

EARLY POSTPARTUM LUTEAL FUNCTION AFTER PROGESTIN  
AND(OR) GONADOTROPIN-RELEASING HORMONE IN DAIRY CATTLE

by

MICHAEL ORVIS MEE

B.S., UNIVERSITY OF WISCONSIN-PLATTEVILLE, 1986

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A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1988

Approved by:

  
Major Professor

## ACKNOWLEDGEMENTS

This thesis is dedicated to my wife, Peggy, and my children, Michele, Dayna, and Brian, whose love and understanding made this experience worthwhile. A sincere thanks and appreciation is extended to my major professor, Dr. Jeffrey Stevenson, for his time, patience, and expertise throughout the experiment and during the preparation of this thesis. I would also like to thank Dr. J. Ernest Minton and Dr. Guy Kiracofe for serving on my graduate committee.

I appreciate the time and assistance of Tami DelCurto whose friendly smile made working in the lab a real joy. In addition, I would like to thank B. Carinder and J. Smith of the KSU dairy and R. Dudley for their time and assistance throughout the experiment. The support and friendship of my fellow graduate students especially R. Stewart, W. McGuire and R. Perry was also appreciated.

Lastly, appreciation is extended to Dr. William Hoffman and Dr. Roy Shaver whose encouragement and friendship during my undergraduate program aided in my decision to attend graduate school.

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## REVIEW OF LITERATURE

### Introduction

A 12-mo calving interval is the optimal economic goal in reproduction of dairy cattle. However, to achieve this goal a cow must conceive by d 85 postpartum. The postpartum interval is characterized by a period of anestrus that differs between suckled and milked cows. Normally, there is a restoration of ovarian cyclicity in dairy (milked) cows within 30 d of parturition, although in some instances this can be delayed (Peters et al., 1981; Peters and Lamming, 1986). In contrast, beef (suckled) cows have a longer and more variable postpartum acyclic period that is particularly pronounced in animals nursing more than one calf (Wettemann, 1980). Therefore, extended periods of anovulation can increase the calving interval beyond 365 d and reduce potential economic profits (Haresign et al., 1983). Delayed ovulation could be regarded as a strategy for survival on the part of the cow to delay conception during periods of environmental or physiological stress (Peters and Lamming, 1986). Therefore, it is necessary to understand the physiological mechanisms involved in the reestablishment of normal ovarian activity.

Results of research have shown that the first corpus luteum (CL) formed in pubertal heifers and postpartum dairy or beef cows is frequently shortlived (Macmillan and Watson, 1971; Lauderdale, 1986) and unable to maintain pregnancy (Ramirez-Godinez et al., 1986a). Thus, understanding both the luteotropic and

luteolytic mechanisms regulating lifespan of the CL are necessary to explain the sequence of events occurring during the restoration of ovarian cyclicity. This understanding might lead to practical development of hormonal treatments that reduce the anovulatory period in cattle, since more ovulations and estrous activity occurring prior to d 60 postpartum have a positive effect on reproductive performance (Thatcher and Wilcox, 1973; BenMrad and Stevenson, 1986).

### Onset of Luteal Activity

The early postpartum period in cattle is characterized frequently by ovarian inactivity. The average interval to first ovulation is approximately 3 wk in milked dairy cattle (Marion and Gier, 1968; Stevenson and Britt, 1979), whereas in suckled beef cows, this period ranges from 36 to 71 d (Wettemann, 1980). The growth and persistence of the first CL may be subnormal (Garverick and Smith, 1986) because the interval from the first to the second ovulation averaged less than 15 d, whereas the interval from the second to third ovulation averaged 21 d (Marion and Gier, 1968). Thus, hormonal balances established to support pregnancy are readjusted gradually after parturition in order to reestablish normal luteal function. However, the time required for this readjustment is variable and appears to be influenced by environmental and genetic factors (Marion and Gier, 1968; Wettemann, 1980; Kiracofe, 1980; Dunn and Kaltenbach, 1980; Edgerton, 1980; Christenson, 1980; Hanzen, 1986).

### Factors Influencing Onset of Luteal Function Postpartum

Feto-placental Unit. The conceptus and maternal units interact throughout

gestation in order to provide for fetal development. Therefore, it may be possible for the fetus to exert carry-over effects into the postpartum period of its dam. Guilbault et al. (1985) demonstrated that Holstein heifers bred to genetically different sires (Holstein, Brahman or Angus) differed in endocrine patterns and rates of uterine involution after parturition. Daily reduction in the diameters of the cervix and uterine horns were greater in heifers mated to Holstein and Brahman sires than to Angus sires and also were associated with higher postpartum concentrations of the stable plasma metabolite of prostaglandin- $F_{2\alpha}$ ; 13,14-dihydro-15-keto prostaglandin- $F_{2\alpha}$  (PGFM). However, concentrations of progesterone in serum were higher and more synchronous in the Angus-sired group after a challenge with GnRH (d 10 postpartum). From these results, Guilbault et al. (1986) suggested that postpartum ovarian activity and uterine involution in Holstein heifers may be affected by the genotype of the fetus carried in utero during pregnancy.

Previously Gravid Horn. The inhibitory action of pregnancy on follicular development in cattle is maintained after parturition by a mechanism believed to involve the previously gravid horn and(or) the ovary bearing the corpus luteum of pregnancy (Kiracofe, 1980; Dufour and Roy, 1985; Spicer and Echterkamp, 1986). This inhibitory action can be maintained during the first 20 d postpartum and reduce the frequency of ovulation from the ovary ipsilateral to the previously gravid horn (Kiracofe, 1980; Dufour and Roy, 1985).

Abnormal Uterus. Conditions of the genital tract have been associated with differences in the resumption of postpartum ovarian cyclicity (Callahan et al., 1971,

Kiracofe, 1980). Approximately 85 to 95% of postpartum cows acquire uterine infections immediately after calving (Peter and Bosu, 1987). A majority of these infections are not serious; however, infections associated with twinning, dystocia and(or) retained placenta detrimentally alter measures of reproductive efficiency. Cows diagnosed with metritis have first postpartum ovulations that are delayed by 29 to 33 d (Callahan et al., 1971). It is believed that cellular debris and fluids (lochia) in the involuting uterus may inhibit feedback mechanisms regulating endocrine control of normal estrous activity and(or) stimulate increased synthesis of prostaglandins that may lyse luteal tissue (Callahan et al., 1971; Peter and Bosu, 1987). An abnormal uterus may delay follicular development and ovulation, but it is doubtful that a normal involuting uterus plays a major role in reestablishing estrous cycles (Kiracofe, 1980). However, it may have an effect on the lifespan of the first CL or luteal tissue (Copelin et al., 1987).

Mammary Gland. It appears the ruminant mammary gland has both an endocrine and exocrine function (Peters and Lamming, 1986). The mammary gland secretes prostaglandin- $F_2\alpha$  and estradiol- $17\beta$  during the periparturient period (Maule-Walker et al., 1983). However, an endocrine role for the mammary gland in controlling postpartum ovarian function has not been demonstrated. Short et al. (1982) showed that when beef cows were mastectomized prior to parturition, they returned to estrus on the average 12 d after calving. The mechanism whereby mastectomy shortened the interval to first estrus is not known, but may involve direct lactational effects or indicate an endocrine role for the mammary gland.

Lactation: Suckled vs Milked. Dairy cows, although selected for high milk yield, may ovulate within 2 wk of parturition, whereas suckled beef cows may not show estrus until 2 mo after parturition (Edgerton, 1980). Studies demonstrating the effect of milking versus suckling within breeds suggested that suckling rather than breed differences is the major factor responsible for delayed ovulation (Peters et al., 1981; Stevenson et al., 1983). A few studies have shown a possible relationship between postpartum intervals and increased milk production in dairy cows (Stevenson and Britt, 1979; Edgerton, 1980; Hanzen, 1986). Suckling increased the postpartum intervals to ovulation and to first estrus (Short et al., 1972; Wettemann et al., 1978; Wettemann, 1980; Edgerton, 1980). This increase was proportional to the number of calves suckled (Wettemann et al., 1978). Suckling reduced the pulsatile release of luteinizing hormone (LH) and mean circulating concentrations of LH in beef cows (Walters et al., 1982; Garcia-Winder et al., 1984) and in dairy cows (Peters et al., 1981; Stevenson and Britt, 1979). Pituitary concentrations of LH and hypothalamic concentrations of GnRH are similar in both milked and suckled cows (Carruthers et al., 1980; Hanzen, 1986). Therefore, suckling appears to interfere with the hypothalamo-pituitary axis by altering the secretion and/or actions of LH and GnRH rather than their synthesis (Carruthers et al., 1980; Hanzen, 1986). Removal of the calf for 48 h induced an acute increase in plasma concentrations of LH (Walters et al., 1982). Thus, the suckling stimulus and its effects on the brain may be a key source of the inhibition that delays the onset of cyclic ovarian function and not lactation per se. However, numerous

hormones are released during suckling and milking and may precipitate the delay in ovarian cyclicity. One of those milking-induced hormones, oxytocin has been shown to be luteolytic when administered early in the estrous cycle (Armstrong and Hansel, 1959). Recent research has demonstrated that multiple daily injections of oxytocin through d 28 postpartum failed to delay the onset of estrous cycles in periparturient dairy cows (Stewart and Stevenson, 1987).

Nutrition. Extended periods of over or underfeeding cattle before or after calving can affect ovarian function (Hanzen, 1986). Folman et al. (1973) showed that limiting available energy in the diet to at least 25% below estimated requirements prolonged the postpartum interval to first ovulation. The postpartum interval to first observed estrus of primiparous dairy cows was increased approximately 21 d (67 vs 88) by restricting energy and protein during gestation (Dunn and Kaltenbach, 1980). Feeding high levels of protein (19.3% crude protein) to high milk-producing cows shortened the postpartum interval to first estrus by 18 and 9 d compared with cows receiving 16.3% and 12.7% crude protein, respectively (Jordan and Swanson, 1979). Dairy cows are normally maintained on a high plane of nutrition in order to achieve maximum milk yield and the problem with undernutrition occurs less frequently than in beef cows. Furthermore, with regard to initiating first estrous cycles, dairy cows may not be as sensitive to losses of body weight often observed after parturition as suckled beef cows (Dunn and Kaltenbach, 1980). The effect of nutrition on the onset of luteal activity in beef cows has been related to prepartum body condition (Dunn and Kaltenbach, 1980), because cows

that are in good body condition at parturition are not affected by changes in pre- or postpartum body weight and normally have shorter intervals to first ovulation than cows in poorer body condition at calving (Dunn and Kaltenbach, 1980).

**Environment.** Changes in season or photoperiod altered the duration of anestrus after parturition in cattle. Cows calving in winter had delayed intervals to first ovulation compared with cows calving in the summer (Hansen, 1985). Likewise, primiparous cows exposed to a 18 h of light and 6 h of darkness (18 L : 6 D) had reduced intervals to first ovulation and first estrus (Hansen, 1985). There also appears to be a direct influence of high ambient temperature and high humidity on ovarian cyclicity after parturition because high temperature and high humidity alter endocrine and uterine function resulting in delayed postpartum ovarian function (Christenson, 1980).

**Age.** Primiparous cows have longer intervals to first ovulation than multiparous cows (Dunn and Kaltenbach, 1980).

#### Patterns of Hormonal Secretion Associated with Onset of

#### Luteal Function

#### Gonadotropins

**Luteinizing Hormone.** In dairy cows milked twice daily, peripheral serum concentrations of LH are low after parturition, but began to rise within 1 wk postpartum, which consists of an increasingly frequent pulsatile secretory pattern of LH (Stevenson and Britt, 1979; Peters et al., 1984; Schallenberger, 1985; Hanzen, 1986). The sensitivity of the pituitary to hypothalamic GnRH also increases at this

time (Hansen, 1986). However, the time and appearance of the pulsatile release of LH and increased sensitivity of the pituitary to GnRH are delayed in suckled beef cows. This period can be extended up to 20 to 30 d after parturition (Humphrey et al., 1983; Hansen, 1986). It has been demonstrated in dairy cows that the greater the frequency of LH pulses between 10 to 17 d postpartum, the shorter the period to the onset of luteal function (Stevenson and Britt, 1979; Peters et al., 1981). Moreover, studies with suckled beef or dairy cows have shown that the sooner after parturition the LH pulses are detected the quicker ovarian cyclicity is resumed (Carruthers et al., 1980; Peters et al., 1981; Peters and Lamming, 1986). In dairy cows, pulses of LH increased from 1 or 2 per 6 h during the early postpartum period to 3 to 8 pulses per 6 h prior to first ovulation (Kesler et al., 1977; Schallenberger, 1985; Webb et al., 1980; Peters et al., 1981). However, in suckled beef cows, frequencies of pulses of LH during postpartum anestrus ranged from 1 to 2 pulses per 6 h (Walters et al., 1982; Convey et al., 1983; Humphrey et al., 1983). In dairy cows, the ability to release LH in response to exogenous GnRH is restored by 7 to 10 d postpartum (Fernandes et al., 1978; Kesler et al., 1977; Kesler et al., 1978), whereas this response was not fully restored until approximately d 20 postpartum in suckled beef cows (Irvin et al., 1981; Peters et al., 1981). Therefore, the most consistent endocrine change reported to precede ovulation in postpartum cows is the onset and increased frequency of pulsatile LH secretion (Peters and Lamming, 1986). In addition, LH is the major luteotropin in cattle and adequate concentrations are necessary to maintain the corpus luteum after ovulation

(Smith, 1986).

Follicle-Stimulating Hormone (FSH). Concentrations of FSH in serum or plasma vary during the postpartum period, but tend to increase during the first few days after calving (Schams et al., 1978; Webb et al., 1980; Peters et al., 1981). Follicle-stimulating hormone is secreted in pulses during the early postpartum period (Peters et al., 1981) and shares the same frequency and magnitude as observed during late gestation (Schallenberger, 1985). However, concentrations of FSH remained relatively constant through d 30 postpartum and then decreased slightly (Peters and Lamming, 1986). It is believed the role of FSH during the early postpartum period is one of synergism with estradiol-17 $\beta$  to induce the formation of LH receptors on the membranes of follicular granulosa cells (Webb et al, 1980; Hanzen, 1986).

#### Steroid Hormones

Estradiol-17 $\beta$ . Serum concentrations of estradiol increase in cows during late gestation and reach a peak (113 pg/ml) just before parturition (Wettemann, 1980; Humphrey et al., 1983; Hanzen, 1986). After parturition and during the early postpartum period, concentrations of estradiol in serum are at basal levels (less than 7 pg/ml) and are similar to those during the luteal phase of a normal estrous cycle (Wettemann, 1980; Humphrey et al., 1983). Average concentrations of estradiol increase throughout the postpartum interval (Kesler et al., 1977; Fernandes et al., 1978) and reach their highest concentrations (approximately 10 pg/ml) 2 to 3 d before the first ovulation (Fernandes et al., 1978). Pulses of estradiol occur after

parturition in cattle (Peters and Lamming, 1984; 1986). Early in the postpartum period when hypothalamic activity is minimal, the hypothalamus is sensitive to the negative feedback effects of estradiol (Haresign et al., 1983). However, as the postpartum interval increases with higher concentrations of estradiol in serum, a positive effect is observed on secretion of LH from the pituitary (Stevenson et al., 1983; Haresign et al., 1983). This positive feedback mechanism is inhibited longer in suckled cows (Stevenson et al., 1983; Garcia-Winder et al., 1986a; Peters and Lamming, 1986). Suckling also inhibits synthesis of estradiol by follicular cells (Hanzen, 1986).

At the pituitary, suckling diminishes the LH response to exogenous estradiol (Stevenson et al., 1983; Garcia-Winder et al., 1986a; Azzazi et al., 1983). However, there is a rapid and sustained rise in the concentration of estradiol in serum within 2 h of an exogenous GnRH injection. This rise in estradiol stimulates the positive effect on peak amplitude and release of LH (Peters et al., 1985). Likewise, the responsiveness of the hypothalamo-pituitary axis to large doses of estradiol increases with stage postpartum (Azzazi et al., 1983; Stevenson et al., 1983). Therefore, these studies demonstrated that the ovary is sensitive to GnRH-induced gonadotropin release and responded by secreting estradiol, producing a positive feedback loop on LH secretory activity (Peters and Lamming, 1986).

Cortisol. Concentrations of cortisol in serum are highest at parturition and decrease to basal levels within 4 wk of parturition (Stevenson and Britt, 1979; Humphrey et al., 1983). Cortisol fluctuates periodically throughout the postpartum

period, but tends to be highest preceding the first postpartum ovulation (Humphrey et al., 1983). High concentrations of corticosteroid can inhibit the secretion of LH and may be a factor in delaying normal ovarian cycles (Peters and Lamming, 1986).

Progesterone. Concentrations of progesterone in cows are high throughout gestation, but diminish rapidly during 2 wk prior to parturition (Hanzen, 1986). After parturition, concentrations of progesterone in serum are generally low (less than 1 ng/ml) until after the first postpartum ovulation (Wettemann, 1980; Humphrey et al., 1983). Blood concentrations of progesterone are good indicators of luteal function because progesterone is the major steroid secreted by the corpus luteum. Concentrations of progesterone greater than 1 ng/ml are associated characteristically with the presence of either a functional CL or luteinized follicle (Wettemann, 1980). Progesterone increases slightly in beef cows 2 to 4 d prior to the onset of ovarian activity (Wettemann, 1980; Humphrey et al., 1983). Similarly, ovarian cyclicity commences in dairy cows approximately 9 d after an acute rise in serum concentrations of progesterone (Wettemann, 1980). The reason for this transient increase is not known but appears necessary for normal luteal activity (Wettemann, 1980).

Three types of luteal activity occur after establishment of ovarian cyclicity (Schams et al., 1978; Ramirez-Godinez et al., 1981; Hanzen, 1986; Garverick and Smith, 1986; Smith, 1986). The luteal phase can be characterized as normal, short, or inadequate (Schams et al., 1978; Hanzen, 1986). The short luteal phase and inadequate luteal phase are commonly referred to as subnormal luteal function

(Garverick and Smith, 1986) and will be discussed later. Thus, progesterone contributes to the preovulatory endocrine milieu in the cow and may alter subsequent luteal function.

#### Prostaglandin- $F_{2\alpha}$ (PGF).

Prostaglandin- $F_{2\alpha}$  plays a major role during parturition in cattle, and a sustained release of PGF during the postpartum period is associated with uterine involution (Madej et al., 1986). Concentrations of PGFM, are highest about d 3 postpartum and decline to basal concentrations within 3 to 4 wk after parturition. There appears to be a correlation between declining concentrations of PGF and the interval to be first ovulation (Kindahl et al., 1982). Measurement of PGFM is a good indicator of the relative daily production of PGF in postpartum cows (Madej et al., 1986). Therefore, in early ovulating cattle, the first postpartum CL could be destroyed prematurely by high levels of PGF secreted from the uterus (Madej et al., 1986).

#### Endocrine Model

Peters and Lamming (1986) proposed the following hypothetical sequel of endocrine secretions, necessary to reestablish cyclic ovarian activity in the "normal" cow after parturition: 1) Some GnRH apparently is secreted immediately postpartum, but the quantity and frequency at which it is secreted is inadequate to elicit sufficient gonadotropin secretion to induce cyclic ovarian activity; 2) Concentrations of FSH rise rapidly after parturition and stimulate early follicular development; 3) There is a gradual increase in concentrations of LH in serum and

increased frequency of LH pulses; 4) Secretion of gonadotropins stimulate follicular growth and the secretion of estradiol. Follicular secretions, possibly inhibin, appear to modulate further secretions of FSH; and 5) There is a gradual recovery of the positive feedback mechanism of estradiol on secretion of LH so that ovarian cycles can begin approximately 2 to 3 wk postpartum in non-suckled cows. Although this model presents a logical order to endocrine events necessary for the restoration of ovarian cyclicity, it fails to provide insight into endocrine events related to the reestablishment of normal luteal function (i.e., it does not account for the differences in why normal, short, or inadequate luteal phases occur).

#### Characteristics of Luteal Function

The corpus luteum is a transient endocrine gland that develops from residual thecal and granulosa cells of the Graafian follicle following ovulation. It secretes progesterone and has an important role in regulating the duration of estrous and menstrual cycles (Smith, 1986).

#### Normal Luteal Function

Typically cows with normal luteal phases have elevated concentrations of progesterone for about 14 d and subsequent estrous cycles ranging from 18 to 24 d in length (Schams et al., 1978; Hanzen, 1986). Peak concentrations of progesterone range from 4 to 8 ng/ml during a normal luteal phase (Schams et al., 1978). Characteristically, only 20 to 50% of the first spontaneous or induced ovulations resulted in a normal luteal phase (Hanzen, 1986). Schams et al. (1978) observed that 7 of 25 or 28% of dairy cows had a normal luteal phase after a

spontaneous ovulation.

### **Subnormal Luteal Function**

Subnormal luteal function has been documented in humans (Strott et al., 1977), primates (Wilks et al., 1976), sheep (McLeod and Haresign, 1984) and cattle (Odde et al., 1980; Pratt et al., 1982). Abnormal luteal function has been reported following spontaneous and gonadotropin-induced ovulations in sheep and cattle (Garverick and Smith, 1986). Subnormal luteal function has been classified into two types; namely, the short luteal phase and inadequate luteal phase (Hanzen, 1986).

**Short Luteal Phase.** A short luteal phase (SLP) appears to be commonplace in cattle after the first postpartum ovulation (McMillan and Watson, 1971; Schams et al., 1978; Kesler et al., 1980; Odde et al., 1980; Pratt et al., 1982). The SLP results in a detectable short estrous cycle that generally ranges from 7 to 12 d in duration (Odde et al., 1980; Kesler et al., 1980; Ramirez-Godinez et al., 1982b). Elevated concentrations of progesterone are detected for 3 to 5 d after ovulation, but decline rapidly between d 5 and 7 (Rutter et al., 1985; Smith et al., 1985). Secretion of progesterone after ovulation was similar for corpora lutea anticipated to have a short or normal lifespan (Smith et al., 1985). The incidence of short estrous cycles resulting from SLP ranged from 50 to 80% after an induced or spontaneous ovulation (Hanzen, 1986).

**Inadequate Luteal Phase.** The inadequate luteal phase (ILP) is characterized by a luteal phase of normal duration, lasting about 14 d, but is associated with concentrations of progesterone in serum lower than normal (Hanzen, 1986). Similar

to the SLP, concentrations of progesterone increase after ovulation (d 1) until d 5 to 7 at which time concentrations remain at subnormal (approximately 50% of normal) and constant levels until luteal regression (Pratt et al., 1982; Schams et al., 1978). Although ILP has been shown in cattle (Pratt et al., 1982; Schams et al., 1978), it appears more common in primates (Wilks et al., 1976; Strott et al., 1970; Smith et al., 1985). This may be due in part to the extended follicular cycle in primates in which the FSH to LH ratios are important for adequate follicular development and necessary in preventing luteal dysfunction (diZerega and Hodgen, 1981; Smith et al., 1985).

Causes of Subnormal Luteal Function. Garverick and Smith (1986) proposed that subnormal luteal function may be due to: 1) inadequate stimulation of the preovulatory follicle by gonadotropins; 2) an inadequate luteotropic stimulus and(or) the inability of the CL to respond to that luteotropic stimulus; 3) an increased and(or) premature release of a luteolysin, or 4) an increased sensitivity of the CL to a luteolysin. In addition, Manns et al. (1983) indicated that the demise of the first short-lived CL does not occur according to the usual luteolytic process, but rather the CL simply loses the ability to synthesize progesterone and no longer responds to LH stimulation. Although these proposals provide some insight into luteal dysfunction, the mechanisms associated with normal or subnormal luteal function are not understood fully. However, various hormonal treatments have been attempted to induce ovulation and further alter luteal function.

Use of Exogenous Hormones in Establishing Luteal Function

Attempts have been made to induce ovarian activity in anestrus cows by human chorionic gonadotropin (hCG), pregnant mare serum gonadotropin (PMSG), gonadotropin-releasing hormone (GnRH), estrogens, and progesterone. However, none of these hormones has been completely effective and resulted frequently in short-lived corpora lutea. Although many of these treatments had been empirical, they provided sound endocrine knowledge and fundamental information regarding the onset and subsequent luteal function in postpartum cows.

#### **Treatments Utilizing GnRH or Gonadotropins**

Gonadotropin-releasing hormone is a naturally occurring hypothalamic decapeptid that elicits release of LH and FSH from the pituitary of cattle and sheep (Leslie, 1983). Injections of GnRH cause rapid increases in peripheral concentrations of LH and FSH in cattle, peaking within 1 h, before returning to preinjection concentrations by 4 to 6 h (Leslie, 1983). The first therapeutic use of GnRH in cattle was for the treatment of cystic ovaries (Leslie, 1983). The release of LH in response to treatment with GnRH is not restored fully until d 7 to 12 postpartum in dairy cows (Fernandes et al., 1977; Foster et al., 1980; Kesler et al., 1977; Peters et al., 1981; Peters and Lamming, 1984; Peters et al., 1985) and d 20 in suckled beef cows (Irvin et al., 1981; Walters et al., 1982). A study of ovulation, estrous, and endocrine responses after GnRH treatment in postpartum dairy cows was the initial experiment utilizing GnRH in cattle (Britt et al., 1974). They demonstrated that GnRH administered in gelatin capsules at 2 wk postpartum induced LH release (within 1 to 2 h) and subsequent ovulation (within 24 h). They

concluded that early postpartum therapy with GnRH may benefit reproductive performance. Likewise, early postpartum administration of GnRH (200  $\mu\text{g}$ ) improved fertility in dairy cows, especially those that experienced periparturient problems (BenMrad and Stevenson, 1986). Various studies have shown that a smaller dose of GnRH (100  $\mu\text{g}$ ) at 2 wk postpartum to induce ovulation resulted in short estrous cycles (Kesler et al., 1978; Garverick et al., 1980). However, before spontaneous ovulations, an increase in the frequency of secretion of LH precedes the preovulatory surge of LH and FSH (Walters et al., 1982). Likewise, multiple small-dose (.5 to 5  $\mu\text{g}$  each) injections of GnRH induced regular synchronous pulses of LH in serum (Riley et al., 1981).

As a result, recent studies have focused on methods to mimic the pulsatile release of LH that is observed prior to ovulation (Mollett et al., 1983; Foster et al., 1980; Riley et al., 1981; Skaggs et al., 1986; Vorstermans and Walton, 1985; Jagger et al., 1987; Wildeus et al., 1987) or continuous infusion of GnRH (Skaggs et al., 1986; Jagger et al., 1987). Injections of GnRH have been applied most commonly at doses ranging from .5 to 5  $\mu\text{g}$ , at intervals of 1 to 2 h, extended over 48 to 96 h. In studies with anestrus beef cows, intermittent low doses of GnRH triggered a pulsatile pattern of secretion of LH and increased release of FSH (Riley et al., 1981; Walters et al., 1982; Wildeus et al., 1987). However, with the exception of one study (Wildeus et al., 1987), transitory concentrations of progesterone were observed, suggesting the occurrence of subnormal luteal function. Similar observations were made in seasonally anestrus ewes given small doses of GnRH

(McNeilly et al., 1981).

A study involving Brahman cattle suggested that reduced intervals to first ovulation after small doses of GnRH were not due to a direct induction of ovulation, but rather to an indirect effect on the sensitivity of the pituitary to GnRH because no increases in concentrations of progesterone were observed after treatment (Wildeus et al., 1987). Recent studies involving continuous infusion or intermittent injections of GnRH in prepubertal beef heifers (Skaggs et al., 1986) or suckled beef cows (Jagger et al., 1987) also have demonstrated short luteal phases. In both cases, pulse frequency and amplitude of LH were greater in animals treated with pulses of GnRH than in those infused continuously with GnRH. Continuous infusion of GnRH in anestrus cows induced LH surges sooner than injected animals. However, estrous cycles tended to be delayed by exposure to continuous infusion of GnRH in prepubertal beef heifers. These results indicated that the method of administration of GnRH influences pituitary responses.

In contrast, different results were observed in dairy cattle in response to injections of GnRH. Cows treated with either 5  $\mu\text{g}$  GnRH every 4 h or 15  $\mu\text{g}$  GnRH every 12 h from d 5 to 10 postpartum had normal estrous cycles (Vorstermann and Walton, 1985). Mollett et al. (1983) demonstrated that anestrus postpartum dairy cows pretreated with 2  $\mu\text{g}$  pulses of GnRH for 72 h prior to a challenge with GnRH (50  $\mu\text{g}$ ) had normal estrous cycles, whereas a challenge with GnRH after pulses of saline resulted in short estrous cycles. Furthermore, more LH was released after the challenge with GnRH in those cows pretreated with

pulses of GnRH.

The reasons for the differences in luteal function between dairy and beef cows after pulses of GnRH are not known, but might be related to an earlier recovery of the hypothalamo-pituitary axis in milked than suckled cattle. Furthermore, since injections of exogenous GnRH elicits an increase in concentrations of estradiol (Peters et al.,1985) and research has demonstrated that estradiol increases secretion of LH in dispersed cultures of pituitary cells (Padmanbhan et al.,1978), pulsatile GnRH may be increasing the sensitivity of the pituitary to the positive feedback of estradiol, allowing for greater release of LH, which alters growth and maturation of ovarian follicles in such a way to prevent the short cycle.

A few studies have examined the use of hCG (Pratt et al., 1982; Garcia-Winder et al., 1986b) and PMSG (Mulvehill and Sreenan, 1977; Wettemann et al., 1982). Both of these gonadotropins are glyco-proteins and are found naturally in the urine and serum of pregnant mares (PMSG) and pregnant women (hCG). However, when given exogenously, these gonadotropins evoke different responses. Pregnant mare serum gonadotropin has mostly FSH-like properties and stimulates ovarian follicular growth, whereas hCG has mostly LH-like properties that can induce ovulation and stimulate progesterone synthesis. Wettemann et al. (1982) demonstrated that treatment of anestrous beef cows with PMSG at d 45 postpartum resulted in increased concentrations of estradiol, probably resulting from ovarian follicular growth without subsequent ovulations. In contrast, a short-term treatment

with progestin preceding PMSG, resulted in increased calving rates in anestrus beef cows (Mulvihill and Sreenan, 1977).

Studies utilizing hCG to induce ovulation in postpartum cattle resulted in responses similar to those after GnRH treatment. Anestrus beef cows were injected with hCG at d 30 postpartum to induce ovulation (Pratt et al., 1982; Garcia-Winder et al., 1986b). Cows responding with an hCG-induced ovulation in both studies had resulting abnormal luteal function. All cows in the latter study had short estrous cycles, whereas subnormal concentrations of progesterone were observed in cows of the former study.

#### **Treatments with Progestins**

Since small, transient increases in serum progesterone are often observed before postpartum estrous cycles of normal duration, exogenous short-term treatments with progestin have been given in attempt to shorten the interval to first ovulation and increase the frequency of normal luteal function. The most successful method of inducing normal luteal function in anestrus beef cows was demonstrated by Ramirez-Godinez et al. (1981). Cows (ranging from d 27 to 67 postpartum) were assigned to three groups: 1) calves were weaned from cows with no further treatment (controls); 2) cows were implanted with 6 mg norgestomet for 9 d prior to weaning; or 3) cows were implanted at weaning with 6 mg of norgestomet for 9 d. All cows exhibited estrus within 25 d of treatment. Treatment with norgestomet in the absence of suckling (Group 3) completely prevented the short estrous cycle and was effective in initiating a normal luteal phase. They suggested

that the presence of norgestomet enhanced the ability of the pituitary to accumulate LH in the absence of suckling, which allowed for pituitary synthesis and release of LH. A recent study also has shown similar luteal responses in weaned anestrus beef cows treated with norgestomet (Garverick et al., 1988). Mean concentrations and frequency, amplitude and duration of pulses of LH and FSH were similar between cows having short estrous cycles (controls) or cows having normal-length estrous cycles (norgestomet implant). Thus, subnormal luteal function does not appear to be related to inadequate gonadotropin stimulation.

Most studies involving treatments with progesterone in dairy cattle have focused on synchronization techniques or inducing cyclicity after d 35 postpartum (Roche et al., 1981; Ball and Lamming, 1983). Administering progesterone by intravaginal devices at various times in seasonally anestrus ewes prior to ram exposure increased the incidence of corpora lutea with normal lifespan (Pearce et al., 1987).

#### **Effects of Progestin Priming and Gonadotropins**

Studies designed to utilize the combination of progestins and gonadotropins could be useful to determine the mechanisms regulating luteal lifespan. However, limited research is available investigating combinations of progestins and gonadotropins in postpartum cows. Previous work has shown that pretreatment of postpartum anestrus beef cows with norgestomet increased the proportion of cows that form corpora lutea of normal lifespan in response to injections with hCG (Pratt et al., 1982; Sheffel et al., 1982). Recently Garcia-Winder et al. (1986b) compared endocrine profiles and luteal function in suckled beef cows implanted with 3 mg

norgestomet for 9 d with no implant prior to inducing ovulation with hCG on d 35 postpartum. All norgestomet-treated cows had normal estrous cycles after induced ovulation, whereas all cows given only hCG had short cycles. Although concentrations of FSH were similar between the two groups, concentrations of FSH tended to be higher in norgestomet-treated cows. Likewise, higher concentrations and more frequent pulses of LH were detected during the norgestomet treatment period. These authors suggested that treatment with norgestomet may play a role in the selection and(or) development of ovarian follicle destined to ovulate via enhanced secretion of LH in an environment of progestin. Furthermore, dairy cows given a single injection of progesterone (100 mg) on d 12 postpartum (Williams et al., 1982) and beef cows given daily injections of progesterone (100 mg) on d 25 through 28 postpartum (Rutter et al., 1985) had normal estrous cycles after a GnRH-induced ovulation. In both studies, 200  $\mu$ g of GnRH was given 2 d after the last progesterone injection. Therefore, a short-lasting treatment with progesterone influenced luteal function. Williams et al. (1982) suggested that progesterone priming occurs via an ovarian mechanism that may be independent of pituitary gonadotropin secretion. Furthermore, it is still unknown whether the effect of progestin (via the norgestomet implant or injections of progesterone) is due to altered preovulatory gonadotropin secretion or a direct effect on the preovulatory follicle.

#### Summary

More research is needed to understand the mechanisms associated with luteal

lifespan and function. It is known that transient increases in progesterone occur prior to the first postpartum ovulation and pretreatment with progestin prior to an induced ovulation results in normal estrous cycles. Likewise, treatments designed to mimic the increased frequency of pulses of LH observed before ovulation also have resulted in normal estrous cycles. Therefore, this thesis focuses on the effect of progestin and(or) pulses of GnRH (enhanced LH secretion) on the lifespan and characteristics of the first CL formed after a GnRH-induced ovulation and characterize the secretory patterns of postpartum hormones associated with the onset of luteal function. These results should provide further insight into the mechanisms associated with the onset and function of the first CL.

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EARLY POSTPARTUM LUTEAL FUNCTION AFTER PROGESTIN  
AND(OR)GONADOTROPIN-RELEASING HORMONE IN DAIRY CATTLE

ABSTRACT

A 2x2 factorial experiment was designed to test the effect of progestin and(or) pulses of GnRH on the lifespan of the first corpus luteum induced by GnRH. Four treatment groups (eight/group) were: 1) blank implant and saline infusion (B+S); 2) norgestomet (6 mg) implant and saline infusion (N+S); 3) blank implant and GnRH infusion (B+G); and 4) norgestomet and GnRH infusion (N+G). Four primiparous and four multiparous cows were assigned to each treatment. Cows were implanted s.c. for 6 d beginning 2 to 5 d postpartum. At implant removal, cows were fitted with two indwelling jugular catheters and were infused 24 h later with saline or 2  $\mu$ g GnRH i.v. over 10 min every 2 h for 48 h via an automatic infusion pump. Blood was collected at 15-min intervals during the first and last 6 h of the infusion period. Following the infusion period, cows were challenged i.v. with 50  $\mu$ g GnRH and blood was collected for an additional 6 h. Blood was collected daily from all cows until 30 d and then thrice weekly until d 60 postpartum. Mean concentrations of LH tended to be higher ( $P<.10$ ) in cows infused with GnRH during the first 6 h. During the last 6 h, B+G cows had fewer ( $P<.05$ ) LH peaks than the B+S group, but overall LH tended to be higher ( $P<.10$ ) in N+G cows. Mean concentrations of FSH were higher ( $P<.05$ ) in the N+G group than in other treatment groups during the 48-h infusion period. After

the 50  $\mu$ g-GnRH challenge, LH release was similar between B+S and N+S cows, but greater ( $P < .05$ ) than that of B+G and N+G cows, whereas mean FSH tended to be higher ( $P < .10$ ) in N+G cows than remaining cows. Interval to ovulation and duration of the first luteal phase and first cycle were unaffected by treatments. Normal cycle duration (18 to 24 d) was observed in more ( $P = .07$ ) N+S cows (7/8) than in B+S (4/8), B+G (4/8), or N+G (3/8) cows and in all multiparous cows ( $n = 4$ ) receiving B+G. These data demonstrated that incidence of short estrous cycles was prevented in early postpartum dairy cows pretreated with norgestomet and in multiparous cows infused with GnRH prior to a GnRH-induced ovulation. Our results suggest that norgestomet and GnRH prevented the short-lived corpus luteum by altering gonadotropin secretion and probably follicular development before ovulation.

### Introduction

The early postpartum period in cattle is characterized by ovarian inactivity. On the average, dairy (milked) cows begin to cycle within 14 to 28 d after parturition (Marion and Gier, 1968; Stevenson and Britt, 1979), whereas beef (suckled) cows have longer intervals that range from 36 to 71 d (Wettemann, 1980). The first corpus luteum formed in postpartum milked and suckled cows is frequently short-lived, resulting in estrous cycles of shorter than normal duration (Odde et al., 1980; Lauderdale, 1986). Incidence of estrous cycles less than 17 d was reported in milked (Macmillian and Watson, 1971; Stevenson and Britt, 1979) and suckled cows (Odde et al., 1980; Ramirez-Godinez et al., 1981; Garcia-Winder et al., 1986).

The mechanisms regulating lifespan and function of the corpus luteum are not completely understood. However, several possibilities have been proposed to account for the short luteal phase suggesting that endocrine changes during follicular growth and development might impact the subsequent function of the first corpus luteum (Smith, 1986; Garverick and Smith, 1986).

Mollett et al. (1983) observed that treating milked cows (d 8 postpartum) with repeated injections of GnRH (2  $\mu$ g every 2 h) for 72 h prior to a 50- $\mu$ g challenge with GnRH resulted in a first cycle of normal duration (19.1 d), whereas a GnRH challenge preceded by pulses of saline resulted in short first estrous cycles (10.3 d). This study suggested that pulses of GnRH apparently altered follicular development and subsequent luteal function through GnRH-induced secretion of gonadotropins. Furthermore, implanting suckled cows with a progestin (norgestomet; 6 mg) for 9 d beginning at weaning resulted in estrous cycles of normal duration (Ramirez-Godinez et al., 1981). Likewise, implanting suckled cows with norgestomet (3 mg) for 9 d prior to a hCG-induced ovulation (d 35 postpartum) increased the duration of the first luteal phase (Garcia-Winder et al., 1986). These studies suggested that a short-term environment of progestin produced by the norgestomet implant also may have altered follicular development and subsequent luteal function. Therefore, pulses of GnRH or pretreatment with progestin prior to a gonadotropin challenge ensured an estrous cycle of normal duration.

The present study was conducted to determine the effect of progestin and(or)

pulses of GnRH on: 1) the lifespan and characteristics of the first corpus luteum (CL) formed after a GnRH-induced ovulation; and 2) to characterize the secretory patterns of FSH, LH, estradiol, progesterone, cortisol, and a serum stable metabolite of prostaglandin  $F_{2\alpha}$  (13,14-dihydro-15-keto prostaglandin  $F_{2\alpha}$ ; PGFM) associated with the onset of luteal function.

### Materials and Methods

**Experimental Design.** Thirty-two lactating Holstein cows of mixed parity were assigned to four treatments at parturition making up a 2x2 factorial experiment (Table 1). There were two main effects; namely, norgestomet and GnRH. Cows (n=16) were implanted in the ear with 6 mg norgestomet for 6 d, whereas their respective controls (n=16) received blank implants containing no norgestomet. One-half of the cows in each of the previous groups were given either 2  $\mu$ g infusions of GnRH every 2 h for 48 h beginning 24 h after implant removal or infusions of saline of similar volume for the same duration. Cows were distributed evenly across treatments with four primiparous and four multiparous cows represented in each group. Treatments were abbreviated as: 1) blank implant and saline infusion (B+S); 2) norgestomet implant and saline infusion (N+S); 3) blank implant and GnRH infusion (B+G); and 4) norgestomet implant and GnRH infusion (N+G). Treatments were designed to expose cows to a transient increase in progestin that is often observed before the first normal estrous cycle, in addition to the increased frequency of pulses of LH observed before first postpartum ovulations in cattle.

**Animal Handling.** Cows were housed on concrete in a free-stall confinement

facility exposed to the environment. A total mixed diet consisting of corn silage, chopped alfalfa hay and grain was fed ad libitum. Additional concentrate (50% corn, 50% sorghum grain) was fed according to level of milk production via an electronic self-feeder. Cows were milked twice daily at 0930 and 2130.

Approximately 2 d postpartum (range d 2 to 5), all cows were implanted with either a norgestomet or blank implant for 6 d. At the time of implant removal, all cows were fitted with two indwelling jugular catheters (one catheter was used for blood collection, whereas the other was connected to an infusion pump). Cows were relocated to an enclosed barn and confined to individual tie stalls and allowed to acclimate before infusions of either saline or GnRH were initiated 24 h after explant. Infusions were given for 48 h via an automatic infusion pump (Auto Syringe, Travenol Laboratory Inc., Hooksett, New Hampshire) that was programmed to deliver 1 ml saline or 1 ml saline containing 2  $\mu$ g GnRH during a 10-min period every 2 h. Cows remained in the tie stalls for approximately 72 h, during which time, they were fed and milked as described above. Within 30 min of the last infused dose of either saline or GnRH, all cows were challenged i.v. with 50  $\mu$ g GnRH to induce ovulation. All cows were returned to free-stall lots 6 h after the GnRH challenge. A scheme depicting the experimental protocol is illustrated in Figure 1.

**Blood Collection.** Blood was collected daily (at 0800 h) via coccygeal venipuncture during the first 30 d postpartum and thrice weekly (Monday, Wednesday, Friday) from d 30 to 60 postpartum. Blood also was collected via

jugular catheters at 15-min intervals during the first and last 6 h of the 48-h infusion period. Blood was collected for an additional 6 h after the 50- $\mu$ g challenge of GnRH at 15-min intervals during the first 2 h and then every 30 min thereafter. In addition, blood was collected at 12 h intervals from the initiation of the infusion period until 24 h after the GnRH challenge. Blood was chilled on ice after collection and held at 4 C for 24 h until serum was obtained by centrifugation. Serum was frozen at -20 C until assayed. All samples collected daily through d 30 after implant and thrice weekly until d 60 were assayed for progesterone. Serum from these same daily samples (through d 20 after implant) also were assayed for PGFM, cortisol, and LH. Samples of serum collected during the frequent sampling periods were assayed for LH and FSH. Samples of serum collected at 12-h intervals were assayed for estradiol-17 $\beta$ .

**Radioimmunoassays.** Concentrations of progesterone, cortisol, estradiol (Skaggs et al., 1986), and PGFM (Stewart and Stevenson, 1987) were quantified according to procedures previously validated in our laboratory. Intra- and inter-assay coefficients of variation of eight assays for progesterone averaged 7.7% and 13.6%, respectively. Cortisol was quantified in five assays with average intra- and inter-assay coefficients of variation of 11.6% and 19.5%, respectively. Concentrations of estradiol were quantified in three assays with average intra- and inter-assay coefficients of variation of 4.3% and 12.5%, respectively. Concentrations of PGFM were quantified in three assays with average intra- and inter-assay coefficients of variation of 6.1% and 18.5%, respectively.

Concentrations of LH in serum were determined by a double-antibody RIA according to Skaggs et al. (1986) with some modifications. Purified bovine LH (USDA-bLH-I-1) was used as the radioligand. Crossreactivity of the antiserum (Chemicon International, Inc., El Segundo, CA; Lot #0986) was less than 0.5% for each USDA-FSH-BP3, USDA-bGH-I-1, and NIADDK-bTSH-I-1 and less than .001% for USDA-bPRL-I-1. Standard reference preparation was bovine LH (USDA-bLH-I-1). Serial dilutions (10, 20, 50, 100 and 200 ml) of bovine serum displaced  $^{125}\text{I}$ -labeled bovine LH from the antiserum to produce a binding curve parallel to the standard curve. Sensitivity of the assay was .04 ng/assay tube. All samples were quantified in five assays and average intra- and inter-assay coefficients of variation were 2.7% and 3.9%, respectively.

Concentrations of FSH in bovine serum were determined by a double-antibody RIA similar to that described by Newton et al. (1987) for porcine FSH with modifications. Purified bovine FSH (USDA-FSH-BP3) was used as the radioligand. Albumin from chicken eggs was added to a pool of filtered bovine serum to give a 5% solution (EA-FBS). Standard curves were prepared in EA-FBS ranging from .1 to 12.8 ng USDA-FSH-BP3/200 ul EA-FBS. Cross-reactivity of the antiserum (Rabbit anti-bovine FSH; Chemicon International, Inc., El Segundo, CA; Lot #09288) was less than .01% for each USDA-bLH-I-1, USDA-bGH-I-1, NIASDDK-bTsh-I-1, and USDA-bPRL-I-1. Increasing volumes of bovine serum (100, 200 and 300 ml) displaced  $^{125}\text{I}$ -labelled bovine FSH from the antiserum to produce a binding curve that was parallel to the standard curve. When 1.07,

2.13, 4.27, 8.53 and 17.07 ng USDA-FSH-BP3/ml were added to EA-FBS, 1.20, 1.74, 4.84, 7.20 and 20.84 ng, respectively, were recovered (average recovery 104%). Sensitivity of the assay was .29 ng/assay tube when incubated for 4 d prior to the addition of  $^{125}\text{I}$ -bFSH. All samples were quantified in 4 assays and intra- and inter-assay coefficients of variation were 2.3% and 3.7%, respectively.

**Definitions.** Concentrations of progesterone in serum (ordinate) for each cow were plotted against days after implant (abscissa) to determine frequency of ovulation and characteristics of estrous cycles. Criteria described by Stevenson and Call (1983) were utilized to estimate days to ovulation. Ovulation after GnRH challenge was indicated when serum progesterone increased ( $> 1$  ng/ml) within 3 to 5 d after the GnRH challenge. If concentrations increased after 5 d, cows were assumed to have ovulated spontaneously. An increase in serum LH was designated as a pulse according to our previous definitions (Skaggs et al., 1986). Pulse magnitude was defined as the maximum level of LH associated with an LH pulse, and pulse amplitude was the concentration of LH resulting from the difference between basal concentrations and pulse magnitude. Overall average concentrations of LH in serum included all values, whereas average baseline concentrations excluded all values associated with a pulse of LH.

Short ovarian cycles were defined according to the following criteria: 1) serum progesterone greater than 1 ng/ml for at least 3 consecutive d after an induced, or spontaneous ovulation or 2) ovarian cycles less than 17 d in duration.

**Statistical Analyses.** Data were subjected to least-squares ANOVA using the

GLM procedure of the Statistical Analysis System (SAS, 1982). A split-plot ANOVA for repeated measurements was used to test the significance of treatments. Analysis of variance for all hormonal data included treatment (n=4), parity (primiparous vs multiparous cows), and their interaction. Treatment and parity were tested using the cow within treatment x lactation variance (Gill and Hafs, 1971). Other characteristics of LH and progesterone were analyzed with a similar model without repeated measurements. Other comparisons among treatment means were made by Bonferroni t-tests and Scheffe's interval (SAS, 1982). Percentage data were tested by Chi-square.

## Results

### Concentrations of Gonadotropin Before GnRH Challenge

Luteinizing Hormone. Average concentrations of LH in serum during the first and last 6 h of the 48-h infusion period are illustrated in Figure 2. An episodic pattern of LH release was detected after each delivered dose of GnRH in cows infused with GnRH. Overall mean concentrations of LH tended to be higher ( $P < .10$ ) in all cows receiving GnRH during the first 6 h of the infusion period. Treatment had no effect on mean pulse frequency of LH (pulses/6 h) or duration of pulses. However, mean magnitude and amplitude of pulses of LH tended to be higher ( $P < .10$ ) in cows treated with blank implants and infusion with GnRH (B+G).

During the last 6 h of the infusion period, overall mean concentrations of LH tended to be higher ( $P < .10$ ) in N+G treated cows. Mean magnitude and duration

of LH pulses were similar between treatments. However, fewer ( $P < .05$ ) pulses of LH were detected in B+G treated cows during the last 6 h of the infusion period.

**Follicle-stimulating Hormone.** During the first and last 6 h of the infusion period, overall mean concentrations of FSH were higher ( $P < .05$ ) in N+G treated cows (Figure 3). There was a significant parity effect in which multiparous cows had higher ( $P < .01$ ) concentrations of FSH in serum than primiparous cows. A pulsatile pattern of FSH release was not observed.

#### Concentrations of Gonadotropin After GnRH Challenge

**Luteinizing Hormone.** Release of LH was detected in all cows after the GnRH challenge. Overall mean concentrations of LH in serum were lower ( $P < .05$ ) in B+G treated cows than in the other groups (Figure 4). Cows treated with norgestomet and infusion of saline (B+S) had the greatest ( $P < .05$ ) release of LH after the GnRH challenge when measured by the magnitude of LH release. However, cows treated with N+S and B+S had more ( $P < .05$ ) release of LH than B+G and N+G treated cows.

**Follicle-stimulating Hormone.** Overall concentrations of FSH in serum after the GnRH challenge tended to be higher ( $P < .10$ ) in N+G treated cows than in other treated cows (Figure 5). Average concentrations of FSH were higher ( $P < .01$ ) in multiparous cows than primiparous cows.

#### Concentrations of Estradiol-17 $\beta$ .

Concentrations of estradiol in serum (Figure 6) were not affected by treatment. However, it is obvious that overall concentrations of estradiol were

affected similarly by the gonadotropin secretion following the GnRH challenge. Primiparous cows tended to have higher ( $P < .10$ ) concentrations of estradiol in their serum than multiparous cows.

#### Daily Concentrations of Hormones After Implant

Cortisol and LH. Average concentrations of cortisol and LH in serum from blood samples collected daily during the first 20 d after implant are summarized in Table 2. Treatment had no effect on concentrations of cortisol or LH. However, there was a tendency for primiparous cows to have lower ( $P < .10$ ) daily concentrations of LH than multiparous cows.

PGFM and Progesterone. Serum PGFM was measured because it was believed that postpartum uterine changes might be different in those cows subjected to the progestin treatment. However, mean concentrations of PGFM in samples collected during the first 30 d after implant were similar between treatments (Figure 7). Likewise, average concentrations of progesterone were similar during this 30-d period (Figure 8). However, the initial rise in progesterone appeared to be delayed longer in the N+G treated cows than in those of the other treatment groups. Primiparous cows had lower ( $P < .05$ ) mean daily concentrations of progesterone than multiparous cows.

#### Ovarian Function and Estrous Cycles

Although all cows responded to the GnRH challenge with release of LH and FSH, more ( $P < .05$ ) N+G treated cows had delayed ovulations than B+S treated cows (Table 3). In fact, two N+G treated cows failed to have first ovulations until

late in the experiment (d 53 and 56 postpartum, respectively). However, all other cows ( $n=5$ ) that failed to ovulate after the GnRH challenge had spontaneous ovulations 10 to 20 d later.

Intervals to first ovulation, duration of the first luteal phase and of the first cycle also are summarized in Table 3. Treatment had no effect on any of these postpartum characteristics. Average interval to first ovulation ranged from 14 to 22 d across treatments. Duration of the first luteal phase ranged from 13 to 17 d, with primiparous cows ( $12\pm 2$  d) having shorter ( $P<.05$ ) luteal phases than multiparous cows ( $18\pm 2$  d). Likewise, duration of the first ovarian cycle differed ( $P<.05$ ) between primiparous ( $19\pm 2$  d) and multiparous ( $25\pm 2$  d) cows.

Further examination of the duration of the first ovarian cycle is in Table 4. Short cycles ( $<17$  d) ranged from 9 to 15 d, whereas extended cycles ( $>24$  d) ranged from 25 to 33 d. Short cycles were prevented in all 16 cows treated with norgestomet. All but one (7/8) of N+S treated cows had normal cycles resulting in proportionally more ( $P=.07$ ) N+S treated cows having first cycles of normal duration than those of other groups. Furthermore, all multiparous cows ( $n=4$ ) treated with blank implants and infused with GnRH (B+G) had normal cycles, whereas 75% of the primiparous cows given the same treatment had short cycles.

#### Discussion

This study is unique in milked cows because it demonstrated that short-term treatment with norgestomet prevented short ovarian cycles. This is in agreement with previous work in postpartum anestrous suckled cows in which norgestomet

treatment beginning at weaning (Ramirez-Godinez et al., 1981) or prior to an induced ovulation (Pratt et al., 1982; Sheffel et al., 1982; Garcia-Winder et al., 1986) reduced the proportion of cows exhibiting short estrous cycles. It is apparent from these findings that treatment with norgestomet influences the lifespan and function of the first CL formed after ovulation. Garcia-Winder et al. (1986) suggested that pre-treatment with norgestomet enhanced lifespan of induced corpora lutea by influencing secretion of LH and development of follicles. In our study, the magnitude and duration of the GnRH-induced release of LH in N+S treated cows was associated with enhanced function and lifespan of the CL, which agrees with observations of Troxel et al. (1984) in norgestomet-treated suckled cows. Since concentrations of FSH tended to be higher in norgestomet-treated cows, adequate concentrations of FSH may be necessary to prevent estrous cycles of short duration because low concentrations of FSH were associated with short-lived CL (Ramirez-Godinez et al., 1982; Garcia-Winder et al., 1986), whereas higher concentrations are associated with normal luteal function (Garcia-Winder et al., 1986).

Recently, Inskeep et al. (1988) observed that norgestomet-treated cows have increased receptors for LH in both follicular granulosa and thecal cells and higher concentrations of estradiol in follicular fluid. In our study, concentrations of estradiol did not differ between treatments, but appeared higher in norgestomet-treated cows. However, previous studies (Pratt et al., 1982; Sheffel et al., 1982; Garcia-Winder et al., 1986) reported higher concentrations of estradiol in norgestomet-treated cows. Perhaps peripheral concentrations of estradiol do not

accurately depict concentrations of estradiol produced by the ovary since pulses in concentrations of estradiol are observed in the ovarian vein (Peters and Lamming, 1984).

Since the role of FSH during the early postpartum period is believed to be one of synergism with estradiol to induce the formation of receptors for LH in granulosa cells (Webb et al., 1980) and in lieu of the data of Inskeep et al. (1988), the interaction of estradiol and FSH could be one of the factors whereby norgestomet aids in the development of a "normal" preovulatory follicle destined to ovulate and form a "normal" CL with a normal lifespan.

Our study also demonstrated that short cycles were prevented in all multiparous cows receiving blank implants and infusion with GnRH (B+G). This result verified earlier work in which multiparous cows given repeated injections of GnRH before a GnRH-induced ovulation had normal luteal function (Mollet et al., 1983). However, in our study, primiparous cows receiving blank implants and infusion with GnRH failed to have estrous cycles of normal duration. Perhaps the hypothalamo-pituitary axis of the primiparous dairy cow has not recovered fully as has the multiparous cow, thus preventing adequate luteotropic support to the preovulatory follicle and subsequent CL.

In the current study, release of LH in response to pulses of GnRH decreased gradually during the 48-h infusion period in B+G treated cows. Likewise, the release of LH following the 50- $\mu$ g challenge with GnRH was less in those cows that received GnRH than saline infusions. This response was not expected because

Mollett et al. (1983) observed an enhanced release of LH when using similar small priming doses of GnRH prior to a 50- $\mu$ g challenge of GnRH. It was suspected that the method of administering repeated GnRH doses influenced the pituitary response to the subsequent challenge of GnRH. Since the automatic infusion pump used in this experiment delivered a small dose of GnRH over a 10-min interval, the pituitary was exposed to lower concentrations of our GnRH dose over a longer period of time, perhaps allowing more GnRH to bind to pituitary GnRH receptors. This might have accounted for the seemingly larger release of LH after each dose of GnRH early in the infusion period. However, as GnRH receptors of the pituitary became saturated, down regulation may have occurred resulting in a reduced release of LH in response to the GnRH challenge. These decreased responses of LH to the GnRH challenge were less dramatic in N+G treated cows suggesting that pretreatment with norgestomet may modulate the down regulation of GnRH receptors because concentrations of LH tended to be higher in cows treated with N+G during the 48-h infusion period, especially during the last 6 h.

Peters and Lamming (1986) suggested that follicles of the ovary are sensitive to GnRH-induced gonadotropin release because they respond by secreting estradiol. Therefore, the higher concentrations of LH during the first 6 h of the pulsing period in cows infused with GnRH may have influenced ovarian secretion, particularly estradiol. We suggest that increased levels of FSH observed in N+G and multiparous B+G treated cows in addition to the estradiol secreted from the ovary might have increased the number of receptors for LH in the follicle destined to

ovulate. Although the LH response to the GnRH challenge was less in cows infused with GnRH than those infused with saline, it is possible that the LH released was sufficient to provide luteotropic support for the induced CL, thereby preventing a short luteal phase especially in multiparous B+G cows.

However, more delayed ovulations were observed in cows treated with N+G than cows in other treatments. Rutter et al. (1982) suggested that high concentrations of progesterone at the time of a GnRH challenge delayed ovulation. This observation was not detected in N+G cows. In fact, concentrations of progesterone were  $< 1$  ng/ml at the time of GnRH challenge. Strott et al. (1970) and Smith et al. (1985) suggested that adequate FSH to LH ratios are necessary for normal luteal function in primates because low concentrations of FSH during the follicular phase tend to increase the incidence of short menstrual cycles. It is possible that high concentrations of FSH may alter follicular development. Since high concentrations of FSH were detected before and after the GnRH challenge in N+G treated cows, these cows may have experienced extended follicular phases because there were no follicles primed for ovulation. With the exception of two cows, all other cows treated with N+G had delayed ovulations after the GnRH challenge. These data suggested that increased concentrations of FSH associated with the N+G treatment might have induced a longer follicular phase and delayed ovulation.

In summary, short estrous cycles were prevented in all cows treated with norgestomet and in all multiparous cows given blank implants and repeated small

doses of GnRH. It is believed that a common mechanism exists whereby norgestomet and GnRH alter follicular growth in order to prevent the short-lived CL. However, it is still unknown whether the effect of norgestomet or GnRH is due to altered preovulatory secretion of estradiol and gonadotropin or a direct effect on the preovulatory follicle.

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TABLE 1. ASSIGNMENT OF DAIRY COWS TO TREATMENT GROUPS<sup>a</sup>

Treatment group <sup>b</sup>	No. cows <sup>c</sup>
Blank/Saline (B+S)	8
Norgestomet/Saline (N+S)	8
Blank/GnRH (B+G)	8
Norgestomet/GnRH (N+G)	8

<sup>a</sup>Thirty-two cows were assigned to four treatments.

<sup>b</sup>Cows were implanted with either 0 or 6 mg norgestomet for 6 d. Twenty-four h after explant, GnRH (2  $\mu$ g) or saline was infused i.v. every 2 h for 48 h before a 50- $\mu$ g GnRH challenge to induce ovulation.

<sup>c</sup>Four primiparous cows and four multiparous cows were assigned to each treatment group.

TABLE 2. CONCENTRATIONS OF LH AND CORTISOL IN SERUM DURING THE FIRST 20 D AFTER IMPLANT<sup>a</sup>

Item	Treatment <sup>b</sup>					Parity <sup>c</sup>
	B+S	N+S	B+G	N+G	SE	
Cortisol, ng/ml						
Primiparous	7.9	7.2	6.9	7.4	.4	7.3 ± .2
Multiparous	7.8	6.3	6.3	6.1	.4	6.6 ± .2
Treatment	7.9	6.7	6.7	6.8	.3	
LH, ng/ml						
Primiparous	.8	.8	.8	.9	.3	.8 ± .1 <sup>d</sup>
Multiparous	.8	.9	1.0	1.3	.3	1.0 ± .1
Treatment	.8	.9	.9	1.1	.3	

<sup>a</sup>Cows were implanted in the ear at d 2 postpartum (range d 2 to 5).

<sup>b</sup>Cows were implanted with either 0 or 6 mg norgestomet for 6 d. Twenty-four h after explant, GnRH or saline was infused i.v. every 2 h for 48 h before a 50- $\mu$ gGnRH challenge to induce ovulation.

<sup>c</sup>Mean  $\pm$  SE.

<sup>d</sup>Different from multiparous cows ( $P < .10$ ).

TABLE 3. CHARACTERISTICS OF OVARIAN FUNCTION AFTER CHALLENGE WITH GNRH<sup>a</sup>

Characteristics	Treatment <sup>b,c</sup>				Parity <sup>c</sup>
	B+S	N+S	B+G	N+G	
No. cows ovulating 3 to 5 d after GnRH					
GnRH-induced (%)	6 (75) <sup>d</sup>	5 (62)	5 (62)	2 (25)	
Delayed (%)	2 (25)	3 (38)	3 (38)	6 (75) <sup>e</sup>	
Days to first ovulation					
Primiparous	14 ± 3	19 ± 5	22 ± 7	26 ± 7	20 ± 3
Multiparous	14 ± 2	16 ± 4	13 ± 2	18 ± 5	15 ± 3
Treatment	14 ± 3	17 ± 3	17 ± 3	22 ± 4	
Duration of first luteal phase, d					
Primiparous	11 ± 2	15 ± 2	10 ± 7	12 ± 2	12 ± 2 <sup>f</sup>
Multiparous	18 ± 5	18 ± 5	15 ± 2	19 ± 3	18 ± 2
Treatment	15 ± 3	17 ± 3	13 ± 3	16 ± 3	
Duration of first cycle, d					
Primiparous	18 ± 2	21 ± 1	17 ± 5	20 ± 2	19 ± 2 <sup>f</sup>
Multiparous	24 ± 5	26 ± 5	22 ± 1	27 ± 4	25 ± 2
Treatment	21 ± 3	25 ± 3	20 ± 3	24 ± 3	

<sup>a</sup>All cows were challenged with 50 µg GnRH to induce ovulation.

<sup>b</sup>Cows were implanted with either 0 or 6 mg norgestomet for 6 d. Twenty-four h after explant, GnRH (2 µg) or saline was infused i.v. every 2 h for 48 h before a 50-µg GnRH challenge to induce ovulation.

<sup>c</sup>Mean ± SE.

<sup>d</sup>Different from N+G (P<.05).

<sup>e</sup>Two cows failed to ovulate until late in the experiment.

<sup>f</sup>Different from multiparous cows (P<.05).

TABLE 4. DURATION OF FIRST CYCLES<sup>a</sup>

Treatment <sup>b</sup>	Cycle duration, d		
	<17 (%)	18-24 (%)	>24 (%)
B+S	2 (25)	4 (50)	2 (25)
N+S	0 (0)	7 (88) <sup>c</sup>	1 (12)
B+G	3 (38)	4 (50) <sup>d</sup>	1 (12)
N+G	0 (0)	3 (38)	3 (38) <sup>e</sup>

<sup>a</sup>The duration of estrous cycles was classified as short (<17 d), normal (18-24 d) or extended (>24 d).

<sup>b</sup>Cows were implanted with either 0 or 6 mg norgestomet for 6 d. Twenty-four h after explant, GnRH (2  $\mu$ g) or saline was infused i.v. every 2 h for 48 h before a 50- $\mu$ g GnRH challenge to induce ovulation.

<sup>c</sup>Different from other treatment groups (P=.07).

<sup>d</sup>All multiparous cows treated with blank implants and infused with saline had normal cycle duration.

<sup>e</sup>Duration of estrous cycles was not determined for two cows because they failed to ovulate until late in the study.

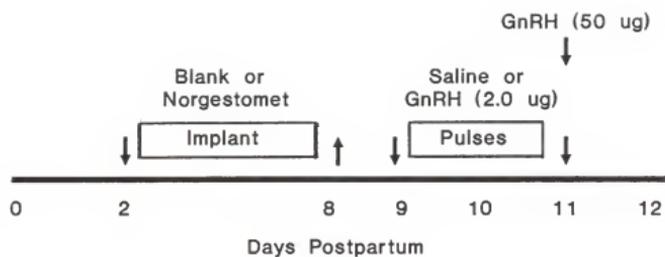


Figure 1. The scheme depicting the experimental protocol.

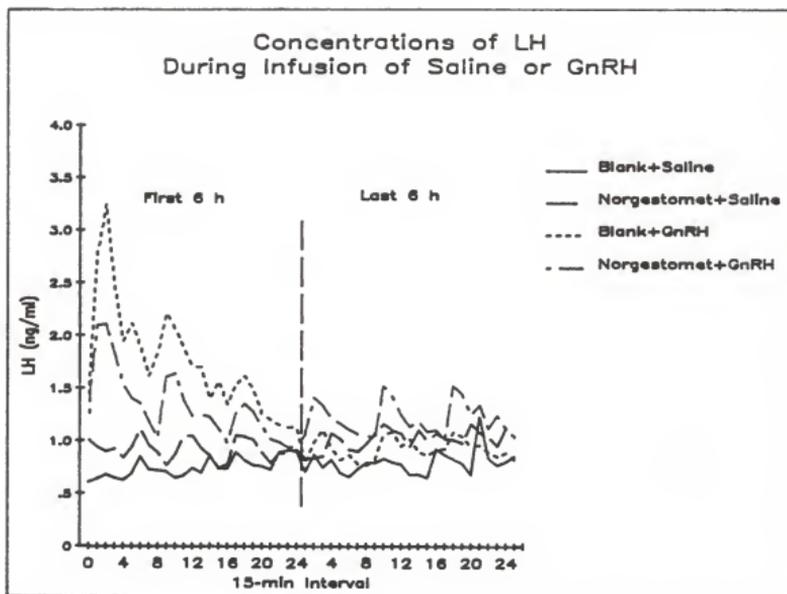


Figure 2. Average concentrations of LH in serum during the first and last 6 h of the 48-h infusion period.

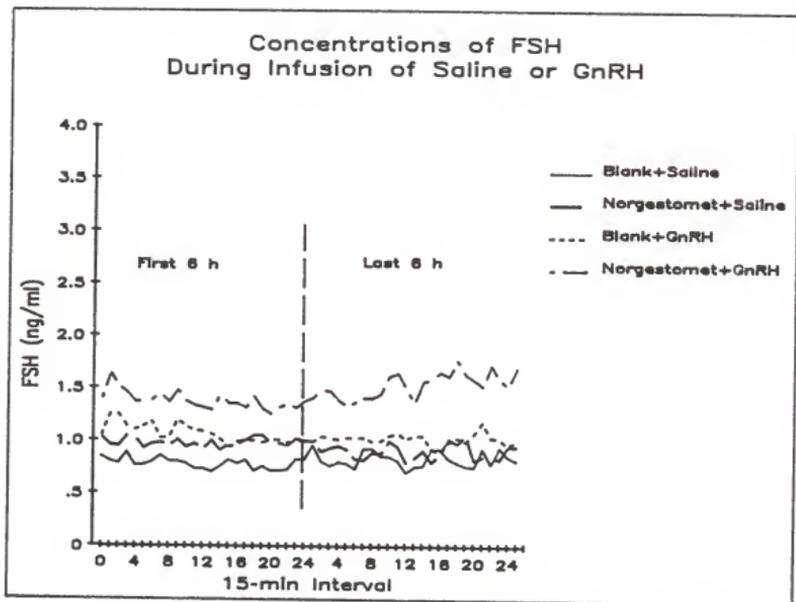


Figure 3. Average concentrations of FSH in serum during the first and last 6 h of the 48-h infusion period.

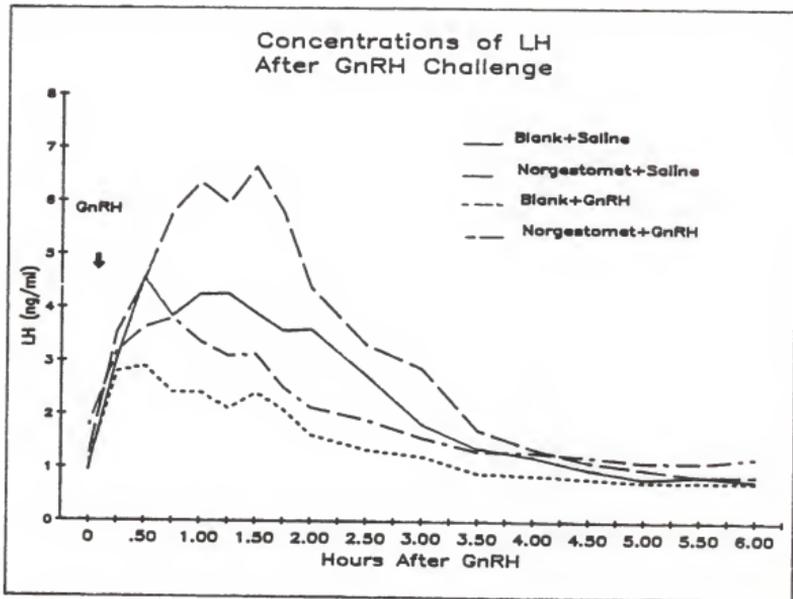


Figure 4. Average concentrations of LH in serum after the challenge with GnRH.

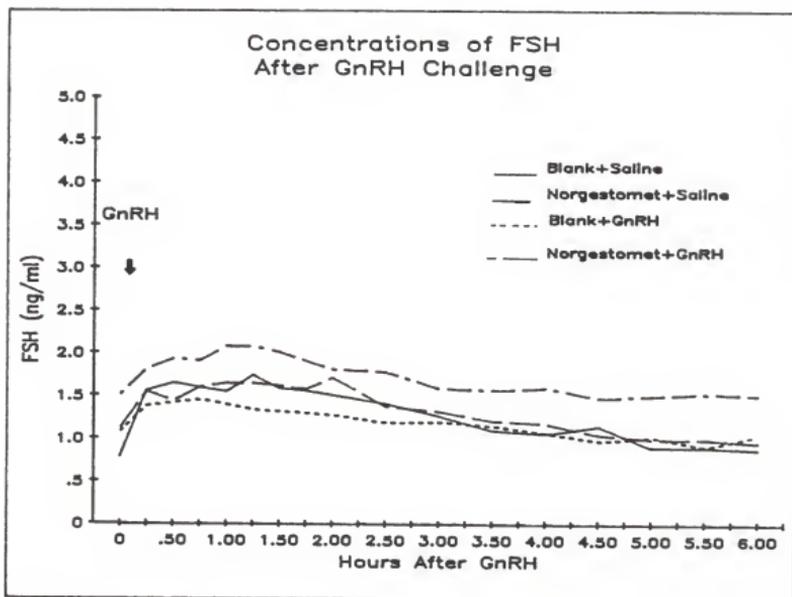


Figure 5. Average concentrations of FSH in serum after the challenge with GnRH.

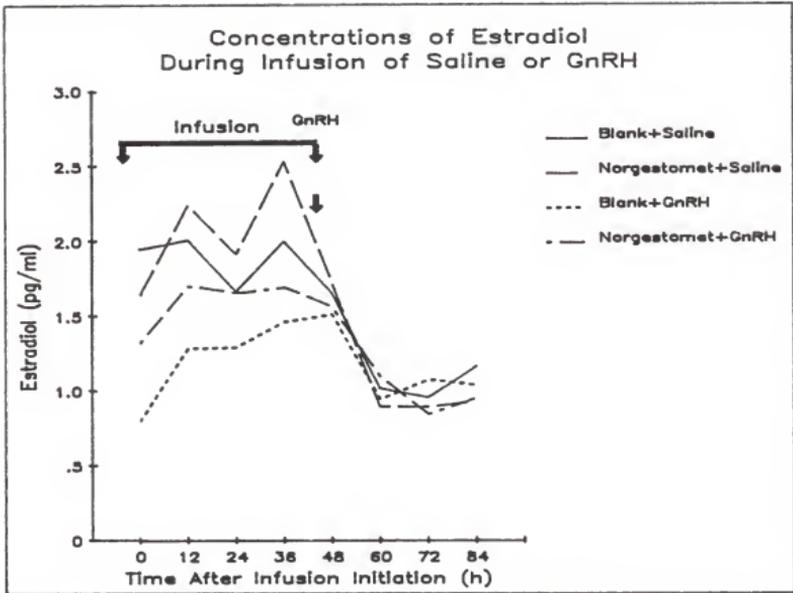


Figure 6. Average concentrations of estradiol in serum after the initiation of the infusion period.

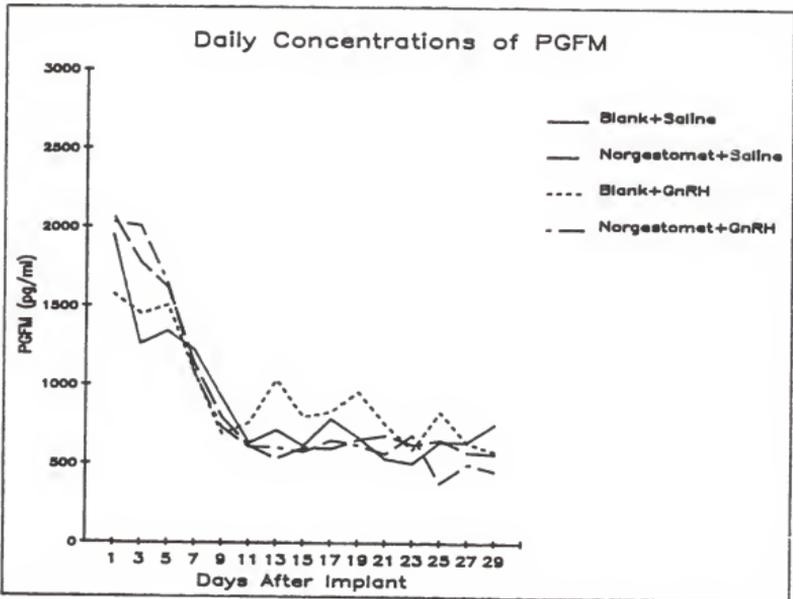


Figure 7. Average daily concentrations of PGFM in serum during the first 30 d after implant.

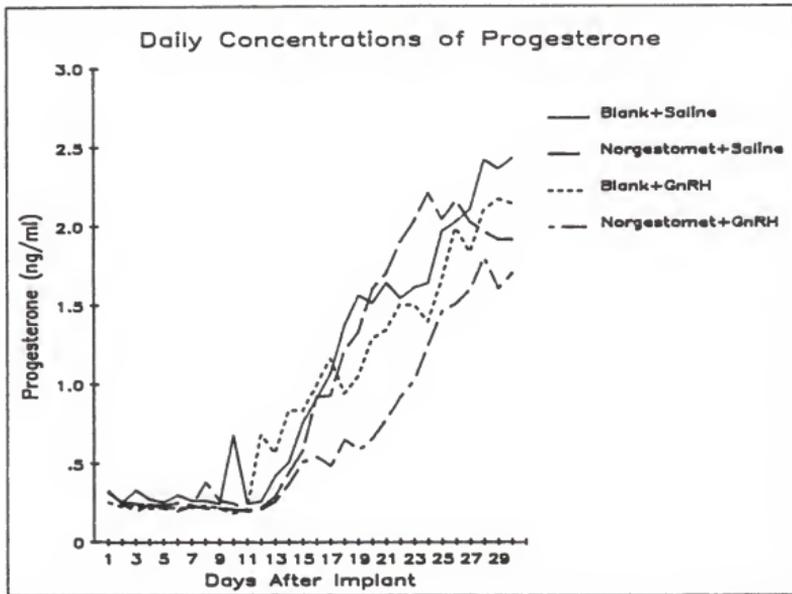


Figure 8. Average daily concentrations of progesterone in serum during the first 30 d after implant.

## Appendix

Characteristics of LH and FSH in Serum During the First 6 h of Pulses of Saline or GnRH

Characteristics	Blank/ Saline	Norgestomet/ Saline	Blank/ GnRH	Norgestomet/ GnRH	SE
Overall FSH, ng/ml	.8 <sup>a</sup>	.9 <sup>a</sup>	1.0 <sup>ab</sup>	1.4 <sup>b</sup>	.2
Overall LH, ng/ml	.8 <sup>c</sup>	.9 <sup>c</sup>	1.3 <sup>cd</sup>	1.7 <sup>d</sup>	.3
Baseline LH, ng/ml	.7 <sup>c</sup>	.8 <sup>c</sup>	1.6 <sup>d</sup>	1.2 <sup>cd</sup>	.2
No. of LH peaks/6 h	1.4	1.5	1.1	1.3	.3
Magnitude of LH, ng/ml	1.2 <sup>c</sup>	1.6 <sup>c</sup>	4.1 <sup>d</sup>	1.9 <sup>c</sup>	.6
Amplitude of LH, ng/ml	.6 <sup>c</sup>	.9 <sup>c</sup>	1.9 <sup>d</sup>	1.1 <sup>c</sup>	.5
Duration of LH pulses, h	1.3	1.3	1.5	1.2	.1

<sup>a,b</sup> Means with superscripts without a common letter differ (P<.05).  
<sup>c,d</sup> Means with superscripts without a common letter differ (P<.10).

Characteristics of LH and FSH in Serum During the Last 6 h of Pulses of Saline or GnRH

Characteristics	Blank/ Saline	Norgestomet/ Saline	Blank/ GnRH	Norgestomet/ GnRH	SE
Overall FSH, ng/ml	.8 <sup>a</sup>	1.0 <sup>ab</sup>	1.0 <sup>ab</sup>	1.5 <sup>b</sup>	.2
Overall LH, ng/ml	.8 <sup>c</sup>	.9 <sup>c</sup>	1.0 <sup>cd</sup>	1.2 <sup>d</sup>	.1
Baseline LH, ng/ml	.7 <sup>c</sup>	.9 <sup>cd</sup>	.9 <sup>cd</sup>	1.1 <sup>d</sup>	.2
No. of LH peaks/6 h	1.6 <sup>a</sup>	1.5 <sup>a</sup>	.3 <sup>b</sup>	.9 <sup>ab</sup>	.4
Magnitude of LH, ng/ml	1.6	2.1	1.6	1.9	.5
Amplitude of LH, ng/ml	1.1	1.3	.8	.8	.5
Duration of LH pulses, h	1.3	1.2	1.2	1.4	.1

<sup>a,b</sup> Means with superscripts without a common letter differ (P<.05).  
<sup>c,d</sup> Means with superscripts without a common letter differ (P<.10).

Characteristics of LH and FSH in Serum After GnRH Challenge<sup>1</sup>

Characteristics	Blank/ Saline	Norgestomet/ Saline	Blank/ GnRH	Norgestomet/ GnRH	SE
Overall FSH, ng/ml	1.3 <sup>c</sup>	1.3 <sup>c</sup>	1.2 <sup>c</sup>	1.7 <sup>d</sup>	.2
Overall LH, ng/ml	2.5 <sup>a</sup>	3.4 <sup>a</sup>	1.6 <sup>b</sup>	2.2 <sup>a</sup>	.2
Baseline LH, ng/ml	1.2	1.4	1.1	1.6	.2
Area, sq cm	18 <sup>a</sup>	24 <sup>a</sup>	8 <sup>b</sup>	10 <sup>b</sup>	.4
Magnitude of LH, ng/ml	5.3 <sup>a</sup>	7.6 <sup>b</sup>	3.8 <sup>a</sup>	4.6 <sup>a</sup>	.9
Amplitude of LH, ng/ml	4.6 <sup>a</sup>	6.4 <sup>b</sup>	2.6 <sup>a</sup>	3.2 <sup>a</sup>	.9
Duration of LH, h	3.5 <sup>cd</sup>	3.6 <sup>c</sup>	2.6 <sup>d</sup>	2.7 <sup>d</sup>	.4

<sup>1</sup>Cows were challenged with 50 µg GnRH within 30 min of the last pulse.

<sup>a,b</sup>Means with superscripts without a common letter differ (P<.05).

<sup>c,d</sup>Means with superscripts without a common letter differ (P<.10).

Concentrations of Progesterone and 13,14-Dihydro-15-Keto-Prostaglandin F<sub>2α</sub> (PGFM) in Serum During the First 30 d Postimplant<sup>1</sup>

Item	Blank/ Saline	Norgestomet/ Saline	Blank/ GnRH	Norgestomet/ GnRH	SE	Parity
Progesterone, ng/ml						
Primiparous	.8	.7	.7	.6	.2	.7 ± .1 <sup>a</sup>
Multiparous	1.3	1.2	1.2	.8	.2	1.1 ± .1
Treatment	1.1	1.0	1.0	.7	.2	
PGFM, ng/ml						
Primiparous	.9	.9	.9	.8	.1	.9 ± .1
Multiparous	.8	.9	.9	.9	.1	.9 ± .1
Treatment	.9	.9	.9	.9	.1	

<sup>1</sup>Cows were implanted with either 0 or 6 mg Norgestomet from 2 to 5 d postpartum.

<sup>a</sup>Different from multiparous cows (P<.05).

Concentrations of Estradiol-17β During Pulses of Saline or GnRH<sup>1</sup>

Item	Blank/ Saline	Norgestomet/ Saline	Blank/ GnRH	Norgestomet/ GnRH	SE	Parity
Estradiol-17β						
Primiparous	2.1	1.9	1.3	1.4	.4	1.7 ± .2 <sup>a</sup>
Multiparous	1.0	1.3	1.0	1.3	.4	1.1 ± .2
Treatment	1.6	1.6	1.2	1.4	.3	

<sup>1</sup>Samples collected at 12-h intervals.

<sup>a</sup>Different from multiparous cows (P<.10).

EARLY POSTPARTUM LUTEAL FUNCTION AFTER PROGESTIN  
AND (OR) GONADOTROPIN-RELEASING HORMONE IN DAIRY CATTLE

by

MICHAEL ORVIS MEE

B.S., UNIVERSITY OF WISCONSIN-PLATTEVILLE, 1986

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the  
requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1988

## ABSTRACT

A 2x2 factorial experiment was designed to test the effect of progestin and(or) pulses of GnRH on the lifespan of the first corpus luteum induced by GnRH. Four treatment groups (eight/group) were: 1) blank implant and saline infusion (B+S); 2) norgestomet (6 mg) implant and saline infusion (N+S); 3) blank implant and GnRH infusion (B+G); and 4) norgestomet and GnRH infusion (N+G). Four primiparous and four multiparous cows were assigned to each treatment. Cows were implanted s.c. for 6 d beginning 2 to 5 d postpartum. At implant removal, cows were fitted with two indwelling jugular catheters and were infused 24 h later with saline or 2  $\mu$ g GnRH i.v. over 10 min every 2 h for 48 h via an automatic infusion pump. Blood was collected at 15-min intervals during the first and last 6 h of the infusion period. Following the infusion period, cows were challenged i.v. with 50  $\mu$ g GnRH and blood was collected for an additional 6 h. Blood was collected daily from all cows until 30 d and then thrice weekly until d 60 postpartum. Mean concentrations of LH tended to be higher ( $P < .10$ ) in cows infused with GnRH during the first 6 h. During the last 6 h, B+G cows had fewer ( $P < .05$ ) LH peaks than the B+S group, but overall LH tended to be higher ( $P < .10$ ) in N+G cows. Mean concentrations of FSH were higher ( $P < .05$ ) in the N+G group than in other treatment groups during the 48-h infusion period. After the 50  $\mu$ g-GnRH

challenge, LH release was similar between B+S and N+S cows, but greater ( $P < .05$ ) than that of B+G and N+G cows, whereas mean FSH tended to be higher ( $P < .10$ ) in N+G cows than remaining cows. Interval to ovulation and duration of the first luteal phase and first cycle were unaffected by treatments. Normal cycle duration (18 to 24 d) was observed in more ( $P = .07$ ) N+S cows (7/8) than in B+S (4/8), B+G (4/8), or N+G (3/8) cows and in all multiparous cows ( $n=4$ ) receiving B+G. These data demonstrated that incidence of short estrous cycles was prevented in early postpartum dairy cows pretreated with norgestomet and in multiparous cows infused with GnRH prior to a GnRH-induced ovulation. Our results suggest that norgestomet and GnRH prevented the short-lived corpus luteum by altering gonadotropin secretion and probably follicular development before ovulation.