ATTEMPTS TO INDUCE PUBERTY IN BEEF HEIFERS WITH LUTEINIZING HORMONE-RELEASING HORMONE

by

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REVIEW OF LITERATURE

Definition of Puberty

"Gonadostat" Theory.

Puberty may be defined as a sequence of events leading to first estrus and ovulation in young females. The "gonadostat" hypothesis proposed by Ramirez and McCann (1963) has been one of the most widely accepted theories regarding endocrine control of puberty. The hypothesis proposes that the prepubertal state is characterized by low levels of gonadotropin release because of hyper-responsiveness of the hypothalamic-pituitary axis to the inhibitory action or negative feedback of estradiol from the ovary. Failure of ovarian follicles to ovulate is related to low or inadequate levels of serum gonadotropins. Hyper-sensitivity to low levels of estradiol decrease during puberty and concentrations of serum gonadotropins increase with initiation of events responsible for first ovulation. The "gonadostat" hypothesis can be applied to humans (Weitzman et al., 1975), lambs (Foster and Ryan, 1979; Ryan and Foster, 1980), pigs (Lutz et al., 1984), rats (Ojeda et al., 1980), and heifers (Day et al., 1982).

Test of "Gonadostat" Theory in Heifers.

Day et al. (1982) and Moseley et al. (1984) utilized prepubertal heifers to test the gonadostat hypothesis. Day et al. (1982) used 266-d old heifers and Moseley et al. (1984) used 60 and 200-d old heifers assigned to be intact controls (C), ovariectomized (OVX), and ovariectomized plus estradiol implants (OVX-E). Ovariectomy resulted in increased luteinizing hormone (LH) pulse frequency to one peak per hour by d 36 (Day et al., 1982) and by d 49 (Moseley et al., 1984) illustrating the inhibitory effect of ovarian secretions on LH release before puberty.

Time is required for the negative feedback control of LH secretion to become functional. Rats (Yamamoto et al., 1970), monkeys (Dierschke et al., 1974b), ponies (Wesson and Ginther, 1979), and lambs (Foster et al., 1972; Foster et al., 1975) exhibited a delay between removal of the gonads and increased gonadotropin secretion. Treatment of OVX heifers with estradiol suppressed LH secretion (Day et al., 1982; Moseley et al., 1984). In addition, LH levels remained undetectable in OVX heifers until LH completely escaped from the inhibitory feedback of estradiol implants on d 139. The timing of this escape was correlated to commencement of estrous cycles for control heifers. A second study (Day et al., 1982) attempted to suppress LH release with a second estradiol implant in OVX-E heifers that had escaped estradiol inhibition and the second implant failed to depress LH release. These two studies concurred that a marked decrease in estradiol feedback was present at puberty (Day et al., 1982).

Pulsatile release of LH resumed more rapidly after ovariectomy of pubertal heifers than of prepubertal heifers (Kiser et al., 1981). The effectiveness of negative feedback of estradiol to control LH secretion was age dependent (Staigmiller et al., 1979). Schillo et al. (1982) found that a single injection of estradiol-17β sufficient to suppress LH secretion declined in effectiveness as age increased from 4 to 12 mo. A 50% increase in LH concentration in untreated ovx lambs occurred simultaneously with escape of LH release from estradiol inhibition in ovx-estradiol treated ewes (Ryan and Foster, 1980). The same increase in circulating LH has been established in guinea pigs (Donovan and Kilpatrick, 1978) and man (Winter and Faiman, 1972). Consequently, increased levels of circulating LH at puberty were due to a combination of increased serum LH concentration and a reduction in negative feedback control of estradiol on LH release.

Factors Altering Age of Puberty

Genetics or Breed.

Genetic factors affect age at puberty in beef heifers. Breed of sire and dam also influence age of heifer at puberty (Laster et al., 1976). Age of dam affect most growth traits and fewer heifers from 2-yr old dams reached puberty by 390 d (Laster et al., 1976). However, percentage of heifers reaching puberty by 390 d increased as age of dam exceeded 2 yr. Proportion of heifers reaching puberty can be influenced by within breed sire selection (Laster et al., 1976). Wiltbank et al. (1966) suggested significant heterosis effects on age at puberty in addition to increased average daily gain. Heterosis reduced age at puberty by 19.5 d in Hereford-Angus crosses over the average of straightbreds (Laster et al., 1976).

Plane of Nutrition.

Plane of nutrition influences puberty. Underfeeding increased age at puberty and reduced conception rates (Sorensen et al., 1954; Wiltbank et al., 1966; Dufour, 1975). While overfeeding resulted in weak signs of estrous behavior, lowered conception rates, increased embryonic mortality and decreased milk production (Moustgaard, 1969; Arnett et al., 1977). Short and Bellows (1971) demonstrated daily gain in body weight was associated closely with attainment of puberty as the percentages of heifers reaching puberty before onset of breeding season were 5, 24 and 83 for heifers gaining .28, .45, and .68 kg per head per d, respectively. Ferrell (1982) fed heifers to gain .4 (L), .6 (M), and .8 (H) kg per d post-weaning. The L heifers were older and lighter at puberty than H heifers. The M heifers were youngest at puberty and weighed intermediate between mean body weights of L and H heifers. Grass et al. (1982) reported that heifers fed diets low in energy reached puberty later than those fed diets high in energy. Monensin elevated rumen propionate concentrations in

heifers and hastened onset of puberty without increasing average daily gain (McCartor et al., 1979; Moseley et al., 1982). Day et al. (1984) noted dietary energy restriction inhibited attainment of sexual maturity by delaying prepubertal rise in LH secretion and reducing responsiveness of the pituitary to LHRH.

Photoperiod and Temperature.

Environmental conditions affect age at puberty. Exposure to colder temperatures (Ames and Brink, 1977) and shorter photoperiods (Peters et al., 1978) resulted in slower rate of gain compared with warmer temperatures and longer photoperiods. Schillo et al. (1983) concluded that 6-mo old heifers exposed to temperature and photoperiod conditions of spring to fall reached puberty at an earlier age than those exposed to conditions of fall to spring. Differences were attributed mainly to length of photoperiod. Peters and Tucker (1978) showed three of 10 heifers exposed to 16 h light per d attained puberty before 10.5 mo of age compared to zero of 19 reared in natural photoperiods of autumn and winter. Consequently, pubertal age was altered by photoperiod duration of photoperiod regardless of the nonseasonal breeding status of heifers. Hansen et al. (1983) concluded that supplemental lighting after 22 or 24 wk of age accelerated time of first ovulation and estrus in February to July born heifers. Likewise, Petitclerc et al. (1983) suggested that 16 h light and 8 h darkness improved feed efficiency, stimulated weight gain, and hastened puberty of heifers fed moderately restricted or ad libitum diets.

Social Rearing.

Social environment was shown to affect age at puberty. Izard and Vandenbergh (1982) reported that bull urine contained a priming pheromone that accelerated puberty in beef heifers as 67% urine-treated and 32% water-treated heifers reached puberty during experimental period. Likewise,

gilts reared in confinement (Thompson and Savage, 1978) and gilts not reared in confinement (Zimmerman et al., 1969, 1974) exposed to a boar reached puberty earlier than gilts reared without this exposure. Presence of older cows influenced age at puberty among heifers (Nelsen et al., 1984). Hereford and Tarentaise-sired heifers in the presence of mature cows were compared with heifers maintained in heifer-only groups. Heifers maintained with older cows were 15.9 and 26.4 kg lighter at puberty and puberty occurred 26 d and 40 d earlier, respectively. However, similar effects failed to occur in Charolais-sired heifers.

Endocrine Events Associated With Puberty

Estradiol.

Onset of puberty is dependent on numerous interrelated maturational events. Many of the processes responsible for attainment of puberty were functional at an early age. The hypothalamic-hypophyseal mechanism responsible for estradiol-stimulated LH release was functional between 3 and 5 mo of age in calves that do not ovulate until 12 to 16 mo of age (Staigmiller et al., 1979). Rapid pubertal growth which started at 7 mo of age was finished by 10 mo of age in heifers (Desjardins and Hafs, 1969). Lambs responded to the positive feedback of estradiol within a few weeks after birth (Foster and Ryan, 1979). Furthermore, the prepubertal heifer released large quantities of LH and follicle stimulating hormone (FSH) in response to iv injections of 200 µg luteinizing releasing hormone (LHRH) and the response was similar at 3, 6, or 9 mo of age (Barnes et al., 1980). The preovulatory LH surge in cattle (Gonzalez-Padilla et al., 1975b), sheep (Foster and Karsch, 1975) and rats (Caligaris et al., 1972) was dependent on the ovary to produce the estradiol responsible for triggering LH release rather than the inability of hypothalamic-hypophyseal system to respond to such a stimulus. In contrast, humans (Grumbach et al., 1974),

monkeys (Dierschke et al., 1974b), and pigs (Foxcroft et al., 1975) failed to respond to estradiol stimulation at younger ages.

Gonadotropins.

Since the hypothalamic-hypophyseal system was functional in immature cattle and sheep, endocrine changes were responsible for initiation of puberty. Gonzalez-Padilla et al. (1975a) reported that FSH, prolactin, and LHRH levels associated with puberty and first estrous cycles were relatively stable. Desjardins and Hafs (1968) recognized that FSH concentration was highest at 1 mo, declined at 2 mo, and remained relatively constant from 2 to 12 mo of age. They concluded that no change was associated with serum FSH during the onset of puberty. Gonzalez-Padilla et al. (1975a) noted that LH fluctuated markedly during the prepubertal period and was of higher concentration than levels associated with cycling heifers and cows. Higher LH levels in prepubertal animals were consistent with results for cattle (Odell et al., 1970; Swanson et al., 1972), rats (Kragt and Masken, 1972), sheep (Leifer et al., 1972) and swine (Chakraborty et al., 1973). Data for cattle (Gonzalez-Padilla et al., 1975a), swine (Lutz et al., 1984), and sheep (Ryan and Foster, 1980) showed that LH secretion immediately preceding puberty was characterized by increased frequency of low amplitude LH pulses. Gonzalez-Padilla et al. (1975a) observed two peaks of LH associated with puberty which included a priming LH peak 9 to 11 d preceding the second or pubertal peak. Low levels of progesterone were associated with the prepubertal period and two elevations were closely linked with LH peaks. The first elevation of progesterone preceded the priming LH peak while the second preceded the pubertal LH peak. The preovulatory rise in serum progesterone concentrations have been found to originate in the ovary (Berardinelli et al., 1979; 1980). Estradiol was not elevated in association with LH peaks. Although circulating hormone levels were sufficient to induce estrous

cycles, puberty was delayed due to lack of cyclic LH release (Gonzalez-Padilla et al., 1975a).

Several similarities existed for endocrine control of final stages of ovarian follicular development in both the cow and ewe. Maturation of preovulatory follicles in both species was controlled by episodic patterns of LH secretion (Yuthasastrakosol et al., 1977; Baird, 1978; Ryan and Foster, 1980). Low frequency LH pulses resulted in ovarian inactivity in the form of seasonal anestrous in ewes and postpartum anestrous in cows (Lamming et al., 1981; McLeod et al., 1982). Onset of puberty in heifers and ewes relied on increased frequency of LH pulses (Gonzalez-Padilla et al., 1975a; Ryan and Foster, 1980).

The decapeptide LHRH of hypothalamic origin was a potent stimulator of LH and FSH release from the pituitary in many species. Exogenous LHRH has been used extensively to increase LH pulse frequency artificially in a variety of species. In addition, LHRH has been investigated in regard to pattern and frequency of secretion and dosage necessary for induction of ovarian cycling (Knobil, 1980).

Luteinizing Hormone-Releasing Hormone

Pulse Frequency.

Nakai et al. (1978) concluded the hypothalamic-hypophyseal control system responsible for directing gonadotropin secretion was obligatorily intermittent. Knobil (1980) determined that the arcuate nucleus was instrumental in the pulsatile release of LHRH into the hypophyseal portal circulation of rhesus monkeys. Radiofrequency lesions placed on the arcuate nucleus reduced serum LH and FSH and abolished the positive feedback of estradiol in the ovariectomized adult rhesus monkey. Luteinizing hormone and FSH levels were restored with chronic intermittent infusions (iv) of LHRH at rate of 1 μg per min for 6 min every h. Frequency of one pulse per h was important in the

monkey because increasing the number of pulses per h to 2, 3, or 5 resulted in suppressed gonadotropin secretion. Frequency of one pulse per h reestablished suppressed LH and FSH levels. Reducing pulse frequency from one pulse per h to one pulse per 3 h altered the LH to FSH ratio by reducing LH concentration and increasing FSH levels. The shift in LH and FSH ratios was attributed to lower metabolic clearance rates of FSH compared with LH. Also, magnitude of LHRH-induced pulses was larger at lower frequency than with one pulse per h. Rate of reduction from the peak was more rapid for LH than FSH. Follicle stimulating hormone declined 21% from peak FSH levels and LH declined 57% within 1 h illustrating FSH accumulation due to a longer half life. Reducing the concentration of infused LHRH from 1 µg per min for 6 min per h to .1 or .5 µg per min for 6 min every h failed to elicit detectable LH and FSH responses. Increasing the infusion rate to 10 µg per min, a tenfold increase in standard dose, did not alter LH concentrations but suppressed FSH levels.

Pulsatile release of LH in anestrous suckled cows was characterized by frequencies of 0 to .5 pulses per h initially, increasing to .25 to 1.25 pulses per h a few days before first ovulation (Carruthers and Hafs, 1980; Peters et al., 1981; Riley et al., 1981). Intermittent injections of LHRH in postpartum beef cows produced distinct LH release with doses of 1, 2.5, 3, or 5 µg LHRH per pulse. However, LH release failed to occur with lower dosage levels of .25 and .5 µg (Riley et al., 1981; Edwards et al., 1983). Pund and Amoss (1982) discovered iv injections of 2.5 µg LHRH per pulse produced a 2 to 4 ng/ml increases in LH that mimicked natural LH pulses in prepubertal heifers. McLeod et al. (1984) reported iv injections of 2 and 5 µg LHRH consistently induced LH release in prepubertal heifers, but .5 µg LHRH produced a response to some of the challenges.

Pulsed LHRH in Postpartum Anestrous Cows and Seasonally Anestrous Ewes.

Increasing low frequency of LH pulses in postpartum anestrous beef cows by intermittent low doses of exogenous LHRH successfully initiated follicular growth and ovarian cyclicity. Riley et al. (1981) injected LHRH (5 µg iv) every 2 h for 48 h in five postpartum anestrous beef cows (20 to 40 days postpartum). Pulsatile LH release occurred in response to LHRH injections and four of five treated cows subsequently ovulated and completed one estrous cycle earlier than control cows. Walters et al. (1982) injected 500 ng LHRH (iv) every 2 h for 4 d to postpartum cows. The LHRH-induced LH pulses shortened postpartum interval over controls. However, Edwards et al. (1983) treated 97 anestrous beef cows (30 days postpartum) with doses of .25, .5, 1, 2.5, 3 or 5 µg LHRH per pulse for a period of 2 to 4 d at 1-h or 2-h intervals. Three experiments demonstrated a failure of the treatments to increase ovulations over untreated control cows. Nevertheless, LHRH injections stimulated follicular development and ovulation in some beef cows. Luteinizing hormone was released synchronously after large LHRH doses while .25 or .5 µg doses induced limited LH release. A correlation existed between the interval from start of LHRH treatment to LH peak and number cows ovulating in the second study. Only one of nine cows ovulated when the LH peak occurred within 48 h after initiation of the treatment whereas, six of the nine ovulated when the LH peak occurred more than 48 h after first injection.

Intermittent injections of LHRH have been successful in inducing cycling in seasonally anestrous ewes. McLeod et al. (1982a) treated anestrous ewes with 75, 125, 250, or 500 ng of LHRH at 2 h intervals for 48 h. A preovulatory LH surge was observed after LHRH and 19 of 20 treated ewes ovulated. McLeod et al. (1982b) injected anestrous ewes with 250, 500, or 1000 µg LHRH at 2 h intervals for 8 d. Plasma LH concentrations increased in response to LHRH

treatments with preovulatory LH peaks occurring 17 to 48 h after first treatment in all ewes and a second preovulatory peak 106 to 133 h later in ewes injected with 500 or 1000 ng of LHRH. Furthermore, ovulation and ovarian cyclicity commenced in all ewes. Seasonal anestrous ewes with ovaries autotransplanted to the neck were injected (iv) with 10 µg of LHRH at rates of one injection per 3 h, one per 2 h, or one per h for a 24-h period (McNeilly et al.,1980). An LH release and increased estradiol secretion resulted from each injection. Luteinizing hormone peaked 52 to 57 h after the first injection and all ewes ovulated.

Induction of Puberty.

Puberty was characterized by identical increased LH frequency required to initiate cycling in anestrous cows and ewes. Gonzalez-Padilla et al. (1975b) reported that progesterone followed by estrogen stimulated LH release in prepubertal heifers. Prepubertal heifers were induced to cycle in response to a progestogen implant of 6 d followed by an injection of estrogen, but first service conception rates were low (Short et al., 1976). Gonzalez-Padilla et al. (1975c) successfully induced puberty in prepubertal heifers using 5 mg estradiol valerate (im) and 3 mg norgestomet in conjunction with a 6 mg norgestomet implant. Four trials showed this treatment induced estrus in 94, 93, 79 and 89% of heifers within 4 d. Pregnancy rates associated with induced estrus in the three of the trials were 50, 56, and 43%, respectively. Intermittent doses of LHRH also have been used to mimic patterns of endogenous LH release at puberty, and consequently induced ovarian cyclicity (Knobil, 1980a; Ryan and Foster, 1980; McLeod et al., 1984).

Pulsed LHRH in Prepubertal Monkeys, Ewes and Heifers.

Knobil (1980) worked with adult female arcuate-lesioned rhesus monkey and demonstrated that hourly LHRH infusion (iv) reestablished 28-d menstrual

cycles. Knobil (1980) followed with a similar treatment regimen initiating menstrual cycles in immature monkeys. A study was conducted using six female prepubertal monkeys (11 to 15 mo of age, 20 mo from commencement of ovulatory menstrual cycles). Exogenous LHRH was infused at a rate of 1 μg per min for 6 min per h and continued for 93 to 253 d. Luteinizing hormone and FSH levels increased within days after initiation of LHRH treatment similar to levels during the follicular phase of the adult monkey. Initial estradiol peaks failed to induce preovulatory-like gonadotropin surges. Three of the six monkeys responded with gonadotropin surges to the second estradiol peak, two to third peak and one to the fourth increase in estradiol. Ovulation and menstrual cycles were induced in all six monkeys. Three of the four females having more than one cycle demonstrated 27 to 31-d cycles which were comparable with the duration of normal adult menstrual cycles. All monkeys reverted to prepubertal state of undetectable gonadotropin levels when LHRH infusion ceased. Estradiol administration after ceasing the LHRH infusion failed to elicit LH and FSH surges. These results indicated the competency of the pituitary and ovary to respond to exogenous LHRH in the immature monkey.

Ryan and Foster (1980) increased the LH frequency in prepubertal ewes (17 to 19 wk of age) by intermittent LHRH pulses. Purified ovine LH (15.5 μg/injection) was administered (iv) once every h or once every 3 h for 48 h. Hourly injections initiated a greater frequency of LH pulses and induced preovulatory surges of LH in two of the three lambs. One preovulatory surge occurred at 24 h and the other at 36 h, and both ewes ovulated. A subsequent experiment by Ryan and Foster (1980) confirmed these results as four of six prepubertal lambs ovulated in response to similar treatments. However, ewes injected once every 3 h failed to ovulate because LH dropped to low levels between two LH challenges.

Prepubertal beef heifers (Hereford x Friesian), six (4 mo of age, 111.7±3.5 kg) and six (10 mo old, 208.3±8.8 kg), were used to determine plasma LH and FSH concentrations before and after nine consecutive injections of .5, 2, or 5 μg LHRH at 2 h intervals (McLeod et al., 1984). Pulsatile pattern of LH release was evident in all 12 heifers and a well defined rise in plasma LH occurred in response to the injections of 2 or 5 μg LHRH. One heifer responded with a definite LH pulse to each injection of .5 μg LHRH, while the other three responded sporadically to .5 μg injections. Response to the LHRH challenges were similar between age groups, but a dose-response relationship occurred as the mean area of LH pulses were different between .5 μg and 5 μg dosage levels. Mean FSH concentrations were unchanged in the LHRH treatment period when compared with pretreatment levels. In addition, one heifer was detected in estrus during study and no change in plasma FSH concentrations was associated with estrus. McLeod et al. (1984) speculated that a higher dosage of LHRH was necessary to achieve the threshold level necessary for FSH release.

Continuous LHRH Infusion.

Continuous infusion of exogenous LHRH has not been successful as intermittent presentation of LHRH for inducing LH release. Continuous infusion caused adenohypophysial refractoriness in rats and sheep (Chakraborty et al., 1974; Piper et al., 1975; and Shuilling et al., 1976). Varying infusion rates from 1 ng to 1 μg per min in a continuous mode were ineffective in initiating LH and FSH release in arcuate-lesioned monkeys. Down regulation resulted from prolonged exposure to high hormone concentration in extracellular fluid. A target tissue response followed with reduction of available LH receptors (Hsueh et al., 1976; Conti et al., 1977; Hsueh et al., 1977; Ryan et al., 1977). Amundson and Wheaton (1979) implanted four anestrous ewes sc with Alzet® osmotic minipumps containing 1.7 mg synthetic LHRH in 170 μl saline. Every

ewe was reimplanted with a new minipump weekly for 4 wk. Theoretically, pumps were to deliver 10 μg LHRH per h. Each ewe exhibited a rapid release of LH in response to the first minipump implant and LH reached peak levels within 3 h. A smaller magnitude of LH release was associated with the second minipump and no LH response was elicited by the third and fourth minipump. Lack of LH release in response to continuous LHRH administration was attributed to 95% depletion of LH reserves in anterior pituitary at the end of fourth week. Postovulatory luteal function was observed in three of four luteinizing hormone releasing hormone (LHRH)-treated ewes. They concluded that initial continuous LHRH administration was sufficient to induce ovulation in ewes, but extended administration minimized ovarian follicular growth. Schanbacher (1984) used pulsatile iv presentation of LHRH at a rate of 500 ng per 2 hours for 4 wk to secure discrete LH pulses in estradiol 17β implanted prepubertal bulls. Continuous infusion of the same dosage to the implanted bulls was ineffective in altering low gonadotropin levels present. Likewise, continuous daily (sc) treatments of 200 μg LHRH per day for 12 d were unsuccessful in initiating episodic LH release and ovulation in prepubertal heifers (Mellin et al., 1975).

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ATTEMPTS TO INDUCE PUBERTY IN BEEF HEIFERS WITH LUTEINIZING HORMONE-RELEASING HORMONE

Introduction

Age at puberty is an important reproductive phenomenon in the beef heifer. Heifers are expected to calve first as 2-yr olds and should wean more calves during their lifetime than those calving first as 3-yr olds or older (Pope, 1967). Heifers attaining puberty before or at onset of breeding season have greater opportunity to conceive during the breeding season. Heifers calving early as 2-yr olds should do so throughout their productive lives and thus contribute greater profit to the cow-calf operation (Short et al., 1971; Lesmeister et al., 1973). However, a large percentage of yearling heifers fail to reach puberty at the start of the first breeding season (Wiltbank et al., 1969; Arije and Wiltbank, 1971). These heifers will probably cycle and conceive late in the breeding season resulting in late calving or fail to breed resulting in decreased lifetime productivity. Postpartum intervals are longer for first calf heifers compared with cows (Wiltbank et al., 1969). Thus, late calving reduces the time available for heifers to rebreed. Puberty is influenced by several genetic and nutritional factors. However, changing these factors may not be a practical or economical approach for most cattlemen.

Endocrine events associated with puberty have been described by Gonzalez-Padilla et al. (1975a). By using combinations of estrogen and progestogens to mimic normal blood hormone changes at puberty, successful

induction of ovulation and sychronization of pubertal estrus resulted for heifers of normal age and weight (Gonzalez-Padilla et al., 1975b,c; Short et al., 1976). Intermittent LHRH injections have also induced ovarian cycling in prepubertal monkeys (Wildt et al., 1980), prepubertal lambs (Ryan and Foster, 1980), anestrous ewes (McLeod et al., 1982a,b) and anestrous cows (Riley et al., 1981; Walters et al., 1982).

The purpose of this study was to determine if low doses of exogenous LHRH administered intermittently or continuously could induce pulsatile LH and FSH release and estrous cyclicity in prepubertal heifers.

Materials and Methods

Exp. 1.

Prepubertal Hereford heifers (n=38), 12 to 14 mo of age, from one ranch at Cassoday, KS, were maintained at the Kansas State University Beef Research Unit (May, 1983). Heifers were checked twice daily (30 min/check) for estrous activity 4 wk preceding onset of treatment (d 1). Ovaries of heifers were palpated per rectum and serum progesterone concentrations were monitored 4 d before beginning Exp. 1. Heifers with progesterone concentrations less than 1 ng/ml and were not observed in estrus during 4-wk pretreatment period were used in experiment. Twenty-two heifers were selected and assigned randomly to one of three treatment groups. Luteinizing Hormone-Releasing Hormone (Cystorelin®, CEVA Laboratories, Overland Park, KS)-pulsed (P) treatment group (n=8) received 500 ng LHRH in 2 ml sterile physiological saline (9 g NaCl/l) through jugular cannulae at 2-h intervals for 96 h (treatment period). An LHRH-infused (I) treatment group (n=7) received a continuous infusion of LHRH by Alzet® osmotic minipumps implanted (sc) in the neck for 96 h. Minipumps contained 53.7 µg synthetic LHRH diluted in 2 ml sterile saline. Pumping rate of

minipumps was 9.31 µl/h or 250 ng LHRH/h for 96 h. A control group (C) consisted of seven heifers with four heifers from this group receiving pulsatile injections (iv) of 2 ml saline every 2 h for 96 h.

Heifers selected were weighed 2 d prior to treatment and body weights are shown in table 1. Fifteen heifers (eight P, five I, four C) were cannulated nonsurgically via jugular venipuncture 2 d preceding treatment and cannulae were filled with a sodium (.9%)-citrate (3.5%) solution containing 3000 U penicillin G/ml. Heifers were haltered and restrained in an outdoor concrete-floored facility during the 96-h treatment period. Heifers were untied twice daily for feed and water from 0800 to 1000 h and from 1800 to 2000 h. All heifers were fed diets of milo grain and prairie hay.

Injections of LHRH every 2 h were initiated at 0600 h on d 1 and terminated at 0600 h on d 5. Alzet® osmotic minipumps were implanted in I heifers from 0600 to 0800 h on day 1 and a 2-h blood collection began after pump insertion in I heifers. Minipumps were removed starting at 0600 h on d 5. Blood was collected at 30-min intervals for 2 h (0600 to 0800 h) and at 1800 h (one sample) on d 1, 2, 3, and 4. On d 5, a 4-h window at 30-min intervals (0600 to 1000 h) and a 1600-h sample were collected. Blood was collected before LHRH and injections via the same jugular catheter. Sodium citrate solution (3 to 4 ml) was used to thoroughly flush cannulae following blood collection and LHRH or saline injections. Blood was refrigerated at 4 C for 24 h before serum was obtained by centrifugation at 1500 x g for 20 min. Serum was frozen at -20 C until assayed. Serum progesterone from daily samples and LH from window samples were quantified by radioimmunoassay according to procedures described in Exp. 2. Cannulae were removed after last sample and heifers were returned to drylot. Heifers were checked twice daily (30 min/check) for estrus for 45 d after treatment and were inseminated artificially when detected in estrus.

TABLE 1. BODY WEIGHT OF HEIFERS ASSIGNED TO TREATMENT GROUPS (EXP. 1)[a]

Treatment	No. of frequently bled heifers	Body weight (kg)	Total no. heifers	Body weight (kg)
Control	4	282.9 ± 7.8	7	287.5 ± 7.2
Infused	5	285.8 ± 7.0	7	277.1 ± 7.2
Pulsed	5	297.6 ± 7.0	8	290.9 ± 6.7

[a]Least-squares means ± SE.

Limited blood samples were collected during 40 d after treatment to determine when first pubertal ovulations occurred after treatment.

Data were analyzed by analysis of variance using the General Linear Model procedure of the Statistical Analysis System (SAS, 1982). Hormone concentrations were analyzed by analysis of variance as a pseudo split-plot design with repeated measurements. Treatment, day, and treatment x day effects were examined. Treatment was tested by the between animal variance (animal within treatment). Preplanned orthogonal contrasts were made to compare C vs I + P and I vs P. Percentage data were tested by Chi-square.

Exp. 2.

Experimental design. Prepubertal heifers (n=57, 12 to 14 mo of age) from one ranch at Cassoday, KS, were maintained at the Kansas State University Beef Research Unit (May, 1984). All heifers were bled once weekly by jugular venipuncture for 4 wk prior to beginning Exp. 2 to eliminate pubertal heifers. Heifers with serum progesterone concentrations exceeding 1 ng/ml in any sample were eliminated. Using this criteria, 33 heifers were classified as prepubertal and were allotted randomly to one of three treatment groups. Body weights of heifers are shown in table 2. The treatment period consisted of 4 d.

An LHRH-pulsed treatment (P) received (iv) injections via jugular cannulae of 2.5 µg LHRH in 2 ml sterile physiological saline (9 g NaCl/l) at 2-h intervals for 72 h. Six heifers were assigned to this group including three Herefords, one Brangus x Hereford, one Red Angus x Hereford and one Polled Hereford. An LHRH-infused treatment (I) received (sc) an Alzet® osmotic minipump implanted in the neck region of each heifer on d 1. Osmotic minipumps were filled with 241 mg synthetic LHRH (Sigma L 7134, St. Louis, MO) diluted in 2 ml sterile saline. Pumping rate of minipumps was 10.3 µl/h or 1.25 µg of LHRH per h for 72 h. Eleven heifers were assigned to the I group including

three Herefords, two Brangus x Hereford, three Red Angus x Hereford and three Polled Herefords. Control (C) heifers were untreated (n=16) including seven Herefords, three Brangus x Hereford, three Red Angus x Hereford and three Polled Herefords.

Sampling Protocol. Eighteen heifers were selected (six from each treatment) to be cannulated for frequent blood collection. Heifers were haltered and tied in box stalls from 0900 to 1700 h for 4 d prior to beginning Exp. 2 to acclimate heifers to new surroundings. Heifers were jugular-cannulated 1 d prior to treatment, cannulae were filled with a sodium (.9%)-citrate (3.5%) solution containing 3000 U penicillin G/ml, and were returned to their box stalls after cannulation. Heifers were restrained by rope halters from 0900 to 1700 h during blood collection for 4 d and were unrestrained in box stalls for the remainder of the day except for P heifers that were restrained to receive pulsatile LHRH injections every 2 h. Heifers were fed diets of prairie hay and milo grain at 0800 and 1800 h daily. Body weights for heifers in each treatment are in table 2. Those heifers not frequently bled were maintained in drylot and received similar diets.

Blood was collected from cannulated heifers at 20-min intervals from 0900 to 1700 h during treatment (d 1, 2, 3, and 4). Pulsatile LHRH injections for P group were initiated at 0900 on d 1 and continued at 2-h intervals for 72 h until d 4 at 0900. Infused heifers were implanted with Alzet® osmotic minipumps from 0800 to 1000 h on d 1. The I heifers in drylot were implanted with osmotic minipumps starting at 1800 h on d 1. Osmotic minipumps were removed beginning at 0800 h on d 4 from heifers in box stalls and at 1800 h on d 4 from heifers in drylot. Blood sampling continued for 8 h (d 4) after terminating of LHRH injections and removing minipumps. Blood was collected immediately preceding each LHRH injection during daily 8-h sampling periods. Cannulae were flushed

TABLE 2. BODY WEIGHT OF HEIFERS ASSIGNED TO TREATMENT GROUPS (EXP. 2)[a]

Treatment	No. jugular cannulated heifers	Body weight (kg)	Total no. heifers	Body weight (kg)
Control	6	272.7 ± 8.7	16	281.2 ± 5.3
Infused	6	272.2 ± 8.7	11	275.5 ± 6.2
Pulsed	6	277.0 ± 8.7	6	277.0 ± 8.7

[a]Least-squares means ± SE.

with 3 to 4 ml of sodium citrate solution following blood collection and LHRH injections. Estrous activity was monitored twice daily (30 min/check) for 49 d after treatment. Heifers were inseminated artificially when detected in estrus. Blood collection continued from all heifers on Monday, Wednesday, and Friday for 7 wk after treatment to determine when first pubertal ovulations occurred.

All blood samples were placed on ice immediately after collection and were stored at 4 C overnight. Serum was harvested by centrifugation at 1500 x g for 20 min. Serum was maintained at -20 C until assayed. All individual serum samples (25 per d) from 18 heifers housed in box stalls during the 4-d treatment period were radioimmunoassayed for luteinizing hormone (LH) and hourly samples (nine per d) for 4 d were assayed for FSH. Daily serum pools from each of 18 heifers during the treatment period were assayed for cortisol, estradiol-17β, and progesterone. All thrice-weekly serum samples collected after treatment also were assayed for progesterone.

Gonadotropins. Serum concentrations of LH were measured by double-antibody radioimmunoassay (RIA) according to Niswender et al. (1969) with some modifications. Sodium-^{125}I was used to replace Na-^{131}I and purified bovine LH (LER 1716-2) was iodinated by chloramine-T method (Greenwood et al., 1963). Rabbit anti-ovine LH (#15) was donated by G.D. Niswender. Standard reference preparation was bovine LH (NIH-LH-B10). Intraassay coefficient of variation was 4.7% and interassay coefficient of variation was 2.1% with a sensitivity of 50 pg/tube. FSH concentrations in hourly samples were determined by RIA according to Akbar et al. (1974) by T. M. Nett of Colorado State University.

Progesterone. Serum progesterone concentrations were quantified by RIA according to Stevenson et al. (1981). Serum progesterone was measured using a highly specific antiserum obtained from immunizing rabbits against progesterone-11-hemisuccinate: BSA (Purchased from Steraloids Inc., Wilton, NH.)

Tritiated-progesterone extracted from bovine serum with ethyl acetate averaged 85% in four assays. Progesterone was recovered quantitatively when added to serum (r=.99). Serum curves paralleled progesterone standards. Variable volumes of serum (.1, .15 and .2 ml, n = 4 each) from estrous cows and luteal-phase cow measured .49, .34, .40 ng/ml and 3.24, 3.34, 3.43 ng/ml. Assay sensitivity was 25 pg/tube. Intraassay coefficient of variation was 7.7% and interassay coefficient of variation was 6.6%.

Estradiol. Serum estradiol-17ß was quantitated in one RIA using antiserum (Estradiol-6 #3) donated by Dr. Norman Mason, Eli Lilly and Company, Indianapolis, IN. Antiserum specificity was tested against five chemically related substances to estradiol-17ß at 50% binding inhibition of the labelled estradiol. No crossreactivity of estradiol-17ß was significant with estradiol-17α (<.001), estrone (<.001), estriol (<.001), testosterone (<.001) and androstenedione (<.001). Recovery of tritiated-estradiol-17ß extracted from bovine serum with ethyl acetate was 64% in one assay. Addition of 25 pg, 50 pg, and 100 pg estradiol-17ß added to 5 ml bovine serum yielded 28, 49, and 87 pg recovery (r = .986). Parallelism existed between serum and standard estradiol curves. Assay sensitivity was 5 pg/tube and intraassay coefficient of variation was 3.6%.

Cortisol. Serum cortisol was measured in one RIA using a specific antiserum obtained from immunizing rabbits against cortisol-3-hemisuccinate: BSA (Purchased from Western Chemical, Fort Collins, CO). Specificity of antiserum was tested against 14 different steroids and only crossreacted slightly (at 50% binding the inhibition of labeled cortisol) with 11-deoxycortisol (7.5%), cortisone (0.6%), and progesterone (2.4%). Cross reactivity with corticosterone (<.1%), deoxycorticosterone (<.01), 21-deoxycortisone (<.1%), 11-α-hydroxy-progesterone (<.1%), 11-ß-hydroxy-progesterone (<.1%), 17α-hydroxy-progesterone

(<.1%), 20α-dihydro-progesterone (<.1%), 20ß-dihydroxy- progesterone (<.1%), pregnenolone (<.1%), testosterone (<.1%), and androstenedione (<.1%). Tritiated-cortisol extracted from bovine serum with ethyl acetate was 87% in one assay. Cortisol was recovered quantitatively. When 50, 60, 80, 200, 400, 600, and 800 pg cortisol were added to .1 ml bovine serum, cortisol recovered was 51, 63, 80, 190, 379, 592, and 702 pg (r = .997). Serum curves paralleled cortisol standards. Variable volumes of serum (.1, .15, and .2 ml, n = 4 each) from a cow measured 12.9, 12.7, and 8.9 ng/ml. Assay sensitivity was 20 pg/tube and intraassay coefficient of variation was 12.1%.

Definitions. A rise in LH concentration was defined as a pulse using criteria previously established (Riley et al., 1981; McLeod et al., 1982a). An increase in serum LH was designated as an LH pulse when 1) the highest LH concentration attained was 50% above the preceding baseline; 2) at least two consecutive LH concentration values were between peak value and the following baseline value; and 3) rate of decline from LH peak values was not greater than the half-life of LH which is approximately 35 min in bovine serum (Schams and Karg, 1969). Pulse amplitude was defined as the maximum LH level associated with an LH pulse. Pulse duration was defined as the interval (min) from the rise in LH concentration to 50% above baseline until its return to baseline. Preovulatory-like LH surges were defined as a distinct elevation in LH (>10 ng/ml) with a duration of greater than 200 min.

Serum progesterone during 53-d was used to determine first progesterone rise, first ovulation, and duration of first estrous cycle. A progesterone rise was defined as an increase in serum progesterone to greater than 1 ng/ml for 2 or more d. The following criteria described by Stevenson and Call (1982) were utilized to estimate day of ovulation: 1) ovulation occurred on the d following observed estrus if serum progesterone was less than 1 ng/ml at estrus, 2) if

estrus was unobserved, ovulation occurred 2 d prior to an increase in progesterone exceeding .5 ng/ml but below 2 ng/ml; or 3) if estrus was unobserved, ovulation occurred 4 d prior to an increase in progesterone above 2 ng/ml.

Statistical Analyses. Data were analyzed using least-squares procedures of General Linear Model procedure of the Statistical Analysis System (SAS, 1982). Effects of treatment, day, and treatment x day interaction were examined in a pseudo split-plot design for repeated measurements of hormone data. Preplanned orthogonal contrasts were used to compare treatment means. Heterogeneous treatment variance of hormone data occurred for the analysis of LH and cortisol requiring log transformation of data. Proportions and percentage data were tested by Chi-square.

Results

Exp. 1.

Mean serum LH concentrations during 2-h windows for treatment groups are shown in table 3. Average serum LH ranged from .2 to 1.3 ng/ml across days for controls (C), .2 to 1.5 ng/ml for pulsed (P), and .2 to 1.1 ng/ml for infused (I) heifers. Composite treatment means indicated that heifers were similar in their response to treatments (P=.20). Although infused and P treatments of LHRH were similar to controls for LH concentrations, there was a tendency (P<.10) for LH to be greater for P than for I heifers. Treatment with LHRH increased LH concentrations on days 2, 3 and 4 resulting in day effect (P<.001). Consequently, LH concentrations (ng/ml) on d 1 (.2) and d 5 (.2) were less (P<.001) than those on d 2 (.9), 3 (1.1), and 4 (.9). Pulses of 500 ng LHRH produced sporadic LH pulses as heifers exhibited LH release to some but not all LHRH pulses. LH pulse frequency, pulse amplitude, and pulse duration were similar among treatments and no preovulatory-like LH surges were observed in daily 2-h windows for each heifer.

Number and percentage of heifers beginning estrous cyclicity during 45 d after treatment are shown in table 4. A higher percentage (P<.10) of C (86%) and P (88%) exhibited estrus than I (43%) heifers. However, overall conception rate showed an advantage (P<.10) for P (100%) over C (71%) heifers.

Exp. 2.

Luteinizing Hormone. Serum LH concentrations over the 4-d treatment for each jugular-cannulated heifer are plotted in appendix figures 1 to 18. Mean serum LH concentrations for treatments, days, and treatment x day interactions are summarized for control (C), infused (I) and pulsed (P) groups (table 5). Means for treatment x day interaction (P<.05), treatment (P<.01) and day

TABLE 3. SERUM LH CONCENTRATIONS (NG/ML) OF CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS DURING TWO-HOUR WINDOWS (EXP. 1)[a]

Treatment	No. heifers	Day 1	2	3	4	5	Treatment means[b]
Control	4	.1 ± .3	.2 ± .3	.9 ± .3	1.3 ± .3	.2 ± .3	.6 ± .2
Infused	5	.1 ± .3	.8 ± .3	.9 ± .3	.6 ± .3	.2 ± .3	.5 ± .2
Pulsed	5	.4 ± .3	1.4 ± .3	1.5 ± .3	1.1 ± .3	.2 ± .3	.9 ± .2
Day means		.2 ± .2[c]	.9 ± .2[d]	1.1 ± .2[d]	.9 ± .2[d]	.2 ± .2[c]	

[a] Least-squares means ± SE.

[b] Orthogonal contrast: Pulsed vs Infused (P<.10).

[c,d] Means within row with different superscripts differ (P<.001).

TABLE 4. REPRODUCTIVE PERFORMANCE OF CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS (EXP. 1)

Treatment	No. heifers	No. exhibiting estrous cycles within 45 d (%)	45-d conception rate (%)	Overall conception rate (%)
Control	7	6 (86)[a]	3 (43)	5 (71)[a]
Infused	7	3 (43)[b]	2 (29)	6 (86)[ab]
Pulsed	8	7 (88)[a]	4 (50)	8 (100)[b]

[a,b]Means within rows with different superscripts differ (P<.10).

TABLE 5. SERUM LH (NG/ML) FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS DURING EIGHT-HOUR WINDOWS (EXP. 2)[a,b]

Treatment	No. heifers	Day[c] 1	2	3	4	Treatment means[d]
Control	6	.4 ± 1.0	.7 ± 1.0	.8 ± 1.0	1.0 ± 1.0	.7 ± .4
Infused	6	.6 ± 1.0	3.1 ± 1.0	3.1 ± 1.0	.3 ± 1.0	1.8 ± .4
Pulsed	6	1.2 ± 1.0	2.0 ± 1.0	2.0 ± 1.0	.9 ± 1.0	1.5 ± .4
Day means		.7 ± .6[e]	1.9 ± .6[f]	2.0 ± .6[f]	.7 ± .6[e]	

[a]Blood was collected at 20-min intervals for 8 h on each of 4 d. LHRH treatments began at 0900 on d 1 and terminated 72 h later on d 4.

[b]Least-squares means ± SE.

[c]Treatment x day interaction (P<.05).

[d]Orthogonal contrasts: Control vs Infused + Pulsed (P<.01)
Infused vs Pulsed (P<.05).

[e,f]Means within row with different superscripts differ (P<.001).

(P<.01) were significant. Luteinizing hormone-releasing hormone treated heifers (I = 1.8 ng/ml and P = 1.5 ng/ml) had higher (P<.01) LH concentrations than C heifers (.7 ng/ml). Infused heifers had higher (P<.05) LH than P heifers. Means for d 1 (.7 ng/ml) and d 4 (.7 ng/ml) were similar as were means for d 2 (1.9 ng/ml) and 3 (2 ng/ml), but means for d 1 and d 4 were less (P<.001) than those of d 2 and 3.

Preovulatory-like surges of LH occurred in three heifers from I group, two on d 2 (appendix figures 7b and 9b) and one on d 3 (appendix figure 11c). Two heifers from the P group exhibited LH surges on d 3 and 4 (appendix figures 1d and 6c). Large standard errors for treatment x day means indicated a large variation among LH concentrations in blood samples collected at 20-min intervals. Due to this observation, heifers were divided further into groups according to their LH response. The resulting five response groups were controls (C), LHRH-infused heifers with no LH surge (I-NS), LHRH-infused with LH surge (I-S), pulsed heifers with no LH surge (P-NS), and pulsed heifers with an LH surge (P-S). Means for LH concentrations of these groups are in table 6. Similar results occurred as with the three treatment groups. Treatment x day (P<.10) and treatment (P<.01) means for LH were significant. Infused heifers (I-S) with an LH surge and P-S groups showed elevated LH concentrations compared with C + I-NS + P-NS heifers (P<.01) and I-NS + P-NS heifers (P<.05). Control LH concentrations were lower (P<.001) compared with LHRH treatments. Infused and I-S heifers had higher (P<.01) LH concentrations than P + PS groups.

LH pulse frequencies (no. pulses per 8-h window) are in table 7. Pulsatile release of LH was evident in response to LHRH pulses every 2 h. An average of four LH pulses occurred during 8-h sampling windows in response to four LHRH injections. Increased pulse frequency in P heifers resulted in treatment x day interaction (P<.001), treatment (P<.001), and day (P<.001) effects. Pulsatile

TABLE 6. SERUM LH (NG/ML) FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS WITH (S) AND WITHOUT (NS) LH SURGES (EXP. 2)[a]

Treatment	No. heifers	Day[b] 1	2	3	4	Response means[c]
Control	6	.4 ± 1.0	.7 ± 1.0	.8 ± 1.0	1.0 ± 1.0	.7 ± .3
I-NS	3	.6 ± 1.4	.9 ± 1.4	.6 ± 1.4	.4 ± 1.4	.7 ± .4
I-S	3	.6 ± 1.4	5.3 ± 1.4	5.6 ± 1.4	.1 ± 1.4	2.9 ± .4
P-NS	4	1.1 ± 1.2	1.7 ± 1.2	1.5 ± 1.2	.5 ± 1.2	1.2 ± .3
P-S	2	1.4 ± 1.7	2.4 ± 1.7	3.0 ± 1.7	1.7 ± 1.7	2.1 ± .5

[a]Least-squares means ± SE.

[b]Treatment x day interaction (P<.10).

[c]Orthogonal contrasts: I-S + P-S vs Control + I-NS + P-NS (P<.01)
Control vs I-NS + I-S + P-NS + P-S (P<.001)
I-S + P-S vs I-NS + P-NS (P<.05)
I-NS + I-S vs P-NS + P-S (P<.01).

TABLE 7. SERUM LH PULSE FREQUENCY FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS (EXP. 2)[a,b]

Treatment	No. heifers	Day[c] 1	2	3	4	Treatment means[d]
Control	6	1.2 ± .2	1.8 ± .2	2.0 ± .2	2.0 ± .2	1.8 ± .1
Infused	6	1.5 ± .2	1.3 ± .2	1.0 ± .2	.8 ± .2	1.2 ± .1
Pulsed	6	4.2 ± .2	4.2 ± .2	4.0 ± .2	1.5 ± .2	3.5 ± .1
Day means		2.3 ± .1[e]	2.4 ± .1[e]	2.3 ± .1[e]	1.4 ± .1[f]	

[a]Pulse frequency = no. LH pulses per 8 h on each of 4 d.

[b]Least-squares means ± SE.

[c]Treatment x day interaction (P<.001).

[d]Orthogonal contrast: Infused vs Pulsed (P<.001).

[e,f]Means within row with different superscripts differ (P<.001).

release of LH in P heifers was responsible for greater (P<.001) LH pulse frequency for P than for I and C heifers, as well as greater (P<.001) pulse frequency on d 1, 2, and 3 compared with d 4.

Pulse frequency for groups with LH surges was similar (I-S + P-S) to nonsurge groups (C + I-NS + P-NS), but a tendency existed (P=.14) for groups with surges to have reduced pulse frequency than for I-NS + P-NS heifers (table 8). Pulsed heifers (P-NS + P-S) displayed greater LH pulse frequency (P<.001) than infused (I-NS + I-S) groups. A definite reduction in pulse frequency (P<.01) occurred in I-S compared with I-NS heifers due to the occurrence of preovulatory-like LH surges.

Amplitude of LH pulses was elevated in I heifers due to LH surges on d 2 and 3 (table 9) resulting in a treatment x day interaction (P<.001). Higher (P<.01) pulse amplitudes of LH in I heifers were associated with significant elevations on d 2, d 3, and d 4 contrasted with d 1. Control and LHRH-treated heifers had similar treatment means. Pulse amplitude for I heifers was greater (P<.05) than P heifers.

Surges of LH in the I group also influenced pulse duration, hence a treatment x day interaction (P<.001) (table 10). Shorter pulse durations were observed (P<.10) for controls compared with LHRH groups. Infused heifers had longer LH pulse duration than C (P<.10) and P (P<.01) groups. Pulse durations also were longer (P<.01) on d 2, 3, and 4 than d 1.

Follicle Stimulating Hormone. Individual serum FSH concentrations for frequently bled heifers are plotted for the 4 d in appendix figures 1 to 18. Mean serum FSH concentrations for treatment, day, and treatment x day interactions are in table 11. Treatment and day means were similar among treatment groups. A treatment x day interaction was suggested (P=.15). FSH concentrations for LHRH-treated heifers tended to parallel one another with

TABLE 8. SERUM LH PULSE FREQUENCY FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS WITH (S) AND WITHOUT (NS) LH SURGES (EXP. 2)[a,b]

Treatment	No. heifers	Day 1	2	3	4	Response means[c]
Control	6	1.2 ± .2	1.8 ± .2	2.0 ± .2	2.0 ± .2	1.8 ± .1
I-NS	3	1.3 ± .3	2.0 ± .3	1.7 ± .3	1.7 ± .3	1.7 ± .1
I-S	3	1.7 ± .3	.7 ± .3	.3 ± .3	0.0	.7 ± .1
P-NS	4	4.3 ± .3	4.3 ± .3	4.3 ± .3	1.5 ± .3	3.6 ± .1
P-S	2	4.0 ± .4	4.0 ± .4	3.5 ± .4	1.5 ± .4	3.3 ± .2

[a]Pulse frequency = no. LH pulses per 8 h on each of 4 d.

[b]Least-squares means ± SE.

[c]Orthogonal contrast: I-NS + I-S vs P-NS + P-S (P<.001).

TABLE 9. AMPLITUDE (NG/ML) OF SERUM LH PULSES FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS DURING EIGHT-HOUR WINDOWS (EXP. 2)[a]

Treatment	No. heifers	Day[b] 1	2	3	4	Treatment means[c]
Control	6	4.1 ± 1.0	3.9 ± .5	4.3 ± .3	5.1 ± .4	4.4 ± .3
Infused	6	3.3 ± .4	8.4 ± 4.6	8.7 ± 5.1	2.9 ± .7	5.8 ± 1.7
Pulsed	6	3.3 ± .3	4.9 ± .3	3.7 ± .5	3.1 ± .7	3.9 ± .2
Day means		3.4 ± .3[d]	5.3 ± .8[e]	4.6 ± .8[e]	4.0 ± .4[e]	

[a]Means ± SE.

[b]Treatment x day interaction (P<.001).

[c]Orthogonal contrast: Infused vs Pulsed (P<.05).

[d,e]Means within row with different superscripts differ (P<.01).

TABLE 10. DURATION (MIN) OF SERUM LH PULSES FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS DURING EIGHT-HOUR WINDOWS (EXP 2)[a]

Treatment	No. heifers	Day[b] 1	2	3	4	Treatment means[c]
Control	6	57.1 ± 6.8	62.0 ± 5.5	69.1 ± 4.9	76.6 ± 8.1	67.5 ± 3.4
Infused	6	71.1 ± 16.0	187.5 ± 65.0	140.0 ± 68.7	58.4 ± 4.9	115.0 ± 25.3
Pulsed	6	58.4 ± 4.2	76.0 ± 4.3	80.0 ± 8.2	86.7 ± 20.3	73.0 ± 3.4
Day means		61.0 ± 4.1[d]	93.5 ± 13.7[e]	85.9 ± 11.1[e]	74.6 ± 8.2[e]	

[a]Means ± SE.

[b]Treatment x day interactions (P<.001).

[c]Orthogonal contrasts: Control vs Infused + Pulsed (P=.10)
Infused vs Pulsed (P<.01)

[d,e]Means within row with different superscripts differ (P<.01).

TABLE 11. SERUM FSH (NG/ML) FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HE IFER DURING EIGHT-HOUR WINDOWS (EXP. 2)[a,b]

Treatment	No. heifers	Day 1	2	3	4	Treatment means
Control	6	53.3 ± 2.2	54.6 ± 2.2	57.7 ± 2.2	48.6 ± 2.2	53.5 ± 4.8
Infused	6	47.8 ± 2.2	46.4 ± 2.2	48.7 ± 2.2	50.4 ± 2.2	48.4 ± 4.8
Pulsed	6	56.9 ± 2.2	54.8 ± 2.2	54.8 ± 2.2	56.7 ± 2.2	55.8 ± 4.8
Day means		52.7 ± 1.3	51.9 ± 1.3	53.7 ± 1.3	51.9 ± 1.3	

[a]Blood was collected at 1-h intervals for 8 h on each of 4 d. LHRH treatments began at 0900 h on d 1 and terminated 72 h later on d 4.

[b]Least-squares means ± SE.

FSH declining from d 1 to d 2, and increasing slightly on d 3 and d 4. Follicle-stimulating hormone in controls tended to increase on d 2 and d 3, then declined on d 4. Distributing heifers into five response groups (table 12) reiterated previous trends existing among treatments, days, and treatment x day means. Heifers with surges (I-S + P-S) had similar FSH concentrations compared with C + I-NS + P-NS groups and I-NS + P-NS groups. Serum FSH concentrations were not elevated by pulsatile injection or continuous infusion of LHRH. Three heifers in the I-S group exhibited similar declines in FSH concentration before increasing to higher concentrations during the LH surge (appendix figures 7b, 9b, and 11c).

Estradiol. Serum estradiol concentrations summarized in table 13 demonstrated a treatment effect ($P<.05$) as C heifers had lower ($P<.01$) estradiol during the 4 d when compared with LHRH-I + P treated heifers. A tendency ($P=.15$) existed for P heifers to have higher estradiol levels than I heifers. In addition, d 2 and 3 means were elevated ($P<.10$) above those of d 1 and 4.

Response mean comparisons emphasized differences in estradiol release among groups (table 14). Surge groups (IS + PS) demonstrated higher ($P<.001$) estradiol levels than nonsurge groups (C + I-NS + P-NS and I-NS + P-NS). Further contrasts displayed that LHRH-treated heifers had elevated ($P<.001$) estradiol concentrations over controls, and pulsed (P-NS + PS) heifers released more ($P<.01$) estradiol than infused (I-NS + I-S) counterparts.

Cortisol. Serum cortisol was similar in concentration and pattern during 4 d in all three treatments (table 15). Peak values occurred on d 1, declined on d 2, and remained unchanged on d 3 and 4. Day 1 cortisol concentrations were elevated ($P<.001$) above the other 3 d. No difference in cortisol concentrations was detected between LHRH and control heifers as well as between P and I groups. Large standard errors were associated with treatment and treatment x

TABLE 12. SERUM FSH (NG/ML) FOR CONTROL, LHRH-INFUSED, LHRH-PULSED HEIFERS WITH (S) OR WITHOUT (NS) LH SURGES (EXP. 2)[a]

Treatment	No. heifers	Day 1	2	3	4	Response means
Control	6	53.3 ± 2.2	54.6 ± 2.2	57.7 ± 2.2	48.6 ± 2.2	53.5 ± 5.1
I-NS	3	53.6 ± 3.1	46.8 ± 3.1	49.5 ± 3.1	56.0 ± 3.1	51.5 ± 7.2
I-S	3	42.1 ± 3.1	46.1 ± 3.1	47.9 ± 3.1	44.8 ± 3.1	45.2 ± 7.2
P-NS	4	58.6 ± 2.7	55.2 ± 2.7	56.3 ± 2.7	58.1 ± 2.7	57.1 ± 6.3
P-S	2	53.3 ± 3.8	53.8 ± 3.8	51.7 ± 3.8	53.9 ± 3.8	53.2 ± 8.9

[a]Least-squares means ± SE.

TABLE 13. SERUM ESTRADIOL (PG/ML) FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS DURING TREATMENT (EXP. 2)[a]

Treatment	No. heifers	Day 1	2	3	4	Treatment means[b]
Control	6	2.3 ± .8	2.2 ± .8	2.6 ± .8	2.1 ± .8	2.3 ± .8
Infused	6	2.7 ± .8	4.3 ± .8	3.8 ± .8	2.4 ± .8	3.3 ± .8
Pulsed	6	2.6 ± .8	5.2 ± .8	5.3 ± .8	3.3 ± .8	4.1 ± .8
Day means		2.5 ± .5[c]	3.9 ± .5[d]	3.9 ± .5[d]	2.6 ± .5[c]	

[a]Least-squares means ± SE.

[b]Orthogonal contrast: Control vs Infused + Pulsed (P<.01).

[c,d]Means within row with different superscripts differ (P<.10).

TABLE 14. SERUM ESTRADIOL (PG/ML) OF CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS WITH (S) AND WITHOUT (NS) RESULTING LH SURGES (EXP. 2)[a]

Treatment	No. heifers	Day 1	2	3	4	Response means[b]
Control	6	2.3 ± .8	2.2 ± .8	2.6 ± .8	2.1 ± .8	2.3 ± .8
I-NS	3	2.5 ± 1.1	2.9 ± 1.1	3.5 ± 1.1	2.1 ± 1.1	2.8 ± 1.1
I-S	3	2.9 ± 1.1	5.7 ± 1.1	4.0 ± 1.1	2.6 ± 1.1	3.8 ± 1.1
P-NS	4	2.7 ± .9	5.3 ± .9	3.6 ± .9	1.8 ± .9	3.3 ± .9
P-S	2	2.3 ± 1.3	5.0 ± 1.3	8.8 ± 1.3	6.2 ± 1.3	5.6 ± 1.3

[a]Least-squares means ± SE.

[b]Orthogonal contrasts: I-S + P-S vs Control + I-NS + P-NS (P<.001)
Control vs I-NS + I-S + P-NS + P-S (P<.001)
I-S + P-S vs I-NS + P-NS (P<.001)
I-NS + I-S vs P-NS + P-S (P<.01).

TABLE 15. SERUM CORTISOL (NG/ML) FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS DURING TREATMENT (EXP. 2)[a]

Treatment	No. heifers	Day 1	2	3	4	Treatment means
Control	6	16.5 ± 2.9	6.9 ± 2.9	4.8 ± 2.9	4.6 ± 2.9	8.2 ± 2.8
Infused	6	24.7 ± 2.9	9.3 ± 2.9	5.5 ± 2.9	7.4 ± 2.9	11.7 ± 2.8
Pulsed	6	22.9 ± 2.9	8.0 ± 2.9	8.2 ± 2.9	5.7 ± 2.9	11.2 ± 2.8
Day means		21.3 ± 1.7[b]	8.0 ± 1.7[c]	6.2 ± 1.7[c]	5.9 ± 1.7[c]	

[a]Least-squares means ± SE.

[b]Means within row with different superscripts differ (P<.001).

day means.

No treatment differences were associated with the five response groups (table 16). Surge groups (I-S + P-S) were generally lower in cortisol concentration than nonsurge (I-NS and P-NS) groups. Within the Hereford breed, four heifers having surges had average serum cortisol of 9 ng/ml and were comparable to 8.1 ng/ml for five heifers without surges.

Progesterone and Estrous Cycles. Progesterone concentrations throughout the 53 d for each individual heifer are in appendix figures 19 to 29. Concentrations of progesterone were consistently low for all heifers and no difference in treatment means were observed during LHRH treatment (table 17).

Synchronized appearance of progesterone rises (defined as progesterone concentration exceeding 1 ng/ml with duration of 2 or more days) occurred after LHRH treatment (table 18). Interval to first progesterone rise along with duration and magnitude of first rises were similar for all treatments. More ($P<.05$) I heifers (45%) had progesterone rises within 10 d after the onset of treatment compared with C group (6%). More ($P<.05$) heifers given LHRH exhibited progesterone rises within 10 d than C heifers.

Average days to first observed estrus and days to first ovulation determined from serum progesterone were similar among treatments (table 19). A tendency ($P=.15$) existed for C heifers to ovulate earlier than P heifers.

Estrous cycle data are in table 20. There was a tendency ($P=.12$) for more heifers to cycle in groups C (75%) and P (83%) during the 53-d period after onset of treatment than for I heifers (45%). Treatments with LHRH failed to initiate earlier onset of estrous cycles as 0/6 P and 2/11 I heifers began cycling within 21 d compared with 5/16 C heifers. Infused heifers that cycled earliest included one with an LH surge that ovulated by d 12 after onset of LHRH treatment and one without an LH surge ovulated by d 16. Four C heifers that

TABLE 16. SERUM CORTISOL (NG/ML) FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS WITH (S) AND WITHOUT (NS) LH SURGES (EXP. 2)[a]

Treatment	No. heifers	Day 1	2	3	4	Response means
Control	6	16.5 ± 2.9	6.9 ± 2.9	4.8 ± 2.9	4.6 ± 2.9	8.2 ± 2.9
I-NS	3	32.2 ± 4.1	11.7 ± 4.1	6.5 ± 4.1	5.9 ± 4.1	14.1 ± 4.1
I-S	3	17.2 ± 4.1	6.9 ± 4.1	4.6 ± 4.1	8.9 ± 4.1	9.4 ± 4.1
P-NS	4	25.4 ± 3.6	9.6 ± 3.6	7.7 ± 3.6	5.9 ± 3.6	12.2 ± 3.6
P-S	2	17.8 ± 5.1	4.9 ± 5.1	9.2 ± 5.1	5.1 ± 5.1	9.2 ± 5.1

[a]Least-squares means ± SE.

TABLE 17. SERUM PROGESTERONE (NG/ML) FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS DURING TREATMENT (EXP. 2)[a]

Treatment	No. heifers	Day 1	2	3	4	Treatment means
Control	6	.2 ± .01	.2 ± .01	.2 ± .01	.2 ± .01	.2 ± .02
Infused	6	.2 ± .01	.2 ± .01	.3 ± .01	.3 ± .01	.2 ± .02
Pulsed	6	.2 ± .01	.2 ± .01	.2 ± .01	.2 ± .01	.2 ± .02
Day means		.2 ± .01	.2 ± .01	.2 ± .01	.2 ± .01	

[a]Least-squares means ± SE.

TABLE 18. CHARACTERISTICS OF FIRST RISE IN PROGESTERONE (P) OBSERVED IN CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS (EXP. 2)[a,b]

Treatment	No. heifers	No. heifers with P rise within 10 d (%)[c]	Days to P rise (no. heifers)	Duration of P rise (d)	Magnitude of P rise (ng/ml)
Control	16	1 (6)[d]	26 ± 9 (4)	3.3 ± .2	2.2 ± .2
Infused	11	5 (45)[e]	21 ± 6 (8)	3.3 ± .2	2.3 ± .3
Pulsed	6	2 (33)[de]	12 ± 10 (3)	3.7 ± .3	1.7 ± .3
I + P	17	7 (41)	19 ± 5 (11)	3.4 ± .2	2.1 ± .2

[a]Progesterone rise was defined as an increase in serum progesterone greater than 1 ng/ml during 2 or more d.

[b]Least-squares means ± SE where applicable.

[c]Orthogonal contrast: Control vs Infused + Pulsed (P<.05).

[d,e]Means within column with different superscripts differ (P<.05).

TABLE 19. INTERVAL (D) TO FIRST OBSERVED ESTRUS AND FIRST OVULATION FOR CONTROL, LHRH-INFUSED , AND LHRH-PULSED HEIFERS (EXP. 2)[a]

Treatment	No. heifers	Days to first observed estrus (no. heifers)	Days to first ovulation (no. heifers)
Control	16	34.4 ± 3.0 (10)	27.9 ± 4.0 (12)
Infused	11	38.3 ± 4.8 (4)	30.2 ± 6.1 (5)
Pulsed	6	36.3 ± 5.5 (3)	38.8 ± 6.1 (5)
I + P	17	37.4 ± 3.5 (7)	34.5 ± 4.3 (10)

[a]Least-squares means ± SE.

TABLE 20. ESTROUS CYCLE TRAITS FOR CONTROL, LHRH-INFUSED, AND LHRH-PULSED HEIFERS (EXP. 2)[a]

Treatment	No. heifers	Duration of first estrous cycle (no.)	No. heifers cycling in 53 d (%)	Conception rate in 53 d (%)	Overall conception rate (%)
Control	16	16.3 ± .8 (8)	12 (75)	7 (44)	11 (69)
Infused	11	16.7 ± 1.2 (3)	5 (45)	5 (45)	10 (90)
Pulsed	6	20.0 ± 2.2 (1)	5 (83)	3 (50)	4 (66)
I + P	17	17.5 ± 1.1 (4)	10 (59)	8 (47)	14 (82)

[a]Least-squares means ± SE where applicable.

cycled earliest ovulated on d 6, 8, 13 and 18, respectively. Conception rates for 53 d and overall conception rates were similar among treatments.

Breed Comparisons. Luteinizing hormone surges were more predominant in Herefords (H, 4/9) than in Polled Herefords (PH, 0/3), Brangus x Hereford (BxH, 1/3), or Red Angus x Hereford (RAxH, 0/3) crosses (table 21). Breed effect on characteristics of progesterone (P) and estrous cycles are in table 22. Interval to the first progesterone rise was shorter ($P<.05$) in H (7.5 d) than PH (32.7 d) and RAxH (33.3 d), but similar to BxH heifers (14.8 d). A tendency ($P<.10$) existed for more H (31%) and BxH (33%) to have P rises by 10 d after onset of treatment when compared with PH (14%) and RAxH (14%). Duration and magnitude of progesterone rises, days to first observed heat, days to ovulation, and duration of first estrous cycles were similar among breeds. A greater proportion ($P<.10$) of BxH (100%) exhibited estrous cycles within 53 d than H (54%) and RAxH heifers (57%), but comparable with PH heifers (71%).

TABLE 21. PROPORTION OF HEIFERS EXHIBITING PREOVULATORY-LIKE LH SURGES AMONG BREEDS (EXP. 2)

Treatment	Breed[a]				Treatment totals[b]
	B X H	H	PH	RA X H	
Control	0/1	0/3	0/1	0/1	0/6
Infused	1/1	2/3	0/1	0/1	3/6
Pulsed	0/1	2/3	0/1	0/1	2/6
Breed totals	1/3	4/9	0/3	0/3	

[a]B = Brangus, H = Hereford, PH = Polled Hereford, and RA = Red Angus.

[b]Control vs Infused + Pulsed ($P<.10$).

TABLE 22. EFFECT OF BREED ON CHARACTERISTICS OF PROGESTERONE (P) AND ESTROUS CYCLES (EXP. 2)[a,b]

Item	B X H	H	PH	RA X H
No. heifers with P rise within 10 d, %	2/6 (33)[c]	4/13 (31)[cd]	1/7 (14)[d]	1/7 (14)[d]
Days to P rise, no.	15 ± 10 (3)[cd]	7 ± 7 (6)[c]	33 ± 9 (5)[d]	33 ± 9 (4)[d]
Duration of P rise, d	3.3 ± .3	3.5 ± .3	3.3 ± .3	3.4 ± .3
Magnitude of P rise, ng/ml	1.7 ± .3	2.3 ± .2	2.1 ± .3	2.0 ± .3
Days to first estrus	36 ± 5.3	35 ± 4.4	39 ± 4.9	36 ± 8.2
Days to first ovulation	30 ± 6.0	31 ± 5.5	39 ± 6.7	31 ± 7.8
No. exhibiting estrous cycles within 53 d, %	6/6 (100)[c]	7/13 (54)[d]	5/7 (71)[cd]	4/7 (57)[d]
Duration (d) of first estrous cycle, no.	18.5 ± .9 (5)	16.0 ± 1.2 (4)	15.3 ± 2.1 (1)	18.8 ± 1.6 (2)

[a]Least-squares means ± SE where applicable.

[b]B = Brangus, H = Hereford, PH = Polled Hereford, RA = Red Angus.

[c,d]Means within row with different superscripts differ (P<.10).

Discussion

Exp. 1. The LH response in prepubertal Hereford heifers indicated that pulsatile administration of 500 ng LHRH approximated a threshold dosage necessary for LH release. Response to intermittent LHRH injections was inconsistent as LH release followed only some of the injections. Edwards et al. (1983) concluded that 500 ng LHRH per injection in suckled beef cows resulted in limited LH release. Continuous LHRH infusion in P heifers established that the dose of LHRH released was insufficient for elevating serum LHRH to trigger LH release. Likewise, continuous infusion of LHRH was ineffective in altering low gonadotropin levels present in prepubertal bulls (Schanbacher, 1984) and rhesus monkeys (Knobil, 1980). Luteinizing hormone release in P heifers on d 2, 3, and 4 elevated LH concentrations above day means of d 1 and 5. However, possible LH release in P heifers on d 1 was undetected due to short sampling periods (2 h). Nevertheless, LHRH enhanced LH concentrations in P heifers, illustrated by the decline in serum LH to baseline immediately upon cessation of LHRH pulses. A difference existed in the number of heifers showing estrus during 45 d as more P (88%) and C (86%) heifers were in estrus than I heifers (43%). A detrimental effect of continuous LHRH infusion was apparent on the initiation of estrous cyclicity.

Pulsatile LHRH administration iv successfully initiated puberty in infantile female rhesus monkeys (Wildt et al., 1980) and prepubertal ewe lambs (Ryan and Foster, 1980). Likewise, intermittent exogenous LHRH injections stimulated follicular growth and ovarian cyclicity in seasonally anestrous ewes (McLeod et al., 1982a,b; McNeilly et al., 1980) and postpartum anestrous beef cows (Riley et al., 1981; Walters et al., 1982). Continuous iv infusion of LHRH resulted in down regulation of LHRH receptors because prolonged exposure to high

concentrations of LHRH reduced available LH receptors (Conti et al., 1976; Hsueh et al., 1976; Hsueh et al., 1977; Ryan et al., 1977). Down regulation of LHRH receptors could deter onset of estrous cyclicity. However, it is improbable in this situation because heifers were never exposed to concentrations of LHRH necessary to elevate serum LHRH. Consequently, the mechanism responsible for reduced estrous cyclicity for the LHRH-infusion treatment is unexplained.

Exp. 2. Onset of puberty rests with timely endocrine changes because the hypothalamic-hypophyseal system becomes functional early in immature cattle and sheep. Prepubertal females responded to positive feedback of estradiol within weeks after birth in sheep (Foster and Ryan, 1979) and from 3 to 5 mo in cattle (Staigmiller et al., 1979). Prepubertal heifers at 3, 6, and 9 mo of age responded to 200 μg LHRH releasing LH and FSH (Barnes et al., 1980). Luteinizing hormone concentrations were elevated and more variable during the peripubertal period than after onset of puberty for heifers (Gonzalez-Padilla et al., 1975a) and ewe lambs (Ryan and Foster, 1980). Luteinizing hormone secretion immediately preceding puberty was characterized by an increased frequency of low amplitude pulses (Gonzalez-Padilla et al., 1975a; Ryan and Foster, 1980). Pulsatile LHRH stimulation of anterior pituitary initiated pulsatile LH release required for onset of puberty. Similar pattern of stimulation occurred when postpartum dairy cows resumed ovarian cyclicity (Stevenson and Britt, 1979; Carruthers and Hafs, 1980). Intermittent exogenous LHRH injections mimicked pulsatile LH release and successfully induced ovulation in rhesus monkeys (Knobil, 1980), lambs (Ryan and Foster, 1980), postpartum anestrous cows (Riley et al., 1981; Walters et al., 1982), and seasonally anestrous ewes (McNeilly et al., 1980; McLeod et al., 1982a,b). Intermittent low doses and continuous infusion of LHRH were utilized in the present study with the

objective of inducing puberty and earlier ovarian cyclicity.

Serum LH concentrations (table 5), LH pulse frequency (table 7), LH pulse amplitude (table 8) and LH pulse duration (table 10) were depicted for three treatments. Luteinizing hormone pulses for control heifers ranged from one to three pulses per 8-h window. Similarly, McLeod et al. (1984) observed one to four pulses per 24 h in prepubertal heifers. Pulsatile pattern of LH release in C heifers agreed with findings of Gonzalez-Padilla et al. (1975a) and Schams et al. (1981). Infused heifers demonstrated one to two pulses per 8-h period and three heifers exhibited preovulatory-like LH surges; two on d 2 and one on d 3. Luteinizing hormone concentration declined to baseline immediately following LH surges. Response of continuously infused heifers was consistent with studies in ewes. A question has emerged over the reliability of the dosage delivery by minipumps. Amundson and Wheaton (1979) found considerable disagreement in the theoretical and actual dosage of LHRH entering circulation. Nevertheless, they reported that continuous administration of LHRH induced ovulation in three of four seasonally anestrous ewes. In contrast, Knobil (1980) concluded that continuous infusion inhibited LH release, regardless of concentration, in arcuate-lesioned adult female rhesus monkeys.

The LHRH dosage rate utilized in this trial was derived from previous research. Pund and Amoss (1982) discovered that iv injections of 2.5 μg LHRH produced LH pulses of 2 to 6 ng/ml similar to naturally occurring pulses in prepubertal heifers. Pulse frequency of LH in anestrous suckled cows ranged from .25 to 1.25 pulses per h preceding first ovulation (Carruthers and Hafs, 1980; Peters et al., 1981). Riley et al. (1981) successfully found that the frequency of one LH pulse per 2 h in anestrous cows was sufficient for LH release. Pulsed heifers exhibited definitive LH pulses in response to intermittent 2.5 μg LHRH injections. Four of the six heifers responded to every injection. Of

the two nonresponding heifers, one responded to all but one LHRH pulse while the other heifer displayed a preovulatory-like LH surge during one injection masking any further response. Preovulatory-like LH surges occurred in two heifers on d 3 and d 4.

Pulsatile LH release induced by LHRH in P heifers was consistent with other studies in prepubertal heifers (McLeod et al., 1984), prepubertal bulls (Schanbacher, 1984), prepubertal lambs (Ryan and Foster, 1980), postpartum anestrous beef cows (Riley et al., 1981; Walters et al., 1982; Edwards et al., 1983), and seasonally anestrous ewes (McNeilly et al., 1980; McLeod et al., 1982a,b). Luteinizing hormone surges in heifers B24 (figure 1d) and Y17 (figure 6c) paralleled results in prepubertal lambs (Ryan and Foster, 1980), anestrous ewes (McNeilly et al., 1980;McLeod et al., 1982a,b) and post partum cows (Riley et al., 1981; Walters et al., 1982; Edwards, 1983). Amplitude (Pund and Amoss, 1982) and duration (McLeod et al., 1984) of LH pulses in P heifers were similar to previous observations.

Follicle stimulating hormone maintained constant levels during prepubertal to pubertal transition in heifers (Gonzalez-Padilla, 1975a) and ewe lambs (Foster and Karsch, 1975). Serum FSH concentrations were not affected by treatment regimens in this study. Mean levels of FSH were similar to concentrations previously reported in heifers (Gonzalez-Padilla et al., 1975a; Schams et al., 1981). Similarity of response for LHRH-treated heifers indicated that LHRH first suppressed and then increased serum FSH.

Dosage of LHRH may have been insufficient for stimulating pulsatile FSH release in P heifers or FSH pulses were of shorter duration than 1 h and missed by the sampling schedule used for FSH. Dosage of LHRH also failed to significantly elevate FSH for I heifers. Pulsatile mode of FSH secretion was not apparent in any heifer and random fluctuations of FSH agreed with data in

other LHRH-pulsed heifers (McLeod et al., 1984), LHRH-pulsed anestrous cows (Riley et al., 1981), and prepubertal heifers (Gonzalez-Padilla et al., 1975a). Luteinizing hormone-releasing hormone can stimulate FSH release in dairy cows (Foster et al., 1980) and prepubertal heifers (Barnes et al., 1980), but more than 200 µg LHRH was required. Reduced FSH response to LHRH administration occurred after 5 mo of age in bull and heifer calves (Schams et al., 1981). A characteristic decline and rise in FSH appeared to occur for I heifers during their LH surges.

Serum estradiol-17ß concentration has been observed to remain constant in heifers in days preceding estrus with no distinct rise associated with LH peaks (Gonzalez-Padilla et al., 1975a). However, ewes and gilts displayed increased serum concentrations of estradiol-17ß approaching puberty (Ryan and Foster, 1980; Diekman and Trout, 1984). Prepubertal heifers, 3, 6, or 9 mo of age, injected iv with 200 µg LHRH failed to release estradiol-17ß coincident with LH surges (Barnes et al., 1980). In this study, distinct differences in estradiol-17ß were evident. The LHRH-treated heifers demonstrated higher serum estradiol-17ß concentrations than controls and heifers exhibiting surges had elevated serum estradiol as compared with nonsurging counterparts. These results indicated LHRH-induced estradiol release and serum estradiol concentrations peaked during preovulatory-like LH surges.

Serum cortisol levels were similar among treatments with elevated levels on d 1, possibly corresponding to initial stress of blood sampling and minipump implantation for I heifers. Even though heifers were maintained in box stalls for 3 d prior to treatment, elevated cortisol was apparent on d 1. Cortisol declined thereafter returning to baseline by d 2. No correlation existed between cortisol levels and the number of heifers with preovulatory-like LH surges. Diekman and Trout (1984) reported that cortisol levels were similar between gilts approaching

puberty and those exhibiting pubertal estrus.

Serum progesterone was expectedly low during treatment. Rises of progesterone within 10 d of treatment occurred more frequently in LHRH-treated (P and I) heifers suggesting possible luteinization of ovarian follicles. Number of heifers cycling within the 53-d period showed similarity between control (75%) and pulsed (83%) heifers with a distinct reduction in I (45%) heifers. Amundson and Wheaton (1979) reported that continuous infusion of LHRH in ewes for 4 wk decreased number of ovarian follicles greater than 2 mm in diameter. Continuous infusion could have minimized development of larger ovarian follicles in I heifers and consequently delayed onset of puberty. Also, serum FSH levels tended to be reduced in I heifers compared to C and P heifers during treatment and may have resulted in decreased follicular growth. Pulsed heifers failed to show a decline in FSH levels suggesting that administration of LHRH mimicked natural conditions and therefore, did not inhibit follicular growth or serum FSH levels. Consequently, resulting estrous cyclicity was not hindered.

Age and weight at puberty have been reported for Hereford-Angus crosses (357 d, 282.7 kg) and Brahman crosses (429 d, 323.6 kg) heifers (Gregory et al., 1982). Heifers used in this study were generally lighter than the average pubertal weights. Four H and one B x H demonstrated LH surges during treatment. Hereford heifers that had LH surges were similar in weight to those without LH surges. The only responding B heifer was heavier and probably closer to puberty (321 kg) than herdmates in P (275 kg) and C (267 kg) groups.

Results of two experiments confirmed the ability of LHRH to induce LH release when administered iv or sc. Dose response to LHRH was evident as 500 ng per 2 h in Exp. 1 failed to consistently trigger LH release, but release was induced in response to 2.5 µg LHRH per 2 h in Exp. 2. Continuous infusion of

LHRH did not increase LH pulses; nevertheless, three heifers from this group exhibited preovulatory-like LH surges. Two heifers in the P treatment also had LH surges. Presence of preovulatory-like LH surges in both P and I groups of Exp. 2 suggested that both continuous and intermittent administration of LHRH were capable of inducing LH surges. Elevated serum estradiol-17β concentrations were apparent during LH surges and LHRH-treated groups had higher estradiol levels than controls. Serum FSH levels were similar among all three groups and pulsatile release of FSH was not detected in the sampling protocol employed. Progesterone rises within 10 d were prevalent in groups after LHRH treatment. Progesterone rises suggested luteinizaton of ovarian follicles in response to the treatment regimen. Interestingly, in both trials, estrous cycle results suggested an inhibitory effect of continuous LHRH administration on cyclicity. Continuous mode of LHRH presentation possibly depressed follicular growth enough to delay onset of puberty. Although the pituitary was responsive to LHRH, LHRH stimulus failed to induce earlier estrous cycles.

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APPENDICES

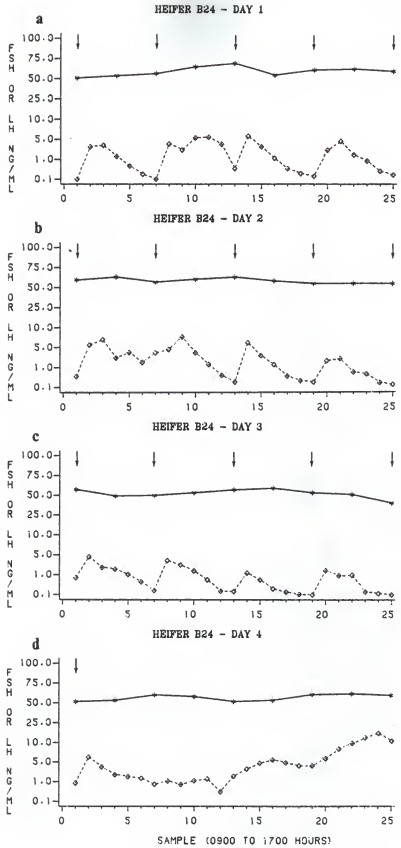

Figure 1. Serum FSH (*——*, 1-h intervals) and LH (◇---◇ , 20-min intervals) concentrations for heifer B24. Blood was collected for 8 h on each of 4 d. Arrows indicate LHRH administration (2.5 μg, iv) every 2 h for 72 h.

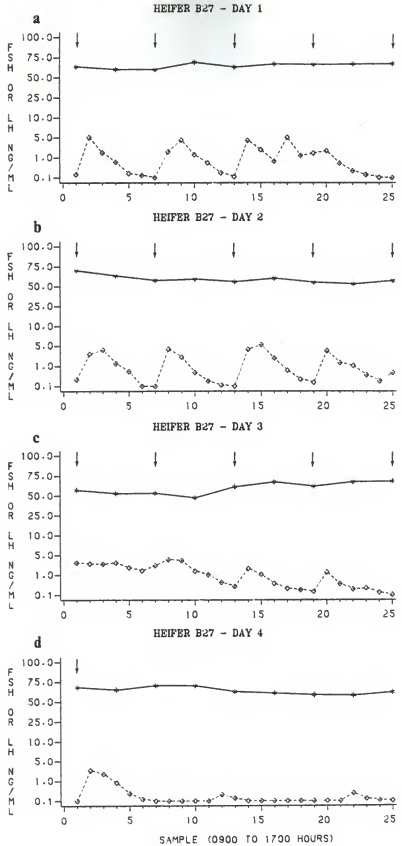

Figure 2. Serum FSH (*——*, 1-h intervals) and LH (◇----◇ , 20-min intervals) concentrations for heifer B27. Blood was collected for 8 h on each of 4 d. Arrows indicate LHRH administration (2.5 μg, iv) every 2 h for 72 h.

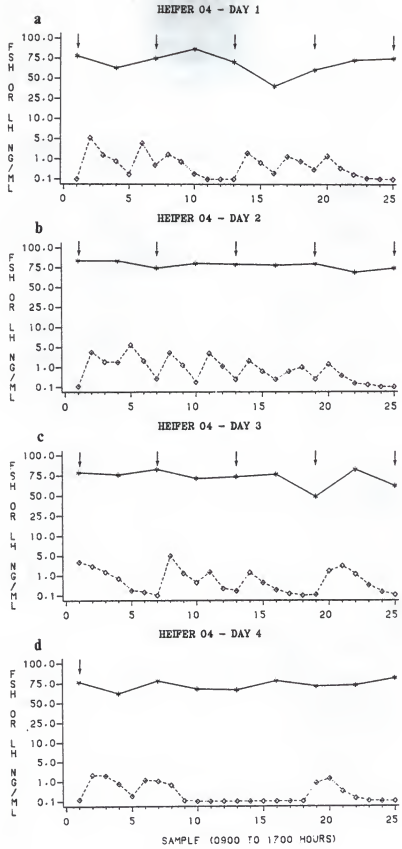

Figure 3. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer O4. Blood was collected for 8 h on each of 4 d. Arrows indicate LHRH administration (2.5 µg, iv) every 2 h for 72 h.

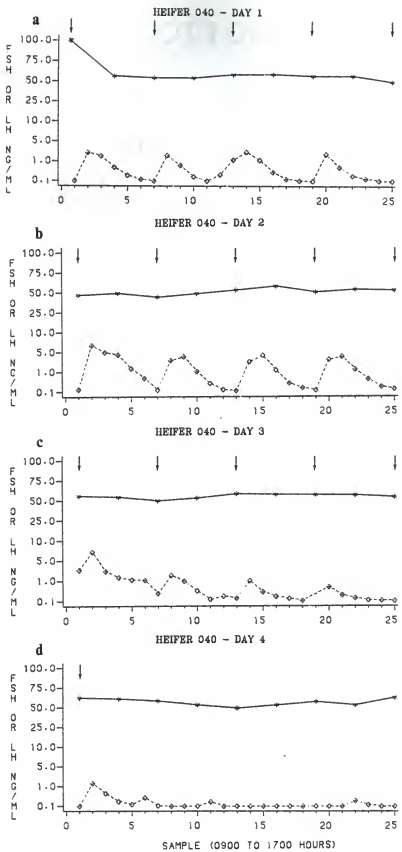

Figure 4. Serum FSH (*——*, 1-h intervals) and LH (◇---◇, 20-min intervals) concentrations for heifer O40. Blood was collected for 8 h on each of 4 d. Arrows indicate LHRH administration (2.5 µg, iv) every 2 h for 72 h.

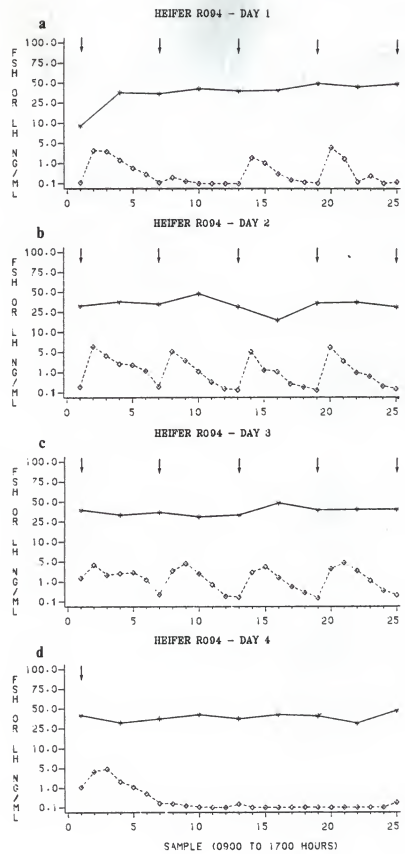

Figure 5. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer RO94. Blood was collected for 8 h on each of 4 d. Arrows indicate LHRH administration (2.5 μg, iv) every 2 h for 72 h.

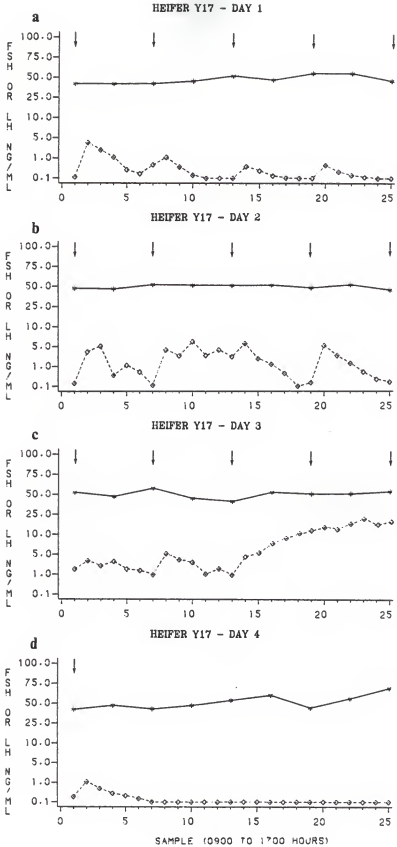

Figure 6. Serum FSH (*——*, 1-h intervals) and LH (◇---◇, 20-min intervals) concentrations for heifer Y17. Blood was collected for 8 h on each of 4 d. Arrows indicate LHRH administration (2.5 μg, iv) every 2 h for 72 h.

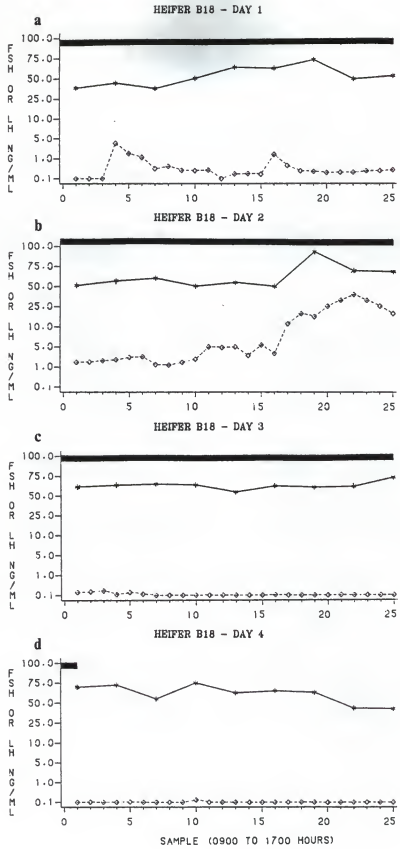

Figure 7. Serum FSH (*——*, 1-h intervals) and LH (◇---◇, 20-min intervals) concentrations for heifer B18. Blood was collected for 8 h on each of 4 d. Continuous bar indicates period of LHRH infusion (1.25 μg/h, sc).

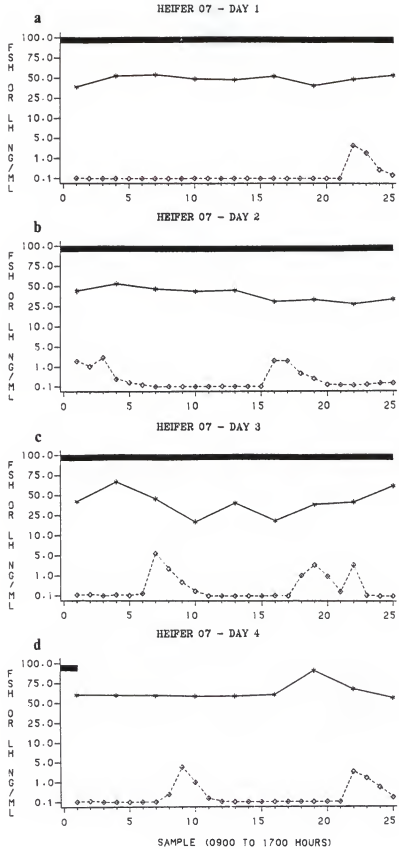

Figure 8. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer 07. Blood was collected for 8 h on each of 4 d. Continuous bar indicates period of LHRH infusion (1.25 μg/h, sc).

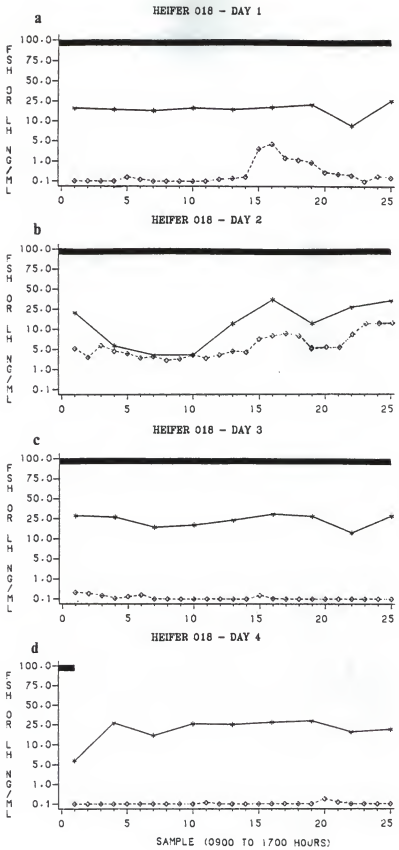

Figure 9. Serum FSH (*------*, 1-h intervals) and LH (◇---◇, 20-min intervals) concentrations for heifer 018. Blood was collected for 8 h on each of 4 d. Continuous bar indicates period of LHRH infusion (1.25 µg/h, sc).

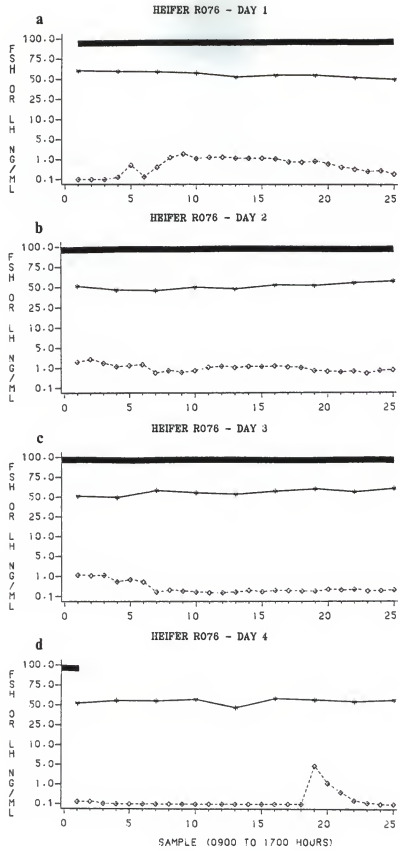

Figure 10. Serum FSH (*-----*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer RO76. Blood was collected for 8 h on each of 4 d. Continuous bar indicates period of LHRH infusion (1.25 μg/h, sc).

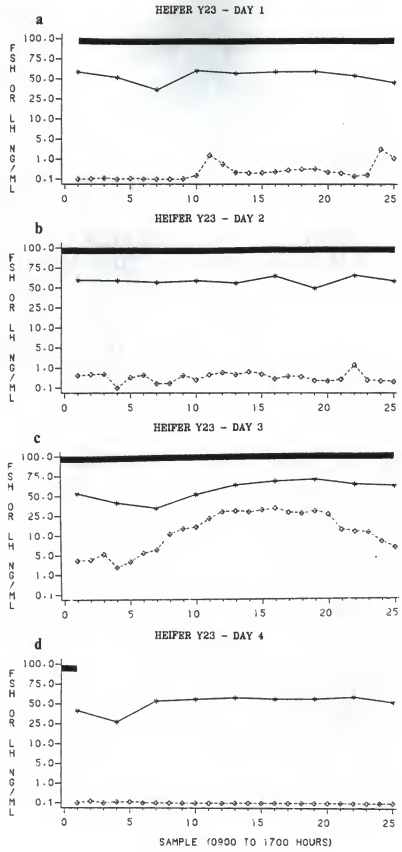

Figure 11. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer Y23. Blood was collected for 8 h on each of 4 d. Continuous bar indicates period of LHRH infusion (1.25 µg/h, sc).

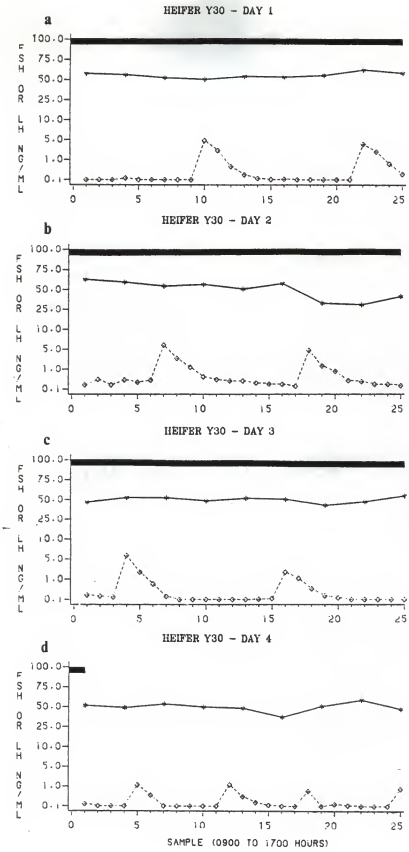

Figure 12. Serum FSH (*——*, 1-h intervals) and LH (◇---◇, 20-min intervals) concentrations for heifer Y30. Blood was collected for 8 h on each of 4 d. Continuous bar indicates period of LHRH infusion (1.25 μg/h, sc).

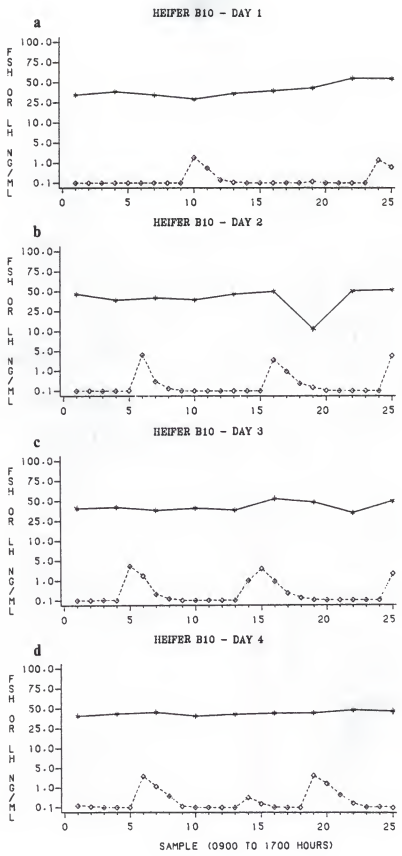

Figure 13. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer B10. Blood was collected for 8 h on each of 4 d.

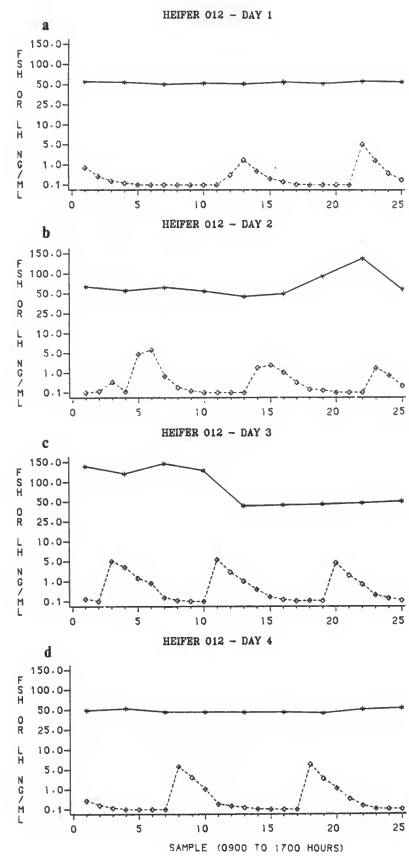

Figure 14. Serum FSH (*-----*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer O12. Blood was collected for 8 h on each of 4 d.

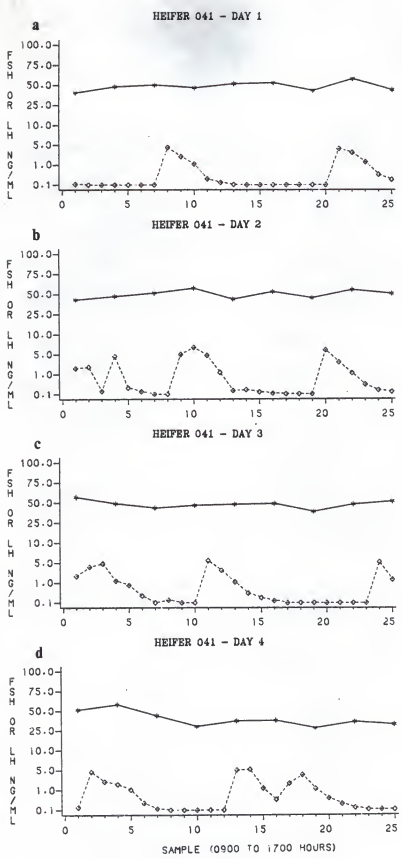

Figure 15. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer 041. Blood was collected for 8 h on each of 4 d.

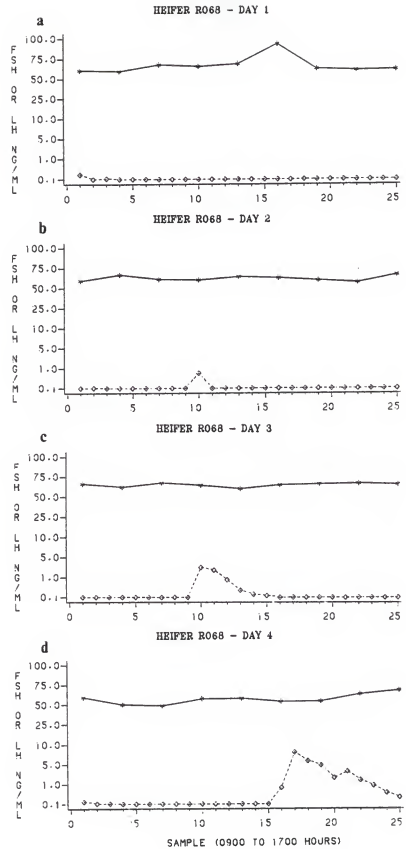

Figure 16. Serum FSH (*——*, 1-h intervals) and LH (◇---◇, 20-min intervals) concentrations for heifer RO68. Blood was collected for 8 h on each of 4 d.

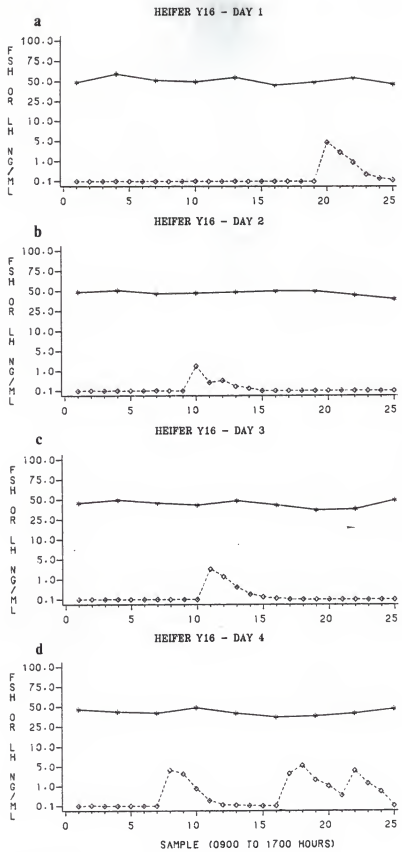

Figure 17. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer Y16. Blood was collected for 8 h on each of 4 d.

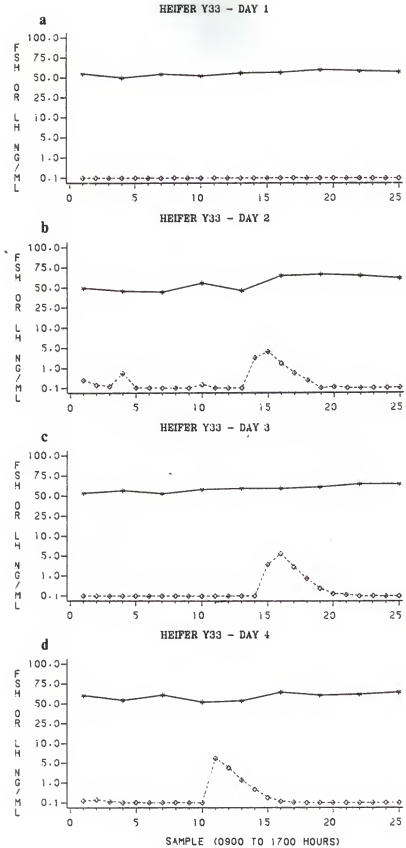

Figure 18. Serum FSH (*——*, 1-h intervals) and LH (◇----◇, 20-min intervals) concentrations for heifer Y33. Blood was collected for 8 h on each of 4 d.

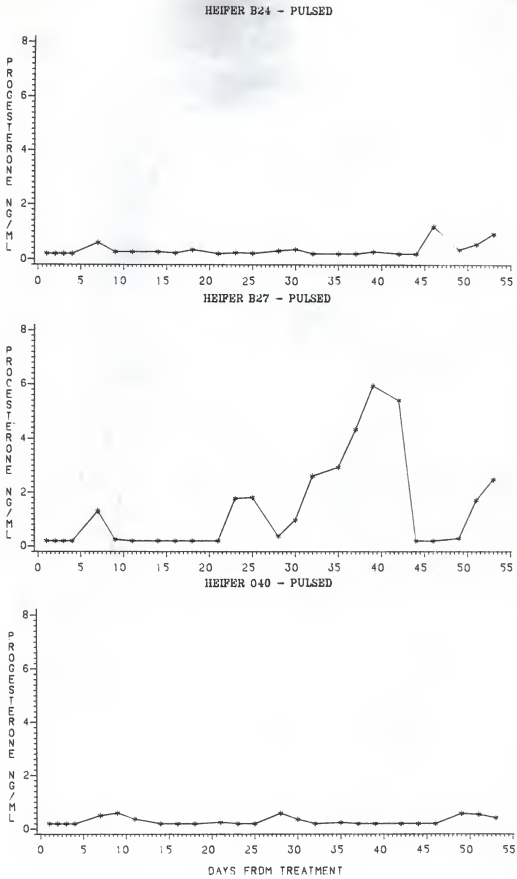

Figure 19. Serum progesterone concentrations in three LHRH-pulsed heifers (B24, B27 and 040) from d 1 (onset of treatment) to d 53.

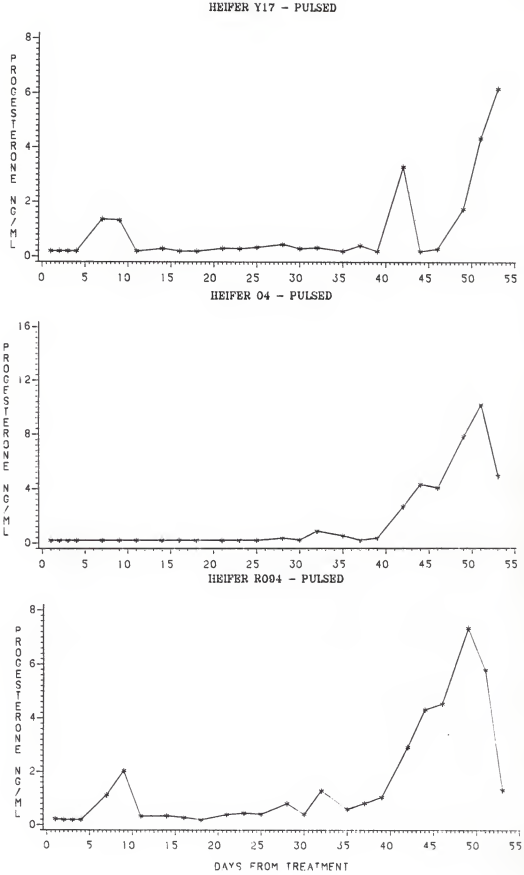

Figure 20. Serum progesterone concentrations in three LHRH-pulsed heifers (Y17, 04 and R094) from d 1 (onset of treatment) to d 53.

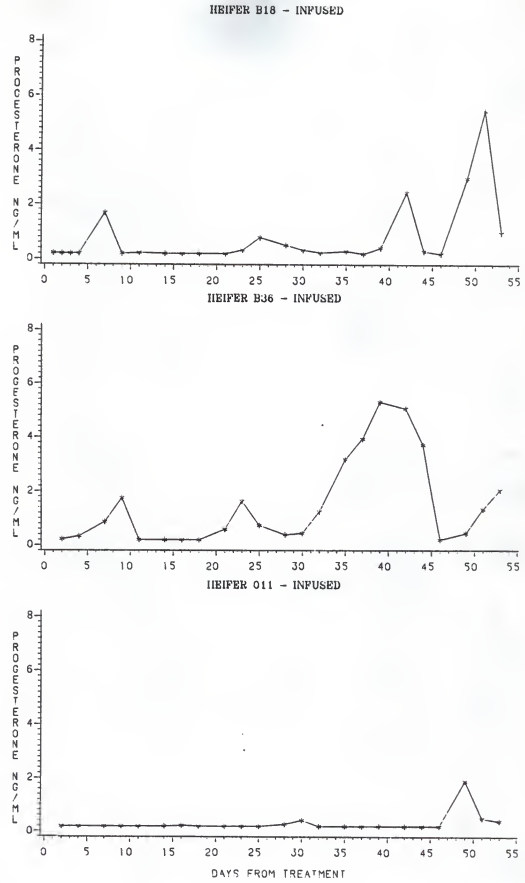

Figure 21. Serum progesterone concentrations in three LHRH-infused heifers (B18, B36 and 011) from d 1 (onset of treatment) to d 53.

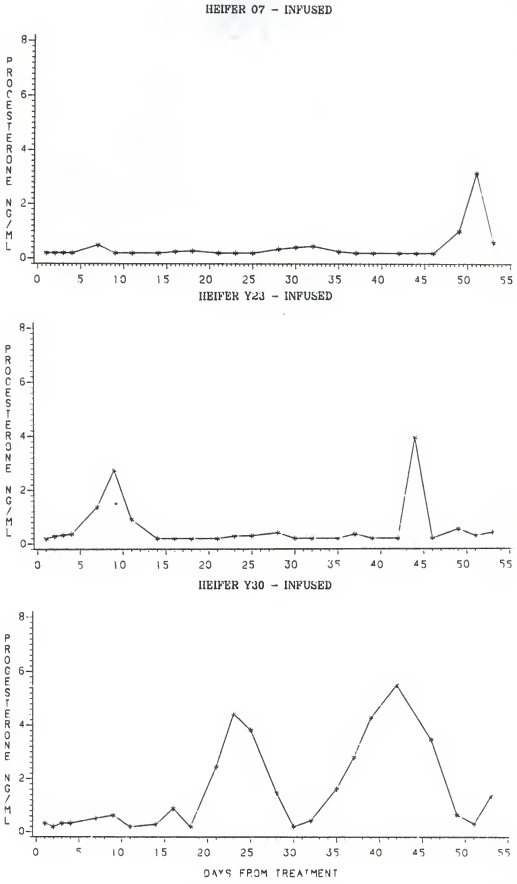

Figure 22. Serum progesterone concentrations in three LHRH-infused heifers (07, Y23 and Y30) from d 1 (onset of treatment) to d 53.

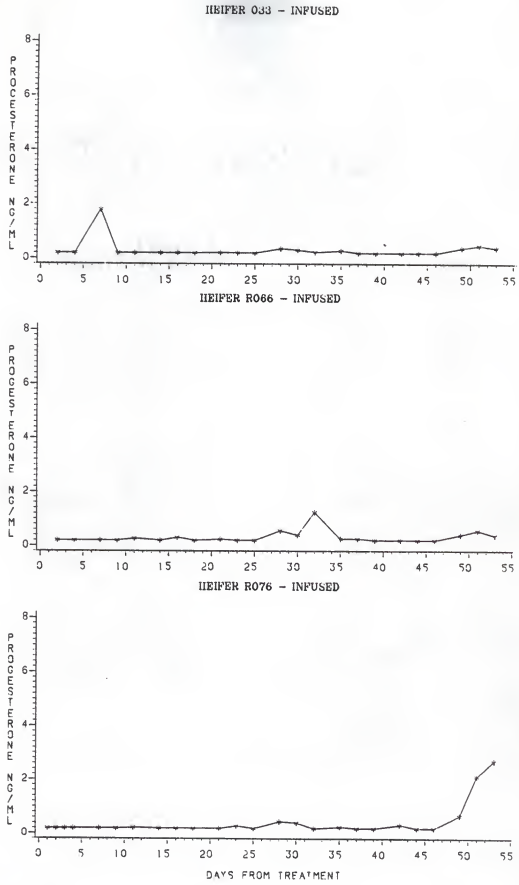

Figure 23. Serum progesterone concentrations in three LHRH-infused heifers (033, R066 and R076) from d 1 (onset of treatment) to d 53.

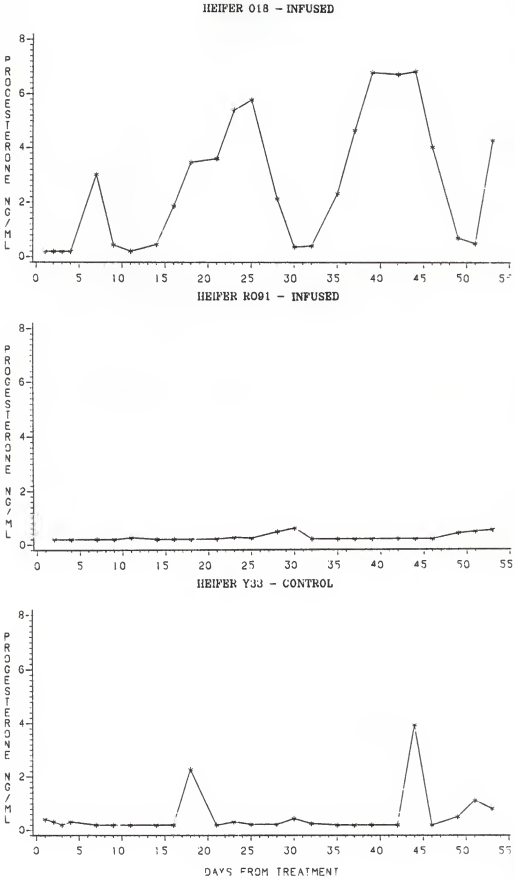

Figure 24. Serum progesterone concentrations in two LHRH-infused (018 and R091) and one Control (Y33) from d 1 (onset of treatment) to d 53.

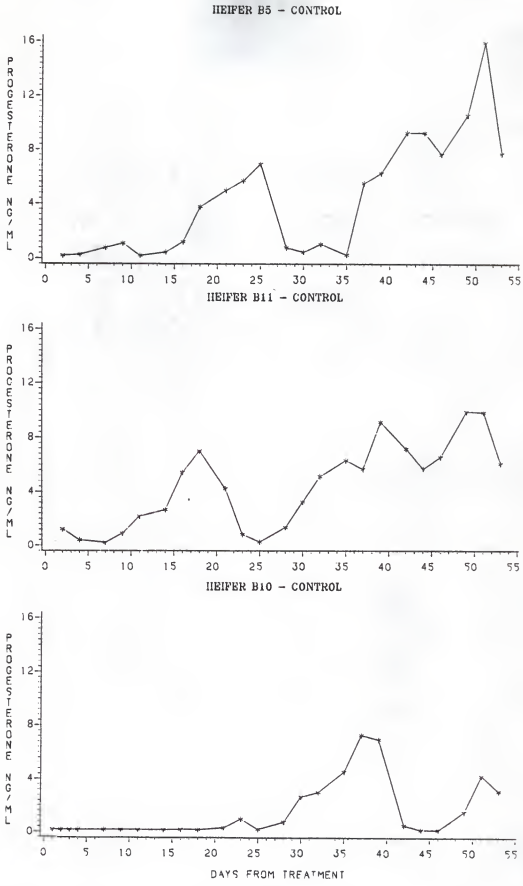

Figure 25. Serum progesterone concentrations in three Control heifers (B5, B11 and B10) from d 1 (onset of treatment) to d 53.

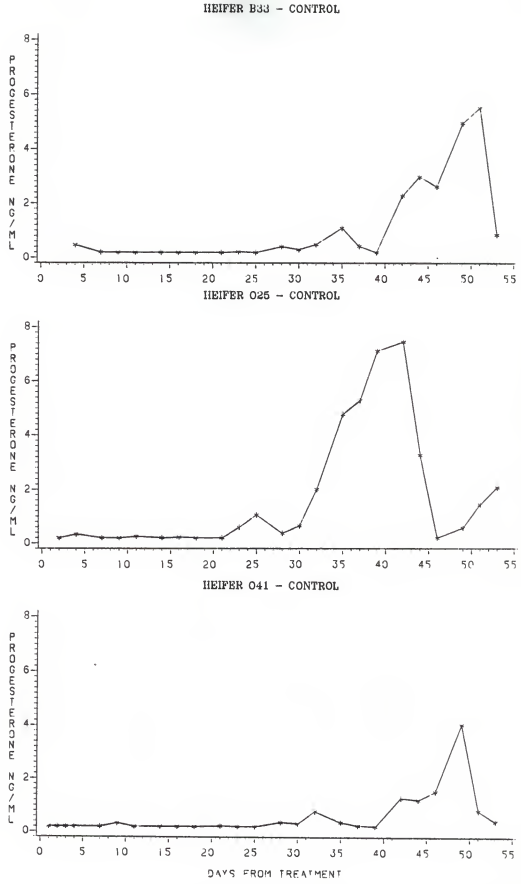

Figure 26. Serum progesterone concentrations in three Control heifers (B33, 025 and 041) from d 1 (onset of treatment) to d 53.

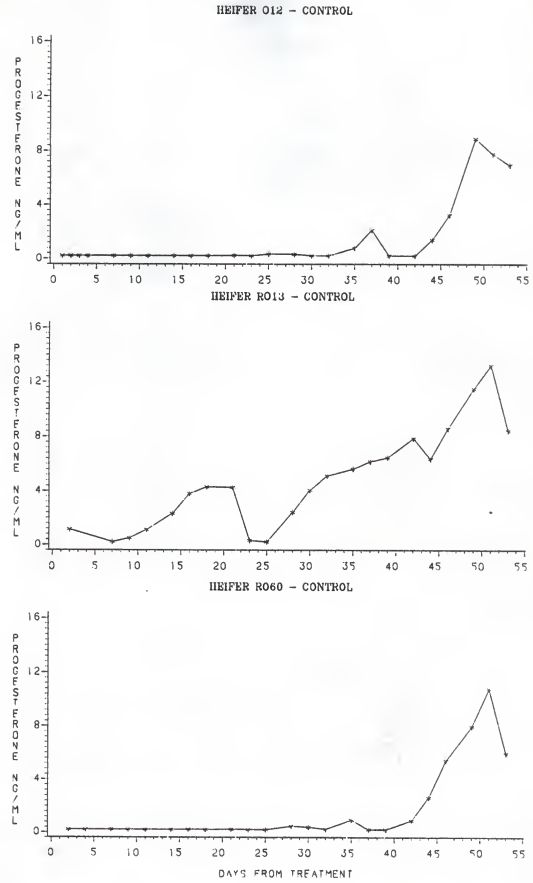

Figure 27. Serum progesterone concentrations in three Control heifers (012, R013 and R060) from d 1 (onset of treatment) to d 53.

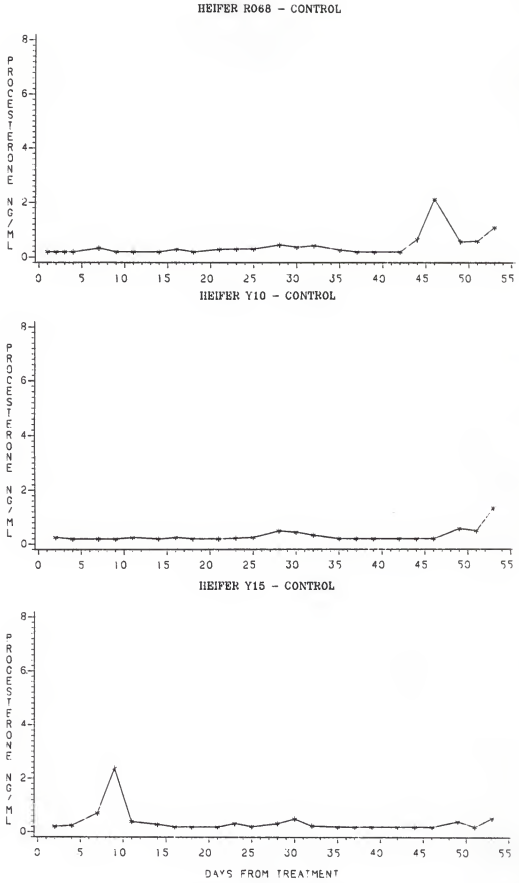

Figure 28. Serum progesterone concentrations in three Control heifers (R068, Y10 and Y15) from d 1 (onset of treatment) to d 53.

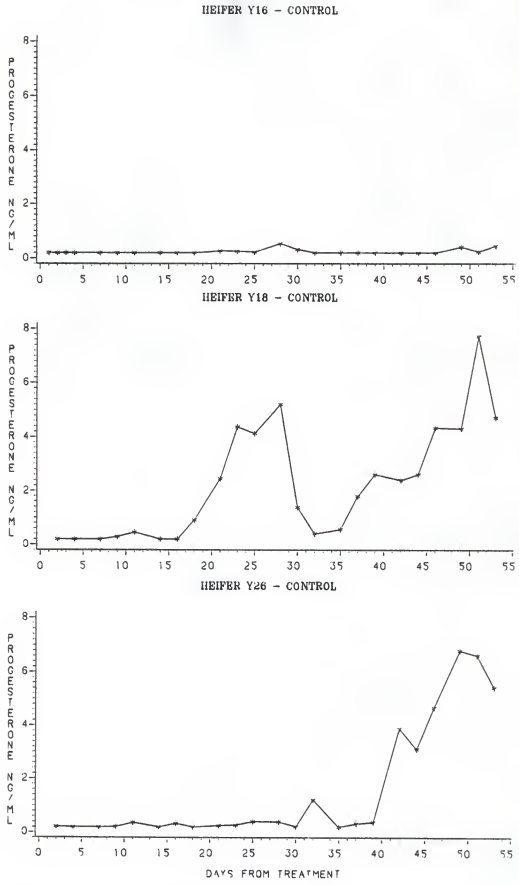

Figure 29. Serum progesterone concentrations in three Control heifers (Y16, Y18 and Y26) from d 1 (onset of treatment) to d 53.

ATTEMPTS TO INDUCE PUBERTY IN BEEF HEIFERS WITH LUTEINIZING HORMONE-RELEASING HORMONE

By

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Two experiments were conducted to determine if low doses of exogenous LHRH administered intermittently (iv) or by continuous infusion (sc) could induce gonadotropin release and hasten estrous cyclicity in prepubertal heifers. Heifers in Exp. 1 were assigned randomly to be pulsed (P, n=8), infused (I, n=7), or controls (C, n=7). Pulsed heifers received iv injections of 500 ng LHRH every 2 h for 96 h. Infused heifers were implanted sc with Alzet® osmotic minipumps which delivered LHRH (250 ng/h) by continuous infusion. One-half (n=4) of control heifers received iv injections of physiological saline every 2 h for 96 h and the remainder were untreated. Blood was collected at 30-min intervals for 2 h (0600 to 0800 h) from five P, five I, and four C heifers on d 1 to d 5. Luteinizing hormone concentrations (LH) and LH pulse frequencies were similar among treatments. Greater proportion ($P<.10$) of C (86%) and P (88%) heifers exhibited estrous cycles during 45 d than I (43%) heifers.

Exp. 2 followed similar procedures outlined for Exp. 1. Pulsed heifers (n=6) were subjected to 2.5 µg LHRH at 2-h intervals for 72 h, I heifers (n=11) received continuous infusion of LHRH (1.25 µg/h for 72 h), and C heifers (n=16) were left untreated. Blood was collected at 20-min intervals for 8 h (0900 to 1700 h) on d 1 to d 4. Heifers treated with LHRH (I=1.8 $\pm$ .4 ng/ml and P=1.5 $\pm$ .4 ng/ml) had elevated ($P<.01$) LH concentrations over C heifers (.7 $\pm$ .4 ng/ml). Preovulatory-like LH surges occurred in three I heifers and two P heifers. Pulse frequencies of LH (no. pulses per 8-h window) were greater ($P<.001$) for P heifers (3.5 $\pm$ .1) than for I (1.2 $\pm$ .1) or C (1.8 $\pm$ .1) heifers due to pulsatile LHRH stimulation. Mean FSH concentrations were similar among three treatments and episodic release of FSH was not observed. Serum estradiol-17β levels (pg/ml) were elevated ($P<.01$) in I (3.3 $\pm$ .8) and P (4.1 $\pm$ .8) heifers than

for C (2.3 $\pm$ .8) heifers. Heifers exhibiting LH surges had higher ($P<.001$) estradiol-17ß concentrations than those without LH surges. Serum cortisol concentrations were similar among treatments. Peak values of cortisol occurred on d 1 but declined ($P<.001$) to baseline by d 2. Progesterone was low and similar for all heifers during 4 d of treatment. Characteristic progesterone rises (serum progesterone concentration exceeding 1 ng/ml with 2 or more d duration) occurred within 10 d in more ($P<.05$) LHRH-treated heifers (I=45%, P=33%) than C (6%) heifers. Days to first observed estrus and first ovulation were similar among treatments. A tendency ($P=.12$) existed for more heifers in groups C (75%) and P (83%) to cycle within the 53-d period than I heifers (45%).

These results suggest that LHRH successfully induced LH and estradiol-17ß release as well as preovulatory-like LH surges in some heifers, but failed to initiate earlier estrous cyclicity. Constant infusion of LHRH appeared to delay onset of puberty in heifers.

KEY WORDS: LHRH, LH, FSH, Estradiol-17ß, Cortisol, Progesterone, Puberty