EFFECTS OF PRENATAL ANDROGEN EXPOSURE ON POSTNATAL GROWTH, ESTROUS CYCLICITY AND BEHAVIOR IN FEMALE BEEF CATTLE

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(ABSTRACT)

This study assessed the effects of prenatal androgen exposure during three periods of gestation on the external genitalia, estrous cyclicity, postnatal growth, social dominance and sexual behavior of female beef cattle. Pregnant cows recieved 17α methyl-testosterone (250 mg/d, sq) on d 40 to 100 (group 1), 70 to 130 (group 2) or 100 to 160 (group 3) of gestation. Control cows (group 0) received no treatment.

Group 1 females exhibited completely masculinized external genitalia. No vulval opening was present and the ano-genital distance (A-g) was similar to that of control male calves. Group 2 females exhibited small vulval openings and enlarged clitoral structures while group 3 females exhibited normally appearing female external genitalia. Ano-genital distances for the heifers in groups 2 and 3 were similar to those of the control heifers. Androgen exposure during the three periods of gestation did not affect age at

puberty (P<.80), estrous cycle length (P<.63) or postnatal growth (P<.60) of the heifers.

At 9, 16 and 21 mo of age, social dominance values (SDV) were determined for each heifer by 3 min random pair contests for a restricted feed source. The animal with the greatest feed source control time was awarded a win. Social dominance value was calculated as 100 times the number of wins divided by the number of competitions for each animal. Group 3 heifers had significantly greater SDV values than group 1 and 2 females (P<.03). SDV did not differ among groups at 16 mo of age (P<.59). Group 1 females had greater SDV than group 2 females at 21 mo of age (P<.04).

At 9, 16 and 21 mo of age, sexual behavior of the heifers was characterized by exposure of the heifers to a teaser female in estrus. Sexual behavior, as indicated by the number of mounts, head placements and interest time, was lower for group 3 females compared to females in groups 1 and 2 at 9 mo of age (P<.04). There were no treatment differences for any sexual behavior variables at 16 or 21 mo of age.

These results indicate that there is little potential for increasing postnatal growth or altering the estrous cyclicity of female cattle by exposure of the fetus to testosterone during the periods of gestation selected in this study. External genitalia of females were masculinized by androgen exposure during d 40 to 100 of gestation. Social

dominance values were increased and sexual behavior was reduced in females by exposure to androgen during d 100 to 160 of gestation.

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REVIEW OF LITERATURE

The X Chromosome

The X chromosome undergoes a special type of genetic regulation known as X inactivation in mammals (Lyon, 1961). The second X chromosome in females is "turned off" or rendered inactive resulting in appproximately equal levels of X-linked enzyme activity for both sexes. Barr and Bertram's discovery in 1949 of a piece of heterochromatin near the nuclear membrane in the neurons of female cats and the findings of Comings (1966) that labelled RNA precursors were not incorporated by this piece of heterochromatin led to the theory of X inactivation.

There is evidence that certain genes on the second X chromosome escape inactivation. Fellous et al. (1975) used cloned cells that were heterozygous for surface antigens to demonstrate the existence of active loci on the distal Xp arm of the inactivated X chromosome. Ferguson-Smith (1965) proposed that a portion of the Xp arm of the second X chromosome escaped inactivation and further that the loss of this genetic material results in monosomy and infertility. XO human females are sterile with streak gonads. Deletions of the Xp arm of the second X chromosome result in gonadal dysgenesis very similar to that seen in the XO condition (Disteche et

al., 1972). Davis et al. (1976) reported that loss of the Xp arm from p11 to the terminal end results in gonadal dysgenesis, whereas loss from p21 to the end does not affect fertility (Bartsch-Sandhoff et al., 1976). Similarly, translocations (Lucas and Smithies, 1973) or deletions (Hecht et al., 1970) of the Xq arm also can result in gonadal dysgenesis, suggesting that there are genes on the Xq that also escape inactivation.

A second explanation for the required presence of the Xp arm of the second X chromosome has been presented. Oocytes and their associated follicular cells are required for ovarian differentiation (Boczykowski, 1973). Mammalian oocytes are the only cells in the adult where both X chromosomes are active. In mice, XX oocytes have twice the X-linked enzyme activity as do XO oocytes (Epstein, 1969). XO oocytes can be fertile in mice and survive to fertilization, but develop abnormally during subsequent cleavage (Burgoyne and Biggers, 1976). Human females bearing both alleles of a dimeric enzyme, glucose-6-phosphate dehydrogenase, have oocytes that contain a hybrid isozyme, proving that both X chromosomes are active in the oocyte (Gartler et al., 1973). Since oocytes are required for ovarian development, the absence of X chromosomal material could result in the failure of oogenesis and subsequent ovarian development. The X chromosome contains many genes that affect gonadal develop-

ment and gametogenesis (Gordon and Ruddle, 1981). The specific genes that must be present in two copies for successful oogenesis remain to be determined.

The Y chromosome, due to its expression of H-Y antigen, holds the key to sexual differentiation, since under normal conditions an embryo without a Y chromosome will develop as a female. Genetic regulation of the Y chromosome directs formation of a testis while the absence of a Y chromosome leaves the gonad in its undifferentiated or female pattern of development. In mammals, gonadal sex is critical since the gonads are of primary importance in subsequent sexual differentiation. Mammalian gonadal sex differentiation is relatively independent of hormonal or environmental factors which can affect the development of gonadal sex in lower vertebrates.

Two X chromosomes function from fertilization until after blastocyst formation in female embryos, whereas in the male there is one functional X during the same period. After formation of the female blastocyst, one X chromosome is inactivated resulting in suppression of its gene products. This inactivation approximately equalizes the amount of functional X material in both sexes. Inactivation of the X chromosome occurs between the time of blastocyst formation and uterine implantation in female embryos (Short, 1972). Two X chromosomes are not required for early development in

females since in many species XO females are fertile adults. The paternal X in trophoblastic cells of female mice is preferentially inactivated, while the cells of the inner cell mass inactivate a parental X chromosome randomly. This preferential X inactivation by trophoblast cells does not occur in humans (Gordon and Ruddle, 1981).

McLaren and Monk (1981) reported that X chromosome inactivation occurs during germ cell migration in mouse oocytes, with reactivation following colonization of the genital ridge and preceding the onset of meiosis. During meiosis in humans both X chromosomes have been shown to be active (Epstein, 1969). In male embryos, the Y chromosome first functions at the eight-cell stage with the production of H-Y antigen, a male specific cell surface protein. The X chromosome in males is active until after spermatogenesis has begun (Lifschytz and Lindsley, 1972).

The Y Chromosome

Mammalian sex determination is the result of a series of developmental events that begin at the time of fusion of male and female gametes (Jost, 1972). A paternally-derived, X-bearing sperm combines with the X-bearing ovum to yield the homogametic female zygote, whereas a Y-bearing sperm combines with an X-bearing egg to yield a heterozygous male offspring.

Ohno (1967) hypothesized that the X and Y chromosome evolved from a common progenitor chromosome. In mammals, evolutionary specialization has caused these two chromosomes to diverge both morphologically and genetically with the concommitant loss of most structural loci on the Y chromosome. This divergence between the X and Y chromosome has rendered the X chromosome essential for life in mammalian species (Naftolin, 1981).

The normal male karyotype consists of one X and one Y chromosome while the female karyotype has two X chromosomes. In theory, there are two possible mechanisms for sex determination arising from these sex chromosome differences. The presence of two X chromosomes could determine female development or conversely, the presence of a Y chromosome may determine maleness. The reported existence of XO female (Lyon and Hawker, 1973) and XXY male mice (McLaren, 1961) demonstrate that the Y chromosome determines maleness in this species. Additional research points to the existence of testicular determining genes on the Y chromosome in other mammals (Ohno, 1967).

Several lines of evidence implicate the short arm of the Y chromosome (Yp) as the location of the testis-determining genes. Chromosomal aberrations resulting in the absence of the Yp arm result in the formation of streak gonads and female phenotypic development typical of an XO karyotype

(Rosenfeld et al., 1979). Failure of testicular formation results from duplication of the long arm (Yq), and deletion of the Yp arm (Book et al.,1973). Deletion of the Yq arm with an intact Yp arm results in the presence of testicular tissue (Tyrkus et al., 1979). Phenotypic males with an XX karyotype have resulted from the presence of Yp material on the X chromosome (Ferguson-Smith, 1966). The existence of testicular tissue in individuals deficient in Yp genetic material (Buhler, 1980) as well as an individual with Yp genetic material who lacked testes both detract from the Yp testis determining theory (Siebers and Vogel, 1973). A possible explanation for this dichotomy is the observed frequency of the Y chromosome to pericentric inversions (Gordon and Ruddle, 1981).

A male specific cell surface protein called H-Y antigen has been linked to mammalian testicular determination (Wachtel et al., 1975). Abnormally high titers of H-Y antigen in XYY individuals point to the Y chromosome as the location of genes coding for H-Y antigen. Y chromosome translocation studies have implicated the proximal arm of the Yp chromosome as the location of the H-Y locus. However, the presence of Yq genetic material on the Yp arm was not ruled out and in one H-Y positive individual Yq material was the only positively identified Y chromosome material (Koo et al., 1977).

XY females with varying levels of H-Y antigen expression have been reported. The absence of testicular development in the presence of H-Y antigen suggests that there is a defect in the H-Y receptor protein. The complete or partial absence of H-Y antigen in these phenotypic females could also be due to abnormal gene regulation, a mutation at the HY locus or an X-linked gene suppressing H-Y antigen (Ghosh et al., 1978). Reports of XY females with abnormal X chromosomes having a duplication of the Xp arm lend support to this theory (Bernstein et al., 1980). A dose-dependent suppressor gene located on the Xp arm in these females could cause the failure of H-Y antigen expression when the Xp arm is duplicated (Wachtel et al., 1978). Research on aberrant Y chromosomes with deletions of distal segments on both arms implicates the pericentric region of the chromosome as being involved in gonadal determination (Maeda et al., 1976). It has been further postulated that these testis determining genes are present in many copies in this centromeric region (Buhler, 1980).

The Y chromosome plays two roles in the process of spermatogenesis. Indirectly, through the action of H-Y antigen, the Y chromosome causes the testes to differentiate and provide the required environment for the support of spermatogenesis. A direct role is also attributed to the Y chromosome in spermatogenesis. The Y chromosome appears to

be essential for viability of spermatogonia. In some marsupials, selective nondisjunction can result in an XO karyotype in somatic tissues. Spermatogonia are always XY however, suggesting that spermatogonia which have lost their Y chromosome are inviable and never present in gonads. Similarly, in a 39X / 41XYY mosaic mouse, only XYY spermatogonia were observed. Spermatogonia were inviable and eliminated when they possessed the XO genotype (Ford, 1970). Deletions of the distal Yq chromosome result in normal testicular formation but cause failure of spermatogenesis suggesting that loci on the distal long arm of the Y chromosome have effects on gamete production (Tiepolo and Zuffordi, 1976). Considering the critical relationship between germ cells and somatic cells in the testis, it is also possible that Y chromosomal aberrations could cause infertility by altering gonadal somatic cell function.

H-Y Antigen

At an early stage of development, the indifferent gonad undergoes differentiation in either an ovarian or testicular pattern. Testicular differentiation occurs earlier in embryonic development than does ovarian differentiation. Research aimed at elucidating a testis-inducing factor focused on H-Y antigen, a minor histocompatibility antigen re-

sponsible for the rejection of male skin grafts by females. H-Y antigen has been detected on somatic cells of all normal male mammals. Such widespread distribution and evolutionary conservatism suggested that H-Y antigen had an important function in mammals (Haseltine and Ohno, 1981). The presence of H-Y antigen in all cases of heterogametic gonads led to the postulation of its role as the director of gonadal differentiation. Further evidence of H-Y antigen's role in testicular differentiation was the demonstration of its presence in XX males possessing testes, as well as in freemartin cattle and sex-reversed mice and goats (Haseltine and Ohno, 1981). In Moscona reaggregation experiments, XY testicular cells from newborn rats or mice were dispersed and incubated in normal female rat serum and permitted to spontaneously reaggregate in culture. The XY testicular cells formed testicular structures. Dispersed ovarian cells developed into follicular aggregates. When dispersed testicular cells were cultured with H-Y antibody they formed follicular aggregates. Dispersed ovarian cells that were cultured with H-Y antigen formed tubular structures containing oocytes (Zenzes et al., 1978a). Ohno et al. (1979) cultured undifferentiated XX fetal calf gonads with human H-Y antigen and observed complete testicular differentiation.

The earliest stage of development where H-Y antigen is serologically detectable is the eight-cell embryo (Muller and

Schindler, 1983). In mice, H-Y antigen is not demonstrable by histocompatibility until after germ cell migration, but is present by the time the genital ridge differentiates. Grafted fetal rat ovaries undergo testicular development when placed beneath the testicular capsule, but not when placed beneath the kidney capsule of a male. This finding suggests that the gonadal masculinizing factor is present in rat testes (Haseltine and Ohno, 1981). A substance similar to H-Y antigen is secreted by the Sertoli cells and bound by mature Leydig cells (Zenzes et al., 1978b).

Freemartins are XX female cattle that are born following a multiple pregnancy with at least one male. Placental fusion with vascular anastomosis permits the exchange of blood between fetuses. H-Y antigen is present in the ovaries of these females and the ovaries have an organization more typical of the male gonad (Ohno et al., 1976). The extent of internal and external genital masculinization is proportional to the amount of testicular tissue present in the ovary (Jost et al., 1972). In turn, the amount of testicular tissue present in the ovary is proportional to the amount of H-Y antigen to which the ovary was exposed.

Daudi cells are a line of human male lymphoma cells capable of producing histocompatibility antigens that, when cultured with normal female gonads, induce the formation of seminiferous tubules and a tunica albuginea in the female

gonad. (Nagai, 1979). When bovine ovarian cells are dispersed and reaggregated in the presence of Daudi secreted proteins, the ovarian cells form structures that are similar to seminiferous tubules. Epididymal fluid also reorganizes ovarian cell cultures into tubules and induces formation of LH receptors characteristic of male fetal Leydig cells (Muller et al., 1978). Both H-Y antiserum and fetal ovarian secretions can block masculine development in the male gonadal ridge and allow ovarian differentiation (Wachtel and Hall, 1979).

H-Y antigen is normally found if a Y chromosome is present in the genome of an animal. Gene mapping has located the regulator gene for H-Y on the short arm of the Y chromosome (Koo et al., 1977). Buhler (1980) postulated that the testis-determining genes are present in many copies and are located in the centromeric region of the Y chromosome. Interestingly, the same DNA sequence found in this region of the Y chromosome in mammals is also found on the W chromosome in female birds (Singh et al., 1976). This DNA sequence and H-Y antigen are both found in the heterogametic sex of birds and mammals. Males are heterogametic and are the induced sex in mammals whereas in birds, it is the female which is heterogametic and is the differentiated sex. This conserved DNA sequence may not code for a protein but may have a regu-

latory function and evoke its effect through the H-Y structural gene.

Germ Cells

Primordial germ cells are first observed outside the developing embryo in the epithelium of the dorsal yolk sac endoderm (Setchell, 1978). These diploid primordial germ cells are formed during early embryogenesis. In both Drosophila and frogs, a granular cytoplasm called the polar cytoplasm is found at the earliest cleavage of the embryo. These granules fragment at cleavage and associate with ribosomes. The granules are believed to contain messenger RNA coding for the synthesis of proteins necessary for cell differentiation. Polar cells located at the posterior end of the embryo are formed around this granular cytoplasm and subsequently form germ cells. Rat and mouse embryos likewise have a dense material comprised of DNA and protein which is incorporated into their primordial germ cells.

Germ cells migrate by amoeboid movement along the hindgut of the embryo until their arrival at the ventral aspect of the mesonephros where the gonad will develop. The primitive gonadal ridge is a thickening of the coelomic epithelium on the mesonephros. A chemotaxic substance called telepheron is secreted by the epithelial layer of the devel-

oping gonad and is believed to specifically attract germ cells to the prospective gonad (Baker, 1972). Approximately 100 germ cells begin migration and increase mitotically during migration until they reach the gonadal ridge. At this stage of development the indifferent gonad is bipotential, that is, capable of differentiating into either an ovary or a testis. Four tissues make up the indifferent gonad : medullary tissue which develops into medullary cords in the testis; cortical tissue derived from coelomic epithelium which develops into secondary sex cords in the ovary; mesenchyme which is the progenitor of thecal and interstitial tissue; and the primordial germ cells (Setchell, 1978). The interaction between mesonephric somatic cells and primordial germ cells leads to gonadal development (Baker, 1972). In the undifferentiated gonad, germ cells and somatic cells are randomly distributed throughout the tissue and there is no evidence of histological differentiation.

Male Gonadal Development

The male gonad begins sexual differentiation during the first third of gestation when the germ cells move to the medulla where cords of cells begin to form. These developing seminiferous tubules enclose the germ cells along with somatic cells, called Sertoli cells. In the periphery of the

gonad, mesonephric cells form cords that grow inwards to connect with the seminiferous tubules. These cell cords form the rete testis and connect the seminiferous tubules to the epididymis. Formation of a connective tissue membrane called the tunica albuginea, beneath the coelomic epithelium inhibits the development of the cortex in the male gonad. Steroidogenesis immediately follows testicular differentiation in the male, as Leydig cells form from mesenchymal cells located between the seminiferous tubules and begin testosterone synthesis (Wilson et al., 1981).

Female Gonadal Development

Female gonadal differentiation begins during the second trimester of gestation with the development of ovarian follicles. Gonadal differentiation in the female occurs in one of two characteristic patterns depending on the species. In one scheme, germ cells are enclosed in cell cords that are analogous to testicular cords. The alternative pathway does not involve cord formation and results in a more compact ovary. In both patterns of female development, the gonadal mesonephric connections are present only early in gonadal development. Mesonephric cells migrate into the gonad and accumulate in the medullary area. Any germ cells in this area either move to the cortex or degenerate, leaving the

ovary with a sterile medulla and the germ cell population in the cortex (Baker, 1972).

Prepuberal Male Germ Cell Development

Formation of spermatozoa begins at puberty and results from the onset of meiosis. Male germ cells complete the early stages of meiosis in fetal life and are then enclosed in seminiferous tubules and arrested in development after the preleptotene or leptotene stage of meiosis. Germ cells that escape seminiferous tubule enclosure may continue through the zygotene and pachytene stages before they degenerate. Meiosis Inducing Substance is secreted by post-puberal testicular cells derived from the mesonephros (Setchell, 1978). Meiosis Inducing Substance is the testicular factor that is responsible for the stimulation of meiosis of male germ cells in post-puberal males. Meiosis Preventing Substance is produced by fetal and prepuberal testicular cells located in the seminiferous tubules (Setchell, 1978). Meiosis Preventing Substance controls the arrest of meiosis in the developing germ cells at the preleptotene or leptotene stage. The role of Meiosis Inducing Substance is demonstrated by the finding that male fetal germ cells incubated in media derived from puberal testes experience a stimulation of meiosis (Byskov, 1979). Fetal ovaries exposed to culture

media from fetal testes demonstrate arrested germ cell meiosis which illustrates the effects of Meiosis Preventing Substance.

Prepuberal Female Germ Cell Development

Meiosis begins before birth in females of many mammalian species and may immediately follow gonadal differentiation. Conversely, the onset of meiosis may be delayed for a species-specific interval following ovarian differentiation. Species demonstrating the immediate meiotic pattern possess compact ovaries with a uniform distribution of germ cells in the gonadal cortex and have little steroidogenic capability until after meiosis. Species with delayed meiosis have germ cells that are enclosed in cords during the delay interval and are able to produce significant quantities of steroids prior to the onset of meiosis. Sheep, pigs and cattle follow the pattern of delayed meiosis while the germ cells of humans and mice undergo immediate meiosis. Meiosis Inducing Substance is secreted at the onset of meiosis by mesonephric cells which become granulosa cells in females. Meiosis is arrested at prophase in females, due to the actions of a follicular oocyte inhibiting substance (Byskov, 1979).

Sex Reversal

Complete sex reversal in nonmammalian vertebrates includes both germ cells and somatic cells whereas mammalian germ cells lack the ability to reverse their sex. Mammalian germ cells that are found in a sex-reversed gonadal environment degenerate leaving a sterile gonad (Haseltine and Ohno, 1981). Mammalian somatic cells are capable of undergoing sex reversal. Mintz (1959) reported finding a male chimeric mouse with 95% XX somatic cells and XY germ cells. Ford et al. (1975) observed a fertile female mouse with an ovarian follicle whose follicular cells were 98% XY. These two observations illustrate that mammalian somatic cells are capable of sex reversal. Male cells have a competitive advantage over female cells in sex chimeras. A testicular inducing substance produced by a relatively few XY cells can masculinize a large number of XX cells. Thus, in an XX/XY chimera, many XX somatic cells undergo testicular differentiation and the remaining ovarian tissue degenerates later in development (Haseltine and Ohno, 1981).

Differentiation of Genitalia

The testis is the director of sexual differentiation following the induction of its formation by H-Y antigen.

Differentiation of the male from the inherently female pattern of development is stimulated by the onset of androgen production following histological differentiation of the testis. The genitalia of both males and females are equally responsive to these testicular secretions, indicating the presence of androgen receptors in both sexes. Normal male development is dependent on the production and secretion of testicular hormones very early in development. The sex chromosome content of extragonadal cells has no effect on their subsequent pattern of development. In a particular group of marsupials, one sex chromosome is eliminated in all extragonadal cells. The genetic constitution of these cells is XO for both sexes (Ford, 1970). Despite their genetic equality, these cells are able to differentiate in either a masculine or a feminine direction as dictated by the presence or absence of testicular secretions (Hayman and Martin, 1965).

Three components comprise the primordial genital tract in males and females. The first component is the gonads; ovaries in the female and testes in the male. The second component is of two genital duct systems; the Wolffian ducts and the Mullerian ducts. The third component of the primordial genital tract is the opening for the genital and urinary tracts through the genital folds to the exterior. The male genital duct system results from retention of the

Wolffian ducts and regression of the Mullerian ducts. Conversely, in the female, the Mullerian ducts persist and the Wolffian ducts regress. The external genitalia in both sexes develop from the genital tubercle, genital folds and genital swellings (Wilson et al., 1981). Testicular secretions direct male urogenital tract development while female differentiation is independent of hormonal stimulation (Jost, 1972).

The first secretory function of the testis is the production of Mullerian inhibiting hormone (MIH), a protein hormone which causes Mullerian duct regression. Although the exact mechanism of action is unknown, MIH has been identified as a glycoprotein of 70,000 MW that is produced by the Sertoli cells. (Donahoe et al., 1977). Failure of MIH production or tissue insensitivity to MIH results in an XY phenotypic male possessing oviducts and a uterus, as well as normal male internal and external genitalia (Sloan and Walsh, 1976).

The second secretory function of the testis is the production of testosterone by the Leydig cells of the testicular interstitial tissue. Testosterone is responsible for the maturation of the spermatogenic tubules and is also required for virilization of the male genital tract. The upper portion of the Wolffian duct becomes the epididymis and connects the testis with the vas deferens. The vas deferens develops

from the central part of the Wolffian ducts and the terminal portion of the Wolffian ducts gives rise to the ejaculatory ducts and seminal vesicle. Endodermal buds in the urethral lining form the prostate gland (Wilson et al., 1981).

The external genitalia of the male are formed in response to dihydrotestosterone (DHT) stimulation following Wolffian duct virilization. Dihydrotestosterone is a reduced metabolite of testosterone and is produced in the cytoplasm of the cells comprising the primordial external genitalia. The penis forms from the elongation and fusion of the genital folds while the genital swellings give rise to the scrotum. The genital tubercle forms the glans penis. Development of male external and internal genitalia is completed during the first third of gestation. Testicular descent into the scrotum and genital growth occur during the latter two-thirds of gestation.

In female embryos, the absence of MIH results in Mullerian duct development and the lack of androgenic stimulation results in the regression of the Wolffian ducts. This occurs in humans between days 45 and 50 of gestation. Mullerian ducts give rise to the oviducts, the uterus and the anterior vagina during the first third of gestation. The external genitalia of females undergo relatively few morphological changes from the indifferent state. During the first third of gestation the genital tubercle forms the

clitoris, the genital swellings become the labia majora and the genital folds give rise to the labia minora (Wilson et al., 1981).

Cellular Metabolism of Testosterone

Testosterone is produced from cholesterol in the Leydig cells. In the mitochondria, cholesterol is converted into pregnenolone which undergoes further conversion to testosterone in the smooth endoplasmic reticulum of the cell (Setchell, 1978). Testosterone is secreted primarily secreted into the blood although some is also transported by the lymph. Testosterone binds chiefly to albumin and cortisol binding globulin in the blood, with only a small fraction remaining unbound. Testosterone circulating in the blood is subject to one of two fates. It may be metabolized and excreted via the kidney, bile or feces or it may be removed from the circulation by cells of a target tissue (Setchell, 1978).

Testosterone is taken up by cells through passive diffusion and may be converted to DHT by the cytoplasmic enzyme 5α-reductase. A cytosolic androgen receptor can bind either testosterone or DHT and the hormone receptor complex moves inside the nucleus. Alternatively, the testosterone may diffuse directly into the nucleus and be bound by a nuclear

receptor. The hormone receptor complex interacts with chromosomal acceptor sites to increase transcription of specific structural genes resulting in messenger RNA production and subsequently cellular protein synthesis. RNA synthesis in target tissues is stimulated by increases in chromatin template activity and the number of initiating sites on the DNA of the cell as well as the stimulation of RNA polymerase I and II (Mainwaring, 1977). Testosterone also increases the activity of many enzymes in the microsomal, lysosomal and mitochondrial fractions of the cell (Bardin et al., 1978).

Disorders of Testosterone Metabolism

Female embryos possess androgen receptors and are equally capable of exhibiting a masculine response to androgenic stimulation as are males. The absence of sufficient levels of androgens is the only factor that prohibits females from demonstrating male developmental responses. Female embryos that are exposed to androgens will develop in a masculine direction, as exemplified by the congenital adrenal hyperplasia disorder. In this condition, adrenal cortisol synthesis is inadequate in the fetus and the compensatory increase in adrenal androgens causes masculinization of the female genitalia (Finkelstein et al., 1983).

Testosterone is responsible for the stimulation of the embryonic Wolffian ducts while DHT regulates the masculinization of the urogenital sinus and external genitalia. Defects in the 5α-reductase enzyme in males result in the formation of a phenotypic female with normal male internal genitalia and the absence of Mullerian ducts. The internal genitalia are typical of a normal male due to the production of MIH and testosterone by the testis. The external genitalia remain feminine due to the absence of DHT stimulation resulting from the 5α-reductase defect (Imperato-McGinley et al., 1974). The onset of androgen production at puberty causes the external genitalia to become masculinized despite the 5α-reductase deficiency and the absence of DHT.

Five human genetic defects have been identified that result in decreased testosterone synthesis and incomplete masculinization of the embryo (Wilson, 1978). These genetic defects cause the dysfunction or absence of an enzyme required for the conversion of cholesterol to testosterone. The degree of virilization ranges from a phenotypic female with no Wolffian ducts and failure of masculinization in the urogenital sinus and external genitalia, to a phenotypic male with normal Wolffian development and hypospadic penile development (Rosler and Kohn, 1983).

The testicular feminized male syndrome (Tfm) is an X-linked genetic disorder causing a genotypic male with testes to have female external genitalia and secondary sexual characteristics. This condition results from the failure of cytoplasmic receptors to bind androgens in target tissues (Imperato-McGinley, 1974). In addition to defects in hormone and receptor production, failure of virilization can occur due to defects in the nuclear interaction of the hormone receptor complex with the chromosomal acceptor sites (Wilson et al., 1981).

The Onset of Testicular Steroidogenesis

The testis assumes its endocrine function approximately one day after histologically apparent testicular differentiation in mammals (Catt et al., 1975). The ovary begins steroid synthesis at the same time as the testis, but before ovarian histological differentiation occurs. In the rabbit, testicular differentiation occurs on day 16 of gestation. Testosterone synthesis begins on day 17 and genital tract virilization occurs on days 17.5 and 18 of gestation. Testosterone levels in the ovary of the rabbit are very low during this period (George et al., 1978).

There are two differences between the histologically differentiated testis and the undifferentiated ovary in re-

gards to hormone synthesis. First, the testis has 50 times more 3-beta hydroxysteroid dehydrogenase (3βHSD) than does the ovary. At this stage of development, 3βHSD is the rate limiting enzyme for testosterone synthesis. The increased level of 3βHSD results in greater production of testosterone in the testis than in the ovary (Pang and Tang, 1983). The second difference between the testis and ovary during this period of development is the presence of aromatase in the ovary. Aromatase, which converts testosterone into estrogen, is present in the ovary but absent in the testis (Wilson et al., 1981). All other enzymes involved in gonadal steroid production are equal in the ovary and testis during this period. The reduced production of testosterone in the ovary and its subsequent aromatization to estrogen result in higher levels of androgens in males than in females during development. Genetically-programmed differences in only two steroidogenic enzymes during this critical period of development result in the divergence of the masculine and feminine patterns of differentiation.

Regulation of the Onset of Steroidogenesis

One line of evidence points to hormonal regulation of the onset of steroidogenesis in the gonads during early development. Pituitary or placental gonadotropins control

gonadal steroid synthesis both in late embryogenesis and postnatally (Wilson et al., 1981). In rabbits, the fetal anterior pituitary differentiates at about the same time as the onset of testicular steroidogenesis, suggesting that the pituitary controls the onset of testicular steroidogenesis (Schecter, 1970). Humans produce placental gonadotropins that could be involved in the onset of testosterone synthesis (Catt and Dufau, 1976). Gonadotropin receptors are found in the testis at the time of Leydig cell differentiation and steroidogenesis (Catt et al., 1975). These findings provide evidence that supports the hypothesized hormonal regulation of the onset of gonadal steroid synthesis.

Conversely, testes and ovaries both develop steroidogenic capability in culture media in the absence of pituitary hormones (Brinkmann, 1977; George and Wilson, 1980). Gonadotropins have been shown to be unnecessary for testosterone synthesis in fetal testes until late in development when the conversion of cholesterol to pregnenolone becomes rate limiting in testicular steroidogenesis (George et al., 1979). The endocrine differentiation of both ovaries and testes of fetal rabbits occurs in organ culture at the same time that the process occurs in vivo (Brinkman, 1977; George and Wilson, 1980). These observations suggest that gonadal steroidogenesis is regulated by factors that are intrinsic to the gonads themselves. Although testosterone-

mediated events later in fetal development, as well as postnatally, depend on pituitary or placental gonadotropins, the onset of the endocrine function in the gonad appears to be autonomous.

Sexual Dimorphism

Sexual dimorphism is the expression of two forms of a trait, depending on the sex of the individual. Sexual dimorphisms include: body size, appendage size, morphology of internal and external genitalia, patterns of gonadotropin and gonadal steroid secretion, behavioral patterns, and cellular metabolism. Sexual dimorphisms are the result of a cascade of differential development between the sexes, ultimately arising from the presence or absence of a Y chromosome in the genome. The primary function of the Y chromosome is testicular development, because without the Y chromosome ovaries develop. The secretory products of the male gonad determine the occurrence of sexual dimorphism in mammals (Jost, 1972). Testosterone is the primary hormone affecting the growth and differentiation of most reproductive and nonreproductive tissues. Androgenic actions of steroids are associated with the masculinization of the reproductive tract while anabolic actions refer to the stimulatory effects of steroids on nonreproductive tissues. The specific messenger

RNA and subsequent proteins produced following testosterone stimulation are tissue dependent, suggesting that chromatin structure is altered during differentiation to permit tissue specific responses to the steroid receptor complex (Bardin and Catterall, 1981).

Within cells, testosterone may act directly or may be metabolized into other steroids of greater or lesser potency. Enzymes that are responsible for these conversions vary in activity among tissues as do the potencies of the androgen metabolites (Wilson and Walker, 1969). The androgen receptor may be modified in some tissues to favor the binding of a particular androgen or the receptor-steroid complex may have differing affinities for the chromatin binding sites, depending on the specific androgen bound by the receptor (Baker et al., 1977).

Dimorphisms of the Central Nervous System

Central nervous system (CNS) development is intrinsically feminine in mammals. Sexually dimorphic CNS control of endocrine function and behavior are evident in many mammalian species. These sexual dimorphisms result from the influence of gonadal hormones on the indifferent CNS during a critical period of development. Exposure of the CNS to

testosterone during this critical period results in permanent and irreversible changes, termed organizational changes.

Activational effects of hormones differ from organizational effects by their lack of permanence and their reversibility in the absence of hormonal stimulation. Many sexually dimorphic traits depend not only on permanent organizational effects early in development, but also on the activational effects of gonadal hormones later in life (Goy and McEwen, 1980).

Pituitary gonadotropin release occurs in a cyclic feminine pattern unless the CNS mechanism controlling gonadotropin secretion is masculinized by exposure to testosterone during a critical period of CNS development (Pfeiffer, 1936). Experiments demonstrating that genetically-female rats, with postnatally grafted testis exhibited a tonic pattern of gonadotropin secretion and that genetic-males castrated at birth, developed cyclic female patterns of gonadotropin release verified Pfeiffer's hypothesis. Further studies with sheep and guinea pigs showed that exposure of females to testosterone during a critical period of development resulted in organizational alterations of the hypothalamic structures regulating gonadotropin secretion (Clarke et al., 1976; Brown-Grant and Sherwood, 1971). These studies provide evidence that exposure of female mammals to androgen during a critical period of development causes or-

ganizational changes in the CNS centers governing gonadotropin secretion. These organizational changes cause genetic-females to exhibit a tonic pattern of gonadotropin secretion that is typical of males and subsequently, the loss of estrous cyclicity.

The presence of testicular secretions in males directs the developmental pattern of the CNS away from the inherently feminine pattern, resulting in the imposition of a masculine pattern of gonadotropin secretion and the exhibition of male behavior (Gorski, 1971). Exceptions to the concept of CNS defeminization in response to androgen exposure have been reported. Female patterns of gonadotropin release in both humans and cattle have been shown to be unaffected by early androgen treatment (Stearns et al., 1973; Hamernik et al., 1987).

Masculine differentiation of the CNS involves the two processes of masculinization and defeminization. Masculinization is the development of male patterns of neuroendocrine secretion and behavior in the CNS. Defeminization is the suppression of female behavior and neurosecretory patterns (Beach, 1971). These two processes are independent and are influenced by gonadal steroids during development (Goy and McEwen, 1980). Prenatal exposure of female guinea pigs and rhesus monkeys to testosterone results in increased masculine sexual behavior (Goy and McEwen,

1980). Masculinization and defeminization of behavior occurs in hamsters following prenatal exposure to estrogens or aromatizable androgens (DeBold and Clemens, 1978).

Androgens are also present in females during the period of CNS differentiation, but fail to induce masculinization. Two theories have been advanced to explain the lack of masculinization of females despite the presence of androgens in female fetuses during the critical period for sexual differentiation. First, there are periodic differences in the circulating levels of androgen between females and males (Pang and Tang, 1983). Weisz and Ward (1979) suggested that a brief exposure to higher levels of testosterone could sensitize the CNS to subsequent reduced levels of testosterone. Males were exposed to higher concentrations of androgen early in development than females and responded differently than females when both were exposed to equal levels of androgen later in development.

A second explanation for the absence of masculinization in normal females is the hormonal protection hypothesis. The presence of ovaries in rats tends to protect the CNS from the defeminizing effects of neonatal androgens (Blizard and Denef, 1973). Progesterone has been implicated as the protective agent in rats and rhesus monkeys

The mammalian CNS has a critical period for sexual differentiation. This is a period when the CNS is maximally sensitive to the organizing effects of gonadal hormones. Not all sexually dimorphic CNS functions are androgen-sensitive at the same time. In rats, CNS control of cyclic gonadotropin secretion and female sexual behavior are most sensitive to androgens immediately after birth and are insensitive to prenatal androgen. In contrast, CNS control of male sexual behavior in rats is very sensitive to prenatal androgen exposure (Goy and McEwen, 1980).

The critical period for the differentiation of the CNS in the rat begins just after Leydig cell differentiation and the ensuing onset of testosterone secretion. Leydig cells differentiate between day 16 and 18 of gestation, immediately prior to the onset of the critical period. In guinea pigs, testosterone secretion begins on day 29 to 30 of gestation with androgenically-organized neural effects first becoming evident on day 30 of gestation (Goy et al, 1964).

Full development of the differences between the sexes for a given response after androgen exposure during the critical period may require subsequent exposure to gonadal steroids. Female rats exposed as neonates to androgen exhibit several normal post-puberal estrous cycles before becoming anovulatory. Subsequent exposure to ovarian steroids is necessary to cause the loss of the ability of the CNS to

sustain cyclic ovarian activity (Gorski, 1971; Swanson and Van der Werff ten Bosch, 1964).

Specific areas of the developing brain are involved in the uptake of testosterone during the critical period for CNS differentiation. Target cells for testosterone have been located in hypothalamic brain centers of perinatal rats (Sheridan et al., 1975). Testosterone implanted into the dorsal preoptic area of the hypothalamus (POA) of neonatal female rats has been shown to stimulate both masculine and feminine behavior (Hayashi and Gorski, 1974). Implantation of testosterone into the ventromedial hypothalamus (VMH) of neonatal female rats inhibits the expression of female sexual behavior and the capacity to support cyclic ovarian function (Christensen and Gorski, 1978).

The fetal rat brain is able to metabolize testosterone to either DHT or estrogen (McEwen, 1981). Neonatal exposure of female rats to either estrogen or testosterone causes these females to become anovulatory after puberty (Wilson et al., 1941). Three pieces of experimental evidence have implicated estrogen as an important mediator of androgenic developmental effects on the CNS. First, the neonatal brain is capable of aromatizing testosterone to estrogen (Naftolin et al., 1975). Second, estrogen antagonists block the differentiating effects of testosterone on the neonatal brain. And third, DHT, a non-aromatizable androgen, is less potent

than either testosterone or estrogen in defeminizing the neonatal brain (Brown-Grant et al., 1971). Interference with the formation of estrogen from testosterone or interference with formation of the estrogen-receptor complex also decreases the response of the CNS to testosterone (McEwen et al., 1977). Testicular feminized male (Tfm) rats demonstrate masculine brain differentiation, suggesting that the androgen receptor is not required for mediation of androgenic effects in the CNS (Beach and Buehler, 1977).

Maternal and placental estrogens are present in the fetal blood of eutherian mammals. The fetus must be protected from these estrogens to prohibit maternal steroids from directing the sexual differentiation of the fetal CNS. Fetal rats and mice possess an estrogen binding system which is present during late gestation and during early postnatal life (Raynaud et al., 1971). Alpha fetoprotein (αFP) is present in developing neurons of the CNS but is absent in regions of the brain where gonadal steroids are sequestered (Toran-Allerand, 1980). This αFP is produced by the yolk sac and fetal liver and sequesters circulating estrogen in the fetus. Testosterone is unaffected by αFP and remains capable of entering the brain for aromatization to estrogen.

The efficiency of αFP in decreasing the availability of estrogen to the tissues is illustrated by the following two experiments. Administration of αFP antibody to neonatal fe-

male rats resulted in a response that was similar to that of rats receiving estradiol (Mizejewski et al., 1980). Slaughter (1977) used synthetic estrogens with low αFP affinities to demonstrate a greater potency for sexual differentiation of neonatal female rats than that of estradiol. These results support the concept that αFP is one mechanism which protects the female fetus from the differentiating effects of maternal estrogens in rats.

Brain cells of newborn rats possess cytoplasmic receptors for androgens, estrogens and progestins (MacLuskey and McEwen, 1980). These receptors are similar to those found in mature brain tissue suggesting that the organizational effects of gonadal steroids occur via the same mechanism as do the activational effects observed later in life. Two important differences exist between the neonatal steroid receptor system and that of the mature adult. First, the receptor numbers in neonatal brain tissue fluctuate during and following the critical period. Second, the presence or absence of receptors in specific brain centers is subject to change during development. These differences in receptor numbers and their location could be responsible for the differences in timing of the critical period for various sexually dimorphic traits.

Early exposure to gonadal hormones induces both structural and functional alterations in the CNS. Steroid

hormones cause changes in nucleic acid and protein synthesis (Bardin and Catterall, 1981; Wilson et al., 1981). Adminstration of inhibitors to protein or nucleic acid synthesis in neonatal rats decreases the extent of CNS differentiation following androgen exposure (Gorski and Shryne, 1972). Gonadal steroid exposure also affects neurotransmitter function. For example, postnatal male rats have higher serotonin concentrations in the brain than do females (Giulian et al., 1973). Drugs that interfere with neurotransmitter function also have inhibitory effects on sexual differentiation (Sutherland and Gorski, 1972). Thus, administration of an inhibitor to neural functions can cause a loss in responsiveness of the CNS to the differentiating effects of gonadal steroids.

Specific regions of the brain such as the POA respond to estrogen and testosterone with increased growth of neurites (Toran-Allerand, 1976). Estrogen appears to play a major role in this response and the administration of either estrogen antibodies or synthetic estrogen antagonists blocks this response (MacLuskey and Naftolin, 1981). Early gonadal secretions cause morphological sex differences in the CNS such as volume of brain nuclei, size of cellular organelles and synaptic and dendritic branching patterns (Toran-Allerand, 1978).

Sexual Dimorphism of Gonadotropin Secretion

The secretion of LH and FSH is cyclic in mature mammalian females. Increases in blood estrogen levels resulting from the maturation of an ovarian follicle exert a positive feedback effect on the hypothalamo-pituitary axis causing an LH and FSH surge (Pfeiffer, 1936; Scaramuzzi et al., 1971). This gonadotropin surge causes the ovulation of a mature follicle. In males, gonadotropin secretion is tonic and incapable of producing a gonadotropin surge (Gorski, 1979). Development of the hypothalamic center governing cyclic gonadotropin release in male sheep is inhibited by exposure to endogenous testosterone during the critical period (Karsch and Foster, 1975). In females, both the tonic and the cyclic hypothalamic centers develop in the absence of testosterone exposure resulting in a basal level of gonadotropin secretion augmented by surges in response to the positive feedback effect of estrogen.

The timing of the critical period for differentiation of the hypothalamic nuclei controlling gonadotropin secretion is day 1 to 5 postpartum for rats, day 30 to 37 of gestation in guinea pigs and day 30 to 90 of gestation in sheep (McLuskey and Naftolin, 1981; Brown-Grant, 1973; Clarke et al., 1976a). Mammals that are less developed at birth generally have postnatal or late gestational critical periods,

whereas, mammals that are more fully developed at birth tend to have critical periods that occur earlier in gestation.

Several investigators have demonstrated that both males and females of domesticated species that are exposed to testosterone in utero, are incapable of exhibiting a gonadotropin surge in response to elevated estradiol concentrations. Karsch and Foster (1975) reported that sexually mature male sheep were unable to exhibit a gonadotropin surge in response to elevated estradiol concentrations even when castrated postnatally. Short (1974) reported that female sheep which had been exposed to testosterone in utero from either day 20 or 60 of gestation until birth, were also unable to exhibit an LH surge in response to estradiol. Testosterone failed to masculinize the regulatory system for LH secretion in female sheep when administered from day 80 to birth or when administered postnatally. Gilts that were treated with androgen on days 30 to 36 of gestation experienced defeminization of gonadotropin release due to decreased positive feedback effects of estrogen on the brain (Elsaesser and Parvizi, 1979). Prenatal exposure of female sheep and pigs to androgen during a critical period of gestation has been shown to masculinize the pattern of gonadotropin release in these species.

Gonadotropin secretion is a sexually dimorphic trait in rats. Mature female rats exhibit a cyclic pattern of

gonadotropin release in response to the positive feedback effect of estrogen on the hypothalamus. Female rats that are exposed to testosterone on day 2 of life become anovulatory after reaching puberty (Clemens and Gorski, 1968). Male rats that are castrated at birth exhibit a typically female pattern of differentiation of the hypothalamic centers that control gonadotropin secretion (Gorski and Barraclough, 1963). Research has shown that testosterone levels are high in the neonatal male rat during the first few days after birth (Resko et al., 1968), and that the uptake of testosterone by the rat brain is greater immediately after birth than on day 10 or 20 of life (Diamond and Dale, 1967). The findings of the researchers cited above indicate that gonadotropin secretion occurs in two sexually dimorphic patterns in rats and further, that it is the presence or absence of testosterone during the postnatal critical period for gonadotropin secretion that determines which pattern will develop.

In stark contrast to the data which has been reported for rodents, exposure of female fetuses of some species to androgen in utero does not affect CNS control of gonadotropin secretion. Female primates exhibit a normal cyclic pattern of gonadotropin secretion after exposure to high levels of androgen in utero (Forest, 1983). Bovine females that were exposed in utero to androgens on day 40 to 60 of gestation

exhibited normal cyclic patterns of gonadotropin secretion and responded to exogenous estrogen with an LH surge typical of normal females (Hamernik et al., 1987). While prenatal exposure to testosterone has been shown to cause masculinization of gonadotropin secretion in many species, it has not been shown to do so in either primates or in cattle.

Sexually Dimorphic Growth

Males of many mammalian species have greater birth weights than females (Glucksman, 1978). This difference in birth weight between the sexes may be due to the exposure of males to higher levels of androgen in utero than females. Female bovine fetuses that were exposed in utero to testosterone during days 40 to 60 of gestation did not differ in birth weight from untreated control females (Hamernik et al., 1987; Putney, 1984). No difference in birth weight was recorded between control female calves and females that were exposed to testosterone on days 80 to 110 or 110 to 140 of gestation (DeHaan et al., 1987a). Guinea pigs that were exposed in utero to testosterone on days 33 to 37 of gestation did not differ in birth weight from control females (Brown-Grant and Sherwod, 1971). Conversely, DeHaan et al. (1987b) reported a significant decrease in birth weight of female

lambs that were exposed to testosterone in utero on days 40 to 60 of gestation when compared to untreated females. These data suggest that in utero androgen treatment of fetal mammalian females does not result in increased birth weights compared to untreated controls.

Intact males have greater postnatal growth rates than castrated males, suggesting that androgens have activational effects on growth (Berg and Butterfield, 1987). Castrated males have a growth advantage over females which implies that androgens also have an organizational effect during development. Exposure of females to androgen during a critical period for the differentiation of the CNS centers that control growth, could alter the growth of females in a more masculine direction. Female rodents that were exposed to testosterone during the perinatal critical period showed greater postnatal growth and mature size than control females (Tartellin et al., 1975; Swanson and van der Werff ten Bosch, 1963; Beal and Brower, 1982). A single perinatal injection of testosterone increased body weight in female mice over that of untreated control females (Harris and Levine, 1965). This evidence suggests that the administration of testosterone to female rodents during the critical period for differentiation of the CNS mechanisms controlling growth results in increased postnatal growth.

Female bovine fetuses that were exposed to testosterone in utero on d 80 to 110 of gestation tended to have increased adjusted 205-day weaning weights (DeHaan et al., 1987a). This increase was not apparent when the treatment was administered on days 110 to 140 of gestation. Adjusted weaning weights for female calves that were exposed to testosterone on either days 40 to 60 of gestation or on days 40 to 80 of gestation were similar to those of intact male calves and were greater than those of control females (Putney, 1984). Conversely, Hamernik et al., (1987) reported significantly decreased weaning weights for heifers that were exposed in utero to testosterone on days 40 to 60 of gestation compared to control heifers. These data suggest that there may be a potential for increasing weaning weights of female calves by exposing the female fetus to testosterone during an as yet undetermined period of development.

Differences in post-weaning growth are apparent between the sexes in many species. Castrated males have an advantage in growth over castrated or intact females demonstrating the organizational effects of gonadal steroid exposure (Berg and Butterfield, 1987). Adjusted yearling weights for heifers that were exposed to testosterone on days 80 to 110 of gestation were greater than those of untreated heifers. No difference in adjusted yearling weights were noted for heifers treated during days 110 to 140 of gestation (DeHaan

et al., 1987a). Yearling weights were also not different for heifers exposed in utero to androgen on days 40 to 60 or days 40 to 80 of gestation compared to untreated heifers (Putney, 1984; Hamernik et al., 1987). These data may support the hypothesis that in utero exposure of female bovine fetuses to testosterone between days 80 and 110 of gestation results in increased adjusted yearling weights over those of untreated females. However, the majority of the data reported for cattle would seem to indicate that prenatal exposure to androgen does not affect postnatal growth in heifers.

Females of other mammalian species experience enhanced postnatal growth in response to testosterone exposure during development. Female sheep exposed in utero to testosterone during days 32 to 88 of gestation showed increased growth rates compared to untreated females (Jenkins et al., 1987). Similarly, DeHaan et al. (1987b) exposed female lambs to testosterone in utero for 68 days beginning on day 40 to 60 of gestation. Treated females showed significantly greater daily gains than untreated females. Female pigs exposed in utero to androgen on days 35 to 45 of gestation exhibited increased postnatal gains compared to untreated females (Matulis et al., 1987). Results from these experiments indicate that the exposure of female mammalian fetuses of certain species to testosterone during a critical period for the

differentiation of the mechanism for growth regulation, can increase postnatal growth over that of untreated females.

Dimorphism of Sexual Behavior

Differences exist between the sexes in both mating and non-mating behavior. Difficulty in the interpretation of sexual behavior arises from two complicating factors. First, most sexually dimorphic behavioral traits depend on the activational effects of gonadal hormones after the critical period (MacLuskey and Naftolin, 1981). Second, there are many traits that are shared at least to some extent by both sexes and cannot be clearly regarded as dimorphic (Beach, 1968). For example, female rats are bisexual and frequently display male mounting behavior, whereas female hamsters do not display male sexual behavior (Clemens and Coniglio, 1971; DeBold and Whalen, 1975).

Species differ in their responses to androgen exposure during the period of sexual differentiation. Administration of either estrogen or aromatizable androgens to female hamsters during a critical period for CNS differentiation, results in behavioral masculinization and defeminization (Ruppert and Clemens, 1981; Lisk, 1980). Fetal ewes that were exposed to testosterone in utero from day 20 or day 60 of gestation until birth exhibited behavioral defeminization

and masculinization (Short, 1974). Androgenized female dogs show behavioral defeminization, but not masculinization (Beach and Kuehn, 1970). Female rats androgenized prior to day 5 of life, exhibit masculinization of sexual behavior and do not exhibit sexual receptivity as adults when primed with ovarian steroids (Barraclough and Gorski, 1962; Brown-Grant, 1973).

Exposure of adult male rats to ovarian hormones following in utero anti-androgen treatment or perinatal castration resulted in the display of receptive behavior similar to that of females in estrus (Harris, 1964; Ward, 1972; Quadagno and Rockwell, 1972). Castration of male rodents after 30 days of life has no effect on their sexual behavior, whereas neonatal castration results in the display of feminine sexual behavior (Scouten et al., 1975). These results indicate that in rats, sheep and dogs, the presence or absence of testosterone during a critical period for differentiation of sexual behavior determines whether the adult sexual behavior will be masculine or feminine.

Sexual behavior is controlled by a sexually dimorphic center of the hypothalamus (Gorski et al., 1978). This dimorphism results from the androgenic stimulation of differentiation in the male CNS during a critical period of development. Similarly, the absence of this androgenic stimulation in the female results in the development of the

CNS along the inherently feminine pattern. Whalen and Edwards (1967) concluded that sexually dimorphic mating behavior in rats is due to the suppression of the development of the hypothalamic center that controls feminine sexual behavior in males. Corbier et al. (1978) demonstrated the existence of a postnatal testosterone surge in male but not in female rats. Castration just prior to birth prevents this surge and results in feminization of adult males. These results indicate that the presence of testosterone in the male during development causes the differentiation of neural mechanisms that control sexual behavior and further, that the administration of exogenous androgen to females during a critical period of CNS differentiation can cause the alteration of female sexual behavior in a masculine direction.

Sexually Dimorphic Aggression

Aggressive behavior is a sexually dimorphic trait in mammals. Male mice normally fight when paired together whereas paired female mice rarely fight, even under the influence of substantial amounts of androgen (Tollman and King, 1956). These findings indicate that differences exist in the neural mechanisms that regulate aggressive behavior between males and females.

The sexual dimorphism in aggressive behavior in rodents is a result of perinatal androgen exposure in the male and the absence of androgen exposure in the female (Whalen and Nadler, 1965; Edwards and Herndon, 1970; Quadagno et al., 1972). Male mice that were castrated at birth showed less aggression than males castrated on day 10 when both groups received exogenous androgen as adults (Goy, 1966). Female mice exposed to androgen at birth exhibited male levels of aggression when administered androgens as adults (vom Saal, 1978). Prenatal exposure of female lambs to testosterone caused increased levels of aggression later in life (Clarke, 1977). Interpretation of these results leads to the conclusion that aggressive behavior is a sexually dimorphic trait in mammals and results from the exposure of the male to higher levels of androgens during development than the female. In turn, the exposure of females to exogenous androgens during a critical period for the differentiation of the CNS would be expected to result in the exhibition of masculine patterns of aggressive behavior.

Aggressive actions in cattle occur in a characteristic sequence of behaviors described by Schein and Fohrman (1955). When a cow is approached by another cow, the approach may be either active or passive. A passive approach is a coincidental crossing of paths by two animals. An active approach implies an aggressive intent by the approaching cow and may

elicit a flight reaction by the cow being approached. Alternatively, the cow being approached may stand her ground and herself imply an aggressive intent. One or both cows will then assume a threatening posture which may cause the less dominant animal to flee. If both animals refuse to accept the submissive role, active physical contact occurs. These interactions are repeated until one animal finally accepts the submissive role. The sum total of all dominance submission relationships between pairs of animals in a group is the social dominance hierarchy.

Several factors can influence the position of an animal in a social dominance hierarchy including age, weight, presence of horns and the seniority of an animal in a group (Schein and Fohrman, 1955; Blockey, 1979; Bouissou, 1972). Temporary factors may also affect the social rank of an animal such as illness, injury and the occurrence of estrus. Injury and illness have a detrimental effect on social rank while a cow in estrus may disregard the social structure and ignore the aggressive actions of higher ranking animals (Beilhartz and Zeeb, 1982).

Aggressiveness is an important factor in the determination and maintenance of social rank (Syme, 1974). Social rank determines the priority of access to limited resources such as feed in a group of animals. Animals ranking high in the dominance structure have first choice of feed and utilize

aggressive behavior to displace subordinate animals (Craig, 1986).

There are three requirements for the establishment of a stable dominance hierarchy. First, there must be a mutual recognition of rank by the interacting pair of animals. Second, the social rank of other animals must be remembered. And third, there must be continuous reenforcement of dominance by the higher ranking member of the pair (Ewbank and Meese, 1971).

Feed competition trials between pairs of heifers were used from weaning to 18 months of age to determine that social dominance relationships are formed around the time of puberty (Bouissou, 1977). In 70% of all animals tested, all-or-none responses indicating the acceptance of a dominant or submissive role, occurred between 9 and 12 months of age. A reduction in levels of all aggressive behaviors was observed at this age signifying an acceptance of dominant and submissive roles by the animals in the group and the formation of a stable social hierarchy. Seventy-three percent of all encounters between two previously unacquainted animals resulted in the occurrence of agonistic behavior within 40 seconds of their introduction. Thirty-seven percent of these encounters provoked actual fighting which lasted no longer than 30 seconds. Following this initial encounter animals were isolated for several days and then reunited. The level

of agonistic behavior was reduced indicating the development of a dominance order between the animals. Once established, dominance relationships were maintained by threats and seldom involved physical contact.

Social experience is the sum of all permanent behavioral changes in an animal resulting from interactions with the other members of a group. Animals reared in an environment that is devoid of contemporaries are not socially experienced and exhibit different behavior patterns when confronted with strange animals than do socially experienced animals. Social experience was found to have a significant effect on the establishment of social dominance (Bouissou, 1974). Animals were considered to be socially-experienced following their second encounter with a stranger. Socially-experienced animals utilized more threats and retreats and less physical contact in encounters with unfamiliar animals. Sixty-six percent of socially-experienced animals established dominance submission relationships within 10 minutes of introduction, whereas only 13% of socially-inexperienced animals did so.

In total, the available information emphasizes that aggressive behavior is a sexually dimorphic trait in mammals and is enhanced by the organizational effects of gonadal steroids on the CNS of the male during a critical period of development. The activational effects of gonadal steroids

present later in life cause the stimulation of these differentiated neural circuits in the brain centers that control aggression (MacLuskey and Naftolin, 1981). Administration of testosterone to 3 to 6 month old heifers caused the treated heifers to become dominant over untreated controls (Bouissou and Gaudioso, 1982). The elevation in social rank persisted long after levels of testosterone had returned to normal suggesting that the dominant quality imparted to treated heifers did not require the presence of testosterone once dominance was established. The increase in social rank was not a result of higher levels of aggression in the treated animals but rather a reduction in the levels of fear of the other animals (Bouissou, 1978). The activational effects of androgen on heifers was apparently not a stimulation of neural circuits that control aggression but an inhibition of circuits that regulate fear.

MATERIALS AND METHODS

Administration of treatments

Angus or Angus-cross cows were mated to a single Simmental sire. Sixty pregnant cows were randomly assigned to one of four treatments as follows:

Group 0. Fifteen pregnant cows were untreated and served as controls. Nine female calves were born from this group of cows;

Group 1. Fifteen pregnant cows were administered 17α methyl-testosterone daily from d 40 through d 100 of gestation. Nine female calves were born from this group of cows;

Group 2. Fifteen pregnant cows were administered 17α methyl-testosterone daily from d 70 through d 130 of gestation. Eight female calves were born from this group of cows;

Group 3. Fifteen pregnant cows received 17α methyl-testosterone daily from d 100 to d 160 of gestation. Five female calves were born from this group of cows (1 female calf died prior to behavioral tests).

Methyl-testosterone administration consisted of 250 mg of 17α methyl-4 androsten-2-one (Sigma Chemical Co., St.

Louis, Mo.) suspended in 10 ml corn oil injected subcutaneously once daily.

Calves were born in March or April and sexed based on the appearance of the external genitalia and the presence or absence of gonads in the scrotum, if one developed. The following measurements were recorded at birth: weight, head circumference, body length, cannon bone length, cannon bone circumference, shoulder width, hip width, heart girth, ano-genital distance and scrotal circumference.

All cows were managed under standard conditions during pregnancy and after calving. Calves were permitted to nurse at will until weaning at approximately 230 d of age. Post-weaning rations consisted of .91 kg cracked shelled corn and 9.1 kg of corn silage/hd/d until approximately 290 d of age. Calves were then fed a ration consisting of corn silage with 2% soybean meal and .28% urea ad libitum until they were 420 d of age. Calves were also fed at least 200 mg of lasalocid/hd/d (Hoffman-LaRoche, Inc. Nutley, NJ) from 290 to 420 d of age. From 420 to 570 d of age, calves were maintained on mixed grass pasture.

Weights were recorded for calves every 28 d. Weaning weights were recorded and adjusted for age of calf and age of dam. Yearling and final weights were measured and adjusted for age of calf.

Puberty and Estrous Cyclicity

Age at puberty and estrous cycle lengths were determined by analysis of serum progesterone (P_4) concentrations. Blood was collected from all calves twice weekly via jugular venipuncture beginning at 235 d of age and ending at 450 d of age.

At approximately 480 d of age, estrous cycles in heifers were synchronized by administration of norgestomet implants (CEVA Laboratories, Overland Park, KS) for 11 d. Following implant removal, blood was collected daily via jugular venipuncture for 30 d and analyzed for P_4 concentration. Heifers were observed for signs of behavioral estrus for at least 2 h daily (AM and PM) during this 30-d period. KAMAR heat mount detectors (KAMAR Inc., Steamboat Springs, CO) were utilized to facilitate heat detection. Serum P_4 values for each heifer were plotted serially by days of age to estimate estrous cycle length and age at puberty.

After removal, blood was stored at room temperature for three hours until clotted. Blood was centrifuged at 2800 x g for 15 min at 4 C. Serum was decanted and frozen at -20 C until analyzed for P_4 by radioimmunoassay (RIA) as per procedures of Beal et al. (1980). Serum samples were assayed in duplicate and re-evaluated when duplicates differed by

greater than 25%. The intraassay and interassay coefficients of variation were 6.30% and 29.14%.

Determination of Social Dominance

Dominance relationships were determined using a 3-min feed competition trial between pairs of animals as described by Bouissou (1970). Social dominance values were determined for all heifers at 9, 16 and 21 mo of age. These ages were chosen to insure that the heifers were tested both prior to and subsequent to the onset of puberty. Half of all possible pairwise combinations were contested to derive a dominance value for each animal as reported by Blockey (1979).

Heifers were pre-conditioned to the test area by exposing small groups of five or six animals to the pen during a 10 d period prior to the date of the behavior trials. Heifers were removed from pasture and feed was withheld for 36 h immediately prior to the trial date. Trials were held at 3-d intervals, with access to pasture provided between trial days.

Heifers were prepuberal during behavioral trials conducted at 9 mo of age. All heifers had reached puberty for the trials at 16 and 21 mo of age but the effects of variable stages of the estrous cycle on testing were removed by im-

planting each heifer with a norgestomet implant 3 d prior to each series of trials at 16 and 21 mo of age.

On the trial day, pairs of heifers were randomly selected and simultaneously introduced into a 10x10 km pen with a gravel surface. In a corner of the pen opposite and equidistant from the introduction gate for the two contesting heifers was a single feeder containing a grain mix. The access space of the feeder was limited such that only one heifer could obtain feed. Total time for each animal in the feeding position or in control of the feeder was recorded by an unobtrusive observer. The animal with the greatest accumulated time in control of the feeder was designated as the dominant member of the pair. Social dominance values (SDV) were calculated as the number of wins divided by the total number of competitions multiplied by 100 (Blockey, 1979). The average time in control of the feeding position (FEEDTIME) was determined by dividing the total number of seconds in control of the feed source by the number of contests in which the animal participated.

Measurement of Sexual Behavior

At 9, 16 and 21 mo of age, heifers were exposed to an unrestrained female in estrus (teaser) for 10 min in a 10x10 km pen with a gravel surface to evaluate sexual behavior.

For trials at 9 mo of age all heifers were prepuberal, but for trials at 16 and 21 mo of age heifers had reached puberty and were fitted with norgestomet implants 3 d before each series of trials to eliminate the effects of estrous cycles on behavior. Each heifer was tested for 10 min on each of 3 d with at least 1 d between trials. The sexual behaviors that were measured were selected by examining a video recording of the heifers during their exposure to an estrus female prior to the date of the actual sexual behavior trials themselves. The sexual behavior variables that were evaluated are listed in table 1.

Estrus was induced in the teaser females using SYNCRO-MATE-B (implant = 6 mg norgestomet, injection at time of implantation = 3 mg norgestomet and 5 mg estradiol valerate; CEVA Laboratories, Overland Park, KS) prior to the behavior tests. Estradiol valerate (10 mg) was injected 1 d prior to the test day to insure the induction of behavioral estrus. Teaser females were tested on the day of the behavior trials to insure their receptivity to the experimental heifers by exposing the teaser to a heifer and observing the teaser for an immobile standing response to the attempted mounts of the heifer. A minimum of 4 teaser females was used for each day of sexual behavior trials. Test heifers were individually exposed to one teaser female for 10 min using a random pairing method. Teaser females were selected for each

TABLE 1. MEASURES OF SEXUAL BEHAVIOR

Mounts	Abbr.	Description	Measurement
Mounts	M	Mounts of the teaser female by the test heifer	No./10 min
Interval to first mount	IM1	Elapsed time between introduction of test heifer to teaser female and the first mount by the test heifer	Sec
Interval to second mount	IM2	Elapsed time between first and second mounts	Sec
Sniffs	S	Sniffs directed towards the teaser female	No./10 min
Sniffs following first mount	SFM	Sniffs directed towards the teaser female following the occurrence of the first mount	No./10 min
Head placement	H	Placement of the head of the test heifer on the tail, body, flank or between the hind legs of the teaser female	No./10 min
Head placement following first mount	HFM	Placement of the head of the test heifer on the tail, body, flank or between the hind legs of the teaser female following the occurrence of the first mount	No./10 min
Nose to nose	NN	Nose-to-nose contact between test heifer and the teaser female	No./10 min
Nose to nose following first mount	NNFM	Nose-to-nose contact between the test heifer and the teaser female following the first mount	No./10 min
Thrusts	T	Pelvic thrusts directed towards the teaser female by the test heifer subsequent to mount	No./10 min
Interest time	I.T.	Accumulated time of interest in teaser female by test heifer	Sec/10 min

contest in a rotating order as part of the randomization procedure in an attempt to minimize fatigue and sexual nonreceptivity. The teaser females were allowed at least 30 min between contests. Prior to the behavior test each heifer was placed in visual proximity to the test pen for 20 min to observe the sexual activity in the test pen. Behaviors were recorded via audio recording of verbal descriptions of visual observations during the trials.

Statistical Analysis

All data were analyzed by least squares analysis of variance using the general linear models procedure of the Statistical Analysis System (SAS, 1985). Data collected at birth were analyzed for treatment effects as were yearling weight and final weight. Age at puberty was also analyzed with treatment as the main effect in the model.

Weaning weight was analyzed with treatment as a main effect and most probable producing ability of the dam (MPPA) as a covariate in the model. A regression of age on weight was analyzed for effects of treatment and linear and quadratic effects of age.

Estrous cycle length was analyzed with treatment and heifer within treatment in the model. The mean square for

heifer within treatment was the error term used to test for effects of treatment.

Social dominance values and FEEDTIME were analyzed using the repeated measures option in the GLM procedure (SAS, 1985). Treatment and the age deviation of individual heifers from the mean age of all heifers were included in the model. The effects of trial (9, 16 and 21 mo of age) were analyzed using this model. Orthogonal contrasts were computed to separate the trial effects and the effects of the treatment by trial interaction. The orthogonal contrasts were: trial 1 vs trials 2 and 3, and trial 2 vs trial 3. Trials were analyzed separately to further elucidate the effect of the treatment by trial interaction on SDV and FEEDTIME. The model included treatment as a main effect and age deviation as a covariate. Tukey's procedure for mean separation was utilized when there was a significant difference among treatments ($P<.05$). Orthogonal contrasts were computed if the difference among treatments approached significance ($P<.25$). The contrasts were: treatment 0 vs treatments 1, 2 and 3, treatment 3 vs treatments 1 and 2, and treatment 1 vs treatment 2.

A multivariate analysis of variance was utilized to compute the partial correlation coefficients between the SDV and FEEDTIME values for the trials at 9, 16 and 21 mo of age. Treatment served as a main effect with age deviation as a

covariate in the model. Partial correlation coefficients were also computed between the SDV or FEEDTIME and age or weight for the trials at 9, 16 and 21 mo of age. Treatment was the main effect in the model with age or weight as a covariate.

An upset was defined to have occurred if an animal of lesser SDV defeated an animal of greater SDV. The percentage of upsets was calculated as the number of upsets for an animal divided by the number of competitions the animal was involved in multiplied by 100. A repeated measures analysis of variance was performed to test the effects of trial on upsets. Treatment was the main effect in the model. Orthogonal contrasts compared trial 1 vs trials 2 and 3, and trial 2 vs trial 3 as well as the interaction of treatment by trials.

The sexual behavior variables were analyzed with the repeated measures option in the GLM procedure. The values for sexual behavior were summed across days within a trial. Treatment was the only effect in the model. The effects of trial were analyzed following which, orthogonal contrasts were used for mean separation. The contrasts were: trial 1 vs trials 2 and 3, and trial 2 vs trial 3. The treatment by trial interaction was also tested by orthogonal contrasts.

Sexual behavior trials were analyzed separately to examine the effects of treatment and treatment by trial interactions with treatment as the main effect.

A multivariate analysis of variance was performed to compute the partial correlation coefficients for sexual behavior traits with treatment as the main effect. The values for sexual behavior variables were summed across days and trials, resulting in one value for each sexual behavior trait per animal.

The regression of the mean number of mounts on the mean interval between mounts was analyzed for the three trials combined. Treatment was a main effect in the model with linear and quadratic effects of the number of mounts included.

RESULTS AND DISCUSSION

Appearance of External Genitalia

Prenatal exposure of female bovine fetuses to testosterone in utero results in masculinization of external genitalia (Putney, 1984; Hamernik et al., 1987). Females that were exposed to androgen on d 40 to 100 of gestation (Group 1) in the present study had external genitalia that were completely masculinized and a greater mean ano-genital distance than all other groups of females ($P<.0001$). A penis with a prepuce and a bilobed scrotum without gonads were present while a vulval opening was absent. The least squares mean (LSM) for ano-genital distance was 46.2±.7 cm. Male half-sibs had a LSM for ano-genital distance of 49.4±10.1 cm.

Females that were exposed to androgen during d 70 to 130 of gestation (Group 2) were incompletely masculinized, exhibiting small vulval openings and an enlarged clitoral structure. The LSM for ano-genital distance for these females was 4.4±.8 cm. Females that were exposed to androgen during d 100 to 160 of gestation (Group 3) had normally-appearing female external genitalia with some enlargement of the clitoris. The LSM for ano-genital distance was 3.0±1.1 cm. Control females (Group 0) had normally-appearing female

external genitalia and a LSM for ano-genital distance of 4.1±.7 cm.

Masculinization of the external genitalia of female sheep occurs following testosterone exposure on d 40 to 50 of gestation (Clarke, 1977) and on d 40 to 60 in female cattle (Putney, 1984). Data from the present study, in accordance with these findings, indicates that the critical period for complete masculinization of female external genitalia is between d 40 and 70 of gestation. Exposure of female fetuses to testosterone on d 70 to 130 of gestation resulted in incomplete masculinization of the external genitalia, evidenced by the absence of a penis and scrotum, the presence of a vulva and an ano-genital distance that was similar to control females. Testosterone exposure during d 100 to 160 of gestation resulted in females that exhibited normally-appearing external genitalia with some clitoral enlargement.

Analysis of Growth

Body weight was recorded for all calves within 24 h of birth. There were no significant differences in birth weight among untreated females and females that were exposed to androgen on d 40 to 100, 70 to 130 and 100 to 160 of gestation (table 2). Females did not differ among treatments for head

TABLE 2. LEAST SQUARES MEANS (±SEM) FOR BIRTH WEIGHT, WEANING WEIGHT, YEARLING WEIGHT AND 565 D WEIGHT

Treatment	No.	Birth weight (kg)	Adjusted weaning weight[a] (kg)	Adjusted yearling weight[b] (kg)	565-d weight (kg)
0	9	38.3 ± 1.0	212.3 ± 6.6	278.3 ± 7.0	392.3 ± 7.6
1	9	36.7 ± 1.0	212.8 ± 6.8	275.5 ± 7.0	399.8 ± 7.6
2	8	36.3 ± 1.1	214.8 ± 7.0	276.2 ± 7.4	403.1 ± 8.0
3	4	37.4 ± 1.6	221.9 ± 10.0	282.5 ± 10.5	395.0 ± 11.3

[a]Weaning weight adjusted to 205-d equivalent with adjustment for age of dam and dam MPPA.

[b]Yearling weight adjusted to 365-d equivalent.

circumference, body length, cannon bone length and circumference, shoulder width, hip width or heart girth.

Body weight was measured for all calves at weaning (230.7±1.5 d of age) and adjusted for age of calf to a 205-d basis and age of dam. The most probable producing ability (MPPA) of the dam was utilized as a covariate in the statistical analysis of weaning weight. The MPPA had a significant effect on weaning weights of calves ($P<.03$), however, adjusted 205-d weaning weights were similar for all treatment groups (table 2).

Body weight was recorded at approximately one year of age (350.7±1.5 d of age) and adjusted to a 365-d basis (yearling weight). No significant differences were observed for adjusted yearling weight among treatment groups (table 2). Body weight was also measured at the end of the experimental period (564.7±1.5 d of age) and was adjusted for age of calf to a constant 565-d basis (final weight). There were no significant differences among treatments for final weight (table 2).

Analysis of the regression of age on weight for the four periods of growth; birth to weaning, weaning to yearling, yearling to final and birth to final showed significant linear and quadratic effects ($P<.0001$). Examination of the regression of age on weight showed no differences in growth among treatments.

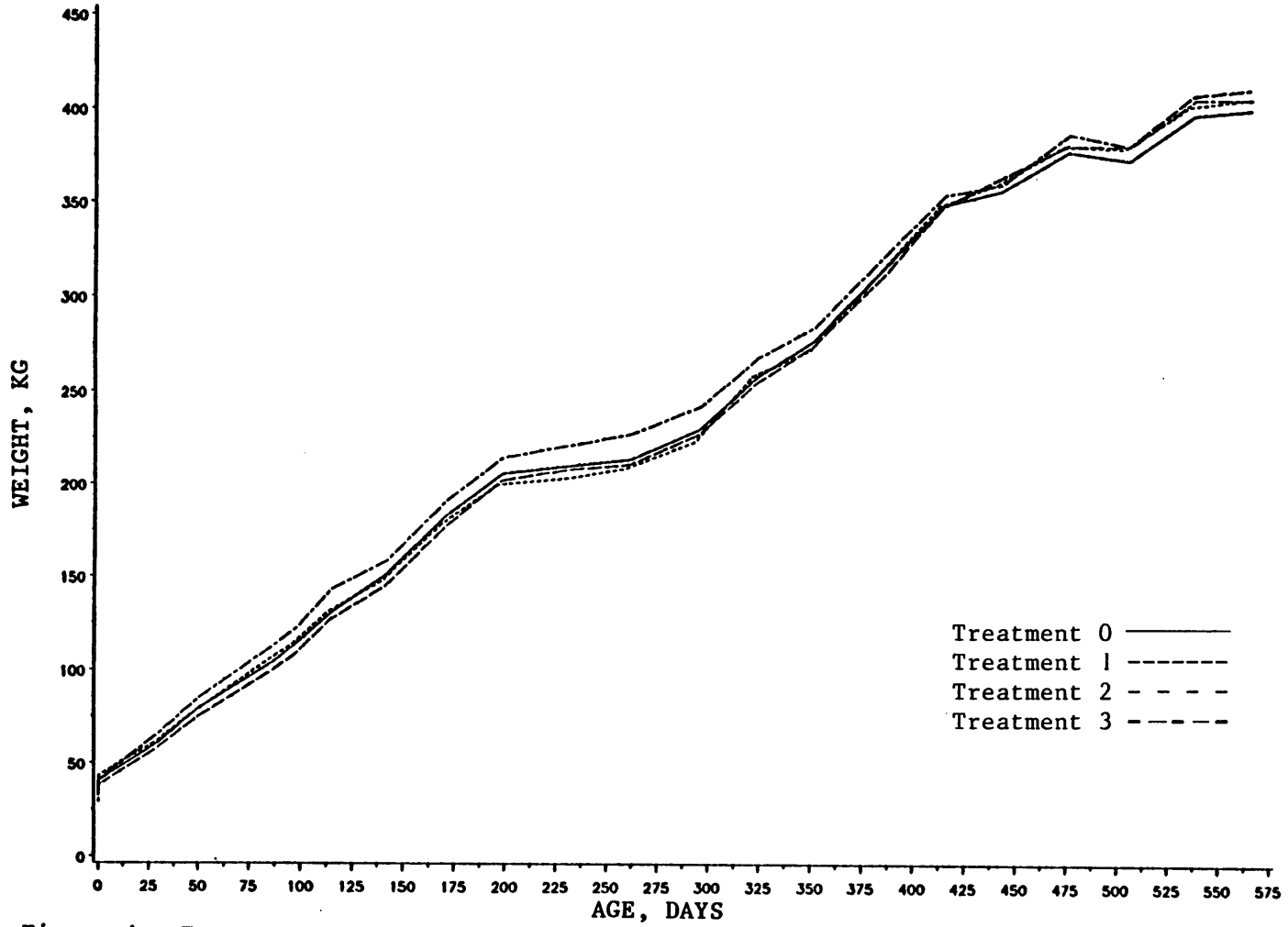

Figure 1. Treatment group means for bodyweight from birth to 565 days of age.

The relationship between weight and age is shown in figure 1. From birth to weaning, the growth of the calves was linear. The primary source of nutrients for calves during this period was milk from the dam which provided a consistent supply of nutrients. From 230 to 290 d of age, calf growth was linear, but the rate of growth was reduced compared to pre-weaning growth. The stress of weaning and the change in diet were probably responsible for the reduction of growth during this period. From 290 to 420 d of age, the growth of calves was linear and increased as calves became accustomed to their diets and the dietary protein and non-protein nitrogen was increased in the ration. Calves were placed on pasture from 420 to 565 d of age and the reduced energy intake caused a reduction in daily gains.

Exposure of female sheep to androgen in utero during d 40 to 60 or d 32 to 88 of gestation has been reported to enhance postnatal growth rates (DeHaan et al., 1986; DeHaan et al., 1987b; Jenkins et al., 1987). Growth rates in cattle have also been reported to be increased when female calves were exposed to androgens in utero on d 40 to 60 (Putney, 1984) or on d 80 to 110 of gestation (Dehaan et al., 1987a). Postnatal growth rates of rodents have been reported to be increased by androgen exposure (Swanson and Van der Werff ten Bosch, 1963; Beatty et al., 1970; Tartellin et al., 1975).

Results of the present study show no differences in 205-d adjusted weaning weight, adjusted yearling weight or final weight (565-d) among females that were exposed to testosterone on d 40 to 100, 70 to 130 or 100 to 160 of gestation, and untreated controls. No differences were recorded in birth weights among treatments which is consistent with previous findings (Putney, 1984; DeHaan, 1987a).

Analysis of the regression of age on weight for the periods: birth to weaning, weaning to yearling, yearling to final and birth to final weight showed that treatment did not significantly affect growth rate during any of these periods. These data suggest that in utero testosterone treatment of female bovine fetuses from d 40 to 160 of gestation does not impart a greater potential for growth from birth to 565 d of age than that of untreated females.

There are several possible reasons for the failure of treatment to increase growth rate. First, the timing of the critical period for differentiation of the proposed hypothalamic mechanism that regulates growth is not known in cattle. The treatment periods utilized in this study may not have have been coincidental with the critical period for the hypothalamic differentiation of growth regulation. Second, the dose of testosterone although sufficient to masculinize the external genitalia of the females, may not have been sufficient to masculinize the hypothesized neural centers

that control growth. Third, the pattern of androgen administration may not mimic the endogenous pattern of androgen secretion in the male. Fourth, there may be a mechanism that protects the female bovine brain from the masculinizing influences of androgens as is seen in human female congenital adrenal hyperplasia patients (Forest, 1983). Lastly, the level of nutrition of the calves in this study may have been too low to permit the expression of an increased growth potential. It is possible that treatment could have had an effect on the neural mechanisms that regulate growth but the lack of availability of sufficient nutrients could have suppressed the potential for enhanced growth, thereby masking any effect of treatment.

The failure of fetal female cattle to exhibit increased growth subsequent to in utero testosterone treatment in this study was most likely due to the absence of androgen during some period of development when its presence is required to cause the expression of increased growth. It is possible that the periods of treatment in this study did not encompass the critical period for the differentiation of the neural control of postnatal growth. The critical period for the differentiation of the hypothalamic nuclei that control postnatal growth may be either earlier or later in development than the treatment periods utilized in the present study. It is also possible that the neural centers control-

ling growth in cattle require a longer period of exposure to prenatal testosterone than was provided by the treatments in this experiment. Despite the findings of Berardinelli et al., (1987) in cattle, that the differentiation of the hypothalamic nuclei controlling LH secretion requires postnatal testosterone exposure, it is unlikely that differentiation of the neural structures that control postnatal growth is dependent on postnatal androgen. It is common knowledge that steers that were castrated at birth exhibit increased postnatal growth rates when compared to heifers. This growth advantage in the absence of postnatal androgen suggests that the differentiation of hypothalamic structures controlling growth has occurred prenatally.

Increased placental aromatization of testosterone to estrogen and rising P_4 levels during gestation both could contribute to the absence of masculinization of the female brain following administration of exogenous androgens (Clarke et al., 1976b). If the required amount of androgen was less for the effective masculinization of the external genitalia than the amount required to induce the masculinization of neural structures then a case could be made for the failure of neural masculinization in this study being a result of an insufficient dosage of androgen. The production of androgen by the fetal testes in the male could provide sufficient levels of testosterone to impose a male pattern of differen-

tiation on the neural centers of the male despite the existence of the hypothesized placental barrier to the entry of androgens.

Age at Puberty

The onset of puberty in bovine females is commonly defined as the first estrus that is accompanied by a spontaneous ovulation (Hafez, 1980). The onset of puberty in heifers is characterized by a pulsatile pattern of luteinizing hormone (LH) secretion which causes the formation of luteinized follicles. The luteinized follicles secrete low levels of progesterone (P_4). The low levels of P_4 appear to be required for the ensuing puberal LH surge which is induced by the positive feedback effects of estrogen secreted by a developing follicle. The LH surge causes the mature follicle to ovulate. Following ovulation, a corpus luteum (CL) is formed which secretes increasing levels of P_4. Progesterone levels plateau when the CL reaches its maximum size and are maintained until the regression of the CL around d 17 of the estrous cycle. The precipitous decline in serum P_4 caused by the regression of the CL, permits estrogen to exert a positive feedback effect on the hypothalamus which responds by secreting increased levels of gonadotropins. The in-

creased gonadotropin secretion causes follicular growth and estrogen secretion and subsequently, ovulation.

Analysis of the serum P_4 concentrations of the females involved in this study enabled the identification of the age at puberty as well as the characterization of the estrous cycle length. Progesterone profiles for two animals in each treatment group that were representative of all the animals in the group were selected and are provided in figures 2 through 5. The average age at puberty was 386±4 d of age and did not differ among treatment groups (table 3).

Weight at puberty was not significantly different among animals in the four treatment groups (table 3). Females that were puberal at 386 d of age were heavier than females that were not puberal at 386 d, although the difference was not significant ($P<.64$; 317.6±4.9 vs 310.5±5.9 kg respectively). Hafez (1980) reported that both weight and age are closely related to the onset of puberty in heifers.

Prenatal androgen treatment during the periods of testosterone exposure utilized in this study did not significantly affect age at puberty which is in agreement with previous research (Zimbelman and Lauderdale, 1973). Treated females failed to reach puberty earlier than control females in this study which refutes the earlier findings of Putney (1984). Treated females had increased growth rates and reached puberty earlier than controls in that study. The

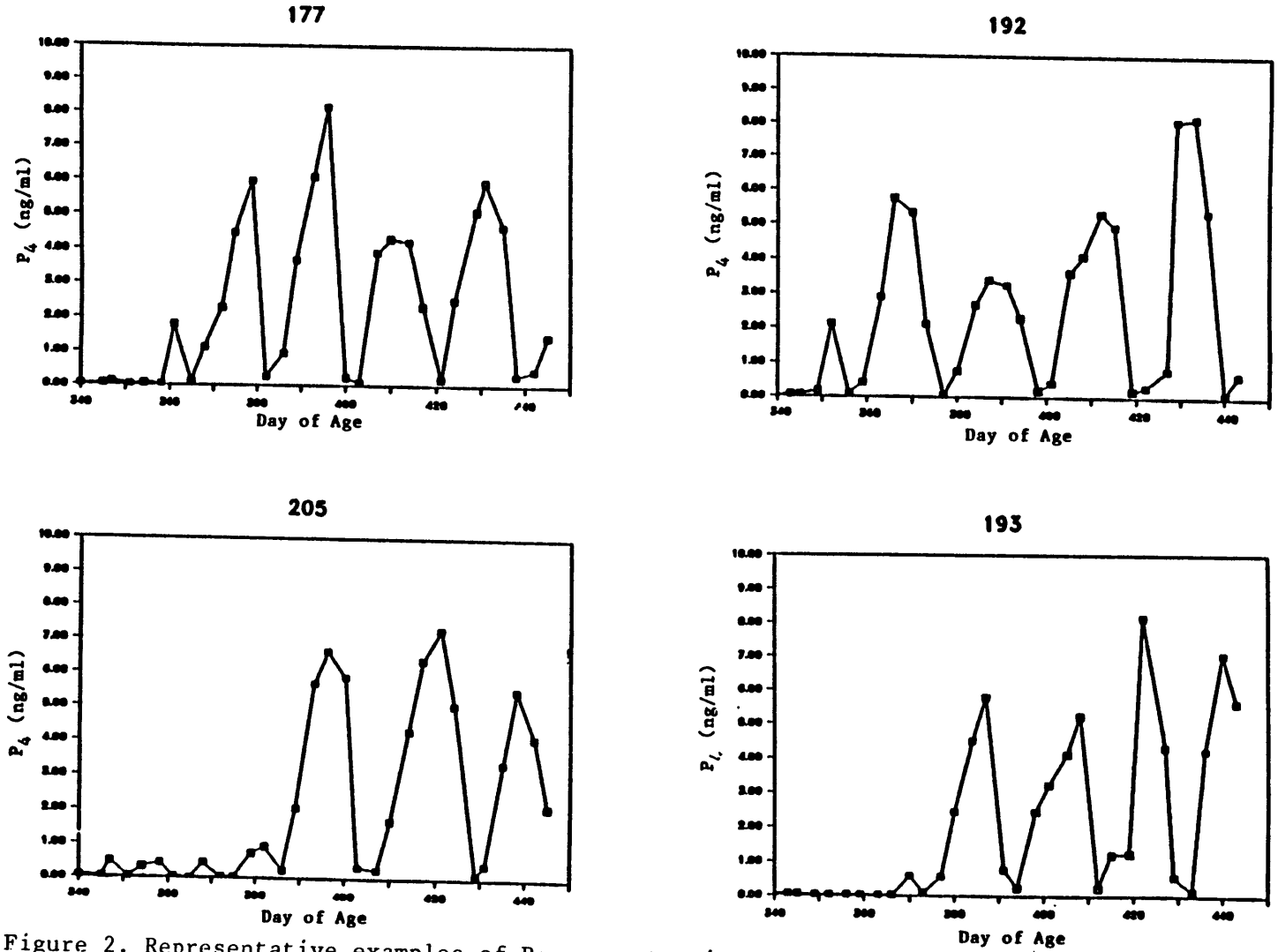

Figure 2. Representative examples of P_4 concentrations for animals in treatment groups 0 (177 and 205) and 1 (192 and 193).

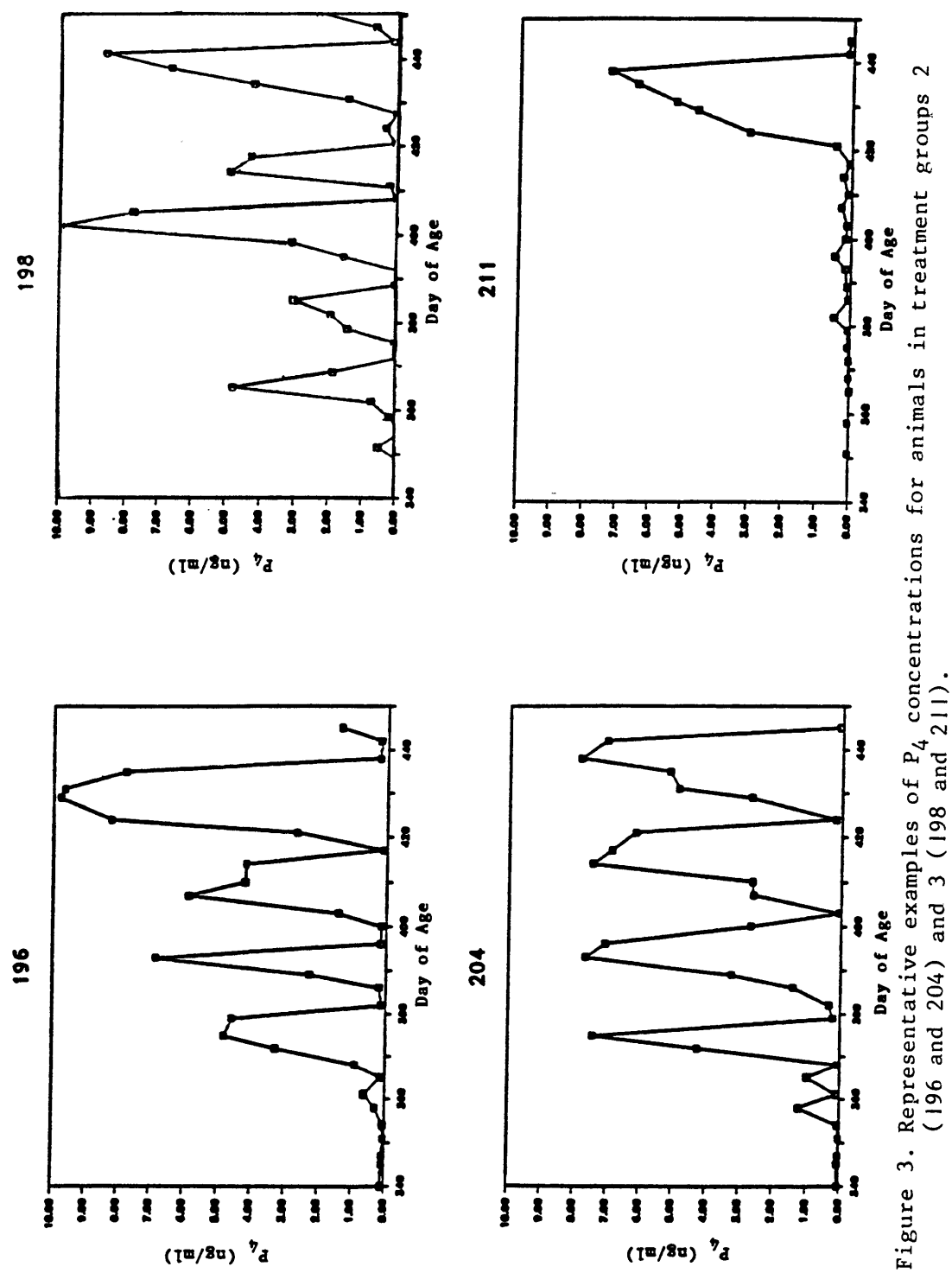

Figure 3. Representative examples of P_4 concentrations for animals in treatment groups 2 (196 and 204) and 3 (198 and 211).

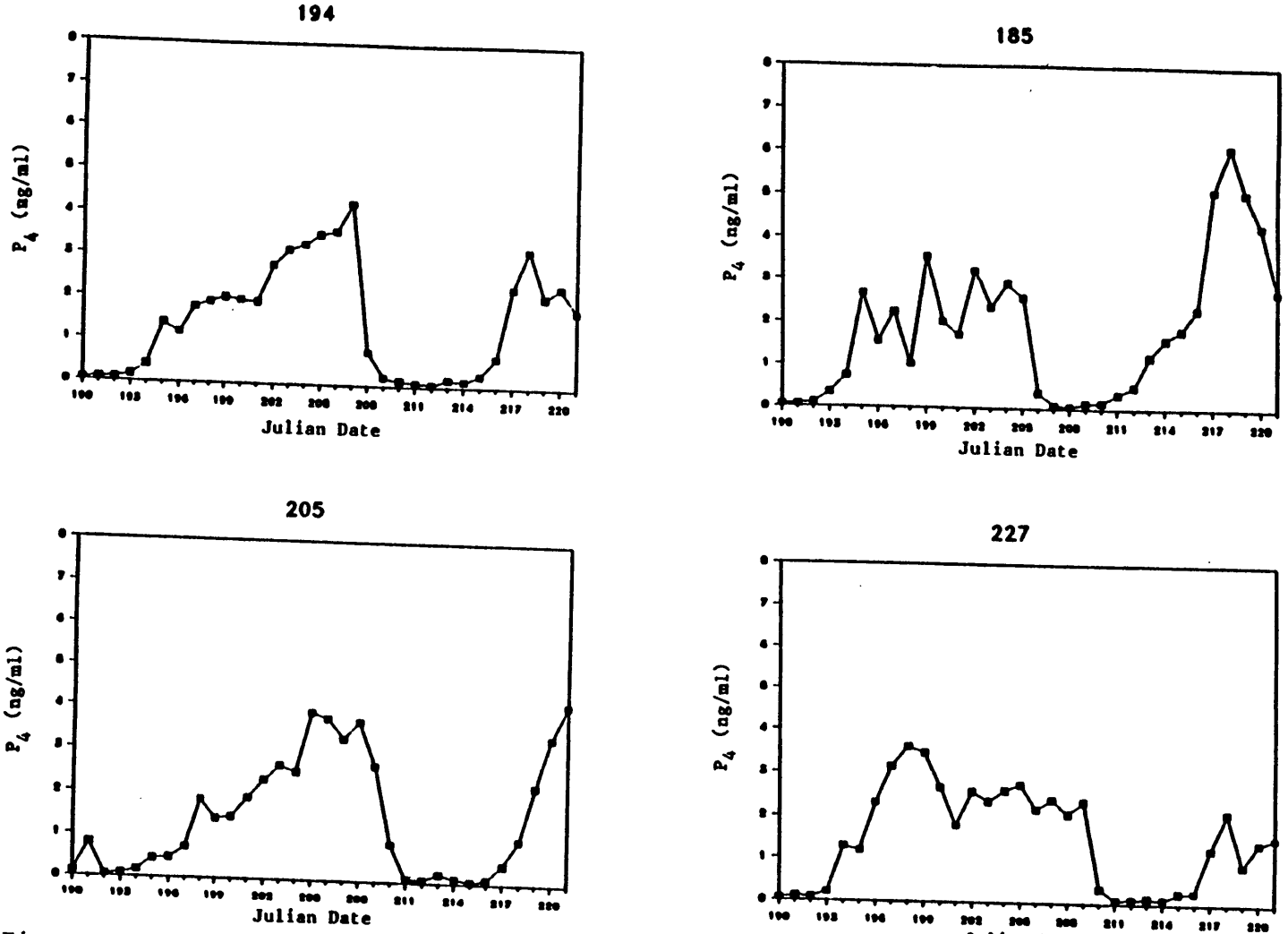

Figure 4. Representative examples of P_4 concentrations following progestin implant removal on d 189 for animals in treatment groups 0 (194 and 205) and 1 (185 and 227).

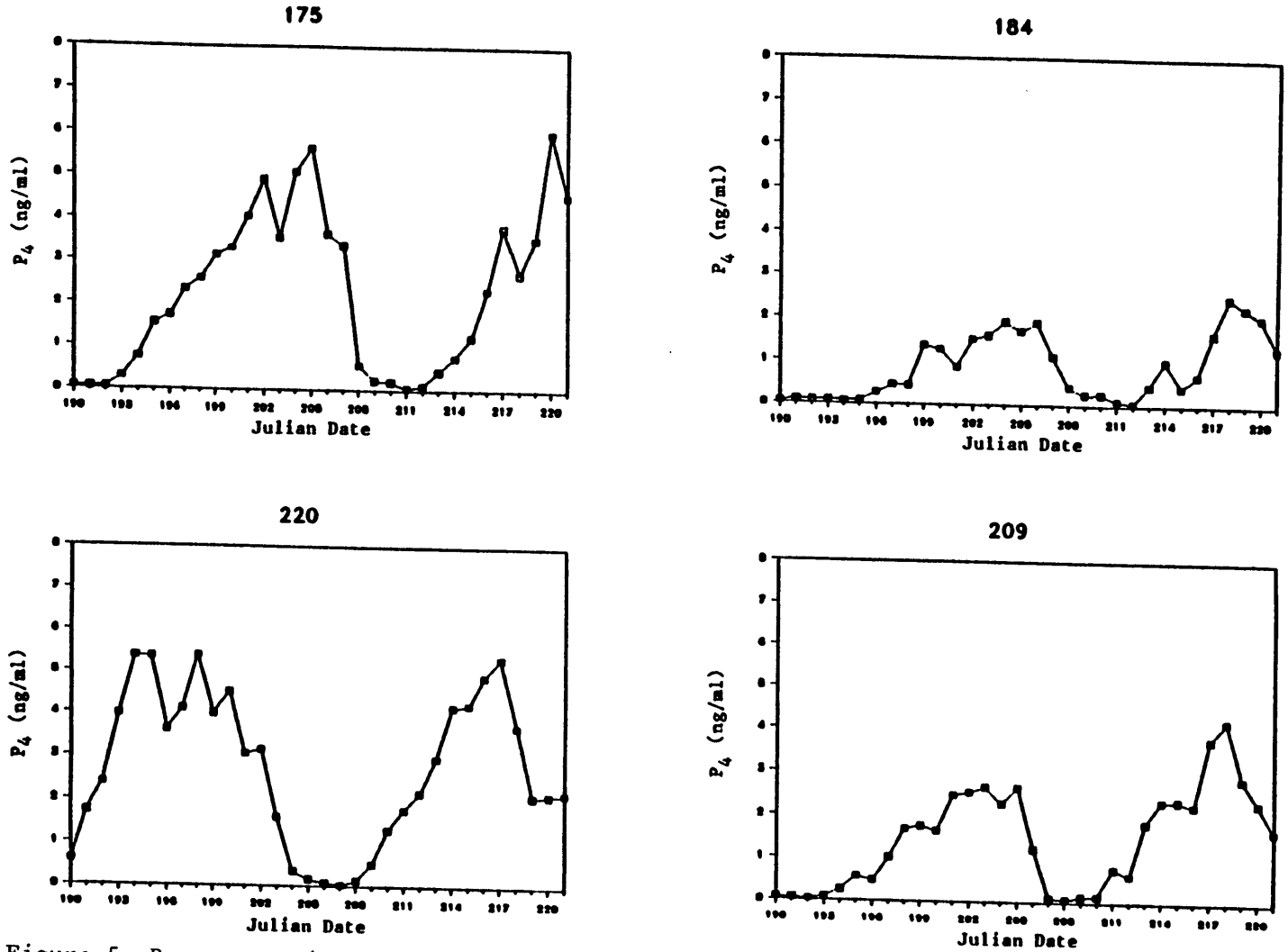

Figure 5. Representative examples of P_4 concentrations following progestin implant removal on d 189 for animals in treatment groups 2 (175 and 220) and 3 (184 and 209).

TABLE 3. LEAST SQUARES MEANS (±SEM) FOR AGE AT PUBERTY, WEIGHT AT PUBERTY AND ESTROUS CYCLE LENGTH FOR TREATED AND CONTROL FEMALES

Treatment	Age at puberty (d)	Weight at puberty (kg)	Estrous cycle length (d)
0	385.8 ± 3.9	331.3 ± 5.9	19.7 ± .6
1	383.2 ± 4.1	333.3 ± 6.3	20.7 ± .5
2	389.4 ± 4.4	323.3 ± 6.4	19.6 ± .6
3	384.7 ± 5.8	332.6 ± 8.6	19.6 ± .9

earlier onset of puberty may have been a result of the enhanced growth of the treated females, since the occurrence of puberty is influenced by body weight (Wiltbank, 1959). Conversely, in the present study, females that were prenatally exposed to testosterone did not show a delay in the onset of puberty as was reported for androgenized female guinea pigs (Brown-Grant and Sherwood, 1971).

Estrous Cycle Length

Treatment did not significantly affect the occurrence or length of estrous cycles in any group of females in this experiment (table 3; figures 2 through 5). All heifers from all treatment groups exhibited regular estrous cycles. The failure of prenatal androgen treatment to alter estrous cyclicity in heifers was previously reported by Zimbelman and Lauderdale (1973) and Putney (1984). The period of androgen administration was d 82 to birth in the first study and d 40 to 60 in the second study. Hamernik et al. (1987) reported that the secretory response of LH to the administration of exogenous estrogen did not differ in heifers that had been exposed to testosterone on d 40 to 60 of gestation compared to control heifers. The LH surge observed in these heifers indicates that treatment did not cause the loss of the abil-

ity to secrete gonadotropins in the cyclic pattern that is necessary for the occurrence of estrous cycles.

Prenatal androgenization on d 20 to 60 of gestation, has been shown to produce irregular cycles in sheep (Clarke, 1977; DeHaan et al., 1986). Brown-Grant and Sherwood (1971) reported the occurrence of irregular estrous cycles in prenatally-androgenized female guinea pigs. Perinatal androgen treatment of female rodents resulted in permanent sterility (Barraclough, 1961; Tartellin et al., 1975). The effect of androgens on estrous cyclicity in rodents and sheep was related to to a neural reorganization of brain centers controlling gonadotropin secretion during a critical period of development (Beatty et al., 1979; Clarke and Scaramuzzi, 1978).

Prenatal exposure of heifers to testosterone failed to cause the masculinization of neural centers controlling estrous cyclicity in this study. There are several possible explanations for the failure of treatment to alter estrous cyclicity in these heifers. The timing of the treatment period may not have been coincidental with the critical period for differentiation of the hypothalamic centers that control gonadotropin secretion. Berardinelli and Adair (1987) suggested that the critical period for control of gonadotropin secretion occurs postnatally in cattle. Second, the dosage administered may be insufficient to masculinize the brain of

the fetal bovine female. Increased placental aromatization of androgens late in gestation could affect the absolute levels that reach the fetus (Clarke et al., 1976a). If the critical period for the differentiation of gonadotropin secretion was late in gestation, the actual amount of androgen that the brain was exposed to could have been reduced to an ineffectual level by aromatization. Third, the pattern of androgen administration may not mimic the endogenous pattern of androgen secretion in males, which could be critical to the effective masculinization of the brain. Finally, a protective mechanism such as high levels of circulating progesterone, could render the female brain immune to the effects of androgen in cattle.

The most probable explanation for the failure of prenatal androgenization to affect the estrous cyclicity of the heifers in this study is that the periods of gestation selected for the administration of testosterone were not sufficient to cause the loss of the positive feedback effect of estrogen on the hypothalamic mechanisms controlling the cyclic pattern of gonadotropin secretion. The recent reports of Berardinelli and Adair (1987) indicate that exposure to androgen between the second and fourth months of postnatal life is necessary to cause the loss of the ability of the bovine hypothalamus to respond to estrogen with an LH surge. The question of whether there is a prenatal androgen re-

quirement in addition to the required postnatal presence of androgen remains unanswered at this time, but is certainly a real possibility. Prenatal testosterone exposure could cause organizational effects in the CNS including the development of the neural structures that inhibit an LH response to estrogen. These structures could develop under the influence of prenatal androgen but remain inoperative until a subsequent postnatal exposure to androgen during a specific period causes their activation. The absence of postnatal androgen during this specific period would cause these inhibitory neural circuits to remain inactive and the positive feedback effect of estrogen on LH secretion would enable the female to exhibit normal estrous cycles. It is also possible that postnatal testosterone exposure is sufficient in itself to cause the imposition of the tonic pattern of gonadotropin secretion and loss of the positive feedback effect of estrogen.

Human females that are exposed to high levels of androgens in utero also exhibit normal menstrual cycles although the onset of puberty is delayed (Forest, 1983). A mechanism for protecting the CNS of the female from the masculinizing influences of prenatal testosterone in cattle and humans could be achieved by this postnatal requirement for androgen.

Social Dominance

Social dominance values were measured at 9, 16 and 21 mo of age. The partial correlation coefficients between SDV and FEEDTIME values measured at these three ages are presented in table 4. The strong correlation between social dominance values (SDV) and the accumulated time in control of the point feed source (FEEDTIME) for trials at all three ages was expected because FEEDTIME was influential in the calculation of SDV. The variable FEEDTIME was used in this study to permit the examination of the extent of dominance of one animal over another. A large difference in the values for FEEDTIME for a contesting pair of animals was indicative of an animal that totally or almost totally dominated the other member of the pair. Similarly, a small difference between the FEEDTIME values for two contestants indicated that the two animals were not completely dominant or submissive to each other.

Hormonal effects associated with sexual maturity are of major importance in causing the onset of aggressive behavior (Guhl, 1958). Aggressive behavior plays an important role in the determination of the social rank of an animal (Schein and Fohrman, 1955; Syme, 1974). The onset of puberty causes the increased levels of aggressive behavior that will determine the social rank of an animal in the dominance structure.

TABLE 4. PARTIAL CORRELATION COEFFICIENTS BETWEEN SOCIAL DOMINANCE VALUES (SDV) AND POINT FEED SOURCE CONTROL TIME (FEEDTIME) FOR TRIALS AT 9, 16 AND 21 MONTHS OF AGE

	SDV2	SDV3	FEEDTIME 1	FEEDTIME 2	FEEDTIME 3
SDV1	.64**	.53**	.91***	.62**	.43**
SDV2		.71***	.58**	.97***	.62***
SDV3			.50**	.70***	.90***
FEEDTIME 1				.56***	.32**
FEEDTIME 2					.57***

**P < .01.
***P < .001.

Formation of the social dominance hierarchy occurs around the time of puberty in heifers (Schein and Fohrman, 1955; Bouissou, 1977).

The heifers in this study exhibited slightly, but not significantly greater correlations between SDV2 and SDV3 than were recorded between SDV1 and either SDV2 or SDV3. These results were probably a refection of the changes in the social dominance structure that occured over time and were probably not a result of the activational effects of gonadal hormones following the onset of puberty. A greater difference in the partial correlation coefficients between the SDV for trials at 9 mo of age and the SDV for trials at 16 and 21 mo of age would suggest that hormonal effects associated with puberal endocrine status had affected the dominance hierarchy. The lesser magnitude of the partial correlation coefficients between the SDV recorded at the three ages in this study would seem to support the concept that puberty did not have an effect on the dominance structure in these cattle. The partial correlation coefficients between FEEDTIME measured at the three ages in this trial also support this hypothesis.

Social dominance values did not differ among trials in this study ($P<.68$), nor was there an interaction of trial and age-deviation for SDV ($P<.48$). Treatment did not affect SDV ($P<.18$) but age-deviation was significant ($P<.05$). A trial

by treatment interaction was observed (P<.09). Orthogonal contrasts revealed a significant treatment effect on SDV for trials at 9 mo compared to 16 and 21 mo of age (P<.01).

Trials were analyzed separately to examine the interaction of treatment and trial. The LSM for treatments are presented in table 5. At 9 mo of age, both treatment and age-deviation had effects on SDV (P<.03). Females in treatment group 3 had greater SDV at 9 mo than groups 1 and 2 (P<.05) but were not different from controls (P>.05). At 16 mo of age SDV was not affected by either treatment or age-deviation (P<.59 and P<.19 respectively). At 21 mo of age, SDV was not affected by age-deviation (P<.12), however, orthogonal contrasts showed a difference between SDV for treatments 1 and 2 (P<.04).

FEEDTIME did not differ among trials at 9, 16 and 21 mo of age (P<.72). The interaction of trial by age-deviation was not significant (P<.99). Treatment and age-deviation did not influence FEEDTIME (P<.24 and P<.06 respectively). The interaction of trial by treatment was not significant (P<.22). Orthogonal contrasts showed a treatment effect for trials at 9 mo compared to trials at 16 and 21 mo of age (P<.04).

Separate analysis of trials revealed that both treatment and age-deviation had significant effects on FEEDTIME at 9 mo of age (P<.03 and P<.05 respectively). The LSM for

TABLE 5. LEAST SQUARES MEANS (±SEM) OF SOCIAL DOMINANCE VALUES (SDV) FOR TREATED AND CONTROL FEMALES AT 9, 16 AND 21 MONTHS OF AGE

Treatment	No.	SDV1	SDV2	SDV3
0	9	54.2 ± 7.2[ab]	49.3 ± 6.9	51.1 ± 5.9[ab]
1	9	39.0 ± 7.2[a]	53.0 ± 6.9	55.6 ± 5.9[a]
2	8	42.8 ± 7.6[a]	40.5 ± 7.3	39.9 ± 6.2[b]
3	4	78.3 ± 10.7[b]	60.7 ± 10.3	55.7 ± 8.8[ab]

[ab]Means in the same column with different superscripts differ ($P < .05$).

TABLE 6. LEAST SQUARES MEANS (±SEM) FOR CUMULATIVE CONTROL TIME OF THE POINT FEED SOURCE (FEEDTIME) AT 9, 16 AND 21 MONTHS OF AGE

Treatment	No.	FEEDTIME 1 (s)	FEEDTIME 2 (s)	FEEDTIME 3 (s)
0	9	92.4 ± 11.5[ab]	85.2 1 13.6	92.3 ± 12.1[ab]
1	9	71.9 ± 11.5[a]	94.2 ± 13.7	99.3 ± 12.1[a]
2	8	77.9 ± 12.2[a]	72.0 ± 14.4	70.0 ± 12.8[b]
3	4	135.6 ± 17.3[b]	104.3 ± 20.4	96.6 ± 18.2[ab]

[ab]Means in the same column with different superscripts differ ($P < .05$).

FEEDTIME are presented in table 6. Heifers in treatment group 3 had significantly greater FEEDTIME values than heifers in groups 1 and 2 ($P<.03$) but were not different from control heifers ($P>.05$). FEEDTIME values at 16 mo of age were not affected by age-deviation or treatment ($P<.16$ and $P<.62$ respectively). At 21 mo of age, FEEDTIME was not affected by age-deviation ($P<.08$), but again, orthogonal contrasts detected a difference between treatments 1 and 2 for FEEDTIME at 21 mo of age ($P<.04$) despite an F test indicating that there were no effects due to treatment ($P<.18$).

The stability of the social dominance structure for the heifers in this study was compared among the three trials. For the trial at 9 mo of age the range of values for SDV and FEEDTIME was greater than that recorded for trials at 16 and 21 mo of age. A large range of SDV in a group of animals is indicative of a greater expression of dominance and increased linearity of the dominance order (Beilharz and Cox, 1967). Both linear dominance order and definitive expression of dominance are related to a high degree of stability in a dominance structure (Schein and Fohrman, 1955). Another measure of stability in a dominance hierarchy is the frequency of reverse decisions (upsets) in pairwise competitions for a limited resource (Ewbank and Bryant, 1972). Upset decisions occurred when an animal with a lower SDV defeated an animal with a higher SDV. The frequency of upsets was

13.1±2.1%, 22.9±2.1% and 32.2±2.1% for trials at 9, 16 and 21 mo of age respectively. Trials had a significant effect on the frequency of upsets (P<.0001). Treatment did not affect the number of upsets and the trial by treatment interaction was also not significant for upsets (P<.58 and P<.52 respectively). Orthogonal contrasts showed that the number of upsets at 9 mo of age was significantly less than the number of upsets at 16 and 21 mo of age (P<.0001). The number of upsets at 16 mo was less than at 21 mo of age (P<.009).

In cattle, the formation of a dominance hierarchy occurs around the time of puberty. In the present study, a dominance order was formed prior to the onset of puberty and the stability of the dominance structure was decreased at 16 mo of age and decreased further at 21 mo of age. The decrease in the range of SDV and the increase in upsets from 9 to 21 mo of age reflects a decay in the stability of the social dominance hierarchy. The formation of a dominance order prior to the onset of puberty and its decay following puberty are opposite to previous reports (Bouissou, 1977; Blockey, 1979; Beilharz and Zeeb, 1982). Results from their studies indicated that prior to puberty, cattle did not form dominance orders and that once formed, dominance orders tended to remain relatively stable unless some unusual event such as sickness or the introduction of strange animals, caused an upset in the existing order.

At 9 mo of age, heifers in treatment group 3 were more dominant than heifers in groups 1 and 2. There are three possible explanations for this effect. First, exposure of female bovine fetuses to androgens during d 100 to 160 of gestation could have resulted in increased aggressive behavior in these females. Exposure to testosterone in utero has been shown to increase aggression in female rats and sheep (Edwards and Herndon, 1970; Quadagno and Rockwell, 1972; Clarke et al., 1976a; Clarke, 1977). Aggressive behavior is an important factor in the determination of the social rank of an animal (Schein and Fohrman, 1955; Meese and Ewbank, 1973; Syme, 1974). An increase in aggressive behavior could have caused the greater SDV and FEEDTIME for the heifers in group 3 at 9 mo of age. The attenuation of this difference at 16 and 21 mo could have been a result of the onset of gonadal hormone secretion at puberty.

A second explanation for the dominance of the heifers in group 3 at 9 mo of age is that the exposure of the female fetuses to testosterone endowed the females with a quality other than aggressiveness that caused them to have greater SDV than other females. Bouissou and Gaudioso (1982) reported that treatment of 3 to 6 mo old heifers with testosterone resulted in an elevation in social rank compared to controls. These authors reported that there was no increase in aggressive behavior observed in the treated heifers

in the study. Reduced levels of fear towards other animals was hypothesized to be the cause of the observed increase in social rank. A similar effect on the heifers in this study is possible despite the differences in the timing of the testosterone administration. The attenuation of the difference in dominance values between heifers in group 3 and heifers in groups 1 and 2 from 9 to 21 mo of age could be related to the decay of the dominance structure. This decay could have been caused by the onset of puberal endocrine events. Alternatively, the decay could have been initiated independently of the influence of gonadal hormones and been enhanced by the gonadal hormones at puberty or the decay could have been totally independent of the effects of puberty.

The significant difference between treatments 1 and 2 at 21 mo of age for SDV and FEEDTIME was considered to be a non-biological effect and not directly related to treatment. The decrease in SDV of the heifers in group 3 between the first and third trials could have caused the increase in dominance values observed in the animals from treatment 1 at 21 mo of age. Examination of the data shows that as the dominance of treatment 3 females decreased from 9 to 16, and 16 to 21 mo of age, the dominance of treatment 1 females increased during these same periods. The changes in dominance value of any group of females will affect the overall

dominance hierarchy because the sum of all dominance-submission relationships in a group of animals comprises the social dominance structure.

The onset of gonadal hormone secretion has been shown to affect social behavior patterns (Allee et al., 1939). The activational effects of gonadal hormones cause increased aggression at puberty (Guhl, 1958). The increase in aggressive behavior is the mechanism by which animals determine their relative social rank, thus creating the dominance hierarchy. Once established, the dominance order reduces the overall levels of aggression in the group. In a stable dominance hierarchy, animals recognize the rank of other animals and the resulting interactions are based on learned responses to either dominant or submissive animals. These responses are generally avoidance or submission by the lower ranking member of an interacting pair, thus precluding the need for aggressive behavior to determine the relative social rank of the pair of animals.

In the present study, the dominance hierarchy was apparently formed without the influence of gonadal hormones. There may have been abnormally higher levels of aggressive behavior prior to puberty which enabled the dominance structure to form. Alternatively, the required level of aggressive interaction necessary for the formation of the dominance structure may have been lower than normal for the heifers in

this study. An increase in aggressive behavior by the heifers in one treatment group could have sparked the precocious development of a social hierarchy, especially considering the small number of interactions that are required for the attainment of social experience in heifers.

Social dominance has been reported to be affected by weight and age in cattle (Schein and Fohrman, 1955; Bouissou, 1972; Blockey, 1979). In the present study, age and weight were moderately correlated with both SDV and FEEDTIME at 9 mo of age. Age had a significant effect on SDV and FEEDTIME at 9 but not at 16 or 21 mo of age. Weight had a significant effect on FEEDTIME at 9 mo of age. Partial correlation coefficients between SDV or FEEDTIME and age or weight are presented in table 7. Age, but not weight was moderately correlated with SDV and FEEDTIME at 16 and 21 mo of age. Weight was slightly correlated with SDV and FEEDTIME at 16 mo and negatively correlated with SDV and FEEDTIME at 21 mo of age. The decrease in correlations of both age and weight with SDV and FEEDTIME along with the loss of the significant effect of age on social dominance after the trials at 9 mo parallels and may be indicative of the decay in the social structure of the animals in this study.

A second possibility for the decrease in the correlation between age or weight and SDV or FEEDTIME is the low variability in age and weight of the heifers in this study. The

TABLE 7. PARTIAL CORRELATION COEFFICIENTS BETWEEN SOCIAL DOMINANCE VALUES (SDV) OR POINT FEED SOURCE CONTROL TIME (FEEDTIME) AND AGE OR WEIGHT FOR TRIALS AT 9, 16 AND 21 MONTHS OF AGE

	SDV-age	SDV-weight	FEEDTIME-age	FEEDTIME-weight
Trial 1	.45*	.38*	.41*	.43*
Trial 2	.26	.09	.28	.14
Trial 3	.29	-.11	.33	-.07

*$P < .05$.

range in age was 32 d while the range in weight was 77.3, 70.5 and 90.9 kg at 9, 16 and 21 mo of age respectively. The relatively small range in age and weight may have caused other unknown factors to assume more prominent roles in the determination of SDV for these animals. As the heifers increased in age, these other factors may have increased in importance for the determination of SDV, thereby decreasing the effects of age and weight on SDV.

The data reported in this study suggest that some factor other than weight or age was of primary importance in the determination of social rank for the animals in this study. Weight and age seem to have played less of a role in the determination of social rank after 9 mo of age and this attenuation of the influence of weight and age on SDV could have been instrumental in the decay of the dominance order observed in this study.

In summary, testosterone administered in utero to fetal bovine females on d 100 to 160 of gestation was associated with increased dominance values at 9 mo of age. This effect could be due to increased levels of aggressive behavior in the heifers or to a reduction in their fear of other animals. The effects of treatment on social dominance variables at 9 mo were not apparent at either 16 or 21 mo of age, possibly due to the endocrine events associated with the onset of puberty in the heifers. The activational effects of gonadal

steroid production after puberty could have masked any organizational effects of treatment on the CNS that were apparent at 9 mo of age.

Sexual Behavior

Sexual behavior variables were measured at 9, 16 and 21 mo of age and analyzed to determine which variables were the most reliable measures of the level of sexual behavior in the heifers. The sexual behavior variables were compared among treatments and trials. Several variables (HFM, SFM and NNFM; see table 1) were calculated for the period of time subsequent to the first mount by a heifer. This measurement enabled the examination of the influence of the first mount, to determine whether it had an effect of sexual satiety on the ensuing sexual behavior of the heifers.

The LSM for the sexual behavior variables that are reported below are the sums of the variables recorded for the 3 d on which each heifer was tested for the trials at each age. The least squares means (LSM) for the number of mounts (M) exhibited by the heifers were 16.8±1.3, 19.6±1.3 and 13.9±1.3 for trials 1, 2 and 3 respectively, and differed among trials ($P<.0002$). The number of M for trial 1 was not different from the number of M for trials 2 and 3 ($P<.98$),

but heifers exhibited a greater number of M in trial 2 than in trial 3 (P<.0001).

The interval to first mount (IM1) differed among trials (P<.04). The LSM for IM1 by the heifers were 107.1±20.7, 30.3±13.4 and 48.9±13.4 s for trials 1, 2 and 3 respectively. Heifers exhibited a greater IM1 for trial 1 than for trials 2 and 3 (P<.05), while the IM1 for trial 2 was not different than IM1 for trial 3 (P<.13).

The LSM for the interval between the first and second mounts (IM2) were 179.4±48.8, 204.0±31.5 and 338.0±33.2 s for heifers during trials 1, 2 and 3 respectively, and were not different among trials (P<.38). The IM2 for the heifers during trial 1 was not different than the IM2 during trials 2 and 3 (P<.31) and the IM2 for the females during trial 2 was not different than the IM2 recorded during trial 3 (P<.16).

Interest time (IT) for the heifers differed among trials (P<.0001). The LSM for IT exhibited by the females were 1229.0±55.6, 1662.5±55.6 and 1531.4±55.6 s during trials 1, 2 and 3 respectively. Heifers exhibited less IT during trial 1 than during trials 2 and 3 (P<.0001) and greater IT was recorded for heifers during trial 2 than during trial 3 (P<.02). There was a treatment by trial interaction noted for IT (P<.05). A significantly lower IT value for the heifers in group 3 compared to the females in groups 1 and 2

at 9 mo of age but not at 16 or 21 mo of age was responsible for the interaction of treatment with trial reported above.

The number of head placements (H) achieved by the heifers was different among trials (P<.04). The LSM for H by the heifers were 59.9±4.5, 66.8±4.5 and 57.0±4.5 during trials 1, 2 and 3 respectively. Heifers exhibited a greater number of H during trial 2 than during trial 3 (P<.02), while the number of H by heifers during trial 1 was not different from the number of H during trials 2 and 3 (P<.74).

The LSM for the number of head placements following the first mount (HFM) exhibited by the females were 54.9±5.3, 61.2±4.4 and 52.9±4.4 during trials 1, 2 and 3 respectively, and differed among trials (P<.02). The number of HFM by the heifers was greater during trial 2 than during trial 3 (P<.005), while the number of HFM for trial 1 did not differ from HFM during trials 2 and 3 (P<.28).

The LSM for the number of thrusts (T) achieved by the females were 2.1±.6, 5.8±.6 and 11.3±.6 during trials 1, 2 and 3 respectively, and differed among trials (P<.0001). Heifers performed fewer T during trial 1 than during trials 2 and 3 (P<.0001) and the number of T recorded during trial 3 was greater than the number of T for trial 2 (P<.0001).

The number of sniffs (S) measured for the animals differed among trials (P<.0001). The LSM for S by the females were 38.4±2.6, 33.9±2.6 and 58.7±2.6 for trials 1, 2 and 3

respectively. The number of S measured for females during trial 1 differed from S for trials 2 and 3 ($P<.03$) and heifers performed a greater number of S for trial 3 than for trial 2 ($P<.0001$).

The LSM for the number of sniffs following the first mount (SFM) by the animals were 34.8±3.0, 30.6±2.5 and 53.9±2.5 for trials 1, 2 and 3 respectively, and differed among trials ($P<.0001$). The number of SFM recorded for the females during trial 1 was not different than the number of SFM measured during trials 2 and 3 ($P<.15$), while the number of SFM achieved by the heifers during trial 3 was greater than the number of SFM during trial 2 ($P<.0001$).

The LSM for the number of nose-to-nose contacts (NN) by the females were .9±.3, 1.6±.3 and 1.5±.3 for trials 1, 2 and 3 respectively, and were not different among trials ($P<.15$). The number of NN recorded for the heifers during trial 1 was less than the number of NN measured during trials 2 and 3 ($P<.05$), while the number of NN by the heifers during trial 2 was not different than the number of NN during trial 3 ($P<.82$).

The LSM for the number of nose-to-nose contacts following the first mount (NNFM) achieved by the animals were .4±.3, 1.3±.3 and 1.5±.3 for trials 1, 2 and 3 respectively, and differed among trials ($P<.007$). The number of NNFM exhibited by heifers in trial 1 was less than NNFM for trials

2 and 3 ($P<.002$), while the number of NNFM was not different between trials 2 and 3 ($P<.45$).

Sexual experience plays an important role in the expression of sexual behavior in cattle (Hurnik, 1987). At 9 mo of age, the heifers in this study were all prepuberal and were considered as being sexually inexperienced. The lower values at 9 mo of age for T, NN, NNFM and IT, as well as the increased IM1 compared to later trials was likely a reflection of this sexual inexperience. The difference in size between the heifers and the teaser females could also have had an effect on the levels of sexual behavior exhibited by the heifers in the three trials. Teaser females were mature cows for trials 1 and 2 when the heifers were relatively young and small in size. Teaser females were heifers that were comparable in age and size to the test heifers for the third trial. The size discrepancy between the test heifers and teaser females during the first trial, was attenuated in the second trial and no longer present in the third trial.

Hurnik (1987) reported that prepuberal heifers exhibit very little sexual behavior. The activational effects of gonadal hormones are responsible for the manifestation of adult levels of sexual behavior in cattle. The females in this study exhibited levels of M, IM2, H, HFM, S and SFM, that were independent of puberal status. In contrast to the findings of Hurnik (1987), the heifers in this study exhib-

ited significant levels of sexual behavior prior to the onset of puberty.

Separate analysis of trials using orthogonal contrasts revealed no differences among treatments for any sexual behavior variables measured at 16 or 21 mo of age. The LSM for sexual behavior variables are presented by treatment and trial in table 8. For the trial at 9 mo of age, females from Group 3 had lower values than females in Groups 1 and 2 for the following variables: IT ($P<.02$), H ($P<.01$) and M ($P<.04$). The significantly lower IT reported for Group 3 heifers at 9, but not 16 or 21 mo of age was responsible for the treatment by trial interaction reported above for IT.

The number of mounts achieved by a heifer was selected as the sexual behavior trait that was most representative of the level of sexual behavior of that heifer. Mounting is the behavioral trait that most researchers have used to measure sexual behavior (Hurnik and King, 1987). Mounts are also considered as being strictly a sexual behavior as opposed to a social behavior (Hurnik, 1987). Sexual behavior traits that correlated strongly with mounts in this study were: head placement (.60), thrusts (.67), interest time (.57), head placement following the first mount (.62) and interval between the first and second mounts (-.73), (table 9). The strong correlations between these variables indicates that these actions are primarily associated with the expression

TABLE 8. LEAST SQUARES MEANS (±SEM) OF SEXUAL BEHAVIOR VARIABLES[1] FOR TRIALS AT 9, 16 AND 21 MONTHS OF AGE

Sexual behavior variable	Trial	Treatment 0	1	2	3	Contrast[2]
M	1	18.00 ± 3.03[ab]	19.67 ± 3.03[b]	20.50 ± 3.21[b]	9.00 ± 4.54[a]	B
M	2	18.89 ± 2.03	18.33 ± 2.03	22.00 ± 2.16	19.00 ± 3.05	
M	3	12.33 ± 1.66	14.89 ± 1.66	14.00 ± 1.76	14.50 ± 2.48	
IM1	1	91.00 ± 40.79	125.00 ± 40.79	189.43 ± 40.79	23.00 ± 107.91	A
IM1	2	15.86 ± 14.15	45.29 ± 14.15	20.00 ± 14.15	25.00 ± 37.45	
IM1	3	25.71 ± 25.04	72.29 ± 25.04	44.00 ± 25.04	35.00 ± 66.24	
IM2	1	154.20 ± 67.49	295.00 ± 61.61	198.83 ± 61.61	89.00 ± 150.91	
IM2	2	221.60 ± 51.88	229.00 ± 47.36	222.00 ± 47.36	135.00 ± 116.00	
IM2	3	314.80 ± 101.05	403.00 ± 92.24	326.00 ± 92.24	144.00 ± 225.95	
S	1	45.22 ± 4.57	36.67 ± 4.57	41.50 ± 4.85	30.25 ± 6.86	A,B
S	2	37.33 ± 3.40	35.89 ± 3.40	31.25 ± 3.61	31.25 ± 5.11	
S	3	56.33 ± 5.21	63.89 ± 5.21	55.25 ± 5.52	59.50 ± 7.81	
SFM	1	43.38 ± 5.17	31.33 ± 4.88	35.00 ± 5.17	29.67 ± 10.35	B
SFM	2	34.13 ± 2.80	32.33 ± 2.64	28.38 ± 2.80	31.00 ± 5.59	
SFM	3	52.75 ± 5.12	58.33 ± 4.82	51.00 ± 5.12	42.50 ± 10.23	
H	1	64.67 ± 8.31[ab]	64.22 ± 8.31[b]	77.63 ± 8.82[b]	33.25 ± 12.47[a]	B
H	2	62.33 ± 6.14	69.56 ± 6.14	65.00 ± 6.51	70.25 ± 9.21	
H	3	53.67 ± 8.39	56.22 ± 8.39	55.00 ± 8.89	63.25 ± 12.58	
HFM	1	59.26 ± 8.74	59.67 ± 8.24	73.00 ± 8.74	24.50 ± 17.48	B
HFM	2	58.25 ± 6.67	64.00 ± 6.29	57.63 ± 6.67	56.50 ± 13.34	
HFM	3	52.88 ± 8.18	51.33 ± 7.71	51.38 ± 8.18	32.00 ± 16.37	
NN	1	1.00 ± .32	1.00 ± .32	.25 ± .34	1.25 ± .48	A
NN	2	1.00 ± .61	1.67 ± .61	1.50 ± .65	2.25 ± .91	
NN	3	1.89 ± .50	1.00 ± .50	1.63 ± .53	1.50 ± .75	
NNFM	1	.75 ± .27	.67 ± .26	0.00 ± 0.00	0.00 ± 0.00	A
NNFM	2	1.00 ± .56	1.33 ± .53	1.38 ± .56	1.50 ± 1.12	
NNFM	3	1.89 ± .54	1.00 ± .50	1.63 ± .53	1.50 ± 1.07	
T	1	2.44 ± .63	2.22 ± .63	2.63 ± .67	1.00 ± .95	A,B
T	2	6.22 ± .94	6.00 ± .94	5.13 ± 1.00	6.00 ± 1.42	
T	3	10.00 ± 1.43	11.89 ± 1.43	11.00 ± 1.51	12.25 ± 2.14	
IT	1	1407.00 ± 133.20[ab]	1294.11 ± 133.20[b]	1412.00 ± 141.28[b]	803.00 ± 199.80[a]	A,B
IT	2	1645.56 ± 57.86	1619.33 ± 57.86	1649.75 ± 61.37	1735.25 ± 86.79	
IT	3	1550.67 ± 80.63	1622.11 ± 80.63	1491.13 ± 85.52	1461.50 ± 120.94	

[1] Values for sexual behavior variables were summed across days within a trial.
[2] Contrasts which are different ($P < .05$) are indicated by
A: trial 1 vs trials 2 and 3
B: trial 2 vs trial 3.
[ab] Means in the same row with different superscripts differ ($P < .05$).

See table 1, page 58 for explanation of abbreviations.

TABLE 9. PARTIAL CORRELATION COEFFICIENTS FOR SEXUAL BEHAVIOR VARIABLES OVER ALL THREE TRIALS

	IM1	IM2	S	SFM	H	HFM	NN	NNFM	T	IT
M	-.30	-.73**	-.25	-.22	.60*	.62*	.12	.09	.67**	.57*
IM1		.43	-.22	-.36	-.30	-.37	-.05	-.25	-.28	-.40
IM2			.18	.09	-.26	-.35	-.15	-.12	-.15	-.31
S				.97***	-.35	-.40	.55*	.58*	-.33	.04
SFM					-.38	-.40	.48	.56*	-.28	.07
H						.99***	.03	-.04	.58*	.77**
HFM							.01	-.04	.57*	.78**
NN								.93***	-.22	.40
NNFM									-.13	.37
T										.53*

*P< .05.
**P< .01.
***P< .001.

of the intensity of sexual behavior and probably do not serve any other behavioral function.

The other behavioral traits measured in this study were not highly correlated with mounts and are probably not reliable measures of the intensity of sexual behavior in heifers. Traits such as sniffs and nose-to-nose contact very likely serve at least partially, as social behaviors rather than sexual behaviors in these heifers. The interval to first mount was only moderately correlated with the number of mounts (-.30) which was contrary to previous reports in other species (DeBold and Clemens, 1978). A highly negative correlation was reported by these authors between the interval to first mount and the number of mounts achieved. This correlation implies that an animal achieving a high number of mounts would have a shorter interval to first mount. Thus, both the interval to first mount and the number of mounts would be accurate indications of the level of sexual behavior of an animal. In the present study, the moderately negative correlation noted between IM1 and M suggests that IM1 is a less accurate measure of the level of sexual behavior of the heifers than was the case in previous studies of other species. The IM1 may be significantly influenced by factors other than the level of sexual behavior of a heifer.

The significantly lower values for mounts, interest time and head placements among heifers in group 3 at 9 mo of age

warrants additional examination. The primary motivating force behind these behaviors is the expression of sexual interest. The significantly lower values recorded for these behaviors by the heifers in group 3 at 9 mo of age may be a reflection of a reduced level of sexual interest. This reduction in female sexual behavior at 9 mo of age by the heifers in group 3 was not seen at 16 or 21 mo of age. The behavioral modification of the heifers in group 3 suggests that exposure of heifers to androgen from 100 to 160 d of gestation may be related to organizational changes in the neural centers that regulate sexual behavior.

Prenatal androgen administration has been reported to suppress the expression of feminine behavior in ewes (Clarke et al., 1976a; Clarke, 1977). Prenatally-androgenized ewes exhibit less feminine sexual behavior in response to estrogen priming and higher levels of aggression and masculine sexual behavior than control ewes (Clarke, 1977). Administration of testosterone to neonatal female rats has been shown to cause defeminization of sexual behavior after puberty (Tartellin et al., 1975). Similarly, prenatal androgen exposure suppresses feminine sexual behavior in adult female guinea pigs (Brown-Grant and Sherwood, 1971).

The reason for the difference in values for sexual behavior variables observed during trials 2 and 3 is not easily explainable because the heifers were puberal at the time of

both trials. One explanation for these differences could be the low ambient temperatures during the third trials. Cold temperature has been reported to inhibit sexual behavior in cattle (Hurnik, 1987).

The expression of sexual behavior in mature male and female mammals is dependent on the presence of estrogen (Katz, 1987). The activational effects of estrogen on existing neural circuits were present in all of the heifers in this study at 16 and 21 mo of age since the heifers had reached puberty by this age. These activational effects following the occurrence of puberty could have resulted in the loss of the differences between the heifers in group 3 and the heifers in groups 1 and 2 that were apparent at 9 mo of age. Activational effects of gonadal hormones on existing neural circuits that are involved with the control of sexual behavior could have masked the organizational effects of treatment on the brain.

Interval between mounts is a commonly used indicator of sexual behavior (DeBold and Clemens, 1978). The relationship between the number of mounts and the interval between successive mounts is shown in figure 6. Analysis of the regression of mounts on the interval between mounts for trials showed linear, quadratic and cubic effects ($P<.0001$). The interval to first mount (IM1) was greater for the trials at 9 mo than for trials at 16 and 21 mo of age (107.1±20.6,

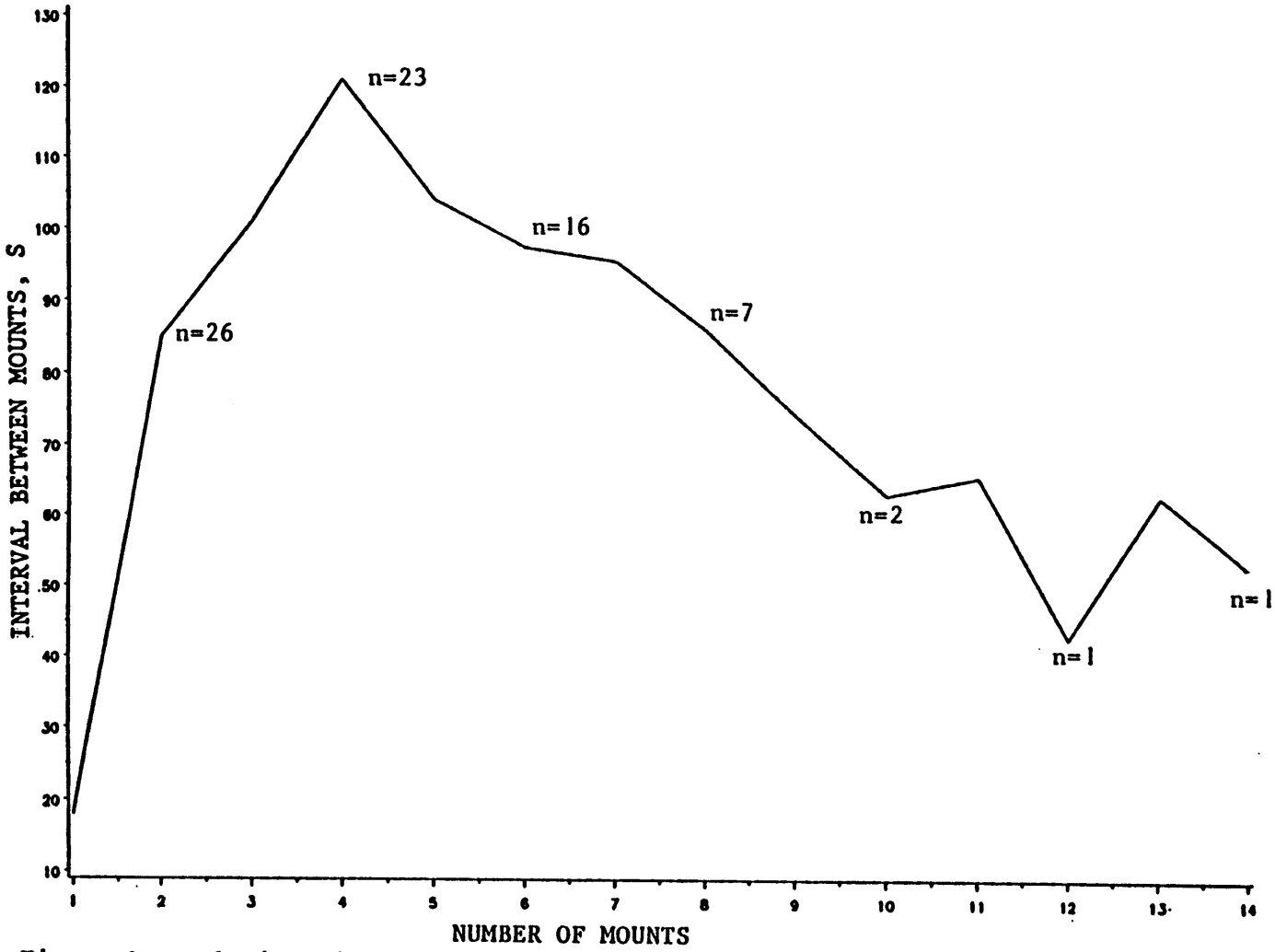

Figure 6. Relationship of the means for the interval between mounts and the number of mounts (n=the number of heifers completing that number of mounts).

30.3±13.4 and 48.9±13.4 s for trials 1, 2 and 3 respectively; P<.05). The IM1 was not different between trials at 16 and 21 mo of age (P<.13). The differences among the IM1 for the 3 trials can be attributed both to the activational effects of gonadal hormones on sexual behavior following the onset of puberty and to the difference in size between the heifers and the teaser females for the three trials. The lack of sexual experience of the heifers may also have had an inhibitory effect on the occurrence of the first mount. All heifers were prepuberal at the time of the first trial and the teaser females were mature, full-sized cows. For trials 2 and 3, heifers were post-puberal and the teaser females were smaller in size and of lesser weight than those employed in trial 1. The difference in size between the heifers and teaser females in trial 1 may have caused both difficulty in mounting and a hesitancy to mount by the heifers. Treatment did not affect IM1 at any age.

The interval between first and second mount (IM2) was not different among trials 1, 2 and 3. This finding indicates that if the difference in size between the heifers and the teaser females was a factor, once a heifer successfully mounted, the size difference had no effect on the subsequent sexual behavior of the heifer. The fact that prepuberal values for IM2 were not different than post-puberal values indicates that the activational effects of gonadal hormones

did not affect the latency between the first and second mounts in this study. The successful achievement of the first mount may encourage the heifer to repeat the behavior. The IM2 was also not different between treatments at any age.

Evaluation of the IM for the first seven mounts suggested that following the first mount, no mounts occurred for a period of about 60 sec. The interval between mounts increased until the fourth mount. The IM declined steadily to the seventh mount as did the number of heifers that acheived a higher number of mounts. The number of heifers that achieved 4 mounts was 23 while only 11 animals achieved 7 mounts. The number of animals that achieved greater than seven mounts was too small to make accurate inferences about sexual behavior. The pattern of IM for mounts one to seven indicated that heifers mounted a teaser female quickly following their introduction. Subsequent to the first mount, the IM increased up to the fourth mount, indicating a satiety of sexual interest. This satiety could involve a mechanism such as an increased threshhold for neural circuits that control mounting. From the fourth to seventh mounts, the IM decreased, which was probably due to the less sexually oriented animals with greater IM failing to achieve more than four mounts and thus being excluded in the calculation of IM subsequent to the fourth mount. This concept is supported

by the observation that only 77% of the heifers achieved more than four mounts.

The sexual behavior data reported above leads to several conclusions regarding sexual behavior in heifers. The variables that were most indicative of the intensity of sexual behavior in the heifers in this study were head placement, mounts, thrusts, interest time and the interval between the first and second mount. The intervals between successive mounts increased following the first mount to a peak value between the third and fourth mounts and then decreased steadily until the seventh mount. Most females (90%) mounted at least once at 9 mos and all heifers mounted at least twice at 16 and 21 mo of age. Heifers tended to mount a teaser female quickly upon their introduction indicating sexual arousal. The increase in IM between the first and fourth mounts could indicate that the neural threshhold for mounting is increasing and requires a greater stimulus to elicit a mount.

Exposure of heifers to testosterone during d 100 to 160 of gestation caused a reduction in interest time, head placement and the number of mounts at 9 mo of age. These variables were among the best indicators of the intensity of sexual behavior in this study. These results suggest that a reduction in the levels of sexual behavior occurred in response to treatment. This difference was no longer apparent

at 16 or 21 mo of age possibly due to the activational effects of gonadal hormones on sexual behavior following puberty.

CONCLUSIONS

The in utero administration of methyl-testosterone between d 40 through 100 of gestation to female fetuses caused complete masculinization of the external genitalia. Exposure from day 70 to 130 of gestation resulted in the incomplete masculinization of the external genitalia. These results indicate that the critical period for the differentiation of the external genitalia in cattle is between d 40 and 70 of gestation.

Neural regulatory mechanisms for growth and gonadotropin secretion were unaffected by exposure to testosterone on d 40 to 100, 70 to 130 or 100 to 160 of gestation. The timing of the treatments may not have been coincidental with the critical period for these traits. The dose may have been insufficient to masculinize the female hypothalamic centers that regulate growth and gonadotropin release or there may be a protective influence in female cattle that prevents the androgenic induction of sexual differentiation in these structures. The CNS of the fetal female bovid may be inherently resistant to the masculinizing effects of androgens during development.

Neural centers controlling factors that determine social dominance and feminine sexual behavior were modified by exposure to testosterone from d 100 through 160 of gestation.

Heifers from this treatment group exhibited lower levels of sexual behavior and had higher SDV at 9 mo of age. The modification of CNS centers that control behavior could be due to a lower androgen requirement for the effective masculinization of these centers than that required by the centers that control growth and gonadotropin secretion. The data from this experiment suggest that modification of hypothalamic structures regulating certain sexually dimorphic traits by prenatal testosterone administration may be possible in female cattle.

Postnatal weight gains were not affected by prenatal androgen treatment in this experiment, which places the potential of this procedure as a profitable method of increasing economic returns for the commercial cattleman in question. Prenatal testosterone treatment also failed to masculinize the neural structures that regulate gonadotropin secretion permitting the occurrence of regular estrous cycles subsequent to the onset of puberty. Prenatal androgen exposure has been shown to eliminate estrous cyclicity and increase postnatal growth in sheep (Clarke et al., 1976a; Jenkins et al., 1987). These reports suggest that in sheep, the critical period for these traits is entirely prenatal and independent of postnatal androgen. The data from the present study indicate that prenatal androgen exposure alone is insufficient to result in the expression of increased postnatal

growth and the elimination of estrous cyclicity in female cattle. If the postnatal androgen effect reported by Berardinelli and Adair (1987) was in fact, an activational effect on preexisting neural structures, then the combination of prenatal androgen exposure with subsequent postnatal exposure may cause the expression of increased postnatal growth and inhibition of estrous cyclicity in female cattle. The failure of treatment to inhibit estrous cyclicity or to increase postnatal growth in of the heifers in this study would suggest that androgen exposure during the periods of gestation used in this study is not advantageous to the commercial cattleman.

Further research into the area of androgen treatment of female cattle could yield valuable insight into the process of sexual differentiation in cattle and as well as in other species. Combining prenatal testosterone administration during various periods of gestation with postnatal testosterone administration from 2 to 4 mo of age could enable the precise definition of the critical periods for various sexually dimorphic traits in cattle. Histological comparison of brain tissue from heifers that were exposed to androgen prenatally with brain tissue from control females and males may permit the identification and location of neural structures that were organized by exposure to androgen during specific periods of gestation.

LITERATURE CITED

Allee, W. C., N. E. Collias and C. Z. Lutherman. 1939. Modification of the social order in flocks of hens by the injection of testosterone propionate. Physiol. Zool. 12:412.

Amrhein, J. A., W. J. Meyer III, H. W. Jones Jr. and C. J. Migeon. 1976. Androgen insensitivity in man: evidence for genetic heterogeneity. Proc. Nat. Acad. Sci. 73:891.

Baker, T. G. 1972. Primordial germ cells. In: C. R. Austin and R. V. Short (Ed.) Reproduction in Mammals. 1. Germ Cells and Fertilization. Cambridge Univ. Press, New York.

Baker, M. W. G., D. J. Bailey, P. D. Feil, L. S. Jefferson, R. J. Santen and C. W. Bardin. 1977. Nuclear accumulation of androgens in perfused rat accessory sex organs and testes. Endocrinology 100:709.

Bardin, C. W. and J. F. Catterall. 1981. Testosterone: A major determinant of extragenital sexual dimorphism. Science 211:1285.

Bardin, C. W., T. R. Brown, N. C. Mills, C. Gupta and L. P. Bullock. 1978. The regulation of the β-glucuronidase gene by androgens and progestins. Biol. Reprod. 18:74.

Barr, M. L. and E. G. Bertram. 1949. A morphological distinction between neurones of the male and female, and the behavior of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature 163:676.

Barraclough, C. A. 1961. Production of anovulatory, sterile rats by single injections of testosterone propionate. Endocrinology 68:62.

Barraclough, C. A. and R. A. Gorski. 1962. Studies on mating behavior in the androgen-sterilized female rat in relation to the hypothalamic regulation of sexual behavior. J. Endocrinol. 25:175.

Bartsch-Sandhoff, M., R. Terinde, W. Wiegelmann and W. Schalz. 1976. Karyotype-phenotype-korrelation bei einem 46,Xdel(X)(p22)-befund. Hum. Genet. 31:263.

Beach, F. A. 1968. Factors involved in the control of mounting behavior by female mammals. In: M. Diamond

(Ed.) Perspectives in Reproduction and Sexual Behavior. pp. 83-131. Indiana University Press, Ind.

Beach, F. A. 1971. Hormonal factors controlling the differentiation, development and display of copulatory behavior in the Ramstergig and related species. In: E. Tobach, L. R. Aronson and E. Shaw (Ed.) Biopsychology of Development. pp. 249-296. Academic Press, New York.

Beach, F. A. and M. G. Buehler. 1977. Male rats inheriting insensitivity to androgen show reduced sexual behavior. Endocrinology 100:197.

Beach, F. A. and R. E. Kuehn. 1970. Coital behavior in dogs X. Effects of androgenic stimulation during development on feminine mating response in females and males. Horm. Behav. 1:347.

Beal, W. E. and R. S. Brower. 1982. Growth of androgenized female mice and attempted androgenization of female calves. J. Anim. Sci. 55 (Suppl. 1):214 (Abstr.).

Beal, W. E., R. A. Milvae and W. Hansel. 1980. Oestrous cycle length and plasma progesterone concentrations following administration of prostaglandin F-2α early in the bovine estrous cycle. J. Reprod. Fertil. 59:393.

Beatty, W. 1979. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organization and activational influences. Horm. Behav. 12:112.

Beatty, W. W., T. L. Powley and R. E. Keesey. 1970. Effects of neonatal testosterone injection and hormone replacement in adulthood on body weight and body fat in female rats. Physiol. Behav. 5:1093.

Beilhartz R. G. and D. F. Cox. 1967. Social dominance in Swine. Anim. Behav. 15:117.

Beilhartz R. G. and K. Zeeb. 1982. Social dominance in dairy cattle. Appl. Anim. Ethol. 8:79.

Berardinelli, J. G. and R. Adair. 1987. Postnatal sexual differentiation of luteinizing hormone (LH) secretion and sexual behavior in male bovine. Soc. Study Reprod. 36 (Suppl. 1):92.

Berg, T. T. and R. M. Butterfield. 1987. New Concepts of Cattle Growth. John Wiley and Sons, New York.

Bernstein, R., G. C. Koo and S. S. Wachtel. 1980. Abnormality of the X chromosome in human 46,XY female siblings with dysgenetic ovaries. Science 207:768.

Blizard, D. and C. Denef. 1973. Neonatal androgen effects on open-field activity and sexual behavior in the female rat: the modifying influence of ovarian secretions during development. Physiol. Behav. 11:65.

Blockey, M. A. de B. 1979. Observations on group mating of bulls at pasture. Appl. Anim. Ethol. 5:15.

Boczykowski, K. 1973. Male and female differentiation of the human gonad. Clin. Genet. 4:213.

Book, J. A., B. Eilon, J. Halbrecht, L. Komlos and F. Shabtay. 1973. Isochrome Y (46,X,i(Yq)) and female phenotype. Clin. Genet. 4:410.

Bouissou, M.-F. 1970. Technique de mise en evidence des relations hierarchiques dans un groupe de bovins domestiques. Rev. Comp. Anim. 4:66.

Bouissou, M.-F. 1972. Influence of body weight and presence of horns on social rank in domestic cattle. Anim. Behav. 20:474.

Bouissiou, M.-F. 1974. Etablissement des relations de dominance-soumission chez les bovins domestiques. I. Nature et evolution des interactions sociales. Ann. Biol. Anim. Bioch. Biophys. 14:383.

Bouissou, M.-F. 1977. Etude du developpement des relations de dominance-subordination chez les bovins, a l'aide d'epreuves de competition alimentaire. Biol. Behav. 2:213.

Bouissou, M.-F. 1978. Effect of injections of testosterone propionate on dominance relationships in a group of cows. Horm. Behav. 11:388.

Bouissou, M.-F. and V. Gaudioso. 1982. Effect of early androgen treatment on subsequent social behavior in heifers. Horm. Behav. 16:132.

Brinkman, A. O. 1977. Testosterone synthesis in vitro by the fetal testis of the guinea pig. Steroids 29:861.

Brown-Grant, K. 1973. Recent studies on sexual differentiation of the brain. In: K. S. Comline (Ed.) Foetal

and Neonatal Physiology. pp. 527-545. Cambridge University Press, New York.

Brown-Grant, K. and M. R. Sherwood. 1971. The 'early androgen syndrome' in the guinea pig. J. Endocrinol. 49:277.

Brown-Grant, K., A. Munck, F. Naftolin and M. R. C. Sherwood. 1971. The effects of the administration of testosterone and related steroids to female rats during the neonatal period. Horm. Behav. 2:173.

Buhler, E. M. 1980. A synopsis of the human Y chromosome. Hum. Genet. 55:145.

Burgoyne, P. S. and J. D. Biggers. 1976. The consequence of X-dosage deficiency in the germ line: impaired development in vitro of preimplantation embryos from XO mice. Develop. Biol. 51:109.

Byskov, A. G. 1979. Regulation of meiosis in mammals. Annal. Biol. Anim. Biochem. Biophys. 19:1251.

Catt, K. J. and M. L. Dufau. 1976. Basic concepts of the mechanism of action of peptide hormones. Biol. Reprod. 14:1.

Catt, K. J., M. L. Dufau, W. B. Neaves, P. C. Walsh and J. D. Wilson. 1975. LH-HCG receptors and testosterone content during differentiation of the testis in the rabbit embryo. Endocrinology 97:1157.

Christensen, L. W. and R. A. Gorski. 1978. Independent masculinization of neuroendocrine systems by intracerebral implants of testosterone or estradiol in the neonatal female rat. Brain Res. 146:325.

Clarke, I. J. 1977. The sexual behavior of prenatally androgenized ewes observed in the field. Reprod. Fert. 49:311.

Clarke, I. J. and R. J. Scaramuzzi. 1978. Release of luteinizing hormone in androgenized ewes after prostaglandin-induced luteolysis or luteinizing hormone releasing factor. J. Endocrinol. 77:261.

Clarke, I. J., R. J. Scaramuzzi and R. V. Short. 1976a. Sexual differentiation of the brain: endocrine and behavioral responses of androgenized ewes to oestrogen. J. Endocrinol. 71:175.

Clarke, I. J., R. J. Scaramuzzi and R. V. Short. 1976b. The effects of testosterone implants in pregnant ewes on their female offspring. J. Embryol. Exp. Morph. 36:87.

Clemens, L. G. and L. Coniglio. 1971. Influence of prenatal litter composition on mounting behavior of female rats. Amer. Zool. 11:617.

Clemens, L. G. and R. A. Gorski. 1968. Induction and facilitation of female mating behavior in rats treated neonatally with low doses of testosterone propionate. Endocrinology 84:1430.

Comings, D. E. 1966. Uridine-5-H^3 radioautography of the human sex chromatin body. J. Cell Biol. 28:437.

Corbier, P., B. Kerdelhue, R. Picon and J. Roffi. 1978. Changes in testicular weight and serum gonadotropin and testosterone levels before, during and after birth in the perinatal rat. Endocrinology 103:1985.

Craig, J. V. 1986. Measuring social behavior: social dominance. J. Anim. Sci. 62:1120.

Davis, J. R., M. W. Heine, E. S. Lightner, H. R. Giles and R. F. Graap. 1976. X-short arm deletion gonadal dysgenesis in two siblings due to unique translocation (Xp-;16p+). Clin. Genet. 10:202.

DeBold, J. F. and L. G. Clemens. 1978. Aromatization and the induction of male sexual behavior in male, female, and androgenized female hamsters. Horm. Behav. 11:401.

DeBold, J. F. and R. E. Whalen. 1975. Differential sensitivity of mounting and lordosis control systems to early androgen treatment in male and female hamsters. Horm. Behav. 6:197.

DeHaan, K. C., L. L. Berger, D. J. Kesler, F. K. McKeith and D. L. Thomas. 1986. Prenatal androgenization of sheep to influence their postnatal growth rate and estrus behavior. J. Anim. Sci. 63 (Suppl. 1):213.

DeHaan, K. C., L. L. Berger, D. J. Kesler, F. K. McKeith, D. B. Faulkner and G. E. Cmarik. 1987a. Effect of prenatal androgenization on performance of steers and heifers. J. Anim. Sci. 65 (Suppl. 1):242.

DeHaan, K. C., L. L. Berger, D. J. Kesler, F. K. McKeith, D. L. Thomas and T. G. Nash. 1987b. Effect of prenatal androgenization on lamb performance and carcass composition. J. Anim. Sci. 65 (Suppl. 1):85.

Diamond, M. and E. Dale. 1967. Distribution of radiolabelled steroid after administration to the neonatal rat. Anat. Rec. 157:234.

Disteche, C., A. Hagemeller, J. Frederic and D. Prognaux. 1972. An abnormal large human chromosome identified as an end to end fusion of two X's by combined results of the new bonding techniques and microdensitometry. Clin. Genet. 3:388.

Donahoe, P. K., Y. Ohto, J. M. Price and W. H. Hendron. 1977. Mullerian inhibiting substance activity in bovine fetal, newborn and prepubertal testis. Biol. Reprod. 16:238.

Edwards, D. A. and J. Herndon. 1970. Neonatal estrogen stimulation and aggressive behavior in female mice. Physiol. Behav. 5:993.

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

Epstein, C. J. 1969. Mammalian oocytes: X chromosome activity. Science 163:1078.

Ewbank, R. and M. J. Bryant. 1972. Aggressive behavior amongst groups of domesticated pigs kept at various stocking rates. Anim. Behav. 20:21.

Ewbank, R. and G. B. Meese. 1971. Aggressive behavior in groups of domesticated pigs on removal and return of individuals. Anim. Prod. 13:685.

Fellous, M., P. L. Pearson, A. G. J. M. van der Linden, P. Meera Khan and A. Hagemeijer. 1975. Mapping the Xg^a red blood cell antigen in human-Chinese hamster cell hybrids. The Xg^a locus is possibly located on the short arm of the X chromosome. Cell Cytogenet. 14:293.

Ferguson-Smith, M. A. 1965. Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. J. Med. Genet. 2:142.

Ferguson-Smith, M. A. 1966. X-Y chromosomal interchange in the etiology of true hermaphroditism. Lancet. 2:475.

Finkelstein, M., Y. Litvin, Y. Mizrachi and G. Neiman. 1983. Apparent double defect in C11β and C21-steroid hydroxylation in congenital adrenal hyperplasia. J. Steroid Biochem. 19:675.

Ford, C. E. 1970. Genetic activity of sex chromosomes in germinal cells (Discussion). Philos. Trans. R. Soc. London Ser. B 259:53.

Ford, C. E., E. P. Evans, M. D. Burtenshaw, H. M. Clegg, M. Tuffrey and R. D. Barnes. 1975. A functional 'sex-reversed' oocyte in the mouse. Proc. Royal Soc. London B 190:187.

Forest, M. G. 1983. Role of androgens in fetal and pubertal development. Horm. Res. 18:69.

Gartler, S. M., R. M. Liskay and N. Grant. 1973. Two functional X chromosomes in human fetal oocytes. Exp. Cell Res. 82:464.

George, F. W. and J. D. Wilson. 1980. Endocrine differentiation of the fetal rabbit ovary in culture. Nature 283:861.

George, F. W., K. J. Catt, W. B. Neaves and J. D. Wilson. 1978. Studies on the regulation of testosterone synthesis in the fetal rabbit. Endocrinology 102:665.

George, F. W., E. R. Simpson, L. Milewich and J. D. Wilson. 1979. Studies on the regulation of the onset of steroid hormone biosynthesis in fetal rabbit gonads. Endocrinology 105:1100.

Ghosh, S. N., P. N. Shah, H. M. Gharpure and U. Athreya. 1978. H-Y antigen in human intersexuality. Clin. Genet. 14:31.

Giulian, D., L. A. Pohorecky and B. S. McEwen. 1973. Effects of gonadal steroids upon brain 5-hydroxytryptamine levels in the neonatal rat. Endocrinology 93:1329.

Glucksman, A. 1978. Sex Determination and Sexual Dimorphism in Mammals. Wykeham Publications Ltd., London.

Gordon, J. W. and F. H. Ruddle. 1981. Mammalian gonadal determination and gametogenesis. Science 211:1265.

Gorski, R. A. and C. A. Barraclough. 1963. Effect of low dosages of androgen on the differentiation of hypothalamic regulatory control of ovulation in the rat. Endocrinology 73:210.

Gorski, R. A. and J. Shryne. 1972. Intracerebral antibiotics and androgenization of the female rat. Neuroendocrinol. 10:109.

Gorski, R. 1979. The neuroendocrinology of reproduction: an overview. Biol. Reprod. 20:111.

Gorski, R. A. 1971. Gonadal hormones and the perinatal development of neuroendocrine function. In: L. Martini and W. F. Ganong (Ed.) Frontiers in Neuroendocrinology. p. 237-282. Oxford University Press, New York.

Gorski, R. A., J. H. Gordon, J. E. Shryne and A. M. Southam. 1978. Evidence for a morphological sex difference within the medial preoptic area of the rat brain. Brain Res. 148:333.

Goy, R. W. 1966. Role of androgens in the establishment and regulation of behavioral sex differences in mammals. J. Anim. Sci. 25:21.

Goy, R. W., W. E. Bridson and W. C. Young. 1964. Periods of maximal susceptibility of the prenatal female guinea-pig to masculinizing actions of testosterone propionate. J. Comp. Physiol. Psychol. 57:166.

Goy, R. W. and B. S. McEwen (Ed.). 1980. Sexual Differentiation of the Brain. MIT press, Cambridge, Mass.

Guhl, A. M. 1958. The development of social organization in the domestic chick. Anim. Behav. 6:92.

Hafez, E. S. E. 1980. Reproduction in Farm Animals (4th Ed.). Lea and Febiger, Philadelphia.

Hamernik, D. L., S. Y. McFarland, D. de Avila, S. R. Becker and J. J. Reeves. 1987. Endocrine and body growth traits in heifers exposed to testosterone-propionate during early fetal development. J. Anim. Sci. 64:858.

Harris, G. W. and S. Levine. 1965. Sexual differentiation of the brain and its experimental control. J. Physiol. 181:379.

Harris, G. W. 1964. Sex hormones, brain development and brain function. Endocrinology 75:627.

Haseltine, F. P. and S. Ohno. 1981. Mechanisms of gonadal differentiation. Science 211:1272.

Hayashi, S. and R. A. Gorski. 1974. Critical exposure time for androgenization by intracranial crystals of testosterone propionate in neonatal female rats. Endocrinology 94:1161.

Hayman, D. L. and P. G. Martin. 1965. Sex chromosome mosaicism in the marsupial genera Isodon and Perameles. Genet. 52:1201.

Hecht, F., D. L. Jones, M. Delay and H. Klevit. 1970. Xq- Turner's Syndrome: reconsideration of the hypothesis that Xp- causes somatic features in Turner's Syndrome. J. Med. Genet. 7:1.

Hurnik, J. F. 1987. Sexual behavior of female domestic mammals. Vet. Clinics N. America: Food Anim. Practice 3:423.

Hurnik, J. F. and G. J. King. 1987. Estrous behavior in confined beef cows. J. Anim. Sci. 65:431.

Imperato-McGinley J., L. Guerrero, G. Teofilo and R. E. Peterson. 1974. Steroid 5 α-Reductase deficiency in man: an inherited form of male psuedohermaphroditism. Science 186:1213.

Jenkins, T. G., J. Klindt and J. J. Ford. 1987. Effect of alteration of sexual differentiation upon growth, feed efficiency and empty body composition. J. Anim. Sci. 65 (Suppl. 1):248.

Jost, A. 1972. A new look at the mechanisms controlling sex differentiation in mammals. Johns Hopkins Med. J. 130:38.

Jost, A., B. Vigier and J. Prepin. 1972. Freemartins in cattle: the first steps of sexual organogenesis. J. Reprod. Fertil. 29:349.

Karsch, F.J. and D.L. Foster. 1975. Sexual differentiation of the mechanism controlling the preovulatory discharge of luteinizing hormone in sheep. Endocrinology 97:373

Katz, L. S. 1987. Endocrine systems and behavior. Vet. Clinics N. America: Food Anim. Practice. 2:393.

Koo, G. C., S. S. Wachtel, K. Krupen-Brown, L. Mittl, R. W. Berg, M. Genel, I. M. Rosenthal, D. S. Borgaonakar, D. A. Miller, R. Tantravahi, R. R. Schreck, B. F. Erlanger and O. J. Miller 1977. Mapping of the locus of the H-Y gene on the human Y chromosome. Science 198:940.

Lifschytz, E. and D. L. Lindsley. 1972. The role of X-chromosome inactivation during spermatogenesis. Proc. Nat. Acad. Sci. USA. 60:182.

Lisk, R. D. 1980. Masculinized female hamsters do not require steroid treatment when adult for activation of the male copulatory response pattern. Psychoneuroendocrinol. 5:305.

Lucas, M. and A. Smithies. 1973. Banding patterns and autoradiographic studies of cells with an X-autosome translocation. Ann. Hum. Genet. 37:9.

Luine, V. N., R. I. Khylchevskaya and B. S. McEwen. 1975. Effect of gonadal hormones on enzyme activities in brain and pituitary of male and female rats. Brain Res. 86:283.

Lyon, M. F. and S. G. Hawker. 1973. Reproductive lifespan in irradiated and unirradiated chromosomally XO mice. Genet. Res. 21:185.

Lyon, M. F. 1961. Gene action in the X chromosome of the mouse (Mus musculus L.). Nature 190:372.

MacLuskey, N. J. and B. S. McEwen. 1980. Progestin receptors in the developing rat brain and pituitary. Brain Research. 189:262.

MacLuskey, N. J. and F. Naftolin. 1981. Sexual differentiation of the central nervous system. Science 211:1294.

Maeda, M., S. Ohno, A. Ishihashi, M. Samejima and K. Sasaki. 1976. Ring Y chromosome: 45,X/46,X,r(Y) chromosome mosaicism in a phenotypically normal male with azoospermia. Hum. Genet. 34:99.

Mainwaring, W. I. P. 1977. The Mechanism of Action of Androgens. Springer, New York.

Matulis,R. J., F. K. McKeith, P. J. Bechtel, J. E. Novakofski and D. G. McLaren. 1987. The effect of prenatal androgenization on the growth and performance of pigs. J. Anim. Sci. 65 (Suppl. 1):249.

McEwen, B. S., I. Lieberberg, C. Chaptal and L. C. Krey. 1977. Aromatization: important for sexual differentiation of the neonatal rat brain. Horm. Beh. 9:249.

McEwen, B. S., P. Davis, B. Parsons and D. W. Pfaff. 1979. The brain target for steroid hormone action. Annu. Rev. Neurosci. 2:65.

McEwen, B. S. 1981. Neural gonadal steroid actions. Science 211:1303.

McLaren, A. 1961. New evidence of unbalanced sex-chromosome constitution in the mouse. Genet. Res. 1:253.

McLaren, A. and M. Monk. 1981. X-chromosome activity in the germ cells of sex-reversed mouse embryos. J. Reprod. Fert. 63:533.

Meese, G. B. and R. Ewbank. 1973. The establishment and nature of the dominance hierarchy in the domesticated pig. Anim. Behav. 21:326.

Mintz, B. 1959. Continuity of the female germ cell line from embryo to adult. Arch. d'Anat. Micro. Morph. Exp. 48:155.

Mizejewski, G. J., M. Vonnegut and R. Simon. 1980. Neonatal androgenization using antibodies to α-fetoprotein. Brain Res. 188:273.

Muller, U. and H. Schindler. 1983. Testicular differentiation - a developmental cascade. Morphogenetic effects of H-Y antigen and testosterone in the male mammalian gonad. Differentiation 23:99.

Muller, U., I. Aschmoneit, M. T. Zenzes and U. Wolf. 1978. Binding studies of H-Y antigen in rat tissues. Indications for a gonad-specific receptor. Hum. Genet. 43:151.

Naftolin, F., K. J. Ryan, I. J. Davies, V. V. Reddy, F. Flores, Z. Petro and M. Kuhn. 1975. The formation of estrogens by central neuroendocrine tissues. Rec. Prog. Horm. Res. 31:295.

Naftolin, F. 1981. Understanding the bases of sex differences. Science 211:1263.

Nagai, Y., S. Ciccarese and S. Ohno. 1979. The identification of human H-Y antigen and testicular transformation induced by its interaction with the receptor site of bovine fetal ovarian cells. Differentiation 13:155.

Ohno, S. 1967. Sex Chromosomes and Sex Linked Genes. Springer-Verlag, New York.

Ohno, S., Y. Nagai, S. Ciccarese and R. Smith. 1979. In vitro studies of gonadal organogenesis in the presence and absence of H-Y antigen. 1979. In Vitro. 15;11.

Ohno, S., L. Christian, S. Wachtel and G. Koo. 1976. Hormone-like role of H-Y antigen in bovine free-martin gonad. Nature (Lond.) 261:597.

Pang, S. F. and F. Tang. 1983. Sex differences in the serum concentrations of testosterone in mice and hamsters during their critical periods of neural sexual differentiation. J. Endocrinol. 100:7.

Pfeiffer, C. A. 1936. Sexual differences of the hypophyses and their determination by the gonads. Amer. J. Anat. 58:195.

Putney, D. J. 1984. The effect of prenatal androgen exposure on sexual differentiation and postnatal growth in beef cattle. M.S. Thesis. Virginia Polytechnic Institute and State Univ., Blacksburg.

Putney, D. J., W. E. Beal and G. A. Good. 1984. Effects of prenatal androgen exposure on sexual development and estrous cycles in female calves. J. Anim. Sci. 59 (Suppl. 1):339.

Quadagno, D. M. and J. Rockwell. 1972. The effect of gonadal hormones on maternal behavior in the adult rat. Horm. Behav. 3:55.

Quadagno, D. M., J. Shryne, C. Anderson and R. A. Gorski. 1972. Influence of gonadal hormones on social, sexual emergence, and open field behaviour in the rat. Anim. Behav. 20:732.

Raynaud, J., C. Mercier-Bodard and E. Baulieu. 1971. Rat estradiol binding plasma protein (EBP). Steroids 18:767.

Resko, J. S., H. H. Feder and R. W. Goy. 1968. Androgen concentrations in plasma and testis of developing rats. J. Endocrinol. 53:604.

Rosenfeld, R. G., L. Luzzatti, R. L. Hintz, O. J. Miller, G. C. Koo and S. S. Wachtel. 1979. Sexual and somatic determinants of the human Y chromosome: studies in a 46,XYp- phenotypic female. Amer. J. Hum. Genet. 31:458.

Rosler, A. and G. Kohn. 1983. Male psuedohermaphroditism due to 17β-hydroxysteroid dehydrogenase deficiency: studies of the natural history of the defect and effect of androgens on gender role. J. Steroid Biochem. 19:663.

Ruppert, P. H. and L. G. Clemens. 1981. The role of aromatization in the development of sexual behavior in the female hamster (Mesocricetus auratus). Horm. Behav. 15:68.

SAS. 1985. SAS User's Guide: statistics. Statistical Analysis Systems Inst., Cary, NC.

Scaramuzzi, R. J., S. A. Tillson, I. H. Thorneycrop and B. V. Caldwell. 1971. Action of exogenous progesterone and estrogen on behavioral estrus and luteinizing hormone levels in the ovariectomized ewe. Endocrinology 88:1184.

Schecter, J. 1970. A light and electron microscopic study of Rathke's Pouch in fetal rabbits. Gen. Comp. Endocrinol. 14:53.

Schein, M. W. and M. H. Fohrman. 1955. Social dominance relationships in a herd of dairy cattle. Brit. J. Anim. Behav. 3:45.

Scouten, C. W., L. K. Groteleuschen and W. W. Beatty. 1975. Androgens and the organization of sex differences in active avoidance behavior in the rat. J. Comp. Physiol. Psychol. 88:264.

Setchell, B. P. 1978. The Mammalian Testis. Cornell University Press, New York.

Shapiro, B. H., A. S. Goldman and H. F. Steinbeck. 1976. Is feminine differentiation of the brain hormonally determined?. Separation Experientia 32:650.

Sheridan, P. J., M. Sar and W. E. Stumpf. 1975. Autoradiographic localization of ^{3}H-testosterone or its metabolites in the neonatal rat brain. Amer. J. Anat. 140:589.

Short, R. V. 1972. Sex determination and differentiation. In: C. R. Austin and R. V. Short (Ed.) Reproduction in Mammals. 2. Embryonic and Fetal Development. Cambridge Univ. Press, New York.

Short, R. V. 1974. Sexual differentiation of the brain of the sheep. In: M.G. Forest and J. Bertrand (Eds.) Sexual Endocrinology of the Perinatal Period, Colloque International INSERM, Lyon. pp. 121-142. INSERM, Paris.

Siebers, J. W. and W. Vogel. 1973. Structural aberrations of the Y chromosome and the corresponding phenotype. Humangenetik. 19:57.

Singh, L., I. F. Purdom and K. W. Jones. 1976. Satellite DNA and evolution of sex chromosomes. Chromosoma 59:43.

Slaughter, M., R. Wilen, K. J. Ryan and F. Naftolin. 1977. The effects of low dose diethylstilbesterol administration in neonatal female rats. J. Steroid Biochem. 8:621.

Sloan, W. R. and P. C. Walsh. 1976. Familial persistent Mullerian duct syndrome. J. Urol. 115:459.

Stearns, E. L., J. S. D. Winter and C. Faiman. 1973. Positive feedback effect of progestin upon serum gonadotropins in estrogen-primed castrate men. J. Clin. Endocrinol. Metab. 37:635.

Sutherland, S. D. and R. A. Gorski. 1972. An evaluation of the inhibition of androgenization of the neonatal rat brain by barbiturate. Neuroendocrinol. 10:94.

Swanson, H. H. and J. J. van der Werff ten Bosch. 1963. Sex differences in the growth of rats and their modification by a single injection of testosterone shortly after birth. J. Endocrinol. 26:197.

Swanson, H. E. and J. J. van der Werff ten Bosch. 1964. The "early androgen" syndrome: differences in response to pre-natal and post-natal administration of various doses of testosterone propionate in female and male rats. Acta Endocrin. (Copenh.) 47:37.

Syme, G. J. 1974. Competitive orders as measures of social dominance. Anim. Behav. 22:931.

Tartellin, M. F., J. E. Shryne and R. A. Gorski. 1975. Patterns of body weight change in rats following neonatal hormone manipulation: A "critical period" for androgen-induced growth increases. Acta Endocrinol. 79:177.

Tiepolo, L. and O. Zuffordi. 1976. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum. Genet. 34:119.

Tollman, J. and J. A. King. 1956. The effects of testosterone propionate on aggression in male and female C57 Bl/10 mice. Br. J. Anim. Behav. 6:147.

Toran-Allerand, C. D. 1978. Gonadal hormones and brain development: cellular aspects of sexual differentiation. Amer. Zool. 18:553.

Toran-Allerand, C. D. 1976. Sex steroid and the development of the newborn mouse hypothalamus and preoptic area in vitro: implication for sexual differentiation. Brain Res. 106:407.

Toran-Allerand, C. D. 1980. Coexistence of α-fetoprotein, albumin and transferrin immunoreactivity in neurones of the developing mouse brain. Nature 286:733.

Tyrkus, M., D. Postellan, W. H. Hoffman, E. Bawle and P. W. Wolley. 1979. Evidence for male determining loci on the Y chromosome. Amer. J. Hum. Genet. 31:113.

vom Saal, F. S. and F. H. Bronson. 1978. Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. Science 208:597.

Wachtel, S. S. and J. L. Hall. 1979. H-Y binding in the gonad: inhibition by a supernatant of the fetal ovary. Cell 17:327.

Wachtel, S. S., G. C. Koo, W. R. Breg, S. Elias, E. A. Boyse and O. J. Miller. 1975. Expression of H-Y antigen in human males with two Y chromosomes. N. Engl. J. Med. 293:1070.

Wachtel, S. S., P. Basrur and G. C. Koo. 1978. Recessive male-determining genes. Cell 15:279.

Ward, I. L. 1972. Female sexual behavior in rats treated prenatally with an antiandrogen. Physiol. Behav. 8:53.

Weisz, J. and I. L. Ward. 1979. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses and neonatal offspring. Endocrinology 106:306.

Whalen, R. E. and D. A. Edwards. 1967. Hormonal determinants of the development of masculine and feminine behavior in male and female rats. Anat. Rec. 157:173.

Whalen, R. E. and R. D. Nadler. 1965. Modification of spontaneous and hormone-induced sexual behavior by estrogen administered to neonatal female rats. J. Comp. Physiol. Psychol. 60:150.

Wilson, J. G., J. B. Hamilton and W. C. Young. 1941. Influence of age and presence of the ovaries on reproductive function in rats injected with androgens. Endocrinology 29:784.

Wilson, J. D. and J. D. Walker. 1969. The conversion of testosterone to 5-androstan-17-ol-3-one (dihydrotestosterone) by skin slices of mice. J. Clin. Invest. 48:371.

Wilson, J. D., G. W. Fredrick and J. E. Griffin. 1981. The hormonal control of sexual development. Science 211:1278.

Wilson, J. D. 1978. Sexual differentiation. Ann. Rev. Physiol. 40:279.

Wiltbank, J. N., W. W. Rowden and J. E. Ingalls. 1959. The age and weight at puberty in hereford heifers. J. Anim. Sci. 18:1562 (Abstr.).

Zenzes, M. T., U. Wolf and W. Engel. 1978a. Organization in vitro of ovarian cells into testicular structures. Hum. Genet. 44:333.

Zenzes, M. T., U. Muller, I. Aschmoneit and U. Wolfe. 1978b. Studies on H-Y antigen in different cell fractions of the testis during pubescence. Hum. Genet. 45:297.

Zimbelman, R. G. and J. W. Lauderdale. 1973. Failure of prepartum or neonatal steroid injections to cause

infertility in heifers, gilts and bitches. Biol. Reprod. 8:388.

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