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Gonadal Hormone Levels Prior to and Following Puberty in Straightbred and Crossbred Beef Heifers

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GONADAL HORMONE LEVELS PRIOR TO AND FOLLOWING PUBERTY IN STRAIGHTBRED AND CROSSBRED BEEF HEIFERS

BY

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GONADAL HORMONE LEVELS PRIOR TO AND FOLLOWING PUBERTY

IN STRAIGHTBRED AND CROSSBRED BEEF HEIFERS

Abstract

WALTER RONALD PARKER

Under the supervision of Professor Christian A. Dinkel

Five heifers from each of four breed groups (Angus x Angus, Charolais x Charolais and the two reciprocal crosses) were used in a study of gonadal hormone levels during the interval from 16 weeks before puberty to puberty (trial I). Puberty was defined as the first standing estrus. Two heifer calves from dams from each of the above breed groups and sired by a single Polled Hereford bull were used in a study of gonadal hormone levels from birth to weaning (trial II).

Blood samples were collected at weekly intervals from trial I heifers before puberty and three times weekly during the first post-puberal estrous cycle. Heifer calves in trial II were sampled once weekly. Total progestins were assayed by a competitive protein binding assay. The binding protein was 2.5% dog plasma containing 16 ng corticosterone-1,2-^{3}H (CBG-B^{3}H). Duplicate plasma samples were extracted two times each with hexane for the removal of progestins. Florisil was used for the removal of unbound steroids from the CBG-B^{3}H. The recovery of progesterone added to plasma blanks was acceptable. Extraction of different quantities of plasma yielded comparable values.

Total estrogens were assayed by a radioimmunoassay utilizing an antibody kindly supplied by Dr. R. D. Randel, U. S. Range Livestock Experiment Station, Miles City, Montana. The antibody was prepared in

sheep by using an estradiol-17β, 11 succinyl-bovine serum albumin conjugate. Cross reactivities with other steroids were estrone 100%, estradiol-17α 80%, estriol 60% and none with cortisol, testosterone or progesterone. Estrogens were extracted from plasma with diethyl ether. Recovery of tritiated estrogens added to plasma was 93.9 ± 2.9 percent. A correlation coefficient between duplicate samples of 0.96 (n = 22 pairs) was obtained. Subsequently, singular assays were conducted on each plasma sample.

Separation of bound from unbound estrogens was accomplished with charcoal and dextran T70. The average blank from the method was 10.0 ± 6.0 picograms. Recovery of estradiol-17β added to plasma blanks was acceptable. Extraction of varying amounts of plasma yielded comparable values. The correlation between values for samples assayed in this laboratory and in the laboratory of Dr. M. L. Hopwood, Colorado State University, was 0.80 (54 pairs).

No differences for hormone levels between breed groups were detected in either trial. Therefore, for further analyses breed groups were pooled.

Breed groups did not differ significantly for date of birth, actual and adjusted weaning weights, date of puberty and age at puberty (trial I). Straightbred Charolais heifers were significantly heavier at birth ($P < .01$) and at puberty ($P < .05$) than straightbred Angus heifers. The two reciprocal crosses did not differ significantly from each other or from straightbreds for these traits.

Average total progestin levels decreased from approximately 1.5 ng/ml at 112 days before puberty to less than 1.0 ng/ml at 70 days before puberty and then gradually rose to approximately 3.0 ng/ml the last 2 weeks before puberty. Average plasma total estrogen levels in the prepuberal heifer decreased from approximately 1000 pg/ml at 16 weeks before puberty to approximately 200 pg/ml during the last 3 weeks before puberty. The regression of age and number of days before puberty on estrogen levels accounted for 31.7 and 29.6% of the variability in estrogen levels, respectively. In a multiple regression analysis these two variables accounted for 34.2% of the variation.

Average plasma total progestin levels during the first postpuberal estrous cycle rose from 1 ng/ml on day 1 to a peak of 6 ng/ml on day 13 and declined from day 14 to approximately 2 ng/ml on day 19. Total estrogen levels during the first postpuberal estrous cycle ranged from 100 to 220 pg/ml. The variation within samples taken on the same day was large. Average estrogen levels fluctuated between 100 and 220 pg/ml from day 1 through day 11. Levels rose steadily from 114 pg/ml on day 12 to 220 pg/ml on day 18 and then dropped to 119 pg/ml on day 19. Samples obtained on the day of estrus were not included.

Average progestin levels in heifer calves from birth to 28 weeks of age remained below 1 ng/ml. Estrogen levels were high during the first 2 days of life (300 pg/ml) but decreased to less than 32 pg/ml on day 3.

Elevated estrogen levels (approximately 40 pg/ml) were observed during the sixth to tenth weeks of life. During the 12th to 23rd weeks

levels remained below 20 pg/ml and rose gradually from the 24th week to 58 pg/ml during the 27th week of life. The discrepancy between estrogen levels in heifers nearing puberty in trial I and the heifer calves in trial II is unexplained.

GONADAL HORMONE LEVELS PRIOR TO AND FOLLOWING PUBERTY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Thesis Adviser Date

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LIST OF ABBREVIATIONS AND SYMBOLS

A x A, A x C, C x A, C x C	denotes breed of animal, breed of sire listed first. A = Angus, C = Charolais
CBG	corticosteroid binding globulin
CBG-B^{3}H	corticosteroid binding globulin-corticosterone-1,2-^{3}H
c/mMole	curies per millimole
FSH	follicle stimulating hormone
FSH-P	follicle stimulating hormone-pituitary
HCG	human chorionic gonadotropin
IU	international unit(s)
LH	luteinizing hormone
ng	nanogram(s) = gram x 10^{-9}
PBS	phosphate buffered saline
pg	picogram(s) = gram x 10^{-12}
PMS	pregnant mares serum
PMSG	pregnant mares serum gonadotropin
R	multiple correlation coefficient
uCi	microcurie(s)
ul	microliter(s)

INTRODUCTION

The beef heifer must reach puberty and conceive by 15 months of age if she is to calve at 2 years of age. It is generally recognized that calving heifers first at 3 years of age is not economically feasible. Many studies have been reported examining the effects of different nutritional regimes on age and weight at puberty in heifers of the British breeds. However, there are indications that some of the later maturing, so-called exotic breeds reach puberty at a later age. Thus, with increased usage of these breeds in the livestock industry, it becomes increasingly important that we understand the mechanisms controlling the onset of puberty.

In recent years a great deal of research effort has been directed toward mapping and understanding the endocrine events which occur during the bovine estrous cycle and during pregnancy. Yet, little research emphasis has been placed on the endocrine events controlling the onset of puberty.

Estimates of the heritability of reproductive traits have generally been low, such that improvement in age at puberty by selection is apt to be slow. Therefore, the most logical method of hastening puberty in the beef heifer is by altering her environment. This may include altering her endocrine environment by the administration of hormones. A greater understanding of the endocrine events occurring prior to puberty will aid in the development of treatments designed to induce precocious puberty.

Differences in the level of fertility are known to exist for different breeds of cattle. Different levels of fertility have been observed for crossbred and straightbred cattle. It has been demonstrated that the crossbred cow excels over the straightbred cow in many reproductive traits. It would seem that measurable differences in certain physiological parameters would exist which would be indicative of these differences in fertility. However, little information of this type is available.

The studies reported herein were conducted to (1) examine levels of gonadal hormones prior to and during puberty and (2) to compare these levels in straightbred and crossbred heifers.

REVIEW OF LITERATURE

The age at which a beef heifer first conceives and thus delivers her first calf has a pronounced effect upon her lifetime production (Webb et al., 1955; Zimmerman et al., 1957; Cundiff, 1973). Webb et al. (1955) and Zimmerman et al. (1957) compared the lifetime performance of beef heifers bred first as yearlings to those bred first as 2-year-olds and concluded that about one extra calf was produced during their lifetime by cows calving first as 2-year-olds as compared to cows calving first as 3-year-olds. Zimmerman et al. (1957) has shown that cows calving first as 2-year-olds had a higher percent calf crop weaned (91.1 vs. 88.2%) and lower cow cost per hundred pounds calf weaned ($10.02 vs. $11.73) when compared to cows calving first as 3-year-olds. An examination of data from the Fort Robinson Beef Cattle Research Station by Cundiff (1973) showed that calving first at 2 years of age as compared to calving first as 3-year-olds essentially produced an extra calf as far as lifetime production of the cow was concerned, since production from 3-, 4-, 5- and 6-year-old cows was similar under both management regimes.

Roberts, LeFever and Wiltbank (1970) have shown that selecting replacement heifers for early pregnancy improved reproductive performance in subsequent years and improved weaning weights of calves. Lesmeister, Burfening and Blackwell (1973) reported that cows calving early in the calving season the first time produced more kilograms of calf in their lifetime than cows calving later the first time.

Wiltbank (1971) has shown that late calving 2-year-old heifers have lower conception rates following calving than early calving 2-year-old heifers. He suggested breeding yearling heifers to calve as 2-year-olds 20 days earlier than the cow herd. These data demonstrate the importance of calving beef heifers first at 2 years of age and thus demonstrate the necessity of their reaching puberty at an early age.

In spite of the relative importance of age at puberty in beef heifers, the mechanism of onset of puberty and the endocrine events leading up to puberty are some of the least studied and least understood areas in endocrine physiology. Although a great deal of research has been directed toward mapping endocrine events during the bovine estrual cycle (Dobrowolski, Stupnicka and Domanski, 1968; Pope, Gupta and Munro, 1969; Donaldson, Bassett and Thorburn, 1970; Garverick et al., 1971; Shemesh, Ayalon and Lindner, 1972; Wettemann et al., 1972) and during and following pregnancy (Pope et al., 1969; Donaldson et al., 1970; Hoffman et al., 1973; Wettemann and Hafs, 1973), the endocrine changes prior to and during puberty are not fully understood.

Macroscopic Activity of the Prepuberal Bovine Reproductive Tract

An examination of slaughter house calves by Casida, Chapman and Rupel (1935) suggested that subthreshold cycles of follicular activity occur in prepuberal heifers. They suggested this based on their observations that (1) there was great variability in calf ovaries at different ages, (2) some young calves had greater follicular development than some older calves and (3) the occurrence of blood follicles in

all ages of calves suggested a rather continuous follicular degeneration.

Howe et al. (1962, 1964) slaughtered Holstein calves at various stages from 30 to 180 days of age and noted that, although no heifers had ovulated, a large percent had one or more follicles 7 to 20 mm in diameter and practically all had at least one follicle at least 3 mm in diameter. Howe et al. (1964) observed atretic follicles in all stages of regression and concluded that age did not appear to be the controlling factor in ovarian activity in prepuberal heifers. Weight of both ovaries was not significantly correlated with either age or live weight of prepuberal calves (Foley et al., 1964). They also observed marked variation among calves of approximately the same age and concluded that the physiological stage of development was more important than chronological age in determining size and weight of ovaries in calves.

Erickson (1966) reported that the number of vesicular follicles in calves less than 2 weeks of age was extremely low and that the number of growing follicles (without vesicles) rose rapidly between 50 and 80 days of age and increased gradually to 120 days. The number of vesicular follicles attained a lifetime high at 180 days of age and declined slightly at puberty at approximately 8 months of age.

Ovarian weight increased almost four times faster than body weight in Holstein heifers from birth to 5 months of age, plateaued from 5 to 8 months and then resumed a growth rate comparable to that of body growth from 8 to 12 months of age (Desjardins and Hafs, 1969).

They reported that ovarian follicles were not visible macroscopically at birth, but that follicle numbers increased to a maximum at about 4 months, decreased to 8 months and remained relatively constant thereafter.

Morrow (1969) reported that increased follicular activity was frequently observed 20 days and sometimes 40 days prior to the first ovulation in Holstein heifers. This increase in follicular size and numbers was associated with increased uterine tone, hyperemic vulvas, mucous discharge and mild signs of estrual behavior in some heifers. He observed ovulations without estrus (silent estrus) in 73.6% of heifers at the first ovulation, in 43.4% at the second ovulation and 20.7% at the third ovulation. Swanson, Hafs and Morrow (1972) reported a high incidence of some degree of estrual activity before the first standing estrus in Holstein heifers but could find no evidence of 20- or 21-day cyclic behavior in these nonovulatory prepuberal activities.

The rate of growth of uteri, cervix and vagina of prepuberal heifers was similar to body growth rates up to 6 months and then increased to a more rapid rate of growth thereafter (Desjardins and Hafs, 1968). Uterine RNA and protein reached a plateau after 10 months of age. They suggested that this age marks the end of rapid puberal growth of the uterus. Howe et al. (1964) noted rapid development of uterine glands in the uterus of calves between 1 and 2 months of age.

Ability of the Prepuberal Ovary to Respond to Gonadal Stimulation

Several workers have established that the ovary of the prepuberal heifer will respond to gonadotropic treatment. Casida et al. (1943) stimulated the formation of corpora lutea in calves 17 to 117 days of age by gonadotropin injections. These corpora lutea appeared to persist as morphological structures for as much as 23 days. Fertility of recovered eggs was quite low. They noted that the ability of ovaries to respond to gonadotropin treatment appeared to be dependent upon the presence of vesicular follicles. However, if follicles were destroyed by electrical cautery, a response was still obtained. They suggested, therefore, that the ability to respond was indicated by vesicular follicles but not necessarily directly dependent upon them. Marden (1953) obtained ovulation and corpus luteum development in calves as young as 9 days with injections of crude pituitary extract materials. Calves 3 weeks old responded equally as well as calves 6 to 8 months old, although no behavioral estrus was observed.

Several workers have used combinations of pregnant mares serum (PMS) and luteinizing hormone (LH) or human chorionic gonadotropin (HCG) to achieve superovulation in prepuberal heifers (Jainudeen, Hafez and Lineweaver, 1966; Onuma and Foote, 1969; Onuma, Hahn and Foote, 1970; Seidel, Larson and Foote, 1971).

Injection of 2000 IU PMS followed by LH or HCG in 5 days resulted in 1 to 50 ovulations in calves 4 to 24 weeks old (Jainudeen et al., 1966). The youngest calf treated (4 weeks) responded just as well as older calves. Those calves observed showed behavioral estrus

5 days after the PMS injection. Although all calves were inseminated and 50 ova were recovered from 10 calves, only two calves yielded fertilized eggs. Onuma and Foote (1969) reported considerable follicular development (means of 64 to 69 follicles) and ovulatory response (means of 34 to 37 ovulation points) in heifers treated during the 17th week of age with 2000 IU PMS followed in 5 days by 25 mg LH. They also noted an increase in mucous flow and some mounting activity. They observed no difference in ovarian response in heifers on an elevated level of nutrition and concluded that nutrition was not responsible for the marked individual variation observed. Onuma et al. (1970) also observed much variability in follicular development in 55 heifers treated at 8 to 9 weeks or 17 weeks of age with 1500 or 2000 IU pregnant mares serum gonadotropin (PMSG). They reported an average of 53 developed follicles (follicles greater than 1 cm in diameter plus ovulation points). Ovulation rates of 79% and 16% following treatment with 50 mg LH and 1500 IU HCG, respectively, 5 days after PMSG administration were observed when ovaries were examined at slaughter 3 days post HCG or LH injection.

Seidel et al. (1971) using a similar treatment (1500 IU PMSG followed by 50 mg LH 5 days later) treated calves at birth, at 1 month of age or at 2 months of age. Calves were slaughtered 3 days after LH injection and ovaries were examined. Ovaries of calves treated at birth did not respond and calves treated at 2 months of age responded better than calves treated at 1 month of age. They suggested that ovaries of calves treated at birth were not capable of responding to

superovulation treatment either because of a lack of vesicular follicles or because of the recent maternal hormone influence. They also reported that follicles normally found in 2-month-old calves were not readily ovulated by LH treatment alone. Onuma et al. (1969) reported considerable follicular and ovulatory response in calves 9 to 11 weeks old receiving five daily injections of 10 mg follicle stimulating hormone (FSH).

Spilman et al. (1972) superovulated calves 3 to 5 months of age with PMS and LH and obtained an average of 46 ovulations per animal. Luteal tissue obtained from these animals responded to LH and nicotinamide adenine dinucleotide phosphate (NADPH) administration in vitro by increased progesterone synthesis.

Neville and Williams (1973) attempted to induce estrus, ovulation and conception in prepuberal beef heifers with treatments similar to those employed for estrus synchronization. Heifers averaging 327 days of age were fed either 120 mg 16a, 17a dihydroxyprogesterone acetophenide (DHPA) for 9 days or 180 mg medroxyprogesterone acetate (MAP) daily for 18 days. On the second day of progestin feeding each heifer received an injection of 5 mg estradiol valerate. Near the end of progestin feeding either PMS or FSH-P was injected. Estrus following PMS or FSH-P was observed in 50 to 100% of the heifers in treated groups with the number of corpora lutea per heifer ranging from 0 to 6.3. Pregnancy rates in treated groups were generally low, ranging from 0 to 50 percent. Arije, Wiltbank and Denham (1969) utilized a subcutaneous implant containing 200 mg Norethandrolone in a

treatment for induction of estrus in prepuberal beef heifers. The implant was placed in heifers for 16 days (days 4 to 19) and followed by 1000 IU PMS on day 19. Some groups also received 2 mg estradiol on day 1 and one group received 2 mg estradiol on day 19. Fifty to 78% of the treated heifers showed estrus and ovulated. Age of heifers at the induced estrus ranged from 9.5 to 11.0 months.

These studies indicate that the ovaries of prepuberal heifers have the ability to respond to gonadotropin treatment and that the subsequently formed luteal tissue is capable of progesterone synthesis. Estrus has been reported in some studies. However, in experiments where animals were inseminated, conception rates have generally been low (Onuma et al., 1970; Neville and Williams, 1973).

Hormone Levels of the Prepuberal Heifer

Gonadotropin

Plasma levels of gonadotropins have not been studied extensively in the prepuberal heifer. Randel et al. (1971) have examined LH levels in prepuberal dairy heifers by taking samples at monthly intervals from 6 to 11 months of age. No significant month to month changes were detected and prepuberal levels (<1.0 ng/ml) averaged only slightly higher than during the first estrous cycle, although sampling interval was such that any preovulatory LH surge may have been missed.

Swanson et al. (1972) began monthly sampling of dairy heifers at 7 months of age and found higher serum LH levels before puberty (2.2 ± 0.3 ng/ml) as compared to cows during the luteal phase of the

cycle (Henricks, Dickey and Niswender, 1970) and significantly higher than levels of the same heifers during the luteal phase after puberty (1.4 ± 0.2 ng/ml). Heifers exhibiting some degree of nonovulatory estrual activity before puberty also exhibited elevated serum LH levels. However, there was no evidence of 20- or 21-day cyclic activity as had been noted by Morrow (1969).

Desjardins and Hafs (1968) studied pituitary FSH and LH levels in Holstein heifers slaughtered at monthly intervals from birth to 12 months of age. Average age at first estrus was 31.7 weeks. Pituitary LH concentration increased fourfold from 2.4 mcg equivalents at birth to 9.1 mcg equivalents at 3 months of age, varied considerably from 3 to 7 months and then declined from 10.4 mcg equivalents at 7 months to 4.8 mcg equivalents at 12 months. Pituitary FSH concentrations were 1.7, 2.7 and 1.1 mcg equivalents at 0, 1 and 2 months of age, respectively, and did not change significantly thereafter. They concluded that puberty was associated with decreased pituitary LH levels.

Gonadal Hormones

Common consensus is that there is little endocrine secretory activity of the testis and ovary prior to puberty (Cole and Cupps, 1969). However, there does not appear to be a preponderance of research confirming this opinion. There have been only a limited number of reports in the literature giving prepuberal blood progesterone (Donaldson *et al.*, 1970; Randel *et al.*, 1971) and estrogen (Robic and Car, 1967) levels.

Donaldson et al. (1970) measured plasma progesterone concentration in prepuberal Brahman x Shorthorn heifers 15 to 18 months of age. Plasma progesterone concentration fluctuated in a way suggestive of cyclic ovarian activity in some heifers, but estrus was not detected. Plasma progesterone concentration ranged from less than 1.0 ng/ml to about 8 ng/ml in these heifers. They also observed one animal which showed no evidence of cyclic ovarian function prior to first estrus and whose progesterone levels did not exceed 1.0 ng/ml prior to first estrus.

Randel et al. (1971) quantitated plasma progesterone in blood samples taken at monthly intervals from five dairy heifers from 6 to 11 months of age and noted a significant increase from 5.0 ± 2.4 ng/ml at 6 months to 14.0 ± 3.0 ng/ml at 11 months of age. Three of these five heifers had a corpus luteum detected by rectal palpation during the sampling period. None of these heifers were detected in estrus, however. These investigators found that freemartins with no detectable gonadal tissue had plasma progesterone levels comparable to controls and suggested that progestins from prepuberal heifers may be of adrenal origin. Balfour, Comline and Short (1959) examined the secretion of steroids from the adrenals of young dairy calves. They noted the secretion of large amounts of 20α hydroxypregn-4-en-3-one during the first 58 days of life and a change over to the secretion of progesterone between the 58th to 64th day.

Roberts and Warren (1964) have demonstrated steroidal transformations in vitro by bovine fetal ovaries more than 1 month before

term. Their data indicated that bovine fetal ovarian tissue upon incubation with progesterone-4-^{14}C and androstenedione-4-^{14}C was capable of accomplishing 17α hydroxylation, 16α hydroxylation, 20α reduction, 17β reduction, cleavage of the side chain and aromatization. They suggested that the fetal ovary may be active in utero.

Plasma estrogen levels in the prepuberal heifer have not been extensively studied. Robic and Car (1967) reported plasma estrogen levels of calves up to 6 months of age to be approximately six times higher than in nonpregnant cows and noted only very slight changes in the concentration of plasma estrogens during this time. They reported no detectable difference between estrogen concentrations of male and female calves. Their findings, however, are not entirely in agreement with those of Velle (1958) and Fowler and Reed (1972). Velle (1958) demonstrated the presence of estradiol-17α and estrone in the urine of newborn calves but found that concentrations decreased to very low levels within the first 6 days of life. Since male calves had higher urinary levels than female calves, he suggested urinary estrogens were not of ovarian origin.

Fowler and Reed (1972) analyzed urine samples from male and female calves at 1, 2, 4, 8 and 16 days of age. Excretion of estrone was essentially nondetectable after day 1, while one-half of the calves sampled at 16 days of age still had detectable levels of estradiol-17β. Least squares analysis of variance revealed that the excretion rates of both estradiol-17β and estrone declined exponentially during the first 16 days of life. Significant differences between estrone

concentrations of male and female calves were noted when expressed as ug/liter urine but not when expressed as ug/mg creatinine excreted. However, the investigators noted that male calves tended to have higher concentrations of both estrone and estradiol-17β.

Allaire et al. (1973) reported that the mean estrogen level in Holstein heifer calves 4 to 6 months of age was 42.3 ± 16.4 pg/ml. They obtained a linear regression of estrogen levels on days of age of -.27 ± 0.11 which indicates that estrogen levels decreased as heifers became older.

Hormone Levels During the Estrous Cycle

The investigation of blood hormone levels during the estrous cycle has received much attention since the development and application of radioimmunoassays (Midgley, 1966, 1967; Abraham, 1969; Niswender et al., 1969) and competitive protein binding assays (Murphy, 1964, 1967; Neill et al., 1967; Johansson, 1969). The sensitivity, precision, relative quickness and requirement for small blood samples of these techniques has made possible detailed studies of the blood hormone levels during the estrous cycle of domestic animals.

Progesterone

One of the more detailed studies of blood plasma progesterone levels during the bovine estrous cycle was performed by Dobrowolski et al. (1968). They measured progesterone levels in ovarian venous blood obtained by cannulation of the ovarian vein. Samples were obtained four times daily at 6-hour intervals. Progesterone

concentration was determined in a paper chromatography system as the 2,4 dinitrophenylhydrazone. Lowest levels were observed on days 1 to 4 of the cycle. Levels rose to an average of about 125 ug/100 ml on day 8, fell for 2 to 3 days, followed by a second rapid rise starting on days 10 and 11 and peaking at about 166 ug/100 ml on day 15. This high level was maintained for only about 24 hours when a continuous decline occurred until the day of ovulation.

Erb, Randel and Callahan (1971) reviewed research concerning the growth pattern of the corpus luteum and its progesterone content during the estrous cycle. The corpus luteum grows rapidly from days 3 to 6, more slowly on days 7 and 8 followed by an increase on days 9 and 10, a decline in rate on days 11 to 13 and another increase on days 13 to 15. This is followed by a 70% decrease in weight from days 15 to 20.

That the pattern of progesterone levels in ovarian venous blood (Dobrowolski et al., 1968) is comparable to that of peripheral blood levels has been well substantiated by workers using gas chromatography (Pope et al., 1969) and protein binding assays (Donaldson et al., 1970; Henricks et al., 1970; Garverick et al., 1971; Henricks, Dickey and Hill, 1971a; Christensen, Wiltbank and Hopwood, 1971; Swanson et al., 1972; Wettemann et al., 1972). The single discrepancy between blood progesterone patterns of ovarian venous blood and peripheral blood is that the marked dip in progesterone levels in ovarian venous blood observed by Dobrowolski et al. (1968) has not been observed in most studies of peripheral plasma progestin levels. Plotka et al. (1967) did observe a similar drop in plasma progesterone levels. However, the

dip occurred on day 6 rather than on days 9 to 10 as reported by Dobrowolski et al. (1968). A corresponding decrease in progesterone content of the corpus luteum has been observed (Erb et al., 1971). The failure to detect such a decline in peripheral plasma may be due to too infrequent blood sampling procedures in some experiments.

Estrogens

Very little information concerning estrogen levels in bovine plasma was available prior to 1970. Available assays were simply not sensitive enough to detect estrogens in peripheral plasma during the estrous cycle (Pope, Jones and Waynforth, 1965). However, since the development of radioimmunoassays (Tillson et al., 1970; Mikhail et al., 1970) and protein binding assays (Korenman, Perrin and McCallum, 1969) specific for estrogens, much information has become available.

Peripheral estrogen levels in the bovine were lowest on day 0 to 2 of the estrous cycle, climbed rapidly to a small peak on day 4 or 5, rose less rapidly to a second higher peak on day 11 or 12 and then surged to their highest levels approximately day 19 or 20 of a 21-day cycle (Christensen et al., 1971; Echternkamp and Hansel, 1971; Henricks et al., 1971a; Mason, Krishnamurti and Kitts, 1972; Shemesh et al., 1972; Wettemann et al., 1972). Shemesh et al. (1972) sampled cows every 4 hours around estrus and found that estradiol peaked at 17 ng/100 ml 4 hours before the onset of estrus. Levels then dropped rapidly during the day of estrus to 0.8 ± 0.11 ng/100 ml before the time of ovulation. Christensen et al. (1971) sampled heifers every 4 hours around estrus and reported that total plasma estrogens peaked at

176 pg/ml 24 hours before the LH peak. Echternkamp and Hansel (1971) and Mason et al. (1972) found that both estradiol and estrone peaked on the day of estrus. Henricks et al. (1971a) reported that in heifers the major estrogen peak (15 to 25 pg/ml) exhibited on the day prior to estrus was in all cases preceded by a minor peak (less than 10 pg/ml) on day 2, 3 or 4 preceding estrus. Their data, based on twice daily bleeding, indicated that estrogen levels peaked 12 to 36 hours prior to the onset of estrus and then fell very rapidly to very low levels within 20 to 30 hours. During proestrus the estrogen level did not exceed 10 pg/ml until the progesterone level had dropped to 2 ng/ml or less.

Garverick et al. (1971) found that urinary estrogen excretion was relatively stable from day 2 to 17 of the estrous cycle and climbed to a peak on the day of estrus.

These data indicate that the major estrogen peak does not occur until after progesterone levels have reached low levels and that the LH peak preceding ovulation is itself preceded by an estrogen peak.

Gonadotropins

Development of highly sensitive radioimmunoassays specific for LH (Niswender et al., 1969) has led to substantial knowledge concerning blood levels of this hormone during the estrous cycle. However, only recently has a similar assay for bovine FSH been developed (Akbar et al., 1973) and a scarcity of data on blood FSH levels during the estrous cycle exists.

Peripheral blood LH levels during the bovine estrous cycle remain relatively constant with the exception of a preovulatory peak (Henricks et al., 1970; Echternkamp and Hansel, 1971; Garverick et al., 1971; Snook, Saatman and Hansel, 1971; Christensen et al., 1971; Swanson et al., 1972; Wettemann and Hafs, 1973). Serum LH levels ranged from nondetectable to 1 ng/ml from days 2 to 20 of the cycle in one study where blood samples were taken every 2 to 3 hours (Henricks et al., 1970). The preovulatory LH peak began 2 to 4 hours before estrus, peaked at 40 ng/ml shortly after the onset of estrus and dropped again to basal levels approximately 18 hours after the onset of estrus. Ovulation occurred 22 to 26 hours after the LH peak. Christensen et al. (1971) also took blood samples every 2 hours and reported the preovulatory LH peak of 18 to 86 ng/ml occurred an average of 9 hours after the onset of estrus and approximately 24 hours prior to ovulation. During the remainder of the cycle levels ranged from 0.6 to 1.6 ng/ml.

Snook et al. (1971) reported that of seven heifers sampled all showed a small luteal LH peak and five of seven also showed a second small peak 4 to 7 days before ovulation. Garverick et al. (1971) observed relatively stable LH levels from day 1 to day 15 and a consistent increase in LH values from 0.4 ng/ml on day 15 to approximately 1.2 ng/ml 1 day before estrus. Blood samples were taken once daily.

Hackett and Hafs (1969) have examined pituitary FSH and LH concentrations during the estrous cycle. Five heifers were slaughtered on each of days 0, 2, 4, 7, 11, 18 and 20 of the estrous cycle and the

pituitary assayed by bioassays for FSH and LH. FSH content of the pituitary decreased 73% from day 18 to its lowest levels on the day of estrus. Levels rose on day 2, decreased approximately 50% from day 2 levels on day 4 and then continuously increased to maximum levels on day 18. Greatest follicular growth occurred between day 18 and the day of estrus, the period of greatest decrease in pituitary FSH levels.

LH levels in the pituitary were greatest on day 20 and decreased 89% by day 2. Levels then rose continuously from day 2 to day 11 with a significant reduction occurring on day 18 prior to the preovulatory peak on day 20.

These data are consistent with the theoretical sequence of events presented by Mason et al. (1972) for the endocrine control of the estrous cycle. They suggested that the rapid decline in plasma progesterone levels beginning at about the 15th day of the cycle gave rise to the release of FSH by the anterior pituitary. The preovulatory release of FSH preceded that of LH by about 2 days (Hackett and Hafs, 1969). Mason et al. (1972) suggested that this FSH release resulted in the production of estrogens which reached a peak level in the systemic plasma on days 19 to 20 and that this estrogen peak was then followed by an LH surge at about the time of initiation of estrus with subsequent ovulation, luteinization and progesterone synthesis.

The crossbred cow has been shown to be superior to the straightbred cow in reproduction and maternal ability (Turner, Farthing and Robertson, 1968; Wiltbank et al., 1966; Wiltbank, Kasson and Ingalls, 1969; Cundiff, 1970). Wiltbank et al. (1966, 1969) have shown that the

crossbred heifer reaches puberty at a younger age than the straightbred heifer. Crossbred cows gave birth to 9.6% more calves and weaned 9.5% more calves than straightbred cows in a Louisiana study (Turner et al., 1968). Cundiff (1970) summarized data from five different experiment stations and reported an advantage for crossbred cows from British breeds of 4.7% for calf crop weaned and 5.4% for calf weaning weight. However, essentially no information was available indicating what inherent qualities the crossbred cow has which make her a more productive individual. Vanjonack, Goret and Johnson (1973) have shown differences between different breeds of cows in their changes in glucocorticoid levels in response to heat or cold stress. Abilay, Johnson and Seif (1973) studied plasma cortisol and progesterone levels in Zebu and Scotch Highland heifers exposed to high temperatures. Highlands and Zebus had significantly different basal plasma cortisol levels. Cortisol levels peaked approximately 140 minutes later in Zebus after heat stress and at less than half the peak levels reached by Highland cattle. Plasma progesterone levels in Highland cattle increased significantly in response to heat stress but not in Zebu cattle.

These data are suggestive that differences in reproductive performance of different breeds of cattle and also of crossbreds can be attributed to differences in reproductive hormone levels.

MATERIALS AND METHODS

The animals used in this study were a portion of the calves from phase II of a crossbreeding experiment being conducted by the South Dakota Agricultural Experiment Station. Ninety heifer calves each of the Angus (A) and Charolais (C) breeds were purchased at weaning in the fall of 1968. Angus and Charolais calves were purchased from 25 and 33 different ranches in South Dakota, respectively. These heifers were mated artificially to either a single Angus or Charolais sire such that straightbred Angus, straightbred Charolais and the two reciprocal crosses were produced. A portion of the heifer calves from the 1970 calf crop produced by these matings was used in a study of gonadal hormone levels from weaning to puberty (trial I). The heifers from the 1970 calf crop were bred to a single Polled Hereford bull in 1971. The first two heifer calves born in each of the four breed groups in 1972 were used in a study of gonadal hormone levels from birth to weaning (trial II).

Trial I

Five heifer calves from the 1970 calf crop were randomly selected from each of the two straightbred and two crossbred breed groups for bleeding purposes. Calves had been weaned on November 11. Average actual weaning weights of the selected group were 166 kg compared to 164 kg for the remaining heifers. At weaning these heifers were placed in the drylot and fed good alfalfa hay free choice plus 3 lb daily of a grain mixture consisting of two parts corn and one part oats for a period of 23 days. Beginning December 4 these heifers were fed in

individual pens where they received free choice a pelleted ration containing approximately 50% roughage (corn cobs and alfalfa hay) and 40% grain (corn, oats and soybean meal). In addition, each animal received 4.5 kg corn silage daily. On May 7, 1971, the corn silage was dropped and in its place each animal received 1.4 kg chopped alfalfa hay daily. On May 6, 1971, the roughage in the pelleted ration was lowered to approximately 40% and grain raised to approximately 56 percent. Heifers were fed once daily from December 4 to January 7, twice daily January 8 to May 14 and were then shifted back to once daily feeding. The twice daily feeding schedule was required to obtain the desired feed consumption and weight gains in these heifers. Heifers gained an average of 0.82 $\pm$ 0.12 kg per head per day during the bleeding period.

Beginning December 23, 1970, each heifer was bled once weekly at approximately 1 pm. Checks were made twice daily for signs of estrus with the aid of a sterilized bull (Wiltbank, 1961). When an individual heifer exhibited her first standing estrus (puberty), the bleeding schedule for that animal was shifted to three times weekly (Monday, Wednesday and Friday) and continued until the animal had exhibited her second standing estrus.

At each bleeding approximately 11 ml of blood were taken by jugular puncture and placed in a small tube containing one drop of heparin. The tube was placed in an ice bath. As soon as all heifers were bled (45 to 60 minutes), the blood was taken to the laboratory and centrifuged for 20 minutes at 6250 g (7200 rpm) at 0 to 5 C. The plasma was removed and split into two aliquots and immediately frozen.

Plasma remained in the frozen state until needed for hormone assays. One aliquot of plasma was used in the progestin assay and the other aliquot in the estrogen assay.

Trial II

In 1972 the first two heifer calves born in each of the four breed groups were selected for bleeding from birth to weaning. These calves were kept in individual pens overnight where they had access to a creep ration. During the daytime they were maintained in a group and had access to water. They were kept separated from their dams except for a short period morning and evening when they were placed with their dams in individual pens and allowed to nurse. The creep ration initially consisted of 95% grain (corn and oats), but on July 5 it was changed to 75% grain and 20% alfalfa. These heifers were bled once weekly by jugular puncture. Since all bleedings were performed on the same day of the week, the age of an animal on the day the initial blood sample was taken could range from 0 to 7 days. Blood samples were handled the same as in trial I.

Progesterone Assay

Total progestins were assayed by the competitive protein binding assay as outlined by Murphy (1964, 1967). The method is based upon the competition between progesterone and corticosterone-1,2-^{3}H for binding sites on the corticosteroid binding globulin (CBG) of dog plasma. Corticosterone-1,2-^{3}H is bound to CBG and subsequently

displaced by the addition of unlabeled progesterone to the system. The larger the amount of total progestins in a sample, the greater the displacement of corticosterone-1,2-^{3}H from the CBG and thus the smaller will be the percentage of corticosterone-1,2-^{3}H bound to the CBG. The amount of total progestin is estimated by comparing the percent corticosterone-1,2-^{3}H bound to CBG to a standard curve obtained by additions of known amounts of progesterone.

Reagents and Materials

All test tubes and pipettes used in the assay were soaked for at least 12 hours in a chromic acid bath, rinsed at least six times with tap water and at least six times with distilled water and dried in an oven at approximately 100 C. Test tubes used for plasma extractions were washed with detergent (Alconox) and rinsed with tap water prior to being placed in the chromic acid bath.

Hexane used for extraction of progestins was Fisher certified reagent and was redistilled two times before use. Florisil (activated magnesium silicate, 60 to 100 mesh) was obtained from J. T. Baker Chemical Company. It was washed repeatedly 15 to 20 times by suspending in distilled water and allowing it to settle. Fine particles which did not settle were poured off. This was repeated until the water was essentially clear. Neill *et al*. (1967) washed their florisil only four times. However, in this laboratory a satisfactory standard curve was not obtained unless repeated washing to remove all fines was accomplished. Washed florisil was dried overnight at 50 to 60 C and then heated to 100 C for 1 to 2 hours and stored in a desiccator.

Corticosterone-1,2-^{3}H having a specific activity of 44 c/mMole was obtained from New England Nuclear and diluted to the desired concentration in twice distilled ethanol without further purification. Unlabeled progesterone was obtained from Nutritional Biochemicals Corporation. Other steroids used in the displacement study were obtained from Sigma Chemical Corporation, St. Louis, Missouri.

Dog plasma used was unstressed male dog plasma obtained from Colorado Serum Company. Dog plasma was obtained frozen in 60 ml quantities. Prior to use, it was thawed, aliquoted in quantities needed for individual assays and refrozen until needed.

The scintillation fluid used for radioactivity measurements was composed of 100 g naphthalene (Fisher certified), 7 g 2,5-diphenyloxazole (PPO, scintillation grade, Amersham/Searle), 0.3 g 1,4-bis(2-(5-phenyloxazolyl))-benzene (POPOP, scintillation grade, Amersham/Searle) in 1 liter 1,4-dioxane (Fisher certified).

Radioactivity was counted by liquid scintillation on a Packard Tri Carb Model 3002.

The CBG-corticosterone-1,2-^{3}H (CBG-B^{3}H) was prepared by placing 200 ul ethanol containing 16 ng corticosterone-1,2-^{3}H (5×10^6 dpm) in a 100 ml volumetric flask. The ethanol was evaporated in a water bath at 45 C under a stream of compressed nitrogen gas. To the dried flask was added 2.5 ml dog plasma and this brought to 100 ml with glass distilled water. The solution was mixed thoroughly and placed in the refrigerator until used.

Standard Curve

Progesterone was dissolved in twice distilled ethanol at concentrations such that 50 ul ethanol yielded 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 10.0 ng progesterone. Duplicates of each of these levels were prepared by placing 50 ul of the ethanol-progesterone solution in 15 x 85 mm disposable glass test tubes and evaporating to dryness in a 45 C water bath under a stream of nitrogen gas. These tubes were carried through the protein binding procedure along with extracted unknowns.

Extraction Procedure

Duplicate aliquots of 0.2 ml of plasma to be assayed were placed in 15 x 125 mm glass test tubes. Samples were placed in a refrigerator and removed 12 at a time for extraction. Each tube was extracted by adding 3 ml hexane and shaking 40 seconds at moderate speed on a vortex mixer. Hexane was removed by aspiration with a 9 inch Pasteur pipette and transferred to 15 x 85 mm disposable glass culture tubes. The extraction was repeated a second time and the extracts combined. Hexane was then evaporated in a 45 C water bath under a stream of nitrogen gas.

Recoveries with this extraction procedure were tested by placing a small quantity (approximately 350 cpm) of progesterone-7-^{3}H (specific activity 16 c/mMole) dissolved in ethanol into an extraction tube and evaporating off the ethanol. An aliquot of 0.2 ml plasma was added to the tube, mixed, heated to 45 C in a water bath for 5 minutes, shook and cooled. The plasma containing the progesterone-7-^{3}H was then extracted as outlined above. Hexane from the extraction was placed

directly into a scintillation vial and evaporated to dryness. Scintillation fluid was added and the system counted in a liquid scintillation counter. An equal quantity of progesterone-7-^{3}H was placed in a scintillation vial, evaporated to dryness and counted in the liquid scintillation system. Recovery was expressed as the percent progesterone-7-^{3}H recovered. This method gave an average recovery of 74.9 ± 5.5% (n = 14).

Protein Binding Procedure

The protein binding procedure used was as follows. One ml of CBG-B^{3}H was pipetted by use of a Repipet (1 ml, Labindustries, Berkeley, California) into each unknown and standard tube. All tubes were then mixed gently for exactly 30 seconds on a vortex mixer. They were then placed in a water bath held at 45 C for exactly 5 minutes. Tubes were then placed immediately in an ice bath for 10 minutes. Each tube was mixed gently for 10 seconds during this time.

Two persons were required for the removal of unbound labeled corticosterone-1,2-^{3}H from the CBG-B^{3}H solution. One person removed one tube at a time from the ice bath and added approximately 90 mg florisil to it by use of a small cup calibrated to deliver that quantity. The tube was then mixed at medium speed for exactly 30 seconds on a vortex mixer. The tube was then placed back in the ice bath and remained there until the fifth tube so treated was returned to the ice bath. At this time a second person removed the first tube from the ice bath and by use of a 500 ul Eppendorf pipette (Brinkmann Instruments, Inc.) placed 500 ul of the supernatant into a scintillation

vial. When all tubes had been so treated, 10 ml liquid scintillation fluid were added to each, mixed vigorously for approximately 3 seconds and counted in a liquid scintillation counter.

The standard curve was obtained by plotting ng unlabeled progesterone on the abscissa and percent bound on the ordinate. The "percent bound" refers to the percent of the corticosterone-1,2-^{3}H in the zero tubes which was not displaced by the unlabeled progesterone in the standard tubes. Unbound corticosterone-1,2-^{3}H was removed by the florisil treatment. Thus, the higher the level of unlabeled progesterone in a sample, the more displacement of corticosterone-1,2-^{3}H and the lower the percent bound. A typical standard curve is shown in figure 1.

Percent bound was calculated for all unknown tubes and the progesterone level read from the standard curve. Since only 0.2 ml of plasma was extracted, all values for unknowns have been multiplied by a factor of five to put them on a ml basis.

Validation of Assay

Several tests were performed to determine the specificity and accuracy of the progestin assay. A specificity test was performed by determining the ability of various progestins, corticoids, androgens and estrogens to displace corticosterone-1,2-^{3}H from CBG-B^{3}H. Steroids tested were added directly to assay tubes in ethanol carrier at 0, 1, 2, 3, 4 and 5 ng levels in duplicate. They were evaporated to dryness and used in the protein binding assay. The ability of the steroids tested to displace corticosterone-1,2-^{3}H from CBG-B^{3}H is illustrated

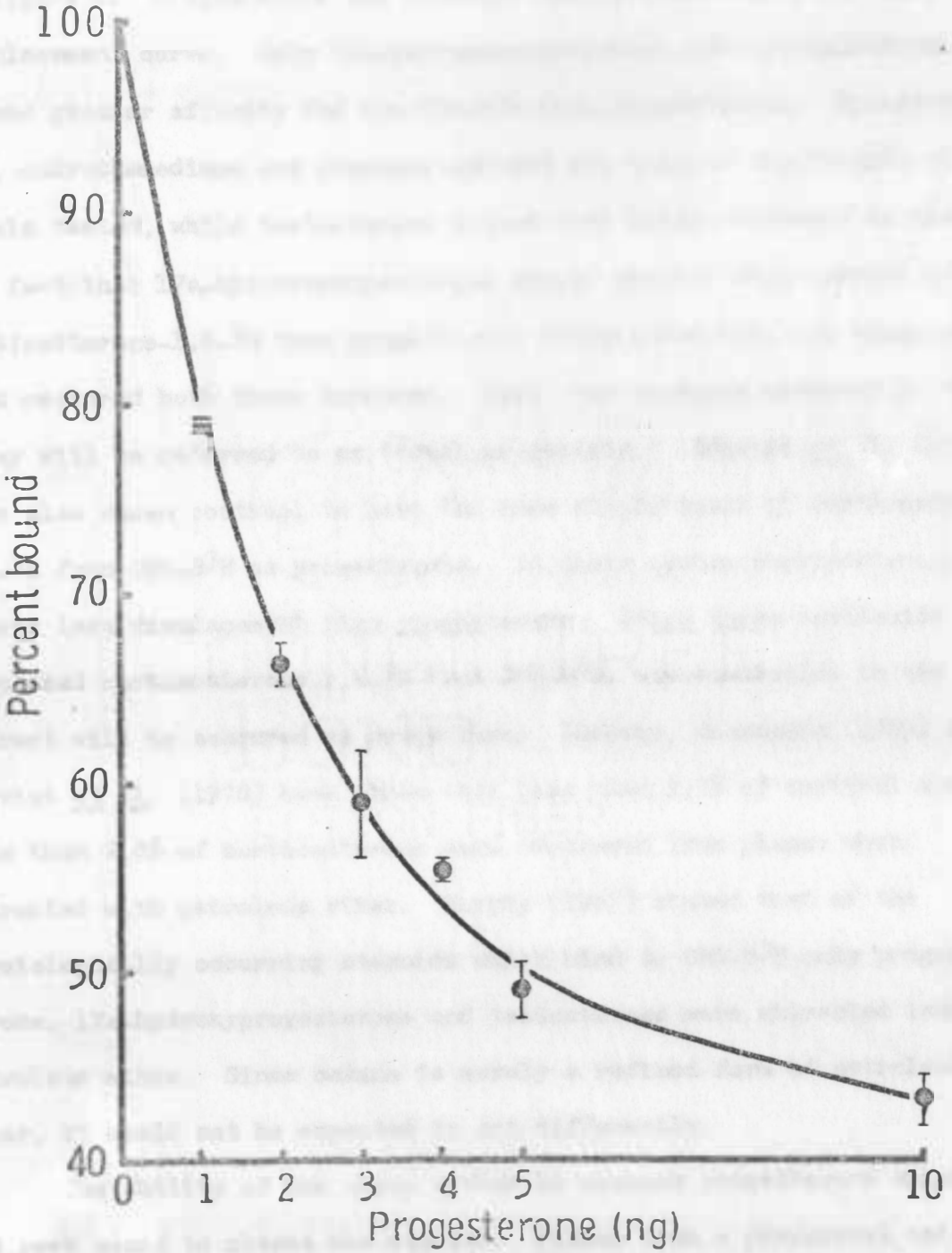

Figure 1. Typical standard curve, progestin assay. Vertical lines represent the range of duplicate samples.

in figure 2. Progesterone and cortisol showed essentially the same displacement curve. Only 17a-hydroxyprogesterone and corticosterone showed greater affinity for the CBG-B^{3}H than progesterone. Estradiol 17B, androstenedione and pregnenolone did not bind to the CBG-B^{3}H at the levels tested, while testosterone showed very little tendency to bind. The fact that 17a-hydroxyprogesterone showed greater displacement of corticosterone-1,2-^{3}H than progesterone illustrated that the assay as used measured both these hormones. Thus, the steroids measured in this assay will be referred to as "total progestins." Edqvist et al. (1970) have also shown cortisol to have the same displacement of corticosterone-1,2-^{3}H from CBG-B^{3}H as progesterone. In their system corticosterone showed less displacement than progesterone. Since these corticoids displaced corticosterone-1,2-^{3}H from CBG-B^{3}H, any corticoids in the extract will be measured as progestins. However, Johansson (1969) and Edqvist et al. (1970) have shown that less than 1.0% of cortisol and less than 2.0% of corticosterone were recovered from plasma when extracted with petroleum ether. Murphy (1967) stated that of the physiologically occurring steroids which bind to CBG-B^{3}H only progesterone, 17a-hydroxyprogesterone and testosterone were extracted into petroleum ether. Since hexane is merely a refined form of petroleum ether, it would not be expected to act differently.

The ability of the assay system to measure progesterone which had been added to plasma was studied. Plasma from a prepuberal heifer which had been previously analyzed and found to be practically devoid of progestins was used. Unlabeled progesterone in ethanol carrier was

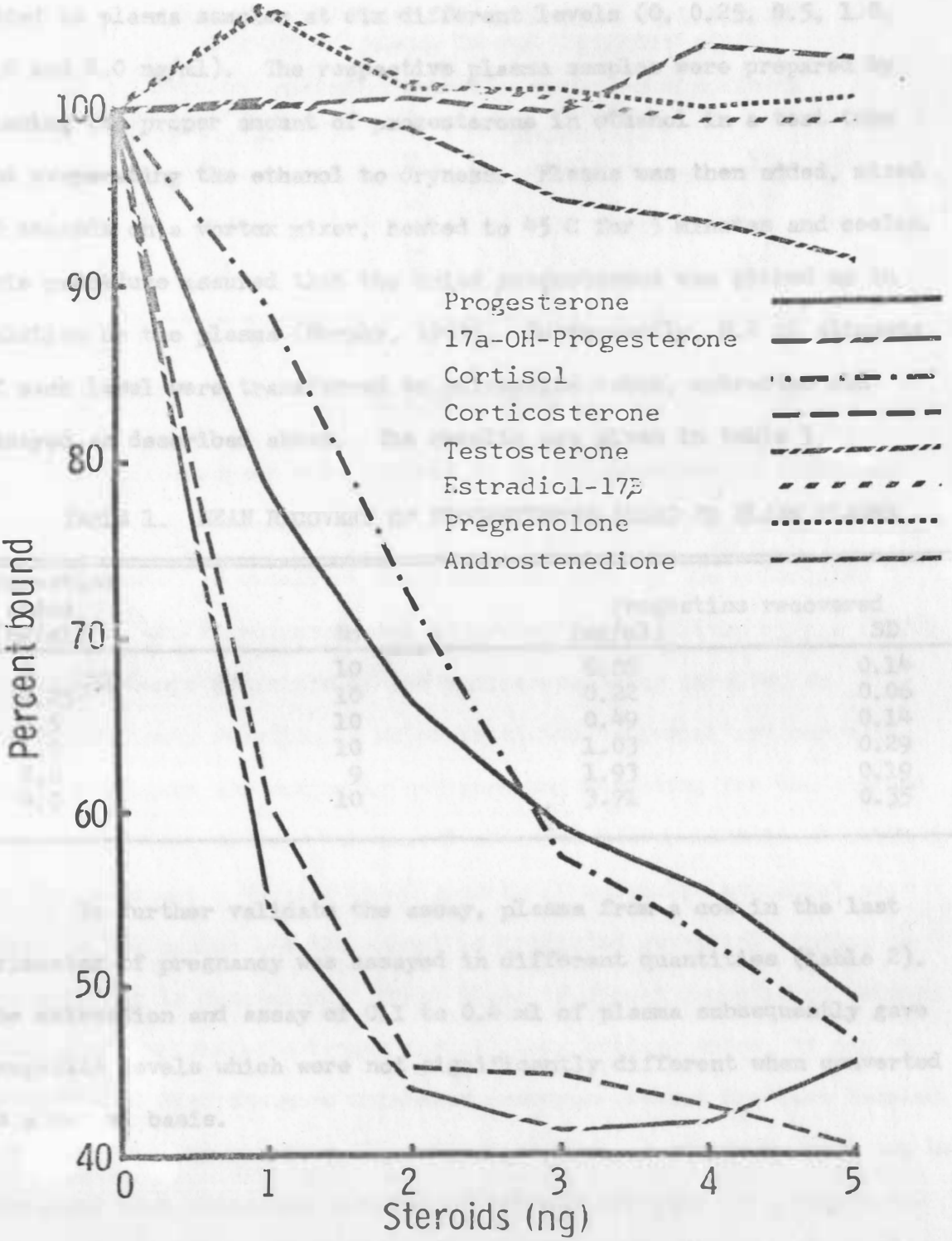

Figure 2. The displacement of corticosterone-1,2-^{3}H from CBG by certain steroids.

added to plasma samples at six different levels (0, 0.25, 0.5, 1.0, 2.0 and 4.0 ng/ml). The respective plasma samples were prepared by placing the proper amount of progesterone in ethanol in a test tube and evaporating the ethanol to dryness. Plasma was then added, mixed 30 seconds on a vortex mixer, heated to 45 C for 5 minutes and cooled. This procedure assured that the dried progesterone was picked up in solution by the plasma (Murphy, 1967). Subsequently, 0.2 ml aliquots of each level were transferred to extraction tubes, extracted and assayed as described above. The results are given in table 1.

TABLE 1. MEAN RECOVERY OF PROGESTERONE ADDED TO BLANK PLASMA

Progestins added (ng/ml)	No.	Progestins recovered (ng/ml)	SD
0	10	0.08	0.14
0.25	10	0.22	0.06
0.5	10	0.49	0.14
1.0	10	1.03	0.29
2.0	9	1.93	0.19
4.0	10	3.71	0.35

To further validate the assay, plasma from a cow in the last trimester of pregnancy was assayed in different quantities (table 2). The extraction and assay of 0.1 to 0.4 ml of plasma subsequently gave progestin levels which were not significantly different when converted to a per ml basis.

TABLE 2. COMPARISON OF EXTRACTION OF DIFFERENT ALIQUOTS OF PLASMA IN THE PROGESTIN ASSAY

Plasma extracted (ml)	No.	Total progestins (ng/ml)	SD
0.1	10	11.2	1.7
0.2	9	11.5	0.9
0.3	10	10.6	1.1
0.4	6	10.6	1.2

Estrogen Assay

Total estrogens were assayed by a radioimmunoassay technique similar to that described by Henricks et al. (1971a) but with several modifications. An excellent discussion of some of the principles involved in the radioimmunoassay is presented by Tillson et al. (1970).

The basic principle of the radioimmunoassay involves an antigen-antibody reaction in which tritiated estradiol and nonradioactive estrogens are acting as antigens and competing for the limited antibody binding sites. The amount of tritiated estradiol and antibody is kept constant. Varying known amounts of unlabeled estradiol are added to the system and the amount of tritiated estradiol binding to the antibody is determined. Since there is direct competition between labeled and unlabeled estrogen for antibody binding sites, it can be demonstrated that the more unlabeled estrogen present the less labeled estradiol will be bound to the antibody. Thus, a standard curve can be developed from which the quantity of unknown estrogen in a sample can be calculated.

Reagents and Materials

All glassware used in the assay was washed as described for the progestin assay.

Phosphate buffered saline (PBS, 0.1 M Phosphate, pH 7.0) was prepared by the addition of 5.38 g NaH_2PO_4-HOH, 16.35 g Na_2HPO_4-7HOH, 9 g NaCl, 1 g Na Azide and 1 g Knox Gelatin to 1000 ml glass distilled water.

Estradiol-17β-6,7-^{3}H having a specific activity of 46.6 c/mMole was obtained from New England Nuclear and used without further purification. Tritiated estradiol was initially received in a quantity of 0.25 ml and was further diluted with twice distilled ethanol to 5.0 milliliters. Aliquots of this solution were further diluted to PBS such that 100 ul of the resulting solution delivered approximately 0.02 uCi per 100 ml PBS.

A suspension of 0.25% activated untreated charcoal powder (Norit A, Sigma Chemical Company) and 0.25% Dextran T-70 (Pharmacia Fine Chemicals) in PBS was used for absorbing estrogens not bound to the antibody.

The antibody used in these studies was kindly supplied by Dr. R. D. Randel, U. S. Range Livestock Experiment Station, Miles City, Montana. This antibody has been characterized and validated by Stellflug (1972). The antibody was prepared in sheep by using an estradiol-17β, 11 succinyl-bovine serum albumin conjugate. The cross reactivity of this estradiol-17B antisera with other steroids is estrone 100%, estradiol-17α 80%, estriol 60% and it has no cross

reaction with cortisol, testosterone or progesterone (R. D. Randel, personal communication). This antibody was used at a dilution of 1:60,000 in PBS.

Standard solutions of estradiol-17β (Sigma Chemical Company) were prepared by dissolving the required amount of steroid in twice distilled ethanol. Concentrations were prepared such that 50 ul of the solution gave the desired quantity of estradiol-17β for construction of the standard curve, i.e., 0, 12.5, 25, 50, 100 and 200 picograms. Standard solutions and all PBS solutions were stored in a refrigerator at 5 C except when in use and were then immediately returned to the refrigerator.

Extraction Procedure

For the extraction of estrogens aliquots of each plasma sample were placed in 18 x 150 mm test tubes. Aliquots of 0.05 to 0.5 ml plasma were used depending upon the level of estrogen expected in each sample. Plasma samples of 0.2 ml or less were extracted with 2 ml freshly opened, anhydrous diethyl ether (Fisher reagent grade). Larger plasma samples were extracted with 3 ml ether.

The detailed extraction procedure was as follows. Ether was added to a group of six samples and each was stoppered with a cork stopper. Tubes were placed at approximately an 80 degree angle in a test tube rack attached to a metabolic shaker and shook for exactly 3 minutes. The base of the test tube was then placed in a dry ice-alcohol bath containing approximately 3/8 inch alcohol. As soon as the plasma was completely frozen, the ether containing the extracted

estrogens was poured into a 15 x 85 mm disposable glass test tube. The ether was evaporated off in a warm water bath under a stream of nitrogen gas leaving the extracted estrogen as a residue.

Recovery of estrogens from plasma samples was determined by including a set of six tubes of plasma to which had been added 12.5 pg estradiol-17β-6,7-^{3}H (approximately 1800 cpm) in each series of samples extracted on a particular day. Estradiol-17β-6,7-^{3}H in 50 ul ethanol was added to a set of test tubes and the ethanol evaporated off under a stream of nitrogen. A corresponding volume of estradiol-17β-6,7-^{3}H was added directly to a set of three liquid scintillation vials. A volume of plasma equal to that of plasma samples being extracted was then added to each test tube, shook gently on a vortex mixer and warmed to 45 C in a water bath for 5 minutes. Following cooling for at least 10 minutes in an ice bath, tritiated samples were extracted as outlined above except that following freezing of plasma in an alcohol-dry ice bath the ether was poured directly into a liquid scintillation vial. Ether was evaporated off in a warm water bath under a stream of nitrogen gas and 10 ml scintillation fluid added to each vial as well as to the three vials to which estradiol-17β-6,7-^{3}H had been added directly. All samples were counted in a liquid scintillation counter. Recovery from the plasma was expressed as a percentage of the estradiol-17β-6,7-^{3}H added directly to scintillation vials.

Percent recovery from nine consecutive assays averaged 93.9 ± 2.9 (range 88.2 to 103.1%). An analysis of variance indicated that assays did not differ in percent recovery ($P \geq .05$).

With each series of samples extracted a set of empty test tubes was extracted to determine the reagent and solvent blank. The average blank over 10 consecutive assays was 10.0 ± 6.0 pg (range 0 to 25). Blanks compared between assays were not significantly different ($P > .10$).

Radioimmunoassay Procedure

The refrigerated centrifuge available had capabilities for spinning only 28 tubes at a time. Each 28-tube assay included a set of standard tubes composed of two zero tubes and one each containing 12.5, 25, 50, 100 and 200 pg estradiol-17β. Standard tubes were prepared by adding 50 ul of the proper standard solution to each tube. Zero tubes received 50 ul ethanol without estradiol-17β. Contents of all standard tubes were then evaporated to dryness in a warm water bath under a stream of nitrogen gas. In addition, each assay usually contained one blank tube and one "total count" tube. Total count tubes gave a measure of the total amount of estradiol-17β-1,2-H^3 added to each tube.

The detailed procedure for each assay consisted of the following steps. To each unknown sample, standard, blank and total count tube was added 100 ul of antibody diluted 1:60,000 in PBS followed by 100 ul of estradiol-17β-6,7-^3H in PBS. All tubes were mixed gently for 20 seconds on a vortex mixer, covered with aluminum foil and incubated in a refrigerator at 5 C for 2 hours. At the end of the incubation period 1 ml of the dextran-charcoal solution was added to all tubes with the exception of the total count tube which received 1 ml PBS. All tubes were mixed at a moderate speed on a vortex mixer for 30 seconds. Immediately following mixing all tubes were centrifuged at

1500 g (2500 rpm) at 4 ± 1 C in an International refrigerated centrifuge, Model PR-2, for 10 minutes. The supernatant was poured into scintillation vials. Ten ml of scintillation fluid were added and each sample was counted for radioactivity. All samples were counted for 5 minutes on a Packard tri-carb liquid scintillation counter, Model 3002.

A standard curve was obtained essentially as described above for the progestin assay by plotting percent bound on the ordinate and pg unlabeled estradiol-17β added to standards on the abscissa. Percent bound is the amount of estradiol-17β-6,7-^{3}H in the standard tubes expressed as a percentage of the estradiol-17β-6,7-^{3}H in zero tubes. It refers to the amount of estradiol-17β-6,7-^{3}H which is bound to antibody during the 2 hour incubation period. Unbound estrogens, labeled and unlabeled, are removed from the system by the dextran-charcoal treatment. Since there is assumed to be direct competition between labeled and unlabeled estrogens for antibody binding sites, it can be seen that higher levels of unlabeled estrogens will result in lower percent bound figures. A typical standard curve is shown in figure 3. Each point is an average of values for that level from 11 consecutive assays. The standard deviations of each point are indicated by vertical lines.

Percent bound was also calculated for unknown and blank tubes and pg of total estrogens read from the standard curve. Since estradiol-17a, estrone and estriol cross react with estradiol-17β and no attempt was made to separate these estrogens, the assay must be considered as measuring "total estrogens."

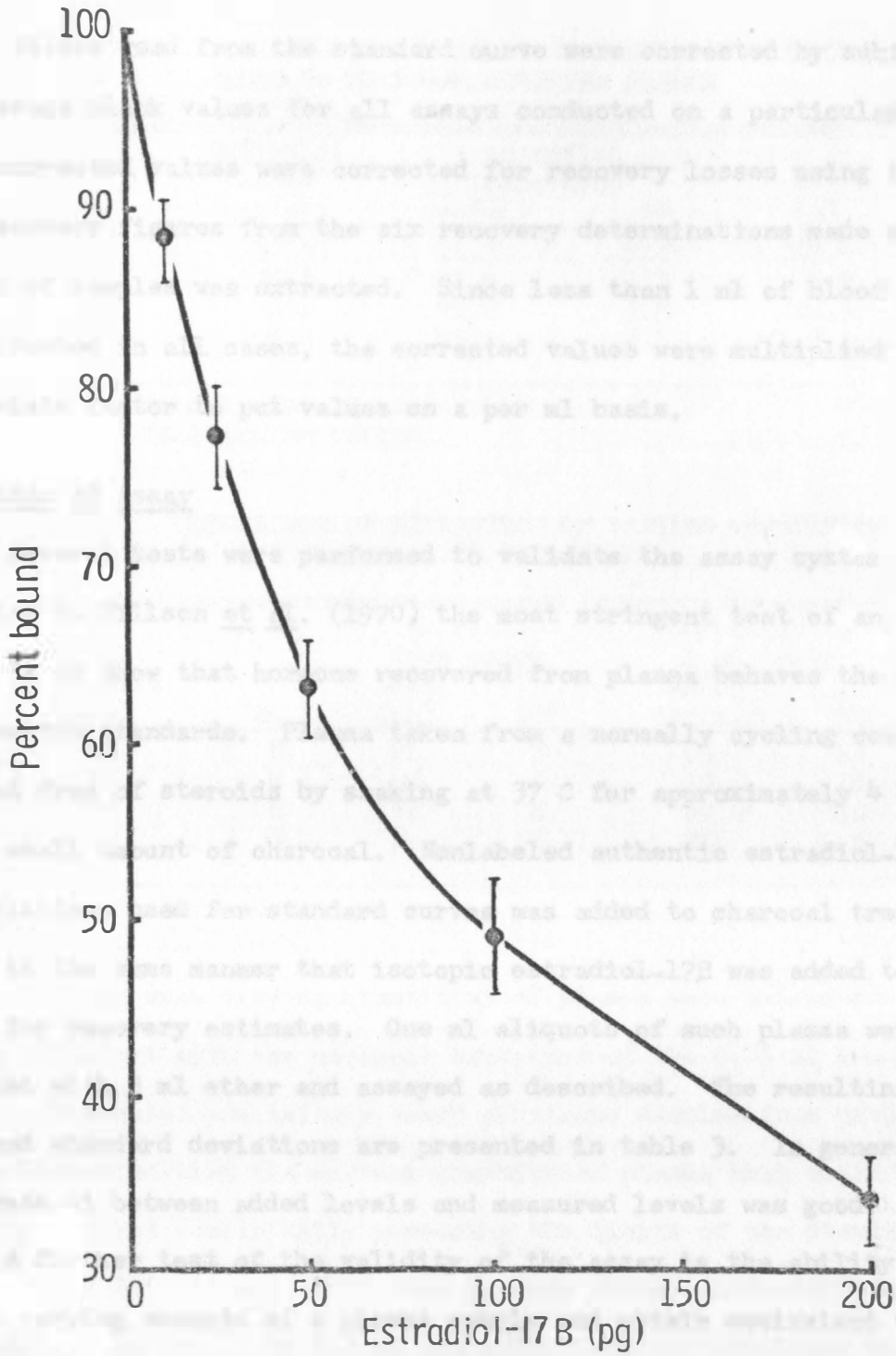

Figure 3. Typical standard curve, estrogen assay. Each point is the average of 11 consecutive assays, vertical lines are standard deviations.

Values read from the standard curve were corrected by subtracting the average blank values for all assays conducted on a particular day. Blank corrected values were corrected for recovery losses using the mean recovery figures from the six recovery determinations made when the set of samples was extracted. Since less than 1 ml of blood plasma was extracted in all cases, the corrected values were multiplied by the appropriate factor to put values on a per ml basis.

Validation of Assay

Several tests were performed to validate the assay system. According to Tillson et al. (1970) the most stringent test of an assay system is to show that hormone recovered from plasma behaves the same as authentic standards. Plasma taken from a normally cycling cow was stripped free of steroids by shaking at 37 C for approximately 4 hours with a small amount of charcoal. Nonlabeled authentic estradiol-17β from solutions used for standard curves was added to charcoal treated plasma in the same manner that isotopic estradiol-17β was added to plasma for recovery estimates. One ml aliquots of such plasma were extracted with 3 ml ether and assayed as described. The resulting means and standard deviations are presented in table 3. In general, the agreement between added levels and measured levels was good.

A further test of the validity of the assay is the ability to extract varying amounts of a plasma sample and obtain equivalent values from each when converted to a per ml basis. Plasma obtained from a normally cycling cow was used. Quantities of plasma varying from 0.25 to 1.0 ml were extracted and assayed. As can be seen in table 4, the

TABLE 3. ABILITY OF THE ASSAY TO MEASURE ESTRADIOL ADDED TO CHARCOAL STRIPPED PLASMA

No.	Estradiol added (pg/ml)	Estradiol recovered (pg/ml)	SD	CV[a] (%)
8	12.5	11.3	3.1[b]	27.4
8	25.0	21.4	1.7	7.9
8	50.0	46.8	5.8	12.4
8	100.0	95.0	8.1	8.5

[a] Coefficient of variability.
[b] Standard deviation.

TABLE 4. COMPARISON OF EXTRACTION OF VARYING QUANTITIES OF PLASMA (0.25 TO 1.0 ML)

Plasma extracted (ml)	No.	As read from curve Mean (pg)	As read from curve SD[a]	Corrected to 1 ml basis Mean (pg/ml)	Corrected to 1 ml basis SD
0.25	8	21	3	85	12
0.50	8	35	5	70	11
0.75	8	51	8	69	10
1.00	8	71	6	71	6

[a] Standard deviation.

values obtained when varying quantities of plasma were extracted are in good agreement with the possible exception of the 0.25 ml group. However, subsequent preliminary assay of plasma samples from prepuberal heifers indicated that the maximum quantity of plasma that could be extracted without consistently exceeding the limits of the standard curve was 0.2 milliliter. With this in mind a comparison of results from extractions of 0.1 to 0.3 ml and of 0.05 to 0.2 ml plasma was obtained. The results are shown in tables 5 and 6, respectively. Plasma used for the comparisons in table 5 was from a normally cycling

TABLE 5. COMPARISON OF EXTRACTION OF VARYING QUANTITIES OF PLASMA (0.1 TO 0.3 ML) FROM A CYCLING COW

Plasma extracted (ml)	No.	As read from curve Mean (pg)	SD[a]	Corrected to 1 ml basis Mean (pg/ml)	SD
0.10	8	6	2	66	21
0.15	8	9	4	62	28
0.20	8	14	3	68	14
0.25	8	19	4	74	14
0.30	8	23	5	76	18

[a] Standard deviation.

TABLE 6. COMPARISON OF EXTRACTION OF VARYING QUANTITIES OF PLASMA (0.05 TO 0.2 ML) FROM A COW IN THE THIRD TRIMESTER OF PREGNANCY

Plasma extracted (ml)	No.	As read from curve Mean (pg)	SD[a]	Corrected to 1 ml basis Mean (pg/ml)	SD
0.05	8	12	6	243	113
0.10	8	27	6	274	61
0.15	8	38	9	251	59
0.20	8	49	6	243	30

[a] Standard deviation.

cow, while that for the comparison in table 6 was from a cow in the third trimester of pregnancy.

In general, there was greater variation where smaller quantities of plasma were extracted.

A random selection of samples from all animals in trial I was assayed following the extraction of 0.2 ml plasma in duplicate. The calculated correlation coefficient between duplicate samples was 0.96 (n = 22 pairs).

All samples from two heifers in trial I were transported to Colorado State University where they were assayed in the laboratory of Dr. M. L. Hopwood, utilizing the solid-phase radioimmunoassay for total estrogens as described by Abraham (1969). Subsequent to the analysis of the same samples at South Dakota State University by the procedure described above, a correlation coefficient between samples assayed at South Dakota State University and Colorado State University was calculated. This value ($r = 0.80$, 54 pairs) is comparable to the value ($r = 0.85$) reported by Wettemann et al. (1972) for the comparison of progesterone levels obtained by two different methods in the same laboratory.

A single sample of 0.2 ml plasma was extracted for the assay of all samples from trial I. Samples from this analysis which exceeded the limits of the standard curve were subsequently reassayed in duplicate following the extraction of 0.1 ml plasma. Finally, if these samples also exceeded the limits of the standard curve, they were reassayed in duplicate following the extraction of 0.05 ml plasma. Average values are reported for samples assayed in duplicate.

All samples from trial II were assayed following the extraction of a single aliquot of 0.5 ml plasma with the exception of one sample. This sample was obtained from a 1-day-old calf and had an extremely high estrogen level (4000 pg/ml) obtained following the extraction of 0.1 ml plasma.

Statistical Analysis of Data

Day of the year of birth and puberty, weights at birth, weaning and puberty and age at puberty for the four breed groups were compared by least squares procedures. A set of orthogonal comparisons was made for these variables only if the least squares analysis revealed significant differences existed. The comparisons which were made were the two straightbred groups to the two crossbred groups and comparisons within these groups, i.e., A x A to C x C and A x C to C x A.

Since animals in trial I reached puberty at different ages and animals in trial II were born on different dates, the number of plasma samples obtained differed for each animal. Therefore, for statistical analysis of plasma hormone levels in trial I the only data used were the 16 weekly samples obtained immediately preceding puberty. Similarly in trial II only data from plasma samples obtained during the first 28 weeks of life were used.

Plasma hormone levels for the four breed groups were compared by the split-plot procedure outlined by Gill and Hafs (1971). In trial I the time element (days before puberty) was considered as weekly periods with the first period being days 1 to 7 before puberty. Samples obtained on the day of first standing estrus were not included. In trial II the time element was days of age with the first period being days 1 to 7 of age. Samples obtained on the day of birth were not included. Since weekly blood samples were taken in both trials I and II, each weekly period comprises one sample per animal.

Polynomial regression was used to determine linearity or non-linearity of the dependent variables (estrogen and progestin levels) when plotted against time. Stepwise multiple regression was used to determine the amount of variation in hormone levels controlled by one or more independent variables.

All animals were weighed every 28 days. For an analysis of the effect of weight upon plasma hormone levels two methods were used. In both trial I and trial II analyses were made using only the hormone levels corresponding to the single plasma sample taken closest to each weigh day. In trial II an analysis was also made using the average of the values of the two hormone values corresponding to the two plasma samples taken closest to each weigh day. Thus, since weekly samples were taken, these were the values from weekly samples taken immediately before and after each weigh day.

For the purpose of analysis the first postpuberal estrous cycles in trial I from which blood samples were collected were standardized to 20 days in length (Garverick et al., 1971). This included 21 days in which blood collections could have been made since both the initial estrus and estrus of the next cycle were included. Two of the 20 heifers sampled were not included in the analysis. One heifer had only a 12-day cycle. The other heifer had insufficient data due to several missing values. The estrous cycles of the remaining heifers ranged from 16 to 39 days in length. The first 15 days of the cycle were numbered consecutively 0 through 14 (day of estrus = day 0).

The second estrus was also numbered day 0 and the five consecutive preceding days numbered 20, 19, 18, 17 and 16, respectively.

In this paper the maximum probability level considered significant was 5 percent.

RESULTS AND DISCUSSION

Trial I

Mean squares for birth date, birth weight, actual and adjusted weaning weight, date of puberty and age and weight at puberty are presented in table 7 for the 20 animals in trial I. The only significant differences between breed groups detected were for birth weight ($P<.01$) and for weight at puberty ($P<.05$). The means for these traits for the four breed groups are presented in table 8.

Orthogonal comparisons made for those traits shown to be significantly different between breed groups indicated that for both birth weight ($P<.01$) and weight at puberty ($P<.05$) the C x C breed group was heavier than the A x A breed group. Sagebiel *et al.* (1973) also reported C x C calves to be heavier at birth than A x A calves ($P<.05$). They also reported C x C calves to be heavier at birth than both C x A and A x C crossbreds and A x A calves to be lighter at birth than the two reciprocal crosses ($P<.05$).

Very little published information is available concerning age and weight at puberty in Angus and Charolais and their reciprocal crosses. Laster, Glimp and Gregory (1972) reported information indicating there was little difference between straightbred Angus and C x A heifers in percent reaching puberty by 15 months of age and in age at puberty in heifers involved in the germ plasm evaluation program at the U. S. Meat Animal Research Center. However, C x A heifers were heavier at puberty than straightbred Angus heifers

TABLE 7. MEAN SQUARES FOR BIRTH, WEANING AND PUBERTY DATA FROM TRIAL I HEIFERS

Source of variation	df	Date of birth	Birth weight	Weaning weight		Date of puberty	Age at puberty	Weight at puberty
				Actual	Adjusted			
Breed group	3	68	145**	95	281	364	277	2062*
Residual	16	284	14	343	385	400	705	498
Total	19							

* $P < .05$.
** $P < .01$.

TABLE 8. MEANS AND STANDARD DEVIATIONS FOR BIRTH, WEANING AND PUBERTY DATA FROM TRIAL I HEIFERS

Breed group	No.	Date of birth[a]	Birth weight (kg)	Weaning weight		Date of puberty[a]	Age at puberty (days)	Weight at puberty (kg)
				Actual (kg)	Adjusted (kg)			
A x A[b]	5	109±18[c]	24.0±2.7[d]	162±23	195±21	129±25	386±23	287±29[e]
A x C	5	115±15	31.0±5.3	161±16	198±11	125±20	374±31	286±22
C x A	5	114±17	33.2±3.8	170±19	208±21	132±12	384±15	315±11
C x C	5	117±17	36.8±2.8[d]	168±14	209±23	145±20	392±33	326±24[e]
Overall	20	114±16	31.3±5.9	165±17	203±19	133±20	384±25	304±27

[a] Day of the year.
[b] A = Angus, C = Charolais, breed of sire listed first.
[c] Standard deviation.
[d] A x A and C x C are significantly different ($P < .01$).
[e] A x A and C x C are significantly different ($P < .05$).

(338.6 ± 10.5 vs. 273.7 ± 8.4 kg). Information was not available on the straightbred Charolais and the A x C cross.

Plasma Hormone Levels Prior to Puberty

Mean squares for total plasma progestins and estrogens for the interval 1 to 16 weeks prior to puberty are presented in table 9. Breed group differences were not detected for either hormone. Therefore, for further analyses hormone levels were pooled across breed groups. The failure to detect breed group differences may mean that differences truly do not exist or that the sampling procedures and hormonal assays involved were too insensitive to detect actual differences. An examination of the power (one minus the probability of making a type II error) of these tests was undertaken utilizing the error mean square used for testing breeds (table 9) as the measure of variance (Kempthorne, 1952). However, in the case of both estrogens and progestins the variance was so large that the resulting values did not fall within the ranges of the corresponding tables for determining the probability of obtaining a type II error. It could only be concluded that the probability exceeded 0.83. A similar analysis indicated that at least 150 replicates of this experiment would be required to obtain a 90% chance of detecting significant ($P < .05$) breed group differences of the magnitude observed in this study.

A significant difference between weeks ($P < .05$) for both estrogens and progestins was detected. However, the breed x week interaction was not significant in either case, indicating a common profile for hormone levels for the four breed groups.

TABLE 9. SPLIT-PLOT ANALYSIS OF TOTAL PLASMA PROGESTINS AND TOTAL PLASMA ESTROGENS UP TO 16 WEEKS BEFORE PUBERTY (TRIAL I)

Source	df	Mean squares	
		Progestins	Estrogens
Between animals	(19)		
Breeds (B)	3	0.47	324314
Animals:breeds (A:B)	16	6.57	410776
Within animals	(300)		
Weeks (W)	15	10.06**	1987226**
B x W	45	1.33	120902
A x W	240	1.65	157477

** $P < .05$ (conservative F).

Progestins. Average total plasma progestins for each week preceding puberty are presented in figure 4. Each point through 16 weeks before puberty is the average of the weekly samples from 20 animals. For weeks 17 to 26 before puberty fewer values were included since some animals reached puberty sooner after the initiation of blood sampling than others. Numbers of samples included in each week are indicated above each point. Vertical lines are the standard deviations around each point. A gradual increase in average plasma progestin levels occurred from a low of approximately 0.75 ng/ml 10 weeks before puberty to 3 ng/ml the last 2 weeks before puberty.

Average weekly total progestin levels remained below 2 ng/ml from the initiation of sampling until 2 weeks prior to puberty (figure 4). The polynomial regression equation obtained for progestin levels for the period from 16 weeks prior to puberty until puberty which most nearly fit the data is plotted in figure 5. Progestin levels decreased from approximately 1.5 ng/ml at 112 days prior to puberty to less than

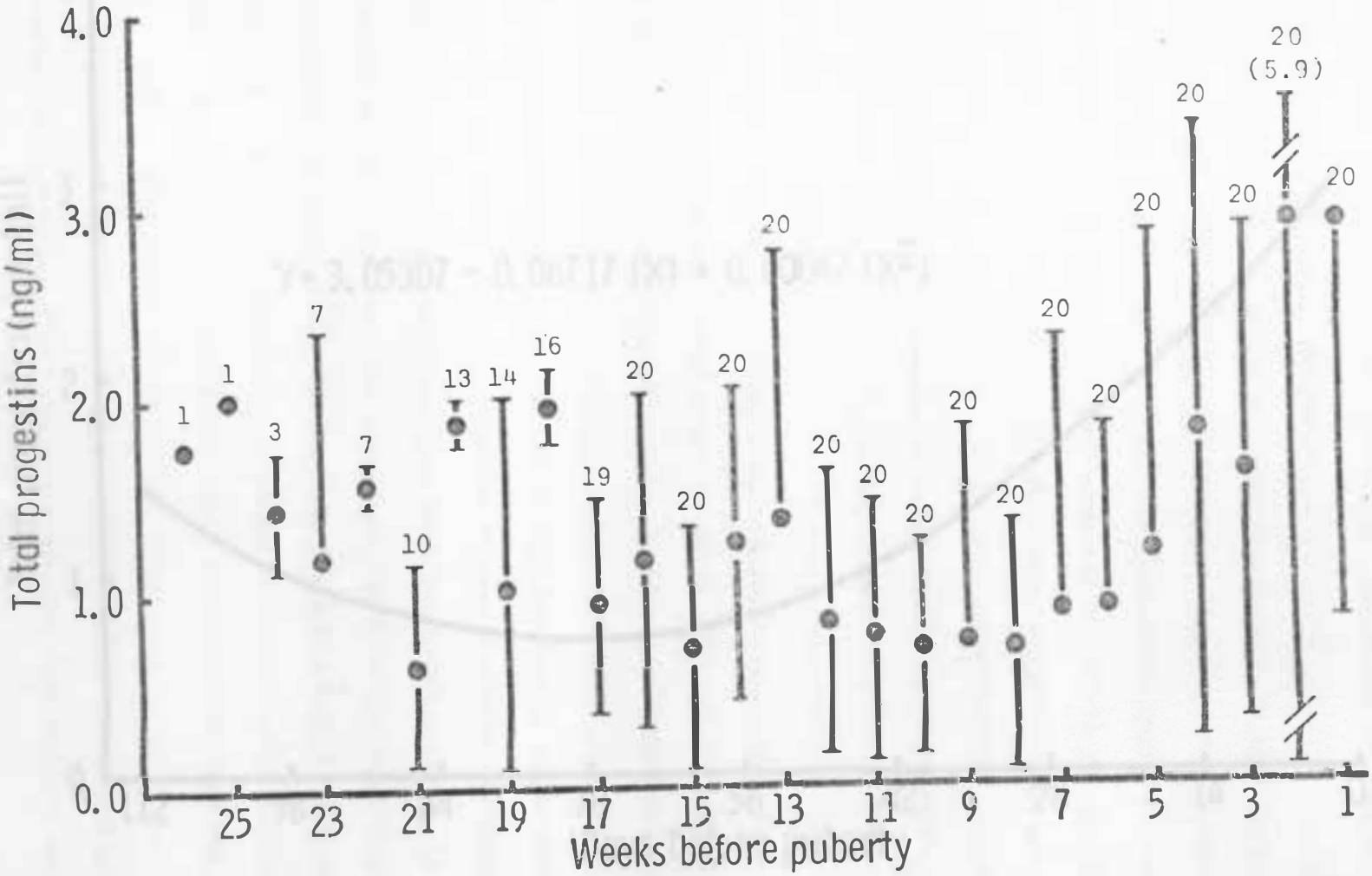

Figure 4. Average plasma total progestins for trial I heifers. Vertical lines are standard deviations. Numbers of samples are indicated above each point.

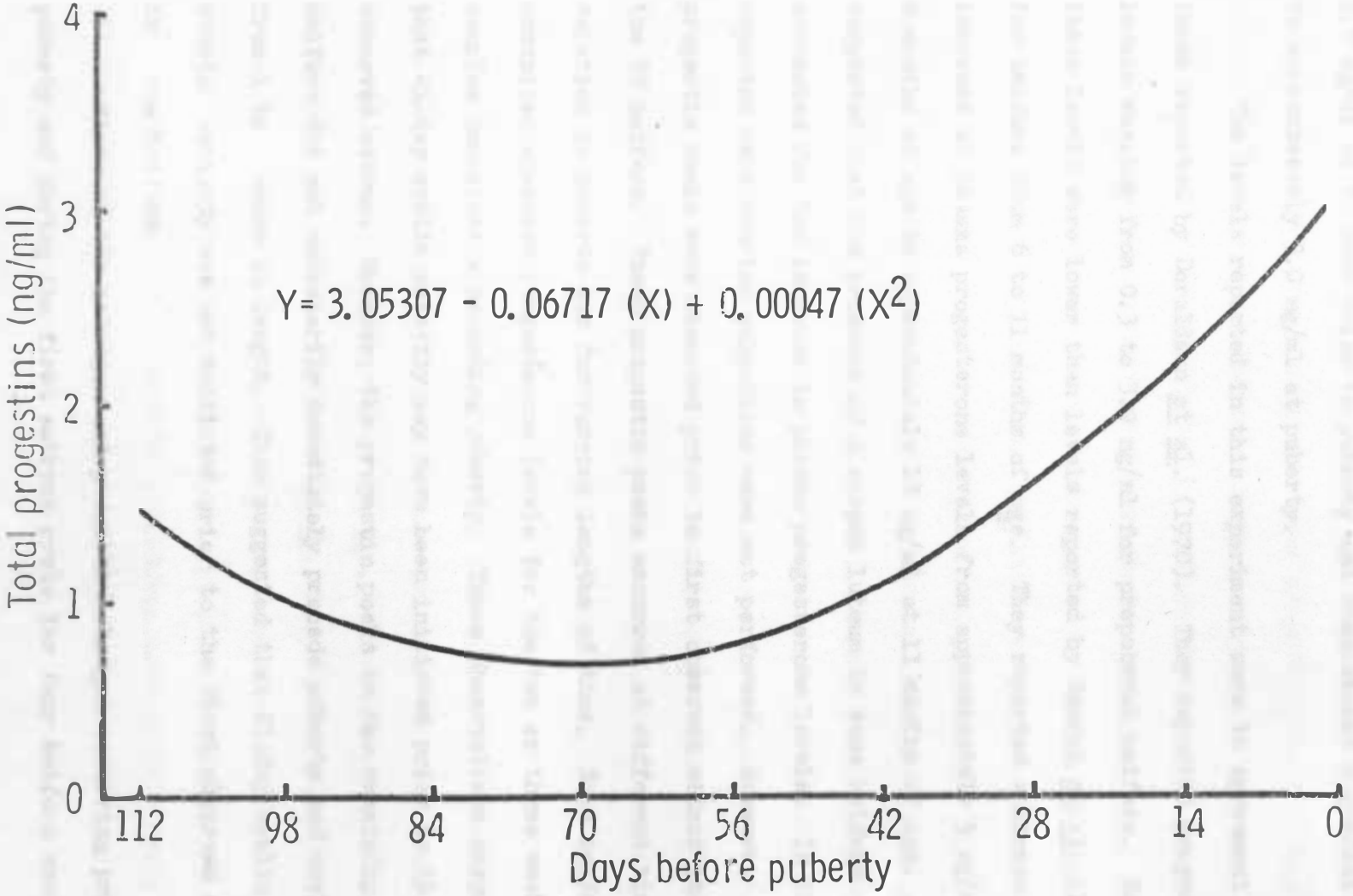

Figure 5. Plot of the polynomial regression equation of plasma total progestin levels up to 16 weeks before puberty, trial I.

1.0 ng/ml at 70 days prior to puberty and then showed a gradual rise to approximately 3.0 ng/ml at puberty.

The levels reported in this experiment were in agreement with those reported by Donaldson et al. (1970). They reported progesterone levels ranging from 0.3 to 3.9 ng/ml for prepuberal heifers. However, these levels were lower than levels reported by Randel et al. (1971) for heifers from 6 to 11 months of age. They reported a linear increase of plasma progesterone levels from approximately 5 ng/ml at 6 months of age to approximately 14 ng/ml at 11 months of age. They suggested that the presence of a corpus luteum in some heifers accounted for the increase in plasma progesterone levels. In the study reported here ovarian palpations were not performed. However, progestin peaks were observed prior to first observed estrus in 15 of the 20 heifers. These progestin peaks occurred at different times in relation to puberty and for varying lengths of time. Nine heifers exhibited elevated progesterone levels for the two or three weekly samples immediately preceding puberty. These observations suggested that 21-day cyclic activity may have been initiated prior to the first observed estrus. However, the progestin peaks in the remaining six heifers did not necessarily immediately precede puberty and varied from 1 to 3 weeks in length. This suggested that 21-day cyclic ovarian activity was not initiated prior to the first observed estrus in these heifers.

Plots of the estrogen and progestin changes occurring prior to puberty and during the first estrous cycle for four heifers are

presented in figure 6. One of these (number 39) is representative of heifers not exhibiting a progestin peak prior to puberty. Another (number 126) is representative of heifers having a 2 to 3 week progestin peak immediately preceding puberty. Numbers 23 and 128 exhibited elevated progestin levels 4 or 5 weeks before puberty.

Possible sources of progestins prior to puberty are the corpus luteum and luteinized follicles. Ovulation and corpus luteum formation prior to the first standing estrus have been reported in 73.6%, 65.0% and 60.0% of dairy heifers observed by Morrow (1969), Morrow, Swanson and Hafs (1970) and Randel et al. (1971), respectively. However, Donaldson et al. (1970) reported that there was no relationship between the concentration of plasma progesterone and the presence or absence of a corpus luteum on the ovary as determined by rectal palpation. They concluded that some luteinization of follicles must have occurred accounting for the source of progesterone prior to puberty in heifers not having a corpus luteum.

That progesterone levels during the estrous cycle decline from a peak during mid-cycle to basal levels prior to the next ovulation has been well documented (Dobrowolski et al., 1968; Pope et al., 1969; Henricks et al., 1971a; Garverick et al., 1971). One would expect to see a similar decline in plasma progestin levels prior to the first standing estrus in heifers exhibiting a prepuberal progestin peak. However, the once weekly sampling procedures used in this study were not frequent enough to detect such a change.

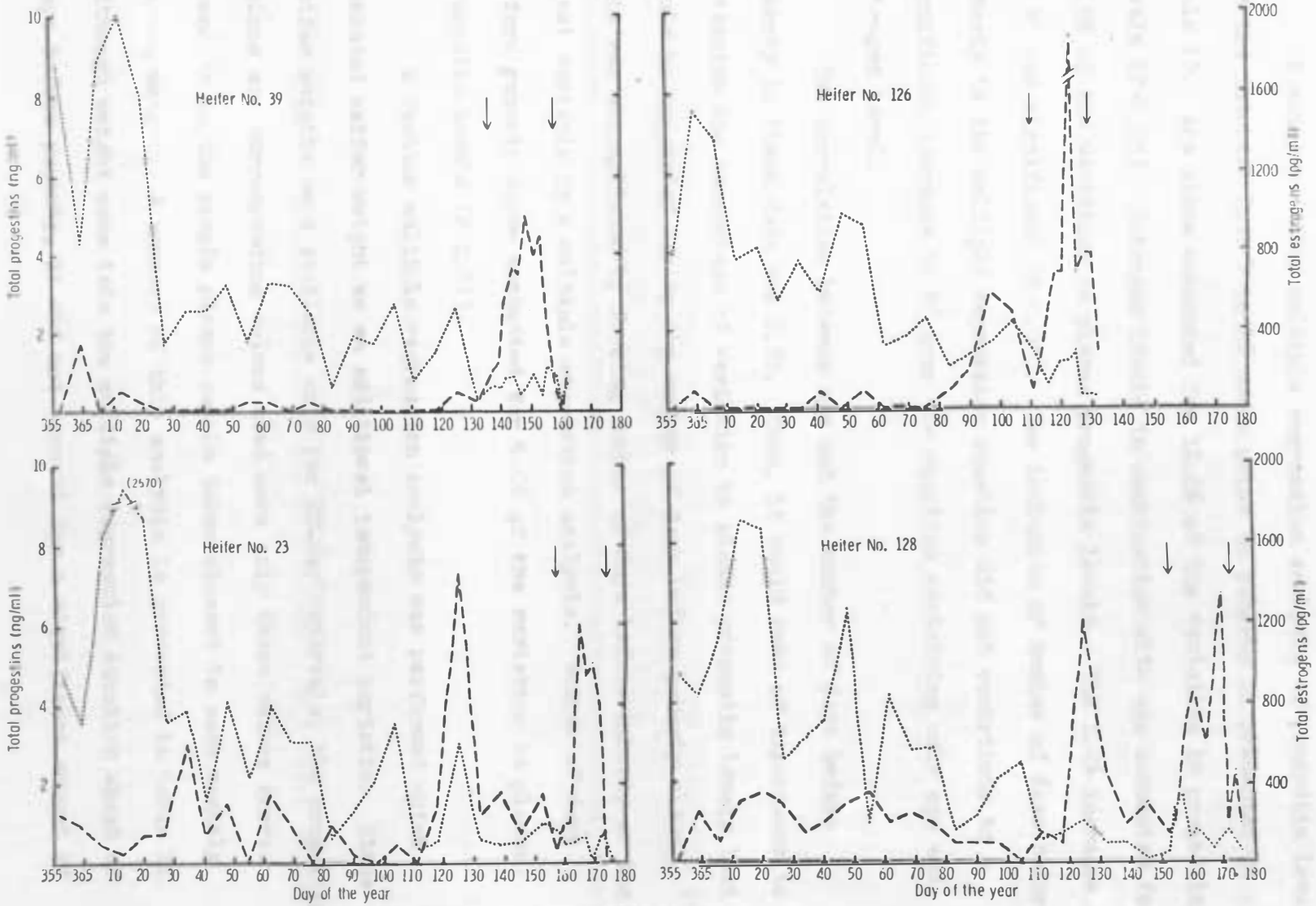

Figure 6. Total plasma progestin (– – – – –) and estrogen (·····) levels for four heifers in trial I. Arrows represent estrus. See text for explanation.

A summary of the multiple regression analysis of progestin levels for the interval from 1 to 16 weeks prior to puberty is presented in table 10. Age alone accounted for 11.6% of the variation in progestin levels ($P<.05$). Estrogen levels in combination with age accounted for 12.8% of the variation in plasma progestin levels. The 1.2% increase in R^2 was significant ($P<.05$). The inclusion of number of days before puberty in the multiple regression equation did not contribute to a significant increase in R^2 over the equation containing only age and estrogen level.

The correlation between age and the number of days before puberty in these data was 0.80. Thus, it would seem of importance to determine the percentage of variation in plasma progestin levels that would be accounted for by the number of days before puberty alone. This was accomplished by forcing number of days before puberty as the first variable in a multiple regression analysis. Number of days before puberty alone accounted for 9.0% of the variation in plasma progestin levels ($P<.05$).

A further multiple regression analysis was performed which included heifer weight as an additional independent variable. Since heifer weights were available only for 28-day intervals, the progestin values and corresponding values used were only those values corresponding to the single plasma sample taken closest to each monthly animal weight. A summary of this analysis is presented in table 11. Although weight came into the multiple regression equation ahead of days before puberty, it did not account for a significant amount of

TABLE 10. MULTIPLE REGRESSION ANALYSIS OF VARIABLES CONTRIBUTING TO VARIATION IN PROGESTIN LEVELS FOR THE INTERVAL 1 TO 16 WEEKS BEFORE PUBERTY, 320 OBSERVATIONS (TRIAL I)

Step	Intercept	Age	Estrogens	Days before puberty	R^2
1	-2.8369	0.0125	--	--	0.116*
2	-3.9415	0.0152	0.0004	--	0.128*
3	-2.6474	0.0122	0.0004	-.0054	0.133

* $P < .05$.

TABLE 11. MULTIPLE REGRESSION ANALYSIS OF TOTAL PLASMA PROGESTINS AS INFLUENCED BY AGE, NUMBER OF DAYS BEFORE PUBERTY, TOTAL PLASMA ESTROGENS AND WEIGHT, 92 OBSERVATIONS (TRIAL I)[a]

Step	Intercept	Age	Plasma estrogens	Weight	Days before puberty	R^2
1	-2.1114	0.0121	--	--	--	0.071**
2	-5.6894	0.0210	0.0010	--	--	0.127*
3	-5.0374	0.0298	0.0009	-.0136	--	0.144
4	-4.7238	0.0293	0.0009	-.0139	-.0013	0.144

[a] Plasma estrogen and progestin values used were only those values from the single plasma sample taken closest to each monthly animal weight.

* $P < .05$.

** $P < .01$.

additional variation in plasma progestin levels over the equation containing age and total plasma estrogens.

Estrogens. Average total plasma estrogens for each week preceding puberty are presented in figure 7. Each point for the interval from 1 to 16 weeks prior to puberty is the average of weekly samples from 20 animals. Numbers of samples are indicated above each point. Vertical lines are the standard deviations around each point. Average total plasma estrogen levels consistently remained above 1000 pg/ml from the initiation of sampling until 14 weeks before puberty. During the interval 3 to 4 weeks before puberty there was a consistent decrease in total plasma estrogen levels. No further decrease was noted for the interval of 1 to 2 weeks before puberty. The equation obtained by polynomial regression analysis for total plasma estrogen levels for the interval 1 to 16 weeks before puberty is plotted in figure 8.

Plots of the actual estrogen and progestin levels of four individual heifers are presented in figure 6.

The total plasma estrogen levels reported here for the pre-puberal heifer are, in general, considerably higher than has been reported for estradiol levels in cycling cows. Echternkamp and Hansel (1971), Wettemann et al. (1972) and Wettemann and Hafs (1973) reported that estradiol levels for the cycling cow generally remained less than 10 pg/ml. Shemesh et al. (1972) reported estradiol levels in the range of 10 to 80 pg/ml with the exception of the pre-estrus peak which reached approximately 170 pg/ml. However, estradiol levels

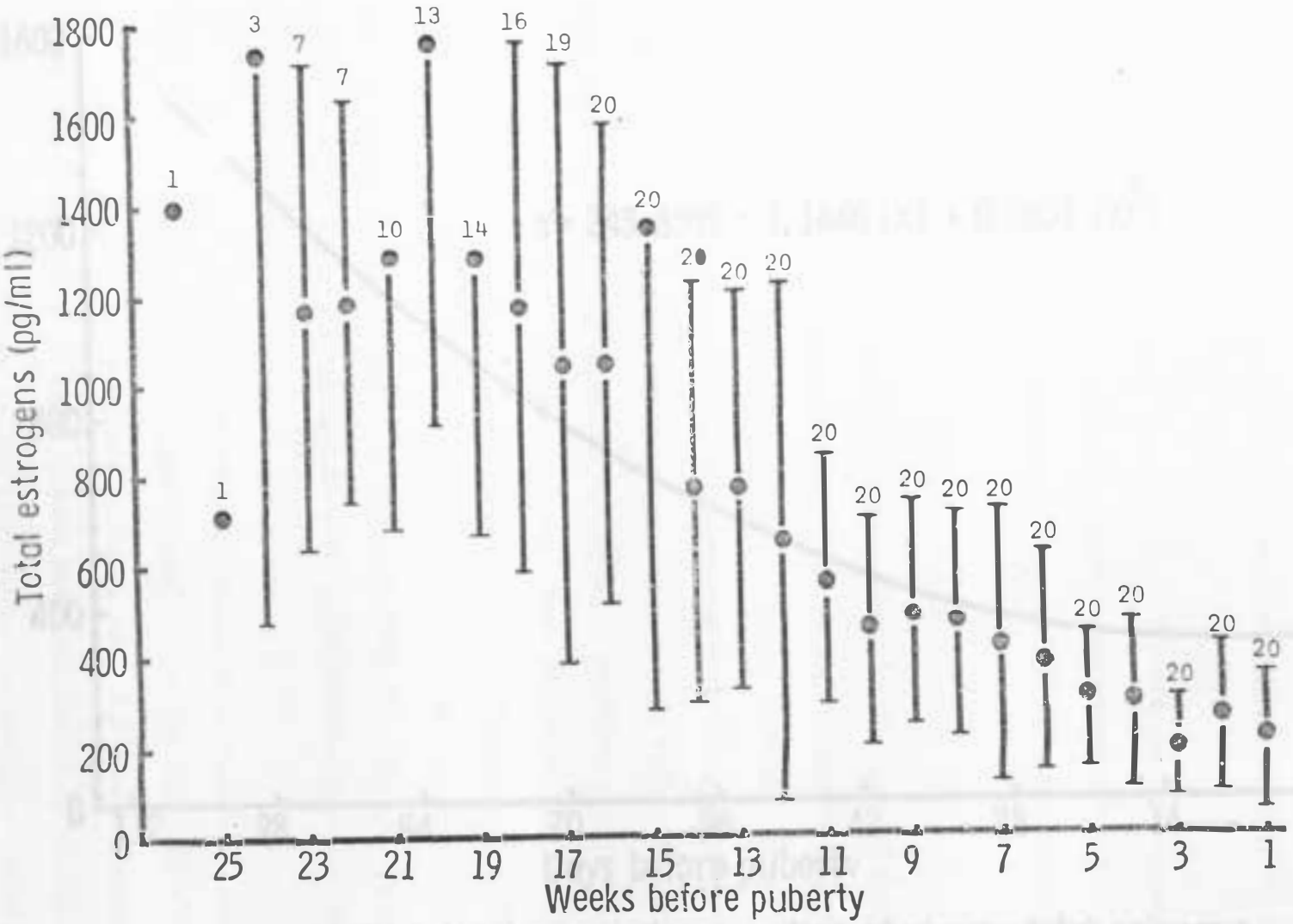

Figure 7. Average plasma total estrogen levels for trial I heifers. Vertical lines are standard deviations. Numbers of samples are indicated above each point.

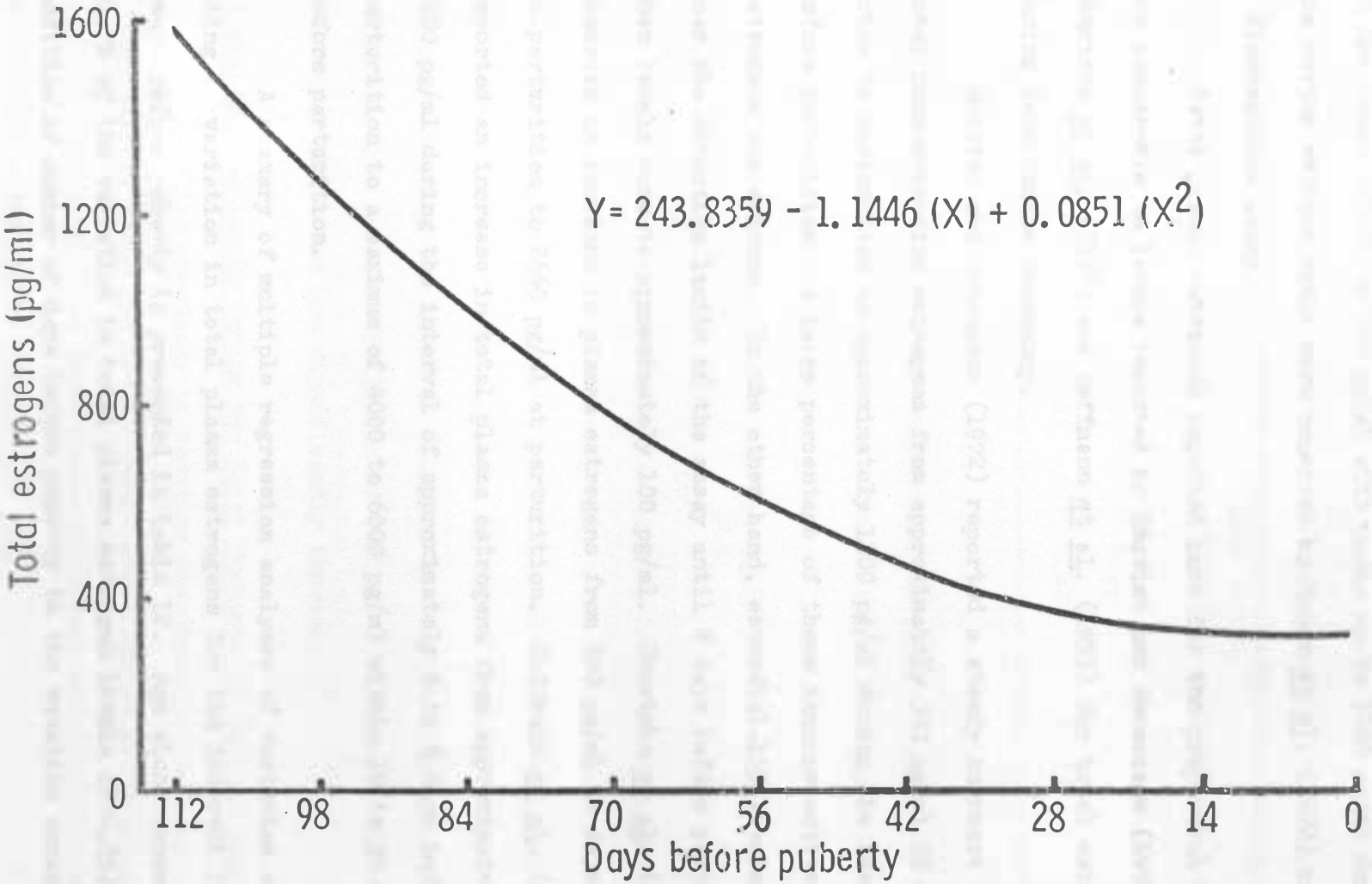

Figure 8. Plot of the polynomial regression equation of plasma total estrogen levels up to 16 weeks before puberty, trial I.

in the range of 200 to 700 pg/ml with peaks up to 1200 pg/ml during the bovine estrous cycle were reported by Mason et al. (1972) using a fluorescence assay.

Total plasma estrogens reported here for the prepuberal heifer are comparable to levels reported by Edqvist and Johansson (1972), Henricks et al. (1972) and Hoffmann et al. (1973) for total estrogens during late bovine pregnancy.

Edqvist and Johansson (1972) reported a steady increase of total immunoreactive estrogens from approximately 300 pg/ml 20 days prior to parturition to approximately 1200 pg/ml during the last 5 days before parturition. A large percentage of these immunoreactive estrogens was estrone. On the other hand, estradiol-17β levels were near the detection limits of the assay until 8 days before parturition when levels rose to approximately 100 pg/ml. Henricks et al. (1972) observed an increase in plasma estrogens from 500 pg/ml 14 days prior to parturition to 2660 pg/ml at parturition. Hoffmann et al. (1973) reported an increase in total plasma estrogens from approximately 2500 pg/ml during the interval of approximately 6 to 8 days before parturition to a maximum of 4000 to 6000 pg/ml within 10 to 50 hours before parturition.

A summary of multiple regression analyses of variables contributing to variation in total plasma estrogens for the interval 1 to 16 weeks before puberty is presented in table 12. Age alone accounted for 31.7% of the variation in total plasma estrogen levels ($P < .05$). The addition of number of days before puberty to the equation accounted for

TABLE 12. MULTIPLE REGRESSION ANALYSIS OF VARIABLES CONTRIBUTING TO VARIATION IN PLASMA TOTAL ESTROGEN LEVELS FOR THE INTERVAL 1 TO 16 WEEKS BEFORE PUBERTY, 320 OBSERVATIONS (TRIAL I)

Step	Intercept	Age	Days before puberty	Progestins	R^2
1	2817.86	-6.96	--	--	0.317*
2	1741.07	-4.37	4.08	--	0.342*
3	1811.04	-4.75	4.22	37.24	0.353*

* $P < .05$.

another 2.5% ($P < .05$) of the variability in plasma estrogen levels, while the additional inclusion of progestin levels added a further 1.1% ($P < .05$). Number of days before puberty alone forced as the first variable in the equation accounted for 29.6% of the variability in total plasma estrogen levels ($P < .05$).

Weight was included as an additional independent variable in a multiple regression analysis using only those hormone values corresponding to the single plasma sample taken closest to each monthly weight (table 13). In the resulting equations numbers of days before puberty accounted for 51.8% ($P < .01$) of the variability in plasma total estrogen levels. Inclusion of further variables in the equation including weight did not significantly increase R^2.

Plasma Hormone Levels During the First Postpuberal Estrous Cycle

Due to the frequency of sampling the number of samples per day of the estrous cycle for the different breed groups was small (1 to 3 samples). Therefore, no breed comparisons were made and data were pooled across breeds.

TABLE 13. MULTIPLE REGRESSION ANALYSIS OF TOTAL PLASMA ESTROGENS AS INFLUENCED BY AGE, NUMBER OF DAYS BEFORE PUBERTY, TOTAL PLASMA PROGESTINS AND WEIGHT, 92 OBSERVATIONS (TRIAL I)[a]

Step	Intercept	Days before puberty	Plasma progestins	Age	Weight	R^2
1	-11.46	10.88	--	--	--	0.518**
2	-96.41	11.16	38.00	--	--	0.533
3	996.98	8.62	47.54	-2.94	--	0.546
4	1123.38	8.28	45.31	-2.07	-1.51	0.548

[a] Plasma estrogen and progestin values used were only those values from the single plasma sample taken closest to each monthly animal weight.

** $P < .01$.

Progestins. Average plasma total progestins for days of the first postpuberal estrous cycle standardized to 20 days in length are presented in figure 9. Vertical lines are the standard deviations around each mean. Numbers of samples represented in each mean are noted above each point. Samples obtained on the day of estrus were not included due to the rapid changes in hormone levels which have been shown to occur around estrus (Henricks et al., 1970, 1971a; Sprague et al., 1971; Mason et al., 1972; Swanson et al., 1972). The data were subjected to polynomial regression analysis. A plot of the polynomial regression equation which best fits the data is presented in figure 10.

Average plasma total progestin levels increased almost linearly from less than 1 ng/ml on day 1 to a peak of approximately 6 ng/ml on day 13 and then declined quite rapidly from day 14 to approximately 2 ng/ml on day 19. Considerable variation between animals was noted as

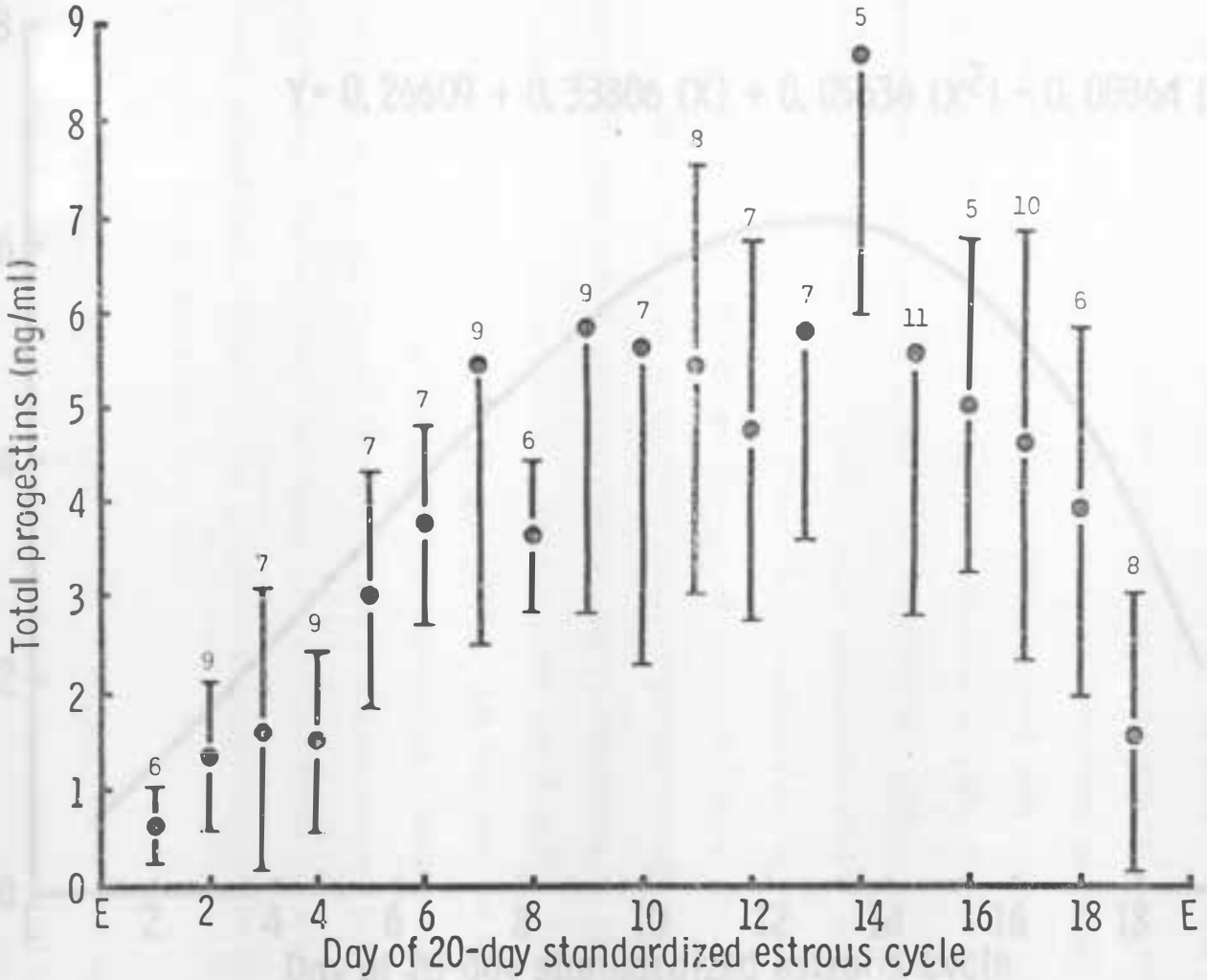

Figure 9. Plasma total progestin levels during the first postpuberal estrous cycle. E= day of estrus. Vertical lines are standard deviations. Numbers of samples are indicated above each point.

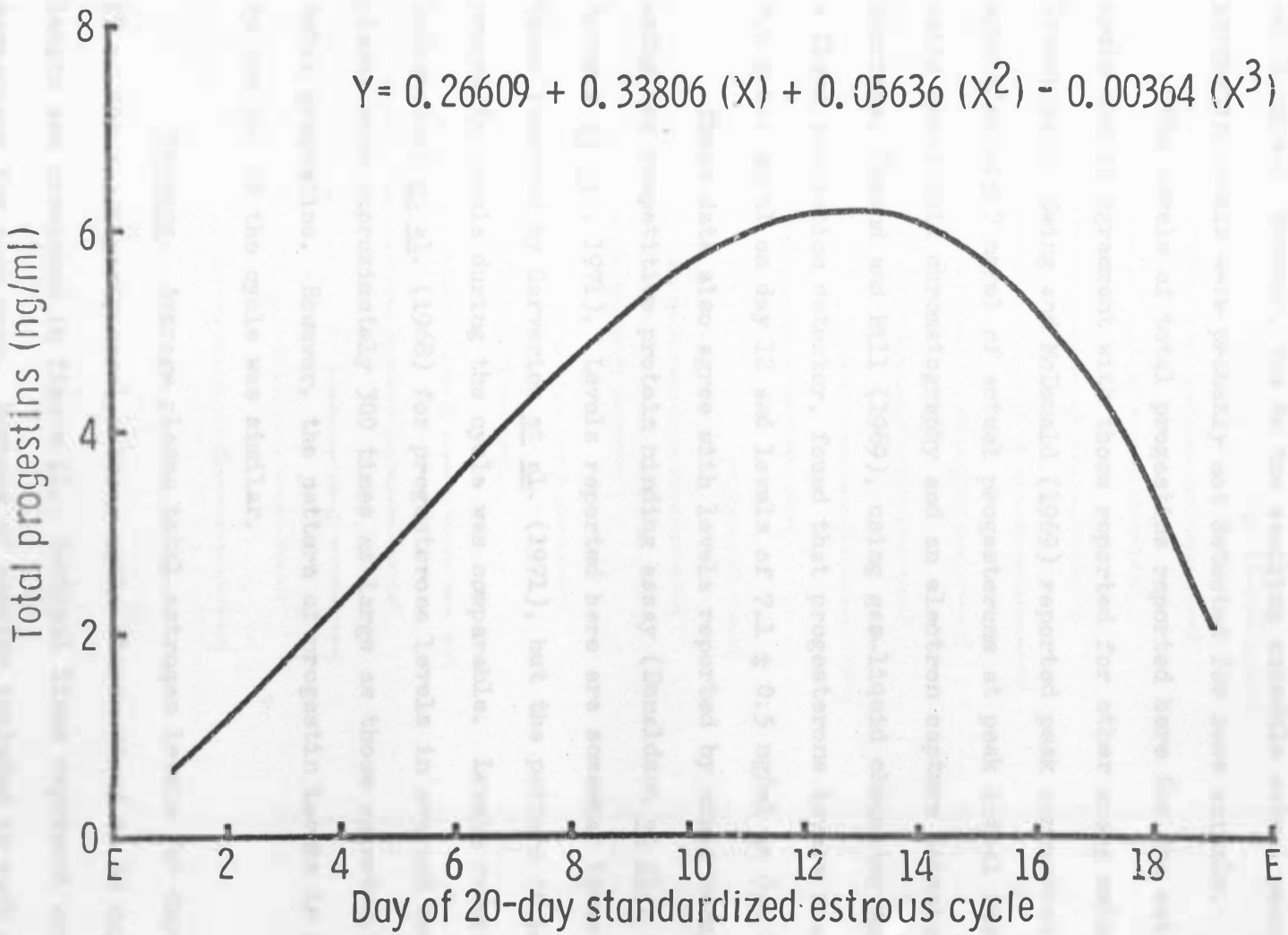

Figure 10. Plot of the polynomial regression equation for total progestin levels during the first postpuberal estrous cycle. E = day of estrus.

indicated by the large standard deviations in figure 9. The peak progestin value detected for individual heifers ranged from 4.0 to 12.75 ng/ml. However, due to the sampling schedule actual peak progestin levels were probably not detected for some animals.

The levels of total progestins reported here for the estrous cycle are in agreement with those reported for other assay methods. Stabenfeldt, Ewing and McDonald (1969) reported peak concentrations of approximately 7 ng/ml of actual progesterone at peak luteal function using gas-liquid chromatography and an electron capture detector. Henricks, Roland and Hill (1969), using gas-liquid chromatography and a flame ionization detector, found that progesterone levels reached 7.9 ± 1.2 ng/ml on day 12 and levels of 7.1 ± 0.5 ng/ml on day 16.

These data also agree with levels reported by other workers using the competitive protein binding assay (Donaldson et al., 1970; Sprague et al., 1971). Levels reported here are somewhat lower than those reported by Garverick et al. (1971), but the pattern of plasma progestin levels during the cycle was comparable. Levels reported by Dobrowolski et al. (1968) for progesterone levels in ovarian venous plasma were approximately 300 times as large as those reported here for total progestins. However, the pattern of progestin levels in relation to the day of the cycle was similar.

Estrogens. Average plasma total estrogen levels for days 1 to 19 of the first postpuberal estrous cycle standardized to 20 days in length are presented in figure 11. Vertical lines represent standard deviations for each mean. Numbers of samples included in each mean are

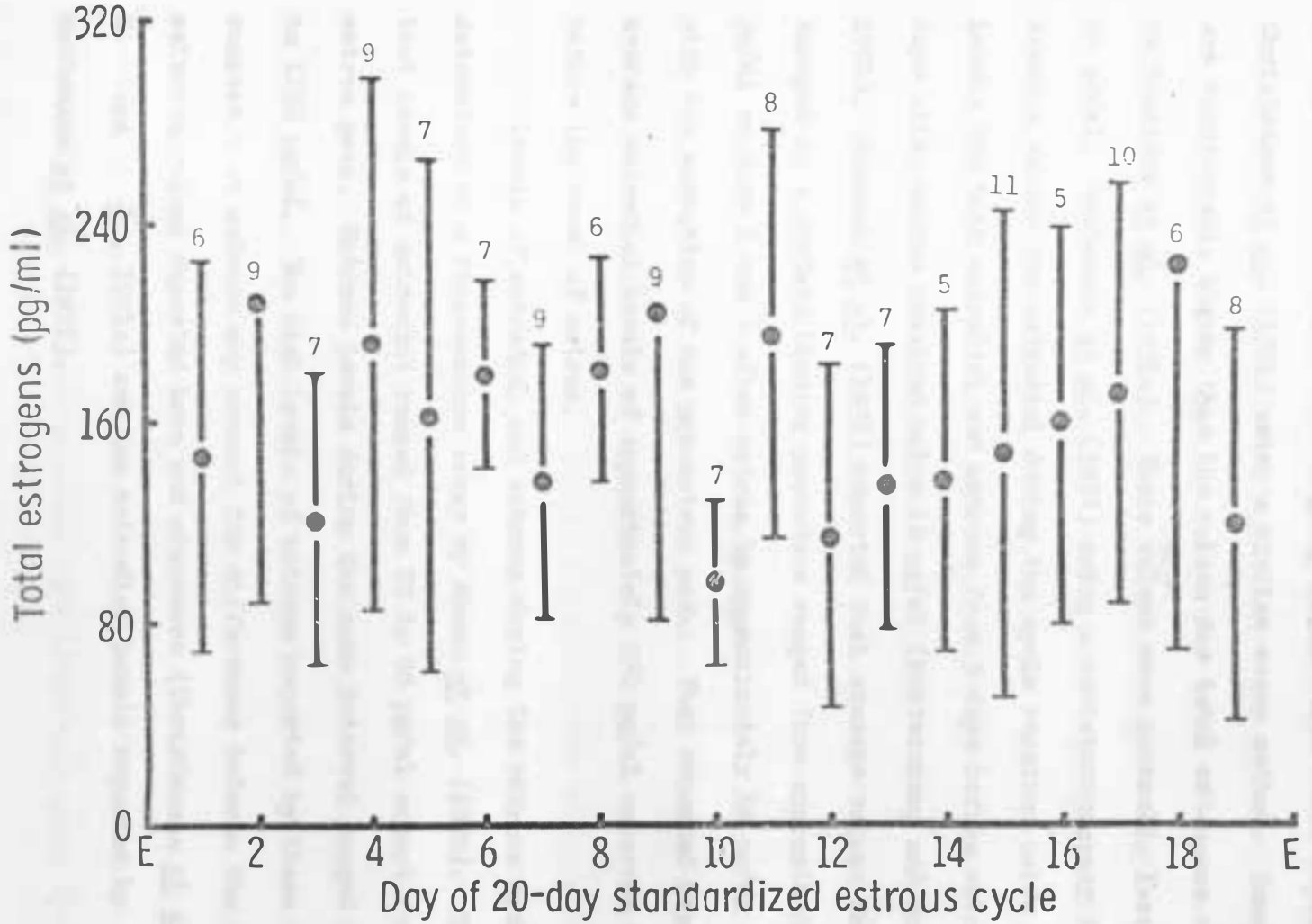

Figure 11. Average plasma total estrogens during the first postpuberal estrous cycle. Vertical lines are standard deviations. Numbers of samples are indicated above each point. E= day of estrus.

noted above each point. Levels reported here for total estrogens during the estrous cycle are slightly higher than those reported by Christensen et al. (1971) using a similar assay method. However, they are considerably higher than the values for total estrogens reported by Henricks et al. (1971a). Their values were generally less than 20 pg/ml. Wettemann et al. (1972) using a radioimmunoassay found that average values for estradiol during the cycle remained below 10 pg/ml. Levels for both estradiol and estrone from 3 days before estrus to 3 days after estrus remained below 10 pg/ml (Echternkamp and Hansel, 1971). Shemesh et al. (1972) reported that average values for estradiol assayed by a protein binding procedure ranged from approximately 10 pg/ml on days 1 and 2 after estrus to approximately 80 pg/ml on day 11 with the exception of the pre-estrus peak. They reported that peak average estradiol levels of approximately 170 pg/ml occurred 4 hours before the onset of estrus.

Levels of estradiol and estrone during the estrous cycle were determined by a fluorescence assay by Mason et al. (1972). They found that levels of estradiol ranged from 20 to 80 pg/ml except for the pre-estrus peak. Estrone levels during the same interval ranged from 250 to 1250 pg/ml. The high levels of estrone reported by these workers suggest that estrone may account for differences between the total estrogen values reported here and elsewhere (Christensen et al., 1971; Henricks et al., 1971a) and the estradiol levels reported by Wettemann et al. (1972).

No discernible pre-estrus estrogen peak was demonstrated in the data reported here (figure 11). Although average total estrogen levels climbed steadily from day 12 through day 18, the standard deviations were quite large and average estrogen levels actually dropped on day 19 when other investigators have shown estrogen peaks to occur (Henricks et al., 1971a; Christensen et al., 1971; Shemesh et al., 1972; Wettemann et al., 1972). However, with the frequency of sampling used in this investigation estrogen peaks could easily have been missed.

No obvious day to day pattern of plasma total estrogens was evident in these data (figure 11). The data were subjected to polynomial regression analysis. The resulting equations including the linear regression equation were not statistically significant. Shemesh et al. (1972) using lactating dairy cows observed a small estradiol peak on day 4 (approximately 45 pg/ml) and another minor peak of slightly greater magnitude on day 11 (approximately 80 pg/ml) of the estrous cycle. Levels declined from day 11 to approximately 20 pg/ml on day 17. Christensen et al. (1971) reported that in beef cows estrogen levels were elevated on days 5 and 6 (141 $\pm$ 44.2 pg/ml) and with the exception of the pre-estrus peak fluctuated between 98 to 133 pg/ml during the remaining days of the cycle.

Urinary excretion of total estrogens during the estrous cycle was examined by Garverick et al. (1971). They reported peak urinary excretion occurred from 2 days before to 1 day after estrus. From day 2 through day 17 excretion rates were lower with minor day to day fluctuations.

Differences between plasma estrogen levels for the data reported here and other published research may be explained by the fact that the hormone levels reported in this paper in all cases pertained to the first postpuberal estrous cycle. Garverick et al. (1971) reported that the rate of urinary excretion of total estrogens, estradiol-17β and estradiol-17α was different for heifers as compared to cows. Randel et al. (1971) noted that the rate of urinary excretion of estradiol-17β, estrone and total estrogens was higher during the second postpuberal estrus as compared to the first estrus. They suggested that these differences indicated a possible hormonal imbalance during the first estrus. Unfortunately, plasma samples were not obtained during subsequent estrous cycles in this study.

Trial II

Trial I indicated a need for information on estrogen levels from heifers of a younger age. For that reason eight heifer calves were used in a second trial in a study of gonadal hormone levels from birth to 28 weeks of age.

Mean squares for total plasma estrogens and total plasma progestins are presented in table 14. No significant differences were detected for breed of dam, weeks of age or the breed of dam x weeks of age interaction for either progestin or estrogen levels. Therefore, data were pooled for the four breed groups.

TABLE 14. SPLIT-PLOT ANALYSIS OF TOTAL PLASMA PROGESTINS AND TOTAL PLASMA ESTROGENS UP TO 28 WEEKS OF AGE (TRIAL II)

Source	df	Mean squares	
		Progestins	Estrogens
Between animals	(7)		
Breed of dam (B)	3	2.06	2728
Animals:breed of dam (A:B)	4	0.93	1757
Within animals	(215)		
Weeks of age (W)	27	0.50	2414
B x W	81	0.20	1965
A x W	107	0.37	1736

Plasma Hormone Levels in Heifer Calves From Birth to Weaning

Progestins. Average weekly plasma progestin levels are presented in figure 12. Each point represents the average of eight samples with the exception of the first week where only seven samples are included. Vertical lines are the standard deviations around each mean. Week 1 included samples collected from animals 1 to 7 days of age. Samples taken from animals on the day of birth are not included in the analysis.

Average progestin levels remained below 1.0 ng/ml from 1 to 28 weeks of age with the exception of the 18th week. These levels were near the lower limits of sensitivity of the progestin assay. These progestin levels were less than those reported for older dairy calves (6 to 11 months) by Randel et al. (1971) but agree with the levels reported by Donaldson et al. (1970) for a prepuberal heifer which showed no evidence of cyclic ovarian activity.

Plasma total progestin levels were subjected to polynomial regression analysis. The equation representing the best fitting line for the data is presented in figure 13.

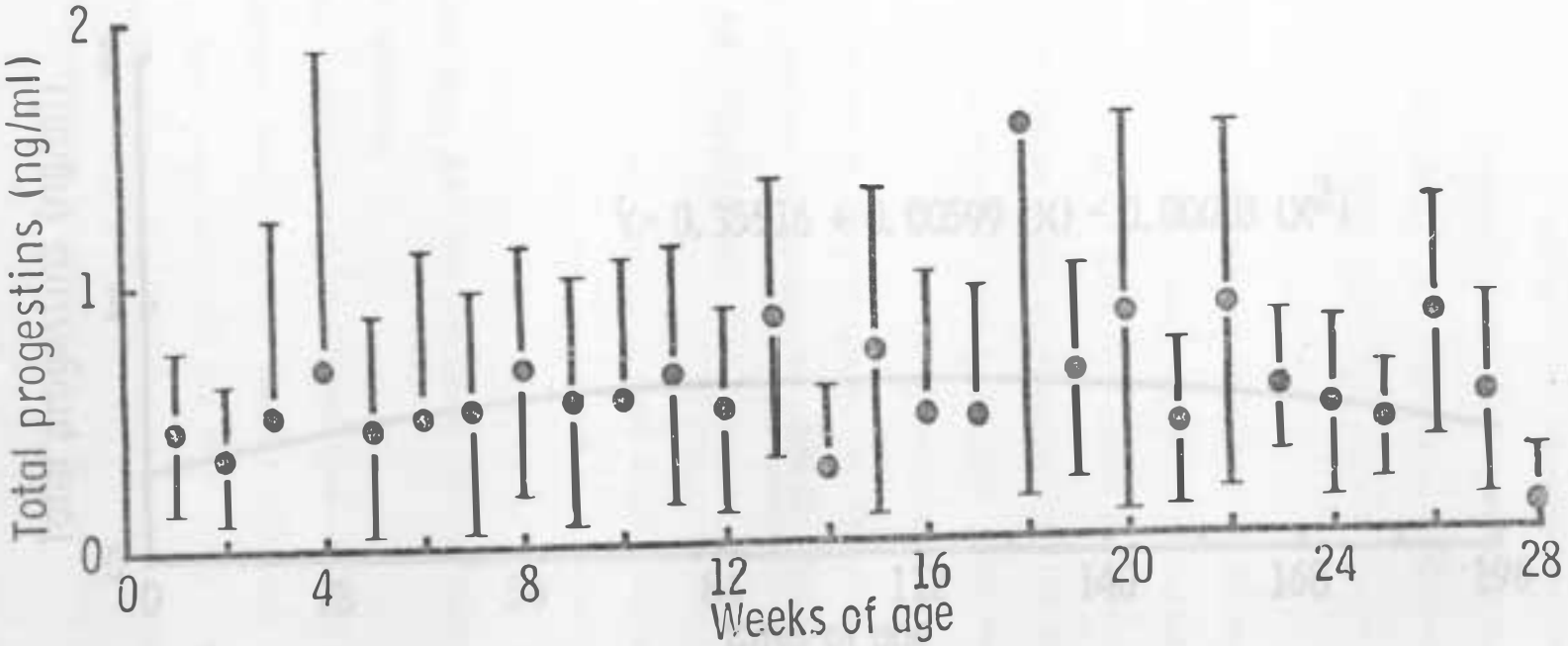

Figure 12. Average weekly plasma total progestin levels for eight crossbred heifer calves, trial II. Vertical lines are standard deviations.

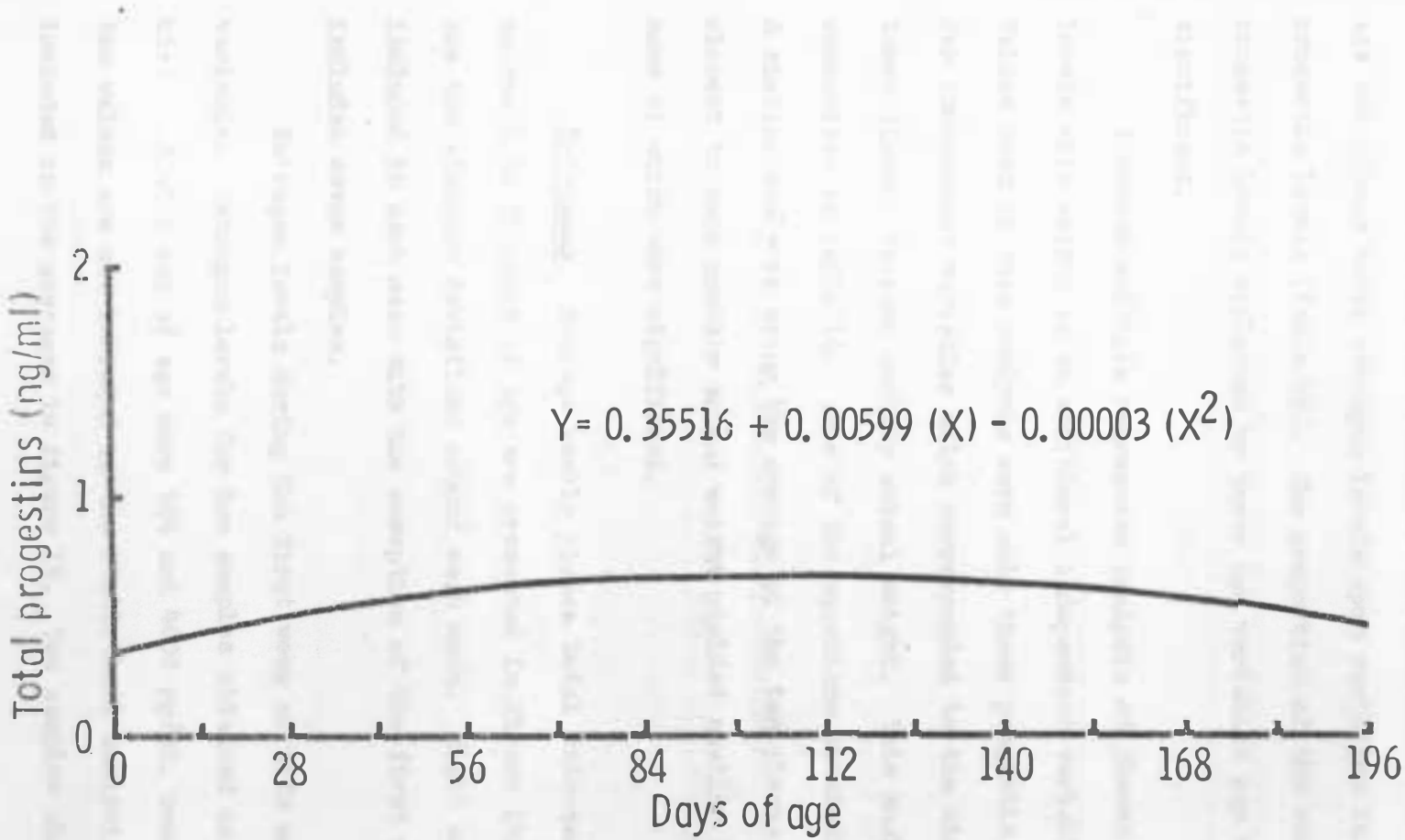

Figure 13. Plot of the polynomial regression equation of plasma total progestins up to 28 weeks of age.

Multiple regression analysis was used to examine the effect of age and plasma total estrogen levels upon variation in plasma total progestin levels (table 15). The proportion of the variation in progestin levels explained by these two variables was small and not significant.

A second multiple regression analysis of plasma total progestin levels with weight as an additional independent variable was obtained. Values used in this analysis were only those progestin values and values for independent variables which corresponded to the single plasma sample taken closest to each monthly animal weight. This analysis is summarized in table 16. None of the equations obtained were significant. A similar analysis using the average of the two plasma samples taken closest to each monthly animal weight yielded smaller values for R^2, none of which were significant.

Estrogens. Average weekly plasma total estrogens for heifer calves 1 to 28 weeks of age are presented in figure 14. Vertical lines are the standard deviations around each mean. Eight samples are included in each mean with the exception of the first week which includes seven samples.

Estrogen levels during the first week of life were highly variable. Estrogen levels for two samples obtained on the day of birth and at 1 day of age were 304 and 4000 pg/ml, respectively. These two values are not included in the statistical analysis and are not included in the averages in figure 14. Two samples obtained from

TABLE 15. MULTIPLE REGRESSION ANALYSIS OF VARIABLES CONTRIBUTING TO VARIATION IN PLASMA TOTAL PROGESTIN LEVELS IN CALVES UP TO 28 WEEKS OF AGE, 223 OBSERVATIONS (TRIAL II)

Step	Intercept	Estrogens	Age	R^2
1	0.6053	-.0007	--	0.002
2	0.5579	-.0006	0.0005	0.004

TABLE 16. MULTIPLE REGRESSION ANALYSIS OF PLASMA PROGESTIN LEVELS AS INFLUENCED BY AGE, PLASMA ESTROGEN LEVELS AND WEIGHT, 50 OBSERVATIONS (TRIAL II)[a]

Step	Intercept	Weight	Age	Plasma estrogens	R^2
1	0.7496	-.0015	--	--	0.025
2	0.7688	-.0062	0.0046	--	0.046
3	0.7467	-.0070	0.0053	0.0014	0.053

[a] Total plasma progestin and estrogen values used were only those values from the single plasma sample taken closest to each monthly animal weight.

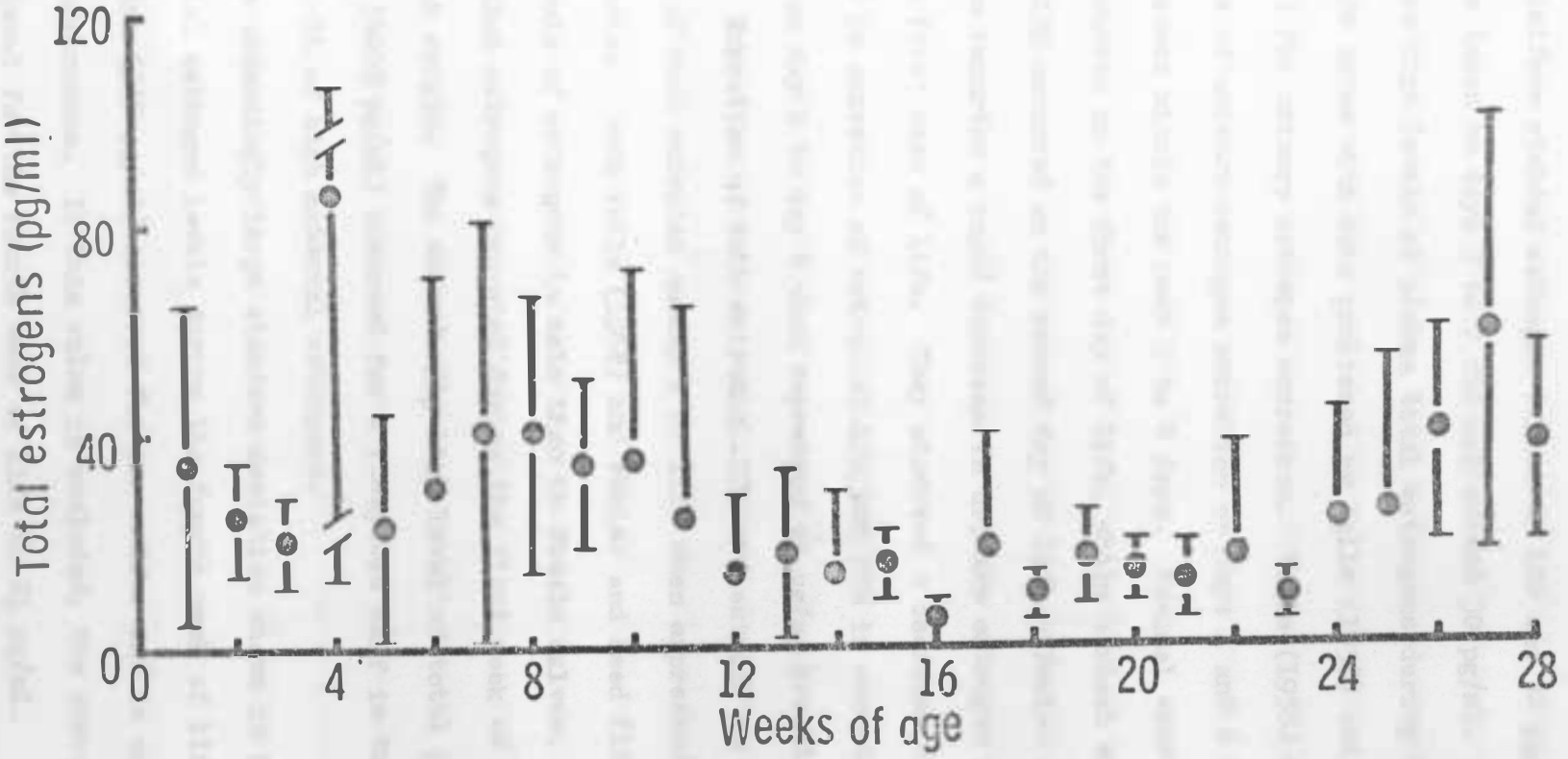

Figure 14. Average weekly plasma total estrogens for eight crossbred heifer calves, trial II. Vertical lines are standard deviations.

2-day-old heifers yielded estrogen values of 100 and 32 pg/ml. Values for samples taken on days 3 to 7 did not exceed 30 pg/ml.

These high levels of plasma total estrogens during the first few days of life agree with data published by Velle (1958) and Fowler and Reed (1972) for urinary estrogen excretion. Velle (1958) reported high levels of urinary estrogen excretion on days 1 and 2 of life with rapid decreases within the next 3 to 4 days. Maximal excretion of estrone occurred on the first day of life, while maximal excretion of estradiol-17β occurred on the second day of life. Fowler and Reed (1972) also reported a rapid decrease in urinary estrogen excretion during the first week of life. They observed a decrease of approximately 79% in excretion of estradiol-17α and 98% in excretion of estrone from day 1 to day 4 when expressed as ug/mg creatinine excreted. Excretion of both estradiol-17α and estrone on day 4 was about 10% of that excreted on day 1 of life when expressed as ug/liter urine. Both Velle (1958) and Fowler and Reed (1972) observed higher levels of estrogens in male than in female calves, which suggests that estrogens excreted during the first week of life may be maternal in origin. The exceedingly high level of total plasma estrogens (4000 pg/ml) observed for a 1-day-old calf in this study may be the result of high maternal estrogens.

The exceedingly large standard deviation shown in figure 14 for plasma total estrogen levels during the fourth week of life is due to a high value (565 pg/ml) observed for one heifer and is assumed to be a chance occurrence. If this value is excluded, the average total estrogen level for the fourth week of life is 21 pg/ml.

A peak of average plasma total estrogen levels occurred during the sixth to tenth weeks of life. However, standard deviations during this period were relatively large. A lower level of average weekly estrogens was observed from 12 to 23 weeks of age followed by a gradual rise in estrogen levels to the 28th week. These changes are illustrated in a plot of the polynomial regression equation representing the best fitting equation to the data (figure 15).

The multiple regression analysis of age and total progestin levels on total estrogen levels is summarized in table 17. The resulting equations were not significant and were associated with small R^2 values.

As with previous variables a second multiple regression analysis was conducted including weight as an additional independent variable. Again, the only values used were those corresponding to the single plasma sample taken closest to each monthly animal weight. The resulting equations are summarized in table 18. Although the R^2 values are considerably larger than for the previous multiple regression analysis (table 17), they are also statistically nonsignificant. An alternative analysis was conducted utilizing the average of the two progestin and estrogen values corresponding to the two plasma samples taken closest to each monthly animal weight. The resulting equations were also not significant and the corresponding R^2 values smaller than those of table 18.

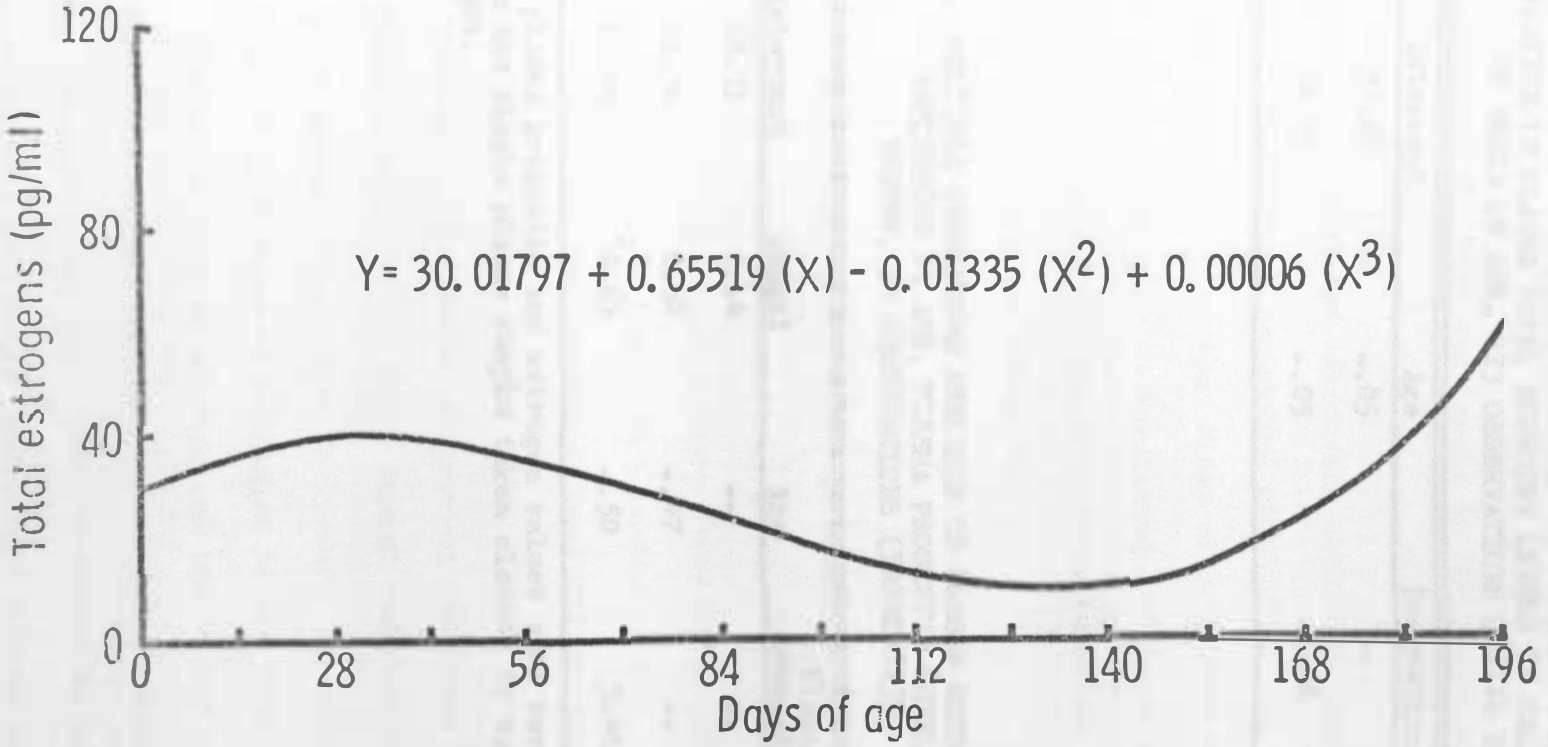

Figure 15. Plot of the polynomial regression equation of plasma total estrogens up to 28 weeks of age, trial II.

TABLE 17. MULTIPLE REGRESSION ANALYSIS OF VARIABLES CONTRIBUTING TO VARIATION IN PLASMA TOTAL ESTROGEN LEVELS IN CALVES UP TO 28 WEEKS OF AGE, 223 OBSERVATIONS (TRIAL II)

Step	Intercept	Age	Progestins	R^2
1	32.26	-.05	--	0.004
2	34.01	-.05	-3.26	0.006

TABLE 18. MULTIPLE REGRESSION ANALYSIS OF PLASMA ESTROGEN LEVELS AS INFLUENCED BY AGE, PLASMA PROGESTIN LEVELS AND WEIGHT, 50 OBSERVATIONS (TRIAL II)[a]

Step	Intercept	Weight	Age	Plasma progestins	R^2
1	18.31	0.14	--	--	0.051
2	16.36	0.62	-.47	--	0.102
3	12.20	0.65	-.50	5.41	0.109

[a] Total plasma progestin and estrogen values used were only those values from the single plasma sample taken closest to each monthly animal weight.

General Discussion

A finding of high estrogen levels in the prepuberal heifer was surprising in view of the fact that the ovaries of the prepuberal heifer have been generally considered relatively quiescent (Lasley, 1968). These findings raise several unanswered questions: (1) what is the source of these estrogens, (2) what class of estrogens do they belong to, (3) what role do they play in regulating the onset of puberty and (4) what is the explanation for the discrepancy between the estrogen levels reported in trial I and trial II?

Although the adrenal has been implicated as a possible source of estrogens (Short, 1960) and progestins (Balfour, Comline and Short, 1957; Klopper, Strong and Cook, 1957), it probably serves as a source of large amounts of these hormones only in abnormal situations. Desjardins and Hafs (1969) noted a sharp increase in paired ovarian weights in Holstein heifers from 4 to 5 months of age. Erickson (1966) reported that paired ovarian weights in beef heifers more than doubled from 8 to 12 months of age. Between birth and 80 days of age he observed a fourfold increase in the number of growing follicles (two or more layers of follicle cells but no fully formed vesicle) and a similar increase in the number of vesicular follicles. He noted a further slight increase in both types of follicles to 180 days of age. These rapid increases in the size of ovaries and the number of follicles suggested that a concurrent increase in ovarian secretory activity might be expected. Desjardins and Hafs (1969) observed an increase in uterine weight proportionately more rapid than that for body weight from 1 to

12 months of age, although the rate of increase in uterine weight increased considerably after puberty (approximately 7 months of age). The increased rate of uterine growth relative to body weight changes prior to puberty may be the result of ovarian estrogen secretion.

There is no evidence available concerning the class of estrogens represented in the prepuberal heifer. Edqvist and Johansson (1972) reported that the high levels of estrogens in cows prior to parturition were largely due to estrone, while variations in estradiol-17β were minimal. Mason et al. (1972) reported similar levels of estrone and estradiol during the bovine estrous cycle except that the estrone peak prior to estrus was nearly twice the magnitude of the estradiol peak. In a study involving only four cows van der Walt, Dunn and Kaltenbach (1972) observed estrone levels approximately four times larger than estradiol levels during the estrous cycle. Conversely, Echternkamp and Hansel (1971) reported higher levels of estradiol from 3 days before to 3 days after estrus in cows.

The role of various hormones in regulating the onset of puberty is largely speculative. It has been reported here and elsewhere (Allaire et al., 1973) that prepuberal estrogen levels in the heifer were high and decreased continuously as puberty approached. Plasma LH levels have been shown to be higher before puberty than during the luteal phase of the estrous cycle after puberty with levels almost doubling 1 to 10 days before puberty (Randel et al., 1971; Swanson et al., 1972). Reports of plasma progestin levels here and by Randel et al. (1971) indicated that they were low before puberty and increased

only after ovulation had occurred. However, the possibility that some luteinization of follicles without ovulation occurs has not been ruled out (Donaldson et al., 1970). These observations on LH and progesterone levels were in agreement with the suggestion by Spilman et al. (1973) that progesterone exerted a direct negative feedback on LH production in the pituitary or some higher control center.

Ramirez and McCann (1963) measured plasma LH levels in rats and suggested that puberty was initiated by increased LH secretion from the pituitary. Desjardins and Hafs (1968) examined pituitary FSH and LH levels in Holstein heifers from birth through 12 months of age. They observed a consistent decrease in pituitary LH levels from 7 to 12 months of age. However, little fluctuation in pituitary FSH concentration from 2 to 12 months of age was noted. These observations along with those of Randel et al. (1971) and Swanson et al. (1972) were in agreement with the hypothesis made by Ramirez and McCann (1963) that puberty was associated with an increased rate of LH release from the pituitary. They suggested that puberty was initiated by a change in sensitivity of the hypothalamus to gonadal hormones such that before puberty low gonadal hormone levels were sufficient to inhibit pituitary LH release. Ramirez and Sawyer (1966) later demonstrated a sharp rise in the luteinizing hormone-releasing factor content of the hypothalamus of rats as they approached puberty. The results of the study reported here indicated that in heifers the increased release of pituitary LH at puberty may simply be the result of decreased plasma estrogen levels and thus removal of a negative feedback system on the

hypothalamic-pituitary complex. This idea was consistent with the observations that the prepuberal ovary will respond to gonadotropin administration (Onuma et al., 1970; Seidel et al., 1971; Spilman et al., 1972). Decreased LH levels after puberty may be the result of a negative feedback effect on the hypothalamic-pituitary axis mediated by increased plasma progesterone levels.

Swanson et al. (1972) observed high prolactin levels which decreased within 20 days before first estrus and further decreased following puberty through the sixth to ninth estrous cycles. The role of prolactin in the onset of puberty is as yet unexplained.

An unexplained discrepancy existed between the estrogen levels observed for prepuberal heifers in trial I and the heifer calves in trial II. Estrogen values corresponding to the first plasma samples obtained in trial I were near 1200 pg/ml (figure 7). However, average estrogen values for heifer calves in trial II never exceeded 100 pg/ml (figure 14). It appeared that a dramatic increase in total plasma estrogens occurred sometime between the 28th week of life and the 35th week of life (average age of trial I heifers at the beginning of sampling). However, there are several factors which must be considered in comparing these data.

Blood samples were obtained from trial I animals during the winter and spring of 1970-71. Trial II animals were born and sampled in 1972. Identical procedures were used for obtaining blood samples and storing them in both trials. All samples remained frozen until assayed, although trial I samples were stored more than a year longer

than trial II samples. The author was unaware of any thawing which may have occurred in these samples prior to their being assayed. Separate aliquots of plasma were used for estrogen and progestin assays.

Plasma samples from both trials were included in some assays. Differences in estrogen levels between trial I and trial II animals were obvious when assayed together, indicating that the observed differences between total estrogen levels for the two trials were not due to assay procedures.

Feed regimes differed for the two groups of animals as outlined earlier (pages 21 through 23). Marked undernutrition (50 to 75% of National Research Council requirements) has been shown to alter plasma progesterone and estrogen levels by Donaldson et al. (1970) and van der Walt et al. (1972), respectively. However, the changes in hormone levels were minor and certainly not of the magnitude of the differences in estrogen levels reported here for trials I and II. Obviously, the animals used in this study were on adequate levels of nutrition and it seems highly unlikely that nutritional differences were responsible for the observed differences in estrogen levels.

Trial II heifers were allowed to nurse their dams twice daily. Trial I heifers had been weaned approximately 6 weeks before the initial blood samples were obtained. Although nursing is known to influence the reproductive performance of the dam (Wiltbank and Cook, 1958), it has not been demonstrated to influence the hormonal activities of the nursing calf.

There was no obvious explanation for the dramatic difference in estrogen levels observed for trial I and trial II animals. Whether an actual rise in estrogen levels occurs after weaning in beef heifers must await further investigations.

Trial I heifers were bred artificially to a single Polled Hereford sire. The breeding season began June 20 and lasted for 63 days. Nine of the 20 heifers (45%) conceived at first service with 17 (85%) ultimately conceiving during the breeding season. No heifers were inseminated at the first observed estrus (puberty). Five heifers were inseminated the first time at the second observed estrus, while the remaining heifers were inseminated the first time at the third or later estrus. Thus, it was very difficult to draw any conclusions concerning the effect of hormone levels upon fertility. No obvious differences in hormone levels could be detected for the three heifers which failed to conceive.

Conclusions from the research reported herein can be summarized as follows:

1. Progestin levels in heifers prior to puberty generally remained low with the possible exception of a progestin peak occurring 2 to 3 weeks before first estrus in some animals.

2. Estrogen levels in nursing heifer calves from birth to 28 weeks of age were low as compared to older heifers.

3. Estrogen levels in weaned heifers were comparable to reported levels for cows in late gestation and decreased almost linearly as puberty approached.

4. Breed group differences between hormone levels were not detectable.

5. Age controlled more of the variation in gonadal hormone levels than did the variable number of days before puberty.

6. Weight was not a good indicator of gonadal hormone levels.

The investigations reported herein, in fact, raised more questions than they answered. Further research will be required to answer the following questions:

1. What is the source of estrogens in the prepuberal heifer?

2. What proportion of these estrogens are estrone, estradiol or estriol?

3. Does an actual surge in estrogen levels occur as suggested by differences between estrogen levels in trials I and II and, if so, what triggers this surge?

4. Is the surge in progestin levels prior to puberty the result of "silent" ovulations or luteinization of follicles?

5. What mechanisms trigger the onset of puberty in heifers and what role do the various hormones play in this process?

6. Do prepuberal hormone levels reflect an animal's postpuberal reproductive capabilities?

Answers to these questions will greatly enhance research directed toward the induction of precocious puberty in heifers and may be valuable as a tool for use in selection of replacement heifers.

SUMMARY

Five heifers from each of four breed groups (Angus x Angus, Charolais x Charolais and the two reciprocal crosses) were used in a study of gonadal hormone levels during the interval from 16 weeks before puberty to puberty (trial I). Puberty was defined as the first standing estrus. Two heifer calves from dams from each of the above breed groups and sired by a single Polled Hereford bull were used in a study of gonadal hormone levels from birth to weaning (trial II).

Blood samples were collected at weekly intervals from trial I heifers before puberty and three times weekly during the first post-puberal estrous cycle. Heifer calves in trial II were sampled once weekly. Total plasma progestins were estimated by a competitive protein binding assay. Total plasma estrogens were estimated by use of a radioimmunoassay.

Least squares analysis indicated that breed groups did not differ significantly for date of birth, actual and adjusted weaning weights, date of puberty and age at puberty (trial I). Straightbred Charolais heifers were significantly heavier at birth ($P < .01$) and at puberty ($P < .05$) than straightbred Angus heifers. The two reciprocal crosses did not differ significantly from each other or from straightbreds for these traits.

In trial I average total plasma progestin levels decreased from approximately 1.5 ng/ml at 112 days before puberty to less than 1.0 ng/ml at 70 days before puberty and then gradually rose to approximately 3.0 ng/ml the last 2 weeks before puberty. Fifteen of the 20 heifers

exhibited elevated progestin levels prior to puberty. However, these progestin peaks occurred at different times in relation to puberty and for varying lengths of time. The fact that they did not all occur during a 2 or 3 week period immediately preceding first observed estrus suggests that the source of these progestins may be luteinized follicles and not necessarily corpora lutea resulting from "quiet" ovulations. Ovarian structural changes were not monitored in this study.

Average plasma total estrogen levels in the prepuberal heifer decreased from approximately 1000 pg/ml at 16 weeks before puberty to approximately 200 pg/ml during the last 3 weeks before puberty. Multiple regression analysis indicated that age alone accounted for 31.7% and number of days before puberty 29.6% of the variability in estrogen levels. The two variables together accounted for 34.2% of the variation.

Average plasma total progestin levels during the first postpuberal estrous cycle were comparable to levels previously reported for cycling cows. Progestin levels rose from 1 ng/ml on day 1 to a peak of 6 ng/ml on day 13 and declined from day 14 to approximately 2 ng/ml on day 19. Total plasma estrogen levels during the first postpuberal estrous cycle ranged from 100 to 220 pg/ml. The variation within samples taken on the same day was large. Average estrogen levels fluctuated between 100 and 220 pg/ml from day 1 through day 11. Levels rose steadily from 114 pg/ml on day 12 to 220 pg/ml on day 18 and then dropped to 119 pg/ml on day 19. Samples obtained on the day of estrus were not included in the analysis. The failure of estrogen levels

during the estrous cycle to conform to previously published information may be due to too infrequent sampling procedures. It may also indicate that a degree of hormonal imbalance exists during the first postpuberal estrous cycle.

Average progestin levels in heifer calves from birth to 28 weeks of age generally remained below 1 ng/ml (trial II). Estrogen levels were high during the first 2 days of life (> 300 pg/ml) but decreased to less than 32 pg/ml on day 3. Elevated estrogen levels (approximately 40 pg/ml) were observed during the sixth to 10th weeks of life. During the 12th to 23rd weeks levels remained below 20 pg/ml and rose gradually from the 24th week to 58 pg/ml during the 27th week of life. The discrepancy between estrogen levels in heifers nearing puberty in trial I and the heifer calves in trial II is unexplained.

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