**MONTANA STATE UNIVERSITY**

The influence of biostimulation on the occurrence of puberty in beef heifers
by Mark Stephen Roberson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
Two experiments were conducted to determine the influence of biostimulation on the occurrence of puberty in beef heifers. In Experiment I the objective was to determine if prostaglandin F2α (PGF2α ; 25 mg/head) and/or biostimulation and social interaction of prepuberal heifers with a group of estrous heifers (n=49) could induce first estrus in prepuberal heifers. Angus (A), Hereford (H) and AxH heifers (n=372), approximately 13 months of age,- were stratified within breed by weight to the following treatment groups: I) heifers injected with PGF2α and isolated from estrous heifers (n=91); 2) heifers injected with PGF2α and placed with estrous heifers (n=97); 3) heifers injected with .9% saline and isolated (n=95); and 4) heifers injected with .9% saline and placed with estrous heifers (n=8 9). Isolation groups were placed .5 km away from groups containing estrous females. Heifers were observed for behavioral estrus twice daily fbr 21 days after initiation of treatments. Percentages of heifers exhibiting first estrus within the 21-day observation period were: 26 and 26% for PGF2α and saline- treated groups, respectively (P > .05), and 23 and 28% for biostimulation with estrous heifers and isolation groups, respectively (P > .05). There was no interaction among treatments (P > .05). In Experiment II, the objective was to determine if biostimulation and social interaction with mature bulls would alter the occurrence, age and weight at puberty in beef heifers. Angus (A), Hereford (H), Simmental (S)xA, SxH, Tarentaise (T)xA and TxH heifers (n=109) were stratified within breed by age, weight and origin of heifer and randomly allotted into the following treatments: 1) heifers exposed to the presence of mature bulls (n=54); and 2) heifers isolated from the presence of mature bulls (n=55). Biostimulation group male to female ratio was 1:27. Biostimulation heifers were separated from isolation heifers by .5 km. Heifers were observed for behavioral estrus twice daily for a 152 day observation period. Puberty was defined by three criterion: I) behavioral estrus; 2) presence of a palpable corpus luteum (CL); and 3) rise in serum progesterone above 1 ng/ml. There were no differences (P > .10) between treatments for the proportion of heifers reaching puberty by 11, 12, 13, 14, and 15 months of age. The percentages of heifers reaching puberty were 84.0 and 88.9% for the biostimulation and isolation groups, respectively (P > .10). Age and weight at puberty were 366.7 days and 291.9 kg and 367.1 days and 293.6 kg for the biostimulation and isolation groups, respectively (P > .10). The results from Experiment I indicate that neither PGF2α nor biostimulation of prepuberal heifers, with a group of estrous heifers, induced first estrus in prepuberal beef heifers. The results from Experiment II indicate that biostimulation from mature bulls did not alter the occurrence of puberty or the age or weight at puberty in prepuberal beef heifers.

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APPROVAL

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Mark Stephen Roberson

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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# VITA

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TO MY FOLKS

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# ABSTRACT

Two experiments were conducted to determine the influence of biostimulation on the occurrence of puberty in beef heifers. In Experiment I the objective was to determine if prostaglandin $F_2\alpha$ ($PGF_2\alpha$ ; 25 mg/head) and/or biostimulation and social interaction of prepuberal heifers with a group of estrous heifers (n=49) could induce first estrus in prepuberal heifers. Angus (A), Hereford (H) and AxH heifers (n=372), approximately 13 months of age, were stratified within breed by weight to the following treatment groups: 1) heifers injected with $PGF_2\alpha$ and isolated from estrous heifers (n=91); 2) heifers injected with $PGF_2\alpha$ and placed with estrous heifers (n=97); 3) heifers injected with .9% saline and isolated (n=95); and 4) heifers injected with .9% saline and placed with estrous heifers (n=89). Isolation groups were placed .5 km away from groups containing estrous females. Heifers were observed for behavioral estrus twice daily for 21 days after initiation of treatments. Percentages of heifers exhibiting first estrus within the 21-day observation period were: 26 and 26% for $PGF_2\alpha$ and saline-treated groups, respectively (P > .05), and 23 and 28% for biostimulation with estrous heifers and isolation groups, respectively (P > .05). There was no interaction among treatments (P > .05). In Experiment II, the objective was to determine if biostimulation and social interaction with mature bulls would alter the occurrence, age and weight at puberty in beef heifers. Angus (A), Hereford (H), Simmental (S)xA, SxH, Tarentaise (T)xA and TxH heifers (n=109) were stratified within breed by age, weight and origin of heifer and randomly allotted into the following treatments: 1) heifers exposed to the presence of mature bulls (n=54); and 2) heifers isolated from the presence of mature bulls (n=55). Biostimulation group male to female ratio was 1:27. Biostimulation heifers were separated from isolation heifers by .5 km. Heifers were observed for behavioral estrus twice daily for a 152 day observation period. Puberty was defined by three criterion: 1) behavioral estrus; 2) presence of a palpable corpus luteum (CL); and 3) rise in serum progesterone above 1 ng/ml. There were no differences (P > .10) between treatments for the proportion of heifers reaching puberty by 11, 12, 13, 14, and 15 months of age. The percentages of heifers reaching puberty were 84.0 and 88.9% for the biostimulation and isolation groups, respectively (P > .10). Age and weight at puberty were 366.7 days and 291.9 kg and 367.1 days and 293.6 kg for the biostimulation and isolation groups, respectively (P > .10). The results from Experiment I indicate that neither $PGF_2\alpha$ nor biostimulation of prepuberal heifers, with a group of estrous heifers, induced first estrus in prepuberal beef heifers. The results from Experiment II indicate that biostimulation from mature bulls did not alter the occurrence of puberty or the age or weight at puberty in prepuberal beef heifers.

## INTRODUCTION

In current beef management practices, the onset of puberty in heifers is of physiologic and economic importance. Beef heifers must reach puberty, ovulate, conceive, and become pregnant by 15 months of age if they are to calve at 24 months of age. Heifers that calve at 24 months of age have the potential for greater lifetime productivity, while heifers that become pregnant and calve early in the calving season are allowed a longer post-partum period and tend to calve earlier in subsequent production years (Lesmeister et al., 1973).

Various factors such as genotype of the female, adequate nutrition and other environmental influences play a critical role in the puberal process and the event of first behavioral estrus. The manipulation of some environmental influences that may alter the occurrence of puberty in beef heifers may be of value to the producer. A greater proportion of heifers reaching puberty prior to the breeding season would allow greater use of estrous synchronization programs and the potential for more heifers to become pregnant early in the breeding season.

# REVIEW OF LITERATURE

Many researchers have used different criteria for determining age at puberty in beef heifers. Joubert (1963) defined puberty in the female of domestic ruminant species as the occurrence of first estrus accompanied by ovulation and the potential to reproduce. The criteria used to identify puberty range from first observed behavioral estrus, (Kaltenbach and Wiltbank, 1962; Laster et al., 1972; 1976; 1979; Swierstra et al., 1977; Gregory et al., 1979; Steffen, et al., 1983), to periodic ovarian palpations per rectum to confirm the presence of a corpus luteum (CL) (Arije and Wiltbank, 1971) to more complex criteria incorporating radioimmunoassay for steriod and gonadotropin hormones (Gonzalez-Padilla et al., 1975a; Berardinelli, 1976).

Current information indicates that first observed behavioral estrus or markings of grease paint over the rump and tail head, may be inadequate in establishing the occurrence of puberty. Rutter and Randel (1982) observed that first behavioral estrus in some Brahman and Brahman crossbred heifers was not accompanied by ovulation or luteal formation. T. C. Nelsen (unpublished data) observed this phenomenon in crossbred beef heifers and termed it non-puberal estrus (N.P.E.), i.e., estrous behavior without overt ovarian activity. The fertility of N.P.E. and its relationship to the onset of puberty in beef heifers is unknown. Given that N.P.E. may or may not be an

abnormal phenomenon the use of first behavioral estrus, even with periodic palpations, may be inadequate as a criterion for puberty in heifers.

Recent studies of puberty in heifers use more complex combinations of data in an effort to reduce any bias caused by N.P.E. or other unexplained phenomena. Studies by Gonzalez-Padilla et al. (1975a) and Berardinelli (1976) utilized three specific criteria for establishing age at puberty in heifers. These criteria were: 1) observed behavioral estrus, 2) the presence of a palpable CL, and 3) a rise in serum progesterone above 1 ng/ml. Utilizing these three criteria, researchers have gained considerable confidence in assigning an age at puberty in beef heifers.

Regardless of formal definition, a complete understanding of that process of physiologic development known as puberty, has long been elusive to researchers. Many factors are known to influence the onset of puberty. These factors are associated with genetic and environmental influences. The following sections will discuss factors known to influence the onset of puberty in beef heifers.

## Genetic Influences

Breed Effect. One of the first researchers to review breed differences in age at puberty in beef heifers was Joubert (1963). Since then, age and weight at puberty in beef and dairy heifers have been observed and reported in many breeds in an attempt to characterize genotypic differences. Table 1 lists several breeds of cattle and the mean age and weight at puberty of females in each breed.

Table 1. Age and weight at puberty in several breeds of <u>bos</u>

| Breed | Author | Age at Puberty[a] | Weight at Puberty[b] |
|---|---|---|---|
| Afrikander | Joubert, 1963 | 645.2±41.9 | -- |
| Angus | Gregory et al., 1978 | 364.6±6.3 | 275.6±4.0 |
| | Stewart et al., 1980 | 385±14 | 225±8 |
| | Laster et al., 1972 | 372.2±10.0 | 273.7±8.4 |
| Brahman | Stewart et al., 1980 | 479±14 | 299±8 |
| Charolais X[c] | Laster et al., 1976 | 398±7 | 303±5 |
| Friesian | Joubert, 1963 | 401.0±50.9 | -- |
| Hereford | Arije & Wiltbank, 1971 | 436.4±32.53 | 250.9±26.35 |
| | Laster et al., 1976 | 389.5±12.9 | 269.2±11.0 |
| | Stewart et al., 1980 | 454±10 | 235±5 |
| | Gregory et al., 1978 | 397.2±7.2 | 272.9±4.6 |
| | Burfening et al., 1979 | 385±3 | 296±2 |
| | Steffen, 1983 | 391.54±4.33 | 296.78±4.02 |
| Holstein | Stewart et al. 1980 | 385±14 | 225±8 |
| Jersey | Joubert, 1963 | 359.6±42.8 | -- |
| | Stewart et al., 1980 | 387±19 | 167±11 |
| Limousin X | Laster et al., 1976 | 398±6 | 292±4 |
| Shorthorn | Joubert, 1963 | 336.5±52.4 | -- |
| | Arije and Wiltbank, 1974 | 372.9±49.0 | 235.4±28.2 |
| Simmental X | Laster et al., 1976 | 372±6 | 286±4 |
| Tarentaise | Gregory et al., 1979 | 318±6.3 | 296±4.9 |

[a] represents days ± S.E.M.
[b] represents kg ± S.E.M.
[c] represents breed of sire; dams were either Angus or Hereford

Hereford and Angus are the predominant breeds in which puberal studies have been conducted. Arije and Wiltbank (1971) reported the age and weight at puberty for Hereford heifers. Using age at first behavioral estrus and the presence of a corpus luteum confirmed by 28-day rectal palpation, the mean age and weight at puberty were 436.4 days and 250.9 kg. Laster et al., (1972) reported that age and weight at puberty in Angus heifers, based on first observed behavioral estrus, was 372.2 days and 273.7 kg, respectively. Also, they indicated that fewer straightbred Herefords reached puberty by 15 months of age than straightbred Angus. This was in agreement with Wiltbank et al. (1966) who reported that straightbred Angus heifers attained puberty at a younger age than straightbred Hereford heifers.

Perhaps the most striking breed difference in age at puberty is among breeds of bos taurus and bos indicus. Afrikander and Brahman heifers reach puberty much later than bos taurus breeds (Table 1). Plasse (1968) reported that Brahman heifers exhibited their first CL and presumably first estrus, between 18.9 and 21.3 months of age. This was in agreement with Reynolds et al. (1963) who reported that Brahman and Brahman X Angus heifers reached puberty at 816 and 460 days of age, respectively; straightbred Angus heifers reached puberty at 433 days of age in that study. Results from Gregory et al. (1979) were in agreement with Reynolds et al. (1963) and they reported that bos indicus sired heifers were older and heavier at puberty than bos taurus sired heifers.

Heritability estimates for age at puberty vary greatly from study to study. By definition, heritability estimates are an estimate of

that portion of the phenotypic variation observed in a trait that is due to heredity. The heritability estimate subtracted from 100 is an estimate of the portion of phenotypic variation that is due to the environment (Lasley, 1978). Table 2 lists heritability estimates for age at puberty in heifers from several studies.

Table 2. Heretability estimates ($h^2$) for age at puberty.

| Authors | $h^2 \pm$ S.E.M. |
| --- | --- |
| Arije and Wiltbank (1971) | .20 $\pm$ .16 |
| Smith et al. (1976) | .67 $\pm$ .24 |
| Laster et al. (1979) | .41 $\pm$ .17 |
| King (1983) | .48 $\pm$ .18 |

Arije and Wiltbank (1971) reported that age at puberty was correlated with puberty weight (r=.57; P < .01), average daily gain (ADG) from birth to weaning (r=-.36; P < .01), 205 day weaning weight (r=-.35; P < .01), actual weaning weight (r=-.22; P < .01), weight gain from weaning to puberty (r=.84; P < .01) and ADG from weaning to puberty (r=.65; P < .01). It has been widely reported that both breed of sire as well as sire within breed influenced age and weight at puberty (Menge et al., 1960; Arije and Wiltbank, 1971; Burfening et al. 1979; Grass et al., 1982). The high relationship between growth related traits and the significant influence of sire breed and sires within breed indicate the importantance of growth rate and sire breed selection upon the occurrence of puberty in beef heifers.

Mature Size and Growth. Differences in mature size and growth pattern in cattle has been reported to account for some variation in the occurrence of puberty in heifers. Heifers of breeds whose mature size tends to be larger, such as Limousin and Charolais, reach puberty at later ages and heavier weights relative to their growth rate, compared to either Angus- or Hereford-sired heifers (Laster et al., 1972 and 1976). Gregory et al. (1982) reported that fewer Limousin-sired heifers reached puberty by 15 months of age than Angus-sired heifers (69.2 and 87.1%, respectively). The percentage of Charolais-sired heifers reaching puberty by 15 months of age was similar to Angus-sired heifers (85.2% and 87.1%) but the Charolais sired heifers reached puberty at a later age (Laster et al., 1976). Swierstra (1977) ranked breed of sire for weight at puberty of crossbred beef heifers. Those heifers of larger mature size breeds tended to have heavier birth, weaning and 400 day weights and were heavier at puberty.

Maternal effects also play a significant role influencing age of puberty in heifers. Laster et al. (1972) reported an influence of breed of dam (P < .005) on the percentage of heifers reaching puberty by 15 months of age. Maternal influence can, in part, be attributed to selection for increased milk production such as in dairy breeds (Laster et al., 1972 and 1976; Grass et al., 1982). Gregory et al. (1982) suggested that breeds or individuals within a breed, which have been selected for milk production, appear to reach puberty at younger ages when compared to breeds of similar growth rate and mature size not selected for milk production.

Heterosis. Heterosis or hybrid vigor is the result of crossing two breed types. Heterosis tends to be greatest when crossing breeds of diverse genetic background and can be associated with the compensation of inbreeding depression within the respective breeds (Falconer, 1981). Inbreeding depression has been reported to adversly affect reproductive fitness (Burfening et al., 1979) and compensation or relief of inbreeding depression from heterosis increases reproductive fitness.

Crossbred heifers tend to reach puberty at younger ages, lighter weights, and have a greater percentage attaining puberty by 15 months of age than straightbred heifers (Laster et al., 1972; Wiltbank et al. 1966; Kaltenbach and Wiltbank, 1962; Burfening et al., 1979; Gregory et al., 1978). Utilizing Angus, Hereford, and Shorthorn straightbred heifers and their respective crosses, Kaltenbach and Wiltbank (1962) reported that crossbred heifers reached puberty 27 lbs lighter (523 vs 550 lbs) and 58 days earlier (373 vs 431 days; P < .05) than straightbred heifers. The differences in age at puberty was accounted for, mostly, by the increased growth rate of the crossbred heifers.

Wiltbank et al. (1966) reported a breed of sire by breed of dam interaction (P < .01) and concluded that these interactions reflect the importance of heterosis on reproductive traits. Age at puberty was reduced (P < .01) as a result of heterosis but when age at puberty was adjusted for average daily gain from birth to weaning and weaning to one year of age some of the heterotic influences remained unaccounted for. These data tend to support the concept that growth rate alone does not account for all of the influence of heterosis;

crossbreeding may alter the genotype for age at puberty and increase reproductive potential.

Selection for Early Puberty. Age at puberty in the female is a sex-limited trait and progress through sire selection for increased fertility in female progeny at puberty has been slow. One indicator trait, easily measured in the male, may prove itself worthy as a selection criterion for earlier age at puberty in the female. Scrotal circumference can be easily measured in bulls and has a high negative genetic correlation to age at puberty in heifers (Brinks et al. 1978; King et al. 1983). The heretability estimates for scrotal circumference were reported by King et al. (1983) and Coulter and Foote (1979) as being .26 and .68, respectively. Brinks (1984) suggested that a reasonably high heretability for scrotal circumference coupled with a large within breed variation may offer the opportunity to select for larger scrotal circumference and reduction in age at puberty in female offspring.

## Environmental Influences

Nutrition. First estrus in beef heifers occurs during growth stages of development and there is a high positive correlation between age and weight at puberty (Wiltbank, 1971; Steffen et al., 1983). Both pre- and postweaning nutrition play an important role in allowing a heifer to realize her genetic potential in attaining puberty. However, the mechanism by which nutrition influences puberty in beef heifers remains unclear (Grass et al., 1982; Berardinelli, 1976).

Preweaning Nutrition. Milk production indirectly influences age at puberty in heifers. Heifers with Angus dams reached puberty 22 days earlier ($P < .01$) and weighed more at 200 and 400 days than heifers of Hereford dams (Plasse, 1968; Gregory et al., 1979). These authors suggested that the difference was due to superior milk production of Angus dams to allow for increased preweaning growth and earlier sexual maturity.

This indirect maternal influence was substantiated by the effect of age of dam on milk production and subsequent age at puberty in their offspring. Doornbos et al. (1982) found that milk production in young females (two and three years of age) was less than for cows, five years old or older and Laster et al. (1976) reported that fewer heifers out of two year-old dams reached puberty by 390 days of age and as dam age increased to five years or older the percentage increased. Maternal influence may be exerted through increased preweaning gain through its effect on milk production or possibly some other unknown maternal influence.

Postweaning Nutrition. Dufour (1975) suggested that as the breeding season approaches weight becomes more critical than age in determining the occurrence of puberty in heifers. Restrictive postweaning feeding delays the onset of puberty in beef heifers (Ferrell, 1982). Short and Bellows (1971) reported that feeding either a low-, medium-, or high-energy diet influenced the onset of puberty in beef heifers. In their study, the percentage of heifers reaching puberty prior to the breeding season were 7, 24, and 83% for

the low, medium, and high groups respectively. Also, a greater average age at puberty and lower subsequent reproductive performance were observed in heifers on the low energy diet.

A variation in response to nutritional regimens was observed in crossbred beef heifers. Although little difference was detected in age at puberty between crossbred heifers and straightbred heifers on the high nutritional plane, crossbred heifers on the restricted ration were younger and lighter at puberty that straightbred heifers on the same ration. This was attributed to the influence of heterosis (Wiltbank et al., 1969) but, as yet, the mechanism by which genotype interacts with nutrition to affect the onset of puberty is unknown.

Mosely et al. (1982) observed that adding a propionate enhancer (monensin sodium) to beef heifers growing rations reduced the age at puberty (P < .07). This effect was not entirely accounted for when age at puberty was adjusted for either average daily gain or body weight (Mosely et al., 1982). Randel and Rhodes (1980) reported that prepuberal heifers fed monensin sodium appear to have an increased hypophyseal capability of LH release and this effect may be mediated through alterations in metabolic parameters. The actual mechanism by which monensin sodium alters reproductive performance is unknown.

Season of Birth, Photoperiod, and Temperature. Spring-born heifers tended to reach puberty at younger ages than heifers born during winter months (Hawk et al., 1954; Arije and Wiltbank, 1971; Grass et al., 1980). This difference was attributed to increased forage supply during spring months hence increased preweaning growth

rate. Hansen et al., (1981) reported that fall-born dairy heifers reached puberty at a younger ages (P < .01) than spring-born heifers. The differences reported by Hansen et al., (1981) were attributed to the fact that the spring-born heifers tended to have a higher incidence of scours. Roy et al. (1980) reported that dairy heifers born during periods of increasing day length reached puberty earlier than heifers born during periods of decreasing day length. However, Greer (1984) could not demonstrate a relationship between day length at birth or lunar phase and age at puberty in beef heifers.

Varying temperature may alter the occurrence of puberty in beef heifers. Dale et al. (1959) examined the influence of temperature on Santa Gertrudis (n=11), Brahman (n=9), and Shorthorn heifers (n=10) using three different environmental conditions 50°F, 80°F, and outdoor conditions. Age at puberty in Santa Gertrudis heifers was unaffected. Shorthorn heifers displayed earlier age at puberty in outdoor conditions and puberty was delayed at either 50°F or 80°F. Brahman heifers in 80°F reached puberty at 463 days. While the remainder of the Brahman heifers in outdoor conditions and one Brahman heifer from the 50°F group displayed seasonal monestrous. Temperature may, indeed, influence the onset of puberty but with such small numbers of heifers used by Dale et al. (1959) few conclusions may be drawn.

## Physiologic Factors in Puberty

Endrocrine Changes Associated with Puberty. Several researchers have characterized endocrine changes associated with the onset of puberty in beef heifers (Desjardins and Hafs, 1968; Gonzalez-Padilla

et al., 1975a; Berardinelli et al., 1979). The author knows of no reported literature that links the endocrine occurrences of the hypothalmic-hypophyseal-gonadal axis, associated with puberty, to factors of environmental or genetic origin. Some researchers have linked the vomernasal organ, a pheromone receptor organ found in most mammals to the accessory olfactory bulb and to some hypothalmic nuclei in rodent species (for review; Wysocki, 1979). But, as yet, no definitive link has been reported between such anatomical structures, pheromones, neuroendocrine or endocrine systems in the onset of puberty in mammals.

Early work by Desjardins and Hafs (1968), characterized pituitary gonadotropin levels by using bioassays that measured biologically active lutinizing hormone (LH) and follicle stimulating hormone (FSH). LH concentrations in the pituitary increased considerably up to 3 months of age, fluctuated from 3 to 7 months of age then (P < .05) decreased up to 12 months of age. The concentration of FSH remained relatively constant from 3 to 12 months of age but increases and decreases were evident between 0 to 2 months of age. From these data, it was concluded that puberty was associated with decreased levels of pituitary LH prior to puberty followed by an increased level near the subsequent estrus.

Hypothalamic, pituitary and ovarian hormones were characterized during the prepuberal period in beef heifers by Gonzalez-Padilla et al. (1975a). Systemic blood collection, via jugular cannulation, began 30 to 64 days prior to puberty on six half-sib Angus heifers and the experimental period continued through midcycle of the puberal

estrous cycle. Radioimmunoassay (RIA) techniques were used to measure gonadotropin releasing hormone (GnRH), FSH, LH, prolactin (PRL), progesterone and estradiol 17β .

In general, GnRH, FSH, PRL, and estradiol 17 β concentrations remained relatively constant throughout the prepuberal period. Each heifer tended to have an individual baseline level of GnRH. FSH levels tended to be higher at days -5 to 9 (day 0 equals the end of the preovulatory LH surge) but this was not consistent among all heifers. PRL levels varied considerably and were inconsistent among heifers. The authors concluded that each heifer had an inherent level of PRL. Estradiol 17 β tended to increase in concentration before day -40, then gradually decline for 3 to 4 days to a constant level for the remainder of the sampling period. This trend was evident in all heifers. Estradiol 17β elevations were not found to be associated with any major increase of LH.

The prepuberal period was characterized by distinct elevations of LH and progesterone. LH exhibited a pustile release pattern that fluctuated greatly with a prepuberal concentration higher than that observed after day 0. The prepuberal period had two marked LH peaks. The initial LH peak was observed at day -11 to -9 and was designated as the "priming LH peak". The subsequent LH peak was associated with first estrus and was designated the "puberal LH peak". Both peaks were of similar magnitude, 11 to 52 ng/ml, and were associated with increases of progesterone concentration.

Similar to the prepuberal LH peaks, progesterone was characterized by two distinct and consistent increases. The initial

progesterone rise occurred between days -18 and -11 and the second occurred between days -10 and 0. Both peaks preceded the priming and preovulatory LH peaks. There was no correlation between LH and progesterone. Gonzalez-Padilla et al. (1975a) indicated that the inherent variability of LH secretion accounted for the lack of statistical association. Nevertheless the two hormones did appear to be associated. The level of progesterone after the preovulatory LH peak (i.e. luteal phase of the puberal estrus) and day 0 was always higher than prepuberal progesterone concentrations.

The source of progesterone in the priming progesterone peak in heifers sampled by Gonzalez-Padilla et al. (1975a) was thought to be of adrenal origin while the preovulatory progesterone peak was from ovarian origin. Since that time, Berardinelli et al. (1979) identified luteal tissue embedded within the stroma of the ovary, that could not be palpated recally, and concluded that the source of progesterone during the priming progesterone peak was of ovarian origin. The preovulatory progesterone peak was also found to have a similar origin. Regardless of its source, progesterone is thought to be critical in producing changes which ultimately lead to the establishment of the phasic release of LH in the puberal beef heifer.

Induction of puberty in beef heifers has been attempted by several researchers (Gonzalez-Padilla et al., 1975b; Berardinelli, 1976; Short et al., 1976; and Burfening, 1979). Burfening (1979) reported that puberty can be induced with exogenous progestogens but success of these treatments was clearly dependent upon the age of the heifer. As a heifer approaches the breed average for age at puberty,

she was more likely to respond to exogenous hormone treatment. The response of estrus to exogenous progestogen treatment in young heifers (334 days of age) was less (P < .05) than in older heifers (406 days of age). Those that did respond with an induced behavioral estrus did not continue to cycle and subsequent pregnancy rates were lower (P < .05) than older light weight heifers and control heifers. As the age of heifers increased approaching the breeding season the response to progestogen treatment was greater (P < .05). Once the heifers responded they continued to display behavioral estrus at regular intervals and pregnancy rates were higher than heifers in the younger age group.

Regulatory Factors in Puberty. Given the endocrine events which precede the onset of puberty, one may question whether the occurrence of puberty is an event or process. Researchers that measure the overt signs associated with puberty, such as first observed behavioral estrus followed by CL function, may consider puberty as an event (Foster and Ryan, 1979). Puberty appears to be the culmination of a complex process. To explain the process, the following concepts have been proposed. One concept involves a critical body weight requirement and the other involves an "escape" from the negative feedback of estrogen on LH release.

Undernutrition, as mentioned earlier, delays the onset of puberty in beef heifers as well as other mammals, including humans (Frisch and McArthur, 1974). Consequently, for a given genotype, some factors other than age may be involved in the puberal process. In human females, Frisch and McArthur (1974) suggested that the attainment and

maintenance of a critical body weight for height appears to be required for the onset and maintenance of regular menstrual cycles.

The concept of a critical body weight may be a function of body composition rather than an increase in mass. The degree of fatness has been postulated as a controlling factor in the onset of puberty in human females (Frisch, 1980) and rats (Frisch et al., 1977). The interrelationship between relative fatness and puberty depends upon an animals inherent rate of maturity. Later maturing rats tend to be heavier at first estrus and it has been hypothesized that these rats need more time to attain a particular level of fatness relative to earlier maturing rats (Frisch, 1980). Also, it has been postulated that adipose tissue may be an extragonadal source of estrogen (Frisch et al., 1977) but its importance is currently not known.

The optimum body weight or composition at puberty in livestock species has yet to be ascertained. Body weight can be used as an indicator of growth rate but other factors, such as level of fatness need to be evaluated as they may serve to better understand the role nutrition and the onset of puberty in beef heifers.

Prior to the priming and preovulatory LH surges associated with the event of first estrus, a tonic release of LH is evident. It has been postulated that the prepuberal period in female rats (Ojeda et al., 1980), ewes (Foster and Ryan, 1979), gilts (Berardinelli et al., 1984) and beef heifers (Day et al., 1981) is characterized by increased sensativity of the hypothalamic-hypophyseal axis to the negative feedback of estrogen on LH release.

The "escape" from the negative feedback of estrogen seems to occur as the time of puberty approaches. Evidence for this has been reported in a number of mammalian species. Castration of prepuberal beef heifers and gilts resulted in an increase in LH release and estrogen replacement inhibited LH release (Day et al., 1981; Berardinelli et al., 1984). In both of these species, the response to exogeneous estrogen treatment was age dependent. As the prepuberal heifer or gilt reached the age in which control groups attained puberty, the negative influence of estrogen diminished.

In the castrated ewe lamb, Foster and Ryan (1979) reported that the frequency of LH pulsatile release increased from less than one per hour to greater than one per hour. The authors speculated that the escape of LH from estrogen inhibition associated with the prepuberal condition allows the frequency of LH release to increase, which, in conjunction with FSH, stimulated follicular maturation, while the positive feedback of estrogen, produced by the follicles, elicted an LH surge.

The positive feedback systems associated with normal cyclic reproductive function does not seem to be a limiting factor in the puberal process. Exogenous GnRH and estradiol treatments induced an increase in LH release in four-week-old beef heifers (Williams et al., 1975). Williams et al. (1975) suggested that the endocrine mechanisms typical of a spontaneous ovulator are well developed in the prepuberal beef heifer.

Currently, it is not known what the precise limiting factors are in the onset of cyclic gonadotropin and steroid hormone release

associated with puberty. It is not known if body weight or composition is interrelated with the escape from these limiting factors, such as the postulated estrogen inhibition of LH release. Finally it is not known if other environmental stimuli limit or enhance the puberal process or to what degree these stimuli may be used to manipulate the sexual maturation of prepuberal beef heifers.

## Social Interaction and Biostimulation

In the past three decades, much information has been reported on physiologic changes influenced by social interaction between sexes in several species. In 1968, Fraser (in Chenoweth, 1983) coined the term biostimulation and defined it in terms of the stimulatory effects of the male on estrus and ovulation in the female. Chenoweth (1983) indicated that no definitive evidence had been reported on the neuroendocrine mechanisms involved and postulated that the effect of biostimulation may be caused by: 1) direct genital stimulation, 2) allelomimetic cues, or 3) pheromones or similar biochemical substances. Also, there are data that show that females may have a biostimulatory effect on other females of the same species (T.C. Nelson, unpublished data). Consequently, in the context of this review of literature, the term biostimulation will include the influences of both sexes upon altering the reproductive status in the female of the same species.

From a historical standpoint, three classical studies of the influence of biostimulation of mice will be reviewed. Lee and Boot (1956) reported that female mice, housed as a group, discontinue normal estrous cyclicity and became either pseudopregnant or anestrus.

Whitten (1956) reported that in mice the presence of the male or his soiled bedding, as a novel stimulus, caused females to exhibit an unequal mating frequency in the first four nights after pairing. A greater number ($P < .001$) of vaginal plugs, indicative of mating, were observed in females three days after pairing. Bruce (1959) reported an exteroceptive block to pregnancy in female mice introduced to a strange male 24 hours postcoitus. All three of these studies have been the basis of more recent experimentation on the influence of biostimulation.

Several physiologic events and postulated mechanisms for biostimulation have been studied in depth in rodent species. Humans and livestock species have been studied to a lesser extent and will be discussed later.

Not all rodent species exhibit similar responses to specific biostimulation. Unlike observations made in mice by Lee and Boot (1956), McClintock (1978) reported that the estrous cycles of rats (Rattus norvegicus), housed in group living situations, became more synchronized ($P < .01$) than females housed individually or randomly selected rats from different living situations. Airborne chemical communication, rather than simple physical proximity, was sufficient to induce synchronization of estrus. In group-housed adult female mice, synchrony of estrus will occur if the females are exposed to the urine of an adult male mouse (Bronson and Whitten, 1968). Bruce (1965) indicated that exposure to adult male mouse urine, from a strange male, will induce pregnancy blockage in newly mated female mice. These studies differed from the original studies by Whitten

(1956) and Bruce (1959) in that the male mouse need not be present, just his urine.

Vandenbergh (1967) was one of the first to report the biostimulatory effects of the male mouse on the occurrence of vaginal opening and puberty in the female mouse. The presence of the male, soiled bedding taken from the stud cage (Vandenbergh, 1969) or urine from an adult male (Crowley and Wise, 1972; Colby and Vandenbergh, 1974) accelerated sexual maturity, as indicated by a shortened interval between vaginal opening and first estrus (Colby and Vandenbergh, 1974). The influence of the male tended to be greatest in weaned female mice, although the male effect was still evident in preweaned females (Vandenbergh, 1967). Prepuberal female mice, exposed to the male at 21 days of age, reached first vaginal estrus 20 days earlier (P < .001) than females denied male biostimulation. As the age of prepuberal females increased, at the time of male exposure, the time interval to sexual maturation decreased (Vandenbergh, 1967; Colby and Vandenbergh, 1974). Similar effects of male induced precocial puberty have been reported in a feral population of mice (Massey and Vandenbergh, 1981), laboratory bred female meadow voles (Microtus pennsylvanicus; Baddaloo and Clulow, 1981) and collard lemmings (Dicrostony groenlandicus; Hasler and Banks, 1975).

All of these studies would indicate that some active component(s) present in the urine of adult male rodents, affects the reproductive maturity pattern in females. This concept is in agreement with earlier discussion, in that pheromones or similar biochemical substances may be the cause of biostimulation.

Meredith (1983) defined a pheromone as chemicals emitted by one member of a species which when detected by another member results in behavioral or physiological changes that are likely to benefit both individuals. Traditionally, pheromones have been divided into two subclasses. A signalling pheromone is an olfactory cue that transfers specific information and consequently elicits a specific behavior, while a priming pheromone is an olfactory cue that elicits a measurable physiologic response (Izard, 1983). The response of accelerated sexual maturity to male mouse urine or a specific component of urine would denote a cue which elicits a measurable physiologic response, thus would fall into the priming pheromone subclass. Signalling pheromones will not be discussed.

The urinary phenomone that accelerates age at puberty in mice is androgen-dependent (Crowley and Wise, 1972; Vandenbergh et al., 1975; Lombardi et al., 1976). Pheromonal activity of urine from newly castrated males lost its potency within ten days and treatment of male castrates or females with testosterone propionate resulted in re-establishing pheromonal activity similar to that of intact male urine (Lombardi et al., 1976). Application of urine from adult virgin female donors failed to accelerate puberty and, in fact, delayed sexual maturity (Crowley and Wise, 1972).

Vandenbergh et al. (1975) characterized the urinary pheromone and reported that it was heat labile, non-dialysable, precipitatable with ammonium sulfate, and not extractable with ether. These data would indicate that the pheromone is non-volatile and is most likely associated with the protein component of male urine. A definitive

characterization of this pheromone, or other pheromones possibly associated with synchronization of estrus, estrus suppression and pregnancy block have not been reported.

Chemoreception of priming pheromones has been the source of much speculation. Both Whitten (1956) and McClintock (1971) indicated that an exteroceptive stimulus functions through one or more of the chemical senses. Much attention has been directed toward the olfactory system in detecting non-volatile biochemicals (Meredith, 1983), such as the protein pheromone partially characterized by Vandenbergh et al. (1975). Currently the vomernasal organ (VNO), a chemoreceptor system independent of the main olfactory bulbs (MOB), has been implicated in the reception of pheromones associated with the acceleration of puberty (Lomas and Keverne, 1982; Sanchez-Criado and Gallego, 1979) in mice and rats as well as estrus suppression (Reynolds and Keverne, 1979) and pregnancy blockade (Bellringer et al., 1980) in mice. Bilateral destruction of the VNO reduces the incidence of these three physiologic phenomenon.

The VNO was originally described by the Danish physician, L. Jacobson (Wysocki, 1979) and was termed Jacobson's organ. The mammalian VNO is housed in a cresent shaped tubular cavity located medially in the anterior one-third of the nasal septum. Vomernasal receptor neurons are located in the sensory epithelium on the posterior two- thirds of the lumen wall and the organ has a single opening either into the nasal cavity or via the nasopalatine canal (rodents and livestock species; Wysocki, 1979; Meredith, 1983). The vomernasal lumen is continuously bathed with mucous-like secretions

from the vomernasal glands located near the border of the sensory epithelium (Meredith, 1983). The VNO is the main source of sensory input into the accessory olfactory bulb (AOB; Scalia and Winans, 1975).

The theoretical combination of the Flehman response (Wysocki, 1979) and an autonomically driven vascular pump (Meredith and O´Connell, 1979) have been implicated in the access of stimuli into the VNO of rodent species. Flehman may serve to transport an "important substance" to the anterior opening of the VNO, via the nasopalatine canal, while sympathetic input, via the nasopalatine nerve, stimulates a pumping mechanism within the lumen of the VNO. The pumping mechanism, powered by vasomotor movements, is postulated to circulate those substances to the posterior two-thirds of the lumen to the sensory epithelium. This mechanism of stimulus access seems to be of particular importance to non-volatile urinary components. Wysocki et al. (1980) reported that urine, contaminated with Rhodamine (a non-volatile fluorescent dye), did not reach the olfactory sensory epithelium when presented to female hampsters but did gain access to the VNO. As of yet, the flehman-vascular pump mechanism has not been observed in conscious animals or in animals not undergoing some exogenous stimulatory influences. Consequently, the mechanism described above is a theoretical model for which future experimentation may be based.

The proposed route of neural transmission after stimulus access and chemoreception begins with the vomernasal axons. These axons gather into large discrete bundles, pass beneath the nasal septum and

enter the cribriform plate of the ethmoid bone as two or three nerve bundles on each side of the midline. Vomernasal nerve bundles pass between the main olfactory bulbs and enter the glomeruli of the AOB (Meredith, 1983). Using induced unilateral lesions of the AOB, neural tracts were followed, histologically, via terminal degeneration (Scalia and Winnins, 1975). Terminal degeneration, assayed by the Fink-Heimer method of silver impregnation (Fink and Heimer, 1967), occurred in the medial amygdaloid nucleus. Wysocki (1979) reviewed evidence that the neuroanatomical relationship included the bed nucleus of the stria terminalis and three hypothalmic structures: the ventromedial nucleus, arcuate nucleus and median eminence. This neuroanatomical link between the VNO and the hypothalmus has contributed to much speculation on the mechanism of action between pheromone reception and a measurable physiologic response, such as accelerated puberty.

The relationship between the VNO, pheromone chemorecption and accelerated puberty seems clear. Mice subjected to the ablation of the VNO were older at puberty ($P < .01$) when exposed to male urine than sham-operated or intact control females (Lomas and Keverne, 1982). The relationship between VNO stimulation and its neuroanatomical link to the hypothalmus and endocrine function is less clear.

Currently, no known mechanism of action has been reported between pheromone reception, the VNO and endocrine function. An immediate and sequential release of LH and estrogen has been reported in male induced puberal females of specific body weight in mice (Bronson and

Desjardins, 1974). In an attempt to mimic the influence of male biostimulation, exogenous estrogen treatments were used for two days followed by a single day of male exposure. The prepuberal females responded with an induced ovulation which was presumably preceded by a sequential release of LH (Bronson, 1975). The mechanism remains unclear as to whether exogenous estrogen induced ovulation (via a subovulatory LH release), regardless of the male or whether both estrogen and the male or just the male elicited the subovulatory release of LH which in turn stimulated estrogen release above threshold level sufficient to induce an ovulatory LH surge.

The relationship between the hypothalamus, GnRH, and pituitary function is well established. Pituitary stalk transection results in an inability to ovulate and a dramatic decrease in the pulsatile release of LH associated with GnRH secretion (Halasz et al., 1967). The question that remains is how does the hypothalmic-hypophyseal axis respond to pheromonal stimulation. Any number of neuroendocrine events may occur when stimulated by the puberty accelerating pheromone. At the hypothalamic level, male pheromonal stimuli may induce alterations in neural stimulation, concentration, activity and/or metabolism of neurotransmitters, axonal flow of specific substrates or carriers of specific substrates and/or alter the metabolism of GnRH, its biological activity or GnRH receptor site activity. If an increase in GnRH concentration or biologic activity occurs, a coordinated change in LH secretion can result, eliciting changes that were observed by Bronson and Desjardins (1974). At the hypophyseal level, puberty accelerating pheromone could theoretically

alter LH metabolism, its activity and/or the sensitivity to the threshold level of estrogen sufficient to induce a preovulatory or ovulatory LH surge. Currently, there is no known neuroanatomical link between the VNO and the pituitary, consequently the data seem to support the control hypothesis at the level of the hypothalamus.

It must be stressed that the only conclusions that can be drawn from the literature are that the presence of the male mouse accelerates the onset of puberty in the female; that a partially characterized pheromone, found in the protein component of adult male urine, is responsible for this physiologic response in mice; that the VNO is the chemoreceptor organ which recognizes this non-volatile pheromone and the AOB receives sensory input from the VNO; and that nuclei of the hypothalmus are linked anatomically, via a neural pathway, to the AOB. Presently, there is no known mechanism which explains how puberty is accelerated through male stimulation and the endocrine events discussed here are strictly theoretical.

The majority of literature concerning humans has been of antecdotal and indirect observation with few controlled experiments. Indirect observations of mothers, daughters and sisters, and all female-living groups reveal that synchrony of menstrual cycles often occurs (McClintock, 1971). In a more controlled situation, synchronization of menses was greater in women who were roommates ($P < .0007$) or closest friends ($P < .0003$) than in randomly selected females from different living situations (McClintock, 1971). The author suggested that the mechanism underlying this physiologic

response may be either pheromonal, mediated by simple awareness (allelomimetic cues) or some other unknown process.

Interest concerning biostimulated early sexual maturity in livestock species has grown in the last 15 years since the onset of puberty can be an economically important trait. Swine producers had a particular interest in this phenomenon since stress related delayed puberty had increased with the implementation of total confinement production systems. Christianson and Ford (1979) reported that as many as 40% of the gilts studied in a total confinement situation did not reach puberty by nine months of age. Fifty-five percent of these prepuberal (delayed puberty) gilts had infantile reproductive tracts and the remainder showed evidence of silent estrus (behavioral anestrus) at slaughter. Breed of gilt was an important factor contributing to the incidence of delayed puberty or behavioral anestrus due to stress (Christenson and Ford, 1979).

The use of male biostimulation has been found to alleviate in this management-oriented production loss. Age of puberty was reduced in prepuberal gilts exposed to the presence of a boar compared to isolated controls (Brooks and Cole, 1970; Thompson and Savage, 1978; Patterson and Lindsay, 1980; Alexander and Froseth, 1982). If prepuberal gilts were exposed to several boars, rotated periodically, a greater proportion reached puberty by 220 days of age than if prepuberal gilts were exposed to a single boar. This was attributed to the habituation of gilts to a single boar (Brooks and Cole, 1970). A marked synchrony in attainment of puberty resulted in prepuberal gilts exposed to boars at either 165 or 190 days of age (Brooks and Cole,

1970). Gilts exposed to boars for a limited daily period responded with younger ages at puberty and were similar to gilts undergoing continuous boar exposure. Gilts that were exposed to boars at 160 to 170 days of age optimized the response of early puberty (Kirkwood and Hughes, 1980). Boar exposure accelerated puberty in gilts in a total confinement production situation (Thompson and Savage, 1978) and level of feeding (ad libitum vs limit fed) had no interaction with boar exposure and the attainment of puberty in gilts (Alexander and Froseth, 1982).

The physiologic mechanisms involved in male stimulated precocial puberty in gilts have not yet been fully elucidated. Signoret (1971) concluded that olfactory stimuli, among others, may be involved. Kirkwood et al. (1981) tested this hypothesis by removing the olfactory bulbs from six- to seven-week old gilts. When these gilts reached 160 days of age, they were exposed to males. Age at puberty was similar in boar-exposed bulbectomized gilts and intact gilt receiving no boar stimulation (234 and 230 days, respectively). Whereas, the boar-exposed bulbectomized gilts were older at puberty ($P < .05$) than sham-operated (204 days) or intact gilts (208 days) similarly exposed. It seems reasonable to assume that boar biostimulation is, in part, mediated via the olfactory sense. What is unknown at present is whether the technique for olfactory bulb (MOB) removal, used by Kirkwood et al. (1981), included the removal of the AOB. However, it is known that swine do have a VNO (Wysocki, 1979) but its function as a pheromone receptor and its neuroanatomical relationship to the hypothalmus has not been reported. Lastly, no

specific endocrine events associated with boar-induced precocial

puberty have been documented.

Results of a small pilot trial conducted by Kirkwood and Hughes

(1980) indicated that prepuberal gilts respond with accelerated sexual

maturity if exposed to a vacated boar pen.  The authors conducted a

corrallary study with the objective of testing a known urinary

signalling pheromone, $5\alpha$-androst-16-ene-3-one ("BoarMate", Antec.

A.H. International Ltd.; Melrose et al., 1971) produced by the boar,

in an attempt to mimic the boar effect.  The trial failed to show that

the presence of the boar could accelerate puberty and that "BoarMate"

did not reduce the age at puberty in gilts.  The authors suggested

that the boars used as biostimulators may have been too young to

elicit a response but could not explain how age affected the boars in

that the younger boars exhibited higher testosterone levels (Kirkwood

and Hughes, 1980).

The $5\alpha$-androst-16-ene-3-one signalling pheromone can be found in

boar saliva as well as in the submaxillary gland (Patterson, 1968).

Booth et al. (1973) indicated that a biochemical sexual dimorphism

exists in the submaxillary gland in the pig, in that, the female lacks

the ability to produce the $5\alpha$-androst-16-ene-3-one because it is not

an androgen target organ. Currently, no conclusive evidence has been

reported on the priming effects of this pheromone, from either urinary

or submaxillary salivary gland origin, resulting in accelerated

puberty in gilts.  It would be premature, at this point in time, to

compare any mechanistic similarities between the response of the

prepuberal gilt and rodent female to the presence of the male because

only a few basic similarities exists.  Both mice and swine have a VNO and both mediate the male effect via either the AOB (rodents) or the MOB (swine).  Further experimentation is needed to characterize the boar effect and the mechanism by which puberty is accelerated.

The influence of the male on induced precocial puberty in ruminants is least understood. Rams (Schinckel, 1954) and goat bucks (Shelton, 1960) have been implicated in the synchronization of first estrus following the anestrus period in adult ewes and does. In prepuberal ewe lambs, the sudden introduction of the ram tends to synchronize first estrus but has not been shown to accelerate puberty (Dy´rmundsson and Lees, 1970).

The reported effects of biostimulation in beef cattle have been varied and few. Chenoweth (1983) indicated that genital stimulation may be a form of biostimulation. Clitoral stimulation for several seconds following artificial insemination resulted in an increased artificial insemination (AI) pregnancy rate in mature cows but not in heifers (Randel et al., 1975).  In studying various mating stimuli in cattle, Randel et al. (1973) conclude that cervical stimulation tended to hasten the LH surge while clitoral stimulation hastened ovulation.

T.C. Nelsen (unpublished data) reported that prepuberal heifers of some genotypes, exposed to mature cows, reached puberty at younger ages and lighter weights.  Breed of sire was an important factor in this effect.  Hereford- and Tarentaise-sired heifers exhibited earlier age at puberty at lighter weights but the Charolais-sired heifers were older and heavier at puberty than control heifers.

The effect of the bull in accelerating puberty in beef heifers has not been well documented. Berardinelli et al. (1978) reported that the presence of the bull had no effect on accelerating puberty in beef heifers when exposed for 21 days prior to the expected date of puberty. MacMillan et al. (1979) reported that the presence of the bull did not alter the proportion of crossbred heifers detected in estrus prior to the breeding season. The heifers used in that study were 15 to 27 months of age and age at puberty was not known. Izard and Vandenbergh (1982) reported that weekly oronasal applications of bull urine for an eight-week period increased (P < .05) the percentage of heifers reaching puberty within the experimental period. The authors suggested that bull urine contains a priming pheromone which accelerated puberty in the treated heifers and that this response may depend upon body weight. This approach to beef heifer precocial puberty was similar to that taken by Vandenbergh (1967) with mice. Currently, no male pheromone has been characterized for accelerating age or altering the occurrence of puberty. Because of the economic importance of age of puberty in beef cattle, and the possible existence of a puberty accelerating pheromone produced by the bull, further investigation into the biostimulatory effect of the bull on puberty in heifers is needed.

## STATEMENT OF PROBLEM

Current beef management practices require replacement females to attain puberty and become pregnant by 15 months of age. The development of management tools to reduce age at puberty, that are both economical and easy to implement, may be beneficial to the current beef industry. Heifers which have attained puberty and have a normally functioning corpus luteum prior to the start of the breeding season would lend themselves to an estrous synchronization program and greater potential for artificial insemination.

As stated in the review of the literature social influences and biostimulation by the male and female can alter the physiological maturity of young females of the same species. These data indicate that prepuberal females, in some species, respond to the presence of the male by altered physiological maturity at or around puberty.

Therefore, the objectives of this study were to determine: 1) if a single injection of prostaglandin $F_2\alpha$ and/or biostimulation and social interaction with estrus synchronized postpuberal heifers for the first 21 days of the breeding season would alter the occurrence of first behavioral estrus in straightbred and crossbred beef heifers; and 2) if biostimulation and social interaction with mature bulls would alter the occurrence, age and weight at puberty in straightbred and crossbred beef heifers.

## MATERIALS AND METHODS

Experiment I. A field trial was conducted to determine the influence of female biostimulation and/or prostaglandin $F_2\alpha$ treatment on the occurrence of first estrus in beef heifers. Prior to the start of the experimental period, estrus detection began twice daily (am, pm) on 421 Angus (A), Hereford (H), and AxH beef heifers at the Montana State Prison Ranch, Deerlodge, Montana. Heifers were approximately 13 months of age. An initial observation period lasted 21 days and any heifer observed standing to be mounted by a herdmate was recorded as in behavioral estrus. Heifers observed in estrus in the last five days of the initial observation period were inseminated artificially, to fertile bulls 12 hours after observed estrus. On day 0 of the experimental period, all heifers were weighed individually and palpated per rectum for the presence of a CL on either ovary and the data recorded. Heifers were then classified into one of two categories: 1) non-bred postpuberal; if a heifer was observed in estrus, not bred, and had a palpable CL on either ovary (n=49), or 2) prepuberal; if a heifer had not been observed in estrus and had no palpable CL on either ovary (n=371).

Non-bred postpuberal heifers were designated as the association group and were given an intramuscular (IM) injection of $PGF_2\alpha$ (25 mg, dinoprost tromethamine, 5 mg/ml; Upjohn Company) on day 0. Also, on day 0, prepuberal heifers were stratified within breed by weight and

randomly alloted to one of four treatments; 1) 91 heifers were injected (IM) with $PGF_2\alpha$ (25 mg/head) and isolated from the association group; 2) 97 heifers were injected (IM) with $PGF_2\alpha$ (25 mg/head) and placed with the association group; 3) 95 heifers were injected with .9% saline solution (5 ml/head) and isolated from the association group; or 4) 89 heifers were given saline and placed with the association group.

The isolation groups (treatments 1 and 3) were placed in pastures approximately .4 km away from groups 2 and 4. The biostimulation groups (treatment 2 and 4) were placed in a single pasture with the association group. All groups were then observed for behavioral estrus twice daily for an additional 21 days and estrus dates were recorded.

Differences in the proportion of heifers that were observed to exhibit first estrus during the experimental period were statistically analysed by contingency Chi square analyses.

Experiment II. A study involving 109 straightbred and crossbred beef heifers was conducted at the Montana State University Experimentation Station, Bozeman, Montana, to determine the effect of bull biostimulation on the occurrence, age and weight at puberty. Thirty-four heifers of Angus (A), A x Hereford (H), Simmental (S) x A and S x H breeding from the Montana State University herd, Bozeman, Montana, and seventy-six heifers of A x H, H x A, Tarentaise (T) x A, T x H breeding from the Red Bluff Agricultural Experiment Station herd, Norris, Montana were used in this study.

Prior to the start of the experimental period, heifers were weaned at approximately 200 days of age, separated from the castrated male herdmates, and managed as a single group. All heifers were acclimated to a growing ration of 18% ground barley (12% crude protein, 83% TDN) and 82% grass alfalfa hay (13% crude protein, 55% TDN) fed at approximately 2.5% of body weight. A salt mineral mix was fed ad libitum.

Detection of estrus began 15 days prior to the beginning of the experimental period and heifers were observed twice daily between the hours of 0730-0830 and 1730-1830. These hours were subject to small changes due to changes in daylight hours. Any heifer observed standing to be mounted by a herdmate prior to the experiment was excluded from the study. Two 10 ml jugular venous blood samples were collected from each heifer ten days (January 3) before and on the day of assignment to treatment (day 0; January 13) and an initial body weight was taken at day 0.

All 109 heifers were stratified within breed by age, weight and origin of heifer and were allotted randomly into one of two treatment groups. Treatment group one was designated as the biostimulation group and consisted of heifers exposed to the presence of sterile mature bulls (n=54). Group two was designated as the control group and isolated from any male biostimulation (n=55).

The two treatment groups were physically separated by a distance of .5 km to isolate each treatment group from any auditory, olfactory, or visual stimuli associated with the bulls or each other. Both treatment groups were housed in open dry lot pens of similar

size, each of which were similar in environmental changes, wind breaks, bedding, water, and salt-mineral supplement availability.

On day 0, a biostimulation ratio of one bull: 27 heifers was implemented. The bulls used in this study were mature two-year old Red Angus teaser bulls, whose penises had been deflected. As a result of loss in condition and health problems to the initial three bulls, the last 30 days of the experimental period employed two new yearling Simmental x Red Angus teaser bulls. Bulls were used in a ten-day rotational scheme, with a new bull introduced every ten days and with one of the two previous bulls removed. As a result, each bull was exposed to heifers for 20 days and then rested for ten days away from physical contact with heifers. The two yearling bulls were rotated into this scheme in an uninterrupted fashion.

Detection of estrus continued in the same manner as in the pre-experimental period with both treatment groups being observed twice daily. Bulls were temporarily separated from group one heifers for the duration of the daily estrus detection periods. Heifers were recorded as in estrus when observed standing to be ridden by a herdmate.

The first observed estrus was designated as day 0 for individual heifers recorded as in estrus. On day 9 post-estrus, individual heifers were palpated per rectum for the presence of a CL on either ovary and the data recorded, and a 20 ml blood sample was collected via jugular puncture. Blood samples were collected in properly labeled Monovette[R] 92 x 16.5 mm blood collection devices (Sarstedt; Princeton, N.J.). Each sample was allowed to clot for 60 minutes at

$37^{\circ}C$. Blood samples were then centrifuged at 2500 rpm for ten minutes and the serum fraction was decanted into properly labeled plastic culture tubes, frozen and stored until radioimmunoassay for progesterone could be performed. Blood samples which had been collected during the pre-experimental period were handled in a similar fashion.

After palpation and blood collection, heifers were returned to their respective treatment groups and observed for subsequent estrous activity. Subsequent estrus was not followed by rectal palpation or blood sampling if it occurred between 17 and 25 days after the first estrous period.

During the last 25 days of the experiment, those heifers observed in estrus (either puberal estrus or subsequent estrus) were bred 12 hours after standing heat to fertile bulls by an experienced artificial insemination technician. Those heifers bred on a puberal estrus were palpated and bled similarly to heifers reaching puberty prior to the breeding season.

At any time during the experimental period, those heifers displaying abnormal estrous behavior, such as short cycles (7-10 days) or questionable signs of estrus were palpated and bled the same as those heifers observed as having normal estrous activity. Those heifers displaying abnormal behavior were again palpated and bled on their subsequent normal estrus. Date of puberty was assigned using date of first observed behavioral estrus confirmed by the presence of a CL on either ovary and a rise in serum progesterone concentration above 1 ng/ml (Gonzalez Padilla et al., 1975a).

Those heifers which did not display a behavioral estrus prior to the termination of the study (day 152; midway through a 45-day breeding season) were palpated rectally and blood samples collected to determine reproductive status. These heifers were classified into one of three categories: 1) no behavioral estrus, no rise in serum progesterone and no significant structures palpated on either ovary, 2) no behavioral estrus, a rise in serum progesterone above 1 ng/ml and a CL present on either ovary, and 3) structural abnormalities of the reproductive tract.

All heifers were weighed at 28-day intervals, starting on day 0 of the experimental period, and weights recorded. One exception to this was the final weight period which lasted 44 days to minimize stress during the breeding season. Weight at puberty was interpolated between the two nearest weights.

All serum samples were assayed for progesterone using a single antibody technique as described by Orczyk et al. (1979). The assay was validated by demonstration of parallelism between serum from known luteal phase and ovariectomized (ovx) cows, as well as progesterone standards. Assays included sera from both high ($5.6 \pm 2.0$ ng/ml; luteal phase serum) and low pools ($.59 \pm .29$ ng/ml; ovx serum) and had intra- and interassay coefficients of variation of $18.5 \pm 1.8\%$ and $20.1 \pm 2.5\%$, respectively.

Analysis of variance for unbalanced data was performed according to the General Linear Models Method described by SAS User Guide (1982). The model used included breed-origin of heifer (BL), age of dam, treatment (TRT), and BL by TRT interaction as independent

variables and age and weight at puberty as dependent variables. The BL variable was included due to the confounding between breed and origin of heifer when analyzed independently. Breed classifications were based on sire-breed of heifer with the Simmental-sired heifers removed from the analysis due to small numbers. The remaining sire breeds, within origin, were Angus and Tarentaise crossbred heifers and Angus heifers. Age of dam was classified into 2, 3, 4 to 10, or 11 to 13 years of age. Treatment was classified as biostimulation or isolation groups. All two- and three-way interactions, except BL by TRT, were assumed to be non-significant. The proportion of heifers reaching puberty by 11, 12, 13, 14, and 15 months of age was analyzed by contingency Chi square analysis. Differences between treatment group means for birthweight, weaning weight, average daily gain from birth to weaning, initial age and weight on test, and average daily gain on test were analyzed by all possible pairings using the Student's t statistic (MSUSTAT, 1984).

## RESULTS

Experiment I.  The results from experiment one are shown in Table
3.  There were no differences in the proportion of heifers reaching
puberty (P > .05) between female biostimulation and isolation or $PGF_2\alpha$
and saline treated heifers. There also was no interactions between
treatments (P > .05).

Table 3.  Proportion of heifers reaching puberty during the
experimental period[a]

| Treatments | Biostimulation | Isolation | Total |
|---|---|---|---|
| $PGF_2\alpha$ | 25/97 | 24/91 | 49/188 (26%) |
| Saline Treated | 18/89 | 29/95 | 47/184 (26%) |
| Total | 43/186 (23%) | 53/186 (28%) | 96/372 (26%) |

[a] P > .05 for main effects and interactions

Experiment II.  By the end of the experiment, two heifers from
the biostimulation group died, while two heifers from the
biostimulation group and one heifer from the isolation group had
structural abnormalities of the reproductive organs and were removed
from the experiment.  All heifers exhibiting a normal behavioral
estrus (cycle length of 17 to 25 days) during the experimental period

met all the criteria necessary to assign a date of puberty. Biostimulation of prepuberal heifers by mature bulls, in a 1:27 male to female ratio, did not (P > .10) alter age or weight at puberty in straight and crossbred beefheifers. Analysis of variance for age and weight at puberty are shown in Table 4.

While treatment had no effect (P > .10) on age or weight at puberty, analysis of variance indicated that BL affected age at puberty (P < .10) and BL by treatment interaction affected weight at puberty (P < .05). From a biologic perspective, variation in age at puberty, regardless of treatment due to a non-significant effect, may be due to differences in genotype and origin of heifer. There were no biologic trends in the subcell least squares means for weight at puberty, hence the author has no explanation for a BL by treatment interaction in the analysis for weight at puberty. Perhaps, this effect is due to sampling error.

The proportion of heifers reaching puberty in various age groups are shown in Table 5. There were no differences between treatments in the proportion of heifers reaching puberty at 11, 12, 13, 14, or 15 months of age (P > .10). A total of 84.0 and 88.9% of the heifers in the biostimulation and isolation groups, respectively, reached puberty during the experimental period. There were no differences (P > .10) in the proportion of prepuberal heifers or heifers displaying silent estrus (Table 6). The mean age and weight at puberty for the biostimulation and isolation groups were 366.7 days and 291.9 kg and 367.1 days and 293.6 kg, respectively (Table 7).

Table 4. Analysis of variance for age and weight at puberty

| Source | df | Age at Puberty<br>Mean Square | P Value | df | Weight at Puberty<br>Mean Square | P Value |
|---|---|---|---|---|---|---|
| Treatment (TRT) | 1 | 3862.5 | NS | 1 | 550.2 | NS |
| Breed-Origin of Heifer (BL) | 4 | 11745.3 | * | 4 | 3430.7 | NS |
| Age of Dam | 3 | 512.2 | NS | 3 | 986.9 | NS |
| BL * TRT | 4 | 1972.6 | NS | 4 | 6142.9 | ** |
| Error | 71 | 84015.7 | -- | 71 | 573.4 | -- |

NS represents non-significant (P > .10)

* represents (P < .10)

** represents (P < .05)

Table 5. Proportion of heifers reaching puberty for various age groups and their totals

| | N | Age (Months) | | | | | Total Proportion of Heifers Reaching Puberty |
| | | 11 | 12 | 13 | 14 | 15 | |
|---|---|---|---|---|---|---|---|
| Biostimulation | 50 | 7/50[a] | 17/43[a] | 9/26a | 6/17[a] | 3/11[a] | 42/50 (84.0%) |
| Isolation | 54 | 8/54[a] | 19/46[a] | 9/27[a] | 10/18[a] | 2/8[a] | 48/54 (88.9%) |

[a] different superscripts for either rows or columns indicate differences at $P > .10$

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Table 6.  Proportion and classification of heifers not reaching
          puberty

|  | Pre-Puberal Heifers | Heifers Displaying Silent Estrus[a] |
|---|---|---|
| Biostimulation | 7/50[b] (14.0%) | 1/50[b] (2.0%) |
| Isolation | 4/54[b] (7.4%) | 2/54[b] (3.7%) |

[a] as determined by rectal palpation on day 152
[b] different superscripts for columns indicate differences at $P < .05$

Table 7.  Age and weight at puberty for biostimulation and isolation
          heifers

|  | n | Puberty Age $(d\pm SD)$[a] | Puberty Weight $(kg\pm SD)$[b] |
|---|---|---|---|
| Biostimulation | 42 | $366.7\pm36.2$ (n=42) | $291.9\pm27.3$ (n=42) |
| Isolation | 48 | $367.1\pm34.3$ (n=48) | $293.6\pm24.4$ (n=48) |

[a] $d\pm SD$ represents day $\pm$ standard deviation
[b] $kg\pm SD$ represents kilogram $\pm$ standard deviation

There were no differences ($P > .10$) between treatment groups for birth weight, weaning weight, average daily gain from birth to weaning, initial age and weight at the start of the experiment, or average daily gain on test (Table 8; Appendix).

## DISCUSSION

The results from Experiment I indicate that the presence of estrous heifers or an injection of 25 mg prostaglandin $F_2\alpha$ or their interaction, did not alter the occurrence of first estrus in prepuberal straightbred and crossbred beef heifers. The author knows of no literature reporting the influence of estrous heifers on the induction of puberty in heifers. In rodent species, the influence of virgin female mouse urine delayed the onset of puberty in prepuberal females (Crowley and Wise, 1972).

T. C. Nelson (unpublished data) did observe that prepuberal heifers of certain genotypes, exposed to the presence of mature cows, were younger at puberty than heifers denied similar exposure. At present, it is not known whether the biostimulatory influence of the mature cow is the result of the development and/or maturation of a pheromone metabolic pathway, behavioral cue or some other factor(s) which seem to influence heifers of some breed types.

Currently, it is not known if prostaglandin $F_2\alpha$ is involved in the puberal process in beef heifers. Harms et al. (1973) reported that prostaglandin $E_2$ increased plasma LH concentration if injected into the third ventricle of the brain in ovariectomized rats. Other prostaglandins, including prostaglandin $F_2\alpha$, had no effect. If specific prostaglandins are involved in the puberal process in beef heifers, perhaps the specific type, concentration, or method of administration in Experiment I was inadequate to elicit a response.

The results from Experiment II indicated that the presence of the mature bull, in the biostimulatory ratio of one bull to 27 heifers did not alter the occurrence of puberty or the age and weight at puberty in straightbred and crossbred beef heifers. These results were in agreement with Berardinelli et al. (1978) and MacMillan et al. (1979) who reported that heifers exposed to the presence of a bull did not alter the occurrence of puberty or age and weight at puberty. Izard and Vandenbergh (1981) reported that a greater proportion of prepuberal beef heifers, treated oronasally with bull urine, reached puberty than water-treated controls during the experimental period. The effect of bull urine was suggested to be the result of a urinary pheromone produced by the bull. The response to this theoretical pheromone was dependent upon the body weight of the heifer.

The primary difference between the present study and that of Izard and Vandenbergh (1981) is the type of biostimulatory exposure. In the present study, and in those of Berardinelli et al. (1978) and MacMillan et al. (1979), the physical presence of the bull was used. The response to bull urine may be a function of stimulus concentration and presence of the bull in the imposed biostimulation ratio may have diluted the opportunity of any single heifer to receive adequate exposure to such stimulus.

The reported response to bull biostimulation in the postpartum cow has been contradictory. Similarities exist between the theoretical neuroendocrine mechanisms controlling the onset of puberty and the return to estrus postpartum. Both physiologic processes involve a decrease in sensitivity of the hypothalamic-hypophyseal axis to the

negative feedback of estrogen on LH release (Day et al., 1981; Acosta et al., 1983). MacMillian et al. (1979) reported that a higher proportion of suckled cows were detected in estrus when exposed to a bull 18 days prior to the breeding season. This effect was evident in spring-calving cows but not in fall-calving cows. Fulkerson (1984) reported that estrogen-treated steers did not alter the proportion of postpartum cows detected in estrus nor did the presence of the steers alter first service non-return rate. Fulkerson (1984) suggested that the estrogen-treated steers may have been an inadequate source of biostimulation.

Perhaps the physiologic and behavioral "experience" of the postpartum cow plays a role in the response to biostimulation. Obviously, prepuberal heifers have not experienced the neuroendocrine, endocrine and some possibly unknown metabolic processes associated with puberty. Whereas the postpartum cow has already undergone the processes associated with puberty and numerous other physiologic and behavioral events such as exposure to bulls during the breeding season, maternal recognition of pregnancy, gestation, parturition, distocia and lactation. Through the course of these physiologic and behavioral events, the post-partum cow may have developed or matured systems to receive and respond to a pheromonal or behavorial cue whereas the prepuberal heifer is still in a developmental stage.

Currently, it is not known why the presence of the mature cow alters the occurrence of puberty in prepuberal heifers of certain genotypes. Also, it is not understood why the presence of the bull increases the proportion of postpartum cows detected in estrus.

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It may be concluded from the present two studies and from previously reported literature that biostimulation from estrus synchronized postpuberal beef heifers, an injection of prostaglandin $F_2\alpha$ or mature bulls does not alter the occurrence of puberty or the age or weight at puberty in straightbred and crossbred beef heifers.

# LITERATURE CITED

Acosta, B., G.K. Tarnavsky, T.E. Platt, D.L. Hamernik, J.L. Brown, H.M. Schoenemann and J.J. Reeves. 1983. Nursing enhances the negative effect of estrogen on LH release in the cow. J. Anim. Sci. 57:1530.

Alexander, P.N. and J.A. Froseth. 1983. The influence of level of feeding and boar exposure on reproductive performance of confinement reared gilts. Proc. West. Sec. Am. Soc. Anim. Sci. 34:125.

Arije, G.F. and J.N. Wiltbank. 1971. Age and weight at puberty in Hereford heifers. J. Anim. Sci. 33:401.

Arije, G.F. and J.N. Wiltbank. 1974. Prediction of age and weight at puberty in beef heifers. J. Anim. Sci. 38:803.

Baddaloo, E.G.Y. and F.V. Clulow. 1981. Effects of the male on growth, sexual maturation, and ovulation of young female meadow voles, Microtus Pennsylvanicus. Can. J. Zool. 59:415.

Bellringer, J.F., H.P.M. Pratt and E.B. Keverne. 1980. Involvement of the vomernasal organ and prolactin in pheromonal induction of delayed implantation in mice. J. Reprod. Fert. 59:223.

Berardinelli, J.G., J.J. Ford, R.K. Christenson and L.L. Anderson. 1984. Luteinizing hormone secretion in ovariectomized gilts: Effects of age, reproductive state and estrogen replacement. J. Anim. Sci. 58:165.

Berardinelli, J.G., R.A. Dailey, R.L. Butcher and E.K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276.

Berardinelli, J.G., R.L. Fogwell and E.K. Inskeep. 1978. Effect of electrical stimulation or presence of a bull on puberty in beef heifers. Theriogenology 9:133.

Berardinelli, J.G.. 1976. Induction of puberty in beef heifers. Masters Thesis. West Virginia University, Morgantown, WV.

Booth, W.D., M.F. Hay, and H.M. Dott. 1973. Sexual dimorphism in the submaxillary gland in the pig. J. Reprod. Fert. 33:163.