SYNCHRONIZATION OF ESTRUS IN HEIFERS WITH MELENGESTROL ACETATE (MGA) $\qquad \text{AND PROSTAGLANDIN F}_2 \text{ALPHA}$

by

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LITERATURE REVIEW

Progestogens for Estrus Synchronization

Effective levels and administration

Artificial insemination is a very useful tool for genetic advancement in beef cattle. However, artificial insemination appears to be very costly and time consuming. The research goal on regulation of the estrous cycle is to devise a simple, cost-effective method that will result in synchronization of estrus and ovulation with enough precision to permit insemination at a single preset time, thereby eliminating the need for observation for signs of estrus.

Use of progestogens for synchronization of estrus in cattle has a history of nearly 40 yr. Daily injections of progesterone in oil have been shown to inhibit estrus and ovulation in the ewe, gilt, and cow, respectively, by Dutt and Casida (1948), Ulberg et al. (1951), and Christian and Casida (1948). Estrus occurred in the cow 5 to 6 d after cessation of daily 50 mg injections of progesterone.

Exogenous progestogens inhibit gonadotropin release, which prevents ovulation but allows development of ovarian follicles (Kaltenbach and Dunn, 1980). However, a single injection of progesterone in a starch suspension given to heifers on d 16 or 17 of the estrous cycle had an inhibitory effect on follicular growth and atresia in many follicles (Nellor and Cole, 1957).

Ulberg et al. (1951) utilized heifers on different days of the estrous cycle and administered varying doses of progesterone. A daily dosage of 50 mg suppressed estrus and ovulation, but allowed some follicular growth during treatment. However, the follicle ovulated at the synchronized estrus was in no case the follicle estimated to be largest at the end of treatment. More

follicular growth and one silent ovulation occurred in heifers treated daily with 25 mg progesterone. As dosage fell below 25 mg, greater follicular growth occurred and a higher percentage of heifers ovulated during treatments.

Zimbelman and Smith (1966) reported the mean follicular size at lower ovulation-inhibiting doses of melegestrol acetate (MGA) (.22 or .42 mg per day) was almost always greater than at a higher (.85 mg) dose. The minimal effective dose (.42 mg) for ovulation inhibition in most heifers in a group, produces the condition of greatest follicular development with maximal incidence and size of follicles. A lower dose was associated with a decreased incidence of large follicles and a higher dose was associated with a higher number of follicles, but smaller follicular size.

Adrenal weights of heifers following long-term administration of MGA were significantly heavier than those of control heifers (Zimbelman and Smith, 1966). Researchers also noted MGA tended to decrease adrenal weight in spayed heifers while increasing adrenal weights of intact heifers (Zimbelman and Smith, 1966).

Estrus Synchrony and Interval to Estrus

Degree of estrous synchrony after progestogen has had varying results and is dependent on route of administration. Nellor and Cole (1956) administered single injections containing 540 to 1120 mg crystalline progesterone in a starch emulsion. Estrus occurred 15 to 23 d after progesterone injection. Chakraborty et al. (1971) fed 1 mg MGA daily to dairy heifers for 14 consecutive days. All heifers exhibited estrus 3 to 6 d following MGA removal. Estrus was exhibited by 75% of treated females within 24 h and 83% exhibited estrus within 48 h. These findings agree with those of

Zimbelman and Smith (1966) and Wiltbank et al. (1967). Curl et al., (1968) sc implanted capsules containing 57 to 248 mg norethandrolone (SC-5914) for 16 d. Of the 22 cows that did not lose their capsules and(or) exhibited estrus during treatment, 81% exhibited estrus within 48 h after capsule removal. Zimbelman and Smith (1966) reported as level or daily dosage of progestogen treatment increased, synchrony and interval from last feeding to onset of estrus increased.

Variation in the interval from end of progestogen treatment to the onset of estrus is due to a number of factors (Ulberg and Lindley, 1960). Dosage of progestogen was reported to have a noted effect upon time of onset of estrus. Animals receiving daily subcutaneous injections of 25 mg progesterone injections for 14 d had an average interval to estrus of 4.8 d with a range of 3 to 9 d. This can be compared with the response of animals treated with 50 mg progesterone. The average interval for this treatment was 5.7 d with a similar range. Day of cycle at the beginning of progesterone treatment was not correlated significantly with interval to onset of estrus. However, body weight at the time of treatment was correlated with the length of this interval for cows who were nursing calves. No such correlation was observed in non-lactating animals.

Orally active progestogens

Due to the impracticality of administering daily injections and limited success of single injection compounds, orally active progestogens that can be incorporated into the daily diet seemed to be a more desirable mode of administration. A number of orally active progestogens have been used for estrous control. Nelms and Combs (1961) conducted three trials noting the effect of 6-methyl-17-acetoxyprogesterone (MAP) on estrus and fertility in

beef cows. The studies indicated cows fed 250 mg MAP daily for 14 d exhibited estrus on the second and third day after the MAP removal. No measure of fertility was made. However, others have noted similar data on degree of synchrony of estrus and reported lower fertility when compared with controls (Collins et al., 1961; Hansel et al., 1961; Anderson et al., 1962; Dhindsa et al., 1967). Wagner et al. (1968) fed 10 mg 6-chloro-6-dehydro-17-acetoxy-progesterone (CAP) for 18 d. Heifers treated with CAP had lower fertilization rates (61%) than controls (82%). The researchers also noted embryonic mortality was not greater among CAP-treated heifers than controls that was earlier suggested as the major factor responsible for reducing the pregnancy rate in progestogen-treated cows (Wiltbank et al., 1967). Wiltbank et al. (1967) sychronized estrus in yearling heifers using 500 mg 16-17-dehydroxyprogesterone acelophenide (DHPA) daily for 20 d. Fertility was lowered once again.

Research using 17-acetoxy-6-methyl-16-methylene pregna-4, 6-diene-3, 20-dione (MGA, The Upjohn Company, Kalamazoo, MI) has spanned the last 20 yr. MGA is a potent orally active progestogen that is currently marketed for increased rate of gain, improved feed efficiency, and suppression of estrus in heifers fed for slaughter. Zimbelman and Smith (1966) reported MGA inhibited estrus and ovulation in all heifers except one receiving .5 mg daily for 15 to 18 d. Data also suggested lower doses suppressed estrus but did not uniformly inhibit ovulation. Orally, MGA was about 300 to 900 times more potent than MAP, but only ten times more potent when both were compared after intravenous injection. Since that time the literature has noted numerous studies regarding the use of MGA for estrus control (Rousell and Beatly, 1969; Wilson et al., 1969; Chakaborty et al., 1971; Chupin et al., 1975; Hill et

al., 1971; Hendricks et al., 1973; Moody 1978; Randel et al., 1972; Lamond et al., 1971).

Fertility

Fertility following progestogen administration has been notably lower than untreated controls (Nellor and Cole, 1956; Hansel et al., 1961; Anderson et al., 1962). Since bovine ova travel down the oviduct at a greatly increased rate in the presence of progesterone, asynchrony between development of the embryo and the uterus may have occurred (Rowson, 1951). Hafez (1980) states the disturbance of ova transport is well known in laboratory animals under steroidal hormone treatment. Excessive progesterone production or progestogenic treatments shortly after ovulation may produce a similar result in farm animals, leading to temporary infertility. Ovarian steroidal hormones control the activity of an electrical pacemaker localized in a discrete area around the ampullary-isthmic junction (Talo and Brundin, 1971). In goats, pronounced activity and groups of positive pulses are recorded during estrus. In diestrus, when progesterone is the predominant ovarian secretion, activity is abolished and groups of negative pulses dominate. The electrical pulses and the changes in their direction are assumed to be involved in the underlying control of ova transport in the isthmus.

Ulberg and Lindley (1960) noted lower fertility in progesterone-treated heifers at the synchronized estrus than in untreated controls. Delayed embryo cleavage noted in progesterone-treated heifers was suspected to contribute to these lower conception rates. Ray et al. (1961) looked at histological sections of uterine endometrium and found superficial and glandular epithelial cells to have greater height in control heifers than in heifers treated with progesterone. This indicates that exogenous progestogens reduce the secretory

activity of uterine endometrial glands.

First-service conception rates have generally been lowered to the point of impracticality after prolonged oral progestogen treatment (14 to 18 d) (Willet, 1950; Ulberg et al., 1951; Trimberger and Hansel, 1955; Curl et al., 1968; Whitham et al., 1972; Woody and Pierce, 1974). However, a short term treatment with progestogens does not significantly lower first service fertility. Chupin et al. (1975) found first service fertility rates were 62%, 57.5%, 45.6%, and 26% for treatments with subcutaneous implants of norgestomet (SC-21009) for durations of 7, 9, 11, and 13 to 15 d in lactating cows, respectively. This finding was substantiated by other reports indicating normal fertility was associated with progestogen treatments of less than 10 d (Roche, 1974; Wishart and Young, 1974; Woody and Pierce, 1974; Sreenan and Mulvehill, 1975; Woody and Abenes, 1975). The conception rate at the second post-treatment estrus, regardless of length of progestogen treatment, appeared to be normal indicating the lowered fertility associated with long-term progestogen treatment was short-lived (Ulberg, 1955; Trimberger and Hansel. 1955).

Prostaglandin F₂alpha for Estrus Synchronization

Properties

Prostaglandin F₂alpha (PGF), a very effective means of estrous control, has a shorter history than progestogens. McCracken et al. (1970) reported exogenous PGF caused precocious regression of the corpus luteum (CL) in sheep. Other researchers noted PGF also caused CL regression in rats (Bishop and Flack, 1973), horses (Douglas and Ginther, 1974), swine (Hallford et al., 1974) and cattle (Rowson et al., 1972; Louis et al., 1972; King and Robertson, 1974; Roche, 1974; Hafs et al., 1975).

Prostaglandin F₂alpha is an unsaturated monocarboxylic acid of 20 carbon atoms on a 5-membered ring with two adjacent sidechains. The free form is soluble in alcohol, and the sodium or tromethamine (THAM) salt is soluble and stable in water (Walpole, 1975). There are four major categories of prostaglandins delineated by differences in the cyclopentane ring. These are A, B, E, and F. Further differentiation is achieved by indicating the number of double bonds present with a subscript number in the name. In the F series, the configuration of the C9 hydroxyl group is indicated as a or b. Only the a forms occur naturally.

Prostaglandin F_{2} alpha is biosynthesized from arachidonic acid in many mammalian tissues. Prostaglandin F_{2} alpha is not stored in tissues, but is released directly into the blood where it is rapidly metabolized by the lung and kidney. Over 90% of the circulating PGF is converted to 15-keto-13,14-dihydro-PGF (the main plasma metabolite) during a single passage through the lung (Vane, 1969; Kindahl, 1980).

Luteolysin

Prostaglandin F₂alpha can be used as a method of estrous synchronization in cattle due to its luteolytic properties. In cycling cattle, PGF is ineffective when administered on 0 to 4 d of the estrous cycle (estrus = d 0) (Lauderdale, 1972; Rowson et al., 1972). Neither dairy nor beef heifers responded to treatment of PGF when administered on d 0 to 4, yet treatment was effective on d 5 to 16 of their estrous cycle (King and Robertson, 1974; Ellicott et al., 1974; Lauderdale, 1972). Two injections of 25 mg PGF given on d 4 or twice daily injections on two consecutive days earlier in the cycle will not reduce the life span of a corpus luteum (Beal et al., 1980).

Luteolytic mechanisms cause the CL to regress beginning on d 16 during

a normal estrous cycle in the bovine (Gomes and Erb, 1965). Cattle will show estrus 2 to 4 d after luteolysis. Thus cattle injected with PGF after d 16 will be in estrus 1 to 4 d after treatment. However, the resulting estrus may not be due to PGF treatment, but due to normal spontaneous regression of the CL.

The mode of action PGF takes in the regression of the CL has been studied for the last 12 yr. Goding (1974) summarized the evidence for the uterine production of PGF and its role as a natural luteolytic agent in sheep. Subsequent work has confirmed this finding and shown ovarian estradiol-17 and progesterone regulate the concentration of oxytocin receptors in the endometrium and subsequent release of PGF from the uterus (McCracken et al., 1981).

The route by which PGF is transferred from the uterus to ovary has been controversial (Goding, 1974), but the transfer mechanism hypothesis has gained credence (McCracken et al., 1972; Land et al., 1976).

Heap et al. (1985) reported ³H-PGF placed in the uterine lumen of sheep is transferred into lymph system. This means PGF enters the extracellular compartment of the uterus drained by the lymphatic vessels and is transferred locally from the arterial lymphatic vessel into the adjacent ovarian vasculature. However, reports of previous studies have emphasized the importance of local veno-arterial transfer of the luteolysin without contributions from other local routes (McCraken et al., 1971; Ginther, 1981). Yet, exclusion of the lymphatic route as a means of transfer is difficult to achieve because surgical manipulation of the ovarian and uterine vessels would be accompanied by damage to the lymphatic vessels followed by a rapid regeneration of lymphatic vessels and(or) redistribution of lymph flow through

collateral vessels (Heap et al., 1985). Thus, this route of transfer has important physiological consequences in the regulation of the corpus luteum.

Hormone Profiles

Reproductive events following PGF are similar to what normally occurs 3 d before estrus in untreated cattle (Louis et al., 1974; Swanson and Hafs, 1971; Wetteman, 1972; Chenault et al., 1975). Cows given intrauterine PGF noted progesterone in blood serum declined rapidly to less than .5 ng/ml by 24 h post-treatment. This demonstrates rapid PGF-induced regression of the corpus luteum. After the progesterone decline, estradiol concentrations increased until the occurrence of a preovulatory luteinizing hormone (LH) surge at which time plasma estradiol declined (Chenault et al., 1976).

Interval to Estrus and Estrus Synchrony

Average time from PGF treatment to the onset of estrus is variable and ranges from 45 to 92 h (Hardin et al., 1980; Thimonier et al., 1975). Factors affecting this interval are day of estrous cycle at treatment, season of the year, age and breed of animal. King et al. (1982) found interval to estrus was not different for heifers treated with PGF on d 6 through 9 of their estrous cycle, but the interval increased by 9 h on d 10, and by 14 h on d 11 through 15. Similar results due to the stage of the estrous cycle have been reported (Dobson et al., 1975; Thimoiner et al., 1975; Johnson, 1978; Jackson et al., 1979; Stevenson et al., 1984).

Dobson et al. (1975) proposed shorter intervals to estrus in cattle on d 6 to 8 were due to the mid-cycle follicular growth. Edqvist et al. (1975) agreed that the mid-cycle follicles continue to mature and ovulate. Jackson et al. (1979) found significantly higher levels of FSH in serum of animals treated with cloprostenol (analog of PGF) on d 6 to 9 than those treated on d 11 to

15. Moreover, Jackson et al. (1979) found FSH, but not LH nor estradiol, was different in early-cycle compared with late-cycle heifers at PGF treatment. Others found serum progesterone at the time of cloprostenol admininistration was correlated positively with the interval to estrus (Refsal and Sequin, 1980; Chenault et al., 1976; King et al., 1982). Stevenson et al. (1984) also observed late-cycle heifers had higher concentrations of progesterone than early-cycle heifers $(7.4 \pm .3 \text{ vs } 3.9 \pm .2 \text{ ng/ml})$. However, some researchers noted interval to estrus appeared to be correlated to the decline of progesterone in serum after PGF (Stevenson et al., 1984).

Season of year affects interval to estrus (Jaster et al., 1982; Britt et al., 1978). However, more recent data suggested when stage of cycle is accounted for, season of year has no effect on interval to estrus (Stevenson et al., 1984). The interval to estrus is significantly shorter for both beef (Burfening et al., 1978; King et al., 1982) and dairy heifers (Plunkett et al., 1984) when compared with lactating cows.

Time of day (Britt et al., 1978) and dose of PGF (Stellflug et al., 1973; Hafs et al., 1975; Louis et al., 1975) appear to have no effect on interval to estrus.

The degree of estrous sychrony after PGF treatment has been variable. A single injection of PGF given to beef cows and heifers which had a palpable CL exhibited estrus over a 7 d period. Eighty-eight percent were in estrus on d 3, 4, and 5 after treatment (Lauderdale et al., 1974; Sequin et al., 1978). The degree of estrous synchrony after the second of two injections of prostaglandin given 11 d apart is greater than after a single injection. Many researchers suggest the more precise onset of estrus after the second injection is due to a greater number of animals being at a similar stage of the

estrous cycle (Johnson, 1978; Refsal and Sequin 1980). Cooper and Rowson (1975) obtained a high degree of synchrony after a second injection of PGF. During a 24-h period, 91% of the heifers were in estrus, whereas 48% of the cows were in estrus during a 24-h period following a second injection (Hafs et al., 1975).

Failure to Synchronize

Unobserved estrus or ovulation without estrus (Lauderdale, 1975; Cooper and Rowson, 1975; Thimonier et al., 1975; Sequin, 1978) and incomplete or delayed regression of the corpus luteum (King and Robertson, 1974; Refsal and Sequin, 1980; Chenault et al., 1976) are reasons for failure to synchronize estrus. Roche and Prendiville (1979) reported 16% of the dairy cows which showed estrus after the first injection of cloprostenol responded 1 to 6 d post treatment. At the second injection, these cows were on d 4 or 5 of an estrus cycle and failed to respond to PGF.

Fertility

Conception rates after estrus resulting from PGF treatment appear to be equal to nonsynchronized cattle. Lauderdale et al. (1973, 1974) found no difference in conception rate between nontreated cows (Group I) and two groups of PGF-treated cows. Both treatments were administered im 30 mg PGF, one group being inseminated by estrus (Group II) and the other (Group III) being inseminated at two predetermined times (72 h and 90 h post-injection). All cows had a functional corpus luteum at treatment and conception rates were 53.3%, 52.2%, and 55.8% respectively for groups I, II, and III. These findings were later substantiated by Roche (1974), Cooper (1974), and Edqvist et al. (1975).

Lauderdale et al. (1981) administered seven injections of PGF at 10 to

12 d intervals to beef heifers. After the final treatment, heifers were inseminated as they showed estrus. Fertility of treated animals was not significantly different from untreated controls.

Insemination by appointment

Estrous detection and artificial insemination requires much labor and may be quite costly. Moreover, a poor manager may be ineffective in detecting estrus. One possibility with estrous synchronization is timed inseminations may be possible and estrous detection eliminated. Burfening et al. (1978) compared two groups of cows that received two im injections of 25 mg PGF im to untreated controls bred 12 h post-estrus. Treated cows were bred either at 72 and 96 h, or only once at 80 h. No differences in conception were found. Similar results have been obtained by Hafs et al. (1975), Louis et al. (1975), Chipepa et al. (1977), and Wilson et al. (1978).

Conception rates in late cycle heifers tend to be higher than for early cycle heifers (Stevenson et al., 1984). They also reported the stage of cycle had a tremendous effect upon rates of conception after timed inseminations at 80 h post injection. Conception rate for early cycle heifers (d 5 to 8) bred according to estrus was less when compared to early cycle heifers inseminated 12 h after the onset of estrus or late cycle heifers timed inseminated at 80 h. Eighty hours post-injection was too late to maximize conception of early cycle heifers.

Estrus Synchronization Schemes

As previously stated, PGF is ineffective in causing luteolysis during the first 4 to 5 d of the bovine estrous cycle. It becomes necessary to prevent bovine females from being in this stage of cycle in order to synchronize successfully estrus in a herd. One method involves the use of two injections

of PGF given 10 to 12 d apart. King and Robertson (1974) used a synchronization program in which Holstein heifers received two injections of 30 mg PGF 10 d apart. All heifers were inseminated by estrus and no differences were found in conception rates compared with untreated controls.

A second approach to overcoming the problem of the inactive period of PGF has involved the use of progestogens prior to the PGF administration. Thimonier et al. (1975) used a 10 d sc implant of 12 mg norgestomet followed by a 500 g im injection of cloprostenol at implant removal. synchronization was similar to cows given two injections of 500 g cloprostenol 10 d apart. Hershee et al. (1979) reported treatment with a 7 d 6 mg implant of norgestomet followed by im injection of PGF, given at either 24 h before or time of implant removal, were effective methods of synchronizing estrus in beef heifers. Similar results were reported by Whishart (1974), Deletang (1975) and Beal et al. (1984). Smith et al. (1984) reported the interval to estrus and degree of estrus synchrony associated with a progesterone-releasing intravaginal device (PRID), in situ for 6 or 7 d, plus administration of PGF on the day of PRID removal was not significantly different from control heifers. However, conception rates were greater in heifers receiving the PRID than in heifers treated with 2 injections of PGF with an 11 d interval (66% vs 52%).

A more practical approach to the combination of progestogen plus PGF treatment has been the utilization of orally active progestogens because of the added labor and cost required in administering implants and PRIDs. Moody et al. (1978) reported no difference in first-service conception of untreated controls compared with cows which were fed 1.0 mg MGA daily for 5 d followed by 25 mg PGF administered on the last day of MGA feeding. Beal

and Good (1985) compared cyclic and anestrous cows treated with a norgestomet implant (6 mg for 9 d) and PGF (1 d prior to implant removal) or cows fed MGA (.6 mg MGA/d for 9 d) and given PGF on the last day of feeding. They noted conception rates did not differ by pretreatment reproductive status (cyclic vs anestrus) and MGA combined with PGF was an effective, economical method of estrous synchronization.

A third method by which the period of the immature CL can be circumvented involves using estrus detection in combination with PGF. Estrus is detected for 4 or 5 d prior to injection of PGF. All females that are within 1 to 4 d of estrus can be inseminated early during this period and not injected with PGF. Those cows not showing estrus after the first 4 or 5 d of breeding season receive PGF and are then inseminated post-injection. First-service conception rates did not differ between this management scheme, cows receiving two injections of PGF 11 d apart, and untreated controls when all cows were inseminated according to estrus (Lauderdale et al., 1980; Wilson et al., 1981).

Factors Altering Age of Puberty

Age at puberty in heifers is affected by breed (Gregory et al., 1979; Laster et al., 1979; Nelsen et al., 1982), nutrition (Joubert, 1963; Wiltbank et al., 1969; Short and Bellows, 1971), and metabolic-endocrine interactions induced by growth promotants (McCartor et al., 1979; Mosely et al., 1982).

Puberty may be defined as a sequence of events leading to first estrus and ovulation in young females. Heifers were not deficient in circulating levels of pituitary or hypothalamic hormones 2 mo preceding the onset of puberty (Gonzalez-Padilla et al., 1975a). Concentrations of progesterone in serum were low (300 pg/ml) in the prepubertal period, but there were two

distinct elevations in every heifer before estrus (Gonzalez-Padilla et al., 1975a). The return of first progesterone elevation to baseline was always followed by the priming peak of lutenizing hormone (LH), while the second preceded the pubertal peak of LH (Gonzalez-Padilla et al., 1975a). The first prepubertal increase in progesterone was produced by luteal tissue embedded within the ovary, but not palpable or observable grossly on the ovarian surface (Berardinelli et al., 1979).

Gonzalez-Padilla et al. (1975b) noted an increased response to endogenous LH by prepubertal ovaries primed with progesterone. Short et al. (1976) induced estrus in prepubertal heifers in response to a short-term progestogen implant and an estradiol valerate injection 24 h after implant removal. A 5-mg estradiol valerate injection and 3-mg norgestomet implant successfully induced puberty in heifers (Gonzalez-Padilla et al., 1975b).

Beal et al. (1984) noted estrus was detected in a greater proportion of cyclic animals prior to treatment (88%) than among those anestrous prior to treatment (77%) with a 9-d sc norgestomet implant and either a 5-mg estradiol valerate and 3-mg norgestomet im injection at the time of implant removal or 25-mg PGF injection 24 h before implant removal. Pregnancy rates after 5 d were similar between cyclic (42%) or anestrous (47%) heifers prior to treatment.

Prostaglandins F_2 alpha (PGF and its analogs) will not initiate ovarian activity in acyclic bovine females (Roche et al., 1978). Roche (1976) palpated ovaries of heifers before giving two im injections of cloprostenol (ICI 80,966) ll d apart. Heifers with inactive ovaries at the beginning of treatment did not show synchronized estrus.

SYNCHRONIZATION OF ESTRUS IN HEIFERS WITH MELENGESTROL ACETATE (MGA) AND PROSTAGLANDIN F_2 alpha

Introduction

The goal of estrous cycle regulation is to devise a simple cost-effective method that will result in synchronization of estrus and ovulation with enough precision to permit insemination of animals at a single preset time, thereby eliminating the need for observation of animals for signs of estrus.

Prostaglandin F₂alpha (PGF) causes luteolysis and return to estrus in bovine females when administered on d 5 to 16 of the estrous cycle (Lauderdale, 1972; Rowson et al., 1972). One treatment of PGF will not effectively synchronize estrus in an entire herd of randomly cycling cattle since PGF is only effective in regressing the corpus luteum on d 5 to 16 of the estrous cycle. Fertility of cattle at the induced estrus after PGF is normal (Inskeep, 1973; Lauderdale et al., 1974; Roche, 1974). Two injections of PGF given 10 to 12 d apart should synchronize all cycling animals after the second injection (King and Robertson, 1974; Lauderdale, 1974). This method overcomes the problems posed by the fact a single injection of PGF given during d 1 to 5 of the estrous cycle is ineffective in producing luteolysis. When the two injection method was combined with fixed time inseminations at 72 and 96 h after the second injection, conception rates were similar to those in untreated control animals bred at a naturally occurring estrus. Later, Cooper and Rowson (1975) reported acceptable conception rates

could be obtained after two injections of PGF with a single insemination at 80 h after the second injection. However, results of some trials (Roche, 1977; Hansel and Fortune, 1978; Roche et al., 1981) suggested acceptable pregnancy rates are not always obtained by using the two dose method.

Interval from PGF to estrus is influenced by the stage of the estrous cycle at the time of PGF administration (King et al., 1982; Tanabe and Hann, 1984). Therefore, if one could manipulate all heifers to be in a similar stage of the estrous cycle, one could expect a higher degree of synchrony. In addition, conception rates in late cycle heifers tend to be higher than for early cycle heifers (Stevenson et al., 1984).

Research to develop improved methods of estrous cycle control has continued. One approach to improve synchronization of estrus has been to combine PGF treatment with an orally active progestogen (Moody et al., 1978; Fitzgerald et al., 1985). Beal et al. (1985) reported that cows fed melengestrol acetate (MGA, .6 mg/d for 9 d) and injected with PGF (last day of MGA feeding) had acceptable conception rates; however, estrous synchrony spanned a 168 h period following the last MGA feeding.

The objectives of our experiments were to further study a method of estrous synchronization combining short-term MGA treatment with a single dose of PGF and to compare pregnancy rates and interval to estrus among various treatments. Further attempts to manipulate an entire herd of heifers into a similar stage of the estrous cycle for comparison of conception rates at a fixed insemination time after PGF administration also were conducted.

Materials and Methods

Exp. 1

Pubertal heifers (n=43, 12 to 14 mo of age) from one ranch at Cassoday, KS, were maintained at the Kansas State University Beef Research Unit (May, 1985). Heifers were checked twice daily (30 min/check) for estrus during 30 d preceding onset of treatment (d 1). Concentrations of progesterone in serum were monitored 14 d and 4 d before beginning treatment. Only heifers with one or more observations with progesterone greater than 1 ng/ml and those observed in estrus during the 30 d pretreatment period were used in the experiment. Selected heifers were weighed 14 d prior to treatment and body weights are shown in Table 1. Forty-one heifers (10 Hereford, 13 Brangus X Hereford, 12 Red Angus X Hereford, and 6 Senepol X Hereford) were selected and assigned randomly to three treatment groups.

All heifers received approximately 1.0 kg milo carrier. Heifers (n=14, 290 $^{\pm}$ 10.0 kg) in group 1 (MGA-7) were fed .5 mg MGA daily for 7 d in the concentrate and received a single im injection containing 25 mg PGF (Lutalyse, The Upjohn Company, Kalamazoo, MI) on the last day of MGA feeding (d 7). Heifers (n=13, 295.7 $^{\pm}$ 10.4 kg) in group 2 (MGA-6) were fed .5 mg MGA daily for 7 d and received a single im injection of 25 mg PGF on d 6 of MGA feeding. Group 3 (n=14, 290 $^{\pm}$ 10.0 kg) served as controls (C).

Heifers were housed and maintained as three groups until after the last day of MGA feeding. Concentrate was fed and consumed before any additional feed was offered. Feed bunks had sufficient feeding space (minimum 60 cm) to allow all heifers to consume the supplement simultaneously. Concentrate was

spread evenly over the length of the feed bunk to enhance the opportunity for equal consumption by all heifers. On the seventh (last) day of MGA feeding, heifers to receive PGF were injected and then all heifers were combined in a single pen.

All heifers were observed for estrus twice daily to determine the suppression of estrus during MGA feeding. All heifers were observed for estrus six-times daily at 4-h intervals until 8 d following last MGA feeding (0200, 0600, 1000, 1400, 1800, and 2200 h) for breeding purposes. Following the frequent period of estrous detection all heifers were observed twice daily for estrus until 45 d following last MGA feeding. All heifers detected in estrus following the last MGA feeding were inseminated artificially 12 hr after the onset of estrus. Semen from 8 bulls was used and balanced across all treatment groups. Conception rates were determined by date of parturition.

Blood was collected by jugular venipuncture at 0 and 48 h following PGF. Blood collection continued from all heifers on Monday, Wednesday, and Friday for 4 wk after treatment to determine luteal function. All blood samples were placed on ice immediately after collection and stored at 4 C overnight. Serum was harvested by centrifugation at 1500 x g for 20 min. Serum was maintained at -20 C until assayed for progesterone.

Concentrations of progesterone in serum were quantified by radioimmunoassay according to Skaggs et al. (1986). Progesterone was measured using a highly specific antiserum obtained from rabbits immunized against progesterone-11-hemisuccinate: BSA. Tritiated-progesterone extracted from bovine serum with ethyl acetate averaged 90% in four assays. Progesterone was recovered quantitatively when added to serum (r=.99). Serum curves paralleled progesterone standards. Variable volumes of serum (.1, .15,

and .2 ml, n=4 each) from estrous cows and luteal-phase cows measured .49, .34, .4 ng/ml and 3.24, 3.34, 3.43 ng/ml. Assay sensitivity was 25 pg/tube. Intraassay coefficient of variation was 12.5% and interassay coefficient of variation was 16.2%.

Comparison of progesterone in serum at 0 and 48 h after PGF was used to determine luteolytic success. Effective corpus luteum (CL) regression was defined as a decline in progesterone from greater than 1 ng/ml at 0 h to less than 1 ng/ml 48 h later. Progesterone in serum during 45 d following inseminations was used to determine duration of estrous cycles. Duration of the estrous cycle was determined by time interval from first progesterone rise to second progesterone rise. Data on the interval from last MGA feeding to onset of estrus also were recorded.

Conception rates were defined as the number of heifers pregnant/number inseminated. Synchronized pregnancy rates were defined were defined as the number to conceive during 3 to 8 d following MGA/number treated. Pregnancy rate for controls was defined as the number of heifers pregnant during the first 21 d/number treated. Data were analyzed using least-squares procedures of General Linear Model procedure of Statistical Analysis System (SAS, 1982). Effects of breed, treatment, and concentration of progesterone in serum were examined. Preplanned orthogonal contrasts were used to compare treatment means. Percentage data were tested by Chi-square (Snedecor and Cochran, 1980).

Exp. 2

Yearling heifers were used to study the results of artificial insemination at a predetermined time following MGA and PGF without regard to estrus.

Pubertal Holstein heifers (n=19, 14 to 16 mo of age) from the Kansas State University Dairy herd were housed in drylot conditions at the KSU Dairy Teaching and Research Center (October, 1985). Yearling Angus (n=12) and Hereford (n=12) heifers, 12 to 14 mo of age, from the Kansas State University Purebred Teaching Herd were housed in drylot conditions during the MGA treatment period at the KSU Purebred Beef Unit (April 1986). All beef heifers were moved to a small, lush brome pasture following the last MGA feeding. Pubertal status, as described in previous experiment was determined by serum progesterone concentration. All heifers were bled by coccygeal venipuncture 20, 10, and 1 d prior to the beginning of the first MGA feeding (d 1). A concentrate carrier containing 1.1 mg MGA/kg of milo was fed on top of the normal diet at a daily rate of .454 kg per head. Administration of MGA lasted for 17 d. All heifers were weighed and received an intramuscular injection of PGF, as described in Exp. 1, 16 d following the last MGA feeding (d 33). Semen from three sires was utilized and distributed evenly across all treatments. Heifers were allotted and assigned randomly to treatment groups. Group 1 (P-E, n=16, 390.0 \pm 8.7 kg) were puberal and inseminated artificially approximately 12 h following the onset of estrus. Group 2 (P-80 h, n=16, 396.0 $^{\pm}$ 8.7 kg) were inseminated artificially approximately 80 h following PGF. The prepuberal heifers, which were Angus (n=15) and Hereford (n=6) were selected and randomly assigned to be inseminated according to estrus (PP-E, n=5, 362.7 $^{\pm}$ 15.6 kg) or inseminated by appointment at 80 h following PGF (PP-80 h, n=6, 372.7 \pm 15.6 kg). Luteolytic success of PGF was verified as described in Exp. 1. All heifers were observed for estrus at 4-h intervals during the daylight hours (0600, 1000, 1400, 1800, and 2200 h) for 6 d after PGF. Interval from injection to onset of estrus was recorded. Pregnancy was determined at 40 to

60 d after insemination by palpation of the uterus per rectum.

Rates of conception are defined as the number of heifers pregnant/number inseminated. Pregnancy rates are defined as the number of heifers pregnant/group. Data were analyzed using least-squares procedures of General Linear Model of the Statistical Analysis System (SAS, 1982). Effects of treatment and puberty were examined. Percentage data were tested by Chi-square (Snedecor and Cochran, 1980).

Results

Exp. 1

Synchrony. Twenty of the 27 MGA treated heifers were in estrus 3 to 8 d after MGA feeding. Six of the remaining MGA-treated heifers had concentrations of progesterone in serum <1 ng/ml at the time of PGF injection. Two heifers not detected in estrus during the study were observed in estrus 23 and 28 d following the last MGA feeding. These heifers apparently ovulated after MGA feeding but were not detected in estrus. One heifer apparently ovulated during MGA administration and was not receptive to PGF treatment. They had normal luteal phases as indicated by concentrations of progesterone in serum preceding detected estrus and conception 17 d following MGA. Three heifers had serum progesterone <1 ng/ml continuing for 11, 14, and 17 d following last MGA feeding. Estrus was detected in each of those three heifers on 11, 12, and 16 d following last MGA feeding. One heifer not detected in estrus exhibited a short (7 d) luteal phase and then apparently became anestrous as serum progesterone never exceeded 1 ng/ml for the remainder of the sampling period.

Interval to Estrus. Interval to estrus was shorter (P<.05, Table 1) in heifers given PGF 24 h prior to the last day of MGA (MGA-6) when compared with heifers receiving PGF following the last MGA feeding (MGA-7). Rate of conception tended to be greater in heifers that exhibited estrus after 95 h after the last MGA feeding (5/15=33%) when compared with heifers exhibiting estrus before 95 h following MGA feeding (0/5=0%).

TABLE I. INTERVAL TO ESTRUS FOLLOWING LAST MELENGESTROL ACETATE (MGA) FEEDING AND FERTILITY FOR CONTROL, MGA-6, AND MGA-7 HEIFERS (EXP. 1).

Treatment	No. of heifers synchronized/group	Interval to estrus, h	First service conception rates, %	Pregnancy rate, %
MGA-6	11/13	94.6 ± 7.0 ^b	27.3	23.0 ^d
MGA-7	9/14	122.3 ± 9.7 ^C	22.2	14.3 ^d
Combined (MGA-6+MGA-7)	20/27	ı	25.0	18.5 ^d
Control	ı	1	41.6	35.7e

"Least-squares means ^T SE. b,^CMeans within column with different superscripts differ (P<.05).

dpregnancy rate = Number of heifers pregnant/number treated.

^eControl pregnancy rate = Number of heifers pregnant in the first 21 d/group.

<u>Fertility.</u> First-service conception and pregnancy rates were not different among treatments. However, first service conception rates tended to be affected by concentrations of progesterone in serum (Table 2). Breed and initial weight had no effect on interval to estrus or conception rates.

Two heifers exhibited short cycles with durations of 10 and 11 d following synchronized estrus.

Exp. 2

Synchrony. Forty-one of 43 heifers in this study exhibited estrus within 100 h following PGF injection. One heifer was detected in estrus during the MGA feeding period as well as 3 d prior to the PGF. Prostaglandin F_2 apparently did not cause CL regression in one heifer as serum progesterone did not fall below 1 ng/ml within 48 h.

Interval to Estrus. Twenty-nine of the 32 puberal heifers showed estrus within 24 h (47 h to 71 h) following PGF. Three puberal heifers had intervals to estrus of 80, 96, and 100 h. Nine of 11 prepuberal heifers showed estrus within a 20 h period (36 h to 56 h) following PGF. Two prepuberal heifers had intervals to estrus of 71 h. Interval to estrus was not affected by treatment or body weight. Heifers that were prepuberal prior to the first MGA feeding had shorter (P<.05, Table 3) intervals to estrus following PGF (49.3 \pm 4.5) than puberal heifers (61.8 \pm 2.7).

Fertility. First-service conception was not different between heifers inseminated according to estrus (15/20=75%) and inseminated 80 h following PGF administration (17/22=77%). Three of the four Angus and Hereford heifers failed to conceive were prepuberal prior to treatment. Five heifers did not conceive to insemination by appointment; Two beef heifers were prepuberal

TABLE 2. INTERVAL FROM LAST MELENGESTROL ACETATE (MGA) FEEDING TO ESTRUS AND FIRST SERVICE CONCEPTION RATES FOR MGA-6 AND MGA-7 HEIFERS AND PROGESTERONE CONCENTRATIONS IN SERUM (EXP. 1).

Treatment	Progesterone level, ng/ml	No. of heifers synchronized/group	Interval to estrus, h	First service conception rate, %
MGA-6		9/#	84.6 ± 10.7 ^b	0
MGA-7	7	2/6	131.0 ± 17.0 ^C	0
MGA-6	71	7/7	104.7 ± 9.1 b,c	42.8
MGA-7	~!	7/8	113.5 ± 9.1 ^C	28.6

b, C. Means within column with different superscripts differ (PC,05).

FERTILITY FOR PUBERAL AND PREPUBERAL HEIFERS INSEMINATED BY APPOINTMENT OR TABLE 3. INTERVAL TO ESTRUS FOLLOWING PROSTAGLANDIN \mathbf{F}_2 (PGF) INJECTION AND ACCORDING TO ESTRUS (EXP. 2).

Puberal	synchronized/group	Interval to estrus following PGF ^a , h	First service conception rate, %	Pregnancy rate, %
	30/32	61.8 ± 2.7 ^C	80.0	75.0
P-80 h	16/16	60.3 ± 3.7	81.3	81.3
P-E	14/16	63.2 ± 3.5	78.6	68.7
Prepuberal	11/11	49.3 ± 4.5d	72.7	72.7
PP-80 h	9/9	49.5 - 5.7	9*99	9.99
PP-E	5/5	49.2 ± 6.2	80.0	80.0
Overall	41/43	1	78.0	74.4

bregnancy rate = Number of heifers pregnant/number treated.

^aLeast-squares means ⁺ SE.

C,dMeans within column with different superscripts differ (PC,05).

prior to treatment, one dairy heifer did not exhibit estrus, and two dairy heifers exhibited estrus before 47 h following PGF.

Discussion

Exp. I

Heifers that received PGF on d 6 of MGA feeding had shorter intervals to estrus than heifers receiving PGF following the last MGA feeding. Corpus luteum regression initiated 24 h prior to the last MGA feeding should decrease endogenous progesterone prior to heifers receiving PGF after the final MGA feeding. Heifers under stimulation of endogenous progesterone from their own corpus luteum in addition to the MGA administration would be expected to have longer intervals to estrus than heifers without a CL because as dose of progesterone increases, interval from cessation of treatment to estrus also increases (Ulberg and Lindley, 1960). High doses of progesterone allow little follicular growth during treatment (Ulberg et al., 1951), folliculogenesis would take more time after treatment.

There appeared to be no difference in interval to estrus among heifers when concentrations of progesterone in serum were less than 1 ng/ml when compared with heifers with \geq 1 ng/ml serum progesterone. A greater difference might have been expected as heifers beginning a 7 d MGA treatment on d 11 to d 21 of the estrous cycle have a shorter interval to estrus than heifers on d 0 to d 10 of the estrous cycle at the beginning of MGA treatment (Patterson, unpublished data, Kansas State University). Beal et al. (1985) reported that cyclic cows had intervals to estrus of 78 ± 3 h when cows were fed .6 mg/d MGA for 9 d and given PGF on d 9. The

difference in average interval to estrus among MGA-6 and MGA-7 heifers with serum progesterone <1 ng/ml is not a valid comparison. Heifers in MGA-7 (<1 ng/ml) group is comprised of only two heifers with intervals to estrus of 103 and 159 h.

Treatment variance revealed no difference in hours to estrus for MGA-6 and MGA-7 treatment groups indicating estrus synchrony did not differ between treatments. Improved synchrony of estrus is obtained when PGF or its analogue is administered 2 d before progestogen removal were compared with those using injections of the PGF following progestogen withdrawal (Thimonier et al., 1975; Chupin et al., 1977). The longer intervals to estrus in heifers given PGF prior to progestogen removal was due to the ability of the progestogen to maintain plasma progesterone concentrations in the absence of a corpus luteum (Mauer et al., 1975; Hansel and Beal, 1979). Maintenance of plasma progesterone for 24 h after PGF injection would improve synchrony by delaying the onset of estrus in heifers that would have responded within 60 to 86 h after simultaneous PGF injection and progestogen removal. Hansel and Beal (1979) speculated the improved synchrony in heifers receiving PGF prior to progestogen removal might be due to: 1) a decrease in the variability of the rate at which progesterone concentrations decline after PGF, 2) 'priming' effects of PGF before progesterone withdrawal on follicle growth and(or) estrogen secretion or 3) a combination of these and(or) other unidentified factors.

No difference in first-service conception rate among treatments was found. This finding agrees with other researchers using similar short term progestogen/PGF synchronization methods (Roche, 1976; Hansel and Beal 1979; Hershee et al., 1979). Patterson et al. (1986) noted lower first-service

conception rates when comparing MGA-treated heifers (.5 mg MGA for 7 d and 25 mg PGF on d 7) with control heifers (54.8 vs 79.7%).

Interval to estrus appeared to have no effect on first service conception rate. Serum progesterone concentration tended to affect conception. No heifers with <1 ng/ml serum progesterone at the time of PGF administration conceived. Heifers are assumed to have experienced normal luteal regression but ovulation was inhibited during MGA administration. Patterson et al. (unpublished data, Kansas State University) noted that heifers that were in later stage of the estrus cycle at the onset of MGA treatment had lower fertility following treatment when compared with early and mid-cycle heifers. Heifers on d 0 to 5, 6 to 11, 12 to 16, or 17 to 20 at the start of MGA administration (7 d) had different (P<.05) rates of conception 82%, 69%, 43%, and 20% respectively. After analyzing these results, pregnancy rates (heifers pregnant/group) following a 7 d MGA, PGF (im, d 7) estrous synchronization scheme may be no greater than pregnancy rates following insemination after a single PGF injection when administered to cycling virgin heifers.

Theoretically, following one injection of PGF, all females, except those in d 0 to 5 of the estrous cycle, should exhibit estrus within 90 h following PGF injection. This would be approximately 75% of all females within a herd. Assuming a first service conception rate of 66%, the percentage of heifers pregnant would be roughly 50% after one service. Fifty percent first service conception is very similar to rates of conception found by other researchers using a short term MGA treatment with an injection of PGF at the end of treatment (Boyd, et al., 1986; Patterson et al., 1986; Beal and Good, 1986). Further research comparing these systems of estrous synchronization is needed.

Exp. 2

After 17 d MGA administration, heifers should be in estrus an average of 5 d (3 to 8 d) following MGA removal. Sixteen days following MGA removal PGF is administered and all heifers are in d 8 to 13 of the estrous cycle. Theoretically, heifers in estrus 7 and 8 d following MGA removal are on d 9 and 8, respectively, of the estrous cycle at the time of PGF injection. Heifers which were prepuberal prior to treatment exhibited shorter (49.3 vs 61.8) intervals to estrus than puberal heifers. Prepuberal heifers are responding to PGF similar to heifers that are early in the estrous cycle (d 5 to 9). This response has not been reported in previous literature. More intensive studies are needed on the interval to estrus among puberal and prepuberal heifers in this treatment.

Ninety percent of the puberal heifers exhibited estrus within a 24 h (47 to 71 h) and 82% of the prepuberal heifers exhibited estrus within a 20 h (36 to 56 h) period, respectively, following PGF administration. Fourty-one heifers responded to estrous synchronization within a 64 h period, 36 to 100 h following PGF. The degree of synchronization among animals at similar stages of the estrous cycle agrees with previous work (Johnson, 1978; Refsal and Sequin, 1980; King et al., 1982).

Thirty-two of 43 heifers conceived after the first service. Treatment, breed, and pubertal status did not significantly affect first service conception rate. Eight of 11 heifers which were prepuberal prior to MGA administration conceived after first service. Similar rates of conception among heifers on d 10 to 16 of the estrous cycle at the time of PGF injection has been reported (King et al., 1982; Johnson, 1978).

Prepuberal heifers responded with shorter intervals to estrus when compared with puberal heifers (49.3 vs 61.8 h). Four of six PP-80 h heifers conceived following first service. Early cycle heifers have shorter (12.1 h) intervals to estrus than late cycle heifers and previous work has indicated higher rates of conception may be possible in early cycle heifers by time inseminating near 70 h rather than 80 h after PGF injection (Leaver et al., 1975; Burfening et al., 1978; Johnson, 1978). Thirteen of 16 (81.3%) puberal heifers conceived following PGF. These results indicate that 80 h after PGF administration is an appropriate time to inseminate heifers that were late cycle at the time of PGF and this agrees with data of King et al. (1982.)

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Ву

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Two experiments were conducted to determine the efficacy of melengestrol acetate (MGA) coupled with prostaglandin F_{2} alpha (PGF) as a method of estrous synchronization. Heifers in Exp. 1 were assigned randomly to be fed MGA (.5 mg for 7 d) and injected with PGF (25 mg, im) following the last MGA feeding (MGA-7, n=14), fed MGA (.5 mg for 7 d) and injected with PGF 24 h prior to the last MGA feeding (MGA-6, n=13), and controls (C, n=14). Twenty of the 27 MGA-treated heifers were in estrus 3 to 8 d after the last MGA feeding. Intervals to estrus following MGA removal were shorter (P<.05) for MGA-6 heifers than for with MGA-7 heifers (94.6 vs 122.3 h). Conception rates did not differ among treatment groups.

Exp. 2 was conducted to determine the efficacy of insemination by appointment in a MGA-PGF estrous synchronization scheme. Heifers received MGA (.5 mg for 17 d) and were injected witth PGF (25 mg, im) 16 d following the last MGA feeding. Heifers were assigned randomly to treatments: puberal and inseminated according to estrus (P-E, n=16), puberal and inseminated 80 h following PGF (P-80 h, n=16), prepuberal and inseminated according to estrus. (PP-E, n=5), and prepuberal and inseminated 80 h following PGF (PP-80 h, n=6). Prepuberal heifers had shorter (P<.05) intervals to estrus following PGF administration. Conception rates did not differ among treatments and were 68.7, 81.3, 80.0, 66.6% for P-E, P-80 h, PP-E, and PP-80 h respectively. Forty-one of the 43 heifers in Exp. 2 exhibited estrus within a 64 h period (36 to 100 h) following PGF administration. Ninety percent of the puberal and 82% of the prepuberal heifers exhibited estrus within a 24 h (47 to 71 h) and 20 h (36 to 56 h) period following PGF administration.

These results suggest MGA used in conjunction with PGF will successfully synchronize estrus in heifers. Degree of estrus synchronization and rate of conception may vary among MGA-PGF estrus synchronization schemes.

KEY WORDS: MGA, PROSTAGLANDIN F_{2}^{alpha} , SYNCHRONIZATION, PUBERTY, BOVINE.