

ARTIFICIAL INSEMINATION AT VARIOUS INTERVALS
AFTER ONSET OF SYNCHRONIZED ESTRUS AND
INDUCED PUBERTY IN BEEF HEIFERS

by

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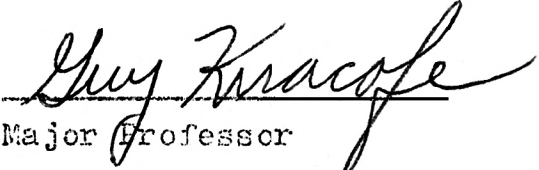
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LITERATURE REVIEW

Estrus Synchronization. Synchronization of estrus means that estrus cycles within a group of animals are altered so they occur over a short predetermined period of time. This procedure is a management tool used to breed a large number of animals in a short predictable period of time.

Successful estrus synchronization could make artificial insemination a wide spread practice in beef cattle production. It concentrates breeding and reduces the length of breeding season, thus decreases the number of days required to check heat. Subsequent calving will also be concentrated allowing for more efficient use of time and facilities.

Control of the estrus cycle is essential for commercial success of such contemporary techniques as superovulation and embryo transfer. Induced calving would become a more appealing technique if cows were bred as a group and could be calved as a group.

Adequate knowledge of the action of endogenous hormones in the cows reproductive processes is essential for control of breeding. The estrus cycle has been reviewed by Geschwind (1972), Hansel and Echterkamp(1972), Hansel and Schechter(1972), Hansel et al. (1973), and Lamming (1973). However, the full story of the hormonal factors involved in estrus and ovulation in cattle is far from complete. A brief summary was given by Gordon (1976) which also discussed different approaches and methods for control of breeding in cattle.

The estrous cycle can be controlled by both inhibiting estrus and ovulation until the corpus luteum has regressed and by causing

the corpus luteum to regress and thus stopping progesterone production. Effective methods for the control of cycles have been discussed by Robison (1975) as follows:

- A. Prolongation of the normal luteal phase of the cycle or establishment of an artificial luteal phase by exogenous progestogen.
- B. Shortening of the normal luteal phase by exogenous prostaglandin.
- C. Control of the breeding cycle by artificial lighting.
- D. Induction of ovulation.

The application of controlling artificial light is limited mostly to the ewe (Robison et al., 1975; Ducker and Bowman, 1970) and avian reproduction (Donald and Follett, 1966).

Progesterone and Analogues. Many different methods are employed in administering progesterone or one of its analogues for estrus control in cattle. The methods are initially aimed at keeping a sustained blood level of progestogen for a period of about 18 days and thus prolonging the normal luteal phase of the cycle or establish an artificial luteal phase.

Various progestogens, such as CAP (Chlormadinone acetate, Syntex), MAP (Melengestrol acetate, UpJohn), DHPA (Dihydroxy-progesterone acetophenide), Norethandrolone, SC 9880 and SC 21009, etc., have been administered by vaginal sponge, intramuscular injection, orally or subcutaneous implants to synchronize estrus.

Since progesterone has an extremely short biological half life in the cow, repeated frequent administration has been essential. Daily injections of progesterone have successfully controlled the estrus cycle in cattle (Ulberg et al., 1951; Trimberger and

Hansel , 1955) but low fertility frequently resulted. Thimonier et al. (1975) reported that 100% of the cows treated with a daily injection of norethandrolone showed signs of estrus within 5 days after the end of the treatment, however, fertility at the synchronized estrus was low. Gordon (1976) concluded that the injection routine may be applicable under certain experimental conditions but because of the time and labor involved, it appears to be impractical in commercial applications.

During the 1960's, emphasis and interest centered almost exclusively on the use of orally active progestogens, especially MAP, CAP and melengesterol acetate (MGA). Treatment at the appropriate dose level for a period approximating one cycle (18 days) was shown to be effective in controlling heat periods that most subsequently occurred within the space of 3 days. Administering an oral progestational compound to cattle has been shown to block heat and ovulation in many reports (Hansel et al., 1961; VanBlake et al., 1963; Zimbelman, 1963; Lamond, 1964; Hansel et al., 1966), but the fertility at estrus following the treatment has been low.

MGA is a synthetic progestogen used to improve efficiency of gain in feedlot heifers (Bloss et al., 1966; O'Brien et al., 1968). Estrus of heifers fed MGA was suppressed and corpora lutea were absent. Young et al., (1969) found that 0.4 mg MGA per head per day was the minimum effective dosage to suppress estrus. Chakraborty et al., (1971) fed MGA (1 mg/head/day) for 14 days. All heifers were in heat 3 to 6 days after MGA withdrawal, however, the first service conception rate was low (8.3%).

Oral administration of MAP at various stages of the estrus

cycle completely inhibited estrus during a 20-day feeding period (Anderson et al., 1962). All animals exhibited estrus 3 to 6 days after end of treatment. Conception rate of the treated animals was lower than the control (55% vs. 60%), although it was not significantly different. Dhindsa et al., (1967) fed MAP (180mg) daily for 18 days. Estrus synchrony was 55%-87% between 18 to 78 hours after withdrawal of MAP. First service calving rate was 33% and 37% for treated and control, respectively.

Grunert (1975) fed heifers 10 mg of CAP daily for 18 days and the conception rate ranged from 33.3% to 36.4% when artificially inseminated without regard to estrus on day 3 and 4 following withdrawal of CAP. Wiltbank et al. (1967) found 96% of the heifers in heat during a 48-hr period after 20-day of DHPA feeding (500 mg per head per day). Fifth-six % of the synchronized heifers had fertilized eggs 48 hrs after ovulation, while the percentage was 83 for the controls.

The technique of administering hormone intravaginally in cows by using CAP, MGA, FGA (Fluorogestone acetate, Searle Co.), etc., has been tried. Carrick and Shelton, (1967), and Shimizu et al., (1967) reported considerable variation of effectiveness in control of estrus. Apart from the problem of retention of sponges, treatment was not always effective in blocking estrus in a proportion of the animals.

Implants of silicone rubber impregnated with progestogens applied subcutaneously for 18 days, Proved highly effective in grouping heat periods in cattle (Roche and Crowley, 1973; Roche 1974b). Eighty-seven percent of the heifers implanted subcutane-

ously with 17-ethyl-19-nortestosterone for 16 days showed estrus in a 96-hr period (Wiltbank et al., 1971). Thirty-eight percent of the heifers conceiving at the synchronized estrus, compared with 65% for the controls.

Despite the success in controlling the breeding cycle in cattle, conception rates after full-length progestogen treatments (16, 18 or more days) were generally depressed, regardless of the methods administered. Some causes for the subfertility often associated with the long-term use of progesterone or its potent analogues in cattle were suggested. Van Niekerk and Belonje (1970) and Henricks et al., (1973) suggested there may be occasional abnormalities in the eggs released after treatment. Wishart (1974) reported that in cattle treated with SC 21009 during a complete cycle, cleavage of fertilized eggs was slower than normal. Minor changes have occurred in the characteristics of cervical mucus after progestogen (Boyd et al., 1972; 1973). Progestin treatment may affect the duration of estrus and the time of ovulation in heifers. Wiltbank et al. (1967) reported a significantly shorter estrus and a significantly longer of the interval from estrus to ovulation in heifers fed 500 mg DHPA for 20 days. An abnormal timing of ovulation relative to estrus can be expected to result in reduced fertility. Rodeffer et al. (1972) reported an occasional abnormal timing of LH release pattern in cattle. Hormonal imbalance either during the period of progestogen treatment or about the time of estrus have also been reported (Hill et al., 1971; Randel et al., 1973).

Regression of the natural bovine corpus luteum has been

readily induced by the injection of estradiol (Wiltbank et al., 1961; Kaltenbach et al., 1964; Wiltbank, 1966). This allows short-term application of progestogens in conjunction with estrogens. Wiltbank and Kasson (1968) reported successful estrus control after a relative short-term (9 days) progestogen regime (400 mg DHPA daily for 9 days) combined with an estrogen injection (5 mg estradiol valerate administered on day 2 of the oral treatment). Seventy-four and seventy-seven percent of the treated animals were in estrus in a 3-day period and conception rate after one breeding at the synchronized estrus was 62% and 59%. Subcutaneous implants of 17-ethyl-19-nortestosterone (Nilevar) were either implanted for 16 days alone or implanted for 9 days with an injection of estradiol valerate on day of implantations. Eighty-seven percent and 93% of heifers showed estrus in a 96-hr period for the two treatments, respectively. Conception rate at the synchronized estrus was 38% and 61% for two treatments, respectively, compared with 65% for the controls (Wiltbank et al., 1971). Lemon (1975) reported the effect of the estrogens, estradiol benzoate and estradiol valerate, is evident only when they are administered during the luteal phase and further corpus luteum development is inhibited if the corpus luteum has not attained its maximum activity. The combination of progestogen (12 mg of SC 21009 implanted subcutaneously for 10 days or norethandrolone 7 mg/day injected im for 10 days) with an injection of estradiol valerate modified the action of estrogen when administered early in the cycle, especially with SC 21009. Mauleon (1974) and Wiltbank and Gonzalez-Padilla (1975) reported effective estrus control in

cyclic cattle by an ear implant containing 6 mg of SC 21009. Chupin *et al.*, (1975) reported that when using SC 21009, a higher degree of synchronization could be obtained with a 12 mg implant for 9 days than a 6 mg implant for 11 days.

A combined progestogen/oestrogen injection at the time of implantation rather than using estrogen alone has been used by Wiltbank and Gonzalez- Padilla (1975). This combination permitted adequate control in cattle that were in the late stages of the cycle or had recently ovulated. An injection of estrogen alone early in the cycle (Day 3 and 5) had no luteolytic effect. It appeared that when progestogen was given in association with estrogen, there was an inhibitory effect on the development of the corpus luteum (Lemon 1975) and also a substantial progestogen dosage resulted in the antiluteotropic property. Combined progestogen/oestrogen injections have also been employed in conjunction with progestogen-impregnated intravaginal sponges and intravaginal silastic coils. Sreenan and Mulvehill (1975) reported that a combination of 250 mg progesterone and 7.5 mg estradiol benzoate can be employed successfully at the commencement of intravaginal sponge treatment. Roche (1975a; 1976) used intravaginal silastic coils (12 days) with an injection of 5 mg estradiol benzoate and 50 mg progesterone at the time of insertion. Ninety-three percent of the coils were retained and 91% of cows with coils were observed in estrus 2 to 6 days post-removal. Fertility was not different between treated and controls in the same herd.

To avoid detecting heat, precise control of ovulation is

necessary. If such a technique were available, artificial insemination during a predetermined period of time without regard to the detection of estrus could be accomplished. Estrogen, GnRH or gonadotropin has been employed in conjunction with the synchronizing treatment.

The use of oestrogen as a means of improving the precision of the estrus response has been based on the fact that preovulatory LH release, and consequently ovulation itself, is triggered by estrogen (Hobson and Hansel, 1972). The precision of synchronization response was improved by administering 2 mg estradiol 24 hours after progestogen withdrawal (Wiltbank *et al.*, 1971), but conception rate was depressed. Roche (1974c), using a dose of 400 ug of estradiol benzoate 16 hours after progestogen withdrawal, also found a depression of conception rate.

GnRH can cause the release of endogenous gonadotropins (Mauer and Ripple, 1972). Injecting 100 ug GnRH to Friesian dairy cows 30 hours after removal of the silastic coil following 12-day progesterone treatment, Roche (1975a) obtained a high synchronization rate (90%) 35 hours post GnRH. Fertility following a fixed time insemination at 48 hours was not significantly different from controls. Similar result in heifers was also reported by Roche (1975b). After 21-day application of intravaginal progesterone, Mauer *et al.*, (1975) gave an intramuscular injection of 100 ug of GnRH 28 to 30 hours after device removal. The cows were inseminated 18 to 24 hours after GnRH injection and conception rate was comparable to controls.

Several investigators have employed PMS/HCG in combination

with progestogen in an attempt to increase the precision of response in cyclical cattle to permit artificial insemination at a predetermined time (Baker and Coggins, 1968; Boyd and Tasker, 1971; Grunert, 1975). However, the use of gonadotropins cannot be regarded as a desirable feature, particularly in view of the considerable variation in superovulatory response.

Prostaglandin F_{2α} and Analogues. It has been known for years that the corpus luteum plays an important role not only in supporting early pregnancy, but also in controlling the length of the ovarian cycle and the time of ovulation. Loeb (1932) made the original observation that the total hysterectomy of the guinea pig during luteal phase of the estrous cycle prolonged the life span of the corpus luteum. Similar effects of hysterectomy on corpus luteum persistence were demonstrated in sheep and cows (Wiltbank and Casida, 1956), pigs (Du Mesnil Du Buisson and Dauzier, 1959), mares (Ginther, 1971) and has been reviewed by Anderson et al., (1969). The results of hysterectomy suggest that the uterus of most cyclic mammal species produces a luteolysin which causes luteal regression and hence the initiation of a new cycle.

PGF_{2α} is notable for its capacity to induce luteolysis. when it was administered in vivo, a depression of progesterone output was observed in the rabbit and hamster (Duncan and Pharriss, 1970), primate (Kirton et al., 1970) and rat (Behrman et al., 1973; Pharriss and Wyngarden, 1969)

The natural prostaglandins (PGS) are all unsaturated hydroxy

acids of 20 carbon atoms based on a 5-membered ring with an adjacent sidechain. There are four main series, designated E, F, A and B, denoting differences in the ring. PGS are formed in many tissues. Members of the different series exhibit different specificities of action. The pharmacological responses are often species-dependent. (reviewed by Walpole, 1975).

The in vivo luteolytic effects of PGS appear to be limited to compounds of the F series in the ewe (Carlson et al., 1972). Wilson et al., (1972) demonstrated that, in the sheep, $\text{PGF}_{2\alpha}$ is produced by the endometrium in increasing quantities towards the end of the estrous cycle. $\text{PGF}_{2\alpha}$ concentration also increased in the ovine uterine venous blood (Bland et al., 1971; McCracken et al., 1971; Thorburn et al., 1972) and peripheral blood (Condert et al., 1972). Endogenous $\text{PGF}_{2\alpha}$ apparently is transferred locally from the uterine venous system to the ovarian arterial system in sheep (Kiracofe et al., 1966; Baird et al., 1973a; 1973b; Ginther et al., 1973; Baird and Scaramuzzi, 1975). The biochemical effects of $\text{PGF}_{2\alpha}$ on luteal cells may be responsible for the initiation of functional luteal regression (Behrman et al., 1971). $\text{PGF}_{2\alpha}$ inhibits cholesterol ester synthetase available for synthesis of progesterone. Another hypothesis is that $\text{PGF}_{2\alpha}$ exerts its luteolytic action by reducing total ovarian blood flow but it needs further confirmation (summarized by Baird and Scaramuzzi, 1975).

In the case of the cow, there is relatively little data concerning levels of prostaglandins in the endometrium or uterine venous plasma. Higher levels of $\text{PGF}_{2\alpha}$ (131 ± 9.0 ng/g dry tissue)

were found in bovine endometrium at day 15 until day of estrus as compared with the lower levels (45 ± 12 ng/g dry tissue) on day 1-14 of cycle (Schemesh and Hansel, 1975). Arachidonic acid, the immediate precursor of $\text{PGF}_{2\alpha}$, was isolated from bovine endometrial tissue and the corpus luteum by Hansel *et al.*, (1973) and Scott *et al.*, (1968), respectively. It was proposed by Inskeep (1973) that the bovine endometrium produces only precursors which are transformed to the active principle in the ovary, or that substances other than prostaglandins are directly luteolytic. This hypothesis was supported indirectly by Wilks *et al.*, (1972) and Chasalow and Pharriss, (1972). They found that the ovary in the rabbit and rat can synthesize prostaglandin. Also, when locally applied, arachidonic acid is capable of causing luteolysis in pseudopregnant hysterectomized hamsters (Hansel *et al.*, 1973).

Exogenous $\text{PGF}_{2\alpha}$ causes luteolysis from various methods of application. $\text{PGF}_{2\alpha}$ placed non-surgically into the uterine horn ipsilateral to the corpus luteum has been shown to cause luteolysis in cattle (Liehr *et al.*, 1972; Louis *et al.*, 1972; 1974; Rowson *et al.*, 1972; Hafs *et al.*, 1974). An intravaginal treatment with 30 mg $\text{PGF}_{2\alpha}$ resulted in luteolysis, but it was more variable and retarded (Hafs *et al.*, 1974). Cows treated with $\text{PGF}_{2\alpha}$ on day 5 to 18 of the estrous cycle exhibited a decrease in size of the corpus luteum followed by a decrease in serum progesterone (Louis *et al.*, 1973) resulting in a return to estrus in 3 days (Louis *et al.*, 1973; Lauderdale, 1972; Rowson *et al.*, 1972; Inskeep, 1973). Henricks *et al.*, (1974) achieved 100%

synchronization in heifers treated intrauterinely with 2 mg of PGF_{2α} after day 5 of the estrous cycle, however, low fertility was reported.

The uterine luteolytic factor acts locally in cattle (Anderson et al., 1969), however, the systemic route of administering is effective if large doses of PGF_{2α} are given so that enough will persist in the blood stream to have an effect on its target organ (Horton, 1969). A single injection (subcutaneous or intramuscular) using 20 to 30 mg of the PGF_{2α} THAM salt (trimethanine) preparation was as effective as intrauterine infusion (Lauderdale, 1972; Lauderdale et al., 1974; Edqvist et al., 1975) when treatment was given after day 5 of the estrous cycle. Two potent analogues, ICI 79,939 and ICI 80,996 have been used in cattle. Tervit et al., (1973) described the induction of fertile estrus in cattle by intramuscular injection of a low dose (1 mg) of ICI 79,939. Cooper (1974) and Cooper and Furr (1974) reported the successful use of a single dose of 500 ug of ICI 80,996, and that fertility after artificial insemination was normal. Roche (1974) reported that a single injection of either 20 mg or 30 mg of PGF_{2α} was effective in synchronizing estrus only when injected from day 5 through 20 of the estrous cycle with fertility equal to that of controls. A single injection of 30 mg PGF_{2α} successfully synchronized estrus when a functional corpus luteum was present (Turman et al., 1975). Eighty-eight percent of those cows with a palpable corpus luteum showed estrus on day 2, 3, and 4 after a single injection of 30 mg PGF_{2α} (Lauderdale et al., 1974). Similarity of fertility between controls and cows inse-

minated at an appointed time suggests that $\text{PGF}_{2\alpha}$ may be useful to allow breeding with normal fertility at predetermined times, independent of estrus detection. $\text{PGF}_{2\alpha}$ (25 mg) injected intramuscularly or subcutaneously on day 8 or 18 of the estrous cycle caused standing heat 48 to 96 hours after treatment (Edqvist et al., 1975). Sixty-nine percent of those treated conceived to the synchronized estrus. Fertility was similar to controls when cows with palpable CL were given 30 mg $\text{PGF}_{2\alpha}$ and artificially inseminated 12 hours after the onset of estrus or 72 or 90 hours after the injection (Lauderdale, 1975). Under range management (Lambert et al., 1975), a single injection of $\text{PGF}_{2\alpha}$ brought 74% of treated cows to estrus. The fertility was equal between the controls and treated cows and $\text{PGF}_{2\alpha}$ treatment resulted in a higher percentage of cows conceiving early in the breeding season. Hafs et al., (1975) reported two injections of $\text{PGF}_{2\alpha}$ given two days apart synchronized 68% of the treated heifers. Administering of $\text{PGF}_{2\alpha}$ on two consecutive days to cows was no more effective than a single injection (Moore, 1975).

Neither $\text{PGF}_{2\alpha}$ nor analogues given in a single dose at any one time induced estrus in all cattle in a herd since animals treated before day 5 of the estrous cycle will not respond. (Lauderdale, 1972; Liehr et al., 1972; Rowson et al., 1972; Hill et al., 1973; Cooper and Furr, 1974). To overcome this problem, some type of prostaglandin schedule must be employed other than a single injection. A regimen of two doses of $\text{PGF}_{2\alpha}$ or its analogues given 10 to 12 days apart was described by Cooper (1974), Cooper and Furr (1974) and Roche (1974a).

Two injections of $\text{PGF}_{2\alpha}$ given 12 days apart resulted in sufficient synchrony of estrus and ovulation after the second injection (Hafs et al., 1975), and insemination at 70 and 88 hours after the second injection without regard to estrus resulted in fertility equivalent to that in untreated controls. Lauderdale (1975) also reported that two injections of $\text{PGF}_{2\alpha}$ 11 days apart were effective for estrus synchronization and fertility was not affected. Cooper (1974), and Cooper and Rowson (1975) treated heifers with two intramuscular injections of 500 ug of ICI 80,996 11 days apart. Estrus occurred somewhat earlier and more closely synchronized than that following the first injection. A large majority (90 percent) of the heifers exhibited estrus between 48 and 72 hours after the second treatment. The subsequent response of the ovary with follicular development and ovulation is similar both morphologically and endocrinologically to the response following natural luteal regression. With two injections of 750 ug ICI 79,939 given 10 days apart, Dobson et al. (1975) reported that progesterone, LH, and estradiol levels were formed around the induced estrus periods in a manner similar to that seen during natural estrus. The data also indicated induction of estrus and ovulation was rapid and precise and corpus lutea formed following such a regimen were secreting normal quantities of progesterone during the early stages of development and had a normal life-span.

The onset of the preovulatory surge of LH after synchronization of estrus with $\text{PGF}_{2\alpha}$ analogues ranges from 60 to 104 hr after treatment (Fernandez-Limia et al., 1977), 71 ± 4 hr after

intrauterine administration (Louis et al., 1974), and 62 to 103 hr after the first injection and 48 to 62 hr after the second injection (Cooper and Furr, 1974). Louis et al., (1975) reported estrus began at 72 hr and ovulation occurred at 95 hr post-injection.

Information cited above demonstrated that two injections of $\text{PGF}_{2\alpha}$ about 10-12 days apart effectively synchronize estrus during a predefined interval of 4 days in cycling cows and fertility should be normal when the cows are inseminated at a predetermined period of time without regard to estrus.

Another approach of administering progestogens and $\text{PGI}_{2\alpha}$ has also been sufficient in synchronizing estrus in cattle. Heersche et al., (1974) reported that administration of a Synron-Mate B implant for 7 days with $\text{PGF}_{2\alpha}$ injected at implant removal resulted in a high degree of estrus synchronization and normal fertility. Wishart (1974) concluded that the combination of SC 21009 with $\text{PGF}_{2\alpha}$ to synchronize estrus does not adversely affect the duration of estrus, the time of ovulation and the fertility of heifers.

Progesterone is known to suppress both estrus and ovulation and therefore can be used to bridge the period during which $\text{PGF}_{2\alpha}$ is ineffective. Thimonier et al., (1975) reported that treatments of 500 ug ICI 80,996 in two injections 10 days apart or 12 mg SC 21009 implanted for 10 days plus an injection of ICI 80996 at implant removal gave a similar degree of synchronization. (70% in estrus during a 48 hr period). Thimonier et al., (1975) also found the degree of synchronization was increased

(92% in estrus during a 48-hr period) with the prostaglandin analogue was injected 48 hours before SC 21009 implant removal. Chupin et al. (1977) reported that the best synchronization was obtained with a 9-day SC 21009 implant plus an ICI 80996 injection 2 days before implant removal. It was significantly different ($P < .01$) from giving two intramuscular injections of ICI 80996 11 days apart. The pregnancy rate was not statistically different, however, the progestogen-prostaglandin group had a slightly higher conception rate than the prostaglandin-prostaglandin group (48.7% vs. 43.5%). Graves et al. (1975) reported estrus occurred within 48 hours in cows implanted with norgestomet for 7 days and given $\text{PGF}_{2\alpha}$ 24 hours before implant removal. Administering GnRH shortened the interval from implant removal to ovulation which appeared to have a practical merit for pre-determined insemination time.

Breeding by Appointment after Synchronized Estrus. Hammond (1929) in his classical study on bovine reproduction, recorded the average duration of estrus as 18 hours. Most subsequent reports have generally agreed the figure somewhere between 12 and 22 hours. Ovulation is thought to occur some 10-12 hours after the end of the estrus (see review by Gordon, 1976). Information about estrus phenomena in cattle is limited due to time-consuming heat checks and the fact that values vary with the method of checking and the interval between checking. (Asdell, 1964).

Conception at a single estrus in cattle averages 70 percent

for natural mating (Turman et al., 1971) and 60 percent for artificial insemination (Laster et al., 1972). The timing of ovulation in relation to preovulatory events is an important factor associated with infertility. Hafez (1967) made an extensive review of the factors contributing to infertility in domestic animals.

Infertility experienced during normal reproduction has markedly increased following most attempts to synchronize estrus. Synchronization of estrus and superovulation frequently result in a decrease in fertilization and implantation and an increase in embryonic death (Butcher, 1972). The causes are not fully understood but several factors were reviewed by Butcher. Gonadotrophins and/or steroids used altered gamete transport (Lauderdale, 1974; Hawk and Conley, 1974). Environment within the reproductive tract (Laster, 1977) and time of ovulation in relation to oocyte maturation or estrus directly affect the intrafollicular oocyte. Phillippo (1968) warned that exogenous gonadotropins shorten the time to ovulation and may alter maturation processes which could lead to defective zygotes. It seems that some treatments could shorten the time to ovulation and still produce a mature egg by initiation of more rapid biochemical reactions within the oocyte. Only limited data are available to either implicate or reject delayed ovulation as a factor in decreased fertility following suppression or induction of ovulation.

There is evidence that progestin treatment may affect the duration of estrus and the time of ovulation in heifers (Wiltbank, et al., 1967). A significant shortening of estrus and a signi-

ficant lengthening of the interval from estrus to ovulation were found in heifers fed 500 mg DHPA for 20 days in the study.

The literature cited above suggests some proper predetermined schedule for AI following various techniques of synchronization should be beneficial. Since different techniques cause different problems, it seems that a specific predetermined breeding schedule may be needed for each technique used.

INTRODUCTION

Reproduction is one of the most important considerations in the economics of cattle production. In beef production, especially under range condition, estrus detection for artificial insemination is a time-consuming task. Although Foote (1975) summarized various aids for estrus detection, it seems missed heats are unavailible due to human error. Barr (1975) also reported that non-observed estrus is a major cause of long calving intervals. Zemjanis et al., (1969) found that approximately 90% of the cows reported as being anestrus were actually having regular estrous cycles. For purposes of both saving time and improving the fertility, a proper predetermined AI schedule following effective estrus synchronization is demanded.

Estrous cycles can be controlled by either inhibiting estrus and ovulation until the corpus luteum has regressed, or by causing the corpus luteum to regress and thus stopping progesterone production. Administering progestogens for the length of an estrous cycle to suppress estrus and ovulation, without reducing the functional life span of the corpus luteum, has been the most widely used method of synchronization. Following this method fertility at the controlled estrus has been low (Jöchle, 1972; Hansel et al., 1961; Anderson et al., 1962; Zimbelman, 1963; Chakraborty et al., 1971; Grunert, 1975). Short-term administering progestogen in combination with estrogen has synchronized estrus and fertility is improved (Ulberg and Lindley, 1960; Wiltbank and Kasson 1968).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is noted for its capacity to induce luteolysis in the ewe (Carlson et al., 1972; Wilson et al., 1972) and cow (Schemesh and hansel, 1975; Hansel et al., 1972). Exoge-

nous $\text{PGF}_{2\alpha}$ or its potent analogues (ICI-79939, ICI-80996, etc.), causes luteolysis in the cow when treated after day 5 of the cycle. $\text{PGF}_{2\alpha}$ given either locally into the uterine horn ipsilateral to the CL (Rowson et al., 1972) or systemically (Lauderdale, 1972), is effective in causing luteal regression between day 5 and 16 of the estrous cycle in the cow. Therefore, neither $\text{PGF}_{2\alpha}$ nor its analogues given in a single injection at any one time will induce estrus in all cattle in a herd. With two $\text{PGF}_{2\alpha}$ injections 10 to 12 days apart or one $\text{PGF}_{2\alpha}$ injection after a 7 to 9 day treatment of progestogen, can both synchronize the estrus effectively and achieve a conception rate comparable to the control (Cooper 1974; Cooper et al., 1974; Heersche et al., 1974; Wishart, 1974; Hafs et al., 1975; Chupin et al., 1977). These techniques have been relatively successful, however, estrus is not synchronized closely enough to eliminate checking for estrus. Furthermore, the detection of estrus becomes more difficult in a synchronized group due to the varying degrees of sexual activity being shown. Double inseminations or giving additional hormones (GnRH, gonadotropins, estrogens, etc.) have been used to increase the precision of the ovulatory response (reviewed by Gordon, 1976), however, fertility is usually decreased.

The purpose of this experiment was to determine conception rates in synchronized heifers inseminated at various intervals after the onset of estrus. Such data should be helpful in determining proper timing of insemination both in relation to estrus and following synchronization with a progestogen and prostaglandin.

MATERIALS AND METHODS

One hundred and sixty-two yearling Angus, Hereford, Polled Hereford, and Simmental X Hereford heifers (102 head in 1976 and 60 head in 1977) that had been in estrus or had a palpable corpus luteum before the start of the trial were used. The heifers averaged 341 kg and were 12 to 16 months old. All heifers were implanted subcutaneously with 6 mg of norgestomet (SC 21009, G. D. Searle & Company, Chicago, Ill.) on the posterior side of one ear. Seven days later each heifer was injected intramuscularly with 25 mg of prostaglandin in THAM salt (UpJohn Company, Kalamazzo, Michigan) when the implant was removed from half of the heifers in the morning (7:00 AM) and half in the evening (7:00 PM). All heifers were confined to dry lot and were observed for estrus every 4 hours during a five-day period. Heifers were then bred artificially 6, 10, 14, 18, 22, or 26 hours after being observed in standing estrus in 1976. In 1977, heifers were bred at the same times except two additional groups bred 2 or 30 hours after onset of standing estrus were added. Heifers detected in estrus at each time were divided into AM and PM breeding (bred at 8:00 AM or 8:00 PM). Conception rates were determined by rectal palpation 50 to 85 days after insemination for 1976 heifers and 77 to 124 days after insemination for 1977 heifers.

RESULTS

Ninety-six percent (98/102), 90% (54/60) and 93.8% (152/162) of treated heifers came in estrus within a 5-day period after treatment for 1976, 1977 and 1976 plus 1977, respectively. Heifers that lost their implant were not included in the calculation. One implant was lost in 1976 and three in 1977.

The degree of estrus synchronization is shown in Table 1. Heifers exhibited standing estrus 19 to 119 hours after implant removal. The distribution of onset of estrus is shown in Figure 1.

There was no difference ($P < .1$) between breeds concerning the highest percentage of heifers in estrus within a given 48-hour period. Angus and Polled Hereford heifers had more estrus occurred later than Simmental X Hereford and Hereford heifers. (Table 2.) During the first 48 hours after implant removal, Angus heifers had the lowest percentage (22.72%) come in estrus compared to other breeds (50%, 64.7% and 66.67% for Polled Hereford, Simmental X Hereford, and Hereford, respectively), and the difference was significant ($P < .01$).

Conception rate at the synchronized estrus was 59.2% (90/152) for both years combined; however, conception rate was higher ($P < .01$) in 1976 (64.3%) than in 1977 (50%). No difference was found in the conception rates of heifers bred in the morning (8:00 AM) or evening (8:00 PM). Conception rates did not differ ($P < .5$) among groups inseminated at different time intervals after onset of estrus (Table 3). On the contrary, if the conception rates were calculated on the basis of interval from implant removal to insemination without

regard to the onset of estrus, a difference did exist ($P < .025$) as shown in Table 4.

There were differences ($P < .05$) in conception rates between breeds. Difference in breeds could be due to differences in weight or age of the animals (Table 5).

There was a difference ($P < .05$) between breeds for average days from implant removal to conception of heifers bred during the breeding season (Table 6). Breed, AM-PM breeding, different hour intervals from onset of estrus to insemination, weight, and age did not have a significant effect on it when tested by least square analysis of variance ($P < .05$). For heifers that conceived at the synchronized estrus, the average days from implant removal to conception were not significantly different.

TABLE 1. ACCUMULATIVE PERCENTAGES OF HEIFERS SHOWING
ESTRUS AFTER IMPLANT REMOVAL

Hours after implant removal	1976 (n=102)	1977 (n=60)	1976+1977 (n=162)
48	45.1	66.6	53.1
72	75.5	80.0	77.2
96	90.2	85.0	88.3
120	96.1	90.0	93.8

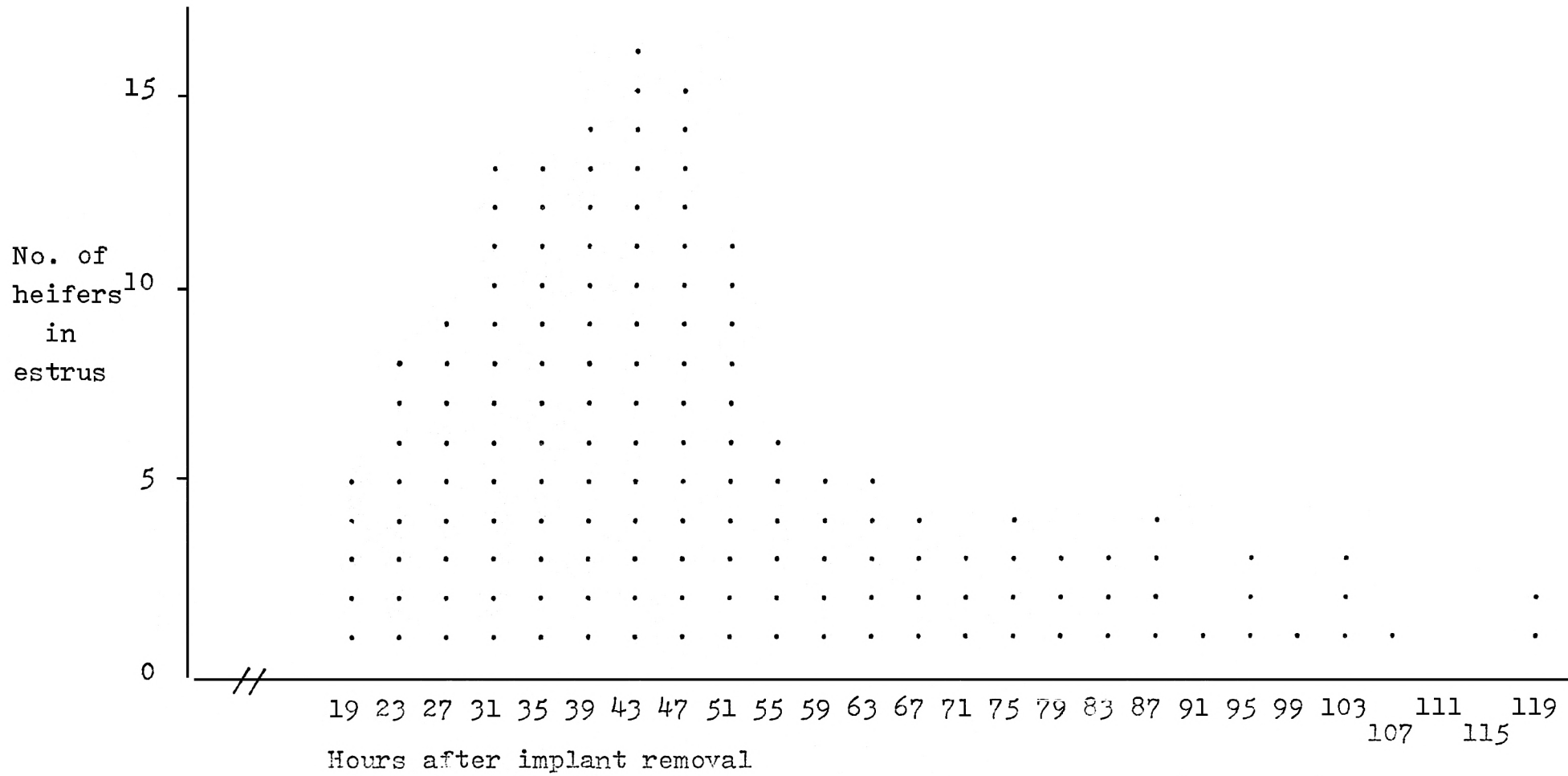


FIGURE 1. DISTRIBUTION OF ONSET OF ESTRUS AFTER IMPLANT REMOVAL

TABLE 2. BREED DIFFERENCE ON ONSET OF ESTRUS AFTER SYNCHRONIZATION

Breed	No. of animals	% in estrus after treatment				Highest % in estrus in a 48 hr-period	
		48 hr. ^a	72 hr.	96 hr.	120 hr.	% in estrus	Hrs after treatment
Simmental X Hereford	51	64.70	82.35	88.23	94.12	72.54	24-72
Angus	22	22.72	63.64	81.82	90.91	59.09	36-84
Hereford	21	66.67	90.47	90.47	95.14	85.71	24-72
Polled Hereford	68	50.00	75.00	89.71	94.12	64.71	36-84
Overall	162	53.09	77.78	88.27	93.83	69.14	24-72 _b or 36-84

^a Significantly different (P<.01) between breeds.

^b The same concentration of heifers in estrus occurred between 24 to 72 or 36 to 84 hours.

TABLE 3. CONCEPTION RATES IN ESTRUS SYNCHRONIZED HEIFERS INSEMINATED
AT VARIOUS INTERVALS AFTER ONSET OF ESTRUS

	Hours After Onset of Estrus								Overall
	2	6	10	14	18	22	26	30	
A M	16.7 (1/6)	55.5 (5/9)	50.0 (6/12)	66.6 (6/9)	64.3 (9/14)	50.0 (6/12)	84.6 (11/13)	60.0 (3/5)	58.7 (47/80)
P M	100 (3/3)	60.0 (9/15)	66.6 (6/9)	50.0 (7/14)	60.0 (6/10)	40.0 (4/10)	66.6 (4/6)	80.0 (4/5)	59.7 (43/72)
Overall ^a	44.4 (4/9)	58.3 (14/24)	57.1 (12/21)	56.5 (13/23)	62.5 (15/24)	45.5 (10/22)	78.9 (15/19)	70.0 (7/10)	59.2 (90/152)

^a For testing the homogeneity of proportions, Goodness-of-fit-test was applied; no significant difference was found ($P < .5$).

TABLE 4. CONCEPTION RATES OF HEIFERS INSEMINATED AT
VARIOUS PERIODS AFTER IMPLANT REMOVAL

Intervals									
from	25	37	49	61	73	85	97	109	121
implant (hr.)	to	to	to	to	to	to	to	to	to
removal									
to	33	45	57	69	81	93	105	117	139
A. I.									
Conception ^a									
rate (%)	87.5	31.8	53.8	83.3	57.1	60.0	50.0	60.0	50.0
No. / No.									
preg / bred	7/8	7/22	21/39	25/30	12/21	9/15	4/8	3/5	2/4

^a For testing the homogeneity of proportions, Goodness-of-fit-test was applied; there was difference among groups ($P < .025$)

TABLE 6. MEANS \pm STANDARD ERRORS BY BREED FOR DAYS FROM
IMPLANT REMOVAL TO CONCEPTION

Breed	Heifers conceived at the synchronized estrus		Heifers conceived during Breeding season (62 days)	
	No. of animals	Mean \pm SE	No. of animals	Mean \pm SE
Simmental x Hereford	38	2.58 \pm 1.2 ^a	47	5.4 \pm 2.2 ^a
Angus	12	2.92 \pm 0.9 ^a	19	11.3 \pm 3.6 ^{bc}
Hereford	16	3.19 \pm 1.1 ^a	20	6.9 \pm 3.3 ^{ac}
Polled Hereford	35	2.66 \pm 0.9 ^a	64	13.7 \pm 2.1 ^b

Means with the same superscript are not significantly different (P<.05).

TABLE 5. EFFECTS OF BREED, WEIGHT, AND AGE ON CONCEPTION RATE
AT THE SYNCHRONIZED ESTRUS

Breed	No. of animals	Average age (days)	Average weight (kg.)	Conception rate (%)
Simmental x Hereford	48	424.9 ^{bc}	353.3 ^b	72.9 ^a
Angus	20	483.8 ^a	395.2 ^a	75.2 ^a
Hereford	20	449.3 ^{ab}	374.5 ^{ab}	70.0 ^a
Polled Hereford	64	415.7 ^c	305.8 ^c	40.6 ^b

Figures with the same superscript in each column were not significantly different ($P < .05$). Average age and average weight were tested by LSD and conception rates were tested by Goodness-of-fit-test.

DISCUSSION

Administration of a progestogen implant for 7 days followed by an injection of $\text{PGF}_{2\alpha}$ was an effective technique for synchronizing estrus without affecting fertility. The synchronization results in this experiment were comparable to those of Heersche et al. (1974) who used the same procedure (93.8% and 92.4% within 120 hours, respectively). Wishart (1974) reported 82% of the heifers in estrus within 5 days. Thimonier et al. (1975) reported 72.5% of heifers in estrus during a given 48 hours period which was similar to the present results of 69.1% as the maximum percentage of heifers detected in estrus during either 24 to 72 or 36 to 84 hours after $\text{PGF}_{2\alpha}$ injection (or implant removal). With the same procedure, Chupin et al. (1977) obtained a maximum of 63.8% heifers in estrus during a given 48 hours period.

There was no breed difference in degree of synchronization. However, Angus and Polled Hereford heifers had their highest percentage come in estrus during a given 48-hour period later than that of Simmental x hereford and Hereford heifers. During the first 48 hours after implant removal, Angus heifers had the lowest percentage (22.72%) come in estrus compared to other breeds (50%, 64.7% and 66.67% for Polled Hereford, Simmental X Hereford, and Hereford, respectively), and the difference was significant ($P < .01$).

Conception rate at the synchronized estrus was comparable to other reports, although, breeding schedules were somewhat different. Chupin et al., (1977) used double AI schedule at 48 and 72 hr after implant removal which resulted in 48.7% conception. In the present experiment, 59.2% of the heifers conceived to first

service at the synchronized estrus. This percentage is comparable to Heersche (1975), who reported 63.8, 64.7 and 53.8% for two trials and controls, respectively. Sixty-seven percent first service conception was obtained by Wishart (1974) using the same treatment and breeding by estrus.

There was a significant difference in conception rates between heifers grouped at a fixed time basis after implant removal (Table 4). The group which was bred the earliest (25 to 33 hours after implant removal) had a higher conception rate than most of the other groups. Heersche et al. (1974) compared conception rates between heifers that had or did not have a corpus luteum at the time of PGF_{2α} injection. Heifers that did not have a palpable corpus luteum at the time of implant removal and PGF_{2α} injection tended to be in estrus earlier than those that had a palpable corpus luteum. The average intervals from PGF_{2α} injection until estrus in no CL and CL groups were 56 and 65 hours, respectively. Ellicott et al. (1974) also reported a longer interval from PGF_{2α} injection to estrus when heifers were injected at mid cycle. The delay may be due to the less rapid decline of progesterone in animals with a CL. Conception rate of heifers with and without CL at the PGF_{2α} injection were 62.1% and 66.7%, respectively (Heersche, 1975). This is in agreement with the high conception rate of heifers bred earlier after implant removal.

The conception rate of Polled Hereford heifers was significantly lower than that of the other three breeds. The weight of animals at the time of treatment could have been a major factor affecting the conception (Table 5).

In Table 3, the conception did not decline by breeding as early as 2 hours or as late as 30 hours following the onset of the synchronized estrus. This does not agree with the "Timing Guide" for the average cow given by Perry (1960) which showed the optimum time to breed as 9 to 24 hours after the onset of estrus. Many reports support the widely accepted rule of thumb that cows first seen in estrus in the morning should be inseminated the afternoon of the same day, and those first observed in estrus in the afternoon should be inseminated the next morning. McDonald (1975) stated that fertility is highest near mid-heat or the end of heat and then declines soon after the end of heat. Asdell (1964) reported highest conception was obtained when cows were artificially inseminated 7 to 24 hours before ovulation. The time ranges for high conception given by other studies are narrower than intervals used in the present experiment.

Wishart (1974) reported heifers ovulated 30.6 hours after the onset of estrus synchronized when given a 5 day SC 21009 implantation plus transcervical administration of PGF_{2α} at the time of implant removal. This ovulation time is compatible with data for non-synchronized cows of Swanson and Hafs (1971), who reported that the ovulation occurred about 30 hours after the onset of estrus. This suggests that the treatment of progestogen + PGF_{2α} does not change the time interval from onset of estrus to ovulation. If ovulation occurred 30 hours after onset of estrus in our heifers, conception rate did not decline when insemination was 0 to 28 hours before the ovulation.

From past recommendation on insemination time, one might

not expect an acceptable conception rate in heifers inseminated 2 hours after the onset of estrus. Perhaps newly developed extenders used for frozen bull semen have extended the fertile life span of sperm in the female tract. McLaren (1975) reported the fertile life of bovine sperm to be 30 to 48 hours. However, "fertile life" is a relative concept and it has been accepted that fertility declines progressively over a period of hours.

The fertile life span of ova is thought to be short. McLaren (1975) estimated the fertile life of bovine ova to be 8 to 12 hours. McDonald (1975) showed the ova which have aged 6 to 8 hours are becoming quite unfertilizable or at least unable to produce an embryo that will develop properly. For those heifers bred 30 hours after the onset of estrus (estimated time of ovulation), the ova should be fertile if these reports are correct. Capacitation may require several hours in the female tract; however, according to our results, it would not be longer than the fertilizable life of the ovum. Precise data concerning the time needed for capacitation are difficult to obtain in cattle. Capacitation takes about 2 hours in the rat, 4 hours in the rabbit and about 1.5 hours in the sheep (Mattner, 1963). Little or no capacitation may be required of bull sperm in the female tract (Mahajan and Menge, 1966).

Our data do not conflict with expected conception rates in terms of estimated fertilizable life spans of spermatozoa and ova and time of ovulation in respect with the onset of estrus. However, our data indicate that acceptable fertility can be obtained by inseminating over a wider range (2 to 30 hours after the onset of

estrus) than normally considered in practice.

Synchronization in the present experiment did not appear to be good enough to allow insemination at an appointed time without detection of estrus. However, knowing that the conception rate was not decreased by breeding as early as 2 hours or as late as 30 hours following the onset of the synchronized estrus, it may be possible to breed as follows:

Check heat during the first 48 hours after implant removal. In this period, 62% of the synchronized heifers show estrus. A single artificial insemination at 50 hours after implant removal should have provided normal fertility for this portion of heifers. If a second insemination would have been given 80 hours after implant removal, 27% of the heifers synchronized would have been bred at an appropriate time. The 11% left could have been bred according to estrus.

All breeding groups in this experiment had acceptable conception rates. It would be beneficial to determine the maximum interval from onset of estrus to insemination where acceptable conception rates could be maintained.

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LITERATURE REVIEW

Induced Ovulation and Puberty. Puberty is an important reproductive phenomenon in beef heifers. In Foote's (1972) review, puberty is defined as the occurrence of full behavioral estrus and ovulation because both events are minimal requirements for natural conception.

Puberty in beef heifers is influenced by breed, level of nutrition (Hansel, 1959; Joubert, 1963; Bellows et al., 1965; Wiltbank et al., 1969) and growth rate (Reynolds et al., 1963; Wiltbank et al., 1966). In addition, breeding systems and other management factors also influence the age at which heifer achieve puterty.

Heifers calving early as two year olds continue calving early in subsequent years and wean more pounds of calf in their lifetime (Burris and Privde, 1958; Lesmeister et al., 1973). However, a large proportion of heifers do not show heat before the start of the regular breeding season (Bellows, 1968; Arije and Wiltbank, 1971; Wiltbank, 1974). Treatments for estrus synchronization in cycling animals are ineffective in many prepuberal beef heifers. Therefore, techniques involving the administering of additional exogenous hormones to induce puberty and ovulation in non-cycling heifers appears necessary.

Gonadotrophins. As early as 1935, Casida demonstrated that ovulation could be induced in prepuberal gilts by giving multiple injections of pregnant mares' serum gonadotrophin or purified pituitary powder. More recently, combinations of PMS and HCG have synchronized estrus with high fertility in prepuberal gilts

(Schilling and Cerne, 1972; Baker and Rajamahendran, 1973). In sexually immature ewe lambs, 1000 IU PMSG causes a significantly higher ovulation rate (13.2) and heavier ovaries, corpora lutea and reproductive tract (Goerke and Dutt, 1975). Ovulation can also be induced in calves (Black et al., 1953; Casida et al., 1943; Onuma et al., 1970). Various combinations and dosages of FSH or PMS with HCG or LH were tried and the best response was obtained with PMS and LH (Onuma et al., 1969 and 1970). Administering gonadotrophins usually causes superovulation which is essential for the success of egg transfer techniques and multiple births. Fertility in superovulated young calves generally has been low (Avery and Graham, 1962; Foote and Onuma, 1970; Lineweaver and Hafez, 1970). In ovulated or superovulated calves the corpora lutea eventually regress (Casida et al., 1943; Spilman et al., 1970) without substantial evidence of further cyclic behavior. Foote (1972) suggested that poor fertility may result from an immature heifer tract which appeared hostile to sperm and to the developing embryo. On a practical scale, it is unlikely that small heifers could sustain pregnancy, especially for those heifers that ovulation was induced as early as 1 to 2 months of age. Generally, application of exogenous gonadotrophins causes ovulatory response, but the results are highly variable.

Progesterone + Estrogen. For the older heifer approaching puberty, the estrus synchronization technique of combining progestogen and estrogen has proven successful (Wiltbank and Gonzalez-Padilla, 1975) in inducing estrus. An implant of 6 mg of nor-

gestomet in the ear for 9 days combined with an injection of 3 mg of norgestomet and 5 mg of estradiol valerate at the time of implantation induced estrus in some prepuberal yearling heifers without a marked reduction of fertility. Fifty percent of the non-cycling control heifers were in heat during the 45-day breeding season compared to 94% of the non-cycling treated heifers within 4 days period after treatment. Conception rates after 45 days were 7% and 94% for control and treated heifers, respectively. Gonzalez-Padilla et al. (1975a) reported the effect of progesterone priming in heifers reaching puberty. Gonzalez-Padilla et al. (1975b) demonstrated that prepuberal heifers receiving progesterone and estrogen developed CL and cycled. However, those heifers receiving progesterone or estrogen alone did not show behavioral estrus nor the development of CL. Gonzalez-Padilla et al. (1975c) also induced fertile estrus in non-cycling beef heifers by giving the same treatment as Wiltbank and Gonzalez-Padilla (1975). Seventy-nine to 94 percent of the heifers showed estrus within 4 days after implant removal and 43 to 56% of these heifers became pregnant. On the other hand, the control groups had only 0 to 5% in estrus within the same period and only 4% conceived.

GnRH. Isolation of porcine (Schally et al., 1971) and ovine (Amoss et al., 1971) luteinizing hormone/follicle stimulating hormone releasing hormones (LH-RH/TSH-RH) resulted in its rapid structural identification (Matsuo et al., 1971a, and 1971b). The decapeptide, GnRH, both purified natural and synthetic products were shown to induce the release of endogenous gonadotropins in the bovine (Schams et al., 1973; Golter et al., 1973; Kaltenbach

et al., 1973; Zolman et al., 1973). The effect of GnRH on LH release in cattle and sheep was well documented by Convey (1973). For cycling beef heifers, 250 ug of synthetic GnRH given intramuscularly stimulated release of both LH and FSH. (Kaltenbach et al., 1974). They reported that high endogenous levels of progesterone may exert an inhibitory influence and if GnRH is given at or near estrus when peripheral levels of estrogen are relatively high, increases in serum LH are obtained. A positive relationship between serum estrogen concentration and GnRH may exist. Henricks et al. (1971) and Shemesh et al. (1972) showed that the pituitary is more sensitive to GnRH near estrus, probably induced by an elevated estrogen level. Increasing the dose of GnRH from 50 to 1500 ug administered IV or IM caused a linear increase in plasma LH and a curvilinear changes in plasma FSH. Schams et al. (1973) suggested that synthetic GnRH does not evoke a completely normal physiological response. Using a new synthetic GnRH (Hoe 766) intramuscularly, Nawito et al., (1977) found that LH response was always more pronounced and prolonged than the FSH response. Evans and Irvine (1977) reported that in acyclic mares, a combined treatment of GnRH and appropriate progesterone can induce normal cyclic pituitary and ovarian activity and cause ovulation. In the bovine, ovulation can not be induced by treatment with LH-RH if a functional CL is present. Britt (1975) reported that ovulation was induced 1 day after LH-RH administration in cows after parturition. Cows with ovarian follicular cysts treated with LH-RH have the cysts luteinized and the estrous cycles initiated. In diestrus heifers and cows

or proestrus heifers; however, LH-RH was not effective. Mellin et al. (1975) found that GnRH generally stimulated follicular growth in calves, but in most instances did not result in ovulation except in those calves that were PMS primed.

In Robinson's colloquium (1975), he stated the difficulty of assessing the potential of synthetic GnRH in the overall context of control of sexual cycle in domestic animals. It can only operate when the pituitary is "loaded" and so in many situations is no substitute for PMS and HCG. The study of the characteristics and potential of the releasing factors is still in its infancy.

INTRODUCTION

Age at puberty in heifers is an important factor in determining the efficiency of beef production. In the studies on the age at puberty in beef cattle, Wiltbank et al., (1966) drew attention to the fact that profitability of beef-calf production under range conditions depends basically on the reproductive rate of the cow. Ideally, the maximal lifetime performance of beef cows could be achieved by calving at 2 years of age (Webb et al., 1955; Zimmerman et al., 1957) which requires the heifers to breed by 15 months of age.

Age of puberty is variable. Morrow (1969) reported that 74% of Holstein heifers have their first ovulation accompanied by silent estrus and 13% of those in first standing estrus did not ovulate. A large proportion of beef heifers did not show estrus the first 21 days of breeding season (Wiltbank et al., 1961; Wiltbank, 1974). Therefore, age at puberty becomes increasingly critical if heifers calving as early as 2 years old is demanded.

Studying the interrelationship in levels of major reproductive hormones in the blood occurring before and at the onset of puberty, Gonzalez-Padilla et al. (1975a) suggested that the establishment of the mature pattern of LH secretion in heifers is not a sudden event but gradual. The change may be the result of a stepwise increase in the levels of progesterone with each elevation serving as a primer for further maturation of the hypothalamo-pituitary-ovarian axis.

In female rats, estrogen injections during the prepuberal

period increase the release of LH and advance the onset of puberty (Ramirez and McCann, 1963; Ramirez and Sawyer, 1965). A progesterone-estrogen interaction facilitated induced ovulation in prepuberal rats (Caligaris et al., 1972 and 1973). Short et al. (1976) reported that an implant of progesterone (2.1 g) for 6 days plus an injection of $E_2\beta$ (5 mg) 24 hours after implant removal induced more prepuberal heifers to show heat and ovulate within 4 days compared to the treatment of $E_2\beta$ alone.

Gonzalez-Padilla et al. (1975b) proposed utilizing estrogen and progesterone to mimic the changes in blood hormone levels near puberty. One intramuscular injection of 20 mg progesterone followed by 2 mg of estradiol-17 β 48 hours later induced CL formation and cycling in prepuberal heifers. Gonzalez-Padilla et al. (1975c) also induced puberty in heifers with an injection of norgestomet (3 mg) that was removed 9 days later. Estrus was detected in 94% of the treated heifers with 50% fertility at the first service. DeBenedette (1977) applied the same procedure and successfully induced puberty (93 to 96% of heifers). However, the incidence of repeated estrus or short estrous cycles was noted (38 to 43% of heifers) and the first service conception rate was low (23 to 40%). Low conception, short estrous cycles and split estrus also reported by Short et al. (1976).

The purpose of the present research was to determine if priming with gonadotrophins would improve fertility after progesterone-estrogen treatment in prepuberal beef heifers.

MATERIALS AND METHODS

Thirty-three yearling Simmental X Hereford, Hereford and Polled Hereford heifers that had not cycled by the beginning of the breeding season were used in this trial. Heifers ranged from 370 to 440 (avg, 410) days of age and weighed 240 to 358 kg (avg. 291 kg). Heifers were checked for signs of estrus for 25 days and ovaries were palpated 7 days before and at the time of implantation to insure that they had not ovulated. In Group I, 17 heifers were implanted subcutaneously with norgestomet (SC 21009, 6 mg) on the posterior side of the ear for 9 days and 3 mg norgestomet plus 6 mg estradiol valerate was injected intramuscularly at the time of implantation. In Group II, 7 heifers were treated the same as Group I except they received an intramuscular injection of 2 cc. Godin-5 and 1500 IU HCG at 7 and 4 days before implantation, respectively. Eight heifers in Group III received no treatment and served as controls. After implant removal all three groups were confined to dry lot. Heifers were checked for estrus every 4 hours for a 5 day period then twice a day for 16 days. If found in estrus, heifers were bred artificially approximately 12 hours later. Heifers were then placed with a bull and allowed to breed naturally for another 41 days. Conception rates were determined by rectal palpation 73 to 88 days after insemination and again 60 days after the end of the breeding season. The results were analyzed by Goodness-of-fit-test for testing the homogeneity of proportions.

RESULTS

All prepuberal heifers treated with either Syncro-Mate B (Group I) or a combination of Syncro-Mate B plus gonadotrophin (Group II) exhibited estrus within 36 hours after the implant was removed. Only 2 of 8 controls showed heat during this time. The average interval from implant removal to onset of estrus was 19.2 and 24.6 hours for Group I and II, respectively. The effect of both treatments on occurrence of estrus differed from controls ($P < .01$); however, fertility at the synchronized estrus was low (Table 1).

Pre-treatment with gonadotrophin did not improve conception rate at the synchronized estrus and there was no difference ($P < .1$) among the three groups. Conception rates during the breeding season were not different ($P < .5$), which were 53.9, 42.9 and 37.5% for Group I, II, and III, respectively.

Although all heifers in the treated groups exhibited estrus within 36 hours after implant removal, some did not continue cycling during the breeding season. The effect of treatment on inducing cycling is shown in Table 2. No differences ($P < .1$) existed among the three groups; however, the Syncro-Mate B treatment tended to result in a higher percentage of heifers cycling.

The time interval from beginning of the breeding season (the day of implant removal in treated heifers) to conception among the three groups was not different ($P < .1$), however, Syncro-Mate B treated heifers tended to breed earlier (Table 3).

Weight and age did not affect any of the parameters measured and shown in Tables 1, 2 and 3. Average weight and average age

were not different ($P < .5$) among the three groups except for the age of those heifer that never cycled (Table 4).

Two heifers in Group II showed estrus after gonadotrophin pretreatment and two more exhibited estrus before the implant was removed.

TABLE 1. PERCENT OF SYNCHRONIZATION AND CONCEPTION RATE
IN PREPUBERAL BEEF HEIFERS BY TREATMENTS

Treatment	No. of animals	Percent in estrus after implant removal		Conception rate (%) after implant removal	
				36 hours	62 days
		36 hours ^c	62 days ^d	36 hours	62 days
I Syncro-Mate B ^a	17	100 (17/17)	100 (17/17)	17.6 (3/17)	52.9 (9/17)
II Godin-5 + HCG + Syncro-Mate B ^b	7	100 (7/7)	100 (7/7)	0 (0/7)	42.9 (3/7)
III Control	8	25 (2/8)	75 (6/8)	0 (0/8)	37.5 (3/8)

^aSix mg norgestomet ear implant for 9 days plus 3 mg norgestomet and 6 mg estradiol valerate injected intramuscularly at time of implantation.

^b+Two cc. of A. P. Godin-5 (Haver-Lockhart Lab.) each cc. contains 25 Fevold-Hisaw-Rat Unit of gonadotrophins) injected 7 days before Syncro-Mate B procedure plus 1500 IU HCG 3 days after Godin-5.

^cTreatment groups different (P<.01) from control.

^dTreatment groups different (P<.05) from control.

TABLE 2. INDUCTION OF CYCLING IN PROPUBERAL HEIFERS

Treatment	No. of animals	Percent of cycling after impl. removal ^a		Interval from impl. removal to cycling(days)	
		25 days ^b	62 days	Average	Range
I Syncro-Mate B	17	64.7 (11/17)	88.2 (15/17)	18.1	15-38
II Godin-5 + HCG + Syncro-Mate B	7	28.6 (2/7)	71.4 (5/7)	23.4	15-27
III Control	8	37.5 (3/8)	75.0 (6/8)	18.8	21-37

^aData refers treated heifers continued cycling or controls showing their first estrus. No difference ($P < .1$) among groups were detected.

^bThree heifers in Group I that concieved at the synchronized estrus were considered cycling and were included in the calculation as cycling. No heifers in Group II and III concieved the first 25 days.

TABLE 3. EFFECT OF TREATMENTS ON DAYS TO CONCEPTION

Treatment	No. of animals	Mean \pm S E ^a
I Syncro-Mate B	8	16.63 \pm 18.96
II Godin-5 + HCG + Syncro-Mate B	3	31.00 \pm 6.93
III Control	3	26.67 \pm 17.35

^aOne-way analysis of variance showed no difference ($P < .1$) among means.

TABLE 4. EFFECT OF WEIGHT AND AGE ON THE ABILITY OF PREPUBERAL HEIFERS TO CYCLE AFTER INDUCTION OF PUBERTY

Treatment	Average days of age			Average weight (kg.)		
	Treated heifers	Cycling	Non- ^a cycling	Treated heifers	Cycling	Non-cycling
I Syncro-Mate B	410.8 (17)	413.5 (15)	390.5 (2)	296.6 (17)	295.1 (15)	308.2 (2)
II Godin-5++ HCG + Syncro-Mate B	403.0 (7)	406.8 (5)	395.5 (2)	279.4 (7)	285.8 (5)	263.2 (2)
III Control	415.5 (8)	409.5 (6)	433.5 (2)	293.5 (8)	293.5 (6)	293.6 (2)

^aNo significant difference except for average days of age for non-cycling heifers among three groups ($P < .01$).

DISCUSSION

Syncro-Mate B with and without gonadotrophin pretreatment was highly successful (100%) in inducing puberty and synchronizing estrus in prepuberal beef heifers. The Syncro-Mate B results are in agreement with those reported by Wiltbank *et al.* (1975), Gonzalez-Padilla *et al.* (1975c) and DeBenedette (1977). Pretreatment with gonadotrophin had no beneficial effect on Syncro-Mate B treated heifers.

First service conception was extremely low for treated heifers (17.6% and 0% for Group I and II, respectively). However, no control heifers conceived the first 25 days of the breeding season. Wiltbank and Gonzalez-Padilla (1975) reported 43% and Gonzalez-Padilla *et al.* (1975c) reported 43 to 56% first service conception after treating prepuberal heifers with Syncro-Mate B. Low first service conception at the synchronized estrus has been generally acknowledged as the major detriment to inducing puberty. There was a tendency for Syncro-Mate B treated heifers to cycle earlier in the breeding season and for conception rates to be higher especially early in the breeding season. Gonzalez-Padilla *et al.* (1975c) reported 71% of heifers were pregnant after a 28-day breeding period in one trial, and in another trial, the pregnant rates were 11 and 58% after 25 days of breeding and 27 and 73% after 48 days of breeding for the control and treated groups, respectively. In the present experiment, some benefit resulted from the Syncro-Mate B treatment. During a 62-day breeding season, 52.9 and 42.9% conception rates were obtained in Group I and II, as compared to 37.5% in the controls.

Various stages in approaching puberty may be one of the factors

contributing to the variability between reports in heifers exhibiting estrus and in the fertility. Some heifers used in this experiment were apparently close to puberty at the time of treatment, because 37.5% of the control were in estrus the first 25 days of the breeding season. Wiltbank *et al.* (1975) reported 50% of the control heifers had been in estrus after 45 days of breeding compared to 94% of treated heifers after 4 days of breeding. The same situation was reported by Gonzalez-Padilla *et al.* (1975c), where 38 and 84% were in estrus within 48 days for control and treated heifers, respectively.

The whole story of hormonal mechanism in approaching puberty in cattle is far from complete. Gonzalez-Padilla and co-workers (1975a) described some hormonal changes associated with puberty and demonstrated that blood level of LH changed strikingly as puberty approached in heifers. There appeared to be a close association between LH and progesterone. The establishment of the mature pattern of LH secretion is not a sudden event but gradual. Progesterone levels were low 20 days prior to puberty and then, there were two periods of 2-5 days durations of elevations of progesterone between 18 to 11 and 7 to 1 days before puberty. With each elevation, progesterone serves as a primer for further maturation of the hypothalamo-pituitary-ovarian axis and causes an ovulatory surge of LH. The extremely detrimental effect of treatment on first service conception at induction of puberty in the present experiment could be partially due to the exogenous hormonal distribution which was given to the heifers while most of them were in their critical stage of approaching puberty.

Pretreatment with Godin-5 and HCG prior to Syncro-Mate B

(Group II) did not improve the fertility or induction of puberty over Syncro-Mate B alone.

Induced puberty appears to be a feasible technique in beef heifers since all treated heifers showed estrus and approximately one-half continued to cycle after treatment. However, conception rate at the induced puberty must be improved for this to become a practical and economically feasible procedure. Gonadotrophin treatment as administered in this experiment did not appear to be a suitable procedure for increasing fertility in heifers in which puberty was induced.

Whether or not Syncro-Mate B will be an economical procedure for inducing puberty lies in the cost of semen and labor required to breed all heifers at the induced estrus.

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ARTIFICIAL INSEMINATION AT VARIOUS INTERVALS
AFTER ONSET OF SYNCHRONIZED ESTRUS AND
INDUCED PUBERTY IN BEEF HEIFERS

by

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AN ABSTRACT OF A MASTER'S THESIS

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Syncro-Mate B implants (6 mg) were placed in 162 cycling Angus (A), Polled Hereford (PH), Hereford(H), and Simmental x Hereford (SxH) heifers and removed 7 days later (AM or PM of day 0). Each heifer was then injected intramuscularly with 33.3 mg of prostaglandin (PGF_{2x}) THAM buffer in 5 ml of sterile water. Heifers were confined to dry lot and observed for estrus every 4 hours during a five-day period. Heifers in 1976 (98 head) were artificially inseminated 6, 10, 14, 18, 22, or 26 hours after being observed in standing estrus. Heifers in 1977 (54 head) were bred at the same times except two additional groups bred 2 or 30 hours after onset of estrus were added. Heifers detected in estrus at each time period were divided into AM and PM breeding groups (bred at either 8:00 AM or 8:00 PM).

Ninety-six percent, 90%, and 93.8% of treated heifers were in estrus within a five-day period after treatment for 1976, 1977, and 1976+1977, respectively. First service conception rates at the synchronized estrus were 64.3, 50.0, and 59.2% for 1976, 1977, and 1976+1977, respectively. Conception rates were 44.4, 58.3, 57.1, 56.5, 62.5, 45.5, 78.9, 70.0, and 59.2% for heifers bred at 2, 6, 10, 14, 18, 22, 26, 30 hours after the onset of synchronized estrus, and overall, respectively. No significant difference was found among breeding groups ($P < .5$). There was no difference in conception rates between AM and PM breeding (58.7% and 59.7%, respectively; $P < .5$).

Thirty-three yearling H, PH, and SxH heifers that were not cycling at the beginning of the breeding season were used to determine the effectiveness of two treatments for inducing puberty.

Seventeen heifers received the Syncro-Mate B treatment which consists of an implant of norgestomet (6 mg) for 9 days and an injection of 3 mg norgestomet plus 6 mg estradiol valerate im at the time of implantation (Group I). In Group II, 7 heifers received an im injection of 2 ml Godin-5 (contains 50 Fevold-Hisaw-Rat Units of gonadotrophins) and 1500 IU HCG at 7 and 4 days before the Syncro-Mate B treatment, respectively. Eight heifers were not treated and served as controls (Group III).

The percentage of animals exhibiting estrus within 36 hours after the implant removal was 100, 100, and 25% for Group I, II, and III, respectively. Fertility at the synchronized estrus was 17.6, 0, and 0% for Group I, II, and III, respectively. Conception rate during the breeding season (62 days), the percentages that continued cycling within 25 days after implant removal, and the percentages cycling within 62 days after implant removal were 52.9, 64.7, and 88.2 for Group I, 42.9, 28.6, and 71.4 for Group II, and 37.5, 37.5, and 75.0 for Group III, respectively. Syncro-Mate B treatment (Group I) resulted in a higher percentage of the heifers cycling and conceiving, although there was no significant difference. Pretreatment with gonadotrophin prior to Syncro-Mate B (Group II) did not improve the fertility or induction of puberty over Syncro-Mate B alone.