IMMUNIZATION OF HEIFERS AGAINST GONADOTROPIN RELEASING HORMONE: EFFECTIVENESS OF ADJUVANTS

By

SHELIA LAVON DUGGAN

Bachelor of Science in Agriculture

University of Arkansas at Fayetteville

Fayetteville, Arkansas

1989

Submitted to the Faculty of the
Graduate college of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1993

OKLAHOMA STATE UNIVERSITY

IMMUNIZATION OF HEIFERS AGAINST

GONADOTROPIN RELEASING HORMONE:

EFFECTIVENESS OF ADJUVANTS

Thesis Approved:

Robert P. Wettemann

Thesis Advisor

Tony Spicer

Keith Lusby

Thomas C. Collins

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to thank Dr. R.P. Wettemann for giving me the opportunity to further my education under his expert and patient guidance. I also thank Dr. Keith Lusby and Dr. Leon Spicer for serving on my graduate committee.

Special thanks are extended to Mark Anderson, David Cox and David Gay for their cooperation and assistance in collecting data and care for the research animals. Assistance in the laboratory was directed by LaRuth Mackey.

I wish to thank my fellow graduate students for their moral support and friendship, but especially to David Bishop and Gary Ziehe, who were never to busy to answer my many questions.

I will always be grateful to Brock Karges. His words of encouragement and confidence in my abilities helped me obtain my goals.

Words cannot express my love and appreciation for my parents. Thank you, Mom, Dad, Mark and family for your unending support throughout my life and education. I would like to dedicate this manuscript in loving memory to my grandfather, W. Earl Duggan, who perhaps is the proudest of my endeavors.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Gonadotropin releasing hormone (GnRH) controls the synthesis and secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary. These hormones are necessary to initiate and maintain estrous cycles. Neurons in the hypothalamus synthesize GnRH which is released into the hypophyseal portal vessels in pulses, and stimulate the anterior pituitary to secrete LH and FSH. The ability to manipulate the hormones regulated by GnRH can be achieved by immunization. Immunization against GnRH has been successful in many species including rats (Arimura et al., 1973), sheep (Lincoln & Fraser, 1979; Adams & Adams, 1986), horses (Garza et al., 1986), pigs (Esbenshade & Britt, 1985; Falvo et al., 1986) and cattle (Robertson et al., 1981; Johnson et al., 1988). Males immunized against GnRH typically have decreased sperm production and decreased testicular size (Schanbacher, 1984) due to reduced concentrations of LH and testosterone. Immunization of females against GnRH will prevent ovulation and estrous cycles (Jeffcoate & Keeling, 1984).

Immunization of male calves or sexually mature bulls against GnRH would eliminate the surgical procedure of castration. This would eliminate stress related weight losses, hemorrhage and infection which can be associate with castration.

Heifers growing in pastures and in feedlots are less efficient than steers due to excess activity associated with estrous cycles and due to pregnancy. If puberty could be delayed by immunization against GnRH, these problems could be reduced and efficiency of production would be increased.

The objectives of this experiment were to determine the ability of four adjuvants to stimulate antibody production against GnRH in heifers and to determine which adjuvant had the least inflammatory response at the injection site.

CHAPTER II

REVIEW OF LITERATURE

Puberty in Heifers

Puberty can simply be defined as when an animal is capable of reproducing itself (Robinson, 1977). A more complete definition is the time at which a heifer first exhibits an increase in progesterone followed by a normal luteal phase (Moran et al., 1989).

Puberty occurs at approximately three to four months of age in rabbits, at six to seven months of age in sheep, goats and swine, at twelve months in cattle, and at fifteen to eighteen months in horses. The age at puberty can be influenced by many factors such as breed, nutrition, social environment, season and body condition.

The time at which an animal attains puberty is related to its lifetime productivity. Heifers bred to calve at two years of age wean more calves in their lifetime than heifers that calve at three years of age (Short and Bellows, 1971; Nunez-Dominguez et al., 1991). In addition, heifers that calve early in the breeding season continue to do so

throughout their lifetime and wean heavier calves (Short and Bellows, 1971; Lesmeister et al., 1973). Results from Lusby et al. (1979), demonstrated that breeding heifers at 30 vs 24 mo of age improved conception at breeding, rebreeding percentages and increased weaning weight of the first calf crop.

Factors influencing age at puberty in heifers.

Breed. Breeds of cattle differ in the age and weight at which they attain puberty (Wiltbank et al., 1966; Ferrell, 1982; Laster et al., 1976; Gregory et al., 1978; Stewart et al., 1980). Continental European breeds usually reach puberty at a younger age but heavier weights than Hereford or Angus heifers (Laster et al., 1979). Ferrell (1982) also found larger type heifers were younger but heavier at puberty than smaller type heifers. Bos taurus cattle reach puberty at a younger age than Bos indicus or Bos indicus x Bos taurus crosses (Dow et al., 1982; Bolton et al., 1987). Heterosis and maternal effects influence age at puberty but do not affect weight at puberty (Laster et al., 1972). Breeds selected for greater amounts of milk production versus beef production reach puberty at a younger age and lighter weight (Laster et al., 1979; Baker et al., 1989). This difference may be related to direct maternal effects due to greater preweaning gain of calves nursing dams producing more milk (Arije and Wiltbank 1971). Further evidence to support this hypothesis is that the correlation

between milk production of the dam and age at puberty is (-0.88; Laster et al., 1979).

Nutrition. Heifers that are fed to achieve greater daily gains pre- or postweaning, reach puberty at a younger age but are heavier than heifers on a lower plane of nutrition (Arije and Wiltbank, 1971; Short and Bellows, 1971; Grass et al., 1982; Greer et al., 1983; Day et al., 1984). Total body fat, body weight (BW) and body condition score (BCS) at puberty were reported to be greater in full fed heifers vs limited or maintenance fed animals (Yelich et al., 1991). Wiltbank et al. (1969) illustrated that there is an interaction between nutrient intake and breed for age and weight at puberty. Crossbred heifers reached puberty at a younger age on a low plane of nutrition, but were heavier than straightbreeds on greater nutrient intake.

Monensin decreases the age at puberty (McCartor et al., 1979; Bushmich et al., 1980; Mosely et al., 1982). The effect of monensin on puberty may be related to the role it has in providing additional available energy to heifers for growth. Rumen fermentation products, such as acetate, propionate and butyrate, supply energy to the heifer for the onset of puberty (McCartor et al., 1979; Mosely et al., 1978). Monensin sodium ($C_{36}H_{61}O_{11}Na$) is produced by Streptomyces cinnamonensis (Haney & Hoehn, 1967), and functions to shift concentrations of acetic and butyric acid to propionic acid. Propionate is a major precursor of

glucose and therefore increases the available energy to the heifer, acting as a higher plane of nutrition. This increase in nutrients available to the heifer may be the reason for a decrease in age at puberty in monensin fed heifers.

Another method used to change rumen fermentation products is through feeding protein-protected lipids. When an encapsulated oilseed supplement was fed to heifers, they were more efficient in converting energy to weight gain and thus there was a significant increase in the number of heifers that attained puberty during the trial (Rhodes et al., 1978). Oyedipe et al. (1982) fed three different amounts of protein to zebu heifers and found that increasing the amount of protein decreased the age to puberty. When the plane of nutrition is minimal during the prepubertal period, reduced secretion of LH is probably the cause of delayed puberty (Day et al., 1986). Average daily gain (ADG) and IGF-1 concentrations in serum are positively related to energy intake and weight loss is associated with increased growth hormone (GH) concentrations in serum (Granger et al., 1989). This is in agreement with other studies in which animals fed on a reduced plane of nutrition had elevated GH concentrations (Househnecht et al., 1988). Thus, GH concentrations increase when tissue is being mobilized to meet requirements for maintenance or growth. Changing from an ample to minimal nutrient intake can be

detrimental, causing an increase in the age at puberty (Crichton et al., 1959).

The effects of nutrition on attainment of puberty is magnified when prepubertal growth occurs during the winter. Another reproductive trait affected by a reduced plane of nutrition is an increase in dystocia (Reid et al., 1960; Turman et al., 1965). Feeding a greater amount of nutrient causes heifers to come into estrus earlier, become pregnant sooner and wean heavier calves (Wiltbank et al., 1985).

Season and Photoperiod. Reproduction in the bovine species is not limited to any particular time of the year. However, season may influence when heifers reach sexual maturity (Kinder et al., 1987). Heifers born in the spring reached puberty earlier than fall born heifers (Menge et al., 1960; Grass et al., 1982; Schillo et al., 1982). Heifers born in the fall are subjected to an additional winter before puberty compared with heifers born in the spring (Grass et al., 1982). The earlier onset of puberty for spring born heifers may be attributed to photoperiod. Increased daylight by use of supplemental lighting after 22 or 24 weeks of age advanced first ovulation and first estrus in heifers born from February to July (Hansen et al., 1983). Supplemental lighting also stimulated the rate of body weight gain, increased feed efficiency and hastened puberty regardless of whether heifers were on moderately restricted or ad libitum diets (Petitclerc et al., 1983). Hansen et

al. (1982) suggested that photoperiod affects age at puberty by altering the positive feedback actions of estradiol. He found a greater release of LH after estradiol injection in ovariectomized heifers exposed to 18 hours of light/day compared with heifers exposed to natural winter photoperiod.

Concentrations of prolactin in serum of cattle are increased when animals are exposed to increased duration of light (Koprowski and Tucker, 1973). Hansen et al. (1983) indicated that rate of ovarian growth was greater in heifers exposed to supplemental lighting compared with heifers exposed to natural lighting. When ovariectomized prepubertal heifers were exposed to 18 hours light/day during the winter, there was an increase in FSH release and greater estradiol-induced LH release (Hansen et al., 1982).

Roy et al. (1980) suggested that the first estrus was associated with four distinct peaks or times within the lunar cycle. Greer (1984) disproved this theory with data indicating that 556 spring born heifers obtained first estrus throughout the lunar cycle.

These studies suggest that photoperiod and season exerts effects on puberty mainly due to environmental conditions which stimulate rate of body weight gain and secretions of gonadotropins.

Social Environment. Social environmental changes such as presence of males, transportation or relocation of environment, electrical stimulation of the cervix and other

biostimulators may decrease age at puberty or synchronize estrus in many species. Reports concerning how social environment influences age at puberty of heifers have been controversial, but recent studies indicate that this topic may be more important than previously indicated. Biostimulation is the stimulatory effect of a male on estrus and ovulation through pheromones, genital stimulation or other less well-defined external cues (Chenoweth, 1983). Introduction or the presence of a male in a group of females will decrease age at puberty in mice (Vandenbergh, 1967; Colby and Vandenbergh, 1974) ewes (Cushwa et al., 1992); gilts (Brooks and Cole, 1970; Thompson and Savage, 1978) and synchronize the first estrus in ewe lambs (Drymundsson and Lee, 1972). Berardinelli et al. (1978) and Roberson et al. (1987) found that long or short term presence of a bull does not alter age or weight at puberty. Recently, Roberson et al (1991), found that bulls do influence age at puberty of heifers. A greater percentage of heifers exposed to bulls for 70 days (short term) had luteal activity by 14 mo of age compared with heifers that were isolated from bulls. Bull exposure decreased the age to puberty even in heifers growing at moderate to fast rates. This suggests that growth rate interacts with bull exposure to decrease age at puberty in heifers.

It is hypothesized that bull urine will decrease age at puberty due to a positive feedback effect on LH secretion which stimulates ovarian function (Roberson et al., 1991).

Izard and Vandenbergh, (1982) treated heifers oronasally with bull urine or water as a control. The urine treated heifers reached puberty earlier, and therefore calved earlier than controls. It was hypothesized that bull urine contains a priming pheromone much like that of the urinary priming pheromone produced by male mice. An androgen dependent priming pheromone produced by adult male mice accelerates puberty in females (Bronson & Whitten, 1968; Colby & Vandenbergh, 1974 and Lombardi et al., 1976) by shortening the interval between vaginal opening and first estrus.

Serum LH increases within one hour after prepubertal female mice are exposed to males (Bronson and Desjardins, 1974). This increase in LH is followed by an increase in serum estradiol within 3-6 hours after male exposure. By 36-48 hours after male exposure, periovulatory changes in serum gonadotropins and plasma progesterone occur (Bronson and Desjardins, 1974).

Another biostimulation that decreased age at puberty in rats is electrical stimulation of the cervix (Swingle et al., 1951). Electrical stimulation also increased the number of anestrous cows that exhibited estrus and ovulated within 7 days of treatment (Hays & Carlevaro, 1959). It was proposed that the electrical stimulation caused gonadotropin secretion. However, Berardinelli et al. (1978) found that electrical stimulation did not affect puberty in heifers.

Body Weight and Body Condition. Body weight and body condition are important factors that influence when puberty is initiated, but they are not the only factors because they are influenced by nutrition, breed, environment and management. Reaching a certain weight does not automatically initiate puberty. If a critical weight was the primary requirement for puberty, there would be no correlations between age and weight or body composition at puberty. Feeding greater amounts of energy, pre or postweaning to accelerate body growth, will cause heifers to reach puberty at a younger age (Crichton et al, 1959; Arije and Wiltbank, 1971 and Ferrell, 1982). However, heifers reaching puberty due to increased nutrient intake are heavier at puberty (Short and Bellows, 1971). Therefore, weight is not the only limiting or critical factor that determines age at puberty. Frisch and Revelle, (1970) suggest that puberty in humans is associated with a common body weight rather than a specific age.

Changes in body fatness which alter the metabolic rate, have also been suggested to initiate puberty. This concept is based on data from Frishe et al. (1977) where the ratios of body water to body weight and body fat at first estrus were similar between rats fed on high and low planes of nutrition. Siebert and Field (1975) also indicated that initiation of estrous cycles is closely related to content of body fat. They predicted a body fat content of 18.8 to 21.8 kg at puberty based on body weight and total estimated

body water at puberty. Rhodes et al. (1978) fed heifers protected lipid supplement which caused greater subcutaneous and intramuscular fat deposition, and greater body condition scores. Less of these heifers reached puberty than controls. Rhodes suggested that decreased plasma GH could have been the determining factor for delaying puberty. The protected lipid supplement decreased GH levels by decreasing ruminal volatile fatty acids, which increased plasma triglycerides.

Endocrine Changes Prior to Puberty. Puberty is a process that requires gradual and/or sudden changes in an animal's endocrine mechanisms. These changes may begin before birth or directly before puberty occurs. Concentrations of LH and FSH in plasma increase from birth to 3 mo of age in heifers, decrease until 5 to 6 mo of age and then increase until 9 mo of age (Schams et al., 1981). Dodson et al. (1988) found that concentrations of LH in serum decreased from birth to 4 mo of age and then increased until 10 mo of age. Concentrations of LH in the pituitary increased fourfold from birth to 4 mo of age in heifers, varied from 4 to 7 mo and then declined from 7 to 12 mo (Desjardins and Hafs, 1968). Frequency of LH pulses increase prior to puberty in heifers (Day et al., 1984; Day et al., 1987), ewes (Foster & Ryan, 1979) and rats (Bronson, 1986). Increases in LH pulse frequency may be related to increased GnRH secretion (Wantanabe and Terasawa, 1989).

Concentrations of FSH in the pituitary of heifers were increased at 1 mo of age, declined at 2 mo, and then changed very little up to 12 mo of age. Concentrations of prolactin varied greatly between heifers, but were minimal at 40 d before the preovulatory LH peak which was the period at which GnRH and estradiol-17B in plasma and mammary DNA and RNA were greatest (Swanson et al., 1972 and Gonzalez-Padilla et al., 1975). However, there are no marked changed in FSH, prolactin or GnRH before puberty in ewes and cattle (Foster et al., 1975; Gonzalez-Padilla et al., 1975). Progesterone concentrations are less than 1 ng/ml from 1 to 9 mo of age and increase during the peripubertal period (Gonzalez-Padilla et al., 1975; Swanson et al., 1972; Berardinelli et al., 1979).

The ovary is the source of both the first and second increases in concentrations of progesterone prior to puberty (Berardinelli et al., 1979). Ovariectomy of heifers during the first period of elevated progesterone resulted in a decline in peripheral progesterone to basal concentrations. At ovariectomy after the second rise of progesterone, ovaries contained luteal tissue. Therefore, the ovary is the source of both increases of progesterone prior to the initiation of puberty. The significance of the first elevation of progesterone is thought to be a "primer" for the ovulatory system, to insure ovulation at the second LH surge.

The 'gonadostat hypothesis' is the classical explanation of how the pubertal increase in plasma LH is of sufficient magnitude to initiate ovulation (Ramirez et al., 1963). According to this hypothesis, LH in plasma is maintained at a minimal concentration prior to puberty, due to the inhibitory feedback action of estradiol on the hypothalamo-pituitary axis. The power of this negative feedback decreases as the animal approaches the time of puberty. This allows LH secretion to increase to a concentration sufficient to stimulate follicle growth, resulting in ovulation. Ramirez et al. (1963) studied factors that influenced LH secretion and storage in immature and adult rats of both sexes. They found that LH was stored in the hypophysis, and could be secreted in immature rats. In addition, estrogen given to female rats reduced plasma and hypophysial concentrations of LH. They concluded that a reduction in hypothalamic sensitivity to estrogen inhibition could be a factor that controls initiation of puberty. Further research has increased the acceptability of the gonadostat hypothesis. Prepubertal animals contain greater quantities of LH and FSH in their pituitary than adults (Bergman and Turner, 1942; Hollandbeck, 1956; Ramirez et al., 1963; Parlow et al., 1964). Seidel et al. (1971) induced ovulation in 1 month old heifers by administering gonadotropins. Therefore, the lack of ability of the ovary to respond to gonadotropins and the ability of the pituitary to synthesize and/or store gonadotropins is not a factor

that controls puberty. Injections of GnRH stimulate LH release in heifers, with the magnitude of the LH response increasing with age (Schams et al., 1981).

Naloxone, an opiate peptide antagonist, can stimulate LH secretion (Blank et al., 1979). Bhanot and Wilkinson, (1983) hypothesized that hypothalamic opiates cause suppression of LH pulses by sensitizing the hypothalamus to estradiol. In addition, opiate inhibition of LH decreased as rats matured. The exact mechanism by which opioid inhibition on LH decreases with age is unknown, but one theory is that opiate receptors are desensitized as puberty approaches, but there is not a decrease in number or affinity of opiate receptors (Bhanot and Wilkinson, 1983).

Estradiol receptors are present in the hypothalamus of bovine females as early as 1 to 2 weeks of age (Armstrong et al., 1977), but their concentrations decline as puberty approaches (Day et al., 1987). Kato et al. (1974) reported that the estradiol receptors in the hypothalamus of female rats increased from birth to 28 days and then declined until puberty. As puberty approaches, gonadotropin inhibition through estradiol negative feedback declines in heifers (Schillo et al., 1982; Day et al., 1984), ewe lambs (Foster and Ryan, 1979), gilts (Berardinelli et al., 1984) and female rats (Docke et al., 1981; Docke et al., 1984). The mechanism by which the negative feedback of estradiol declines is probably due to a decrease in the number of estradiol receptors.

In summary, the machinery for gonadotropin secretion and secretion of releasing hormones are functional in immature animals. However, there is a strong negative feedback of estradiol at the level of the pituitary/hypothalamus to decrease LH secretion. As the animal matures, there is a reduction in estradiol receptors which allows LH to be secreted at a greater frequency to initiate follicular development. The maturing follicle(s) will produce estrogen which stimulates uterine growth and development. When the follicle reaches a preovulatory stage, estradiol induces a surge of gondaotropin and ovulation occurs.

Functions of GnRH in Reproduction

GnRH is a decapeptide [(pyro) Glu-His-Try-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2] (Matsuo et al., 1971). GnRH is synthesized in pericellular neurons located in various parts of the hypothalamus including the arcuate nucleus, anterior hypothalamus and preoptic area. GnRH is pulsed into capillaries of the hypothalamo-hypophyseal portal blood system located in the median eminence (Clarke and Cummins, 1982). Pulses of GnRH stimulate gonadotroph cells in the anterior pituitary (Noar and Childs, 1986), causing synthesis and pulsatile release of gonadotropins in rats (Levine and Duffy, 1988), sheep (Clarke and Cummins, 1982), rabbits (Pau et al., 1986) and monkeys (Pau et al., 1989).

The anterior pituitary is dependent upon the hypothalamus for GnRH signals. If the pituitary gland is disconnected from the hypothalamus through hypothalamo-pituitary disconnection, LH and FSH concentrations decrease (Clarke et al., 1983), as well as mRNA for the subunits of the gonadotropins (Hamernik et al., 1986). Therefore, pulsatile release of GnRH is essential for maintaining secretions of LH and FSH (Arimura et al., 1972; Belchetz et al., 1978; Gallo, 1980; Clarke and Cummins, 1982).

There is controversy as to whether GnRH is the primary regulator of both FSH and LH, because these two gonadotropins have different secretory characteristics. Concentrations of LH and FSH differ in the circulation, whether it be short term (Lincoln, 1978) or long term (Lee et al., 1976; Lincoln and Peet, 1977). Another example of their differences is seen through their pulsatile release patterns (Arimura et al., 1976; Grady et al., 1985). It has been proposed that GnRH is able to regulate both gonadotropins through its frequency of pulses. A fast frequency stimulates LH secretion, while a slower frequency release of GnRH stimulates FSH release (Wildt et al., 1981; Clarke et al., 1984; Haisenleder et al., 1987; Savoy-Moore & Swartz, 1987; Wu et al., 1987). The pattern of LH release in cows is dependent upon the stage of the estrous cycle (Rahe et al., 1980). In the early luteal stage, minimal amplitude and rapid frequency pulses were observed. During the midluteal stage, pulses were of greater amplitude and

less frequency. Therefore, GnRH frequency of release may differentially regulate gonadotropin subunit mRNA expression to cause secretion of a specific hormone (Dalkin et al., 1989).

Culler and Negro-Vilar (1986) suggested that pulsatile release of FSH is regulated by a separate factor(s) other than GnRH, but agree that GnRH is necessary for maintaining secretion of FSH. Bovine follicular fluid has inhibin-like activity that is capable of suppressing pulsatile FSH secretion while having no effect on LH and prolactin release (de Greef et al., 1987). GnRH has an effect on the anterior pituitary through GnRH receptors located on the plasma membrane of gonadotrophs (Duello and Nett, 1980; Nett et al., 1981; Conn et al., 1987). Wise et al (1985) demonstrated that the number of GnRH receptors and amount of GnRH in circulation is important in determining gonadotroph activation. The number of receptors for GnRH in the anterior pituitary of cattle decrease on day 19 postestrus and remained minimal through day 21 postestrus (Nett et al., 1987). This suggests that synthesis of LH is initiated and the anterior pituitary content is restored before the number of receptors for GnRH returns to normal. There is also an increase in GnRH receptors before the LH surge and a decrease in receptors at the end of the surge (Crowder and Nett, 1984; Leung et al., 1984), due to down regulation of the receptors (Moenter et al., 1989). GnRH receptor numbers can be increased by GnRH itself (Nett et al., 1981; Conn et

al., 1984) or through estradiol (Moss et al., 1981; Laws et al., 1990).

Estradiol acts directly on the pituitary to stimulate and increase the number of GnRH receptors (Gregg and Nett, 1989). There are several mechanisms by which GnRH secretion and/or its actions are regulated. In the ewe, progesterone suppresses LH release by acting at the hypothalamus to decrease the frequency of GnRH pulses. The combination of progesterone suppressing GnRH release and estradiol suppressing the responsiveness of the pituitary to GnRH causes the LH pulse amplitude to be decreased (Goodman and Karsch, 1980). Exposure of the bovine pituitary to GnRH (in vivo) has a self-priming mechanisms, in that a second exposure causes an increase in LH release (Foster, 1978). In vitro. estradiol increases the ability of GnRH to self-prime, but progesterone alone or in combination with estradiol has an inhibitory effect on the self-priming mechanism (Padmanabhan et al., 1982).

Endogenous opioid peptides (EOP) may regulate LH secretion by acting on the hypothalamic GnRH pulse generator (Kesner et al., 1986), in an ovarian steroid-dependent manner (Wu et al., 1991) or a steroid-independent manner (Wu et al., 1991). Morphine, an EOP agonist, suppresses the preovulatory surge of GnRH into the pituitary portal blood of rats (Ching, 1983) and decreases LH pulse frequency (Ebling et al., 1989). Naloxone, an opioid antagonist, can increase gonadotropin pulse frequency in monkeys (Van Vugt

et al., 1984), ewes (Whisnant & Goodman, 1988; Ebling et al., 1989; Wu et al., 1991) and cows (Whisnant et al., 1986; Leshin et al., 1991). Beta endorphins and met-enkephalins are found in the hypophyseal portal blood system in large amounts (Wardlaw et al., 1980). Therefore, EOP could influence the anterior pituitary to decrease LH release by two ways. EOP could inhibit GnRH release through GnRH neurons that have opioid receptors, modulation of other neurons that excite GnRH (Malven, 1986), by affecting pituitary receptors for GnRH (Barkan et al., 1983), or through ovarian steroids (Rund et al., 1989).

Active Immunization

Antibody Production. An animal obtains active immunity when its body develops antibodies or activated lymphocytes in response to an invasion of the body by a foreign antigen. Vaccinations cause acquired active immunity. Organisms such as Mycobacterium tuberculosis in FCA is dried and heat killed, making it no longer harmful. However, it still contains the chemical antigen to stimulate antibody formation. Production of antibodies vary between individuals as well as species.

Antibody production can be measured by an animal's titer against the antigen, and can determined by the ability of serum to bind a radiolabeled antigen. Antibody titers are expressed as the percentage of radioactivity bound at a

particular serum dilution or as a dilution which binds a predetermined quantity of radiolabeled antigen (Jeffcoate & Keeling, 1984).

Booster immunizations can be given to enhance antibody titers, with the number of booster immunizations needed to cause a significant response varying between individuals and species. In studies designed to produce antibodies against GnRH, between 0.2 and 1.0 mg of a GnRH conjugate have been used. While a minimum dose range of GnRH conjugate has not been determined in animals, as little as 50 ug has been successfully used in the immunization of sheep, cattle and horses (Schanbacher, 1982).

Site of injection does not seem to affect antibody *production against* GnRH. Most immunizations utilize the multiple intradermal site technique (Arimura et al., 1973; Fraser et al., 1974; Jeffcoate et al., 1976). Other immunization sites include subcutaneous (Clarke et al., 1978), intramuscular (Rosenblum and Schlaff, 1976) and lymph node (Kerdelhue et al., 1976).

Environmental differences can also affect antibody response to antigens. Changes in temperature, humidity and animal density or social stress alters antibody response in mammals (Kelley, 1980).

Carrier Protein. For a substance to be antigenic, it usually requires a molecular weight (MW) of 8000 kDa or greater. The MW of GnRH (1183) requires that it is attached

to a larger carrier molecule to enhance its antigenicity. An animal will produce the most effective and reliable antisera titers against a protein (Fraser, 1980). Protein and nonprotein carriers that have been used for immunization against GnRH are tetanus toxoid (Ladd et al., 1989; Upadhyay et al., 1989), Alhydrogel (Jeffcoate et al., 1976), polyvinylpyrrolidone (Arimura et al., 1973), charcoal (Pique et al., 1978), thyroglobulin (Bercu et al., 1977), keyhole limpet haemocyanin (KLH; Adams & Adams, 1986), ovalbumin and equine serum albumin (Goubau et al., 1989a), human serum albumin (Schanbacher, 1982), and bovine serum albumin (BSA; Koch et al., 1973; Nett et al., 1973; Fraser et al., 1974; Hauger et al., 1977; Copeland et al., 1979; Garza et al., 1988; Traywick and Esbenshade, 1988). In most of the studies reviewed, BSA was the carrier protein of choice for use in species other than bovine. Ovalbumin, human serum albumin (HSA) and human serum globulin (HSG) are carriers for immunization of the bovine species (Schanbacher, 1984; Hoskinson et al., 1990; O'Connell, 1990). A recent study evaluating ovalbumin and KLH on the antigenicity of LH in heifers demonstrated that ovalbumin was the superior protein based on antibody production (Roberts et al., 1990).

Conjugation. Several methods have been utilized in order to conjugate or join GnRH to a carrier protein. The simplest and most frequently used conjugation method is the carbodiimide reaction (Fraser et al., 1974). The GnRH

molecule does not contain a free amino or carboxyl group, it is attached to a carrier protein at the hydroxyl group on serine or tyrosine (Fraser, 1980).

Two less frequently used techniques are diazotization and the glutaraldyde condensation reaction. In the diazotization technique, the conjugating agent, bis diazotized, incorporates two carbon rings between GnRH and the protein carrier (Fraser et al., 1974). The glutaraldehyde condensation reaction polymerizes GnRH with a protein carrier (Arimura et al., 1974; Jeffcoate and Keeling, 1984).

Adjuvants

An adjuvant will enhance an immune response by slowing the release of an antigen into the body (Tizard et al., 1984). Many different adjuvants have been used to immunize animals against GnRH, but the most successful and consistent adjuvant in eliciting an immune response against a GnRH conjugate is Freund's complete adjuvant (FCA; Johnson et al., 1988; Goubau et al., 1989b). Freund's complete adjuvant is composed of an emulsifier, mineral oil (MO), and dried heat killed Mycobacterium tuberculosis. Unfortunately, there are disadvantages associated with FCA use. A local inflammatory response at treatment sites can result in granuloma production which ranges from small visible granulomas to open lesions. In addition, immunized animals will test positively to the tuberculin test due to

the presence of the mycobacterial cell wall fraction (Robertson et al., 1984). These two major disadvantages of FCA makes it unacceptable for commercial use. Therefore, an adjuvant that will elicit an immune response without a major local inflammatory response must be identified to produce a satisfactory vaccine against GnRH.

Bortella pertussis in combination with FCA has been evaluated as a potential adjuvant (Fraser et al., 1975; Fraser, 1980; Schanbacher, 1982). This combination elicits an immune response, but the response is not comparable to FCA used alone. Eide et al. (1992) modified FCA by utilizing 1 mg/ml sonicated Mycobacterium paratuberculosis added to Freund's incomplete adjuvant (FIA). This same study demonstrated that mixing the antigens diptheria toxoid and HSA, will not elicit a greater antibody response compared with separate injections of the two antigens.

Freund's incomplete adjuvant has the same chemical makeup as FCA, excluding heat killed Mycobacterium tuberculosis. This adjuvant is less potent than FCA and is usually chosen as the booster adjuvant when FCA is used for the primary immunization.

Goubau et al. (1989b) evaluated six adjuvants in the immunization of steers and bulls against GnRH. Adjuvants tested were FCA, Havlogen, Ribi adjuvant system (RAS), dimethyl dioctadecyl ammonium bromide (DDA), alhydrogel or Regressin. Results indicate that alhydrogel and DDA stimulated production of GnRH antibodies, but not to the

degree that FCA caused antibody production. Halvogen, Regressin and RAS were not successful in producing significant titers against GnRH. In contrast, Silversides et al. (1988) found that Havlogen and DDA were effective when used in the immunization of mice against GnRH. However, in this same study, FCA was found to be a poor adjuvant which is inconsistent with most studies (Jeffcoate et al., 1982; Goubau et al., 1989b).

Polycations have a strong affinity for cell surfaces which can result in stimulation of macromolecule uptake, RNA and DNA uptake from virus, increased adhesion to surfaces and increased aggregation of cells. Polycationic compounds such as polylysine, DEAE dextran protamine, methylated HSA, and protamine sulphate were observed to suppress the PHA- and Con A-induced responses of human lymphocytes (Larsen and Heron, 1978). Sixty percent of an antigen in combination with DEAE dextran can be recovered in the lymph after 24 hours. In comparison, 80-90% of antigen injected alone and 15% of antigen and FIA used in combination was recovered in lymph after 24 hours (Beh and Lascelles, 1985). These results suggest that cellular uptake of mitogens may be increased by polycationic compounds (Larsen and Heron, 1978).

The adjuvant M103, which contains 63% oil, 37% water and Quillaha saponin was found to be comparable to FCA in its ability to induce and maintain antibody production against human chorionic gonadotropin (hCG) in heifers

(Johnson et al., 1988). However, this adjuvant was less effective than FCA when used to immunize heifers against LH or GnRH.

Adjuvant B, an experimental adjuvant, is as effective as FCA in inducing an antibody response against GnRH-KLH in mice and rats (Gonzalez et al., 1991). However, these data indicate that FCA does not produce granulomas which is inconsistent with most literature.

In a study by Hoskinson et al. (1990), 2 to 7 wk old rams were immunized against GnRH-BSA emulsified in DEAE dextran, a mineral oil emulsion, or an emulsion of DEAE dextran in mineral oil. A booster immunization was given at 9 wk of age. This study indicated that the mixed adjuvant system stimulated a titer sufficient to reduce testicular weight 40 d postbooster immunization, and was a superior adjuvant system over the separate adjuvants of mineral oil or DEAE dextran.

Various aqueous and MO containing adjuvants were studied to determine what the superior adjuvant was in stimulating antibody production against LH-ovalbumin. Adjuvants with greater than 50% oil produced a greater antibody response than those with less than 50% oil (Roberts et al., 1990).

A stable water-in-oil emulsion is difficult to achieve (Becher, 1966) and requires a thoroughly mixed suspension of the water-in-oil adjuvant and antigen. A suspension is usually achieved by the two syringe method, where two

syringes are joined together via a three-way valve and the mixture is forced expulsed into each syringe until a consistent emulsion is obtained. The emulsion is satisfactorily mixed when a drop of emulsion does not break in water. Instruction sheets for Titer Max (CytRx Corp., Norcross, GA), a newly formulated adjuvant, suggests the following routine for successful emulsification. The first suggestion is to use syringes with hydrophobic surfaces that will bind oil and not water, which induces the aqueous phase to form drops. Secondly, adding water in small aliquots will help the oil surround water. Thirdly, forcing the water phase into the oil phase will also contribute to oil surrounding a water droplet. A fourth suggestion is to *vigorously* mix the suspension to reduce the size of the water drops, which increases the emulsions stability.

Hunter's Titer Max #R-1 is a recently patented adjuvant containing three ingredients: CytRx's block copolymer CRL89-41; squalene; and a microparticulate stabilizer (CytRx corp. Norcross, GA). Copolymers are composed of blocks or chains of hydrophilic polyoxyethylene (POE) and blocks of hydrophobic polyoxypropylene (POP). Varying the length of the POE and POP chains can produce major changes in biological activity (Hunter et al., 1981). The CRL89-41 copolymer used in Titer Max was developed by CytRX corporation. Copolymers were found to stimulate macrophages (Howerton et al., 1990) and to activate complement (Hunter & Bennett, 1984). The activation of complement influences the

localization and retention of an antigen in lymphoid tissue and the activation of immunoreactive cells. A microparticulate stabilizer was incorporated into Titer-Max to stabilized the water-in-oil emulsion (Becher, 1966). Hunter et al. (1989) demonstrated in mice, rats, guinea pigs and rabbits that Hunter's Titer Max #R-1 produced significant titers against GnRH. Even though small laboratory animals may use this as an alternative adjuvant to FCA, the efficiently of Titer Max has not been demonstrated in ruminants.

Active Immunization against GnRH

If an animal is immunized against GnRH, antibody titers will increase and block the endogenous actions of GnRH to release gonadotropins. This will cause the cessation of estrous cycles, gonadal atrophy and degenerative spermatogenesis. Antibody titers that affect reproductive performance can be maintained or re-established after titers decline by booster immunizations. If antibody titers are allowed to decline, reproductive performance can be re-established. Immunization against GnRH could be an effective method to control fertility in males and females or to increase the pasture and feedlot performance of beef heifers due to the lack of estrous cycles and pregnancies.

Active Immunization in the Male

Luteinizing hormone in the male regulates steroidogenesis in the Leydig cells of the testis and stimulates testosterone production which is necessary for maintenance of spermatogenesis (Cunningham & Huckins, 1979; Awoniyi et al., 1989; Zirkin et al., 1989). Active immunization against GnRH has been achieved in male rats (Fraser et al., 1982b), rabbits (Arimura et al., 1973), dogs (Schanbacher et al., 1982; English et al., 1983), pigs (Falvo et al., 1986), sheep (Jeffcoate et al., 1982; Schanbacher, 1982) and cattle (Robertson et al., 1982; Jeffcoate et al., 1982; Schanbacher, 1984). Immunization of males against GnRH typically causes atrophy of the testes and a decrease in plasma concentrations of LH and testosterone (T), decreased libido, decreased GnRH binding to gonadotroph cells and decreased spermatogonial content in the testes. Spermatogenesis is dependent upon intratesticular concentration of T and sperm production is correlated with testis size (Amann & Schanbacher, 1983). Therefore, when immunization causes a decline in concentrations of LH and T, testis size will reduce and decreased spermatogenesis will ensue. Immunization of dogs against GnRH causes not only testicular atrophy, but also a collapse of seminiferous tubules with only a few spermatogonia and primary spermatocytes in the epithelium (Schanbacher et al., 1983). A decrease in libido has also been observed in rats (Fraser et al., 1974; Ladd et al.,

1989), rabbits (Arimura, 1973) and monkeys (Chappel et al., 1980) after immunization against GnRH.

The primary mode of action of antibodies against GnRH is probably through interception of GnRH in the pituitary portal vessels (Schanbacher, 1984). There is only a slight chance that antibodies may not be able to cross the blood-brain barrier to react with GnRH in the hypothalamic region (Hokfelt et al., 1976). Boars immunized against GnRH produced antibody titers that reduced plasma LH and T, resulting in atrophy of the testes and accessory sex glands. No effects were observed on average daily gain, hot carcass weight or loin eye area, but boar taint was reduced (Falvo et al., 1986).

Active immunization could have valuable applications in the field of fertility control of dogs and be an alternative to castration in pigs and cattle. Immunization would provide an alternative to surgical castration and problems associated with it such as hemorrhage, infection and weight loss.

Active Immunization in Females. Immunization against GnRH has been successful in ewes (Clarke et al., 1978; Adams and Adams, 1986; McNeilly et al., 1986), gilts (Esbenshade and Britt, 1985), heifers (Johnson et al., 1988; Wettemann and Castree, 1988; Adams and Adams, 1990a), mares (Garza et al., 1986), mice (Silversides et al., 1988), monkeys (McCormack et al., 1977; Fraser, 1983) and rats (Arimura et

al., 1974; Fraser and Baker, 1978). Immunization of females against GnRH will cause cessation of estrous cycles due to elimination of the preovulatory surge of LH.

Ewes immunized against GnRH had reduced pituitary stores of LH and FSH (Adams and Adams, 1986). Under a continual booster regimen, ovarian cyclicity was inhibited throughout two subsequent breeding seasons in immunized ewes (McNeilly et al., 1986). Active immunization against GnRH in gilts reduced LH, FSH and gonadal steroid levels sufficiently to produce acyclic performance by wk 10 of the experiment (Esbenshade and Britt, 1985). Feedlot heifers immunized against GnRH, had reduced weight gains (Adams and Adams, 1990a). However, it has been demonstrated that this *nonprofitable effect* can be reversed by supplemental implantation of Synovex H (Adams and Adams, 1990b). Mares immunized against GnRH had similar reproductive characteristics as seasonal anestrous mares (Garza et al., 1986). Active immunization of stumptailed macaque monkeys against GnRH produced titers that were capable of causing amenorrhea. Concentrations of luteinizing hormone and FSH declined with increasing antibody titers, causing a reduction in serum concentrations of 17 beta-estradiol and an increase in concentrations of progesterone (Fraser & McNeilly, 1983). The absence of ovulation in rats due to immunization against GnRH was identified by the cessation of regular cyclic patterns of vaginal smears and absence of luteal tissue (Fraser and Baker, 1978). Ovulation was

inhibited for six months in immunized rats, but follicular development was dependent upon titer concentrations (Koch, 1977). After 12 weeks post immunization, rat ovaries contained antral and cystic follicles. This suggests that folliculogenesis occurs up to the antral stage and becomes cystic due to the absence of the ovulatory LH surge.

When the pituitary is separated from hypothalamic stimulation, there is secretion of prolactin and hypertrophy and hyperplasia of mammotrophe cells (Nikitovitch-Winer and Everett, 1959; Clarke et al., 1983). Blockage of the hypothalamus stimulation of the pituitary by antibodies against GnRH does not affect prolactin stores in the rat (Fraser et al., 1975), gilts (Esbenshade and Britt, 1985) and ewes (Adams and Adams, 1986). Seasonal changes in concentrations of prolactin exist in ewes immunized against GnRH (Clarke et al., 1978). Concentrations of prolactin decreased at the onset of estrus in both control ewes and ewes immunized against GnRH. However, concentrations of prolactin in ewes immunized against GnRH increased significantly over control ewes within three months. It is hypothesized that the increased concentration of prolactin observed in ewes immunized against GnRH was due to the minimal concentrations of progesterone and the normal concentrations of estrogen.

Passive Immunization against GnRH. Passive immunization against GnRH can be achieved by infusing anti-

GnRH serum into the systemic circulation of an animal. The anti-GnRH serum can be obtained from actively immunized animals of the same species. Passive immunization is a technique to study the effects of GnRH by acutely blocking its endogenous actions. By use of passive immunization against GnRH, it has been demonstrated that LH secretion is dependent upon GnRH (Lincoln and Fraser, 1979). The estradiol induced surge of LH is a product of estrogenic action at hypothalamic and pituitary loci (Herman and Adams, 1990) and the secretion of estradiol from the preovulatory follicle is dependent upon the secretion of LH (McNeilly et al., 1984). The consequences of passively immunizing animals against GnRH is basically the same as active immunization, except where time of action is concerned. There is an immediate response of LH due to the neutralization of GnRH through passive immunization. Passive immunization of GnRH can prevent the preovulatory surge of LH in rats (Arimura et al., 1974) and ewes (Fraser and McNeilly, 1982 & 1983; Herman & Adams, 1990). Neutralization of GnRH in rams (Lincoln and Fraser, 1979) and hamsters (Bartke et al., 1987) by administration of antibodies against GnRH caused cessation of LH and testosterone secretions. Partial recovery of pituitary LH after administration of antisera against GnRH was observed after 24 days in rams. This is in agreement with studies in rats (Hauger et al., 1977).

FSH, in contrast to LH, is not dependent upon short-term changes in GnRH release. Injection of GnRH antibodies before the preovulatory gonadotropin surge will prevent the surge of FSH and LH in the rat (Arimura et al., 1974) and ewe (Fraser and McNeilly, 1982). The second increase in FSH, occurring on the morning of estrus in the rat and ewe, cannot be prevented by the administration of GnRH antibodies after the first FSH surge (Narayana and Dobson, 1979; Blake and Kelch, 1981). For this reason, most studies indicate that FSH is unaffected by administering antibodies against GnRH (Fraser and McNeilly, 1983; Herman & Adams, 1990). In a study by Herman and Adams (1990), suppression of FSH was a slow process that was not significant until 76 h after infusion of GnRH antiserum. They suggested that GnRH has a permissive role on the secretion of FSH. Bartke (1987) reported a significant reduction of plasma FSH in hamsters passively immunized against GnRH.

Passive immunization against GnRH can be used as a tool to investigate the physiological requirements of GnRH in reproduction. However, the amount of antiserum required and repeated injection make passive immunization unacceptable as a means of fertility control.

Immunization against Luteinizing Hormone

Immunization against LH has been successful in cyclic cows and heifers (De Silva et al., 1986; Johnson et al.,

1988; Roberts et al., 1990), rams (Schanbacher, 1985), bull calves (Schanbacher, 1985), boars (Falvo et al., 1986) and rats (Laurence & Ichikawa, 1968). The estrous cycle of cows was abolished by immunizing against LH without interrupting follicular growth and steroidogenesis (De Silva et al., 1986). Immunization of rats against bovine LH also resulted in suppressed ovulation, but no adverse follicular development (Laurence and Ichikawa, 1968).

Antibodies against LH in the sera of immunized bulls successfully neutralized the actions of endogenous LH (Schanbacher, 1985). Concentrations of testosterone were suppressed, testes size and weight gain were decreased and there was a lack of development of secondary sex characteristics. Only rams with the greatest titers against LH exhibited neutralized LH characteristics, suggesting a booster immunization was needed to reach the goal of immunocastration. Immunization of boars against LH had no effect on testicular weight and average daily gain (Falvo et al., 1986). Plasma LH was not determined, but there was a decline in concentrations of testosterone in plasma with a concomitant reduction in accessory sex gland weight. Boar taint was reduced in immunized animals (Falvo et al., 1986).

Reversibility of immunization against GnRH

Active immunization against GnRH will cause cessation of the estrous cycle in females and testis atrophy and

degenerative spermatogenesis in males. There have been attempts to reverse the consequences of immunization by pulsing GnRH or a GnRH analog into immunized animals.

Pulsatile administration of GnRH induces follicular growth that leads to ovulation in prepubertal gilts (Carpenter and Anderson, 1985; Lutz et al., 1985) as well as lactating and anestrous sows (Cox and Britt, 1982; Armstrong and Britt, 1985). An analog of GnRH causes similar actions as endogenous GnRH. Injections of some GnRH analogs do not cross-react with endogenous antibodies against GnRH produced after immunization and therefore will cause release of LH and FSH. However, levels of LH necessary to initiate ovulation are not achieved by injections of GnRH analogs in gilts immunized against GnRH (Clarke et al., 1978; Esbenshade and Britt, 1985; Traywick and Esbenshade, 1988). Other studies have tested different GnRH analogs, doses and lengths of administration to find a method of reversibility for immunized animals, and most combinations increase LH and FSH levels, but estrous cycles (Traywick & Esbenshade, 1988), steroidogenesis and testis weight (Sabeur & Adams, 1989) can not be restored to normal values. Administration of the GnRH analog, des-Gly^{10} GnRH ethylamide (100 ng/hr) increased LH secretion in ewes immunized against GnRH within two days, with concentrations of LH similar to those in control ewes by six days (Adams and Adams, 1986). Pulsatile GnRH administration for 72 or 144 hr (100 ng/2 hr) did not induce follicular growth or ovulation in gilts immunized

against GnRH (Traywick and Esbenshade, 1988). Pulsatile administration of an analog for 10 days (400 ng/hr) increased concentrations of serum LH and testosterone in rams, but testosterone concentrations were only partially restored with no increase in testicular weight (Sabeur and Adams, 1989). Exogenous gonadotropin administered for 9 d at 6 h intervals was not sufficient to stimulate follicular growth in gilts immunized against GnRH (Traywick and Esbenshade, 1988). Pulsatile administration of a GnRH analog increased the secretion of LH in passive immunized ewes. However, FSH concentrations were not influenced by the GnRH analog (Herman and Adams, 1990). O'Connell et al. (1990) found that pulsatile infusion of an analog to GnRH for 14 d increased concentrations of LH and LH pulse amplitude, but not to a concentration that would reverse the suppressive effects of immunization against GnRH on reproductive function.

Male rat pituitaries require endogenous GnRH exposure to maintain GnRH receptors (Frager et al, 1981; Clayton et al., 1982). A lack of GnRH receptors in animals immunized against GnRH suggests that administration of a GnRH analog may not cause LH to be synthesized and secreted at normal concentrations.

The only known way to reverse the actions of immunization is through a natural decline in antibody titers against GnRH. Estrous cycles in ewes immunized against GnRH were reinitiated at 1 to 2 years after immunization.

Conception and birth followed the onset of regained cycles with no detrimental effects on the offspring (Keeling and Crighton, 1984). Testicular regeneration was reported to occur in rams 1 to 2 years after immunization (Keeling and Crighton, 1984). Wettemann and Castree (1988) demonstrated that after estrous cycles of heifers that were immunized against GnRH were reinitiated, through a natural decline in GnRH antibody titers, booster immunizations caused cessation of estrous cycles for approximately two months. Upadhyay (1989) found that drastic atrophic changes in the epididymal ducts of rats indicate that prolonged immunization could cause irreversible testicular damage even though titers against GnRH declined. In conclusion, reversibility of immunization of animals against GnRH can be achieved through a natural decline in antibody titers. However, continual stimulation of antibody titers through booster immunizations may permanently affect an animals reproductive capabilities.

Many species can be actively and passively immunized against GnRH. Immunization of males against GnRH causes atrophy of the testes and a decrease in plasma concentrations of LH and T, decreased libido and decreased spermatogenesis in the testes. Immunization of females against GnRH causes cessation of estrous cycles due to elimination of the preovulatory surge of LH. Reversibility of immunization of animals against GnRH can only be achieved thorough a natural decline in antibody titers. In addition, continual stimulation of antibody titers through booster

immunizations may permanently affect an animals reproductive capabilities. Additional studies on different analogs, intensity of stimulation, and duration of treatment are needed to determine if administration of an analog can reverse the effects of immunization against GnRH.

CHAPTER III

IMMUNIZATION OF HEIFERS AGAINST GONADOTROPIN RELEASING HORMONE: EFFECTIVENESS OF ADJUVANTS

ABSTRACT

Prepuberal Angus x Hereford heifers at 11 ± 1 mo of age and 212 ± 16 kg BW were used to evaluate the effectiveness of four adjuvants for active immunization against GnRH. Heifers (n=30) were randomly allocated to five treatments. Control heifers were immunized against human serum albumin (HSA) emulsified in Freund's complete adjuvant (FCA). The other four adjuvant treatments contained GnRH conjugated to HSA (GnRH-HSA) emulsified in either FCA, Freund's incomplete adjuvant (FIA), DEAE dextran (DD) + mineral oil (MO) or DEAE dextran + FIA. Heifers were immunized subcutaneously and intradermally at six sites in the mammary gland. Booster immunizations were given at 3 and 12 wk after the primary immunization to enhance antibody production against GnRH. Freund's incomplete adjuvant was used for the booster immunization of the control, FCA and FIA treatment groups. Heifers on DD+MO and DD+FIA treatments were boostered with the same adjuvants used for primary immunizations. Jugular blood samples were obtained weekly for 29 wk from all heifers for determination of antibody titers against GnRH

and concentrations of LH and progesterone. An udder scoring system was devised to evaluate growth of granulomas at injection sites in the udder. Body condition scores (BCS) and body weight (BW; shrunk) were recorded monthly for 30 wk. Within two weeks after each booster, antibody titers against GnRH for heifers on FCA, DD+MO or DD+FIA treatments were greater than titers for heifers on control or FIA treatments ($P<.01$). Mean concentrations of LH in serum were not different between treatment groups. Luteal activity, defined as $\geq$ 2 ng/ml progesterone for two consecutive wk, was not significantly different between treatment groups. At the onset of puberty, titers against GnRH were greater in FCA, DD+FIA and DD+MO treated heifers (17,35,24% respectively) than in control and FIA heifers (4,6% respectively). However, titers against GnRH were insufficient to prevent ovulation. Mean BW was reduced in the FCA treated heifers compared with DD+MO, DD+FIA and FIA heifers. Differences in mean BW was not significant between control and DD+FIA immunized heifers, or between DD+MO and FIA treated heifers. BCS were not influenced by treatment. At wk 30, heifers immunized with DD+MO and DD+FIA had less granulomas at injection sites in the mammary glands than heifers treated with FCA ($P<.001$). We conclude that DD+MO and DD+FIA are effective adjuvants to immunize heifers against GnRH, producing a sufficient antibody response with minimal granuloma production at the injection site.

(Key Words: Adjuvants, Heifers, Immunization, GnRH)

Introduction

Gonadotropin releasing hormone (GnRH) is a decapeptide produced in the hypothalamus which controls the synthesis and secretion of gonadotropins. Immunization of heifers against GnRH results in delayed puberty (Wettemann and Castree, 1988; O'Connell and Wettemann, 1989). Ovulation is blocked in immunized heifers, due to decreased secretion of LH. LH secretion is necessary for the initiation of estrous cycles and the onset of puberty.

Since GnRH is a small peptide, it must be conjugated to a carrier protein and emulsified in an adjuvant to increase its immunogenicity. Carrier proteins enhance the immunogencity of GnRH by increasing its size (Fraser, 1980), while adjuvants enhance the immune response by slowing the release of the antigen into the body (Tizard et al., 1984). Freund's complete adjuvant is a satisfactory adjuvant (Goubau et al., 1989b; Wettemann and Castree, 1988). However, two problems associated with the use of FCA are granuloma production at the site of injection and a positive response to the tuberculin test (Robertson et al., 1984), due to the mycobacterial cell wall fraction in FCA. Use of DEAE dextran in combination with FIA, elicited a greater

response than if each adjuvant was used alone (Hoskinson et al., 1990).

Heifers growing on pasture or in the feedlot have less efficient gains compared with steers due to excess activity associated with estrous cycles or pregnancy. If puberty could be delayed in heifers, these problems could be reduced and efficiency of production would be increased. The objectives of this experiment were to determine the ability of four adjuvants to stimulate antibody production against GnRH and to determine which adjuvant caused the least inflammatory response at injection sites.

MATERIALS AND METHODS

Thirty prepuberal Angus x Hereford heifers, maintained on range conditions, weighing 212 $\pm$ 16 kg at 11 $\pm$ 1 month of age were utilized. Heifers were randomly allocated to five treatments. Control heifers were immunized with human serum albumin (HSA; 1.4 mg in 2 ml of saline/heifer; Sigma Chemical Co., St. Louis Mo.) emulsified in Freund's complete adjuvant (FCA; Sigma Chemical Co.). A 33% solution of DEAE dextran (Sigma Chemical Co., St. Louis Mo.) was prepared in saline. Immunized heifers received GnRH-HSA (50 ug of GnRH; Sigma Chemical Co.; conjugated to 1.4 mg of HSA in 2 ml saline) emulsified in FCA, Fruend's incomplete adjuvant (FIA; Sigma Chemical Co.), DEAE dextran + Mineral Oil (DD+MO) or DD+FIA (2 ml/heifer). Primary immunization were

given on wk 0. Primary immunization injections (a total of 4 ml) were given subcutaneously and intradermally at six sites in the mammary gland. Booster immunizations were given at 3 and 12 wk after the primary immunization to enhance antibody production against GnRH. FIA was used as the adjuvant to booster the control, FCA and FIA treatments. Booster immunizations for heifers on DD+MO and DD+FIA treatments were boostered with the same adjuvants used for the primary immunization.

Blood was obtained (30 ml) weekly by jugular venipuncture for 30 wk. Blood was divided into two tubes. One subsample (15 ml) was used for serum, and plasma was obtained from one tube by adding .1.3 mg of oxalic acid. Both tubes were placed on ice immediately after collection. Serum samples were maintained at 4^{o} C for 24 h, centrifuged (20 min, 2800 x g), and serum was decanted and stored at -20^{o} C until antibody titers against GnRH and LH concentrations (Bishop and Wettemann, 1993) were determined. Plasma samples were centrifuged (20 min 2800 x g) within 3 h after collection, decanted and stored at -20 C until progesterone concentrations were quantified by radioimmunoassay (Bishop and Wettemann, 1993).

BW (12-15 h shrunk) and BCS were recorded monthly. BCS were based on a scale from one to nine, with 1 = emaciated and 9 = obese (Wagner et al., 1988).

An udder scoring system was devised to record the production of granulomas. This system was based on a scale

from one to six; 1 = no granulomas present by sight or touch, 2 = few or small granulomas present by touch, 3 = many or large present by touch, 4 = few or small present by sight, 5 = many or large granulomas present by sight and 6 = open lesions. Mammary gland scores were recorded monthly for each heifer.

Heifers were actively immunized against GnRH that was conjugated to human serum albumin (GnRH-HSA) by the carbodiimide reaction (Fraser et al., 1974). HSA (25 mg) was added to a vial (12 ml) containing GnRH (25 mg; Sigma Chemical Co., St. Louis MO.), followed by 74.4 mg of 1-ethyl-3(3-dimethylamino)-propylcarbodiimide hydrochloride (JBL Scientific Inc., San Luis Obispo CA). GnRH-I^{125} (diluted in water, 82,000 cpm) was added to quantify incorporation of GnRH into the conjugate. The vial was capped, gently rotated and incubated at room temperature for 20 h. The vial was mixed after 8 and 16 h. After incubation, the contents were quantitatively transferred to a 10 inch dialysis tubing (Spectr, Por 3, MW cutoff 3,500, Baxter Scientific), placed in 2 l of deionized water and dialyzed twice for 24 h at 4^{o} C. After dialysis, the inside of the tubing was flushed with water and the conjugate was collected. Incorporation of GnRH in the conjugate was determined by quantifying I^{125} in the conjugate. About 4% of the GnRH used in the conjugation was attached to the HSA and remained in the dialysis tubing. Aliquots of the conjugate were frozen at -20^{o} C until used for immunization.

Antibody titers against GnRH were determined by the method of Esbenshade and Britt (1985). Serum was diluted 1:100 in ethylenediaminetetraacetate (EDTA)-phosphate buffered saline (PBS), (pH 7.0). One hundred ul of serum was placed in a 16 x 125 culture tube and diluted with 10 ml of EDTA-PBS (pH 7.0). Two hundred ul of the diluted serum from heifers or serum with known antibody titers (quality control standards) were added to culture tubes in duplicate. One hundred ul of I^{125}-GnRH (15,000 cpm) diluted in PBS + 0.01% gelatin were added to all tubes and tubes were incubated for 24 h at 4° C. The free ^{125}I-GnRH was separated from the antibody bound ^{125}I-GnRH by adding 1.5 ml of ethanol (4° C) to precipitate protein and bound GnRH. Tubes were then centrifuged (15 min, 2800 x g), and the supernatant was decanted and tubes were inverted to dry at 21° C for 12 h. Radioactivity in the precipitate was quantified with a Packard Multiprias Gamma counter. The percentage ^{125}I-GnRH bound was determined as the average for the duplicate tubes.

GnRH was iodinated with ^{125}I using the chloramine-T procedure. Three micrograms of GnRH suspended in 20 microliters of water and 25 microliters of phosphate buffer (.5 M) were combined with .75 mCi ^{125}I diluted in 7.5 microliters of water). Ten ul of chloramine-T (2mg/ml in H_2O) were added to the mixture and mixed during a 45 second reaction period. The reaction was stopped by the addition of 10 ul of sodium metabisulfite (10 mg/ml in H_2O). The

bound and free ^{125}I were separated by column chromatography. The column used was a 10 cc disposable glass pipette packed with LH-20 swelled in 0.05 M phosphate buffer (pH 7.1). The column was eluted with 0.05 M phosphate buffer (pH 7.1) prior to separation. The mixture was placed on the column in 250 ul of phosphate buffer (0.05 M, pH 7.1), and eluted with phosphate buffer (.05 M, pH 7.1) + 0.1% gelatin. One ml fractions were collected in tubes containing 1 ml PBS + 0.1% gelatin. Ten ul of each tube were counted in a Packard Gamma counter to determine which tubes contained the iodinated preparation.

Progesterone concentrations were quantified through a single antibody RIA in weekly plasma samples (Bishop and Wettemann, 1993). Short peaks of progesterone which are observed before puberty were eliminated from the statistical data set by utilizing concentrations of progesterone $\geq$ 2 ng/ml for two consecutive weeks to confirm onset of luteal activity.

Effects of treatment on the number of weeks to the onset of ovarian activity, BW, BCS and udder scores were analyzed by analyses of variance using the General Linear Models Procedure of SAS (SAS, 1985). Treatment differences at wk was determined through Duncan's t test. Antibody titers and LH concentrations were analyzed as split plot analyses of variance with treatment as the main effect and week as the subplot. Outlying LH concentrations were

identified and deleted through a program based on an outlier test (SAS, 1985).

Results

Immunization against GnRH-HSA induced production of antibodies against GnRH. Within three weeks after each booster, titers against GnRH were greater in heifers on FCA, DD+MO and DD+FIA treatments compared with heifers immunized against HSA (controls) or heifers immunized against GnRH-HSA using FIA ($P<.01$; Figure 1). At four months after the second booster (i.e., at wk 29), only DD+FIA heifers had titers against GnRH that were different ($P<.01$) from control heifers (Figure 1).

Weeks to the onset of puberty were not significantly affected by treatment (Figure 2). At the onset of puberty, titers against GnRH were greater in heifers on the FCA, DD+FIA and DD+MO treatment groups (17,35 and 24%, respectively) than in control and FIA treated heifers (4 and 6% respectively) (Figure 3). Although titers were greater in FCA, DD+FIA and DD+MO heifers, the titers had apparently declined to concentrations that were insufficient to prevent ovulation, so estrous cycles were initiated.

Mean concentrations of LH in serum of immunized and control heifers were not influenced by treatment (Figure 4).

Mean concentrations of LH averaged 1.9 ng/ml in heifers immunized against GnRH and 1.7 ng/ml in control heifers.

At wk 30, there was a significant difference between the weight of the FCA (327 kg) treatment compared with the FIA, DD+MO and DD+FIA (340, 348 and 337 kg, respectively) treatment groups (Figure 4). There was a treatment by wk interaction. This suggests that FCA may have an inhibitory effect on growth rate. Body condition scores were not influenced by treatment (Figure 5) and increased from an average of 4.67 $\pm$ .30 at wk 8 to 5.60 $\pm$ .42 at wk 26 and 5.27 $\pm$ .25 at wk 30.

Treatment influenced the amount of granuloma production at the sites of injection. There was a treatment by wk interaction. At wk 30, there was a significant difference between the four adjuvant groups and the production of granulomas. Heifers on the FIA, DD+FIA and DD+MO treatments had less granulomas than heifers treated with FCA ($P<.005$; Figure 6). However, FIA did not elicit an antibody response, leaving only DD+MO and DD+FIA treatments as satisfying the objective as an effective adjuvant.

In summary, FCA, DD+MO and DD+FIA caused significant production of antibodies against GnRH but heifers treated with DD+FIA or DD+MO had less inflammatory response compared with heifers given FCA. Therefore, we conclude that DD+FIA and DD+MO are effective adjuvants for immunizing heifers against gonadotropin releasing hormone.

DISCUSSION

Antibody production was observed in all heifers immunized against GnRH. Other studies have demonstrated that production of antibodies against GnRH alters gonad funtion. Antibody titers against GnRH ranging from 5 to 55% caused gonadal atrophy and dysfunction in rams (Chase et al., 1988). Heifers immunized against GnRH exhibited a direct relationship between antibody titer and length of delay in puberty (Wettemann & Castree, 1988). Antibody production is dependent upon the individual animal (Keeling and Crighton, 1984), as well as species (Johnson et al., 1988, Clarke et al., 1978; Esbenshade and Britt, 1985). Wide ranges in antibody production observed in ewes immunized against GnRH were attributed to genetic differences between individual animals (Keeling and Crighton, 1984). Heifers immunized against GnRH (Adams & Adams, 1990a; Johnson et al., 1988; Roberts et al., 1990) do not produce antibody titers that are as great as those observed for ewes (Clarke et al., 1978) and gilts (Esbenshade & Britt, 1985).

The onset of puberty was not significantly affected by treatment. This in is contrast to other studies in which puberty was delayed 20.5 wk (O'Connell, 1990) and 11 wk (Wettemann & Castree, 1988). Possible reasons for the lack of delay in puberty in this study, compared with the other studies, could be age and BW of the animals, conjugation and

emulsification variability, and/or the time of a third booster immunization . An additional booster prior to puberty may have increased antibody titers to a concentration that would suppress the onset of luteal activity. Another major difference between O'Connell (1990) and this study was the weight of heifers at the time of immunization. O'Connell (1990) immunized heifers against GnRH at 11 mo of age when they weighed 317 kg. In this study, we used heifers at 11 mo of age that weighed 212 kg. In O'Connell's study, estrous cycles were initiated in control heifers at 5.3 weeks after the primary immunization, compared with 19 weeks after the primary immunization in this study. O'Connell (1990) found that onset of luteal activity was delayed 20.5 weeks in heifers immunized against GnRH, whereas, onset of luteal activity was not delayed in this study. This suggests that weight is an important factor that influences age at puberty and the response to immunization against GnRH.

Mean concentrations of LH were not influenced by treatment. O'Connell (1990) also found that LH concentrations were not affected by immunizing heifers against GnRH. This is in disagreement with other species. Immunization against GnRH was associated with a reduction in LH concentrations in ewes and gilts (Clarke et al., 1978; Esbenshade and Britt, 1985). A possible cause for this difference, is low antibody titer production against GnRH in heifers. If antibodies produced against GnRH were fully

saturated by endogenous GnRH, there may be sufficient GnRH reaching the pituitary gonadotrophs to cause secretion of LH.

The FCA treatment group had reduced body compared with FIA, DD+MO and DD+FIA treatment groups. Adams and Adams (1990) also found a reduction in weight gain of heifers immunized against GnRH compared with control heifers that were not immunized. This suggests that FCA may have an inhibitory effect on growth rate.

Freund's complete adjuvant causes a local inflammatory response at the treatment site which results in the production of granulomas. A commercial vaccine utilizing FCA is not feasible due to the granuloma production associated with its use and positviely testing of the tuberculosis. Significantly less granuloma production was observed in the DD+MO and DD+FIA treatments.

Our results indicate that immunization of heifers against GnRH-HSA utilizing DD+MO or DD+FIA is comparable to FCA in antibody production and produces less granulomas at treatment sites.

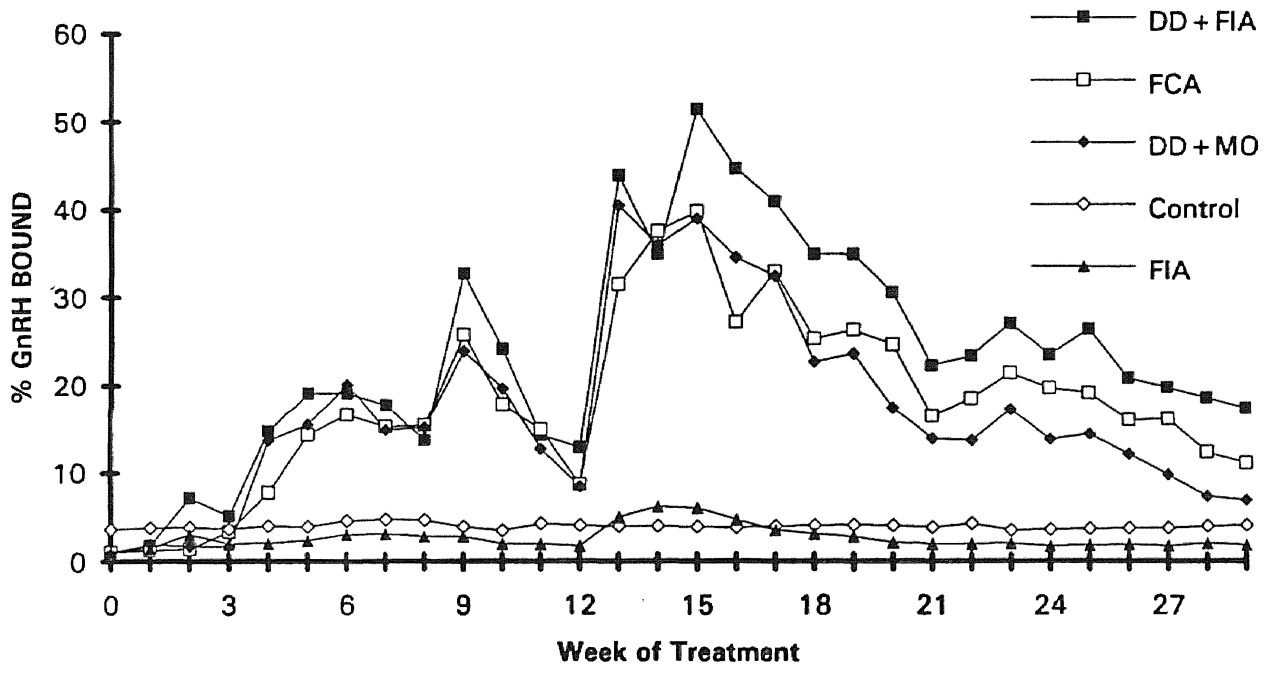

Figure 1. Effect of adjuvants on antisera titers against GnRH.

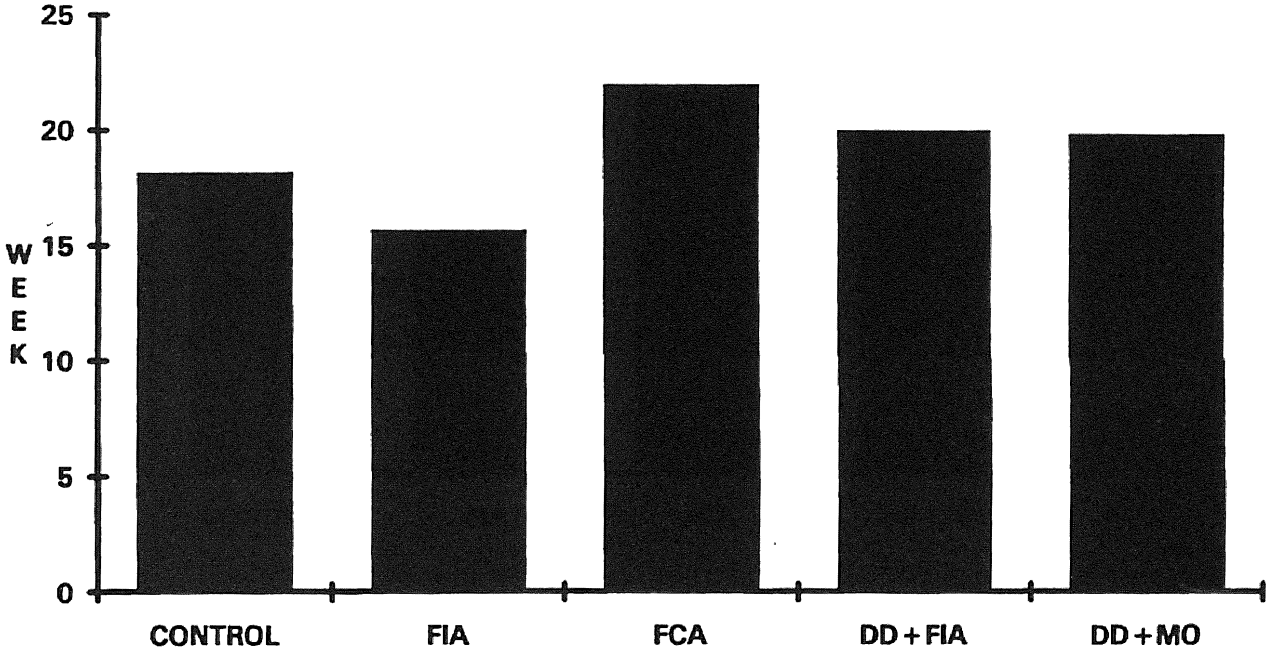

Figure 2. Week at which the onset of puberty was achieved in heifers immunized against GnRH.

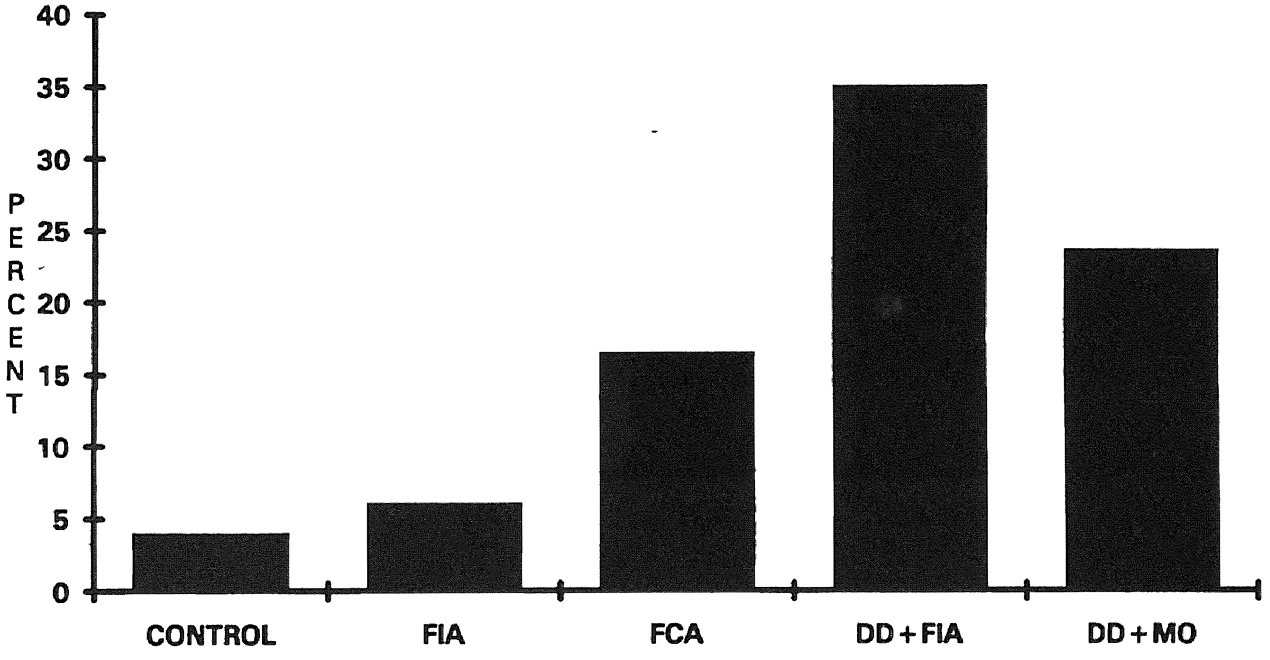

Figure 3. Antisera titers against GnRH at the onset of luteal activity in heifers.

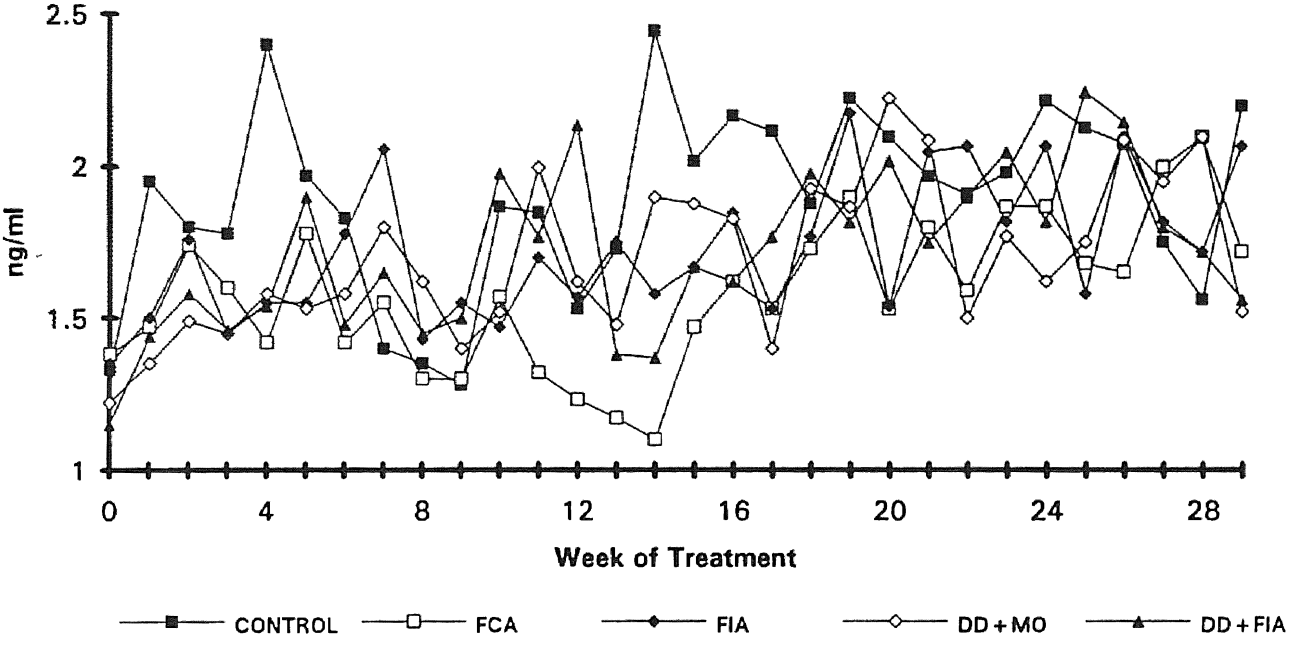

Figure 4. Effect of adjuvants on LH concentrations of immunized heifers.

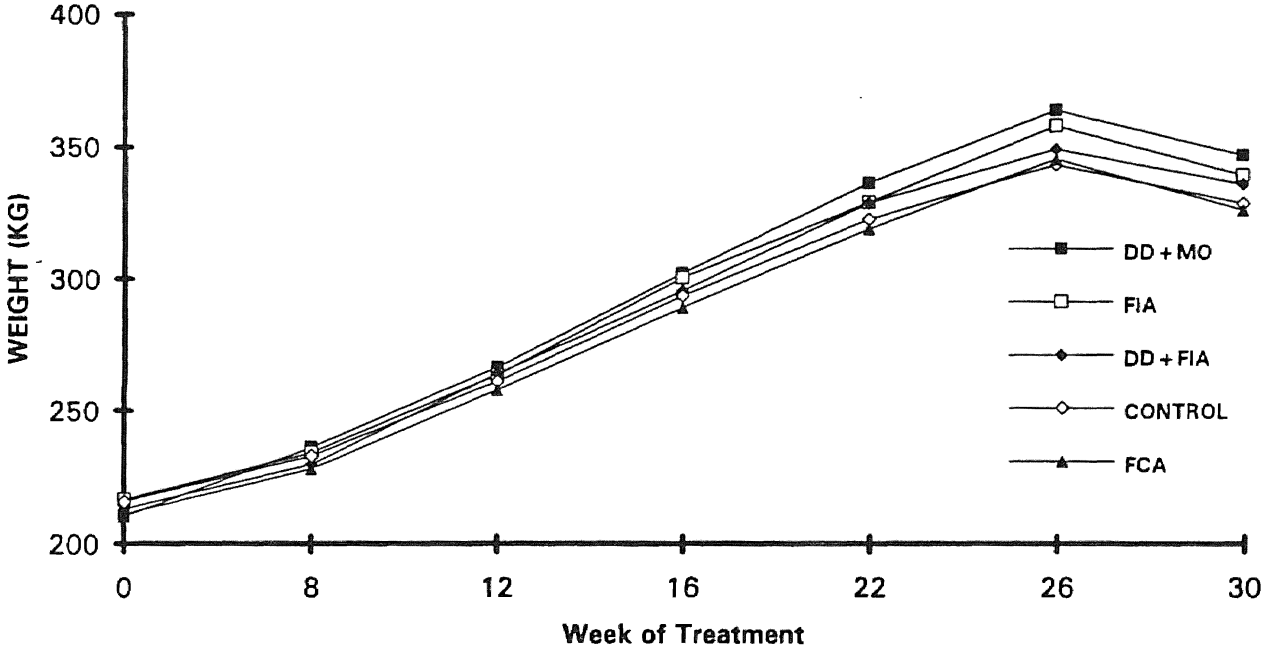

Figure 5. Effect of adjuvants on body weights of immunized heifers.

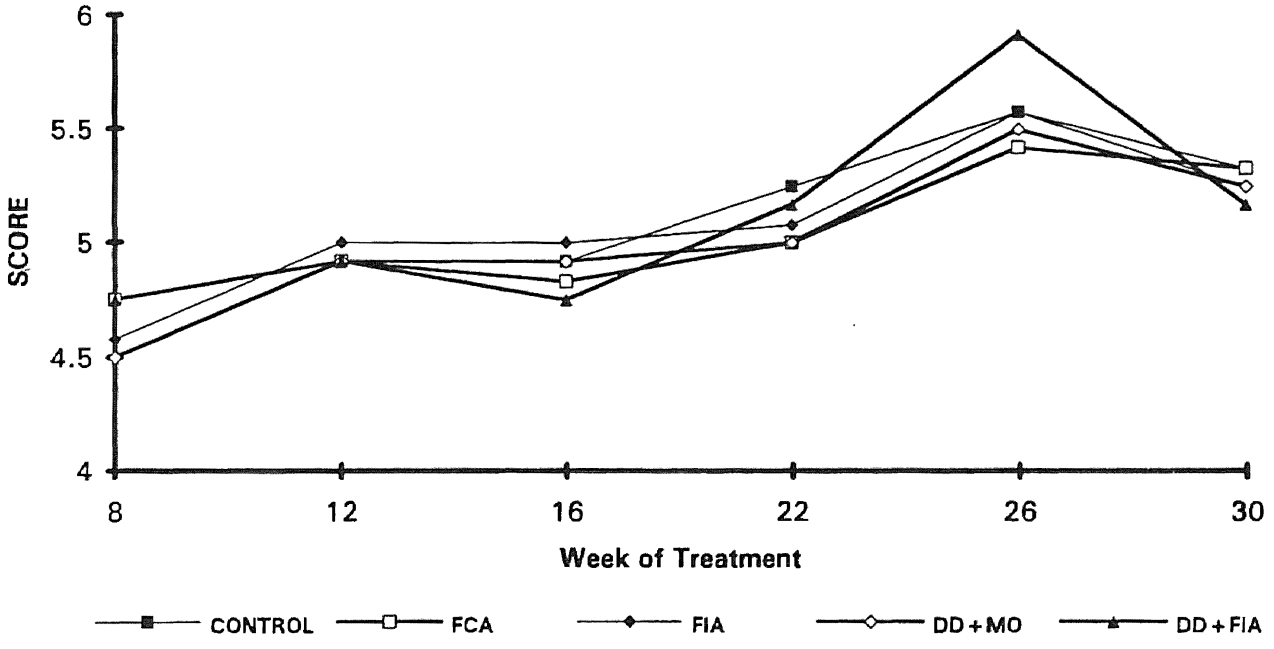

Figure 6. Effect of adjuvants on body condition score of heifers.

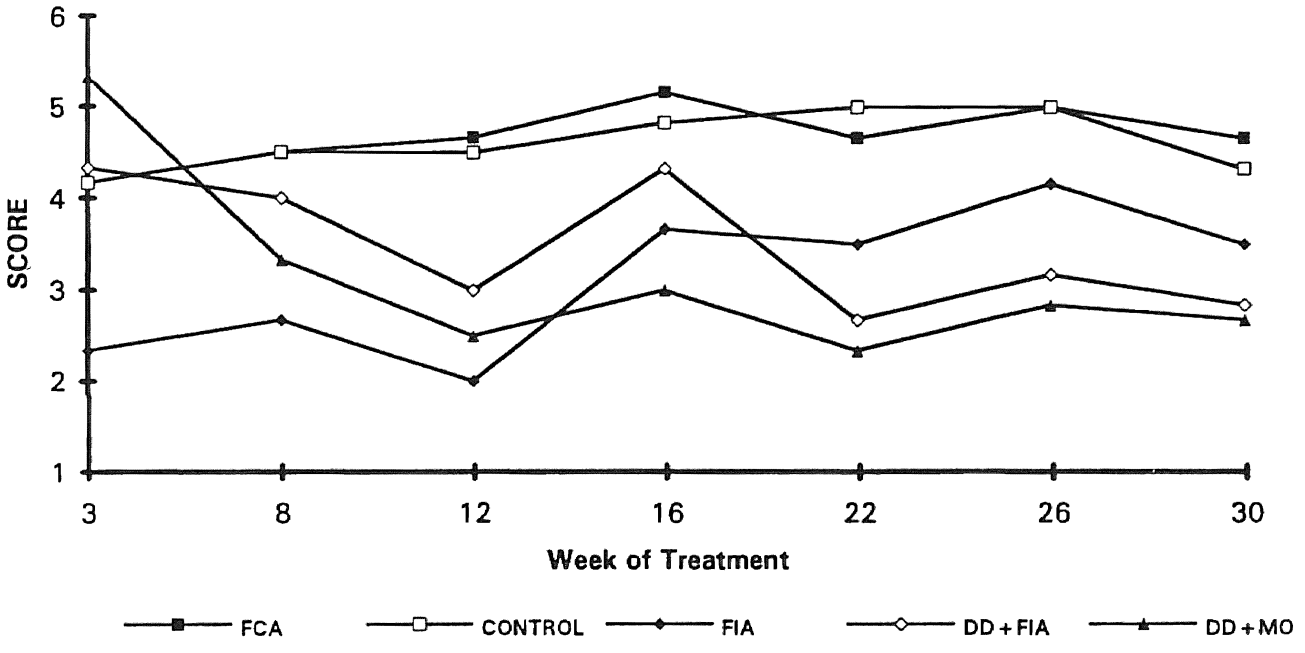

Figure 7. Effect of adjuvants on granuloma production in the mammary gland of control heifers and heifers immunized against GnRH.

IMPLICATIONS

Immunization against GnRH holds potential to induce temporary infertility in heifers. Immunization procedures utilizing FCA as an adjuvant cause granuloma production at treatment sites. Immunization against GnRH utilizing DD+MO or DD+FIA as an adjuvant causes sufficient production of antibodies with less granuloma production than FCA. Further studies are required to determine if immunization against GnRH could be an effective method to increase the pasture and feedlot performance in beef heifers by reducing the incidence of estrous cycles and pregnancies.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Gonadotropin releasing hormone is synthesized in the hypothalamus and regulates the synthesis and release of LH and FSH from the anterior pituitary. If the hypothalamus is separated from the pituitary gland, there is reduced LH and FSH secretion (Clarke et al., 1983). Blockage of GnRH function can be accomplished in animals through immunization against GnRH. Immunization of females against GnRH causes cessation of estrous cycles (Fraser et al., 1975). An adjuvant is used for immunization in combination with an antigen. The primary objective of an adjuvant is to cause a slow release of the adjuvant into the body and to stimulate the immune response. Freund's complete adjuvant is the most consistent and effective adjuvant to stimulate production of antibodies against GnRH. However, a major problem associated with the use of FCA is production of granulomas at the treatment sites.

Thirty prepubertal heifers were randomly allocated to five treatments. Control heifers were immunized with HSA emulsified in FCA. Heifers in the other four groups were immunized against GnRH-HSA emulsified in either Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA),

DEAE dextran (DD) + mineral oil (MO) or DD+FIA. Booster immunizations were given at weeks 3 and 12 after the primary immunization. Blood serum and plasma were obtained weekly by jugular venepuncture for 30 wk. Within two weeks after each booster, titers against GnRH were greater in heifers treated with FCA, DD+MO and DD+FIA compared with heifers immunized against HSA or treated with FIA. Only DD+FIA maintained significant antibody titers against GnRH for 29 wk. Weeks to the onset of puberty was not significantly affected by treatment likely due to a decline in antibody titers. There was a reduction in BW of the FCA treatment group compared with FIA, DD+MO and DD+FIA immunized heifers. Body condition scores were not influenced by treatment. Production of granulomas were reduced in the DD+FIA and DD+MO treated heifers compared with heifers treated with FCA (controls and GnRH-HSA).

In summary, FCA, DD+FIA and DD+MO are effective adjuvants to stimulate production of antibody titers against GnRH. However, there is a lower inflammatory response at treatment site for the DD+FIA and DD+MO treatments groups compared with FCA.

In conclusion, DD+FIA or DD+MO are effective adjuvants for immunizing heifers against GnRH, stimulating sufficient antibodies against GnRH with a minimal inflammatory response at injection site.

LITERATURE CITED

Adams, T.E. and B.M. Adams. 1986. Gonadotrope function in ovariectomized ewes actively immunized against gonadotropin-releasing hormone (GnRH). Biol. Reprod. 35:360.

Adams, T.E. and B.M. Adams. 1990a. Reproductive function and feedlot performance of beef heifers actively immunized against GnRH. J. Anim. Sci. 68:2793-2802.

Adams, T.E., J.R. Dunbar, S.L. Berry, W.N. Garrett, T.R. Famula and Y.B. Lee. 1990b. Feedlot performance of beef heifers implanted with Synovex-H: Effect of melengestrol acetate, ovariectomy or active immunization against GnRH. J. Anim. Sci. 68:3079-3085.

Amann, R.P. and B.D. Schanbacher. 1983. Physiology of male reproduction. J. Anim. Sci. 57:380-403 (Suppl. 2).

Arije, G.F and J.N. Wiltbank. 1971. Age and weight at puberty in Hereford heifers. J. Anim. Sci. 33(2):401-406.

Arimura, A., L. Debeljuk and A. Schally. 1972. Stimulation of FSH release by prolonged infusion of LH-RH. Endocrinology 11:2055.

Arimura, A., L. Debeljuk and A.V. Schally. 1974. Blockade of the preovulatory surge of LH and FSH and of ovulation by anti-LHRH serum in rats. Endocrinology 95:323-324.

Arimura, A., N. Nishi and A.V. Schally. 1976. Delayed implantation caused by administration of sheep immunogamma globulin against LHRH in the rat. Proc. Soc. Exp. Biol. Med. 152:71.

Arimura, A., H. Sato, T. Kumasaka, R.B. Worobec, L. Debeljuk, J. Dunn and A.V. Schally. 1973. Production of antiserum to LH-releasing hormone (LH-RH) associated with gonadal atrophy in rabbits: Development of radioimmunoassays for LHRH. Endocrinology 93:1092.

Armstrong, J.D. and J.H. Britt. 1985. Pulsatile administration of gonadotropin releasing hormone to anestrous sows: Endocrine changes associated with

GnRH-induced and spontaneous estrus. Biol. Reprod. 33:375.

Armstrong F.G., C.A. Ville and J.R. Ville. 1977. Characterization and comparison of estrogen and androgen receptors of calf anterior pituitary. J. Steroid Biochem. 8:285-92.

Awoniyi, C.A., R. Santulli, V. Chandrashekar, B.D. Schanbacher and B.R. Zirkin. 1989. Quantitative restoration of advanced spermatogenic cells in adult male rats made azoospermic by active immunization against luteinizing hormone or gonadotropin-releasing hormone. Endocrinology 125:1303-1309.

Baker, J.F., C.R. Long, G.A. Posada, W.H. McElhenney and T.C. Cartwright. 1989. Comparison of cattle of a five-breed diallel: Size, growth, condition and pubertal characters of second-generation heifers. J. Anim. Sci. 67:1218-1229.

Barkan, A., S. Regiani, J. Duncan, S. Papavasiliou and J.C. Marshall. 1983. Opiods modulate pituitary receptors for gonadotropin-releasing hormone. Endocrinology 112:387-392.

Bartke, A., B.D. Schanbacher, A.G. Amador, H.G. Klemcke and B. Chandrashekar. 1987. Effects of immunoneutralization of luteinizing hormone (LH)-releasing hormone on testicular prolactin and LH receptors in the golden hamster and on LH receptros in the Djungarian hamster. Endocrinology 121:2027-2034.

Becher, P. 1966. Emulsions: Theory and Practice, Second Edition. Reinhold Publishing Corporation, New York.

Beh, K.J. and A.K. Lascelles. 1985. The effect of adjuvants and prior immunization on the rate and mode of uptake of antigen into afferent popliteal lymph from sheep. Immunology 54:487-495.

Belchetz, P.E., T.M. Plant, Y. Nakai, E.G. Keogh and E. Knobil. 1978. Hypophysial responses to continuous and intermittent delivery of hypothalamic GnRH. Science 202:631.

Berardinelli, J.G., R.A. Dailey, R.L. Butcher, and E.K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49: 1276-1280.

Berardinelli, J.G., R. L. Fogwell and E. K. Inskeep. 1978. Effect of electrical stimulation or presence of a bull on puberty in beef heifers. Theriogenology. 9:133-141.

Berardinelli J.G., J.J. Ford, R.K. Christenson and L.L. Anderson. 1984. Luteinizing hormone secretion in ovariectomized gilts: Effects of age, reproductive state and estrogen replacement. J. Anim. Sci. 58:165-73.

Bercu, B.B., I.M.D. Jackson, C.T. Sawin, H. Safaii and S. Reichlin. 1977. Permanent impairment of testicular development after transient immunological blockade of endogenous luteinizing hormone releasing hormone in the neonatal rat. Endocrinology 101:1871-1879.

Bergman, A.J. and C.W. Turner. 1942. Gonadotropic hormone in AP of male and female rabbits during growth. Endocrinology 30:11.

Bhanot R., and M. Wilkinson. 1983. Opiatergic control of gonadotropin secretion during puberty in the rat: A neurochemical basis for the hypothalamic Gonadostat?. Endocrinology 113:596-603.

Bishop, D.K. and R.P. Wettemann. 1993. Pulsatile infusion of gonadotropin-releasing hormone initiates luteal activity in nutritionally anestrous beef cows. J. Anim. Sci. (Submitted)

Blake, C.A. and R.P. Kelch. 1981. Administration of antiluteinizing hormone-releasing hormone serum to rats: Effects on periovulatory secretion of luteinizing hormone and follicle-stimulating hormone. Endocrinology 109:2175-2179.

Blank, M.S., E. Panerai and H.G. Friesen. 1979. Opiate peptides modulate luteinizing hormone secretion during sexual maturation. Science 203:1129.

Bolton, R.C., R.R. Frahm, J.W. Castree and S.W. Coleman. 1987. Genotype x environment interactions involving proportion of brahman breeding and season of birth. II. Postweaning growth, sexual development and reproductive performance of heifers. J. Anim. Sci. 65:48-55.

Bronson F.H. 1986. Food-restricted, prepubertal, female rats: Rapid recovery of luteinizing hormone pulsing with excess food, and full recovery of pubertal development with gonadotropin-releasing hormone. Endocrinology 118:2483-2484.

Bronson, F.H. and C. Desjardins. 1974. Circulating concentrations of FSH, LH, estradiol and progesterone associated with acute, male-induced puberty in female mice. Endocrinology 94: 1658.

Bronson, F.H. and W.K. Whitten. 1968. Oestrus-accelerating pheromone of mice: Assay, androgen-dependency and presence in bladder urine. J. Reprod. Fert. 15: 131-134.

Brooks, A.L., R.E. Morrow and R.S. Youngquist. 1985. Body composition of beef heifers at puberty. Theriogenology 24(2):235-250.

Brooks P.H. and D.J.A. Cole. 1970. The effect of the presence of a boar on the attainment of puberty in gilts. J. Reprod. Fert. 23:435-440.

Bushmich, S.L., R.D. Randel, M.M. McCartor and L.H. Carroll. 1980. Effect of dietary monensin on ovarian response following gonadotropin treatment in prepuberal heifers. J. Anim. Sci. 51(3):692-697.

Carpenter, L.S. and L.L. Anderson. 1985. Pulsatile infusion of luteinizing hormone secretion and ovarian function in prepuberal gilts. Anim. Reprod. Sci. 9:261.

Chappel, S.C., W.E. Ellinwood, C. Huckins, D.C. Herbert and H.G. Spies. 1980. Active immunization of male rhesus monkeys against luteinizing hormone releasing hormone. Biol. Reprod. 22:333-342.

Chase, D.J., B.D. Schanbaacher and D.D. Lunstra. 1988. Effects of pulsatile and continuous luteinizing hormone (LH) infusions on testosterone responses to LH in rams actively immunized against gonadotropin-releasing hormone. Endocrinology 123:816.

Chenoweth, P.H. 1983. Reproductive management procedures in control of breeding. Anim. Prod. Aust. 15:28.

Ching, M. 1983. Morphine suppresses the proestrous surge of GnRH in pituitary portal plasma of rats. Endocrinology 112:2209-2211.

Clarke, I.J. and J.T. Cummins. 1982. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology 111:1737-1739.

Clarke, I.J., J.T. Cummins and D.M. de Kretser. 1983. Pituitary gland function after disconnection from direct hypothalamic influences in the sheep. Neuroendocinology 36:376-385.

Clarke, I.J., J.T. Cummins, J.K. Findlay, K.J. Burman and B.W. Doughton. 1984. Effects on plasma luteinizing hormone and follicle-stimulating hormone of varying the

frequency and amplitude of gonadotropin-releasing hormone pulses in ovariectomized ewes with hypothalamic-pituitary disconnection. Neuroendocrinology 39:214.

Clarke, I.J., H.M. Fraser and A.S. McNeilly. 1978. Active immunization of ewes against luteinizing hormone releasing hormone and its effects on ovulation and gonadotropin, prolactin and ovarian steroid secretion. J. Endocrinology 78:39.

Clayton, R.N., R.M. Popkin and H.M. Fraser. 1982. Hypothalamic regulation of pituitary gonadotropin-releasing hormone receptors: Effects of gonadotropin-releasing hormone immunoneutralization. Endocrinology 110:1116.

Colby, D.R. and J.G. Vandenberg. 1974. Regulatory effects of urinary pheromones on puberty in the mouse. Biol. Reprod. 11:268-279.

Conn, P.M., C.A. McArdle, W.V. Andrews and W.R. Huckle. 1987. The molecular basis of gonadotropin-releasing hormone (GnRH) action in the pituitary gonadotrope. Biol. Reprod. 36:17-35.

Conn, P.M., D.C. Rogers and S.G. Seay. 1984. Biphasic regulation of the gonadotropin-releasing hormone receptor by receptor microaggregation and intracellular calcium levels. Mol. Pharmacol. 25:51.

Copeland, K.C., M.L. Aubert, J. Rivier and P.C. Sizonenko. 1979. Luteinizing hormone releasing hormone: Sequential verses conformational specificity of antiluteinizing hormone releasing hormone sera. Endocrinology 104:1504-1512.

Cox, N.M. and J.H. Britt. 1982. Pulsatile administration of GnRH to lactating sows: Endocrine changes associated with induction of fertile estrus. Biol. Reprod. 27:1126-1137.

Crichton, J.A., J.N. Aitken and A.W. Boyne. 1959. The effect of plane of nutrition during rearing on growth, production, reproduction and health of dairy cattle. I. Growth to 24 months. Animal Production 1:145-162.

Crowder, M.E. and T.M. Nett. 1984. Pituitary content of gonadotropins and receptors for gonadotropin releasing hormone (GnRH) and hypothalamic content of GnRH during the periovulatory period of the ewe. Endocrinology 114:234-239.

Culler, M.D. and A. Negro-Vilar. 1986. Evidence that pulsatile follicle-stimulating hormone secretion is independent of endogenous luteinizing hormone-releasing hormone. Endocrinology 118(2):609-612.

Cunningham, G.R. and C. Huckins. 1979. Persistence of complete spermatogenesis in the presence of low intratesticular concentrations of testosterone. Endocrinology 105:177.

Cushwa, W.T., G.E. Bradford, G.H. Stabenfeldt and Y.M. Berger, M.R. Dally. 1992. Ram influence on ovarian and sexual activity in anestrous ewes: Effects of isolation of ewes from rams before joining and date of ram introduction. J. Anim. Sci. 70(4):1195-1200.

Dalkin, A.C., D.J. Haisenleder, G.A. Ortolano, T.R. Ellis and J.C. Marshall. 1989. The frequency of gonadotropin-releasing hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression. Endocrinology 125(2): 917-924.

Day, M.L., K. Imakawa, M. Garcia-Winder, D.D. Zalesky, B.S. Schanbacher, R.J. Kittok and J.E. Kinder. 1984. Endocrine mechanisms of puberty in heifers: Estradiol negative feedback regulation of luteinizing hormone secretion. Biol. Reprod. 31:332-341.

Day, M.L., K. Imakawa, P.L. Wolfe, R.J. Kittok and J.E. Kinder. 1987. Endocrine Mechanisms of puberty in heifers. Role of hypothalamo-pituitary estradiol receptors in the negative feedback of estradiol on luteinizing hormone secretion. Biol. Repro. 37:1054-1065.

Day, M.L., K. Imakawa, D.D. Zalesky, R.J. Kittok and J.E. Kinder. 1986. Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to Luteinizing Hormone-Releasing Hormone in heifers. J. Anim. Sci. 62:1641-1648.

De Greef, W.J., A.M. Eilers, J. de Koning, B. Karels and F.H. de Jong. 1987. Effects of ovarian inhibin on pulsatile release of gonadotropins and secretion of LHRH in ovariectomized rats: Evidence against a central action of inhibin. J. Endocrinology 113:449-455.

De Silva, M., L. Tjoelker, C.F. Chang and J.J. Reeves. 1986. Ovarian response to active immunization against luteinizing hormone in the cow. Theriogenology 26:89.

Desjardins, C. and H.D. Hafs 1968. Levels of pituitary FSH and LH in heifers from birth through puberty. J. Anim. Sci. 27:472-447.

Docke F., W. Rohde, P. Gerber and G. Dorner. 1981. Further studies on the hypothalamic desensitization to estrogen in immature female rats evidence for a possible role in the control of puberty. Endocrinology 77:1-12.

Docke, F., W. Rohde, P. Gerber and G. Dorner. 1984. Evidence that desensitization to the negative estrogen feedback is a prepubertal and not a postpubertal event in female rats. Exp. Clin. Endocrinol. 84:1-6.

Dodson, S.E., B.J. McLeod, W. Haresign, A.R. Peters and G.E. Lamming. 1988. Endocrine changes from birth to puberty in the heifer. J. Reprod. Fert. 82:527-538.

Dow, J.S. Jr., J.D. Moore, C.M. Bailey and W.D. Foote. 1982. Onset of puberty in heifers of diverse beef breeds and crosses. J. Anim. Sci. 55(5):1041-1047.

Drymundsson, O. R. and J.L. Lees. 1972. Effect of rams on the onset of breeding activity in Clun Forest Ewe Lambs. J. Agr. Sci. 79:269.

Duello, T.M. and T.M. Nett. 1980. Uptake, localization and retention of gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs in rats. Mol. cell Endocrinology 19:101-112.

Ebling, J.P., M.L. Schwartz and D.L. Foster, 1989. Endogenous opioid regulation of pulsatile luteinizing hormone secretion during sexual maturation in the female sheep. Endocrinology 125:369-383.

Eide, D.M., T. Adnoy and J.J. Larsen. 1992. Selection for immune response in goats: The effect of immunization procedure on antibody response to diptheria toxoid and human serum albumin. J. Anim. Sci. 70:1432-1438.

English, H.G., B.D. Schanbacher, D. Gross, M.F. Walker, R.E. Falvo and R.J. Santen. 1983. Animal model of isolated gonadotropin deficiency. II. Morphologic responses to LHRH immunoneutralization. J. Androl. 4:233-239.

Esbenshade, K.L. and J.H. Britt. 1985. Active immunization of gilts against gonadotropin-releasing hormone: Effects on secretion of gonadotropins, reproductive function and responses to agonists of gonadotropin-releasing hormone. Biol. Reprod. 33:569.

Falvo, R.E., V. Chandrashekar, R.D. Arthur, A.R. Kuenstler, T. Hasson, C. Awoniyi and B.D. Schanbacher. 1986.

Effect of active immunization against LHRH or LH in boars: Reproductive consequences and performance traits. J. Anim. Sci. 63:986.

Ferrell, C.L. 1982. Effects of postweaning rate of gain on onset of puberty and productive performance of heifers of different breeds. J. Anim. Sci. 55(6):1272-1283.

Foster, D.L., J.A. Lemons, R.B. Jaffe and G.D. Niswender. 1975. Sequential patterns of circulating luteinizing hormone and follicle-stimulating hormone in female sheep from early post-natal life through the first estrous cycles. Endocrinology 97:985-994.

Foster, D.L. and K.D. Ryan. 1979. Endocrine mechanisms governing transition into adulthood: A marked decrease in inhibitory feedback action of estradiol on tonic secretion of luteinizing hormone in the lamb during puberty. Endocrinology 105: 896-904.

Foster, J.P. 1978. Plasma LH concentrations after single or double injections of synthetic LH-RH in dairy cows. J. Reprod. Fertil. 54:119.

Frager, M.S., D.R. Pieper, S. Tonetta, J.A. Duncan and J.C. Marshall. 1981. Pituitary gonadotropin-releasing hormone receptors: Effects of castration, steroid replacement, and the role of gonadotropin-releasing hormone in modulating receptors in the rat. J. Clin. Invest. 67:615.

Fraser, H.M. 1980. In Immunological Aspects of Reproduction and Fertility Control, pp 143-171. Ed. by Hearn, J.P. MTP Press, Lancaster.

Fraser, H.M. 1983. Active immunization of stumptailed macaque monkeys against luteinizing hormone releasing hormone, and its effects on menstrual cycles, ovarian steroids and positive feedback. J. Reprod. Immunol. 5:173-183.

Fraser, H.M. and T.G. Baker. 1978. Changes in the ovaries of rats after immunization against luteinizing hormone releasing hormone. J. Endocrinol. 77:85-93.

Fraser, H.M., A. Gunn, S.L. Jeffcoate, and D.T. Holland. 1974. Effect of active immunization to luteinizing hormone releasing hormone on serum and pituitary gonadotropins, testes and accessory sex organs in the male rat. J. Endocrinol. 63:399-406.

Fraser, H.M., S.L. Jeffcoate, A. Gunn and D.T. Holland. 1975. Effect of active immunization to luteinizing

hormone releasing hormone on gonadotropin levels in ovariectomized rats. J. Endocrinol. 64:191.

Fraser, H.M. and A.S. McNeilly. 1982a. Effect of immunoneutralization of luteinizing hormone and follicle-stimulating hormone surges in the ewe. Biol. Reprod. 27:548-555.

Fraser, H.M. and A.S. McNeilly. 1983. Differential effects of LH-RH immunoneutralization on LH and FSH secretion in the ewe. J.Reprod. Fertil. 69:569-577.

Fraser, H.M., R.M. Sharpe, G.A. Lincoln and A.J. Harmer. 1982b. LHRH antibodies: Their use in the study of hypothalamic LHRH and testicular LHRH-like material, and possible contraceptive applications. In Progress Towards a Male Contraceptive, pp 41-78. Ed. by Jeffcoate, S.L. and Sandler, M. John Wiley and Sons, Bristol.

Frisch, R.E., D.M. Hegsted and K. Yoshinga. 1977. Carcass components at first estrus of rats on high-fat and low-fat diets: Body water, protein and fat. Proc. Nat. Acad. Sci. 74:379.

Frisch, R.E. and R. Revelle. 1970. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science 169:397-398.

Gallo, R.V. 1980. Neuroendocrine regulation of pulsatile LH release in the rat. Neuroendocrinology 35:63.

Garza, F. Jr., D.L. Thompson, Jr., D.D. French, J.J. Wiest, R.L. St. George, K.B. Ashley, L.S. Jones, P.S. Mitchell and D.R. McNeill. 1986. Active immunization of intact mares against gonadotropin-releasing hormone: Differential effects of secretion of luteinizing hormone and follicle-stimulating hormone. Biol. Reprod. 35:347.

Garza, G. Jr., D.L. Thompson, Jr., P.S. Mitchell and J.J. Wiest. 1988. Effects of active immunization against gonadotropin releasing hormone on gonadotropin secretion after ovariectomy and testosterone propionate administration to mares. J. Anim. Sci. 66:479-486.

Gonzalez, A., N.D. Greene and B.D. Murphy. 1991. Active immunization against GnRH in mice and rats: adjuvant B, an alternative to Freund's adjuvant. J. Anim. Sci. 69(supplement 1) 396.

Gonzalez-Padilla, E., J.N. Wiltbank and G.D. Niswender. 1975. Puberty in beef heifers. I. The

interrelationship between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. 40:1091-1104.

Goodman, R.L. and F.J. Karsch. 1980. Pulsatile secretion of luteinizing hormone: Differential suppression by ovarian steroids. Endocrinology 107:1286-1290.

Goubau, S., D.W. Silversides, A. Gonzalez, B. Laarveld, R.J. Mapletoft and B.D. Murphy. 1989a. Immunization of sheep against modified peptides of gonadotropin releasing hormone conjugated to carriers. Dom. Anim. Endocrinology. 6:339-347.

Goubau, S., D.W. Silversides, A. Gonzalez, B. Laarveld, R.J. Mapletoft and B.D. Murphy. 1989b. Immunization of cattle against modified peptides of gonadotropin releasing hormone conjugated to carriers: Effectiveness of Freund's and alternative adjuvants. Theriogenology 32:557.

Grady, R.R., L. Shin, M.C. Charlesworth, I.R. Cohen-Becker, M. Smith, C. Rivier, J. Rivier, W. Vale and N.B. Schwartz. 1985. Differential suppression of follicle-stimulating hormone and luteinizing hormone secretion *in vivo* by a gonadotropin-releasing hormone antagonist. Neuroendocrinology 40:246.

Granger, A.L., W.E. Wyatt, W.M. Craig, D.L. Jr., Thompson and F.G. Hembry. 1989. Effects of breed and wintering diet on growth, puberty and plasma concentrations of growth hormone and insulin-like growth factor 1 in heifers. Dom. Anim. Endocrinology 6(3):253-262.

Grass J.A., P.J. Hansen, J.J. Rutledge and E.R. Hauser. 1982. Genotype x environmental interactions of reproductive traits of bovine females. I. Age at puberty as influenced by breed, breed of sire, dietary regimen and season. J. Anim. Sci. 55;1441-1457.

Greer, R.C. 1984. Effect of daylength at birth and lunar phase on the occurrence of first oestrus in beef heifers. Anim. Prod. 39:59-63.

Greer, R.C., R.W. Whitman, R.B. Staigmiller and D.C. Anderson. 1983. Estimating the impact of management decisions on the occurrence of puberty in beef heifers. J. Anim. Sci. 56(1):30-39.

Gregg, D.W. and T.M. Nett. 1989. Direct effects of estradiol-17beta on the number of gonadotropin-releasing hormone receptors in the ovine pituitary. Biol. Reprod. 40:288.

Gregory, K.E., D.B. Laster, L.V. Cundiff, R.M. Koch and G.M. Smith. 1978. Heterosis and breed maternal and transmitted effects in beef cattle. II. Growth rate and puberty in females. J. Anim. Sci. 47(5):1042-1053.

Grubbs, F.E. Sample criterion for testing outlying observations. In: Annals of Mathematical Statistics, Vol. 21 pp 27-58.

Haisenleder, D.J., S. Khoury, S.M. Zmeili, S. Papavasiliou, G.A. Ortolano, C. Dee, J.A. Duncan and J.C. Marshall. 1987. The frequency of gonadotropin-releasing hormone secretion regulates expression of alpha and luteinizing hormone beta subunit messenger ribonucleic acid in male rats. Mol. Endocrinology 1:834.

Hamernik, D.L., M.E. Crowder, J.H. Nilson and T.M. Nett. 1986. Measurement of mRNA for luteinizing hormone beta-subunit, alpha-subunit, growth hormone and prolactin following hypothalamic-pituitary disconnection in ovariectomized ewes. Endocrinology 119:2704.

Haney, M.E., Jr. and M.M. Hoehn. 1967. Monensin, a new biologically active compound. I. Discovery and isolation. Antimicrob. Agents Chemother 349.

Hansen P.J., L.A. Kamwanja, E.R. Hauser. 1983. Photoperiod influences age at puberty of heifers. J. Anim. Sci. 57:985-991.

Hansen P.J., L.A. Kanwanja and E. R. Hauser. 1982. The effect of photoperiod on serum concentrations of luteinizing and follicle stimulating hormones in prepubertal heifers following ovariectomy and estradiol injection. Theriogenology 18:551-559.

Hauger, R.L., R.P. Kelch, Y.I. Chen and A.H. Payne. 1977. Testicular receptors for luteinizing hormone after immuno-neutralization of gonadotropin releasing hormone in the rat. J. Endocrinol. 75:23-32.

Hays, R.L. and C.H. Carlevaro. 1959. Induction of estrus by electrical stimulation. Am. J. Physiol. 196:899.

Herman, M.E. and T.E. Adams. 1990. Gonadotropin secretion in ovariectomized ewes: Effect of passive immunization against gonadotropin-releasing hormone (GnRH) and infusion of a GnRH agonist and estradiol. Biol. Reprod. 42:273-280.

Hokfelt, T., O. Johansson, K. Fuxe, M. Goldstein, D. Park, R. Ebstein, H.M. Fraser, S.L. Jeffcoate, S. Efendic, R.

Luft and A. Arimura. 1976. Proceedings of the Sixth International Congress of Pharmacology 3:93-99.

Hollandbeck, R., B. Baker, H.W. Norton and A.V. Nalbandov. 1956. Gonadotrophic hormone content of swine pituitary glands in relation to age J. Anim. Sci. 15:418.

Hoskinson, R.M., R.D.G. Rigby, P.E. Mattner, V.L. Huynh, M. D'Occhio, A. Neish, T.E. Trigg, B.A. Moss, M.J. Lindsey, G.D. Coleman and C.L. Schwartzoff. 1990. Vaxstrate: An anti-reproductive vaccine for cattle.

Houseknecht, K.L., D.R. Campion, J.L. Sartin, T.E. Kiser, G.B. Rampacek and H.E. Amos. 1988. Effect of dietary energy source and level on serum growth hormone, insulin-like growth factor 1, growth and body composition in beef heifers. J. Anim. Sci. 66:2916-2923.

Howerton, D.A., R.L. Hunter, H.K. Ziegler and I.J. Clarke. 1990. Induction of macrophage Ia expression in vivo by synthetic block copolymer, L81. J. Immunol 144:1578-1584.

Hunter, R.L. and B. Bennett. 1984. The adjuvant activity of nonionic block polymer surfactants. II. Antibody formation and inflammation related to the structure of triblock and octablock copolymers. J. Immunology 133:3167-3175.

Hunter, R.L., B. Bennett, D. Howerton, S. Buynitzky and I.J. Check. 1989. Nonionic block copolymer surfactants as immunological adjuvants: Mechanisms of action and novel formulations. In Immunological Adjuvants and Vaccines. G. Gregoriadis, AC Allison, G. Poste, editors. Plenum Press, New York. pp133-144.

Hunter, R., F. Strickland and F. Kezdy. 1981. The adjuvant activity of nonionic block copolymer surfactants. I. The role of hydrophile-lipophile balance. J. Immunol. 127:1244-1250.

Izard M.K. and J.G. Vandenbergh. 1982. The effects of bull urine on puberty and calving date in crossbred beef heifers. J. Anim. Sci. 55:1160-1168.

Jeffcoate, I.A. and B.J. Keeling. 1984. Active immunization against LH-RH in the female. In: D.B. Crighton (Ed.) Immunological Aspects of Reproduction in Mammals. pp 363-377. Butterworths, London.

Jeffcoate, I.A., J.M.S. Lucas and D.B. Crighton. 1982. Effects of active immunization of ram lambs and bull

calves against synthetic luteinizing hormone releasing hormone. Theriogenology 18:65-77.

Jeffcoate, S.L., D.T. Holland and H.M. Fraser. 1976. In Physiological Effects of Immunity Against Hormones, pp 121-136. Ed. by Edwards, R.G. and Johnson, M.H. Cambridge University Press, Cambridge.

Johnson, H.E., D.M. DeAvila, C.F. Chang and J.J. Reeves. 1988. Active immunization of heifers against luteinizing hormone releasing hormone, human chorionic gonadotropin and bovine luteinizing hormone. J. Anim. Sci. 66:719.

Kato J., Y. Atsumi and M.Inaba. 1974. Estradiol receptors in female rat hypothalamus in the development stages and during pubescence. Endocrinology 94:309-17.

Keeling, B.J. and D.B. Crighton. 1984. Reversibility of the effects of active immunization against LH-RH. In: D.B. Crighton (Ed.) Immunological Aspects of Reproduction in Mammals. pp 379-397. Butterworths, London.

Kelley, K.W. 1980. Stress and immune function: A bibliographic review. Ann. Rech. Vet 11:445.

Kerdelhue, B., S. Catin, C. Kordon and M. Jutisz. 1976. Delayed effects of in vivo LH-RH immunoneutralization on gonadotropins and prolactin secretion in the female rat. Endocrinology 98:1539-1549.

Kesner, J.S., J.M. Kaufman, R.C. Wilson, G. Kuroda and E. Knobil. 1986. The effect of morphine on the electrophysiological activity of the hypothalamic luteinizing hormone-releasing hormone pulse generator in the rhesus monkey. Neuroendocrinology 43:686-688.

Kinder, J.E., M.L. Day, R.J. Kittok. 1987. Endocrinology of puberty in cows and ewes. J. Reprod. Fertil., Suppl., 34:167-186.

Koch, Y. 1977. Effects of antibodies against luteinizing hormone-releasing hormone on reproduction. In Proc. Vth Int. Congr. Endocrinology, Hamburg, July 18-24, 1976, Vol. 1, pp 374-378. Ed. V.H.T. James. Amsterdam: Excerpta Medica.

Koch, Y., P. Chobsieng, M. Fridkin and H.R. Linder. 1973. Suppression of gonadotropin secretion and prevention of ovulation in the rat by antiserum to synthetic gonadotropin-releasing hormone. Biochem. Biophys. Res. Commun. 55:623-629.

Koprowski, J.A., and H.A. Tucker. 1973. Serum prolactin during various physiological states and its relationship to milk production in the bovine, Endocrinology 92:1480-1487.

Ladd, A., Y.Y. Tsong, G. Prabhu and R. Thau. 1989. Effects of long-term immunization against LHRH and androgen treatment on gonadal function. J. Reprod. Immunol. 15:85-101.

Larsen, B., and I. Heron. 1978. Modification of lymphocyte response to phytomitogens by polycations and polyanions. Experientia 34:9 1224-1227.

Laster, D.B., H.A. Glimp and K.E. Gregory. 1972. Age and weight at puberty and conception in different breeds and breed-crosses of beef heifers. J. Anim. Sci. 34(6):1031-1036.

Laster, D.B., G.M. Smith, L.V. Cundiff and K.E. Gregory. 1979. Characterization of biological types of cattle (cycle II) II. Postweaning growth and puberty of heifers. J. Anim. Sci. 48:500-508.

Laster, D.B., G.M. Smith and K.E. Gregory. 1976. Characterization of biological types of cattle. IV. Postweaning growth and puberty of heifers. J. Anim. Sci. 43:63-70.

Laurence, K.A. and S. Ichikawa. 1968. Effects of active immunization with bovine luteinizing hormone on reproduction in the female rat. Endocrinology 82:1190.

Laws, S.C., J.C. Webster and W.L. Miller. 1990. Estradiol alters the effectiveness of gonadotropin-releasing hormone (GnRH) in ovine pituitary cultures: GnRH receptors versus responsiveness to GnRH. Endocrinology 127:381-386.

Lee, V.W.K., I.A. Cummings, D.M. De Kretser, J.K. Findlay, B. Hudson and E.J. Keough. 1976. Regulation of gonadotropin secretion in rams from birth to sexual maturity. I. Plasma LH, FSH, and testosterone levels. J. Reprod. and Fert. 46:1-6.

Leshin, L.S., L.A. Rund, R.R. Kraeling and T.E. Kiser. 1991. The bovine preoptic area and median eminence: sites of opioid inhibition of luteinizing hormone-releasing hormone secretion. J. Anim. Sci. 69:3733-3746.

Lesmeister, J.L., P.J. Burfening and R.L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. J. Anim. Sci. 36:1.

Leung, K., V.Padmanabhan, E.M. Convey, R.E. Short and R.B. Staigmiller. 1984. Relationship between pituitary responsiveness to Gn-RH and number of Gn-RH-binding sites in pituitary glands of beef cows. J. Reprod. Fertil. 71:267-277.

Levine, J.E. and M.T. Duffy. 1988. Simultaneous measurement of luteinizing hormone (LH)-releasing hormone, LH and follicle-stimulating hormone release in intact and short-term castrate rats. Endocrinology 122:2211-2221.

Lincoln, G.A. 1978. The temporal relationship between plasma levels of FSH and LH in the ram. J. Reprod. and Fert. 73:31-37.

Lincoln, G.A., and M.J. Peet. 1977. Photoperiodic control of gonadotropin secretion in the ram: A detailed study of the temporal changes in plasma levels of follicle-stimulating hormone, luteinizing hormone and testosterone following an abrupt switch from long to short days. J. Endocrinology 74:355-367.

Lincoln, G.A. and H.M. Fraser. 1979. Blockade of episodic secretion of luteinizing hormone in the ram by the administration of antibodies to luteinizing hormone releasing hormone. Biol. Reprod. 21:1239.

Lombardi, J. R., G. Vandenbergh and J. M. Whitsett. 1976. Androgen control of the sexual maturation pheromone in house mouse urine. Biol. Reprod. 15:179.

Lusby, K.S., S.O. Enis and R.W. McNew. 1979. Twenty-four vs 30-month-old calving with Hereford heifers. Animal Science Research Report. Oklahoma State Univ., Stillwater. pp 90-93.

Lutz, J.B., G.B. Rampacek and R.R. Kraeling. 1985. Induction of ovulation in the prepuberal gilt by pulsatile administration of gonadotropin releasing hormone. Dom. Anim. Endocrinol. 2:61.

Malven, P.V. 1986. Inhibition of pituitary LH release resulting from endogenous opioid peptides. Dom. Anim. Endocrinology 3(3):134-144.

Matsuo, H., Y. Baba, R.M.G. Nair, A. Arimura and A.V. Schally. 1971. Structure of porcine LH and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Commun. 43:1334.

Menge A.C., S.E. Mares, W.J. Tyler and L.E. Casida. 1960. Some factors affecting age at puberty and the first 90

days of lactation in holstein heifers. J. Dairy Sci. 43: 1099-1107.

McCartor, M.M., R.D. Randel and L.H. Carroll. 1979. Dietary alteration of ruminal fermentation on efficiency of growth and onset of puberty in Brangus heifers. J. Anim. Sci. 48:488.

McCormack, J.T., T.M. Plant, D.L. Hess and E. Knobil. 1977. The effect of luteinizing hormone releasing hormone (LHRH) antiserum administration on gonadotropin secretion in the rhesus monkey. Endocrinology 100:663-667.

McNeilly, A.S., H.M. Fraser and D.T. Baird. 1984. Effect of immunoneutralization of LH releasing hormone on LH, FSH, and ovarian steroid secretion in the preovulatory phase of the oestrous cycle in the ewe. J. Endocrinology 101:213-219.

McNeilly, A.S., J.A. Jonassen and H.M. Fraser. 1986. Suppression of follicular development after chronic LHRH immunoneutralization in the ewe. J. Reprod. Fert. 76:481-490.

Moenter, S.M., C.J.I. Woodfill, A. Caraty and F.J. Karsch. 1989. The estradiol induced GnRH surge in the ewe. Biol. Reprod. 40(Suppl. 1):165 (Abstr.)

Moran, C., J.F. Quirke and J.F. Roche. 1989. Puberty in Heifers: A Review. Anim. Reprod. Sci. (18):167-182.

Moseley, W.M., M.M. McCartor and R.D. Randel. 1978. Effects of monensin on growth and reproductive performance of beef heifers. J. Anim. Sci. 45(5):961-968.

Moseley, W.M., T.G. Dunn, C.C. Kaltenbach, R.E. Short and R.B.Stagmiller. 1982. Relationship of growth and puberty in beef heifers fed monensin. J.Anim. Sci. 55(2):357-362.

Moss, G.E., M.E. Crowder and T.M. Nett. 1981. GnRH-receptor interaction. VI. Effect of progesterone and estradiol on hypophyseal receptors for GnRH, serum and hypophyseal concentrations of gonadotropins in ovariectomized ewes. Biol. Reprod. 25:938-944.

Narayana, K. and H. Dobson. 1979. Effect of administration of antibody against GnRH on preovulatory LH and FSH surges in the ewe. J. Reprod. Fertil. 57:65-72.

Nett, T.M., A.M. Akbar, G.D. Niswender, M.T. Hedland and W.F. White. 1973. A radioimmunoassay for

gonadotropin-releasing hormone (GnRH) in serum. J. of Clinical Endocrinol. and Metabolism. 36:880-885.

Nett, T.M., M.E, Crowder, G.E. Moss and T.M. Duello. 1981. GnRH-receptor interaction. V. Down-regulation of pituitary receptors for GnRH in ovariectomized ewes by infusion of homologous hormone. Biol. Reprod. 24:1145.

Nett, T.M., D. Cermak, T. Brademe. J. Manns and G. Niswender. 1987. Pituitary receptors for GnRH and estradiol, and pituitary content of gonadotropins in beef cows. I. Changes during the estrous cycle. Dom. Anim. Endocrinology. 4(2):123-132.

Nikitovitch-Winer, M. and J.W. Everett. 1959. Histocytological changes in grafts of rat pituitary on the kidney and upon retransplantation under the diencephalon. Endocrinology 65:357-368.

Noar, Z. and G.V. Childs. 1986. Binding and activation of gonadotropin-releasing hormone receptors in pituitary and gonadal cells. Int. Rev. Cytology 103:147.

Nunez-dominguez, L.V. Cundiff, G.E. Dickerson, K.E. Gregory and R.M. Koch. 1991. Lifetime production of beef heifers calving first at two vs three years of age. J. Anim. Sci. 69:3467-3479.

O'Connell, C.M. 1990. Immunization against gonadotropin releasing hormone alters puberty, secretion of luteinizing hormone and ovarian activity in beef heifers. Masters Thesis. Oklahoma State Univ., Stillwater.

O'Connell, C.M. and R.P. Wettemann. 1989. Effects of active immunization against LHRH in beef heifers on the onset of ovarian activity. Animal Science Research Report. Oklahoma State Univ., Stillwater. MP-127:242-244.

Oyedipe, E.O., D.I.K. Osori, O. Akerejola and D. Saror. 1982. Effect of level of nutrition on onset of puberty and conception rates of Zebu heifers. Theriogenology 18(5):525-539.

Padmanabhan, V., K. Leung and E.M. Convey. 1982. Ovarian steroids modulate the self-priming effect of luteinizing hormone-releasing hormone on bovine pituitary cells in vitro. Endocrinology 110:717-721.

Parlow, A.F., L.L. Anderson and R. M. Melampy. 1964. Pituitary follicle-stimulating hormone and luteinizing hormone concentrations in relation to reproductive stages of the pig. Endocrinology 75:365.

Pau, K.-Y., D.L. Hess, A.H. Kaynard, W.Z. Ji, P.M. Gliessman and H.G. Spies. 1989. Suppression of mediobasal hypothalamic GnRH and plasma LH pulsatile patterns by phentolamine i ovariectomized rhesus macaques. Endocrinology 124:891-898.

Pau, K.-Y., K.M. Ostead, D.L. Hess and H.G. Spies. 1986. Feedback effects of ovarian steroids on the hypothalamic-hypophyseal axis in the rabbit. Biol. Reprod. 35:1009-1023.

Petitclerc D., L.T. Chapin, R. S. Emery and H.A. Tucker. 1983. Body growth, growth hormone prolactin and puberty response to photoperiod and plane of nutrition in holstein heifers. J. Anim. Sci. 57:892-898.

Pique, L., F. Cesselin, G. Strauch, J.C. Valcke and H. Bicaire. 1978. Specificity of anti-LHRH antisera indu ed by different immunogens. Immunochemistry 15:55-60.

Quigley, M.E. and S.S.C. Yen. 1980. The role of endogenous opiates on LH secretion during the menstrual cycle. J. Clin. Endocrinol. Metab. 57:881-884.

Rahe, C.H., R.E. Owens, J.L. Fleeger, J.J. Newton and P.G. Harms. 1980. Pattern of plasma luteinizing hormone in the cyclic cow: Dependence upon the period of the cycle. Endocrinology 107:498-503.

Ramirez, Domingo V. and S.M. McCann. 1963. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology. 72:452-464.

Rayford, P.L., H.J. Brinkley, E.P. Young and L.E. Reichert, Jr. 1974. Radioimmunoassay of porcine FSH. J. Animal. Sci. 39:348.

Reid, J.T. 1960. Effect of energy intake upon reproduction in farm animals. Suppl. J. Dairy Sci. 43.

Rhodes, R.C. III., M.M. McCartor and R.D. Randel. 1978. Effect of feeding protein-protected lipid upon growth and reproductive development of yearling heifers. J. Anim. Sci. 46:769-777.

Roberson, M.S., R.P. Ansotequi, J.G. Berardinelli, R.W. Whitman, and M.J. McInerney. 1987. Influence of biostimulation by mature bulls on occurrence of puberty in beef heifers. J. Anim. Sci. 64:1601-1605.

Roberson, M.S., M.W. Wolfe, T.T. Stumpf, L.A. Werth, A.S. Cupp, N. Kojima, P.L. Wolfe, R.J. Kittok and J.E. Kinder. 1991. Influence of growth rate and exposure

to bulls on age at puberty in beef heifers. J. Anim. Sci. 69:2092-2098.

Roberts, A.J., deAvilia, D.M., J.D. Gerber and J.J. Reeves. 1990. Active immunization of beef heifers against luteinizing hormone: I. Evaluation of protein carriers and adjuvants on antigenicity of LH. J. Anim. Sci. 68:3742-3746.

Robertson, I.S., H.M. Fraser, G.M. Innes and A.S. Jones. 1982. Effect of immunological castration on sexual and reproductive characteristics in male cattle. Vet. Rec. 111:529.

Robertson, I.S., J.C. Wilson, H.M. Fraser, G.M. Innes and A.S. Jones. 1984. Immunological castration of young bulls for beef production. In: Manipulation of Growth in Farm Animals. pp 137-145.

Robertson, I.S., J.C. Wilson, H.M. Fraser and A.C. Rowlands. 1981. Further studies on immunological castration in male cattle. Vet Rec. 108:381.

Robinson, T.J. 1977. Reproduction in cattle. In: H.H. Cole and P.T. Cupps (Eds.) Reproduction in Domestic Animals, 3rd Edition. pp 433-441. N.Y. Academic Press, New York.

Rosenblum, N.G. and S. Schlaff. 1976. Gonadotropin-releasing hormone radioimmunoassay and its measurement in normal human plasma, secondary amenorrhea, and postmenopausal syndrome. Am. J. of Obstetrics and Gynecology, 124:340-347.

Roy, J.H., C.M. Gillies, M.W. Perfitt and I.J. Stobo. 1980. Effect of season of the year and phase of the moon on puberty and on the occurrence of oestrus and conception in dairy heifers reared on high planes of nutrition. Anim. Prod., 31:13-26.

Rund, L.A., L.S. Leshin, F.N. Thompson, G.B. Rampacek and T.E. Kiser. 1989. Influence of the ovary and suckling on luteinizing hormone response to Naloxone in postpartum beef cows. J. Anim. Sci. 67:1527-1531.

Sabeur, K. and T.E. Adams. 1989. Gonadotrope and testicular function in ram lambs: Effect of active immunization against gonadotropin-releasing hormone (GnRH) and pulsatile delivery of a GnRH agonist. Proc. West. Sect. Am. Soc. Anim. Sci. 40:324.

SAS. 1985. SAS. User's Guide: Statistics. SAS Inst. Inc., Cary NC.

Savoy-Moore, R.T., and K.H. Swartz. 1987. Several GnRH stimulation frequencies differentially release FSH and LH from isolated, perfused rat anterior pituitary cells. Adv. Exp. Biol. Med. 219:641.

Schams, D., E. Schallenberger, S. Gombe, and H. Karg. 1981. Endocrine patterns associated with puberty in male and female cattle. J. Reprod. Fert.. 83: 663- 671.

Schanbacher, B.D. 1982. Responses of ram lambs to active immunization against testosterone and luteinizing hormone-releasing hormone. Amer. J. Physiol. 242:E201.

Schanbacher, B.D. 1984. Active immunization against LH-RH in the male. In: D.B. Crighton (Ed.) Immunological Aspects of Reproduction in Mammals. pp 345-362. Butterworths, London.

Schanbacher, B.D. 1985. Effects of active immunization of the ram and bull against luteinizing hormone. Theriogenology 24:59.

Schanbacher, B.D., H.F. English, D. Gross, R.J. Santen, M.F. Walker and R.E. Falvo. 1983. Animal model of isolated gonadotropin deficiency. I. Hormonal responses to LHRH immunoneutralization. J. Androl. 4:233-239.

Schillo, K.K., D.J. Dierschke, and E.R. Hauser. 1982. Regulation of luteinizing hormone secretion in prepubertal heifers: Increased threshold to negative feedback of estradiol. J. Anim. Sci. 54:325-336.

Seidel, G.E., Jr., L.L. Larson, and R. H. Foote. 1971. Effects of age and gonadotropin treatment of superovulation in the calf. J. Anim. Sci. 33: 617-622.

Short, R.E. and R.A. Bellows. 1971. Relationships among weight gains, age at puberty and reproductive performance in heifers. J. Anim. Sci. 32(1):127-131.

Siebert, B.D. and J.B. Field. 1975. Reproductive activity in beef heifers following post-weaning feeding on spear grass hay alone or with supplements. Aust. J. Exp. Agri. and Anim. Hus. 15:12.

Silversides, D.W., A.F. Allen, V. Misra, B.D. Murphy and R.J. Mapletoft. 1988. A synthetic luteinizing hormone releasing hormone vaccine II. Temporal aspects of titer development and formulation trials in BALB/c mice. J. Reprod. Immunol. 14:47-58.

Stewart, T.S., C.R. Long and T.C. Cartwright. 1980. Characterization of cattle of five-breed diallel. III.

Puberty in bulls and heifers. J. Anim. Sci. 50(5):808-820.

Swanson, L.V., H.D. Hafs and D.A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone, and glucocorticoids from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34:284.

Swingle, W.W., P. Seay, J. Perlmutt, E.J. Collins, E.J. Fedor and G. Barlow, Jr. 1951. Effect of electrical stimulation of uterine cervix upon sexual development of prepuberal rats. Am. J. Physiol. 167:599.

Thompson, L.H. and J.S. Savage. 1978. Age at puberty and ovulation rate in gilts in confinement as influenced by exposure to a boar. J. Anim. Sci. 47:1141.

Tizard, I.R. 1984. Foreign antigens and antigenicity. In: I.R. Tizard (Ed.) Immunology, an introduction. pp12-30. saunders College Publishing, Philadelphia, PA.

Tizard, I.R. and K.L. Esbenshade. 1988. Pulsatile administration of gonadotropin-releasing hormone agonist to gilts actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 66:2209.

Traywick, G.B. and K.L. Esbenshade. 1988. Pulsatile administration of gonadotropin-releasing hormone agonist to gilts actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 66:2209-2215.

Turman, E.J., L.S. Pope and D.F. Stephens. 1965. Some factors associated with the rebreeding of two-year-old hereford heifers on high, moderate and low levels of winter supplemental feeding. Animal Science Feeders' Day Report. Oklahoma State Univ., Stillwater. pp 25-31.

Upadhyay, S.N., A. Alam and G.P. Talwar. 1989. Functional morphology of testis and its excurrent ducts in rats immunized with synthetic luteinizing hormone releasing hormone conjugated to tetanus toxoid. J. Reprod. Immunol. 16:151-163.

Vandenbergh J.G. 1967. Effect of the presence of a male on the sexual maturation of female mice. Endocrinology 81:345-349.

Van Vugt, D.A., N.Y. Lam and M. Ferin. 1984. Reduced frequency of pulsatile luteinizing hormone secretion in the luteal phase of the rhesus monkey: Involvement of endogenous opiates. Endocrinology 115:1095-1101.

Wagner, J.J., K.S. Lusby, J.W. Oltjen, J. Rakestraw, W.P. Wettemann and L.E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603.

Wardlaw, S.L., W.B. Wehrenberg, M. Ferin, P.W. Carmel and A.G. Frantz. 1980. High levels of beta-endorphins in hypophysial portal blood. Endocrinology 106:1323-1326.

Watanabe, G. and E. Terasawa. 1989. In vivo release of luteinizing hormone releasing hormone increases with puberty in the female Rhesus monkey. Endocrinology 125:92-99.

Wettemann, R.P. and J.W. Castree. 1988. Immunization of heifers against luteinizing hormone delays puberty and causes anestrus. Animal Science Research Report. Oklahoma State Univ., Stillwater. MP-125:17.

Whisnant, C.S. and R.L. Goodman. 1988. Effects of an opioid antagonist on pulsatile luteinizing hormone secretion in the ewe vary with changes in steroid negative feedback. Biol. Reprod. 39(5): 1032-1038.

Whisnant, C.S., T.E. Kiser, F.N. Thompson and C.R. Barb. 1986. Naloxone infusion increases pulsatile luteinizing hormone release in postpartum beef cows. Dom. Anim. Endocrinol. 3(1):49-54.

Wildt, L., A. Hausler, G. Marshall, J.S. Hutchinson, T.M. Plant, P.E. Belchetz and E. Knobil. 1981. Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. Endocrinology 109:376.

Wiltbank, J.N. , K.E. Gregory, L.A. Swiger and J.E. Ingalls. 1966. Effects of heterosis on age and weight at puberty in beef heifers. J. Anim. Sci. 25:744-751.

Wiltbank, J.N., C.W. Kasson and J.E.Ingalls. 1969. Puberty in crossbred and straightbred beef heifers on two levels of feed. J. Anim. Sci. 29:602-605.

Wiltbank, J.N., S. Roberts, J. Nix and L. Rowden. 1985. Reproductive performance and profitability of heifers fed to weigh 272 or 318 kg at the start of the first breeding season. J. Anim. Sci. 60(1):25-34.

Wise, M.E., J.H. Nilson, M.T. Nejedlik and T.M. Nett. 1985. Measurement of messenger RNA for luteinizing hormone beta-subunit and alpha-subunit during gestation and the postpartum period in ewes. Biol. Reprod. 33:1009-1015.

Wu, F.C.W., D.C. Irby, I.J. Clarke, J.T. Cummins and D.M. deKretser. 1987. Effects of gonadotropin-releasing hormone pulse frequency modulation on luteinizing hormone, follicle-stimulating hormone and testosterone secretion in hypothalamo/pituitary-disconnected rams. Biol. Reprod. 37:501.

Wu, T.J., D.L. Morris, N.H. McArthur and P.G. Harms. 1991. A priming effect of Naloxone on *in vitro* LHRH release from the hypothalamus of mid-luteal ewes. Biol. Reprod. 44:546-549.

Yelich, J.V., R.P. Wettemann, K.S. Lusby, H.G. Dolezal and D.K. Bishop. 1991. Influence of growth rate on body composition of beef heifers at puberty. Animal Science Research Report. June 1991 MP-134 p48-51. Ag. Exp. Station Div. of Ag. OSU.

Zirkin, B.R., R. Santulli, C.A. Awoniyi and L.L. Ewing. 1989. Maintenance of advanced spermatogenic cells in the adult rat testis: Quantitative relationship to testosterone concentration within the testis. Endocrinology 124:3043.

VITA

SHELIA LAVON DUGGAN

Candidate for the Degree of

Master of Science

Thesis: IMMUNIZATION OF HEIFERS AGAINST GONADOTROPIN RELEASING HORMONE: EFFECTIVENESS OF ADJUVANTS

Major Field: Animal Science

Biographical:

Personal Data: Born in DeQueen, Arkansas; September 19, 1967, the daughter of Max S. and Lavon R. Duggan.

Education: Graduated from Gillham High School, Gillham, Arkansas, May 1985; received Bachelor of Science in Agriculture from the University of Arkansas at Fayetteville, May 1989; completed the requirements for the Master of Science Degree in Animal Science at Oklahoma State University, May, 1993.

Professional Experience: Biologist at Merck and Co., Inc. 1989-1990; graduate research and teaching assistant at Oklahoma State University 1990-1992; laboratory technologist I at Oklahoma Animal Disease Diagnostic Laboratory 1992-present.