) to rise (less slope) than when GnRH treatment occurred after a spontaneous LH surge.

Discussion

Use of GnRH during estrus induced preovulatory-like LH surges. Our data suggest that animals having induced LH surges may not display estrus because all six females with GnRH-induced LH surges were not observed in estrus. This

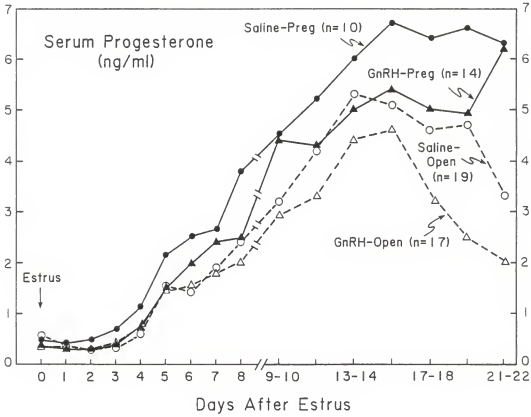

Figure 4. Serum progesterone concentrations for cows and heifers that either conceived (preg) or failed to conceive (open) to a timed insemination (80 h after a luteolytic dose of prostaglandin $F_{2\alpha}$). Gonadotropin-releasing hormone (GnRH) or saline were given 8 h before insemination. Day 0 designates the day of estrus or artificial inseminaton in the absence of observed estrus.

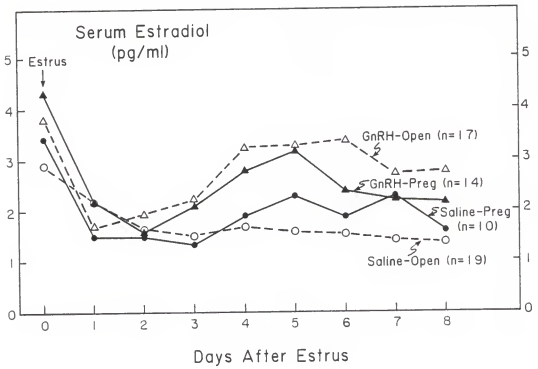

Figure 5. Serum estradiol concentrations for cows and heifers that either conceived (preg) or failed to conceive (open) to a timed insemination (80 h after a luteolytic dose of prostaglandin $F_{2\alpha}$). Gonadotropin-releasing hormone (GnRH) or saline were given 8 h before insemination. Day 0 designates the day of estrus or artificial inseminaton in the absence of observed estrus.

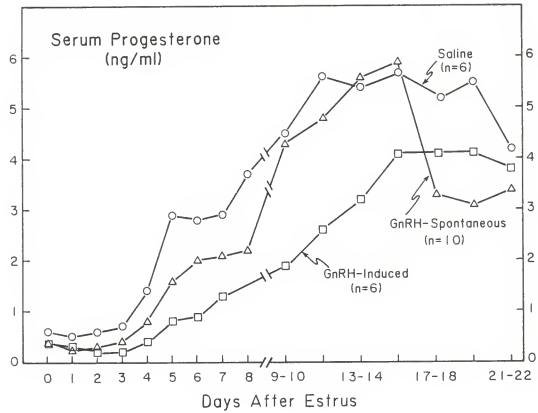

Figure 6. Serum progesterone concentratons in cows and heifers after estrus or artificial insemination in the absence of observed estrus. Females were classified according to their luteinizing hormone (LH) response to saline or gonadotropin-releasing hormone (GnRH) given 72 h after a luteolytic dose of prostaglandin $F_{2\alpha}$ (8 h before insemination). GnRH-treated cattle had either a spontaneous LH surge or one induced by GnRH.

phenomenon was observed by others (Rodriguez et al., 1975). Reasons for no observed estrous behavior may be related to E profiles. Serum E at 72 h was lower in GnRH-induced animals than peak E concentration prior to the LH surge observed for GnRH-spontaneous, GnRH-spontaneous and(or) induced and saline-treated animals. Thus, one reason for no estrous expression might be the lack of adequate E secretion by a mature preovulatory Graffian follicle before the induced LH surge.

Time of the LH surge was dissimilar among GnRH-treated animals. This is understandable due to the nature of treatment, allowing spontaneous surges only to occur before 72 h. A more reasonable estimation of the interval from PGF to the LH-surge is observed in the saline controls. Magnitude of the LH surge was apparently greater in GnRH spontaneous and(or) induced animals. This effect has several plausible explanations. First, exogenous GnRH given at the onset of an endogenous LH surge should be highly potent in terms of its ability to release LH. Pituitary responsiveness to GnRH at or near estrus but before the spontaneous preovulatory LH surge is greater than at any other time during the estrous cycle (Kaltenbach et al., 1974). Thus, GnRH should release a maximum amount of LH in a dose-response manner. A second possibility is that exogenous GnRH is enhancing endogenous LH release, and there is an additive effect between endogenous and exogenous GnRH. Thirdly, this effect could reflect a bias in sampling interval. The numeric magnitude of the LH surge may be misleading when comparing spontaneous with induced or spontaneous and(or) induced animals because peak LH concentration was estimated more accurately in induced and spontaneous and(or) induced animals having an LH surge during the 240-min frequent sampling period. Because peak LH in the animals with spontaneous LH surges was based on one sample every 4 h, detection of peak LH concentration was less accurate.

Preovulatory LH surges of GnRH-induced and GnRH-spontaneous and(or) induced animals, differed greatly in terms of magnitude and duration. This might indicate that the pituitaries of GnRH-induced animals are not primed adequately at the time of exogenous GnRH. During the estrous cycle, ovarian estradiol is thought to be responsible for priming pituitary gonadotrophs (Reeves et al., 1971; Kesner et al., 1981; Padmanabhan et al., 1982) as well as inducing the gonadotropin surge (Beck and Convey, 1977; Martin et al., 1978). Thus, Graffian follicles (with concurrent increases in serum E concentration) may determine time of ovulation, and also ensure maximum release of LH. Exogenous GnRH did not coincide necessarily with the natural timing of endocrine events associated with estrus and may have precluded normal magnitude and duration of the LH surge because the follicle had not matured adequately prior to the induced LH surge. Additional evidence linking E and LH release is apparent if GnRH-induced heifers are considered individually. Magnitude and duration of LH surges following GnRH were directly proportional to serum E concentration at 72 h. This phenomenon was observed by others (Zoleman et al., 1973) and further suggests the importance of adequate serum E preceding the LH surge. The fact that estrus had not yet occurred in the GnRH-induced group suggests that E secretion was insufficient to induce estrus before GnRH treatment.

We administered GnRH at 72 h so that ovulations of animals with an induced LH surge would be timed optimally with insemination at 80 h. However, we observed lower conception following an induced LH surge. This may suggest the absolute necessity of an adequate follicular phase and associated estrous behavior prior to the LH surge and ovulation. If GnRH-induced LH surges are not timed with follicular maturation (as we suggested earlier), then ovulation of immature follicles may occur (as evidenced by aberrant serum P; figure 6), and this could decrease fertility. Magnitude and duration of the LH surge also may

play a role in fertility, and smaller induced LH surges may be contributing to some aspect important for subsequent conception.

When GnRH was administered after an LH surge, the LH release was of lower magnitude and shorter duration. This could be expected and has been attributed to a refractoriness of pituitary cells to GnRH following an LH surge (Kesner and Convey, 1982), and not due to a lack of stored LH in the pituitary gland (Covey et al., 1981). Magnitude of the LH release differed between heifers and cows. Possible responsibility for this fact could lie in the timing of the spontaneous LH surge with respect to GnRH treatment. Heifers had spontaneous LH surges about 10 h earlier than cows, and this could be important with regard to pituitary responsiveness to the exogenous GnRH treatment.

Tendencies existed in both heifers and cows suggesting that serum P during 3 wk after treatment was compromised in pregnant and open animals given GnRH. Therefore, in order to elucidate the GnRH effect, data from the two groups were pooled (figure 4). As expected, serum P was higher in pregnant than open animals. In addition, GnRH-pregnant animals had lower serum P than saline-pregnant animals. In open animals, GnRH treatment also decreased P compared with saline controls.

Three possible reasons may exist for suppressed P after GnRH. First, the effect of a small GnRH-induced LH release after a spontaneous LH surge is not known. Depletion of pituitary LH stores to the extent that LH release during the luteal phase is compromised is unlikely, although some evidence for short-term (12 h) depletion of releasable LH after exogenous GnRH exists (Beck et al., 1983). Second, GnRH-induced animals were shown to have LH surges of reduced magnitude and duration. The resulting effect of suppressed LH surges on the luteinization of cells in the follicle is not known. Conceivably, animals with induced LH surges may have lower P due to reduced

mitoses of thecal and granulosa cells and(or) inadequate luteinization. We found that GnRH-induced animals had lower serum P than GnRH-spontaneous or saline cattle (figure 6). Thus, the presence of a subpopulation of animals with induced LH surges within the GnRH-treated group may be lowering serum P with respect to saline controls. A third possibility, that GnRH is acting directly on the ovary to inhibit granulosa and luteal cell function, is supported by evidence in the rat (Rippel and Johnson, 1976) and human (Sheehan et al., 1982). However, two studies have demonstrated that a direct effect on the bovine ovary in not likely due to a lack of GnRH receptors (Brown and Reeves, 1984) and inablility of GnRH to inhibit P secretion of luteal cells in vitro (Milvae et al., 1984).

Pooling data from cows and heifers and analyzing E during 7 d after estrus revealed no effect of GnRH for pregnant animals. On the other hand, GnRH increased E when given to animals that subsequently failed to conceive. Differences in E after GnRH were probably resulting from the follicular population present on ovaries during d 3 to 7. GnRH administration may have caused a larger follicular population by rescuing atretic-bound follicles, and/or a more active secretion of E by follicles.

Our study indicates that two concepts about the use of GnRH in cattle may be inaccurate. First, the idea that beneficial effects on fertility of GnRH after PGF are derived from inducing ovulations of otherwise late ovulatory ovarian follicles may be untrue. We observed lower fertility in cows with GnRH-induced LH surges and this effect may be the result of poor timing of the induced LH surge with final follicular maturation. This observation is supported by other research, where the possibility of lower fertility in induced animals or animals not showing estrus after GnRH was suggested (Burfening, 1976; Roche, 1977). Second, serum P during the luteal phase following GnRH is not

increased. In fact, our data suggest a suppression of P following GnRH. It appears likely, therefore, that action of GnRH on fertility is mediated by some other means than augmenting serum P or synchronizing ovulations in treated animals.

In sheep, it has been postulated that asynchrony between luteal function and the developing embryo may lead to a premature rise in serum P which causes an accelerated uterine state in relation to the conceptus (Wilmut and Sales, 1981). Asynchronous embryos, although displaying compensatory growth, do not develop the ability to prevent uterine-induced luteolysis (Lawson et al., 1983). Therefore, increased fertility after GnRH in cattle may be a result of a delayed rise in serum P which may reduce early embryonic death due to a progesterone-induced advanced uterine state. If true, decreased serum P after GnRH could indicate one possible mechanism of GnRH action on improved conception.

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CONTROL OF INTERVALS TO FIRST SERVICE AND ATTEMPTS TO IMPROVE FERTILITY IN DAIRY CATTLE USING PROSTAGLANDIN $F_{2\alpha}$ AND GONADOTROPIN-RELEASING HORMONE

by

Matthew Christian Lucy

B. S., Cornell University, 1982

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Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
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ABSTRACT

Lactating Holstein cows (n=283) were inseminated according to estrous detection beginning 6 wk postpartum (control) or timing of first services were controlled using two luteolytic doses of prostaglandin $F_{2\alpha}$ or PGF (PGF-1 and PGF-2) at an 11-day interval beginning 40 to 46 days postpartum (treatment) followed by artificial insemination (AI) by appointment. Timed AI methods after PGF-2 (0 h) were: 1) AI at 80 h, 2) gonadotropin-releasing hormone (GnRH) at 72 h and AI at 80 h, and 3) AI at 72 and 96 h. Blood was collected from 176 lactating cows and 24 virgin heifers to determine luteolytic response to PGF and effect of GnRH on serum concentrations of luteinizing hormone (LH), progesterone (P), and estradiol-17ß (E). Nearly 28% of treated cows failed to have regression of the corpus luteum after PGF-2 due to either PGF inefficacy (14%), or lack of significant P concentrations (<1 ng/ml) because of inappropriate stage of the estrous cycle (10%) or due to postpartum anestrus (5%). Cows not receiving GnRH had higher conception if luteolysis had occurred after both PGF treatments than those with partial or no luteolytic response at either time. Variance and interval to first service (IFS) were reduced for treated cows than controls. Conception at first service was similar for timed AI methods, but inferior to controls. Days from calving to conception (days open) were unaffected by treatment, except for reduced variance in that interval for cows with timed AI at 80 h without GnRH. Gonadotropin-releasing hormone after PGF induced preovulatory LH surges in 33% of cows (n=7) and heifers (n=11) treated during estrus. Cattle with GnRH-induced LH surges had lower E prior to the LH surge, LH release of shorter duration, lower conception, lower serum P during the subsequent luteal phase, and no estrous expression compared with animals having spontaneous LH surges. Serum P during 21 days following treatment was reduced after GnRH treatment for pregnant and nonpregnant

cows. Nonpregnant cattle had higher serum E during 7 days after estrus if previously treated with GnRH at estrus. These data demonstrate that PGF can control IFS, but not reduce the variance in days open for dairy cows. Gonadotropin-releasing hormone does not appear to improve conception by inducing preovulatory LH surges and(or) increasing serum P after insemination.