MAINTENANCE OF PROGESTERONE RELEASING INTRAVAGINAL DEVICES (PRIDs) AFTER $PGF_2\alpha$ INCREASES SYNCHRONIZATION OF LH SURGES AND ESTRUS IN HOLSTEIN HEIFERS

By

Benon M. Kanyima

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1984

ABSTRACT

MAINTENANCE OF PROGESTERONE RELEASING INTRAVAGINAL DEVICES (PRIDs) AFTER $PGF_2\alpha$ INCREASES SYNCHRONIZATION OF LH SURGES AND ESTRUS IN HOLSTEIN HEIFERS

By

Benon M. Kanyima

Objective was to increase precision of LH surges and estrus with reduced progesterone and increased basal LH.

Control and experimental heifers received PRIDs (2% progesterone) for 7 and 10 d. Controls received BLANKs (0% progesterone) for 3 d after PRIDs removal. All received $PGF_2\alpha$ on day 7. After $PGF_2\alpha$, blood from experimental heifers (Exp. I) was collected 4-hourly for 72 h. Then from all heifers, blood was collected 4-hourly for 12 h, 2-hourly for 72 h and 4-hourly for 36 h.

Intervals from PRIDs removal to LH surges in experimentals (32.2 $\pm$ 1.8 h; range 26-38 h) varied less ($p < .05$) than controls (59.0 $\pm$ 6.1 h, range 34-78 h).

Schedules for PRIDs and $PGF_2\alpha$ in Exp. II were as for Exp. I. After PRIDs removal, observations for estrus were every 4 h for 96 h.

Intervals from PRIDs removal to estrus in experimentals (40.0 $\pm$ 1.2 h; range 24-56 h) varied less ($p < .05$) than controls (60.8 $\pm$ 2.2 h; range 36-88 h).

Maintaining PRIDs after $PGF_2\alpha$ synchronizes LH surges and estrus in Holstein heifers.

Dedicated to Mom, Dad,

Brothers, Sisters, and

Brenda

ACKNOWLEDGMENTS

My sincere thanks and appreciation are extended to: Dr. R. L. Fogwell, my major Academic Advisor for his faithful guidance, constructive criticisms and availability to assist me during production of this thesis, Dr. John B. Kaneene and Dr. David A. Morrow, for their dedication to my work while they served as members of my guidance committee; Faculty and Graduate Students particularly Alex Villa-Godoy and William Enright for their collaborative effort and availability to assist me during my experiments; and to the rest of my friends for their moral support during the 2 years of my stay at MSU.

Heartfelt thanks and love are extended to Brenda Mbabazi Kanyima for her patience and understanding during various stressful situations and to Susan Mwebesa, Penina Tukahiirwa, Metu Bagyenyi, Charles Atuheire and Nathan Twinamasiko for their continuous prayers and words of encouragement.

Final gratitude is extended to my parents Rev. Canon K. and Mrs. M. Kanyima, my brothers Kenneth, David and Andrew and my sisters for their continuous prayers, encouragement and rememberance while I was on this mission.

Last but not least, I don't forget the soul of Goretti Turyahabwe. I remember Fred T. Bujuuri for the encouragement he provided me while he lived. May he be in comfort wherever he is? God knows what happened!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Reproduction has been identified as most limiting to efficient production of farm animals (Cartwright, et al, 1980). Consequently, it is justified to study control of reproduction in farm animals in order to achieve or increase their reproductive potentials.

Since domestication, cattle have been an important source of food for man. Ability of cattle to utilize low quality nutrients in forages to synthesize high quality proteins (eg. milk and meat) makes cattle the least-cost and most efficient food resource for humans.

Necessity to control reproduction of cattle arises from the need to increase reproductive efficiency through enhancement of superior genetic potentials for their productivity. This in turn ensures availability of food for humans. Improvements of reproductive efficiency of cattle is limited by biology of gestation. The average gestation period of 283 days means that, the maximal achievable reproductive performance in cattle is limited to annual calving.

Research into breeding and genetics of dairy cattle has enabled the Holstein cattle to produce large quantities of milk at an early age (eg. 24 mo.). There

is great advantage in perpetuating this milk production potential both within and among cattle breeds and successful reproduction is the key to perpetuation of this potential.

Artificial insemination (AI) is presently, the common and most available practical method to perpetuate superior genetic material among cattle. For AI to succeed, cows or heifers ideally, should be inseminated at a stage of estrus which ensures maximal conception. One major obstacle to increased usage of AI is detection of estrus especially in Beef cattle and Dairy heifers. Methods to increase ability of cattlemen to detect estrus accurately or better eliminate the requirement to detect estrus would enhance use of AI in the cattle industry. Fertility range of bovine spermatozoa is 24h; within which ovulation should occur. Thus fixed time AI or AI without detection of estrus is a group of cattle results into fertilization if precision of synchronized estrus or ovulation is witnin a range of 24h. In addition to AI, faster perpetuation of superior genetic material in cattle can be achieved through superovulation and embryo transfer (ET) technologies. However, for successful transfer of embryos to a large number of recipients, stage of an estrus cycle of the donor and recipients needs to be synchronous.

It appears that for full realization of reproductive potentials of cattle, through AI and ET, methods to artificially synchronize estrous cycles have to be employed. Thus increased precision of estrus and ovulation following estrous synchronization would reduce inaccurate timing of AI and ET. This would increase reproductive efficiency and enhance genetic progress of cattle.

It is the intention of this research to investigate if maintaining 1 to 2 ng/ml of progesterone for 3 days after $PGF_{2\alpha}$ will increase precision of preovulatory surges of LH and estrus in Holstein heifers.

REVIEW OF LITERATURE

Introduction

Cattle are polyestrous and capable of reproduction independent of season. Duration of bovine estrous cycles varies within and among cattle but ranges from 18 to 24 days (average = 21 d) Asdell, 1964, Desjardins and Hafs, 1968; Morrow, 1969).

Ovarian steroids (progesterone and estradiol 17β) are two major hormones, controlling bovine estrous cycles. Concentrations of ovarian steroid hormones influence secretory patterns of gonadotropic hormones (luteinizing hormone (LH) and Follicle Stimulating hormone (FSH)). Concentrations of gonadotropic hormones secreted from anterior pituitary gland, are required for growth and development of ovarian follicles (Peters, 1979).

Artificial methods have been developed to mimick and/or modify secretion of reproductive hormones for purposes of controlling reproduction. Progesterone, estradiol 17β and luteolytic prostaglandins can be used for synchronizing estrus and ovulation. Various routes of administration for synchronization treatments are employed for the different regimens used in synchronization of estrus and ovulation of cattle. Currently,

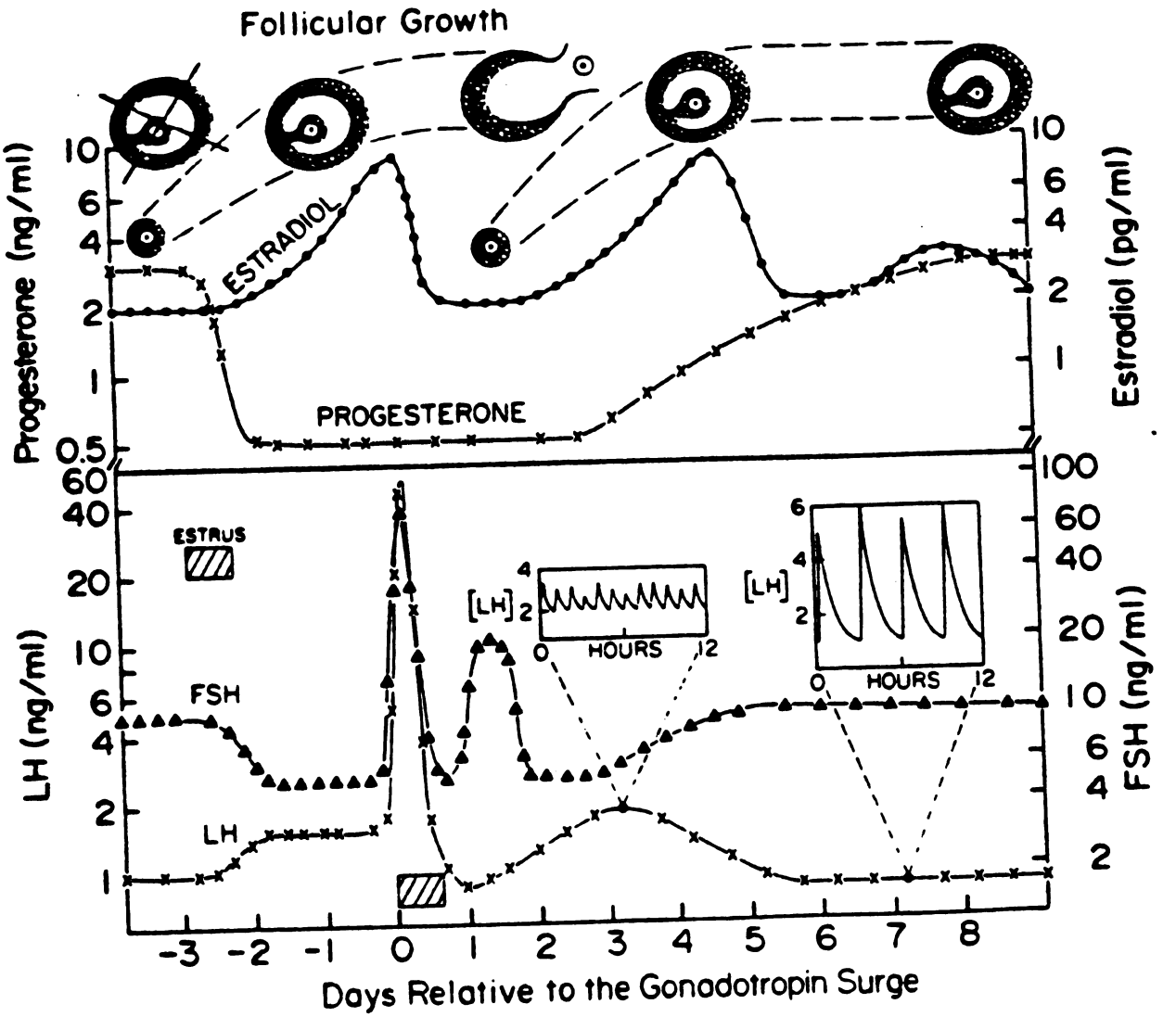
regimens for estrous synchronization, use either progestagens combined with either estradiol 17β (Roche, 1978; Roche, 1979a) or prostaglandin $F_2\alpha$ ($PGF_2\alpha$) or its analogues. Use of combination regimens for estrous synchronization increases percentages of cattle, responding to estrous synchronization treatments (Britt, 1979). In addition, $PGF_2\alpha$ can be administered alone for estrous synchronization of cattle. Single injections or double injections of $PGF_2\alpha$, spaced 11 days apart can synchronize estrus in cattle (Thatcher, 1976).

General Survey of Bovine Estrous Cycles

A diagramatic description depicting hormonal and ovarian follicular changes during bovine estrous cycle is shown in Figure 1 obtained from a review article (Hansel and Convey, 1983). Follicular growth and function occur continuously throughout the bovine estrous cycle. Ovulation takes place 10 to 12 h after surge of gonadotropic hormones (luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH)) when concentrations of progesterone in serum are less than 1 ng/ml.

Concentrations of progesterone in serum of cattle begin to decline on day 16 or 17 of an estrous cycle (Hansel et al, 1973) following endogenous secretion of estrogens by ovarian follicles (Ireland, 1984). Secretion of estrogen by ovarian follicles occurs after middle of

Figure 1.--Follicular and endocrine changes from luteal regression to resumption of luteal function in cows (Hansel and Convey, 1983).


Follicular Growth
Progesterone (ng/ml)
Estradiol (pg/ml)
ESTRADIOL
PROGESTERONE
LH (ng/ml)
FSH (ng/ml)
ESTRUS
FSH
LH
[LH]2
[LH]
HOURS
Days Relative to the Gonadotropin Surge

the bovine estrous cycle (Ford et al, 1975). Estrogens stimulate the endometrium to synthesize and release prostaglandin $F_2\alpha$ ($PGF_2\alpha$) (Horton and Poyser, 1976; Bartol et al, 1980). $PGF_2\alpha$ is locally transferred via utero-ovarian vasculature (Ginther, 1976) to the ovary bearing a corpus luteum (CL) to cause luteal regression. Concurrent with declining concentrations of progesterone, after $PGF_2\alpha$-induced luteal regression, basal concentrations of LH increase (Hobson and Hansel, 1972; Chenault et al, 1975). Increased basal concentrations of LH after luteal regression probably promote follicular growth when concentrations of progesterone in serum, are low (Roche and Ireland, 1981b).

Artificial Control of Bovine Estrous Cycles

Objective of artificial methods for controlling reproduction in cattle, is to synchronize estrous cycles in a group of cattle without impairing fertility so that maximum numbers of synchronized cattle can be bred within a specified time interval. Artificial control of estrous cycles is achieved through regulation of endogenous secretion of reproductive hormones. Ideally, inhibition or induction of follicular growth and ovulation achieved by regulating lifespan of CL, will synchronize bovine estrous cycles (Hafez, 1969). Thus, basis of artificial methods

for controlling bovine estrous cycles is to prevent ovulation by administering progestagens or to induce luteal regression by administering luteolytic substances. Following withdrawal of progestagens or luteal regression, follicular growth and ovulation occur (Hafez, 1969; Roche, 1976b). For purposes of this review, progestagens and luteolytic substances (estrogens and prostaglandins) used for synchronizing estrus of cattle, will be discussed.

Progestagens

Administration of sufficient dose of progesterone or synthetic progestagens to cows, will suppress estrus without affecting lifespan of the CL (Christian and Casida, 1948). To control estrus progestagens can be administered for 18 to 21 days (Long term progestagens) or 7 to 12 days (Short term progestagens). Luteolytic substance can be administered at the beginning (Wiltbank and Kasson, 1968) or the end (Wishart, 1974a; Roche, 1976b) of a short-term progestagen treatment, to enhance estrous response.

Long term progestagens

Several progestagen compounds can be administered to control estrus and ovulation for a duration of 18 to 21 days. In cattle some progestagens can be administered

by oral route (eg. Fluorogestone Acetate (FGA), Chloroacetoxy progesterone (CAP)) but others (eg. norprogesterone (SC 21009) and melangesterol acetate (MGA)) are administered via subcutaneous route (Mauleon, 1974). Intramuscular route for SC 21009 or MGA cannot maintain sufficient concentrations of progesterone in serum of cattle to block estrus (Mauleon, 1974), hence intra muscular route is not commonly used for administering progestagens. Intravaginal route for administering progestagens indicated that FGA could diffuse across vaginal mucosa easily. Thus long term progestagens can be administered intravaginally to control estrus in cattle.

Progestagens are withdrawn after 18 to 21 days after corpora lutea in all treated cows have regressed spontaneously (Britt, 1979). Essentially all treated cows will be in follicular phase of an estrous cycle when progestagens are withdrawn (Roche, 1979a). Given that lifespan of CL is not affected by progestagens duration of exposure to progestagens among follicular phase animals is variable. However, most of the treated cattle manifest behavioral signs of estrus 2 to 6 days after withdrawal of progestagens (Roche, 1976a). The degree of synchronization within a specific period of 24 h to 48 h after withdrawal of progestagens ranges from 68 to 98 percent (Mauleon, 1974).

Non-synchronization occurs in less than 10 percent of the animals (Mauleon, 1974). Theoretically precision of synchronized estrus after long term progestagen treatment would be sufficient for application of artificial insemination (AI) at a fixed time. But fertility of cattle following either natural or artificial insemination was low (Jochle, 1972; Roche, 1974). There was a possibility that high concentrations of progesterone in serum due to administered progestagens, was detrimental to fertility. However, it appeared that detrimental effect was associated with duration of progesterone and variable onset of follicular phase among treated cattle. Residual effect of exposing ovarian follicles to long term progestagens further reduced fertility of treated cattle in the ensuing estrous cycle by 7 to 10 percent (Mauleon, 1974). Long term progestagens have been withdrawn from being used for synchronizing estrous cycles of cattle but MGA can still be administered via subcutaneous implants or feed to prevent occurrence of estrus and ovulation in feedlot heifers.

Short term progestagens

Short term progestagens for synchronizing estrus in cattle have been described (Wiltbank and Kasson, 1968; Mauleon, 1974; Roche, 1974; Sreenan and Mulvehill, 1975b). Subcutaneous route for administering short term

progestagens is commonly used. However, Roche (1975) described a method which used Progesterone Releasing Intravaginal Devices (PRID) to administer short term progestagens. Description of the method for installing PRID's in vagina of cattle was discussed (Roche, 1979a).

Fertility of cattle after synchronized estrus using short-term progestagens for 12 days is not impaired (Mauleon, 1974; Roche, 1974; Sreenan, 1975a; Wiltbank and Gonzalez-Padilla, 1975; Roche, 1976c) in contrast to using short term progestagens for 14 days. Thus impairment of fertility of cattle synchronized with progestagens is alleviated by minimizing duration of treatment with progestagens. However, precision of synchronizing estrus within 24 to 48 h is reduced unlike when long term progestagens are used. Percentage of synchronized cattle, manifesting estrus within a specified period of 48 h after short term progestagens ranged from 45 to 67 percent in contrast to 68 to 98 percent after long term progestagens (Mauleon, 1974). It is evident that despite ability of short term progestagens to maintain fertility of treated cattle, possibility for using short term progestagens for AI at fixed time, is limited because of assynchrony of intervals from withdrawal of progestagens to estrus or ovulation among cattle. Consequently there

is need to increase precision of synchronized estrus and ovulation.

Prostaglandins

Local or systemic injections of $PGF_2\alpha$ or its analogues effectively cause luteolysis between day 5 and 16 or 17 of bovine estrous cycle (Rowson et al, 1972; Lauderdale, 1972; Louis et al, 1972; Cooper, 1974a). $PFG_2\alpha$ or its analogues do not cause regression of CL in cattle during day 0 to 4 or 17 to 21 of an estrous cycle (Rowson et al, 1972; Louis et al, 1973; Hafs et al, 1974). However non responsiveness of CL during metestrus (days 0 to 4) or follicular phase (days 17 to 21) can be overcome if injections of $PGF_2\alpha$ or its analogues are administered using either of the following options:

1. two injections of $PGF_2\alpha$[1] spaced 10 to 12 days apart (Cooper and Furr, 1974b; Heersche et al, 1974; King and Robertson, 1974; Graves et al, 1974; Hafs et al, 1975; Cooper, 1974a).
2. One injection of $PGF_2\alpha$ with modifications in the management as follows:

---

[1] $PGF_2\alpha$ will refer to both $PGF_2\alpha$ and its analogues.

(a) prior ascertainment of CL by palpation of ovaries per rectum before injection of $PGF_2\alpha$, or

(b) breed all animals detected in estrus 4 to 6 days prior to injection of $PGF_2\alpha$ into the remaining animals (Lauderdale et al, 1980).

Two injections of $PGF_2\alpha$ spaced 10 to 12 days apart:

This protocol causes luteal regression in all randomly cycling cows or heifers (Cooper, 1974a; Hafs et al, 1975). Intervals from second injection of $PGF_2\alpha$ to estrus range from 48 h to 120 h (Cooper, 1974a) up to 168 h (Tanabe and Hann, 1984; Smith et al, 1984). Precision of synchronized estrus varies with parity of cattle (Roche, 1979a) and stages of an estrous cycle (Macmillan et al, 1978; Jackson et al, 1979, Refsal and Seguin, 1980; King et al, 1982; Tanabe and Hann, 1984).

Assynchrony of intervals from treatment to estrus does no facilitate AI at fixed time (Britt, 1979). Thus, conception rates after AI performed at fixed time eg 72 h (Cooper, 1976; Roche et al, 1977; Hansel et al, 1978) or 80 h (Thimonier et al, 1975; Smith et al, 1984) are lower than when AI is scheduled by detection of estrus. However, conception rates following two artificial

inseminations at fixed times eg 72 h and 90 h (Lauderdale, 1975) or 72 h and 96 h (Roche, 1976b), are comparable to conception rates after AI scheduled by detection of estrus or spontaneous estrus (Lauderdale et al, 1974; Louis et al, 1975; Hafs et al, 1975).

Single injection of $PGF_2\alpha$

This treatment protocol requires availability of labor as an essential component for it to work. Assynchrony of intervals from single injection of $PGF_2\alpha$ without employing modifications in management as described, only qualifies this technique as aid to detection of estrus rather than a method to use for fixed time AI.

It appears that unless two injections of $PGF_2\alpha$ are to be used to synchronize estrus of cattle for fixed time AI, new methods should be developed to overcome the inability of single injection of $PGF_2\alpha$ to synchronize estrus in randomly cycling cattle.

Combination of Short Term Progestagens with luteolytic substances:

Additional to methods using progestagens or prostaglandins independently, combination of the two synchronization agents may result into synchronization of estrus (Lauderdale, 1975; Thimonier et al, 1975; Nancarrow and Cox, 1976a; Roche, 1976a; DeBenedetti et al, 1977, Chupin and Pelot, 1977).

The major advantage from using combination protocols is that synchronized estrus occurs in all randomly cycling cattle (Wishart, 1974a; Roche, 1976a) and duration of progestagen treatments is reduced (Roche, 1976a; O'Farrell, 1977; Roche, 1978) without impairment of fertility (Thimonier et al, 1975; Roche, 1978). Both estrogens (Wiltbank et al, 1975) and $PGF_2\alpha$ (Thimonier et al, 1975) have been used to reduce duration of short term progestagens to 9 days (eg. with synchromate B) or to 7 days (eg. with PRID-$PGF_2\alpha$).

Short term progestagens combined with estrogens

Administration of estrogens to cattle after middle of spontaneous estrous cycle, causes luteal regression (Wiltbank et al, 1961; Niswender et al, 1965; Brunner, 1969). However, injections of 5 mg of estradiol benzoate at beginning of short term progestagen treatments in cattle, reduce estrous response to synchronization (Roche, 1974). Piper and Foote (1965) reported that estrogens administered early during an estrous cycle of ewe, cause lengthening of the cycle due to increased luteotropic support. However, if 5 mg of estradiol benzoate are administered together with 50 mg of progestagen at beginning of short-term progestagen treatment, estrous response to synchronization is

increased (Roche, 1974; Wiltbank and Gonzalez-Padilla', 1975; Sreenan, 1975a; Webel, 1976). Controversy exists about dosage of estradiol benzoate to use in synchronization regimens. Injection of 5 mg instead of 7.5 mg of estradiol benzoate synchronizes estrus without impairing fertility (Whitman et al, 1972; Burrell et al, 1975). Thus high precision is required for determining dosage of estradiol benzoate which may curtail applicability of short term progestagen-estrogen protocol. However, the protocol is recommended for synchronizing estrus in beef cattle (Wiltbank et al, 1971; Whitman et al, 1972; Burrell et al, 1972; Roche, 1976a; Wiltbank and Gonzalez-Padilla', 1975).

Intervals from removal of short term progestagen (subcutaneous implant) to estrus, range from 1 to 4 days (Wiltbank et al, 1971, D'addammio et al, 1972). Approximately 65 percent of synchronized cattle manifest signs of estrus within 48 h to 72 h of withdrawing progestagens (Wishart and Drew, 1977). Consequently, this synchronization method is recommended for fixed time AI, 48 h to 54 h after withdrawal of progestagen.

In spite of the method of synchronization being recommended for fixed time AI in beef cattle, requirement of high precision in determining dosage of estradiol benzoate probably curtails fertility cattle since

conception rates after fixed time AI are lower than would be expected from estrous response.

Short term progestagens combined with single injection of $PGF_2\alpha$

Concentrations of progesterone released from progestagens block occurrence of estrus and ovulation without affecting lifespan of CL. Injections of 25 mg of $PGF_2\alpha$ into cycling cattle after receiving progestagens for 6 to 7 days, causes luteal regression in all CL-bearing cattle. Cattle originally between days 0 to 5 of an estrous cycle at installment of progestagens would be between day 6 and 12 of an estrous cycle. Consequently protocols using short term progestagens for 6 to 7 days before $PGF_2\alpha$, synchronize estrus in all randomly cycling cattle (Thimonier et al, 1975; Britt, 1979).

Methods for administering short term progestagens have been reviewed. The common method for administering short term progestagens is via PRID (Roche, 1979a; Smith et al, 1980; Smith et al, 1984) especially if synchronization protocol involves $PGF_2\alpha$. Reports indicate that more cows are bred to synchronized estrus with PRID-$PGF_2\alpha$ combinations than by two injections of $PGF_2\alpha$ spaced 11 days apart (Thimonier et al, 1975; Chupin, 1977; Roche, 1979a; Smith et al, 1980; Smith et al, 1984).

Intervals from removal of progestagens to estrus range from 24 h to more than 120 h (Smith et al, 1984; Tanabe and Hann, 1984). Percentage of synchronized cattle 48 h to 72 h after removal of progestagen (40 to 60 percent) is low (Stauffer et al, 1976; Roche, 1979a). Overall, lack of precisely synchronized estrus and ovulation reduces fertility to fixed time AI in contrast to AI, scheduled by detection of estrus (Macmillan et al, 1980).

Variation of intervals from treatment to estrus and ovulation has been discussed (Britt, 1979; Macmillan et al, 1983/84) as a major limitation to successful AI at fixed time (Nancarrow and Cox, 1976a). There are controversial reports regarding conception rates or fertility of cattle inseminated artificially after detection of estrus following synchronization with PRID-$PGF_2\alpha$. Reports agree that conception rates or fertility of cattle after synchronization with PRID-$PGF_2\alpha$ combinations, are similar to those of controls (Wishart, 1974a; Thimonier et al, 1975; Delatang, 1975; Heersche et al, 1979). However, Chupin and Pelot (1976) reported that there is tendency for conception rates to be lower when AI is scheduled by estrus after PRID-$PGF_2\alpha$ (35.6%) than after two injections of $PGF_2\alpha$ (45.9%) or Norgestomet-estradiol benzoate (48.4%). On the contrary, conception rates following AI at a fixed time are not different between treatments

(Chupin and Pelot, 1976). The cause of reduced fertility reported above is not known given that results in a recent report (Smith et al, 1984) favor the opposite view.

To make AI by appointment (AI at fixed time) feasible possible causes of variation in fertility must be identified and understood. Then methods must be developed to control the identified causes, so as to ultimately increase precision of ovulation after treatments used for estrous synchronization.

Causes of Variation in Intervals From PRID-$PGF_2\alpha$ Synchronization Treatment To Estrus in Heifers

In a previous discussion, intervals from removal of PRID to estrus were reported to range from 24 h to more than 120 h, however, Smith et al (1984) has reported that injection of $PGF_2\alpha$, one day before removal of PRID increases precision of synchronized estrus. Similar results were reported by Thimonier et al (1975).

Smith et al (1984) reported that conception rates of heifers synchronized with PRID-$PGF_2\alpha$ and bred to AI at fixed hour of 84 h (66%) were comparable to conception rates of non-synchronized controls, (73%) bred to AI after detection of estrus. It is possible to increase precision of estrus by modifications of PRID-$PGF_2\alpha$

regimen. However, modifications of currently used procedure of PRID-$PGF_2\alpha$ is limited. PRID is required to prevent occurrence of estrus for a minimum of 6 days in order for injections of $PGF_2\alpha$ to induce luteal regression in all randomly cycling cattle (Wishart and Young, 1974b; Roche, 1979a). Duration of PRID in vagina of the cow should not exceed 12 days (Roche, 1979a) otherwise fertility after removal of PRID will be reduced.

To understand variation of intervals from treatment with PRID-$PGF_2\alpha$ to estrus and ovulation, possible causes and variation have been identified:

1. stage of an estrous cycle at injection of $PGF_2\alpha$ and (or) removal of PRID (Roche, 1979a; Tanabe et al, 1984).
2. concentrations of progesterone in peripheral circulation at removal of PRID.

Effect of Stage of an Estrous Cycle on Precision of Synchronized Estrus and Ovulation in Heifers

Possible influence of stage of an estrous cycle on precision of synchronized estrus has been reported (Macmillan et al, 1978; Jackson et al, 1979; Refsal and Seguin, 1980; King et al, 1982; Tanabe and Hann, 1984; Stevenson et al, 1984).

Stage of an estrous cycle at removal of PRID and (or) injection of $PGF_2\alpha$ may be influenced by:

a) function of CL at removal of treatments for estrous synchronization.

b) rate of growth and development (Scaramuzzi et al, 1980) or developmental status of ovulatory follicles (Nancarrow and Cox, 1976 Ireland and Roche, 1982b).

Function of CL at removal of PRID and/or injection of $PGF_2\alpha$

Variation of onset of estrus after injections of $PGF_2\alpha$ on any day of diestrus (King et al, 1982) has been attributed to variable response of CL to luteolytic doses of $PGF_2\alpha$ (Momont and Seguin, 1984). The CL becomes fully functional by day 10 of an estrous cycle. However, estrous response of heifers to luteolytic doses of $PGF_2\alpha$ administered on day 11 of an estrous cycle (13.3%) is less than when $PGF_2\alpha$ is administered on either day 7 (88.4%) or day 15 (73.5%) (Tanabe and Hann, 1984). Similar to the report above, Stevenson et al (1984) observed 97 percent of heifers in estrus if $PGF_2\alpha$ administered on days 5 to 8 in contrast to 83 percent of heifers observed in estrus if $PGF_2\alpha$ is administered on days 14 to 16 of an estrous cycle.

Detection of few heifers in estrus after $PGF_2\alpha$ on day 11 of an estrous cycle is probably related wave patterns of bovine follicular growth. Two periods of

follicular growth and atresia occur in heifers before their midcycle (Ireland and Roche, 1982b). From days 3 to 7, of an estrous cycle a single ovarian follicle develops in heifers but does not ovulate because of inhibitory effect of high (> 1 ng/ml) concentrations of progesterone in peripheral circulation. Atresia of non-ovulatory follicles occurs concurrently with onset of a second wave of non ovulatory follicular growth between day 7 and 13 of an estrous cycle (Ireland and Roche 1982b). Given that around midcycle of heifers, functional transition for estradiol 17β occurs simultaneously with attainment of full functional status of CL; these ovarian changes may affect estrous response to injections of $PGF_2\alpha$ around day 11 of an estrous cycle.

On the contrary, insufficient control of luteal function in heifers (Roche, 1978) by injections of $PGF_2\alpha$ (Nancarrow et al, 1974) may be associated with concentrations of progesterone secreted by CL (Watts et al, 1984). Rates of estrous response after injections of $PGF_2\alpha$ into diestrous heifers were positively correlated to concentrations of progesterone at the time of injections (Watts et al, 1984).

Further evidence is required to define relationship between follicular growth and CL function at midcycle of cattle. In general however, data by Momont

and Seguin (1984), Tanabe and Hann (1984) and Stevenson et al (1984) suggest that maximal precision of synchronized estrus within a 24 h period, can be attained if regimens for synchronization are designed to maximize numbers of cattle receiving $PGF_2\alpha$ on day 8 or 15 of an estrous cycle.

Effect of Rates of Growth and Developmental Status of Ovulatory Follicles

Wave patterns of bovine follicular growth were described (Rajakoski, 1960; Mariana and Nguyen Huy, 1973; Staigmiller et al, 1982). Determination of concentrations of follicular fluid steroid hormones and receptors for gonadotropins in granulosa cells of bovine follicles on different days of bovine estrous cycle, gave further evidence for existence of wave patterns of bovine follicular growth (Ireland and Roche, 1983). Preovulatory follicles develop from small antral follicles into large ovulatory follicles (Richards, 1980). During bovine estrous cycle follicular growth and atresia occur concurrently and continuously independent of stages of an estrous cycle (Choudary et al, 1968). Luteal regression enhances ability of bovine ovaries to develop ovulatory follicles (Matton et al, 1981) and facilitates the largest antral follicle, present on the ovary, 3 days before estrus, to ovulate (Dufour et al, 1972). Developmental

status of ovarian follicles affects precision of synchronized estrus (Nancarrow and Cox, 1976a; Ireland and Roche, 1982b; Macmillan et al 1983/84). On the contrary rate of growth and development of ovarian follicles at the end of treatments for synchronizing estrus, could affect precision of estrus and ovulation (Scaramuzzi et al, 1980). However, Ireland and Roche (1982b) hypothesized that presence or absence of ovulatory follicle on ovary at removal of PRID and (or) injection of $PGF_2\alpha$ may be more critical with respect to precision of estrus and ovulation than just rates of growth and development of ovarian follicles.

In principle, PRID-$PGF_2\alpha$ regimen mimicks hormonal events preceeding occurrence of spontaneous estrus but probably does not ensure homogeneous stages of ovulatory follicles among cattle. Variation in stages of ovarian follicles among synchronized cattle is likely to affect precision of estrus and ovulation. Therefore, failure to precisely synchronize estrus or ovulation in a group of cattle may be inherent in the complexity of bovine ovarian follicular system. Modifications of synchronization procedure so as to homogenize follicular growth among cattle should probably increase precision of ovulation. However, through understanding of involvement of steroid and gonadotropic hormones in control of bovine ovarian follicles is required (Nancarrow and Cox, 1976a).

Effect of Exogenous Concentrations of Progesterone in Peripheral Circulation and Injections of $PGF_{2\alpha}$

In ewes, long term progestagens impair sperm transport and consequently reduce fertility (Quinilivian and Robinson, 1972; Jennings and Crowley, 1972). Short term progestagens are recommended for synchronizing estrus of cattle (Manuleon, 1974) on the basis that fertility is not impaired (Roche, 1979a) but do not induce sufficient estrous response unless combined with either estrogens (Wiltbank and Kasson, 1968) or $PGF_{2\alpha}$ (Roche, 1976b). Pathological effects of progesterone from short term progestagens have not been established in cattle (Gordon, 1976). Manifestation of estrus occurs in presence of less than 1 ng/ml of serum progesterone (Webel et al, 1974; Roche, 1977b). Variation in intervals from injection of $PGF_{2\alpha}$ to estrus is related to decline in serum progesterone. However, other changes associated with decline in serum progesterone and injection of $PGF_{2\alpha}$ are probably important. Injections of $PGF_{2\alpha}$, 2 days before removal of a 9-day subcutaneous implant (SC 80996) increased precision of synchronized estrus (Thimonier et al, 1975; Chupin and Pelot, 1977) because concentrations of progesterone in serum of synchronized cattle at removal of implant were .5 ng/ml (Thimonier et al, 1975). Smith et al (1984) however, observed increased precision of

synchronized estrus, if $PGF_2\alpha$ was injected 1 day before removal of a 7-day PRID.[2] Increased synchrony of estrus was attributed to PRID being able to maintain 3.5 to 4.0 ng/ml of progesterone in serum of heifers, 24 h after $PGF_2\alpha$ as previously suggested (Mauer et al, 1975; Hansel and Beal, 1979). However, mechanism by which presence of progesterone in serum of heifers, after $PGF_2\alpha$ increases precision of estrus, is not known.

Increased precision of estrus and consequent high conception rates following AI at fixed time (Smith et al, 1984) was perhaps due to both decreased variability of rates of decline in postluteolytic progesterone and priming effect of $PGF_2\alpha$ (Hansel and Beal, 1979). However, intervals from removal of PRID after $PGF_2\alpha$ to occurrence of LH surge, vary directly with percentage of progesterone contained in PRID (Roche and Ireland, 1981b) suggesting possible effect of postluteolytic concentrations of progesterone on LH. Given that 75 percent decline of progesterone from CL occurs within 12 h after $PGF_2\alpha$ (Spicer et al, 1981) and luteolysis is possibly complete within 24 h of $PGF_2\alpha$; feasibility of PRID reducing rate of decline in serum progesterone is difficult. Perhaps if difference in progesterone between PRID and serum after $PGF_2\alpha$

---

[2]PRID is 6.75% progesterone by weight of silastic.

is small, retaining PRID for more than 24 h after luteolysis may maintain progesterone in serum, within ranges that are steadily declining. $PGF_2\alpha$ induced priming would possibly manifest via release of GnRH to influence secretion of endogenous gonadotropic hormones from pituitary gland. In turn, gonadotropic hormones, would influence development of ovarian follicles. In contrast to this view, progesterone inhibits secretion of gonadotropic hormones at hypothalamic level (Shoenmann et al, 1983). Given that half-life of $PGF_2\alpha$ is very short (Glew, 1982) and concentrations of both endogenous (CL) and exogenous progesterone (PRID) in serum are likely to still be high enough to inhibit secretion of gonadotropins, influence of $PGF_2\alpha$-induced priming on observed precision of synchronized estrus and ovulation is still to be determined.

Nancarrow and Radford (1976b) hypothesized that induction of follicular diapause for a specific period, in synchronized cattle which normally would manifest estrus and ovulation early after synchronization, could facilitate development of ovulatory follicles in the late responders, such that developmental stages of ovarian follicles will be homogeneous among synchronized cattle. However this hypothesis depends on possibility of increasing basal concentrations of gonadotropic hormones, to prevent normal occurrence of atresia (McNatty et al, 1982) during follicular diapause.

Further understanding of relationship or interaction between progesterone and gonadotropic hormones after luteal regression, is required, before better methods to increase precision of synchronized estrus.

Control of Preovulatory Follicular Development

Follicular development depends on interplay of two major control mechanisms, located in central nervous system and ovary respectively (Peters, 1979). Follicular growth begins with emergence of ovarian follicles from non-growing pre-antral state, via transitory stages of antral follicle and graafian follicle until ovulation or atresia.

Endogenous or exogenous gonadotropic hormones or estradiol 17β do not initiate follicular growth (Ashkol et al, 1970; Peters et al, 1973). However, in mice and rats, gonadotropic hormones are required to support orderly development of growing follicles (Richards and Midgley, 1976). Follicular growth, initiated and established before puberty (Peters et al, 1969) continues throughout all estrous cycles in the animals' lifetime (Govan, 1970; Pedersen and Peters, 1971).

Role of gonadotropic hormones

Influence of FSH and LH during preovulatory phase affects ovulation (Richards and Midgley, 1976; Richards, 1980). In mice, (Peters et al, 1975) and ewe (McNatty et al, 1982), injections of pregnant mare serum gonadotropin (PMSG) prevent atresia of large follicles. In contrast, hypophysectomy in rats, reduces atresia of medium and large follicles (Ingram, 1953). In ewe, injections of PMSG, 10 h after $PGF_2\alpha$-induced heteolysis, prevents occurrence of normal atresia in small antral follicles yet enhances secretion of estradiol 17β by large antral follicles (McNatty et al, 1982). Estradiol 17β, in rats, is required for FSH to increase its receptors on granulosa cells before FSH promotes follicular growth (Tonetta and Ireland, 1983). In prepubertal rats, basal concentrations of LH, sustained by physiological concentrations (50 to 70 ng/ml) of exogenous progesterone in serum, support growth of small antral follicles to preovulatory stage (Richards et al, 1982). In prepubertal heifers, estrus and ovulation are induced following withdrawal of 1 to 2 ng/ml of serum progesterone (Gonzalez-Padilla' et al, 1975; Sheffield et al, 1982). It appears that steroid and gonadotropic hormones interact, to control preovulatory follicular development.

Interaction of ovarian steroid hormones and pituitary gonadotropic hormones during synchronized estrous cycles of heifers

Development of better techniques to control estrous cycles of cattle require understanding of interactions between ovarian steroid and pituitary gonadotropic hormones (Roche and Ireland, 1981a). Role of gonadotropic hormones during follicular development has been discussed. However, regulatory role of ovarian steroid hormones on secretion of gonadotropic hormones requires further understanding.

Progesterone, secreted by CL, is key steroid hormone regulating secretion of LH in sheep (Hauger et al, 1977) and cattle (Convey et al, 1977; Roche and Ireland, 1981b).

Using a 7 day PRID + $PGF_2\alpha$ to synchronize estrus in heifers, Ireland and Roche (1981a) observed a 2 to 3-fold increase in basal concentrations of LH, as concentrations of progesterone in serum declined after $PGF_2\alpha$. Basal concentrations of LH increased in heifers receiving progesterone via 2% PRID (Roche and Ireland, 1981a) suggesting that low but not high (Rahe et al, 1980) concentrations of progesterone do not block increase in basal concentrations of LH in heifers. Tonic LH stimulates follicular growth in metestrus rat (Peluso et al, 1983) but pulses of LH, in ewe (Baird et al, 1976) stimulate

preovulatory follicles to secrete estradiol 17β. Role of estradiol 17β during follicular growth as been discussed. However, increased concentrations of estradiol 17β, also:

1. modulates response of pituitary gland to endogenous or exogenous gonadotropin releasing hormone (GnRH) (Convey, 1973). GnRH stimulates preovulatory gonadotropin surge of LH in cattle (Beck and Convey, 1977; Kesner et al, 1982) ewe (Howland et al, 1977) and gilt (Elsaesser et al, 1979). In turn increased concentrations of LH (preovulatory LH surge) induce final maturation of ovulatory follicles (Richards, 1980).
2. induce behavioral signs of estrus in cattle, sheet and swine (Asdell et al, 1945; Glencross et al, 1980). Peak of behavioral signs of estrus occurs, when concentrations of progesterone in serum are less than 1 ng/ml (Katongole et al, 1971; Lemon et al, 1975; Esslemont et al, 1981).

Rationale for Increasing Precision of Estrus and Ovulation Using PRID-PGF$_{2\alpha}$ to Synchronize Estrus in Cattle

Data reported (McNatty et al, 1982) suggest that LH and FSH interact synergistically to prevent follicular

atresia after $PGF_2\alpha$-induced luteolysis. Changes in secretion of gonadotropic hormones after luteolysis are associated with promotion of 1 to 2 ovarian follicles to ovulation (Brand and deJong, 1973; McNatty et al, 1981). Ovarian steroid hormones may regulate promotory effects of ovarian follicles through modulated secretion of gonadotropic hormones. It appears possible to increase precision of synchronized estrus and ovulation in cattle. However, artificial methods for synchronizing estrus have to be modified such that concentrations of progesterone maintain increased basal concentrations of LH after $PGF_2\alpha$, for a period long enough to facilitate homogeneous stages of follicular development among synchronized cattle. Thus, following removal of exogenous progesterone (PRID) synchronized cattle would manifest preovulatory surges of LH, estrus and possibly will ovulate, with increased precision.

Outline of Review of Literature

1. Introduction to Review of Literature.
2. General survey of bovine estrous cycles.
3. Artificial control of bovine estrous cycles.
4. Causes of variation in intervals from PRID-$PGF_2\alpha$ synchronization treatment to estrus in heifers.

5. Control of preovulatory follicular development.
6. Rationale for increasing precision of estrus and ovulation using PRID-$PGF_2\alpha$ to synchronization of estrus in cattle.

The objective of this thesis was to study if precision of estrus and ovulation is increased by maintaining 1 to 2 ng/ml of progesterone in serum of heifers for 3 days with PRIDs, after injection of 25 mg of $PGF_2\alpha$. Increased precision of estrus or ovulation will be monitored by magnitude of variances for intervals from removal of PRIDs to peaks of preovulatory LH surge and onset of estrus.

MATERIALS AND METHODS

Preliminary Trial

Objectives

1. To identify approximate time required for PRID to remain in the vagina of Holstein heifers before concentrations of progesterone in serum are between 1 and 2 ng/ml.
2. To evaluate whether PRIDs will maintain 1 to 2 ng/ml of progesterone in serum, for at least 3 days after interval defined above (1).

Animals, Treatments, Sampling of Blood, Storage of Sera and Quantification of Progesterone

Reproductive organs of five pubertal Holstein heifers were palpated via rectum for evidence of corpus luteum. Heifers were then injected intramuscularly with 25 mg of prostaglandin $F_2\alpha$ ($PGF_2\alpha$).[3] Immediately after injection of $PGF_2\alpha$ we installed Progesterone Releasing Intravaginal Devices (PRIDs, containing 2% progesterone by weight of silastic, Appendix 1), into the vagina of heifers. Coccygeal arterial (or) venous blood was sampled once, daily, over an interval of 12 days. Blood was

[3]Lutalyse,® Upjohn Co., Kalamazoo, MI USA.

allowed to coagulate at room temperature of approximately 20°C for 8 h before centrifugation at 3000g to separate serum. Sera were stored at -18°C until concentrations of progesterone were determined by radioimmunoassay (RIA) (Louis et al, 1973).

Experiment I

Objectives

1. To determine if basal secretion of LH would change during 3 days when progesterone was 1 to 2 ng/ml.
2. To determine if retention of PRIDs for 3 d after $PGF_2\alpha$ affects precision of preovulatory surges of LH which occur after removing PRIDs.

Design

Seventeen pubertal Holstein heifers were observed for signs of estrus for 30 d and were then synchronized by 3 injections of $PGF_2\alpha$ spaced 11 days apart. At the start of the experiment, heifers ranged between 6 to 15 days of an estrous cycle (mode = day 7). All heifers were in diestrus but at injection of $PGF_2\alpha$ (7 days after installment of PRIDs), PRIDs were assumed to be maintaining at least 2 ng/ml of progesterone in serum of heifers. Therefore all heifers (n = 12) having more than 3 ± 2 ng/ml of progesterone at injection of $PGF_2\alpha$ were presumed

in diestrus and heifers (n = 5) having less than 3 $\pm$ 2 ng/ml of progesterone were in follicular phase of an estrous cycle.

PRIDs were installed into vagina of heifers. Seven (7) days after installing PRIDs (approximately day 14 of an estrous cycle for 17 heifers), 25 mg of $PGF_{2\alpha}$ were injected intramuscularly to cause luteal regression.

In experimental heifers (n = 9) PRIDs remained in the vagina for 3 d after injection of $PGF_{2\alpha}$, thus in experimental heifers, PRIDs stayed for a total of 10 d. In contrast, PRIDs remained in vagina of control heifers (n = 8) for a total of 7 d and was replaced by BLANKs (0% progesterone, Appendix 1) which remained in vagina for 3 d after injection of $PGF_{2\alpha}$ (Figure 2).

Sampling of Blood

Jugular venous canulae (Ico-Rally Corp., Palo Alto, Calif.) were installed into all heifers 6 days after installing PRIDs (one day before injection of $PGF_{2\alpha}$). For experimental and control heifers, blood was sampled immediately before injection of $PGF_{2\alpha}$ (oh). After $PGF_{2\alpha}$, blood was sampled from experimental heifers every 4 h for 72 h (Figure 2). After removal of PRIDs, from both experimental and control heifers, blood was sampled every 4 h for 12 h, then every 2 h for 72 h and finally, every 4 h for 36 h (Figure 2).

Figure 2.--Design of Experiment I: Temporal relationship of Treatments and Sampling of Blood.

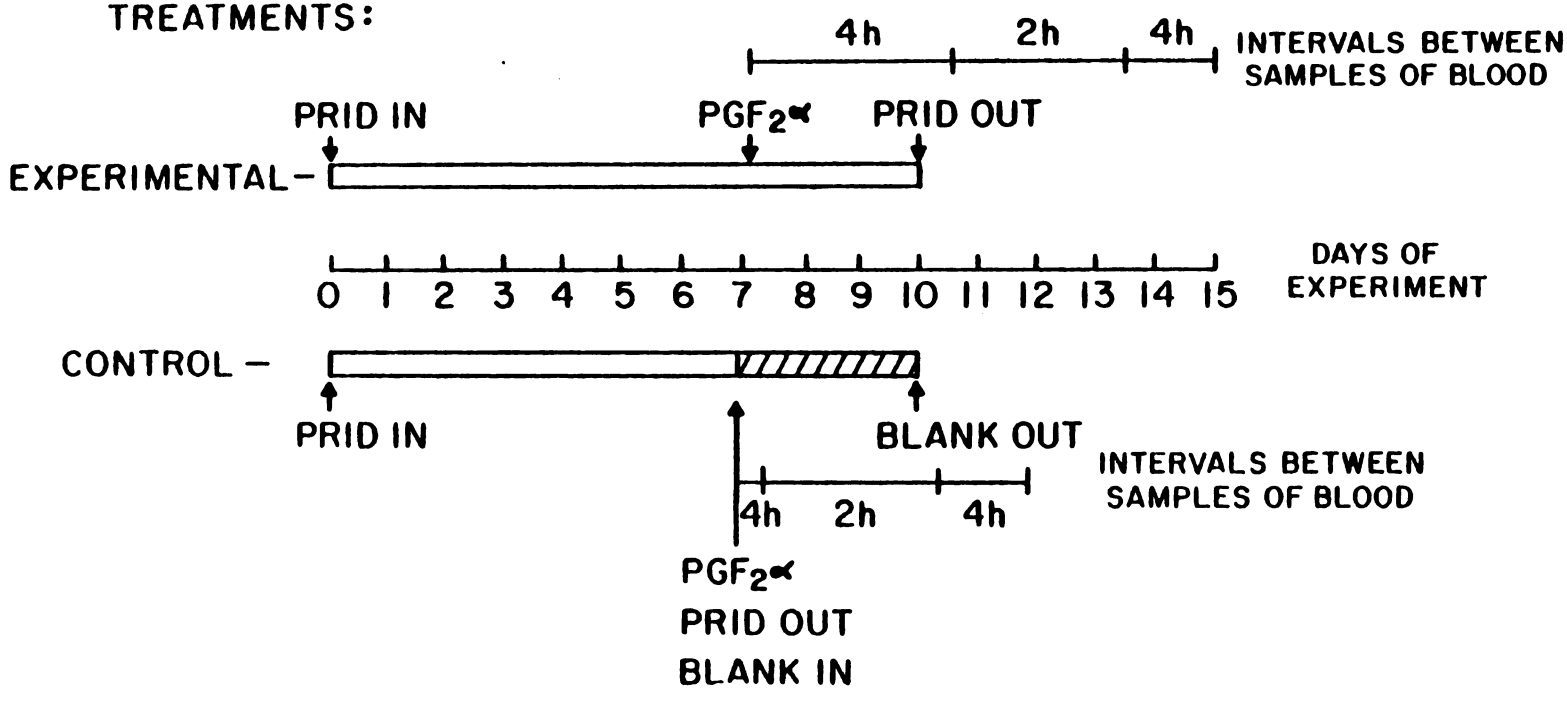
EXPERIMENT I

ANIMALS: DIESTROUS HOLSTEIN HEIFERS

EXPERIMENTAL n=9

CONTROL n=8

TREATMENTS:



Handling of Sera and Quantification of LH and Progesterone

Blood was stored at room temperature (approximately 20°C) for 4 h, to coagulate. Then blood was stored at 4°C for 8 to 12 h prior to centrifugation at 3000g to collect sera. Sera were stored at -18°C until concentrations of LH (Convey et al, 1976) or progesterone (Louis et al, 1973) were determined by RIA. Serum sample collected from both experimental and control heifers before removal of PRIDs, were assayed for progesterone and LH. However, serum from samples collected after removal of PRID was assayed only for LH. Resulting secretory profiles of LH were used to identify intervals from removal of PRIDs to presumed preovulatory surges of LH.

Statistical Analysis

Because animals were at different stages of an estrous cycle at removal of PRIDs, data from heifers that were between day 6 to 8 of an estrous cycle at installment of PRIDs were analyzed separately from data obtained from all diestrous heifers.

1. Data from heifers that were 6 to 8 d when PRIDs were installed:

Data from experimental (n = 7) and control (n = 5) heifers was analyzed to test if basal concentrations of LH increased when PRIDs were maintaining 1 to 2 ng/ml of progesterone in serum for 3 days after $PGF_2\alpha$.

Bartlett's test (Snedecor and Cochran, 1967) performed on data for LH, indicated, presence of heterogeneous variance. Heterogeniety of variance was reduced by transforming LH data to $\log_{10}(LH) + .5$.

2. Data from all diestrous heifers.

Data from experimental (n = 9) and control (n = 8) heifers was analyzed to test if basal concentrations of LH, increased when PRIDs were maintaining 1 to 2 ng/ml of progesterone in serum for 3 days after $PGF_2\alpha$. Bartlett's test (Snedecor and Cochran, 1967) performed on data for LH, indicated presence of heterogenous variance which was reduced by transforming LH data to $(LH)^2$.

Concentrations of LH before injection of $PGF_2\alpha$ (oh) and after $PGF_2\alpha$ were contrasted using Dunnett's test (Gill, 1978). Individual pairs of means of LH were contrasted using Tukey's test (Gill, 1978). Variances of intervals from removal of PRIDs to peaks of LH were contrasted by ratios of variances (Gill, 1978).

Experiment II

Objectives

To determine if 1 to 2 ng/ml of progesterone for 3 days after $PGF_2\alpha$ would affect:

1. percentage of heifers detected in estrus
2. precision of onset of estrus
3. fertility of heifers

Design

Holstein heifers (N = 100) were palpated via rectum to assess ovarian murphology. Data about ovarian morphology indicated that all heifers were pubertal and represented all stages of an estrous cycle. Heifers were assigned equally to experimental (n = 50) and control (n = 50) groups.

Of the control group, 30 heifers were assigned to receive BLANSs at removal of PRIDs and injection of $PGF_2\alpha$; 20 control heifers did not receive BLANKs (Figure 3). Methods for experimental heifers were as described for Experiment I and are illustrated in Figure 3.

Prior to installing PRIDs, samples of blood were collected from medial coccygeal artery (or) vein. Blood was handled as for Experiment I.

Concentrations of progesterone in serum were determined by RIA (Louis et al, 1973) were used to confirm variability of stages of an estrous cycle represented in heifers used for this experiment.

Detection of Estrus

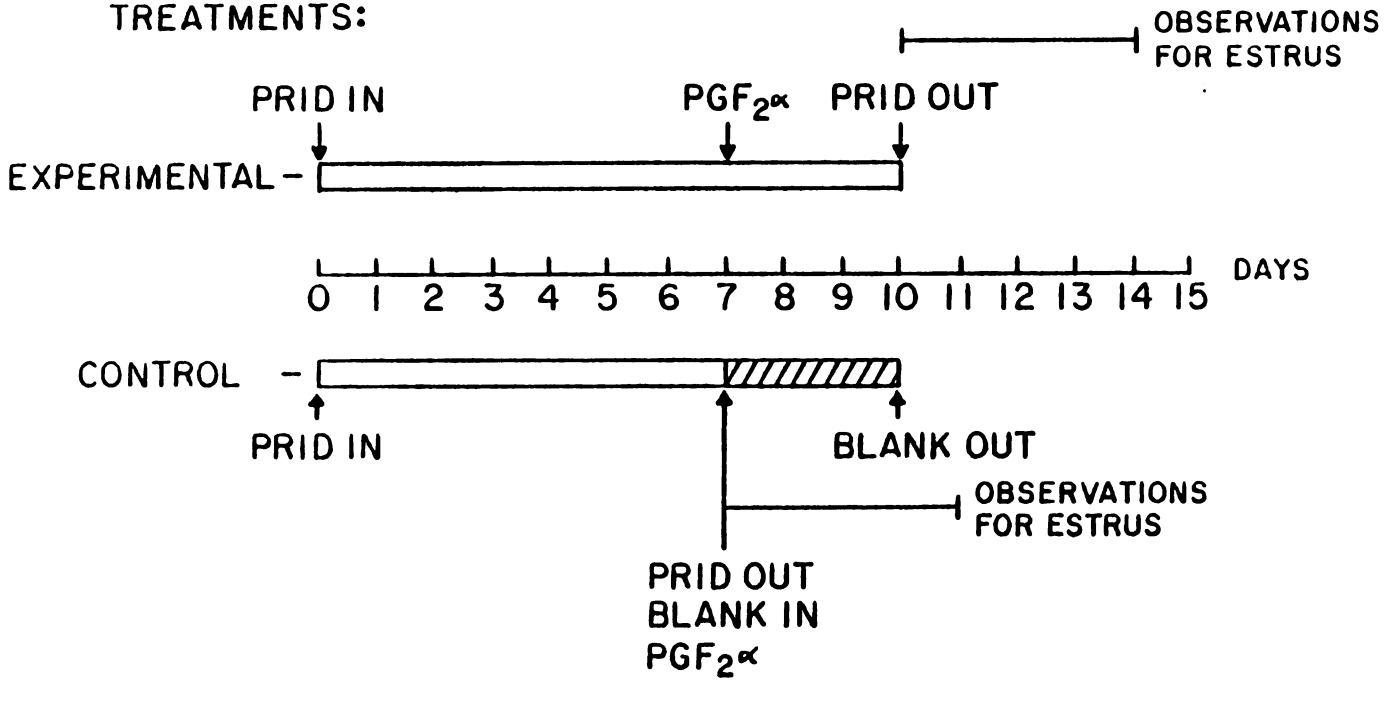
After removal of PRIDs, heifers were observed for estrus for 30 minutes at intervals of 4 h for 96 h. Onset of estrus was defined as first observation of a heifer 'standing to be mounted' by another heifer or if

Figure 3.--Design of Experiment II: Temporal relationship of Treatments and Observations for Estrus.

EXPERIMENT II

ANIMALS: PUBERTAL HOLSTEIN HEIFERS (100)
EXPERIMENTAL (n=50)
CONTROL (n=50)

TREATMENTS:



a heifer that mounted others was observed to discharge clear mucus at the vulva.

Artificial insemination (AI) of heifers was 8 to 12 h after detection of estrus.

Statistical Analysis

Intervals from removal of PRID to onset of estrus were determined from data collected during observations for estrus. Variances of intervals from removal of PRIDs to onset of estrus were contrasted between experimental and control heifers using ratio of variances (Gill, 1978). Chi-square test was used to contrast numbers and percentages of experimental versus control heifers detected in estrus or diagnosed pregnant.

RESULTS

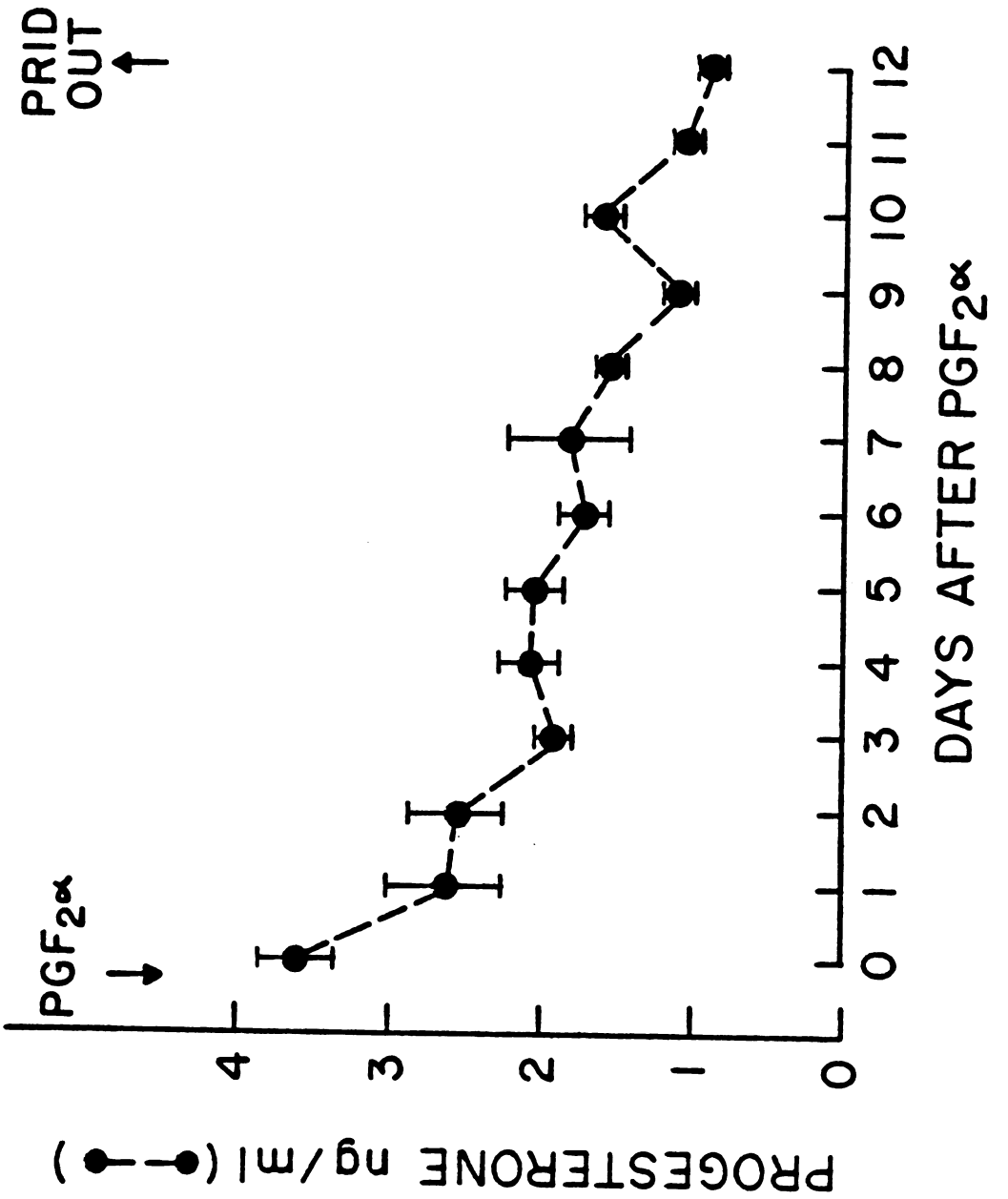
Preliminary Trial

Data from one heifer was eliminated from this study because concentrations of progesterone during the trial indicated that it did not respond to $PGF_2\alpha$, so data presented, is from 4 heifers.

Concentrations of progesterone (Figure 4) during 12 days with PRIDs present, declined from an average of 3.63 $\pm$ .52 ng/ml at injection of 25 mg of $PGF_2\alpha$ to .90 $\pm$ .03 ng/ml at end of trial. Concentrations of progesterone declined ($p < .05$) from 3.63 $\pm$ .52 ng/ml to 2.60 $\pm$ .35 ng/ml within 24 h of installation of PRIDs (Figure 4). Concentrations of progesterone were maintained between 1 and 2 ng/ml (Figure 4) by PRIDs, 3 to 11 days after injection of $PGF_2\alpha$.

Given that injections of $PGF_2\alpha$ do not induce luteolysis between days 0 and 5 of an estrous cycle, installations of PRIDs for a minimum of 6 to 7 days before injection of $PGF_2\alpha$ is necessary to ensure estrous response in all injected cattle regardless of day of an estrous cycle. Thus, for purposes of experiment I and II, installing PRIDs for 7 days prior to $PGF_2\alpha$ was chosen to

Figure 4.--Concentrations of Progesterone maintained by PRIDs after $PGF_{2}\alpha$ in Diestrous Holstein heifers.


PRID
OUT
PGF2α
PROGESTERONE ng/ml (●—●)
0
1
2
3
4
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS AFTER PGF2α

ensure that PRIDs maintained 1 to 2 ng/ml of progesterone in serum for 3 days after $PGF_2\alpha$.

Experiment I

Presynchronization was not successful in all heifers, thus when PRIDs were installed, heifers ranged from 6 to 15 days post-estrus. Consequently, results from experiment I will be presented in two ways:

1. heifers synchronized before installing PRIDs and ranging from 6 to 8 days post-estrus.
2. all diestrous heifers with PRIDs installed, and ranging from days 6 to 15 post-estrus.

1. Data from experimental heifers (n = 7) and controls (n = 5) was used to determine if 1 to 2 ng/ml of progesterone in serum for 3 days maintained by PRIDs for 3 days after $PGF_2\alpha$, increased basal concentrations of LH.

Injection of 25 mg of $PGF_2\alpha$ caused a precipitous decline ($p < .05$) of progesterone (Figure 5) in serum of experimetal heifers, from 10.29 $\pm$ 1.11 ng/ml to 2.64 $\pm$ .57 ng/ml within 24 h. In contrast, concentrations of progesterone in serum of control heifers declined ($p < .05$) from 8.63 $\pm$ .63 ng/ml to 1.18 $\pm$ .11 ng/ml within 24 h of injection of $PGF_2\alpha$.

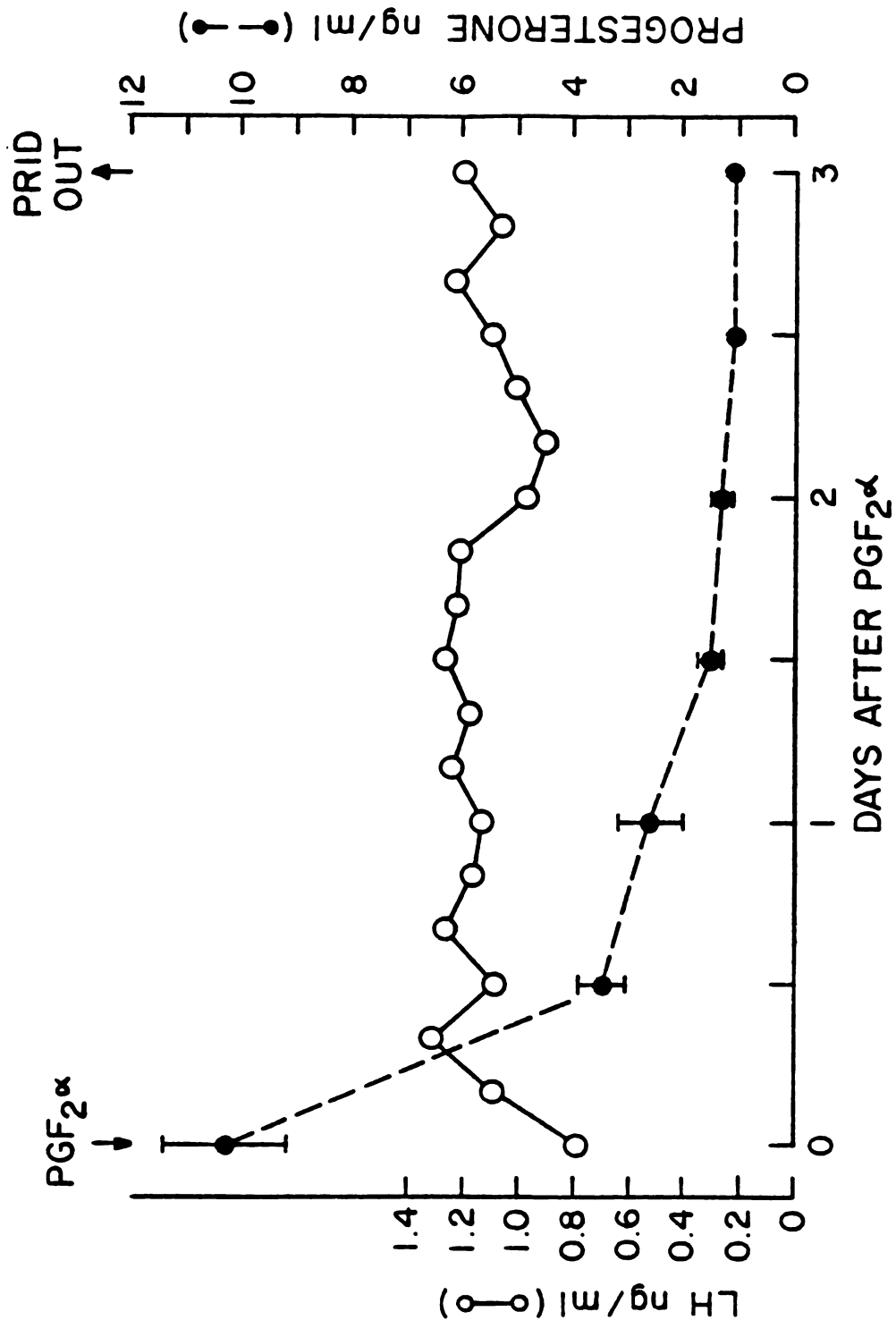
Concentrations of progesterone in serum of experimental heifers (Figure 5) at injection of $PGF_2\alpha$ (10.29 $\pm$ 1.11 ng/ml) were not different ($p > .05$) from those in

control heifers (8.63 ± to .63 ng/ml). However, concentrations of progestrone in serum of experimental heifers (2.64 ± .57 ng/ml) 24 h after $PGF_2\alpha$ were greater ($p < .05$) than in controls (1.18 ± .11 ng/ml). Concentrations of progesterone were maintained between 1 and 2 ng/ml in experimental heifers (Figure 5), 24 h after $PGF_2\alpha$ until removal of PRIDs. In contrast, concentrations of progesterone in control heifers, declined below 1 ng/ml when BLANKs were present. At removal of BLANK from control heifers, concentrations of progesterone (.50 ± .04 ng/ml) were lower ($p < .05$) than in experimental heifers (1.07 ± .02 ng/ml) at removal of PRIDs.

Basal concentrations of LH in experimental and control heifers, increased ($p < .02$) with time after $PGF_2\alpha$. Following injection of $PGF_2\alpha$, and before removal of PRIDs or BLANKs, increase in basal LH among experimental and control groups did no differ ($p > .05$) until 60 h after $PGF_2\alpha$; when preovulatory surges of LH occurred in 4 of 5 control heifers with BLANKs present.

Range of intervals from removal of PRIDs to preovulatory peak of LH in experimental heifers (12 h) was not different from that in controls (14 h). However, preovulatory surges of LH occurred earlier after PRIDs in experimental heifers (26 to 38 h) than in controls (64 to

Figure 5.--Basal concentrations of LH and concentrations of Progesterone maintained during 3 days of PRIDs after $PGF_2\alpha$ in presynchronized Diestrous Experimental heifer


PRID
OUT
PGF2α
PROGESTERONE ng/ml (●—●)
LH ng/ml (o—o)
DAYS AFTER PGF2α
0
2
4
6
8
10
12
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1
2
3

78 h). Mean of intervals from PRIDs to preovulatory peak of LH (Table 1) in experimental heifers (32.9 ± 1.8 h) was shorter ($p < .05$) than in controls (70.8 ± 2.3 h). Variation in intervals from PRIDs to preovulatory peaks of LH (Table 1) in experimental heifers was not different ($p > .05$) from that in controls.

TABLE 1.--Occurrence of preovulatory surges of LH after removal of PRIDs from heifers presynchronized to day 6 to 8 of an estrous cycle

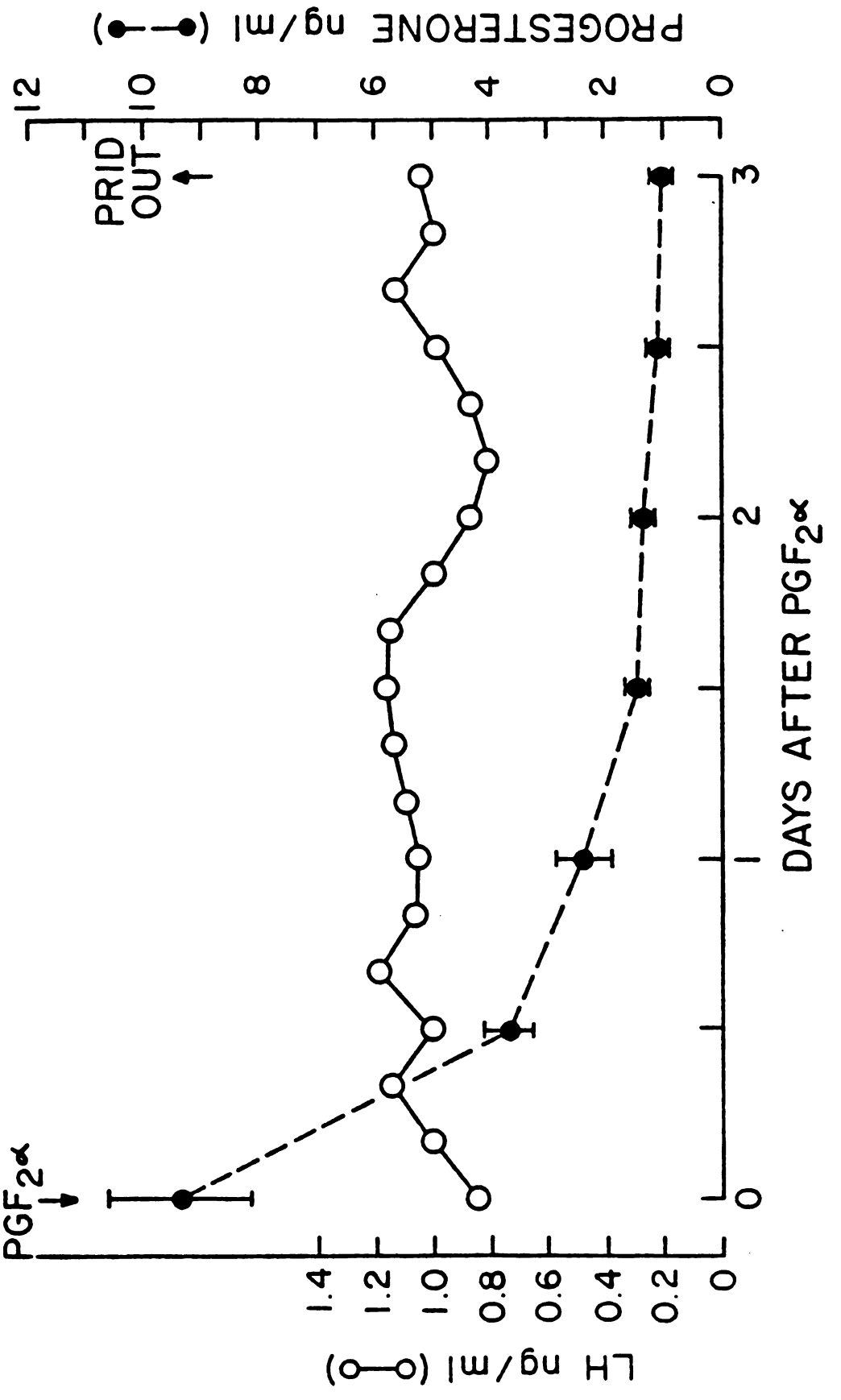
Sources of variation	Groups of heifers	
	Experimental	Control
Number of heifers	7	5
Intervals from removal of PRIDs to peak LH:		
Mean (h)	32.9 ± 1.8[a]	70.8 + 2.3[a]
Standard deviation (h)	4.7[a]	5.2[a]
Variance (h^2)	22.5[a]	27.2[a]
Coefficient of variation (%)	14.4[a]	7.4[a]
Range (h)	26 - 38	64 - 78

[a]Data in a row without common superscripts differ ($p < .05$).

2. Data from all diestrous heifers was used to determine overall effect of 1 to 2 ng/ml of progesterone maintained by PRIDs for 3 days after $PGF_2\alpha$, on basal concentrations LH and precision of preovulatory surges of LH.

After 7 days of PRIDs, injections of 25 mg of $PGF_2\alpha$ caused a precipitous decline ($p < .05$) of progesterone in serum of experimental heifers (Figure 6) from 9.40 $\pm$ 1.32 ng/ml to 2.36 $\pm$ .36 ng/ml, within 24 h. In contrast, concentrations of progesterone in control heifers, declined from 4.63 $\pm$ 1.15 ng/ml to .95 $\pm$ .15 ng/ml within 24 h. Concentrations of progesterone in serum of experimental heifers (Figure 6) were maintained between 1 to 2 ng/ml, 24 h after $PGF_2\alpha$ until removal of PRIDs whilst those in controls were less than 1 ng/ml, 24 h after $PGF_2\alpha$ when BLANKs were present. Concentrations of progesterone in experimental heifers were greater ($p < .05$) than in controls before and after injection of $PGF_2\alpha$. However, decline in concentrations of progesterone was 5-fold in control heifers in contrast to 4-fold in experimental heifers. There was tendency ($p < .10$) for basal concentrations of LH to increase above concentrations at pretreatment time (0h) in experimental heifers (Figure 6) after injection of $PGF_2\alpha$. However, in 2 of 9 experimental heifers, basal concentrations of LH did not increase after $PGF_2\alpha$ but declined below baseline for approximately 24 h and then tended to increase, 12 h before removal of PRIDs. In contrast basal concentrations of LH at pretreatment time (0h) were not different ($p > .05$) among experimental vs controls. However there was

Figure 6.--Basal concentrations of LH and concentrations of Progesterone, maintained during 3 days of PRIDs after $PGF_2\alpha$ in all Diestrous Experimental heifers.


PROGESTERONE ng/ml (●—●)
12
10
8
6
4
2
0
PRID OUT
PGF2α
LH ng/ml (o—o)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
1
2
3
DAYS AFTER PGF2α

tendency for basal concentrations of LH in experimental heifers to be lower ($p < .10$) than controls, 24 h after $PGF_2\alpha$ because preovulatory surges of LH were due to occur in 2 of 8 controls.

Range of intervals from removal of PRIDs to preovulatory peak of LH (Table 2) was shorter in experimental heifers than controls. Mean of intervals from removal of PRIDs to preovulatory peak of LH (Table 2) in experimental heifers was shorter ($p < .05$) than in controls. Variation in intervals from removal of PRIDs (Table 2) in experimental heifers, was less ($p < .05$) than in controls. Percentage of preovulatory peaks of LH occurring within $\pm$ 12 h of mean interval (Figure 7) from removal of PRID to preovulatory surges of LH was greater ($p < .01$) in experimental heifers than in controls.

Experiment II

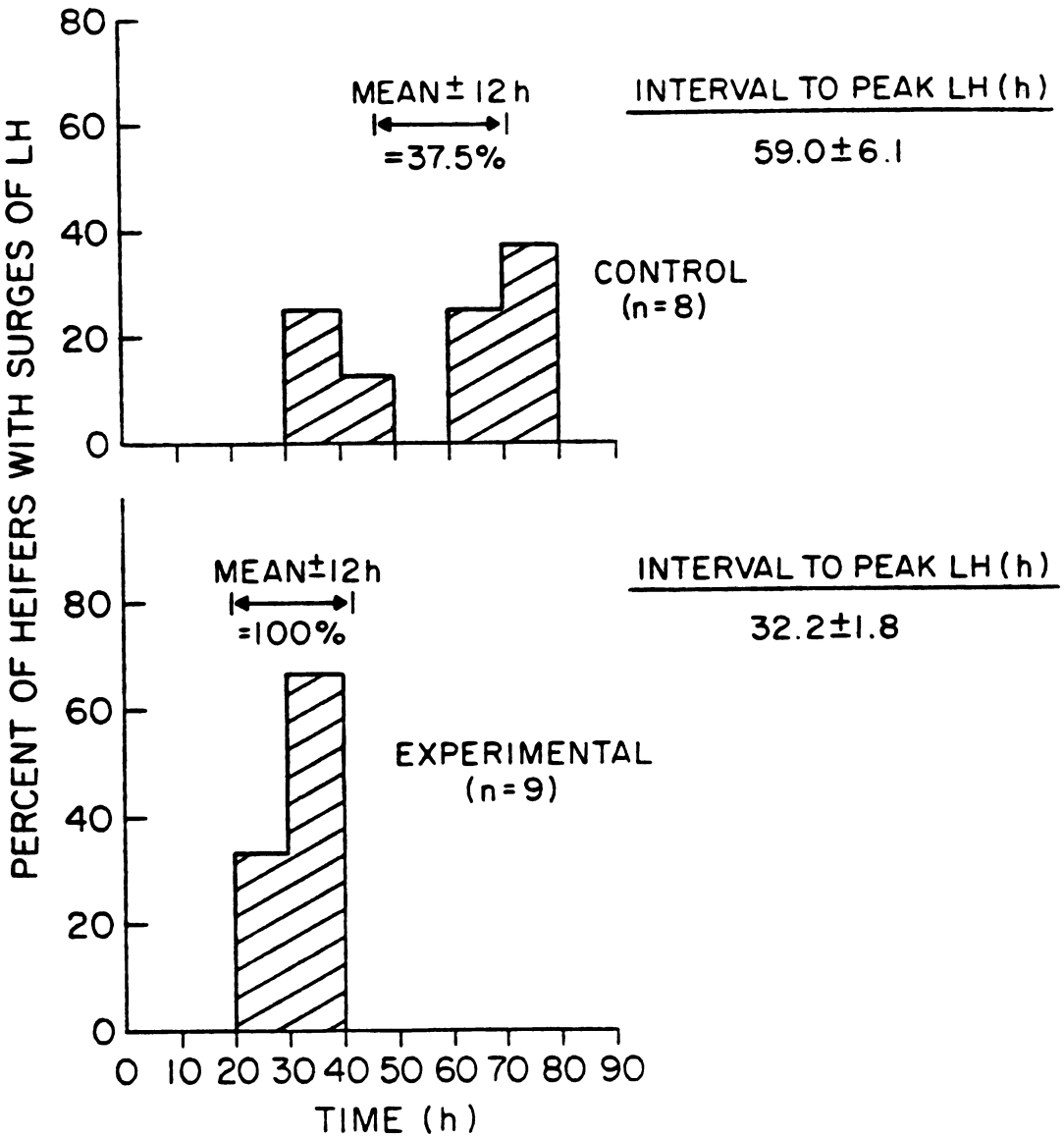
Overall, 5 of 100 heifers lost PRIDs (experimental) n = 2, control, n = 3). Data on estrus from these heifers were excluded from analysis. Estrus was detected in 81 of 95 heifers (85.3%). Therefore all data analyzed and reported in experiment II are derived from these 81 heifers. Based on ovarian norphology assessed rectally and wide range in concentrations of progesterone (0.2 to 11.3 ng/ml) when PRIDs were installed it appeared that

TABLE 2.--Occurrence of preovulatory surges of LH after removal of PRIDs from all diestrous Holstein heifers.

Sources of variation	Groups of heifers	
	Experimental	Control
Number of heifers	9	8
Intervals from removal of PRIDs to peak of LH:		
Mean (h)	32.2 ± 1.8[a]	59.0 ± 6.1[b]
Standard deviation (h)	5.2[a]	17.2[b]
Variance (h^2)	27.4[a]	297.1[b]
Coefficient of variation (%)	16.0[a]	29.0[a]
Range (h)	24 - 38	34 - 78

[ab]Data in a row without common superscripts differ ($p < .05$).

Figure 7.--Distribution of onset of Preovulatory surges of LH after removal of PRIDs from Diestrous Holstein heifers.


PERCENT OF HEIFERS WITH SURGES OF LH
80
60
40
20
0
MEAN ± 12 h
=37.5%
INTERVAL TO PEAK LH (h)
59.0±6.1
CONTROL
(n=8)
MEAN±12h
=100%
INTERVAL TO PEAK LH (h)
32.2±1.8
EXPERIMENTAL
(n=9)
0 10 20 30 40 50 60 70 80 90
TIME (h)

most, if not all, stages of an estrous cycle were represented.

Detection of estrus (Table 3) in experimental heifers was not different ($p > .05$) from that in controls. However percentage of heifers detected in estrus (Table 3) in experimental group, was greater ($p < .01$) than in controls.

TABLE 3.--Detection and onset of estrus after removal of PRIDs from Holstein heifers.

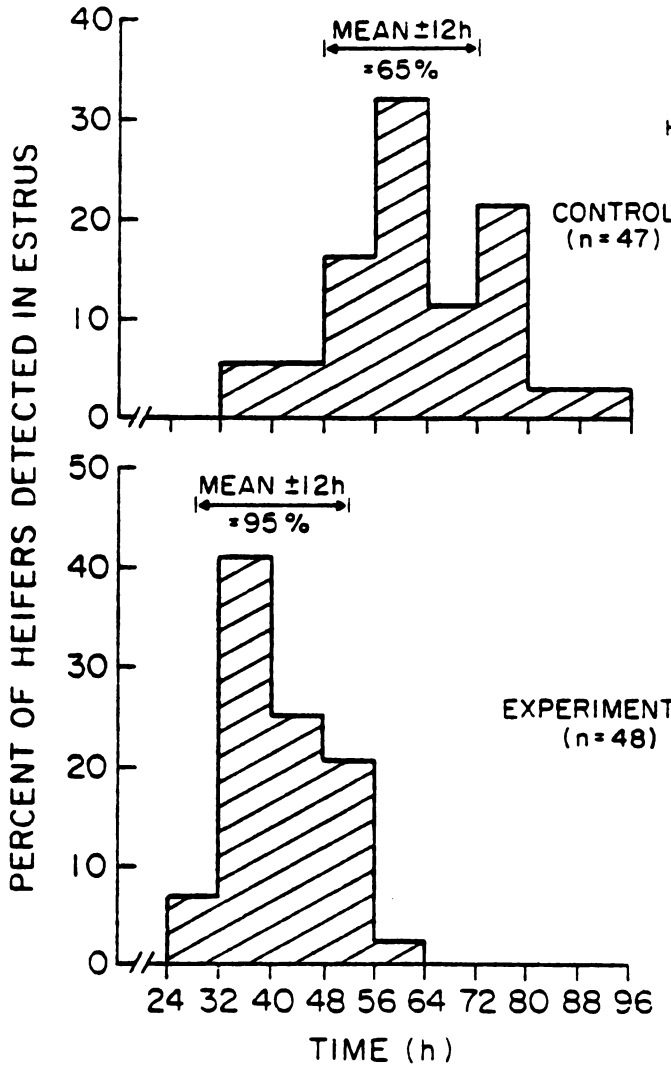
Sources of variation	Groups of heifers	
	Experimental	Control
Number of heifers in estrus	44	37
Percent of heifers detected in estrus	92[c]	79[d]
Intervals from removal of PRIDs to onset of estrus:		
Mean (h)	40.0 ± 1.2[a]	60.8 ± 2.2[b]
Standard deviation (h)	8.0[a]	13.1[b]
Variance (h^2)	64.0[a]	171.7[b]
Coefficient of variation (%)	20.0[a]	22.0[a]
Range (h)	24 - 56	36 - 88

[ab]Data in a row without similar superscript differ ($p < .05$).

[cd]Data in a row without similar superscript differ ($p < .05$).

Range of intervals from removal of PRIDs to estrus (Table 3) was short in experimental heifers in contrast to controls. Mean of intervals from removal of PRIDs to onset of estrus (Table 3) in experimental heifers was lower ($p < .05$) than in controls. Variation in intervals from removal of PRIDs to onset of estrus (Table 3) in experimental heifers was less ($p < .05$) than in controls. Distribution of intervals from removal of PRIDs to estrus (Figure 8) indicated that percentage of experimental heifers detected in estrus within $\pm$ 12 h of mean interval from removal of PRIDs to estrus was greater ($p < .05$) than for control heifers detected in estrus within same range of corresponding mean interval. There was tendency for conception rate (Table 3) in experimental heifers to differ ($p < .10$) from that in control heifers.

Figure 8.--Distribution of Onset of Estrus after removal of PRIDs from Holstein heifers.



	HEIFERS DETECTED IN ESTRUS (%)	INTERVAL TO ESTRUS (h)
CONTROL (n=47)	37(79)	60.8±2.2
EXPERIMENTAL (n=48)	44(92)	40.0±1.2

DISCUSSION

The goal for using PRID to maintain 1 to 2 ng/ml of progesterone is serum of heifers (Preliminary Trail) so that basal concentration of LH could increase was attained (see Exp. I(1)). The main objective for increasing basal concentrations of LH when PRIDs were maintaining 1 to 2 ng/ml of progesterone in serum of heifers was to provide a hormonal milieu facilitatory to follicular development. Thus removal of PRIDs after decreased concentrations of progesterone in serum of heifers for 3 days after $PGF_2\alpha$, could permit occurrence of preovulatory surges of LH and estrus with increased precision. This objective was achieved (see Exp. I and II).

In Experiment I, concentrations of progesterone declined to less than 1 ng/ml in serum of control heifers within 24 h of injecting $PGF_2\alpha$. Changes in concentration of progesterone after $PGF_2\alpha$ in controls were similar to those reported in other studies (Rowson et al, 1972; Louis et al, 1973; Lauderdale, 1975; Renegar, 1978). Thus presence of higher concentrations of progesterone in experimental heifers than in controls, 24 h after $PGF_2\alpha$, was due to presence of PRIDs.

Increased basal concentrations of LH after $PGF_{2\alpha}$ occurred in 7 of 9 experimental heifers which originally were at days 6 to 8 of an estrous cycle before PRIDs were installed (Exp. I(1)). Increase in mean LH, 1 to 3 h after $PGF_{2\alpha}$ was in agreement with earlier studies (Louis et al, 1974, Stellflug et al, 1977, Milvae and Hansel, 1983). After $PGF_{2\alpha}$, decrease in serum progesterone and increase in concentrations of LH are associated with regression of CL (Ireland and Roche, 1982a). Similar results were observed in the present study (Experiment I). Basal concentrations of LH in serum of 7 experimental heifers were maintained for 3 d after $PGF_{2\alpha}$, when 1 to 2 ng/ml of progesterone from PRID maintained in serum of heifers. In contrast, increased basal concentrations of LH in 5 control heifers were maintained despite presence of BLANKs, until preovulatory surges of LH. These results suggest that presence of 1 to 2 ng/ml of progesterone did not prevent post-$PGF_{2\alpha}$ increase of basal LH.

Increased basal concentrations of LH after $PGF_{2\alpha}$ in heifers is not maintained 36 h after injection of $PGF_{2\alpha}$ (Schallenberger et al, 1984). However, the results suggest that in presence of 1 to 2 ng/ml of progesterone, increased basal concentrations of LH were maintained in 7 of 9 experimental heifers up to time of removal of

PRID (72 h after $PGF_2\alpha$) similar to observations during proestrous of cattle (Rahe et al, 1980). However increased basal concentrations of LH, after spontaneous or $PGF_2\alpha$-induced luteal regression (Ireland and Roche, 1982a) normally occurs during decline of progesterone from CL and often preceeds occurrence of preovulatory surges of LH. Thus increased endogenous basal concentrations of LH for 3 days in spite of 1 to 2 ng/ml of progesterone in serum of heifers, suggests that low concentrations of progesterone do not block basal concentrations of LH from increasing after $PGF_2\alpha$ but may block preovulatory surges of LH in diestrous heifers (Short et al, 1979).

Increased basal concentrations of LH do not occur if $PGF_2\alpha$ is administered in absence of CL (Hafs et al, 1975; Furr et al, 1981). Similar results were observed in 2 of 9 experimental heifers in which basal concentrations of LH did not increase after injection of $PGF_2\alpha$. Concentrations of progesterone at injection of $PGF_2\alpha$ indicated that the two heifers were in follicular phase of an estrous cycle. Thus failure to observe increased basal concentrations of LH in the two heifers after $PGF_2\alpha$ was probably due to stage of an estrous cycle.

The largest antral follicles on the ovary regress after injection of $PGF_2\alpha$ on day 8 or 9 of an estrous cycle

of the cow (Ireland and Roche, 1983). Atretic follicles become non-responsive to stimulation by LH due to lack of LH receptors in granulosa and theca layers of the follicles (Ireland and Roche, 1982b). Results from the study suggest that injection of $PGF_2\alpha$ probably caused ovarian follicles to become atretic in the two heifers and presence of 1 to 2 ng/ml of progesterone in serum of heifers caused further decline in basal concentrations of LH observed 36 to 40 h after $PGF_2\alpha$. Progesterone inhibits secretion of basal LH by pituitary cells in response to LHRH challenge in vitro (Padmanabhan et al, 1982; Keech et al, 1984). Concentrations of progesterone, greater than 1 ng/ml in serum of postpubertal heifers (Roche and Ireland, 1981b), suppress pulsatile secretion of LH after mid luteal phase (Rahe et al, 1980). Similar observations have been reported in ewe (Karsch, 1977) and rats (Richards et al, 1982). There is evidence that progesterone treatments result in atresia of follicles in cattle (Maracek et al, 1977) probably because of inhibitory effect of progesterone on FSH-induced increase of estradiol in granulosa cells reported in rats (Schreiber et al, 1981). Consequently, presence of 1 to 2 ng/ml of progesterone in sera of 2 experimental heifers having follicular phase, may have caused atresia of large antral follicles. However, 1 to 2 ng/ml in sera

of the 2 heifers, did not prevent increased basal LH 12 h before removal of PRIDs. Thus, the two heifers manifested preovulatory surges of LH after removal of PRIDs, which suggests a possibility of development of new ovulatory follicles 12 h before removal of PRIDs. Therefore 1 to 2 ng/ml of progesterone maintained in serum of heifers for 3 d after $PGF_2\alpha$ may facilitate follicular growth.

Given that follicular growth and artesia occur continuously throughout an estrous cycle (Rajakoski, 1960; Schams et al, 1977; Matton et al, 1981) results in Experiment I(1) and II suggest that possible follicular growth preceeding preovulatory surges of LH coincided with facilitatory effects of 1 to 2 ng/ml of progesterone and increased basal secretion of LH in serum of experimental heifers.

Sustained secretion of basal LH in ewes (Baird et al, 1981; England et al, 1981) and rats (Richards et al, 1982) stimulates preovulatory follicular growth (McNatty et al, 1982). Thus increase of basal LH observed in diestrous heifers (Exp. I(1)) possibly stimulated ovulatory follicles, to grow in 7 to 9 experimental heifers which were in diestrus at injection of $PGF_2\alpha$. But occurrence of preovulatory surges of LH in all experimental heifers (Exp. I(2)) after removal of PRIDs obviates, stage of diestrus as part of synchronization regimen.

Preovulatory surges of LH (Exp. I) and estrus (Exp. II) occurred with increased precision in experimental heifers than in controls after 3 days of 1 to 2 ng/ml of progesterone. These results suggest that 1 to 2 ng/ml of progesterone and its associated changes in LH (and) or probably over reproductive hormones, were necessary to increase precision of preovulatory surges of LH and estrus regardless of stage of an estrous cycle at time of injecting $PGF_2\alpha$. However, the mechanisms involved for increasing precision of preovulatory surges of LH and estrus using PRID for 3 days after $PGF_2\alpha$ remain to be resolved.

In control heifers, range of intervals from removal of PRIDs and injection of $PGF_2\alpha$, to occurrence of preovulatory surges of LH, were similar to intervals, previously reported (Roche and Ireland, 1981b). Intervals from removal of PRIDs to estrus were similar to intervals reported after 2 injections of $PGF_2\alpha$, spaced 11 days apart (Kinkie et al, 1976; Smith, 1976; Macmillan et al, 1980; Refsal et al, 1980; Tanabe and Hann, 1984; Stevenson et al, 1984). Similar results were obtained when PRIDs were installed for 6 days with $PGF_2\alpha$ on removal of PRIDs (Smith et al, 1984) or PRIDs for 7 days with $PGF_2\alpha$ 1 day before removal of PRIDs (Graves et al, 1975; Stauffer et al, 1976; Smith et al, 1984). Regimens for

synchronizing estrus with PRID and $PGF_2\alpha$ combination or two injections of $PGF_2\alpha$, do not increase precision of preovulatory surges of LH or estrus. Thus, maintaining 1 to 2 ng/ml of progesterone for 3 days after $PGF_2\alpha$ as in experimental heifers was probably a better method to increase precision of preovulatory surges of LH and estrus in cattle.

Preovulatory surges of LH occurred shortly after removal of PRIDs, in experimental heifers. There results suggest that physiological changes associated with 1 to 2 ng/ml of progesterone and increased basal LH probably simulates events of proestrus. Overall, experimental heifers were less variable with respect to occurrence of estrus and preovulatory surges of LH. However, PRIDs maintained for 10 days in contrast to 7 days, (Exp. I(1)) did not decrease variation of intervals from removal of PRIDs to LH surges in diestrous heifers. Thus, increased precision of estrus and surges of LH in experimental heifers was due to possible presence of homogeneous proestrous state rather than disparity in duration of PRIDs.

Synchronization of estrus increases frequency of displaying behavioral signs of estrus because more than one cow are in estrus at the same time (Hurnik et al, 1975; Esslemont et al, 1981; Glencross et al, 1980).

Maintaining 1 to 2 ng/ml of progesterone in serum of heifers for 3 days after $PGF_2\alpha$, increases detection of estrus (Exp. II) in experimental heifers (Table 3). Results indicate that the number of experimental heifers detected in estrus within $\pm$ 12 h of mean interval to estrus after PRIDs was greater than in controls. Thus, better detection of estrus in experimental group versus control group, was due to increased synchronization of estrus.

There was tendency for conception rate in experimental heifers (Table 3) to be lower than in controls. The results suggest that maintaining 1 to 2 ng/ml of progesterone or other experimental conditions could affect fertility of cattle. However, conception rates of experimental and control heifers (Table 3) were comparable to conception rates reported in other studies (Lauderdale, 1975; Roche, 1976a). The fact that two-thirds of this study was carried out during winter could have affected fertility

Normally, cattle in temperate zone have lowest fertility during winter and summer in contrast to spring or fall (Mercier and Salisbury, 1947; Spalding, Everett, and Foote, 1975). In extreme cases of short day light and low temperatures (Sweetman, 1950), lowest fertility occurred during winter. If season was detrimental to

fertility, both experimental and control heifers would have been affected unless subfertility in experimental group was due to interaction between 1 to 2 ng/ml of progesterone or changes associated with it, and environment. However winter season affects fertility of cattle regardless of previous concentrations of progesterone in serum if fixed time AI is performed at 65 h versus 80 h (Jaster et al, 1982). Effects of climatic conditions on plasma progesterone of cattle are conflicting (Wolff Vaught et al, 1977; Rosenberg et al, 1977; Roman-Ponce et al, 1981; Rosenberg et al, 1982). High basal concentrations of LH are associated with high concentrations of progesterone in serum of cattle, subjected to stress of high ambient temperature but occurrence of preovulatory surges of LH is not affected (Roman-Ponce et al, 1981). Given that AI in this study was scheduled by detection of estrus, subfertility observed in experimental heifers was probably due to factors other than season. Subfertility due to long term progestagen (Gordon, 1976) can occur because of:

1. altered sperm transport
2. too rapid sperm capacitation
3. defective fertilization
4. abnormalities in ova

5. reduced embryo survival
6. change in cervical mucus
7. release of LH out of phase with estrus
8. high estrogen during progestagen treatment

However, use of short term progestagen ($\leq$ 12 days) overcomes subfertility previously due to long term progesterone (Roche, 1979a).

Results from the study (Exp. II) suggest that subfertility in experimental heifers was not due to occurrence of preovulatory surges of LH out of phase with estrus (Table 3) since surges of LH occurred within ranges of intervals from PRIDs to estrus. However, synchronized cattle may express varying shades of estrous activity and behavioral signs not necessarily indicative of physiological status (Wishart and Young, 1974b). Thus, subfertility in experimental group could be due to AI of some heifers which probably were manifesting false positive signs of estrus due to increased synchronization. There were changes observed in vaginal mucus which were associated with nylon string used to remove PRIDs. Vaginal mucus in both experimental heifers and controls, became cloudy whenever the nylon string was in contact with vaginal mucosa. However, more heifers in experimental group were affected than in control group. Thus vaginitis due to nylon string could have interferred with

insemination and fertility of heifers. Care to minimize contact between nylon string and vaginal mucosa may reduce vaginitis along with other inflammatory changes in vaginal mucus and probably to increase fertility of heifers.

Major goal of underlying this kind of study is to develop a method to increase precision of estrus and ovulation so that fertility at AI is increased. Information learned from the study along with that reviewed suggests that maintaining 1 to 2 ng/ml of progesterone in serum of Holstein heifers for 3 days after $PGF_2\alpha$ increases precision of preovulatory surges in LH and estrus. Given that 95 percent of heifers or greater, manifested surges of LH or estrus within an interval of 24 h (Figures 4 and 5), maintenance of 1 to 2 ng/ml of progesterone in serum of Holstein heifers, after $PGF_2\alpha$ enhances feasibility of AI at fixed time. However further understanding of physiological status of entire reproductive system during presence of 1 to 2 ng/ml of progesterone in serum of heifers after $PGF_2\alpha$ through to after removal of PRID, is required. Then field application to test this method should follow.

In conclusion, 2% PRIDs, installed in vagina of Holstein heifers for 7 days prior to $PGF_2\alpha$ can be maintained to deliver 1 to 2 ng/ml of progesterone in serum for 3 days after $PGF_2\alpha$.

Concentrations of progesterone (1 to 2 ng/ml) maintained by PRIDs in serum of heifers, for 3 days after $PGF_2\alpha$, allow increased basal concentrations of LH in serum of diestrous heifers which probably facilitate ovarian follicular growth to increase precision of LH surges and estrus. Increased precision of estrus and LH surges occur in all heifers regardless of stage of an estrous cycle and fertility of heifers is not affected.

APPENDIX

APPENDIX

Procedure for Making 2% PRIDs or BLANKs

The materials for making 9 PRIDs are:

450 g Silastic 382® Medical Grade Elastomer (Dow Corning Corp., Midland, MI).

59.4 g Silastic® RTV Thinner (Dow Corning Corp., Midland, MI).

10.46 g P-0130® 4 Pregnene-3, 20-dione (Sigma Chemical Company, St. Louis, MO).

90 drops Catalyst M® (Dow Corning Corp., Midland, MI).

9 strips of stainless steel (27.5 cm long, 3 cm wide, and .02 mm thick) supplied by Dept. of Agric. Engineering, Michigan State Univ., East Lansing, MI).

Food color (Spartan Stores Inc., Grand Rapids, MI)

Silastic 382® Medical Grade Elastomer (450 g.), Silastic® RTV Thinner (59.4 g) and P-0130® Pregnene-3, 20-dione (10.46 g) were mixed together vigorously in a plastic jar (1 litre) for 5 to 10 minutes. Then Catalyst M® (90 drops) was added to the mixture simultaneously with food color (approximately 5 mls). Mixing of the

five ingredients continued for approximately 1 minute. Food color was used to color-code the product regarding percent progesterone, and to indicate homogenity of mixing. Steel strips were centered in the enclosed mold such that a space of 2 mm was left between perimeters of mold and steel strips.

Soon after mixing the thick past was injected under manual pressure, with 50 c.c. Catheter tip-syringes (Plastipak® Disposable syringe Becton-Dickson, Co., Rutherford, NJ) into molds of plexi-glass; so that a thin layer of silastic (1.14 mm thick) coated the flat sides of the stainless steel strips. A layer of Silastic (2.0 mm thick) coated the perimeter of the stainless steel. Total thickness of PRID was about 2.3 mm. The final products (PRIDs) contained approximately 2% progesterone by weight of silastic.

CAUTION: After adding catalyst M® to mixture, further mixing lasts approximately 1 minute so as to avoid vulcanization of silastic mixture before injection into plexiglass molds. Thus working time for injecting silastic mixture was approximately 10 minutes. After injection of silastic mixture into molds, curing of PRIDs occurred after 4 to 8 h, depending on ambient temperature. Ambient temperatures between 21 to 24.5°C were ideal.

Procedure for making BLANK's

Procedure for making BLANKs was same as for PRIDs except no progesterone (P-0130® Pregnene-3, 20-dione), was involved. BLANKs contained 0% progesterone.

After curing and recovery from molds, PRID's or BLANK's were each coiled manually to a final coiled diameter of approximately 5.5 cm and length approximately 10 cm. A piece of nylon cord (100 cm long, folded in half) was fixed at one of the ends of PRID or BLANK.

Cleaning of molds

Following recovery of PRIDs or BLANKs from molds, a blunt instrument (eg. screw-driver) was used to clear out thick remnants of cured silastic from sides and corners of the molds. Then gauze sponges (4 in. by 4 in.) (Parke-Davis and Co., Detroit, MI) impregnated with Xylene (AR® Mallinckrodt Inc., Paris, Kentucky) were used to wipe out remnants of cured silastic until molds were thoroughly clean. Then molds were left to dry in air for at least 2 h before assembling them for new batches of PRIDs or BLANKs.

BIBLIOGRAPHY

BIBLIOGRAPHY

Asdell, S. A., J. DeAlba and J. S. Roberts. 1945. The level of ovarian Hormones required to induce heat and reactions in ovariectomized cow. J. Anim. Sci. 4:277.

Asdell, S. A. 1964. In 'Patterns of Mammalian Reproduction' (2nd Ed.), Cornell Univ. Press.

Ashkol, A., B. Lunefeld and H. Peters. 1970. Ovarian Development in Infant Mice. Dependence on gonadotropic hormones. In 'Gonadotropius and Ovarian Development, W. R. Butt, A. C. Cook and M. Ryle (Eds.) p. 249, Livingstone, London.

Baird, D. T. and R. J. Scaramuzzi. 1976. Changes in the secretion of ovarian steroids and pituitary luteinizing homrone in periovulatory period in the ewe. The effect of progesterone. J. Endocrinol. 70:237.

Baird, D. T. and A. S. McNeilly. 1981. Gonadotropin control of follicular development and function during oestrus cycle of the ewe. J. Reprod. Fert. (suppl.) 30:119.

Bartol, F. E., W. W. Thatcher, G. S. Lewis, E. L. Bliss, M. Drost, F. W. Bazer and E. L. Baco. 1980. Effect of Estradiol on bovine uterine intraluminal content of $PGF_2\alpha$ and total protein. 72nd Ann. Meeting Amer. Soc. Anim. Sci. p. 258 (Abstr.).

Beck, J. W. and E. M. Convey. 1977. Estradiol Control of serum luteinizing hormone concentrations in the bovine. J. Anim. Sci. 45:1096.

Brand, A. and W. H. R. DeJong. 1973. Quantitative and qualitative micronurphological investigations of tertiary follicle population during the oestrous cycle in sheep. J. Reprod. Fert. 33:431.

Britt, J. H. 1979. Prospects for controlling Reproductive processes in cattle, sheep, and swine from Recent Findings in Reproduction. J. Dairy Sci. 62:651.

Brunner, M. A., L. E. Donaldson and W. Hansel. 1969. Exogenous hormones and luteal function in hysterectomized and intact heifers. J. Dairy Sci. 52:1849.

Burrell, C., J. N. Wiltbank, D. G. Lefever and G. Rodefter. 1972. Ear implant (SC 21009) for estrous control in heifers. Proc. Western Sec. Amer. Soc. Anim. Sci. 23:547.

Cartwright, T. C., R. J. Gerrits, C. A. Kiddy, F. Bazer, G. E. Bradford, K. I. Brown, J. Dendell, G. E. Dickerson, N. L. First, R. H. Foote, J. Gorski, H. D. Hafs, C. Kaltenbatch, R. B. Land, E. Lasley, L. D. McGalliard, R. H. Nelson, I. T. Omtredt, H. Schroeder, R. S. Sechrist, G. Seidel, C. R. Shumway, R. W. Touchberry and H. A. Tucker. 1980. Animal Genetics and Reproduction. In 'Proceedings Animal Agriculture Research to meet Human needs in the 21st Century. G. W. Pond, R. A. Merkel, L. D. McGilliard and V. J. Rhodes (Eds.). Westview Press. Boulder, Colorado.

Chenault, J. R., W. W. Thatcher, P. S. Kalre, R. M. Abraus, and C. J. Wilcox. 1975. Transitory changes in plasma progestins estradiol and luteini zing hormone approaching ovulation in the bovine. J. Dairy Sci. 58:468.

Choudary, J. B., H. T. Gier and G. B. Marion. 1968. Cyclic changes in Bovine vesicular follicles. J. Anim. Sci. 27:468.

Christian, R. E. and R. E. Casida. 1948. The effect of progesterone in altering the estrual cycle of the cow. J. Anim. Sci. 7:540 (Abstr.).

Chupin, D. and J. Pelot. 1976. Progestagens and (or) prostaglandins for estrous synchronization in Dairy Cows. VIIIth Int. Cong. Anim. Reprod. and Artif. Insem. III, p. 447.

Chupin, D., J. Pelot and P. Mauleon. 1977. Control of estrous and ovulation in Dairy Cows. Thenogenology. 7:339.

Convey, E. M. 1973. Neuroendocrine relationship in Farm Animals. A review. J. Anim. Sci. 37:745.

Convey, E. M., W. E. Beal, B. E. Sequin, K. J. Tannen and V. C. Lin. 1976. Gonadotropin releasing hormone induced luteinizing hormone release after prostaglandin $F_2\alpha$. Proc. Soc. Exp. Biol. Med. 151:84.

Convey, E. M., T. W. Beck, R. R. Nietzel, E. F. Bostwick and H. D. Hafs. 1977. Negative feedback control of bovine serum luteinizing hormone (LH) concentration from completion of preovulatory LH surge until resumption of luteal function. J. Anim. Sci. 46:792.

Cooper, M. J. 1974a. Control of estrus of heifers with synthetic prostaglandin analogue. Vet. Rec. 95: 200.

Cooper, M. J. and B. A. Furr. 1974b. The role of prostaglandins in Animal Breeding. Vet. Rec. 94:161.

Cooper, M. J. 1976. The use of cloprostenol (Estrumate) in the controlled breeding of cattle. An assessment of European field trials. In 'Oestrus Synchronization in Cattle' C. D. Nancarrow and I. Cox (Eds.). p. 24.

D'Adammio, G. H., W. F. Williams, J. V. DeBarthes, T. Sweeney, G. E. Moor and J. Buric. 1972. Control of estrus in beef cattle with normal ear implants. J. Anim. Sci. 35:290.

De'Benedetti, R. E., G. H. Kiracofe, R. M. McKee and G. Heersche, Jr. 1977. Synchronization of estrus in beef heifers with Norgestoelt implant and $PGF_2\alpha$. 69th Meeting Amer. Soc. Anim. Sci. p. 149 (Abstr.).

Delatang, W. F. 1975. Synchronization of estrus in cattle using a progestagen (21009) and a synthetic analogue of prostaglandin $F_2\alpha$ (cloprootenol). Vet. Rec. 97:453.

Desjardins, C. and H. D. Hafs. 1968. Levels of pituitary FSH and LH in heifers from birth through puberty. J. Anim. Sci. 27:472.

DuFour, J., H. L. Whitemore, O. J. Ginther and L. E. Casida. 1972. Identification of the ovulating follicle by its size on different days of the estrous cycle in heifers. J. Anim. Sci. 34:85.

Elsaesser, F. and N. Parvizi. 1979. Estrogen Feedback in the pig: Sexual differentiation and the effect of prenatal testosterone treatment. Biol. Reprod. 20:1187.

England, G. B., R. Webb and M. K. Dahmer. 1981. Follicular steroidogenesis and binding to ovine follicles during estrous cycle. Endocrinology 109:881.

Esslemont, R. J., R. G. Glencross, M. J. Bryant and G. S. Pope. 1980. A quantitative study of preovulatory behaviour in cattle (British Friesian heifers). Appl. Anim. Ettiol. 6:1.

Ford, S. P., C. W. Weems, R. E. Pitts, J. E. Pexton, R. L. Butcher and E. K. Inskeep. 1975. Effects of Estradio-17β and progesterone on prostaglandin $F_2\alpha$ in sheep uteri and uterine venous plasma. J. Anim. Sci. 41:1407.

Furr, B. J. A., M. J. Cooper, P. S. Jackson, I. C. Hart and G. S. Pope. 1981. Effects of cloprostenol and prostaglandin $F_2\alpha$ on secretion of follicle stimulating hormone prolactin, growth hormone, Thyroxin and cortisol in heifers. Ecta Vet. Scand. Suppl. 77:55.

Gill, J. L. 1978. Design and Analysis of Experiments in Animal Medical Sciences. Iowa State Univ. Press. Ames.

Ginther, O. J. 1976. Comparative Anatomy of uteroovarian vasculature. In 'Veterinary Scope'. Upjohn Co. (publ.).

Glencross, R. G., R. J. Esslemont, M. J. Bryant and G. S. Pope. 1980. Relationship between incidence of preovulatory behavious and the concentrations of oestradiol-17β and progesterone in Bovine plasma. Appl. Anim. Ethol. 7:141.

Glew, R. H. 1982. Lipid metabolism II. Pathways of metabolism of special lipids, prostaglandins. In 'Textbook of Biochemistry with Clinical Correlations'. T. M. Delvin (Ed.). Wiley Medical Publications. Wiley and Sons, New York.

Gonzalez-Padilla', E., R. Ruiz, D. LeFever, A. Denham and J. N. Wiltbank. 1975. Puberty in Beef heifers III. Induction of Fertile estrus. J. Anim. Sci. 40:110.

Govan, 1970. Ovarian follicular activity in late pregnancy. J. Endocrinol. 48:235.

Gordon, I. 1976. Controlled Breeding in Cattle Part I: Hormone in the regulation of Reproduction estrus control and set-time Artificial Insemination. Anim. Breeding Abstr. 44:265.

Graves, N. W., T. G. Dunn, C. C. Kaltenbach, R. E. Short and J. B. Carr. 1975. Estrus and Ovulation with prostaglandin $F_2\alpha$, Norgestomet® and Gonadotropin in cattle. Proc. Western Sec. Amer. Soc. Anim. Sci. 26:193.

Hafs, H. D., T. M. Louis, P. A. Noden, W. D. Oxender. 1974. Control of estrous cycle with prostaglandin $F_2\alpha$ in cattle and horses. J. Anim. Sci. (Suppl.) 38:10.

Hafs, H. D., J. G. Manns and B. Drew. 1975. Onset of estrus after prostaglandin $F_2\alpha$ in cattle. Vet. Rec. 96:134.

Hafez, E. S. E. 1969. Synchronization of estrus. In 'Reproduction in Farm Animals' (2nd Edit.). Lea and Febiger. Philadelphia.

Hansel, W., P. W. Concannon and J. H. Lukaszewska. 1973. Corpora lutea of the large Domestic Animals. Biol. Reprod. 8:22.

Hansel, W. and J. Fortune. 1978. The Applications of ovulation control. In 'Control of ovulation' p. 237. D. B. Crighton, N. B. Hanes, G. R. Foxcroft and G. E. Lemming (Eds.). Butterworths, London.

Hansel, W. and W. E. Beal. 1979. Ovulation Control in Cattle. In Animal Reproduction, p. 91 (H. H. Hawk (Edit.).

Hansel, W. and E. M. Convey. 1983. Physiology of the Estrous Cycle. J. Anim. Sci. (Suppl. 2) 57: 404.

Hauger, R. L., J. F. Karsch, D. L. Foster. 1977. A new concept for control of estrous cycle of the ewe based on temporal relationship between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. Endocrinology. 101:807.

Heersche, G., G. H. Kiracofe, R. D. DeBenedetti, S. Wen, and R. M. Mckee. 1979. Synchronization of estrous in beef heifers with Norgestomet implant and prostaglandin $F_2\alpha$. Theriogenology. 11:197.

Hobson, W. C. and W. Hansel. 1972. Plasma levels after ovariectomy corpus luteum removal and estradiol administration in cattle. Endocrinology. 91: 185.

Horton, E. W. and N. L. Poyser. 1976. Uterine luteolytic hormone. A physiological role for prostaglandin $F_2\alpha$. Physiol. Rev. 56:595.

Howland, B. E., A. M. Akbar and F. Stormshak. 1971. Serum LH levels and luteal weight in ewes following a single injection of estradiol. Biol. Reprod. 5:25.

Hurnik, J. F., G. H. King and H. A. Robertson. 1975. Estrous and related behavious in postpartum Holstein cows. Appl. Anim. Ethol. 2:55.

Ingram, D. L. 1953. The Effect of hypophysectomy on the number of 00cytes in the adult albino rat. J. Endocrinol. 9:307.

Ireland, J. J. and J. F. Roche. 1982a. The effect of progesterone on basal LH and Episodic LH and FSH secretion in heifers. J. Reprod. Fert. 64:295.

Ireland, J. J. and J. F. Roche. 1982b. Development of Antral Follicle s in cattle after prostaglandin induced luteolysis: Changes in serum hormones, steroids in Follicular Fluid and Gonadotropin receptors. Endocrinology. 111:2077.

Ireland, J. J. and J. F. Roche. 1983. Development of Non ovulatory Antral Follicles in Heifers: Changes in steroids in Follicular Fluid and Receptors for gonadotropins. Endocrinology. 112:150.

Ireland, J. J. 1984. Heat: What It means to You and the Cow. In 'Profitable Reproductive Management' p. 1 MABC (publ.).

Jackson, P. S., C. T. Johnson, B. J. Furr and J. F. Beattie. 1979. Influence of Stage of oestrous cycle on time of oestrus following cloprostenol treatment in the Bovine. Theriogenology. 12:153.

Jaster, E. H., B. Broodie and J. R. Lodge. 1982. Influence of season on timed inseminations of dairy heifers synchronized by prostaglandin $F_2\alpha$.

Jennings, J. J. and J. P. Crowley. 1972. Influence of mating management on fertility in ewew following progesterone-PMS treatment. Vet. Rec. 90:495.

Jochle, W. 1972. Pharmacological aspects of the control of the cycle on domestic animals. VIIth Int. Cong. Anim. Reprod. Artif. Insem. p. 97.

Kersch, J. F., S. J. Legan, R. L. Hauger and O. L. Foster. 1977. Negative feedback action of progesterone on tonic luteinizing secretion in the ewe: Dependence on ovaries. Endocrinology. 101:800.

Katongole, C. B., F. Naftolin and E. V. Younglai. 1973. Diurnal variation in ovarian steroids and luteinizing hormone in cows at oestrus. Steroids and Lipids Res. 4:1.

Keech, C. A., K. A. Case, R. D. Randel, N. H. McArthur, J. J. Reeves and P. G. Harms. 1984. Ovarian steroids affect in vitro and in vivo pituitary LH parameters in ovariectomized heifers. 76th Ann. Meeting Amer. Soc. Anim. Sci. p:343 (Abstr.).

Kesner, J. S. and E. M. Convey. 1982. Interaction of estradiol and luteinizing hormone releasing hormone on follicle stimulating hormone release in cattle. J. Anim. Sci. 54:817.

King, G. J. and H. A. Robertson. 1974. A two injection schedule with prostaglandin $F_2\alpha$ for regulation of the ovulatory cycle of cattle. Theriogenology. 1:123.

King, G. J., J. K. Hurnik and H. A. Robertson. 1976. Ovarian function and estrus in dairy cows during early lactation. J. Anim. Sci. 42:688.

King, M. E., G. H. Kiracafe, J. S. Stevenson and R. R. Schalles. 1982. Effect of stage the estrous cycle on interval to estrus after $PGF_2\alpha$ in Beef cattle. Theriogenology. 18:191.

Kinkie, R. A., D. C. Anderson, E. L. Moody and P. J. Burfening. 1976. Breeding heifers by Appointment with $PGF_2\alpha$ and GnRH. Proc. Western Sec. Amer. Soc. Anim. Sci. 26:205.

Lauderdale, J. W. 1972. Effects of $PGF_2\alpha$ on pregnancy and estrous cycle in cattle. J. Anim. Sci. 35:246.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, C. K. Vincent and F. F. Loyancano. 1974. Fertility of Cattle following $PGF_2\alpha$ injection. J. Anim. Sci. 38:964.

Lauderdale, J. W., W. F. McAllister, E. L. Moody and D. D. Kratzer. 1980. Pregnancy rate in cattle injected with $PGF_2\alpha$. 7snd Ann. Meeting Amer. Soc. Anim. Sci. p. 296 (Abstr.).

Lemon, M., J. Pelletier, J. Saumande and J. P. Signoret. 1975. Peripheral plasma concentrations of progesterone, oestradiol-17β and luteinizing hormone around oestrus in the cow. J. Reprod. Fert. 42:137.

Louis, T. M., H. D. Hafs and B. E. Seguin. 1973. Progesterone, LH estrus and ovulation after prostaglandin $F_2\alpha$ in heifers. Proc. Soc. Exp. Biol. Med. 143:152.

Louis, F. M., H. D. Hafs, D. A. Morrow. 1974. Intrauterine administration of prostaglandin $F_2\alpha$ in cows: Progesterone, estrogen, LH oestrus and ovulation. J. Anim. Sci. 38:347.

Louis, T. M., H. D. Hafs, J. N. Steelflug. 1975. Control of ovulation, fertility and endocrine response after prostaglandin $F_2\alpha$ in cattle. Ann. Biol. Anim. Bioch. Biophys. 15:407.

Macmillan, K. L., A. M. Day and J. F. Smith. 1980. Onset of oestrus and fertility in lactating Dairy cows injected with an analogue of prostaglandin $F_2\alpha$, cloprostenol. Anim. Prod. Sci. 3:245.

Maracek, I., M. Tokos and J. Halagan. 1977. Tertiary follicles in heifers treated with melangesterol acetate. Endocrinol. Exp. 11:249.

Mariana, C. and Nguyen Huy. 1973. Folliculogènese chez la vachè Colloque "ovogenèse Folliculogènese". Ann. Biol. Bioch. Biophys. 13:211.

Matton, P., V. Adelakoun, Y. Contrue and J. J. DuFour. 1981. Growth and Replacement of Bovine ovarian Follicles during the estrous cycle. J. Anim. Sci. 52:813.

Mauer, R. E. V., S. K. Webel and M. D. Brown. 1975. Ovulation control in cattle with progesterone intravaginal device (PRID) and gonadotropin releasing hormone (GnRH). Ann. Biot. Bioch. Biophys. 15:369.

Mauleon, P. 1974. New Trends in the control of reproduction in the Bovine. Livestock Prod. Sci. 1:117.

McNatty, K. P., D. M. Smith, A. Makns, R. Osathanondh and K. J. Ryan. 1979. The microenvironment of human antral follicle: Interrelationships among the steroid levels in antral fluid, the population of granulosa cells and the status of the oocyte in vivo and in vitro. J. Clin. Endocrinol. Metals. 49:686.

McNatty, K. P., M. Gibb, C. Dobson, K. Ball, J. Coster, D. Heath and D. Thurley. 1982. Preovulatory follicular development in sheep treated with PMSG and (or) prostaglandin. J. Reprod. Fert. 65:111.

Mercier, E. and G. W. Salisbury. 1947. Seasonal variation in hours of daylight associated with fertility level of cattle under natural breeding conditions. J. Dairy Sci. 30:747.

Milvae, R. A. and W. Hansel. 1983. Luteolytic effect of 13, 14 dihydro-$PGF_2\alpha$ in heifers. J. Reprod. Fert. 67:203.

Momont, H. W. and B. E. Seguin. 1984. Influence of day of estrous cycle on response to $PGF_2\alpha$ products: Implications for AI programs for Dairy cattle. 10th Int. Cong. Anim. Reprod. Artif. Insem. III, p. 336.

Morrow, D. A. 1969. Estrous behaviour and ovarian activity in prepubertal and post pubertal dairy heifers. 1969. J. Dairy Sc. 52:224.

Nancarrow, C. D., H. Hearshaw, P. E. Mattner, P. J. Connell and B. J. Restall. 1974. Hormonal changes in cattle following the administration of prostaglandin $F_2\alpha$. J. Reprod. Fert. 36:484.

Nancarrow, C. D. and R. I. Cox. 1976a. Oestrus synchronization in cattle. In "Evaluation of practical techniques for synchronization of oestrus in cattle with prostaglandins." ICI Australia Ltd. Melbourne.

Nancarrow, C. D. and H. M. Radford. 1976b. Endocrine basis of synchronization techniques. In oestrus synchronization in cattle. C. D. Nancarrow and I. Cox (Edit.) p. 8.

Niswender, G. D., D. E. Suter and H. R. Sawyer. 1981. Factors regulating receptors for LH on ovine luteal cells. J. Reprod. Fert. (Suppl.) 30:183.

O'Farrel, K. J. 1977. Claving rate of dairy cows and heifers following 12 day progesterone and benzoate synchronization treatment. Irish J. Agric. Res. 16:131.

Padmanabhan, V. K. Keung and E. M. Convey. 1982. Ovarian steroids modulate the self priming effect of lutenizing hormone-releasing hormone on bovine pituitary cells in vitro. Endocrinology. 110: 717.

Peluso, J. J., S. Luttmer and M. L. Grueberg. 1983. Effect of LH bulse amplitude on ovarian follicular growth in vitro. Biol. Reprod. 28 (Suppl.) (Abstract).

Peters, H. 1969. The development of the ovary from birth to maturity. Ecta Endocrinol. 62:98.

Peters, H., A. Byskov, S. Lintern-Moore, M. Faber and M. Anderson. 1973. The effect of Gonadotrophin in follicle growth initiation in the neonatal mouse ovary. Reprod. Fert. 35:139.

Peters, H., A. G. Byskov, Shintern-Moore, M. Faber. 1975. Follicular growth: The basic event in the moose and human ovary. Reprod. Fert. 45:559.

Peters, H. 1979. Some Aspects of Early Follicular Development: In "Ovarian Follicular Development and Function", Midgley and Sadler (Edit) Raven Press; New York.

Pedersen, T. and H. Peters. 1971. Follicle growth and cell dynamics in the mouse ovary during pregnancy. Dertil. Steril. 22:42.

Piper, E. L. and W. C. Foote. 1965. A luteolytic effect of Estradiol in the ewe. J. Anim. Sci. 24:920.

Quinilivan, T. D. and Robinson, T. J. 1969. Numbers of Spermatozoa in genital tract after artificial insemination of progestagen-treated ewes. J. Reprod. Fert. 19:73.

Rahe, C. H., R. E. Owens, J. L. Fleeger, H. J. Newton and P. G. Harms. 1980. Patterns of plasma luteinizing hormone in the cyclic cow: Dependence upon the period of the cycle. Endocrinology. 107:498.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal cyclical and left to right variations. Ecta Endocrinol (Suppl. 52) 34:7.

Refsal, K. R. and B. E. Seguin. 1980. Effect of stage of diestrus and number of cleprostenoe (ICI 80996) injections on intervals to Estrus LH peak and ovulation in heifers. Theriogenology. 14:37.

Renegar, R. H., H. D. Hafs, J. H. Britt and T. D. Carruthers. 1978. Luteolysis growth hormone glucocorticoids prolactin and milk production in lactating Dairy Cows given prostaglandin $F_2\alpha$. J. Anim. Sci. 47:532.

Richards, J. S. and A. R. Midgley. 1976. Protein Hormone Action: A Key to understanding ovarian Follicular and luteal cell development. Biol. Reprod. 14: 82.

Richards, J. S. 1980. Maturation of ovarian follicles. Actions and Interactions of Pituitary and Ovarian hormones on follicular cell differentiation. Physiol. Rev. 60:51.

Richards, J. S. and K. Bogovich. 1982. Effect of Human Chorionic gonadotropin and progesterone on Follicular Development in the Immature rat. Endocrinology. 111:1429.

Roche, J. F. 1974. Effect of short-term progesterone treatment on oestrous response and fertility in heifers. J. Reprod. Fert. 40.433.

Roche, J. F. 1975. Synchronization of oestrus in cows using silastic coils containing progesterone. Ann. Biol. Bioch. Biophys. 15:301.

Roche, J. F. 1976a. Synchronization of oestrus in cattle. World Rev. Anim. Prod. XII:79.

Roche, J. F. 1976b. Fertility in cows after treatment with prostaglandin analogue with or without progesterone. J. Reprod. Fert. 46:341.

Roche, J. F., O'Farrel, D. Prendiville, W. Davis and T. Condon. 1976c. Fixed Time Insemination of Cows' following 12-day progesterone treatment with silastic coils. VIIIth Int. Cong. Anim. Reprod. and Artif. Insem. III.

Roche, J. F. and J. Gosling. 1977. Control of Estrus and progesterone levels in heifers given intravaginal proesterone coils and injections of progesterone and estrogen. J. Anim. Sci. 44: 1026.

Roche, J. F. 1977b. Synchronization of Estrus with prostaglandins. Vet. Sci. Comm. 1:121.

Roche, J. F. 1978. Control of oestrus in cattle using progesterone coils. Anim. Reprod. Sci. 1:145.

Roche, J. F. 1979a. Control of oestrus in cattle. World Rev. Anim. Prod. XV:49.

Roche, J. F. and D. J. Prendiville. 1979b. Control of estrus in dairy cows with synthetic analogue of prostaglandin F_2 Alpha. Theriogenology. 11: 153.

Roche, J. F. and J. J. Ireland. 1981a. The differential effect of progesterone on concentrations of luteinizing Hormone and Follicle Stimulating Hormone in Heifers. Endocrinology. 108:568.

Roche, J. F. and J. J. Ireland. 1981b. Effect of Exogenous Progesterone on time of occurrence of LH surge in heifers. J. Anim. Sci. 52:580.

Roman-Ponce, H., W. W. Thatcher, C. J. Wilcox. 1981. Hormonal interrelationships and physiological response of lactating dairy cows to a shade management system in subtropical environment. Theriogenology. 16:139.

Rosenberg, M., Z. Herz, M. Davidson and Y. Folman. 1977. Seasonal variation in postpartum plasma progesterone levels and conception in primiparous and multiparous dairy cows. J. Reprod. Fert. 51: 363.

Rosenberg, M., Y. Folman, Z. Herz, I. Flamebaum, A. Berman and M. Kaim. 1982. Effect of Climatic conditions on peripheral concentrations of LH progesterone and estradiol-17β in high milk yielding cows. J. Reprod. Fert. 66:139.

Rowson, L. E. A., H. R. Teruit and A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. J. Reprod. Fert. 29:145.

Scaramuzzi, R. I., K. E. Turnbull and C. D. Nancarrow. 1980. Growth of Graafian Follicles in cows following luteolysis induced by prostaglandin $F_2\alpha$ analogue, cloprostenol. Austr. J. Biol. Sci. 33:63.

Schallenberger, E., D. Schams, B. Bullerman and D. L. Walters. 1984. Pulsatile secretion of gonadotrioubsm ivaruab steriuds and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J. Reprod. Fert. 71:493.

Schams, D., E. Schallenberger, B. Hoffman and H. Karg. 1977. The oestrous cycle of the cow: Hormonal parameters and time relationships concerning oestrus ovulation and electrical resistance of the vaginal mucus. Ecta Endocrinol. 86:180.

Schoenmann, H. M., W. D. Humphrey and J. J. Reeves. 1983. Pituitary LHRH receptors in ovariectomized cows after challenge estrogens alone or with progesterone. Proc. Western Sec. Amer. Soc. Anim. Sci. 34:270.

Schrieber, J. R., K. Nakamura and G. F. Erickson. 1981. Progestins Inhibit FSH. Stimulated Granulosa Estrogen Production in Post cAMP site. Mol. Cell Endocrinol., 21:161.

Sheiffield, L. G. and A. R. Ellicott. 1982. Effect of low levels of exogenous progesterone on puberty in beef heifers. Theriogenology. 18:175.

Short, R. E., R. D. Randel, R. B. Staigmiller and R. A. Bellows. 1979. Factors affecting estrogen-induced LH release in the cow. Biol. Reprod. 21:683.

Smith, J. F. 1976. Use of Synthetic prostaglandin analogue for synchronization of estrus in heifers. N.Z. Vet. J. 24:71.

Smith, R. D., W. Hansel, G. E. Urban and T. E. Pilbeam. 1980. Use of PRID + $PGF_2\alpha$ to control estrus in lactating Dairy cows. J. Anim. Sci. (Suppl.) p. 392 (Abstr.).

Smith, R. D., A. J. Pomerantz, W. E. Beal, T. E. Pilbeam and W. Hansel. 1984. Insemination of Holstein heifers at a preset time after estrous cycle synchronization using progesterone and prostglandin. J. Anim. Sci. 58:792.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods (6th Edit.). Iowa State Univ. Press. Ames.

Spalding, R. W., R. W. Everett and R. H. Foote. 1975. Fertility in New York artificially inseminated Holstein herds in Dairy Herd Improvement. J. Dairy Sci. 58:718.

Spicer, L. J., J. J. Ireland and J. F. Roche. 1981. Changes in serum LH, progesterone and specific binding of [125_I]-LCG to luteal cells during regression and development of bovine corpora lutea. Biol. Reprod. 25:832.

Sreenan, J. M. 1975a. Effect of long-term and short-term Intravaginal progestagen treatments on synchronization of oestrus and fertility in heifers. J. Reprod. Fert. 45:367.

Stauffer, G. D., E. F. Ellington, and M. Nielson. 1976. Estrous cycle control with progestagen-prostaglandin treatment. Proc. Western Sec. Ameri. Soc. Anim. Sci. 27:201.

Staigmiller, R. B. and B. G. England. 1982. Folliculogenesis in the Bovine. Theriogenology. 17:43.

Stellflug, J. N., T. M. Louis, R. C. Gorewit, W. D. Oxender, and H. D. Hafs. 1977. Luteolysis induced by prostaglandin $F_2\alpha$ Before and after Hysterectomy in heifers. Biol. Reprod. 17:535.

Stevenson, J. S., M. K. Schmidt, and E. P. Call. 1984. Stage of estrous cycle time of insemination and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin $F_2\alpha$. J. Dairy Sci. 67:1798.

Sweetman, W. J. 1950. Artificial breeding in Alaska and effect of extra light during short winter days. J. Dairy Sci. 33:391.

Tanabe, T. Y. and R. C. Hann. 1984. Synchronized estrus and subsequent conception in dairy heifers treated with prostaglandin $F_2\alpha$.I: Influence of stage of cycle at treatment. J. Anim. Sci. 58: 805.

Thatcher, W. W. and J. R. Chenault. 1976. Reproductive physiological responses of cattle to exogenous prostaglandin $F_2\alpha$. J. Dairy Sci. 59:1366.

Thimonier, J., D. Chupin and J. Pelot. 1975. Synchronization of oestrus in heifers and cyclic cows with progestagens and prostaglandin analogue alone on in combination. Ann. Biol. Bioch. Biophys. 15:437.

Tonetta, A. S. and J. J. Ireland. 1983. Is estradiol required for increases of FSH receptors? 16th Ann. Meeting Soc. Study Reprod. p. 61 (Abstr.).

Watts, T. L., J. W. Fuquay and W. R. Hearne. 1984. Response and fertility of Dairy heifers following injection of $PGF_2\alpha$ in early, middle or late diestrus. 79th. Ann. Meeting. Amer. Dairy Sci. Assoc. p. 153 (Abstr.).

Webel, S. K. 1976. Control of the estrous cycle in cattle with progesterone releasing intravaginal device. Proc. VIIIth Int. Cong. Anim. Reprod. and Artif. Insem. III. p. 521.

Whitman, R. W., J. H. Wiltbank, D. G. LeFever and H. A. Denham. 1972. Ear implant (SC 21009) for estrus control in cows. Proc. Western Sec. Amer. Soc. Anim. Sci. 23:280.

Wiltbank, J. H., J. E. Ingalls, and W. W. Rowden. 1961. Effects of various forms and levels of estrogen alone or combination with ganadotrogins on estrus cycle of beef heifers. J. Anim. Sci. 20:341.

Wiltbank, J. H., II, R. Zimmerman, J. E. Ingalls and W. W. Rowden. 1965. Use of progestational compounds alone or in combination with estrogen for synchronization of estrus. J. Anim. Sci. 24:990.

Wiltbank, J. N. and C. W. Kasson. 1968. Synchronization of Estrus in Cattle with an Oral Progestational agent and injection of an estrogen. J. Anim. Sci. 27:113.

Wiltbank, J. N., J. C. Sturges, D. Wideman, D. G. LeFever, and L. C. Faulkner. 1971. Control of estrus and ovulation using subcutaneous implants and estrogens in beef cattle. J. Anim. Sci. 33:600.

Wiltbank, J. N. and E. Gonzalez-Padilla'. 1975. Synchronization and Induction of estrus in heifers with progestagen and estrogen. Ann. Biol. Bioch. Biophys. 15:255.

Wishart, D. F. 1974a. Synchronization of ostrus in cattle using potent progestin SC 21009 and $PGF_2\alpha$. Theriogenology. 1:87.

Wishart, D. F. and I. M. Young. 1974b. Artificial Insemination of progestion (SC 21009) treated cattle at predetermined times. Vet. Rec. 95:503.

Wishart, D. F. and S. B. Drew. 1977. A comparison between pregnancy rates of heifers inseminated once or twice after progestin treatment. Vet. Rec. 101:230.

Wolff Vaught, L., D. E. Monty Jr., C. W. Foote. 1977. Effect of summer heat stress on serum luteinizing hormone and progesterone values in Holstein-Friesian cows in Arizona. Ameri. J. Vet. Res. 38:1027.