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University of Alberta

Effects of Immunocastration on Feedlot Performance in Bulls of Different Breed-types Implanted with Estradiol 17-β

by

Christine Margaret Erichsen 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

in

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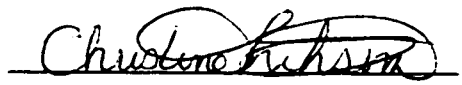

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December 30 1998

DEDICATION

I dedicate this to the people who, through their support, greatly contributed to the accomplishment of this thesis.

For: Mom, Dad, Jonathan, and Mick.

ABSTRACT

The effects of anti-GnRH immunization on reproductive physiology, behavior, production and carcass traits of feedlot bulls were studied and compared with the effects on intact and surgically castrated bulls. Ninety-six bull calves were randomly allocated to 24 pens of four cattle each in a 3x2 factoral design, replicated. Half the calves were implanted with Estradiol-17β. The anti-GnRH vaccine elicited an immediate and high immune response. A significant reduction in serum testosterone concentration followed and in turn, inhibited sexual development and behavior in immunocastrates, but had little effect on growth, feed efficiency and carcass traits. Implanting appeared to complement some effects of immunization. There was a high degree of variation among individual cattle in their response to immunization. Anti-GnRH immunization may be a useful alternative to surgical castration, but not in an environment requiring a 90 day withdrawal period between vaccination and marketing, as was required for this experiment.

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1. DEVELOPMENT OF IMMUNOCASTRATION

1.1 INTRODUCTION

The cattle industry in Canada contributes 5.1 billion dollars to farm cash receipts (Canfax 1997). Almost three million cattle were slaughtered at federally inspected packing plants in 1997 (Canfax 1997). Of these cattle, 1.5 million were steers that generated approximately 1.7 billion dollars (Canfax 1997). Based on these figures which demonstrate the magnitude of raising steers to the industry, the market potential for improving current management practices associated with raising steers could be enormous. One possibility is to find an alternative to the current practice of castrating bulls intended for the feedlot. The following research was conducted to study the effects of immunocastration of feedlot bulls using anti-GnRH immunization.

1.2 PROBLEMS ASSOCIATED WITH RAISING INTACT BULLS

Meat producing animals have been castrated for thousands of years (Trow-Smith 1957), mainly because of management difficulties associated with rearing intact farm animals. In cattle, the problems are mostly related to sexual and aggressive behavior as well as negative carcass attributes. Postpubertal bulls are difficult to manage, cannot be grazed with heifers, cause pasture damage and are a threat to human safety (Price and Tennesson 1981; Gregory and Ford 1983). When heifers reach puberty they become fertile and exhibit estrus behavior every 18 to 24 days (Bonneau and Enright 1995). Estrus behavior results in increased disturbance, stress, risk of injury and unwanted

pregnancies if the females are housed with intact males (Curran et al. 1965; Roche and Crowley 1973). For these reasons, males and females must be fed separately once they reach about nine months of age (Bonneau and Enright 1995).

Intact bulls and heifers also present a higher inherent risk of dark cutting at slaughter than do steers or spayed heifers, and also have a high tendency to yield bruised carcasses (Kenny and Tarrant 1983; Kempster and Lowe 1993; Scanga et al. 1998). Dark cutting results from pre-slaughter stress, which depletes muscle glycogen stores, reducing glycogen needed to produce lactic acid to reduce the pH of postmortem muscle (Scanga et al. 1998). The high pH (>6.0) increases the light-absorption and water-binding capacity of postmortem muscle and results in an undesirable, dark, firm and dry cut lean surface (Lister, 1988).

1.3 PROBLEMS ASSOCIATED WITH TRADITIONAL CASTRATION

Castration of non-breeding bulls is a standard practice in Canada. There are several disadvantages associated with traditional castration techniques. Castration is stressful to the animal and is a costly, laborious procedure. Besides the labor and equipment costs necessary to perform the procedure, there is an associated risk of hemorrhage and infection, which may lead to morbidity or mortality. As well, the welfare and ethical issues associated with castration are a matter of public concern and may potentially affect consumer perception of beef. The biggest economic disadvantage however, is from the loss of growth potential and feed conversion efficiency that are

inherent with intact bulls.

Gonadal steroids play a critical role in mammalian growth and development (Adams and Adams 1992). Entire bulls exhibit higher growth rates, convert feed more efficiently and produce leaner carcasses than steers (Price et al. 1980). Studies show that animals castrated on entry into the feedlot experience significant reductions in final live weight, average daily gain (ADG) and hot carcass weight when compared to intact contemporaries (Gonzalez 1990; Adams and Adams 1992; Huxsoll et al. 1998). Hannon et al. (1991) reported that in animals castrated at 6.5 months of age, feed intake and weight gain was reduced over the subsequent eight months. In North America, steers are commonly implanted with growth promoters in an attempt to reverse the effects of castration on growth rate and feed conversion efficiency. However, this is not an option in the European Union where their use is banned, and does not solve the ethical and welfare problems associated with surgical castration.

1.4 EFFECTS OF CASTRATION

Castration of bulls involves either the surgical removal of the testicles and epididymides, or a treatment that causes degeneration of the testes. The depressing action of castration varies with species, individual, and physiological and behavioral status of the animal at the time of the operation (Hafez, 1993). At puberty, androgens induce male sexual behavior. If the males are castrated prior to puberty, this sexual behavior does not develop to the same extent. Castration of immature males results in sterility, and prevents

the maturation of accessory glands, aggressiveness, and sex drive. Jago et al. (1997) found that prepubertal surgical castration of bulls prevented the development of copulatory (intromission and ejaculation) behaviors and reduced the expression of precopulatory (searching, courtship and mounting) behaviors. However, increasing sexual activity of steers from seven to 17 months of age indicates that removal of the testes does not eliminate the development of all heterosexual behaviors, and in fact some steers will mount estrous cows (Jago et al. 1997).

1.5 CASTRATION METHODS

There are several commonly used methods of castrating bulls. One is surgical castration where a knife is used to cut the scrotum, allowing removal of the testes and part of the spermatic cords. Another surgical method uses an emasculator to crush and ablate the spermatic cord. Two non-surgical methods involve the use of a burdizzo or an elastrator. A burdizzo is a blunt jaw pincher used to crush and ablate the spermatic cord without cutting the scrotum. Castration using an elastrator is performed on young calves by positioning a rubber band at the neck of the scrotum above the testicles, and stopping the blood supply to them and the scrotum.

A variety of alternative methods to surgical castration have been considered and include:

1) injection of lactic acid into the parenchyma of the testicle resulting in sclerosis and atrophy of the testes (Cohen et al. 1991a; Cohen et al. 1991b).

2) down-regulation or interruption of the hypothalamic-pituitary-gonadal axis by chronic administration of gonadotropin-releasing hormone in high doses (Melson et al. 1986, Ronayne et al. 1993). However, these studies have demonstrated that administration of GnRH or a GnRH analogue for short or long periods ultimately increased rather than decreased testosterone concentrations.

3) active immunization against sex steroids in cattle or sheep or against boar taint-related steroids in male pigs (Schanbacher 1982; Price et al. 1987).

All of these alternative methods have significant drawbacks. The efficacy may be insufficient, consumers may have negative attitudes towards them, or their use may be banned in some countries. The most promising alternative castration method is active immunization with an anti-gonadatrophin releasing hormone (GnRH) vaccine. Antibodies raised by vaccination will attenuate the reproductive functions, prevent the development and functioning of the testicles in males and prevent the synthesis of sex steroids (Meloen et al. 1994).

Three important areas where immunocastration could have a major impact in animal production are; to reduce aggressive behavior in bulls, to prevent estrous behavior and fertility in heifers and to decrease the incidence of boar taint in boar meat (Bonneau and Enright 1995; Harland, 1998). This thesis will focus on the effects of immunocastration of bulls using an anti-GnRH vaccine.

1.6 HORMONAL INFLUENCES ON BULL CALF DEVELOPMENT

The decapeptide, GnRH is produced in the hypothalamus and plays a critical role in the endocrine events guiding the reproductive process. From the hypothalamus, GnRH travels through the hypophyseal portal blood system to the anterior pituitary, where it is responsible for the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Fig. 1.1). There is an age-associated increase in the pulsatile release of GnRH from the hypothalamus in pre-pubertal bulls, which results in an increased pulsatile secretion of LH from the anterior pituitary (Rodriguez and Wise 1989). This increase in pulsatile LH secretion initiates the onset of puberty and testicular maturation in bulls by increasing serum testosterone concentrations (McCarthy et al. 1979; Amann et al. 1986). Luteinizing hormone acts upon the Leydig (interstitial) cells of the testes inducing the production of androgens. Follicle stimulating hormone stimulates spermatogenesis by action on both spermatogonia and the Sertoli cells in the testes. The Sertoli cells also convert testosterone to estradiol under the influence of FSH.

1.6.1 *Hormonal Changes at Puberty*

Puberty usually occurs by about four to eight months of age in bull calves (Rodriguez and Wise 1989; Evans et al. 1996). In bull calves, serum concentrations of LH are low after birth, then show an early transient rise between about 10 and 20 weeks of age (McCarthy et al. 1979; Evans et al. 1993; Evans et al. 1996). Circulating FSH concentrations are high initially, but decrease between 14 and 30 weeks of age (Evans et

al. 1996). From about 24 weeks of age, mean LH and FSH concentrations increase to the time of puberty (McCarthy et al. 1979; Evans et al. 1993; Rawlings and Evans 1995). The secretion of testicular androgens occurs in pulsatile peaks, reflecting the pulsatile release of the pituitary gonadatrophins. Circulating testosterone concentrations increase gradually from six to 35 weeks of age and then rapidly to 42 weeks of age (Evans et al. 1996). This suggests that the early rise in LH secretion, with high serum FSH concentrations, appears to stimulate the testes to secrete steroids, and initiates testicular maturation and spermatogenesis (Evans et al. 1996). A study examining the relationship between the early rise in LH secretion and rate of sexual maturation demonstrated that early maturing bulls had a higher early rise in LH than late maturing bulls (Evans et al. 1995). Furthermore, when LH secretion is suppressed during this early period, testicular development and age at puberty is delayed (Chandolia et al. 1994).

Testosterone is the principle androgen in sexually mature males. The role of testosterone includes: (1) development of secondary sex characteristics, (2) maintenance of the male duct system, (3) expression of male sexual behavior (libido), (4) function of the accessory glands, (5) function of the tunica dartos muscle in the scrotum, (6) spermatogenesis and (7) embryonic differentiation of the male duct system and external genitalia (Bearden and Fuquay 1997). Testosterone induces aggressive and sexual behavior in males either directly, or through the action of estradiol that is derived by aromatization of testosterone in upper brain centers (Balthazart 1990). High serum concentrations of testosterone inhibit the secretion of GnRH, FSH and LH, whereas low

levels increase secretion of these hormones. Thus the interrelationships of the hormones regulating male reproduction are controlled by negative feedback mechanisms.

1.7 IMMUNOLOGY ASPECTS

The function of the immune system in animals in general, is to attack foreign substances in the body and destroy them. The immune system can distinguish between 'self' and 'nonself' molecules so that the former are preserved and latter are destroyed. However, there are times when the immune system attacks self molecules or tissues, resulting in damage to the body. The immune system can be manipulated to regulate the immune attack on self molecules that will have beneficial effects. It is on this basis that immunocastration vaccines were developed.

There are two differences in the development of vaccines for disease control and for vaccines for regulating hormone concentrations. First, the latter vaccines are directed against self or self-like target molecules. Second, antibody titres against hormones must remain high to inactivate an endogenous hormone, since it's production will usually increase after immunization because of existing feedback loops (Hage-Van Noort et al. 1992). These have hampered early efforts in immunological control due to problems of self-tolerance and immunological cross-reactivity. The problems early vaccine prototypes had with low immunogenicity of the autoantigens have partly been overcome by introducing suitable carrier molecules to optimize presentation of peptides by major histocompatibility complex class II molecules, and by the introduction of adjuvants that

are designed to act as lymphocyte mitogens (Dirnhofer et al. 1994).

1.8 HOW ANTI-GNRH IMMUNIZATION WORKS IN THE BODY

By regulating a hormone cascade GnRH plays an essential role in reproduction, and manipulation of it interrupts the reproductive process. Immunoneutralization of GnRH suppresses gonadatrophin secretion, decreases testosterone secretion, retards testicular development and suppresses reproductive behavior in males. When injected intramuscularly, antibodies form against the GnRH-protein conjugate. These antibodies then bind to GnRH in the pituitary portal blood vessels, altering it's shape and preventing it from acting on the anterior pituitary to stimulate the secretion of FSH and LH (Schanbacher 1984a) (Fig. 1.2). This inhibits secretion of these three hormones, but their actions will not be entirely prevented. The goal of inducing immunity against GnRH in males is to reduce steroid secretion to a level at which no reproductive function can occur, even though a base amount of testosterone will still be secreted from the Sertoli cells, allowing anabolic effects to occur. It has been projected that long intervals, such as 60 to 90 days, between the primary and secondary (booster) injections would allow the suppresser and cytotoxic T-cells to return to a quiescent stage after their stimulation by the primer injection. Then, in response to the boost, the B cells would rapidly increase until the suppresser and T-cells are reactivated and bring antibody production under control (Morris 1985).

There may be certain circumstances where it is desirable to reverse the effects of

immunocastration. Animals could be returned to a 'normal' bull condition prior to slaughter if a period of leaner carcass gain is considered necessary (Lobley et al. 1992). Reversible castration also allows for the option of grazing young bulls with heifers, without having a problem with unwanted pregnancies. Several methods could be employed to neutralize the effects of immunocastration. Firstly, natural degradation of the antibody could be allowed (Lobley et al. 1992). This would vary for each animal and would be difficult to monitor. Secondly, an agent with greater affinity for the antibodies, or for GnRH itself, could be administered, accelerating the decline in antibody titre and allowing normal mechanisms to be re-asserted (Keeling and Crighton 1984). The third possible method would use a synthetic analogue that has the biological properties of GnRH but is sufficiently immunologically distinct so that it would be active even in the presence of high antibody titres (Keeling and Crighton 1984).

1.9 ANTI-GNRH IMMUNIZATION OF BULLS

1. 9. 1 *Early Work*

Robertson et al. (1979) described a pilot trial in which two three-month-old entire male calves were immunized with GnRH conjugated to tetanus toxoid (first and second immunizations) or thyroglobulin (third and fourth immunizations) and then emulsified with Freund's complete adjuvant. The results showed that inhibition of GnRH by antibodies to GnRH was achieved in calf one with titres above 1 in 1000, causing arrest of testicular growth and prevention of testosterone secretion and sperm production.

Although this was not achieved in calf two there was some evidence that lower titres did have a temporary effect on testis growth and function. In further studies by Robertson et al. (1982, 1984), ten bull calves were immunized with a multiple injection regime against GnRH-human serum albumin in Freund's complete adjuvant and incomplete adjuvant. Half the animals had a good immune response and these had reduced serum testosterone levels, involuted testes, reduced libido and semen production, and docile behavior. The effects of immunocastration lasted approximately six months by which time the calves had reverted to near normal male status. The rate of average daily gain to slaughter was greater in responders and non-responders than in contemporary steers (0.91, 0.91 and 0.81 kg respectively). Carcass fat was lower and carcass lean higher in the immunized bulls than in the steers.

In general, early studies using active anti-GnRH immunization in male cattle (Robertson et al. 1979, 1982, 1984; Jeffcoate et al. 1982; Lobley et al. 1992) show that a percentage of animals that develop significant GnRH antibody titres also express a temporary castration effect lasting six to nine months. This is manifested by low testosterone concentrations, involution of the testes and azoospermia. However, even with repeated booster immunizations in some studies (Robertson et al. 1979, 1982, 1984; Gonzalez et al. 1990), only up to 50 % of the GnRH-immunized animals developed significant antibody titres and had periods of reduced testosterone concentrations and testicular size. Although immunization against GnRH proved to be partially effective, there were problems relating to consistency and longevity of the immune response both

within and between animals in most studies in the literature.

Immunocastration has also produced conflicting results. In young rams, Schanbacher (1982) observed that the immunocastrated male had lower growth performance and feed conversion efficiency than either the untreated rams or wethers, although backfat thickness was similar to that of the former. Conversely, in cattle (Robertson et al. 1982; Robertson et al. 1984), immunocastrated bulls had growth performance superior to that of steers, with carcass composition intermediate between that of steers and bulls. In these cattle studies, the observed behavior of the immunocastrated animals was similar to steers, suggesting, in cattle at least, that the technique offered a possible practical approach to inhibit androgenic actions, including territorial defense and sexual aggression, without severe reductions in anabolic performance (Lobley et al. 1992). However, two limitations were apparent with these last studies (Robertson et al. 1979; Robertson et al. 1982; Robertson et al. 1984; Jeffcoate et al. 1982). First, although they included a comparison between the anti-GnRH bulls and surgically castrated steers, they did not compare them to a group of control bulls. Second, although general observations were made, no formalized behavior studies were conducted.

The results of these experiments on immunocastration emphasize two immediate concerns. Despite the use of repeat booster immunizations, only 50 % of animals became 'good responders' (exhibited high GnRH antibody titres and low serum testosterone concentrations) for significant periods of the experiments (Lobley et al. 1992). Due to

this poor immunogenicity, several repeat boosters were required. As well, in the periods immediately following effective immunocastration, serum testosterone concentrations in the cattle rapidly re-established to greater levels than would normally be observed (Robertson et al. 1982; Robertson et al. 1984). A 'rebound' phenomenon may have occurred, with compensatory effects on body composition.

1.9.2 *Effects of Immunocastration on Testicular Function, Production and Carcass Traits*

Although GnRH antibody titres are interpreted as a direct measure of immune response to a vaccine, there is some question as to how important high antibody titres are in leading to a biological change in the animal. It is difficult to determine a specific GnRH antibody titre for maximum biological effect in bulls. In many studies there is a lack of correlation between the duration of antibody titre persistence and the duration of the biological effect of GnRH antibody titres.

Many authors have reported a significant reduction in serum testosterone concentration and testes weight in immunized bulls compared to control bulls (Jeffcoate et al. 1982; Adams et al. 1993; Finnerty et al. 1996; Jago et al. 1997; Huxsoll et al. 1998). Cook et al. (1998) also reported treatment effects on testicular weight, daily sperm production, and epididymal sperm reserves. On average, the GnRH immunized group's testicular weight was 53% of the weights for control bulls, with daily sperm production at 40% relative to control bulls, and epididymal sperm reserves of 16% (Cook et al. 1998).

Although testicular function appears to be inhibited, most immunocastration studies reported that GnRH-immunized bulls were similar to control bulls for average daily gain, feed efficiency, final live weight and carcass traits (Gonzalez et al. 1990; Adams and Adams 1992; Adams et al. 1993; Adams et al. 1996; Finnerty et al. 1996). However, Finnerty et al. (1996) reported that during a period of suppressed testosterone concentration, a group of bulls with a high response to the vaccine had a lower average daily gain and were lighter than the control bulls. Similarly, Lobley et al. (1992) found that carcass composition was similar to that of steers in high responding immunocastrates and Cook et al. (1998) reported significantly lower carcass weight and grade fat in immunocastrates compared to intact bulls. It has been suggested that the maintenance of a high rate of growth in immunized bulls may be due to the residual serum concentrations of testosterone noted in immunized cattle (Huxsoll et al. 1998).

1.9.3 *Behavior*

Testosterone is responsible for gender-related bull behavior (Dykeman et al. 1982; Katz and McDonald 1992) with aggressive behavior markedly increasing during the peripubertal period (Baker and Gonyou 1986; Price and Wallach 1991). The pattern and type of behavior expressed by bulls appears to vary with time. Finnerty et al. (1996) reported more sexual than aggressive behavior with eight to 13.4 mo old bulls on pasture, but found that prior to slaughter at 22 mo of age, most of the observed behavior was aggressive. Similarly, Reinhardt et al. (1978) and Appleby (1986) recorded a higher

incidence of sexual (mounting) than aggressive behavior in pre-pubertal bulls and steers, while others (Hinch et al. 1982, 1983; Robertson and Lowman 1977) found that aggressive activity was the most prevalent activity for both bulls and steers aged between 10 and 18 mo of age.

Typical male behavior poses management difficulties for feedlot operators, which is why most bulls intended for the feedlot are castrated. In order for an immunocastration vaccine to be commercially acceptable, the behavior of the vaccinated cattle would have to be more similar to that of steers than intact bulls. Although few studies examining the effects of immunocastration on bull behavior have been conducted, in the ones that were, (Finnerty et al. 1996; Jago et al. 1997; Huxsoll et al. 1998), typical bull behavior was found to be suppressed to varying degrees. Finnerty et al. (1996) found that control bulls were more active than immunocastrated bulls (age of primary immunization was 1.5 to 2.5 mo), both at pasture (eight to 10.8 mo of age) and one week prior to slaughter, at approximately 22 mo of age. At younger ages, most of this activity was sexual, but as they grew older it became more aggressive. However, the high titre group of immunized bulls was more sexually active than either the medium titre group or the non-immunized control bulls at 11 to 13 mo of age. The authors suggest that this may reflect delayed sexual maturity and sexual activity in the high titre bulls, due to a delayed pubertal rise in testosterone concentration.

Jago et al. (1997) also found that altering GnRH function during the prepubertal

period changed the development of sexual and social behavior of bulls. However, these effects did not persist, and by slaughter, behavior of intact bulls and immunized bulls was similar. The findings of this study indicate that the effects of immunocastration on male-male mounting behavior and behaviors causing paddock damage reduced with time. In contrast, Finnerty et al. (1996) found that prepubertal immunocastration had a long-term suppressive effect on behavior. They found differences between combined sexual and agonistic behavior of intact and immunized bulls at 20 mo of age, approximately 14 mo after testosterone concentrations of immunocastrates had returned to the levels of intact bulls. In the study conducted by Jago et al. (1997), it was found that even though the effects of immunocastration seemed to diminish with time, the typical pubertal pattern of behavior for bulls was not seen in immunocastrates. The immunocastrates did not display the typical dramatic increase in homosexual mounting behavior, and the level of pasture damage never reached the highest levels seen with bulls, although the level of damage was higher than that of steers. The authors suggested that immunocastration may have changed the normal pubertal development of bull behavior, or that even though the development patterns were normal, duration of suppressed testosterone varied between individuals so animals were effectively going through puberty at different ages which would have affected the average data values.

Jago et al. (1997) also found that prepubertal immunocastration lowered the mean sexual behavior score (searching, courtship, mounting, intromission and

ejaculation) between 10 and 17 mo of age and increased the age at which bulls were first able to copulate. Some immunocastrates would repeatedly mount the cow in estrus but did not protrude the penis so intromission and ejaculation could not occur. It appears that this difference in sexual behavior score for bulls and immunocastrates can be largely attributed to differences in the ability to complete copulation rather than to differences in the sexual motivation of the animals (Jago et al. 1997). The delay in the development of the ability to achieve copulation in comparison with sexual motivation, may be explained by the fact that the ejaculatory mechanisms, in particular separation of the penis from the prepuce, are dependent on a higher level of testosterone than pre-copulatory sexual behaviors (Sodersten et al. 1980; D'Occhio and Brooks 1982).

Huxsoll et al. (1998) also reported changes in typical bull behavior due to immunocastration. They found that the frequency of spars (head to head contact) and butts (head to flank contact) initiated did not differ between steers and immunized bulls and was less than the same measures of aggressive behavior in control bulls. However, the behavior was only evaluated at one age, when cattle were approximately 16 months old, (primary immunization was at one, four or six mo of age) so changes in behavior over the experimental period cannot be compared.

1.9.4 *Immunocastration Vaccine Development and Vaccination Protocols*

1.9.4.1 *Carriers*

Since GnRH occurs naturally in the body and has a low molecular weight; it has

low immunogenicity. Consequently, it needs to be conjugated to a larger protein molecule, usually foreign to the host species, that will function as a carrier for breaking immunological tolerance to GnRH (Fraser 1980; Millar et al. 1984). Immunocastration vaccines are produced using a peptide that elicits anti-GnRH antibodies, neutralizing the biological activity of GnRH. Researchers have experimented with several different carrier proteins, attempting to find high yielding ones. Protein carriers tested include ovalbumin (Gonzalez et al. 1990; Jago et al. 1997), horse albumin (Gonzalez et al. 1990), bovine serum albumin (Jeffcoate et al 1982), human serum albumin (Robertson et al. 1982; Finnerty et al. 1994; Finnerty et al. 1996), avian egg albumin (Lobley et al. 1992), keyhole limpet haemocyanin (Gonzalez et al. 1990; Adams and Adams 1992; Adams et al. 1993; Adams et al. 1996; Huxsoll et al. 1998), tetanus toxoid (Fraser 1983) and thyroglobulin (Robertson et al. 1984). Lobley et al. (1992) proposed that the use of avian albumin as a carrier provides superior stimulation of the immune system compared to human serum albumen. This is because a wider species diversity usually leads to a greater immune response.

1.9.4.2 *Adjuvants*

Adjuvants are added to vaccine preparations to augment the immune response to the peptide-carrier conjugates (Gonzalez 1990). They accomplish this by enhancing the immune system and by prolonging the release of antigen. An adjuvant can enhance immune response by acting in one or more of three ways (Covey et al. 1985). A slow

release of the antigen from the site of deposition may prolong the antigenic stimulus. The adjuvant could also stimulate the mononuclear phagocyte system, causing rapid ingestion or more effective processing of antigen and enhanced lymphocytic response. Finally, adjuvants may evoke a generalized immune response, such as a direct action on lymphocytes or an acceleration of cellular differentiation.

A large number of adjuvants are available for experimental use. These include mineral oils, synthetic adjuvants and soaps (Campos 1992). However, these are unsuitable for use in animals due to severe side effects, including granuloma and ulcer formation (Campos 1992). The classic choice of adjuvant is Freund's Complete Adjuvant (FCA), an oil emulsion containing killed mycobacterium components. To date, the most successful GnRH immunization protocols have included the use of FCA (Silversides et al. 1988; Fraser 1983; Gonzalez 1990; Adams and Adams 1992; Adams et al. 1993; Adams et al. 1996; Huxsoll et al. 1998). However, FCA and non-ulcerative Freund's adjuvant are oil-based, and oil-based adjuvants are not an option in the development of a practical GnRH immunization regimen, owing to poor degradation of the mineral oil base (Langer,1981) and local irritation at the site of injection, resulting in granulomas and abscesses (Chapel and August, 1976; Goubau et al., 1989). Furthermore, the mycobacterial cell wall fractions contained in FCA cause a false positive reading in the tuberculosis test (Goubau et al., 1989). Unfortunately, FCA and Freund's incomplete adjuvant still yield the highest responses among test adjuvants and a suitable effective alternative to the oil-based adjuvants has not been reported.

Finally, a biocompatible vehicle in which the vaccine can be formulated and which persists as a depot at the injection site is needed. The peptide-carrier conjugate and immunostimulant are cleared rapidly if injected in an aqueous medium (Griffin 1993). To ensure that the vaccine persists at the injection site long enough to initiate an immune response, and to maintain antibody production, the vaccine is administered in a water and oil emulsion, that will create a depot to slowly release the immunogen at the injection site. The route of immunogen administration does not appear to be crucial since successful antibody production occurs with subcutaneous, intramuscular and lymph node injections (Crighton 1985).

1.9.4.3 *Dose*

An optimum dose of GnRH conjugate has not been determined in farm animals; however, doses between 0.05 and 5.00 mg GnRH conjugated to various protein carriers have been shown to elicit immune responses of varying efficiency (Schanbacher 1984a; Goubau et al. 1989a; Lobley et al. 1992; Adams and Adams 1992). Carson et al. (1992) reported that 1.0 mg of a GnRH conjugate given to prepubertal bulls was more effective than 4.0 mg.

1.9.4.4 *Primary-Booster Time Interval*

An optimal primary-booster interval has not been determined in any of the cattle studies to date. Lobley et al. (1992) found that greater antibody titres and longer periods

of immunosuppression were achieved with the primer given at four months of age compared with the five and six month initial injection (booster given at eight months of age). Gonzalaz et al. (1990) used a primary-booster interval of five weeks and obtained poorer titres, using similar amounts of immunogen as Lobley et al. (1992). The longer interval (16 weeks) may allow the T-cells to return to a quiescent stage after stimulation by the prime injection and then, in response to the boost, antibody producing cells (B cells) can rapidly increase, until the suppressor and T-cells are reactivated and bring antibody production under control (Morris 1985). Finnerty et al. (1994) found eight weeks between the prime and booster immunizations to be an effective protocol.

1.9.4.5 *Number of Immunizations*

Most immunization protocols for cattle employ a multi-injection regime requiring animals to be handled two or more times. In some experiments several repeat booster vaccinations were given to maintain antibody titres when they started to decline (Jeffcoate et al. 1982; Robertson et al. 1984; Jago et al. 1997). However, the protocol used by Adams et al. (1996) involved a single primary immunization with no subsequent booster. Single immunization with the GnRH-keyhole limpet hemocyanin conjugate in FCA results in a long-lived immune response (Adams et al. 1996). Adams and Adams (1992) also found that single immunization with the GnRH- keyhole limpet hemocyanin conjugate is as effective as multiple immunization in development of anti-GnRH titres. A single dose protocol would be advantageous because cattle would be handled only

once, reducing labor and handling costs associated with castration, and improving the efficacy of immunocastration as a management tool. However, these studies used FCA which is not suitable for use in commercial animal production.

1.9.5 *Age at Vaccination*

One advantage of immunocastration is that it can be invoked when best suited in each management system. However, researchers disagree as to what the best age is for vaccination against GnRH, to achieve optimal results. Generally, vaccination prior to puberty has been more successful, but the optimal length of time before puberty is still unknown. Based on findings showing very few differences between medium and high antibody titre groups of immunized bulls for any variable measured, Finnerty et al. (1996) suggested that level of titre may not be the critical factor in achieving a successful immunization regime, but rather early immunization of bulls relative to the onset of puberty. The study by Robertson et al. (1982) where bulls were immunized before puberty, supports the view that time of immunization relative to the expected onset of puberty affects the subsequent biological response obtained. They noticed that all the 'poor responders' were heavier at primary immunization than the 'good responders'. Therefore, the 'poor responders' may have been more sexually mature than the 'good responders' and that was why they did not respond as well (Robertson et al. 1979).

Adams et al. (1996) found that immunization was most effective when administered at seven months of age, as opposed to 1.5, four or 12 mo. They suggest that

the optimal age for immunization is at weaning. Immunization at later ages may not provide a sufficient interval between immunization and slaughter for the physiological effects of immunization to fully manifest. Immunization at a younger age may result in an attenuated immune response due to the immaturity of the immune system (Adams et al. 1996). Alternatively, the effects of the vaccine may have worn off by slaughter in the 1.5 mo old group because they were not boosted again.

Although Adams et al. (1996) suggested that the age at immunization had a significant influence on the magnitude of immune response, later research by this group demonstrated that the immune response to the vaccine was similar in calves receiving primary immunization at one, four or six mo of age (Huxsoll et al. 1998). Jago et al. (1997) also found that age of primary immunization (two, four or 7.5 mo of age) had no differential effect on behavior. They concluded that it was unnecessary to immunize bulls before 7.5 mo of age to achieve behavioral control, but that it would be necessary to repeat booster immunizations to continually suppress behavior in young bulls.

1.9.6 *Growth Promoting Implants*

The reduced growth efficiency of steers compared to bulls is attributed to an adverse effect of castration on the animal's hormonal status, specifically the inability to produce testosterone. The anabolic effects of implants in steers changes their hormonal status to resemble that of bulls (Lee et al. 1990). Feedlot gain and final live and carcass weights of steers increased markedly when implanted with growth promotors

(Schanbacher 1984b; Perry et al. 1991; Rumsey et al. 1992; Adams et al. 1993). As well, synthetic hormones reduce fat thickness, percentage of internal fat and marbling, while increasing carcass weight and improving carcass conformation in steers (Apple et al. 1991).

There are both estrogenic and androgenic growth promoting implants, and although their mechanisms may differ, both improve growth primarily by increasing muscle protein synthesis (Hayden et al. 1992). Enhanced growth responses are seen in steers when trenbolone acetate, a synthetic androgen, is combined with estrogenic implants (Apple, et al. 1991; Hayden et al. 1992), but little effect is seen in bulls (Henricks et al. 1988). This is in agreement with other reports that zeranol, trenbolone and combined progesterone and estadiol benzoate did not improve feedlot performance or carcass traits of bulls (Silcox et al. 1986; Doornenbal et al. 1987; Jones et al. 1991; Adams and Adams 1992; Adams et al. 1993). This indicates that bulls have sufficient endogenous anabolic steroids for maximum growth (Lee et al. 1990) and administration of supplemental steroid hormones does not markedly improve the anabolic effects (Adams and Adams 1992).

However, even though the administration of steroid implants does not alter growth rate in bulls, it does decrease testosterone secretion, perhaps through a feedback mechanism, (Staigmiller et al. 1985; Lee et al. 1990). This reduction in testosterone secretion appears to lead to suppression of testicular development and function in implanted bulls, as was noted in bulls implanted with estradiol-17β (Schanbacher 1984b;

Calkins et al. 1986), zeranol (Silcox et al. 1986) and progesterone with estradiol benzoate (Adams et al. 1993). Adams et al. (1993) has also shown that along with serum testosterone concentrations, carcass masculinity in bulls is reduced by implantation. The suppression of testicular growth and development in cattle with implants containing anabolic steroids likely results from implant-induced attenuation of episodic secretion of GnRH and an associated reduction in gonadotrophin secretion (Schanbacher 1984). In support of this, the frequency and amplitude of secretory episodes of LH are reduced and testicular growth attenuated in bull calves with implants that contain estradiol (Schanbacher 1984b; Deaver et al. 1988). Moreover, episodic administration of GnRH to calves treated with estradiol reestablished secretion of LH and sustained testicular growth (Schanbacher 1984b).

Adams and Adams (1992) found that the steroid supplementation (progesterone and estradiol benzoate) had no effect on feedlot performance and carcass traits in bulls immunized against GnRH, as these bulls were similar to unimmunized bulls. This study did not find a significant difference in testosterone production between the control and immunized bulls, which may explain why steroid supplementation was ineffective. However, a further study found that even though testosterone secretion was decreased in bulls actively immunized against GnRH, growth and feedlot performance was not improved by implantation (Adams et al. 1993). Huxsoll et al. (1998) implanted one month old calves to determine whether implantation would suppress the early stages of testicular growth and development and thus complement the effect of immunization

against GnRH. They observed that treatment combining early implantation with immunization did not suppress testicular development more effectively than immunization alone and found no additional benefit to a treatment regimen combining both.

Against this background, the following research was conducted to look at the effects of immunocastration using anti-GnRH immunization. Immunocastrated bulls were compared to intact bulls and when appropriate, to surgically castrated bulls for growth, feed intake, feed efficiency, carcass traits, behavior, GnRH titres, scrotal circumference, serum testosterone concentration and other testicular traits.

Fig. 1.1: Endocrine-neuroendocrine relationship among hypothalamus, pituitary gland and testis (adapted from Hafez 1993 p. 68).

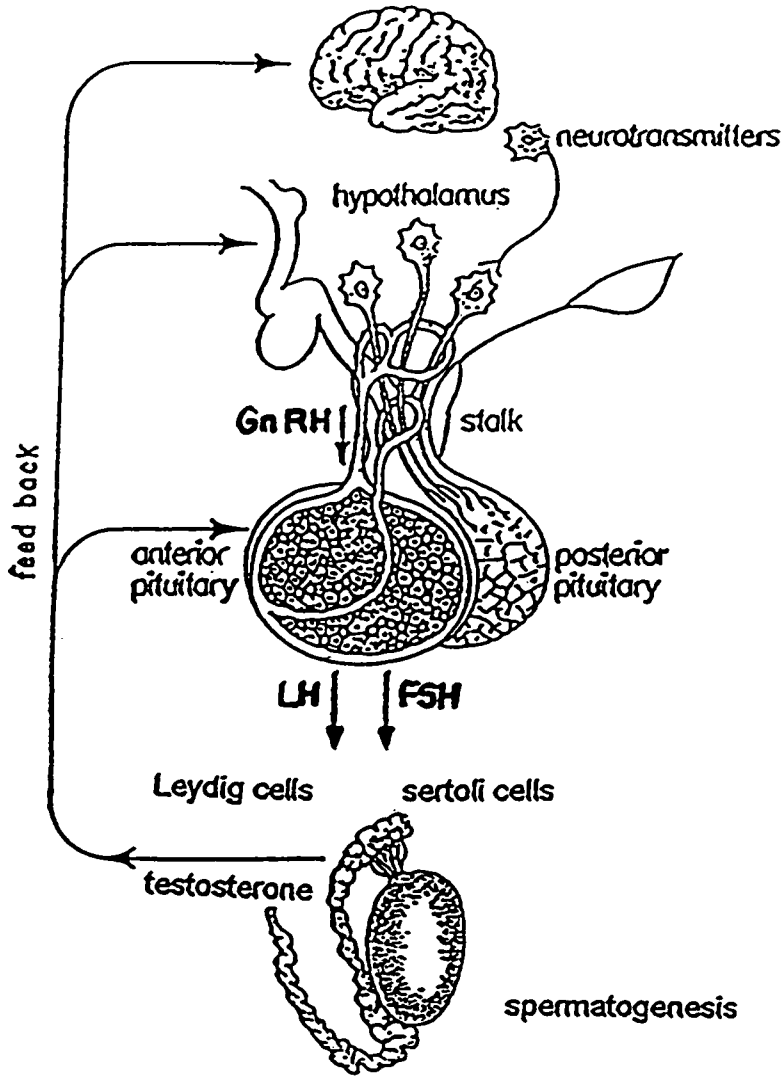
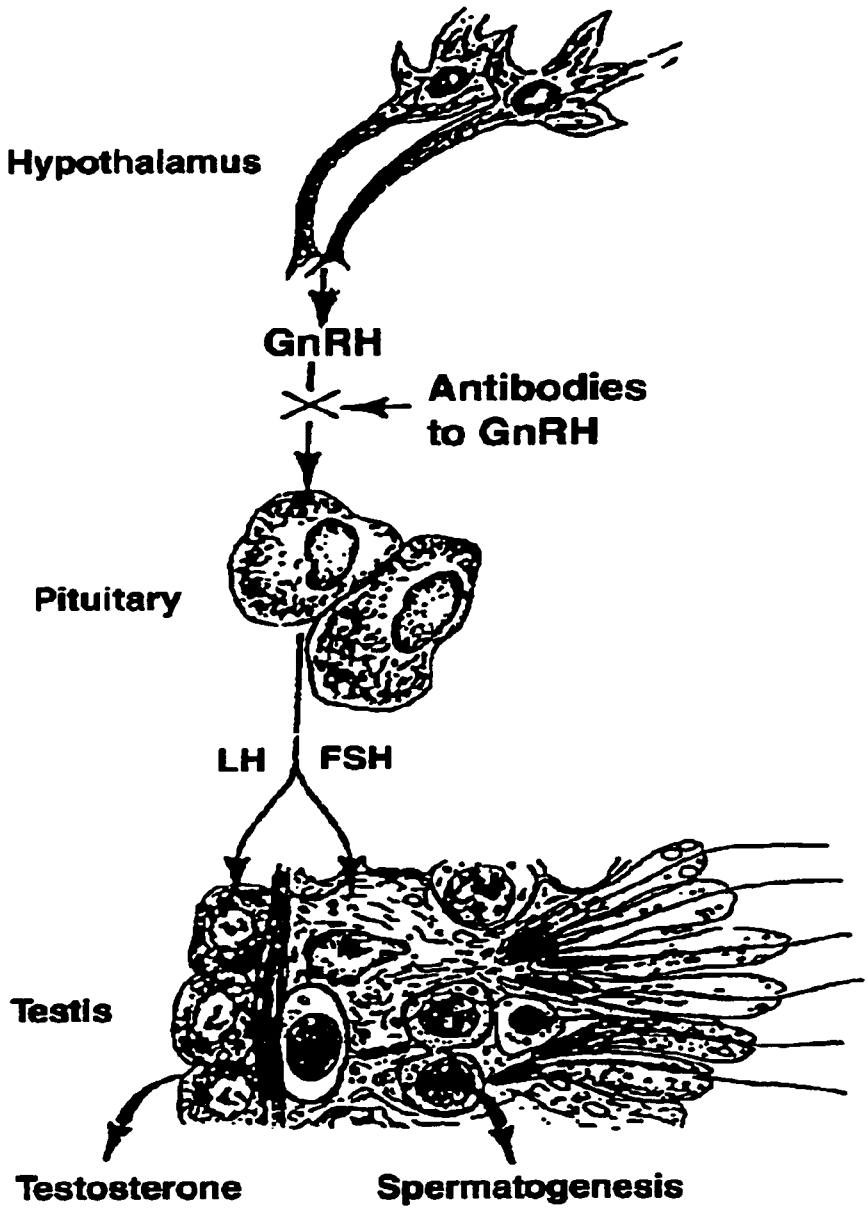


Fig. 1.2: Proposed site at which GnRH antibodies intercept GnRH and inhibit reproductive function in the male (Crighton 1984 p. 345).



1.10 REFERENCES

Adams, T. E., and Adams, B. M. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. **70**:1691-1698.

Adams, T. E., Daley, C. A., Adams, B. M., and Sakursi, H. 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. **71**:811-817.

Adams, T. E., Daley, C. A., Adams, B. M., and Sakursi, H. 1996. Testes function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of age at immunization. J. Anim. Sci. **74**:950-954.

Amann, R. P., Wise, M. E., Glass, J. D. and Nett, T. M. 1986. Prepubertal changes in the hypothalamic-pituitary axis of holstein bulls. Biol. Reprod. **34**:71-80.

Apple, J. K., Dikeman, M. E., Simms, D. D. and Kuhl, G. 1991. Effects of synthetic hormone implants, singularly or in combination, on performance, carcass traits, and longissimus muscle palatability of holstein steers. J.Anim.Sci. **69**:4437-4448.

Appleby, M. C. 1986. Development of sexual and agonistic behavior in bulls and steers. Appl. Anim. Behav. Sci. **15**:190 (abstr.).

Baker, A. M. and Gonyou, H. W. 1986. Effects of zeranol implantation and late castration of sexual, agonistic and handling behavior in male feedlot cattle. J. Anim. Sci. **62**:1224-1232.

Balthazart, J. 1990. Brain aromatization of testosterone regulates male reproductive behavior in birds. Progress of Comparative Endocrinology. Wiley-Liss Inc. Chichester. pp. 92-98.

Bearden, H. J. and Fuquay, J. W. 1997. Applied Animal Reproduction – 4th Ed. Prentice Hall Inc. New Jersey, USA. pp.22.

Bonneau, M. and Enright, W. J. 1995. Immunocastration in cattle and pigs. Livest. Prod. Sci. **42**:193-200.

Calkins, C. R., Clanton, D. C., Berg, T. J. and Kinder, J. E. 1986. Growth, carcass and palatability traits of intact males and steers implanted with zeranol or estradiol early and

throughout life. J. Anim. Sci. **62**:625-631.

Campos, M. 1992. J.Anim.Sci. **70**:231-239.

Canfax Annual Report 1997. Canfax and Canfax Research Services, A Division of Canadian Cattlemen's Association. Calgary, Alberta, Canada.

Carson, A., McCaughey, W. J. and Steen, R. W. J. 1992. Active immunization of young male calves and post-pubertal bulls against LHRH with various conjugate doses. Proc. Brit. Soc. Anim. Prod. **54**:465.

Chandolia, R. K., Rawlings, N. C. and Evans, A. C. O. 1994. Inhibition of the early rise of LH secretion in bull calves suppresses testicular development. Biol. Reprod. **50**:86 (abstr.).

Chapel, H. M. and August, P. J. 1976. Report of nine cases of accidental injury to man with Freund's complete adjuvant. Clin. exp. Immunol. **24**:538-541.

Cohen, R. D. H., King, B. D., Janzen, E. D. and Hunter, P. S. W. 1991a. The efficacy of castration methods and effects of age at castration and implant regime on testicular measurements and testosterone levels of male beef cattle. Can. J. Anim. Sci. **71**: 1-11.

Cohen, R. D. H., King, B. D., Janzen, E. D. and Nicholson, H. H. 1991b. The effect of castration age, method and implant regime on growth and carcass traits of male beef cattle. Can. J. Anim. Sci. **71**: 301-309.

Cook, B., Popp, J., Kastelic, J., Robbins, S. and Harland, R. 1998. Active immunization of bulls against GnRH: effects on testes, feedlot performance and carcass characteristics. J. Anim. Sci. **76(Suppl.)**:277 (abstr.).

Covey, D.C., O'Brien, K.D. and Moore, D.E. 1985. Current trends in antifertility vaccine research. West. J. Med.. **142(2)**:197-201.

Crighton, D. B. 1985. Immunological aspects of reproduction in mammals. Butterworths. England. p. 346.

Curran, S., Crowley, J. P. and McGloughlin, P. 1965. The effect of spaying and hormone implantation on the growth and carcass quality of beef heifers. Ir. J. Agric. Res. 4:93-100.

Deaver, D. R., Glass, J. D., Grieger, D. M. and Reeves, J. J. 1988. Effects of estradiol

on secretion of LH, hypothalamic function and testicular development in bull calves. Domest. Anim. Endocrinol. **5**:307.

Dirnhofer, S., Wich, G. and Berger, P. 1994. The suitability of human chorionic gonadotropin (hCG)-based birth-control vaccines. Immunol. Today. **15(10)**:469-474.

D'Occhio, M. J. and Brooks, D. E. 1982. Threshold of plasma testosterone required for normal mating activity in male sheep. Horm. Behav. **16**:383-394.

Doornenbal, H., Tong, A. K. W., Newman, J. A., Murray, N. L. and Mears, G. J. 1987. Blood and serum components and organ weights in steers, bulls and zeranol-implanted bulls. J. Anim. Sci. **64**:489.

Dykeman, D. A., Katz, L. S. and Foote, R. H. 1982. Behavioral characteristics of beef steers administered estradiol, testosterone and dihydrotestosterone. J. Anim. Sci. **55**:1303-1309.

Evans, A. C. O., Currie, W. D. and Rawlings, N. C. 1993. Opioidergic regulation of gonadotrophin secretion in the early prepubertal bull calf. J. Reprod. Fertil. **99**:45-51.

Evans, A. C. O., Davies, F. J., Nasser, L. F., Bowman, P. and Rawlings, N. C. 1995. Differences in early patterns of gonadatrophin secretion between early and late maturing bulls, and changes in semen characteristics at puberty. Theriogenology **43**:569-578.

Evans, A. C. O., Pierson, R. A., Garcia, A., McDougall, L. M., Hrudka, F. and Rawlings, N. C. 1996. Changes in circulating hormone concentrations, testes histology and testes ultrasonography during sexual maturation in beef bulls. Theriogenology **46**:345-357.

Finnerty, M., Enright, W. J., Morrison, C. A. and Roche, J. F. 1994. Immunization of bull calves with a GnRH analogue-human serum albumin conjugate: effect of conjugate dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. J. Reprod. Fert. **101**:333-343.

Finnerty, M., Enright, W. J., Prendiville, D. J., Spicer, L. J. and Roche, J. F. 1996. The effect of different levels of gonadotropin-releasing hormone antibody titres on plasma hormone concentrations, sexual and aggressive behaviour, testis size and performance of bulls. Anim. Sci. **63**:51-63.

Fraser, H. M. 1980. 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.

Fraser, H. M. 1983. LHRH immunoneutralization: Basic studies and prospects for practical application. Immunological Approaches to Contraception and Promotion of Fertility (ed. G. P. Talwar) pp.125. Plenum Press, New York.

Gonzalez, A., Goubau, S., Allen, A. F., Mapletoft, R. J., Cohen, R., and Murphy, B. D. 1990. Livestock Reproduction in Latin America (IAEA Panel Proceedings Series STI/PUB 833). p.269-284.

Goubau, S., Siversides, D. W., Gonzalez, A., Laarveld, B., Mapletoft, R. J. and Murphy, B. D. 1989a. Immunization of cattle against modified peptides of gonadotropin releasing hormone conjugated to carriers: effectiveness of Freund's and alternative adjuvants. Theriogenology **32(4)**:557-567.

Gregory, K. E. and Ford, J. J. 1983. Effects of late castration, zeranol and breed group on growth, feed efficiency and carcass characteristics of late maturing bovine males. J. Anim. Sci. **56**:771-780.

Griffin, P.D. 1993. Immunization against HCG. Human Reproduction. **9(2)**:267-272.

Hafez, E.S.E. 1993. Reproduction in Farm Animals - 6th Ed. Lea and Febiger, Philadelphia. p. 82.

Hage-van Noort, M., Puijk, W. C., Schaaper, W. M. M., Kuperus, D., Beekman, N. J. C. M., Plasman, H. H., Lankof, H., Wensing, C. J. G., and Meloen, R. H. 1992. Immunomodulation of reproductive systems. J. Anim. Reprod. Sci. **28**:187-193.

Hannon, K., Gronowski, A. and Trenkle, A. 1991. Relationship of liver and skeletal muscle IGF-1 mRNA to plasma GH profile, production of IGF-1 by liver, plasma IGF-1 concentrations, and growth rates of cattle. Proc. Soc. Exp. Biol. Med. **196**:155-163.

Harland, Richard. 1998. Personal communication. Biowest Inc. Saskatoon, Saskatchewan.

Hayden, J. M., Bergen, W. G. and Merkel, R. A. 1992. Skeletal muscle protein metabolism and serum growth hormone, insulin and cortisol concentrations in growing steers implanted with estradiol-17β, trenbolone acetate, or estradial-17β plus trenbolone acetate. J. Anim. Sci. **70**:2109-2119.

Henricks, D. M., Gimenez, T., Gettys, T. W. and Schanbacher, B. D. 1988. Effect of

castration and an anabolic implant on growth and serum hormones in cattle. Anim. Proc. **46**:35.

Hinch, G. N., Lynch, J. J. and Thwaites, C. J. 1982/83. Patterns and frequency of social interactions in young grazing bulls and steers. Appl. Anim. Behav. Sci. **9**:15-30.

Huxsoll, C. C., Price, E. O. and Adams, T. E. 1998. Testis function, carcass traits and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. **76**:1760-1766.

Jago, J. G., Cox, N. R., Bass, J. J. and Matthews, L. R. 1997. The effect of prepubertal immunization against Gonadatropin-Releasing Hormone on the development of sexual and social behavior of bulls. J. Anim. Sci. **75**:2609-2619.

Jeffcoate, I. A., Lucas, J. M. S. and Crighton, D. B. 1982. Effects of active immunization of ram lambs and bull calves against synthetic luteinizing hormone releasing hormone. Theriogenology **18(1)**:65-77.

Jones, S. J., Johnson, R. D., Calkins, C. R. and Dikeman, M. E. 1991. Effects of trenbolone acetate on carcass characteristics and serum testosterone and cortisol concentrations in bulls and steers on different management and implant schemes. J. Anim. Sci. **69:**1363.

Katz, L. S. and McDonald, T. J. 1992. Sexual behavior of farm animals. Theriogenology **38**:239-253.

Keeling, B. J. and Crighton, D. B. 1984. Reversibility of the effects of active immunisation against LH-RH. In Immunological aspects of reproduction in mammals (ed. D. B. Crighton), pp. 379-397. Butterworths, London.

Kempster, A. J. and Lowe, D. B. 1993. Growth performance and carcass characteristics as influenced by genotype and environment. Proc. 44th Annu. Mtg. EAAP, Aarhus, p 2.1:322-323.

Kenny, F. J. and Tarrant, P. V. 1988. The effect of oestrus behaviour on muscle glycogen concentration and dark-cutting in beef heifers. Meat Sci. **22**:21-31.

Langer, R. 1981. Polymers for the sustained release of macromolecules: their use in a single-step method of immunization. Methods in Enzymology **73**:57-75

Lee, C. Y., Henricks, D. M., Skelley, G. C. and Grimes, L. W. 1990. Growth and

hormonal response of intact and castrate male cattle to trenbolone acetate and estradiol. J. Anim. Sci. **68**:2682-2689.

Lister, D. 1988. Muscle metabolism and animal physiology in the dark cutting condition. In: Dark-cutting in cattle and sheep-Proceedings of an Australian workshop. Australian Meat and Live-stock Research and Development Corporation, Sydney South, NSW, Australia.

Lobley, G. E., Connell, A., Morris, B., Anderson, J., Clayton, P. E., Williams, V. and Nevison, I. M. 1992. The effect of active immunization against gonadotropin-hormone-releasing-hormone on growth performance and sample joint composition of bulls. Anim. Prod. **55**:193-202.

McCarthy, M. S., Hafs, H. D. and Convey, E. M. 1979. Serum hormone patterns associated with growth and sexual development on bulls. J. Anim. Sci. **49(4)**:1012-1020.

Meloen, R. H., Turkstra, J. A., Lankhof, H., Puijk, W. C., Schaaper, W. M. M., Dijkstra, G., Wensing, C. J. G. and Oonk, R. B. 1994. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. Vaccine **12(8)**:741-746.

Melson, B. E., Brown, J. L., Schoenemann, H. M., Tarnavsky, G. K. and Reeves J. J. 1986. Elevation of serum testosterone during chronic LHRH agonist treatment in the bull. J. Anim. Sci. **62**:199-207.

Millar, R. P., Tobler, C., King, J. A. and Arimura, A. 1984. Region-specific antisera in molecular biology of new uropeptides: Application in quantitation, structural characterization and metabolism of luteinizing hormone-releasing hormone. Molecular Biology Approach to the Neurosciences (ed. H. Soreq), pp.221. Wiley, New York.

Morris, B. A. 1985. Principles of immunoassay. In Immunoassay in food analysis (ed. B. A. Morris and M.N. Clifford), pp. 28-31. Elsevier Applied Science Publishers, London.

Perry, T. C., Fox, D. G. and Beermann, D. H. 1991. Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of holstein and beef steers. J. Anim. Sci. **69**:4696.

Price, C. A., Morris, B. A. and Webb, R. 1987. Reproductive and endocrine effects of active immunization against a testosterone conjugate in the heifer. J. Reprod. Fertil. **81**:149-160.

Price, E. O. and Wallach, S. J. R. 1991. Development of sexual and aggressive behaviors in Hereford bulls. J. Anim. Sci. **69**:1019-1027.

Price, M.A., Jones, S.D.M., Mathison, G.W. and Berg, R.T. 1980. The effects of increasing dietary roughage level and slaughter weight on the feedlot performance and carcass characteristics of bulls and steers. Can. J. Anim. Sci. **60**:345-358.

Price, M.A. and Tennessen, T. 1981. Preslaughter management and dark-cutting in the carcasses of young bulls. Can. J. Anim. Sci. **61**:205-208.

Rawlings, N. C. and Evans, A. C. O. 1995. Androgen negative feedback during the early rise in luteinizing hormone secretion in bull calves. J. Endocrinol. **145**:243-249.

Reinhardt, V., Mutiso, F. M. and Reinhardt, A. 1978. Social behavior and social relationships between female and male prepubertal bovine calves (Bos indicus). Appl. Anim. Behav. Sci. **4**:43-54.

Robertson, I. S. and Lowman, B. G. 1977. Bull beef production from suckler herds of traditional breeds. Anim. Prod. **24**:144(abstr.).

Robertson, I. S., Wilson, J. C. and Fraser, H. M. 1979. Immunological castration of male cattle. Vet. Rec. **105**:556-557.

Robertson, I. S., Fraser, H. M., Innes, G. M. and Jones, A. S. 1982. Effect of immunological castration on sexual and production characteristics in male cattle. Vet. Rec. **111**:529-531.

Robertson, I. S., Wilson, J. C., Fraser, H. M., Innes, G. M. and Jones, A. S. 1984. Immunological castration of young bulls for beef production. Current topics in Vet. Med. Anim. Sci. **26**:137-145.

Roche, J. F. and Crowley, J. P. 1973. The long-term suppression of heat in cattle with implants of melengestrol acetate. Anim. Prod. **16**:245-250.

Rodriguez, R.E. and Wise, M.E. 1989. Ontogeny of Pulsatile Secretion of Gonadotropin-Releasing Hormone in the Bull Calf During Infantile and Pubertal Development. Endocrinology. **124**:248 256.

Ronayne, E., Enright, W. J. and Roche, J. F. 1993. Effects of continuous administration of gonadotropin-releasing hormone (GnRH) or a potent GnRH analogue on blood luteinizing hormone and testosterone concentrations in prepubertal bulls. Domest. Anim.

Endocrin. **10**:179-189.

Rumsey, T. S., Hammond, A. C. and McMurtry, J. P. 1992. Response to reimplanting beef steers with estradiol benzoate and progesterone: performance, implant absorption pattern, and thyroxine status. J. Anim. Sci. **70**:995-1001.

Scanga, J. A., Belk, K. E., Tatum, J. D., Grandin, T. and Smith, G. C. 1998. Factors contributing to the incidence of dark cutting beef. J. Anim. Sci. **76**:2040-2047.

Schanbacher, B. D. 1982. Responses of ram lambs to active immunization against testosterone and luteinizing hormone-releasing hormone. Am. J. Physiol. 242:E201-E205.

Schanbacher, B. D. 1984a. Active immunization against LH-RH in the male. Immunological Aspects of Reproduction in Mammals (ed. D B. Crighton),. Butterworths. England. pp.345-362.

Schanbacher, B. D. 1984b. Pituitary-testicular responses of estradiol-17β-implanted bull calves to continuous versus pulsatile infusion of luteinizing hormone releasing hormone. J. Anim. Sci. **58**:943.

Silcox, R. W., Keeton, J. T. and Johnson, B. H. 1986. Effects of zeranol and trenbolone acetate on testis function, live weight gain and carcass traits of bulls. J. Anim. Sci. **63**:358-368.

Silversides, D. W., Allen, A. F., Misra, V., Murphy, B. D. and Mapletoft. R. J. 1988. A synthetic LHRH vaccine. II. Temporal aspects of titer development and formulation trials in Balb/C mice. J. Reprod. Immunol. **14**:47.

Sodersten, P. A., Hansen, S., Eneroth, P., Wilson, C. A. and Gustafsson, J. A. 1980. Testosterone in the control of rat sexual behavior. J. Steroid Biochem. **12**:337.

Staigmiller, R. B., Brownson, R. M., Kartchner, R. J. and Williams, J. H. 1985. Sexual development in beef bulls following zeranol implants. J. Anim. Sci. **60(2)**:342-351.

Tarrant, P.V. 1981. The occurrence, causes and economic consequences of dark cutting beef - A survey of current information. Pages 3-34 in D.E. Hood and P.V. Tarrant, eds. The problem of dark cutting beef. Martinus-Nijhoff Publishers, The Hague, The Netherlands.

Trow-Smith, R. 1957. A history of British livestock husbandry to 1700. London: Routledge and Kegan Paul. pp.286.

2 EFFECTS OF SURGICAL, IMMUNO- AND NON-CASTRATION ON FEEDLOT PERFORMANCE IN THREE BREED-TYPES OF BULL CALVES

2.1 INTRODUCTION

Bulls intended for feedlots in North America are usually castrated to suppress testosterone production. Steers are preferred in feedlots because they are more manageable and have a lower incidence of dark cutting when slaughtered (Price and Tennessen 1981; Tarrant 1981). However, gonadal steroids play a critical role in mammalian growth and development (Adams and Adams 1992) and entire bulls have higher growth rates, convert feed more efficiently and produce leaner carcasses than steers (Price et al. 1980). Animals castrated upon feedlot entry experience significant reductions in final live weight, average daily gain and hot carcass weight when compared to intact contemporaries (Adams and Adams 1992; Gonzalez et al. 1990). In addition to the loss of growth potential, there are animal welfare concerns with surgical castration, an associated risk of infection that may lead to morbidity or mortality, and labour and equipment costs necessary to perform the procedure. When older bulls are castrated prior to feedlot entry the effects on their health and growth become even more serious. Research has shown that bulls castrated in the feedlot exhibit poorer gains compared with earlier castrated steers, to 104 days post treatment, and conventional castration is

discouraged for short keep feedlot animals (Hand and Goonewardene 1989).

Testicular function in bulls is primarily regulated by the secretion of LH and FSH, both of which are regulated by the secretion of GnRH from the hypothalamus (Amann et al. 1986; Finnerty et al. 1996). Disruption of testosterone production through active immunization against GnRH could be a viable and practical method of minimizing these disadvantages while achieving some measure of emasculation (Teague et al. 1992; Adams and Adams 1992). There is a suggestion (Lobley et al. 1992) that immunocastration of bulls will reduce aggressive and sexual behavior without severely reducing anabolic performance. Immunoneutralization of GnRH would temporarily suppress pituitary gonadatropin secretion, hence decreasing testosterone production, attenuating growth and development of testicular tissue and inhibiting spermatogenesis (Jeffcoate et al. 1982; Robertson et al. 1984; Finnerty et al. 1994). Since the GnRH molecule is too small to elicit an immune response, GnRH must be conjugated to a protein carrier to inhibit GnRH production and the subsequent hormonal cascade. Nevertheless, an immunocastrated male could still be expected to secrete a base amount of testosterone from the Sertoli cells, allowing some expression of masculinity.

Peptide vaccines are simple to use and inexpensive relative to surgical castration. They are easy to produce, store, transport and modify (Hage-Van Noort et al. 1992), and immunization has a very low associated risk of morbidity and mortality. The primary

objective of this study was to determine the effect of immunocastration with an anti-GnRH peptide on production, behavior and carcass traits in bull calves.

2.2 MATERIALS AND METHODS

This study, conducted in accordance with Canadian Council of Animal Care (1993) guidelines, used 90 male calves born at the University of Alberta Beef Research Ranch at Kinsella. A factoral design with three castration treatments, three breed-types, five calves per pen, and two replications was used. The three castration treatments were non-castration (BULL), immunocastration (IMMUNO) and surgical castration (STEER). The three breed-types which have been fully described by Berg et al. (1986), were Beef Synthetic 1 (BS1), a hybrid of Angus, Galloway and Charolais; Beef Synthetic 2 (BS2), a hybrid containing about 50% Hereford and 50% other beef breeds; and Dairy Synthetic (DS), a hybrid dominated by Holstein, Brown Swiss and Simmental. Thirty calves were randomly allocated at d 0 (July 26, 1994, aged 2.5 to 3.5 mo) to each of the three treatments. Immediately after assignment to treatment, the IMMUNO group was vaccinated with a chemical conjugate of GnRH and mammalian ovalbumin (Sigma Chemical Co., St. Louis, Mo). Prior to vaccination, the GnRH:ovalbumen conjugate was diluted in physiological saline to give a final concentration of 0.5 mg mL-1 GnRH equivalents. The diluted conjugate was then mixed with an equal volume of Emulsigen Plus (MVP Laboratories Inc., Ralston, ME). Both priming and booster vaccinations supplied by the University of Saskatchewan (Saskatoon, Saskatchewan) consisted of 2

mL (0.5 mg GnRH equivalents) of the adjuvanted vaccine delivered intramuscularly to the left hip. The BULLs remained intact and were vaccinated with 2 mL of a placebo consisting of the adjuvant. At the same time, a veterinarian surgically castrated the STEERs using a scalpel to open the scrotum, Ligaclips (Ethicon Inc.) to clip the artery and an emasculator to cut each spermatic cord. Each STEER was given an intramuscular injection of 20 mL of the antibiotic Longisil® and vaccinated with 2 mL of the same placebo as the BULLs.

All 90 calves were weaned on d 100 (Nov. 3; aged 5.5 to 6.5 mo) and the immunity of the IMMUNO group was boosted with the same vaccine as used previously, at this time, and again on d 194 (Feb. 15; aged 8.5 to 9.5 mo). The BULLs and STEERs received placebos at both dates. No further boosters were given since a 90 day pre-slaughter withdrawal period was required by the Bureau of Veterinary Drugs for this vaccine. Live weight, scrotal circumference, and hip height were measured on days 100, 128, 162, 194, 219 and 290. Hip height was measured as the vertical distance from the ceiling to the mid-back above the last sacral vertebrae. This number was subtracted from the ceiling to floor distance to obtain a calculation of hip height. Scrotal circumference was measured using a plasticized cloth tape.

Following weaning, the cattle were allocated to six by eight metre pens, and the quantity of the feedlot diet (Table 2.1) was gradually increased starting at 16 kg/pen/day until all were on ad libitum intake by d 128. Blood was collected by jugular puncture into

10 ml vaccutainer® tubes on days 100 (immediately prior to vaccination), 107, 114, 128, 162, 219 and 290. A minimum of sixty minutes after collection, the blood was centrifuged at 1650 g for 50 minutes and the serum was pipetted into sterile vials and stored at –23 °C until it was assayed for serum testosterone concentration using Coat-A-Count® kit (Diagnostic Products Corporation, Los Angeles, California) and for anti-GnRH antibody titres (Van Kessel, 1998).

2.2.1 *Slaughter*

Cattle were selected for slaughter on a pen basis when they visually appeared to have 10 mm of fat cover. Most were approximately 13 months of age. They were trucked directly from the ranch to a commercial abattoir 300 km away, where they were held for approximately four hours prior to being slaughtered. Warm carcass weight, rib-eye area (REA; measured between the 12^{th} and 13^{th} rib), grade fat, cutability estimate, marbling and quality grade, were measured by Canadian Grading Agency graders at the abattoir on the morning following slaughter. Average fat was calculated as the average of three fat depths over the longissimus dorsi (Agriculture Canada Form ML107, undated). Cutability (percentage of the carcass that is red meat) was calculated as: 57.96 - (0.027 x warm wt.) + (0.202 x REA) - (0.703 x avg. fat) (Agriculture and Agri-Food Canada 1992).

2.2.2 *Behavior*

Behavior of the cattle was observed on two occasions: d 219 (February 28, 1995; 9.5 to 10.5 mo of age) and d 289 (May 10, 1995; 11.5 to12.5 mo). Six animals (two of each breed-type) of the same castration treatment, but from six different pens, were put in a 4.9 by 6.7 metre wood plank pen with three cows and observed for a 15 minute period on d 219 and a 10 minute period on d 289. The cows were injected with depo testosterone to induce estrus and were tied to the fence during the test. At the end of each minute, sexual behavior towards the cows and aggressive behavior towards the other males were recorded as having occurred or not. Sexual behaviors recorded were interest (genital sniffing and flehmen) and mounting. Recorded aggressive behaviors were bunting (one animal bunting another anywhere on the body) and fighting (prolonged head to head pushing). The same two people observed and recorded the behaviors each time.

2.2.3 *Statistical Analysis*

The main effects included in the statistical model were treatment (BULL, IMMUNO and STEER), breed-type (BS1, BS2 and DS), pen (treatment by breed-type), period (1 to 5) and interactions. Data were analyzed using the following model:

$$Y_{ijklm} = \mu + T_i + B_j + TB_{ij} + PN_{k(ij)} + PE_l + PET_{il} + PEB_{jl} + PETB_{ijl} + e_{ijklm},$$

where Y_{ijklm} = animal observation, μ=overall mean, T_i = treatment (i = BULL, IMMUNO, STEER), B_j = breed-type (j = BS1, BS2, DS), TB_{ij} = interaction between treatment and

breed-type, $PN_{k(ij)}$ = pen with treatment and breed-type nested, PE_l = period (l = 1 to 5), PET_{il} = interaction between pen and treatment, PEB_{jl} = interaction between pen and breed-type, $PETB_{ijl}$ = interaction between pen and treatment and breed-type and e_{ijklm} = random error. The dependent variables, repeated over time, included: live weight, average daily gain (ADG), hip height, scrotal circumference, serum testosterone concentration and anti-GnRH titres. These were analysed as a split plot in time (repeated measures) using the General Linear Model (GLM) procedure of SAS (Statistical Analysis Systems Institute Inc.1990). Scrotal circumference was adjusted for differences in live weight. Pen feed intake was recorded daily, starting on d 128, and feed efficiency was calculated as the ratio of gain to feed. Average daily gain was computed as the change in the regression of live weight from d 128 to d 250. Slaughter weight was determined by linear regressions of weight on age. Feed intake and gain:feed were analysed on a pen basis using the same model as before. Day 0 weight, slaughter age, carcass traits and behavior were analysed using the General Linear Models Procedure (GLM) of SAS, and the main effects included in the model were treatment, breed-type, pen (treatment by breed-type) and interactions. Where a significant main effect was detected, at the 0.05 probability level, least square means were separated using the PDIFF option procedure (SAS 1990).

2.3 RESULTS

Two BULL, BS2 calves died from bloat (d 193 and d 279) and their data were not used in

the statistical analyses. The blood samples collected on d 107 and d 114 to be assayed for serum testosterone concentration were misplaced prior to being assayed.

2.3.1 *Antibody Binding and Testicular Traits*

There was a significant ($P<0.01$) interaction between treatment (BULLs, IMMUNOs and STEERs) and period (one to five) for both GnRH antibody titres and testosterone concentration. There was also a significant ($P<0.01$) interaction between treatment and period and between breed-type and period ($P<0.01$) for scrotal circumference. There were no other significant ($P>0.05$) interactions between castration treatment, breed-type and period for GnRH antibody binding, serum testosterone concentration or scrotal circumference.

Following the booster vaccination given on d 100, the IMMUNO cattle showed a large increase in the percent of bound GnRH (Table 2.2). Titre levels decreased 23% between d 107 and d 114 and a further 53% by d 162 (Fig. 2.1). A second booster was given on d 194, and by d 219 the titre level was 23% higher than it had been on d 162. By d 290 the mean bound GnRH levels were 50% of what they had been on d 219, and in twelve of the 30 IMMUNOs, binding could not be detected at 1:1000 dilution. However, a further booster was not given because of an expectation of slaughter within 90 days. At days 107, 114, 128, 162 and 290, 56.7%, 33%, 32%, 3% and 0% of IMMUNO animals respectively showed 80% or higher binding of GnRH at a dilution of 1:1000. The binding levels pooled over time for the three breed-types in the IMMUNO group was BS1=

35.2% ± 4.4, BS2= 43.2% ± 5.6 and DS= 50.1% ± 4.9, P=0.09 (Table 2.2).

Although there was no difference (P<0.05) between BULLs and IMMUNOs for serum testosterone concentration at previously measured times, by d 290 the testosterone concentration of the IMMUNOs was 33% lower (P<0.01) than that of the BULLs (Table 2.2). As GnRH titres declined in the immunocastrates, testosterone concentrations increased by 83% from d 219 to d 290. The correlation coefficients (r) between testosterone concentration and GnRH percent binding (1:1000 dilution) at days 128, 162, 219 and 290 in the IMMUNOs were -0.06 (P=0.74), -0.74 (P<0.01), -0.41 (P=0.04), -0.38 (P=0.07) and -0.39 (P=0.13) respectively.

There was a significant (P<0.01) treatment, breed-type and period effect on scrotal circumference. The scrotal circumference of the IMMUNOs was significantly (P<0.01) smaller than that of the BULLs at all measured times after the first booster vaccination. The final scrotal circumference of the BULLs and IMMUNOs prior to slaughter was 30.8 and 29.3 cm respectively (Table 2.2). DS had significantly (P<0.05) larger scrotal circumferences than BS1, and BS1 had significantly (P<0.05) larger than BS2 (Table 2.2).

2.3.2 *Production and Carcass Traits*

There were no significant (P>0.05) interactions between castration treatment and breed-type for any of the production or carcass traits, with the exception of a significant (P<0.05) interaction for rib-eye area (Fig. 2.2). This interaction shows that STEERs of all

three breed-types have smaller REAs, while DS IMMUNOs and BS1 BULLs have the largest REAs. Castration treatment significantly ($P<0.05$) affected slaughter weight at a constant fatness, ADG, gain:feed, warm carcass weight and dressing percentage, with BULLs and IMMUNOs being not different from each other, but greater ($P<0.05$) than STEERs for all these traits (Table 2.3). For cutability estimate, the BULLs had significantly ($P<0.05$) larger estimates than the STEERs, while the IMMUNOs were not significantly ($P<0.05$) different from either BULLs or STEERs.

The BS1 and BS2 cattle were ready for slaughter at similar ages, while DS animals were about two weeks older ($P<0.05$) when slaughtered. There was no significant ($P<0.06$) breed-type effect on slaughter weight, although it was approaching significance. The BS2 cattle had significantly ($P<0.01$) lower estimated cutabilities, but greater fat cover over the ribeye, than BS1 and DS, which were not different from each other.

Approximately half (53.3%) the STEER and one quarter of the BULL (25.0%) and IMMUNO (23.3%) carcasses received a Canada AA grade, whereas half (53.6%) of the BULLs and two thirds (63.3%) of the IMMUNO carcasses received the Canada A grade (Table 2.4). None of the STEER carcasses graded Canada B4 (dark-cutter), whereas 18% of the BULL and 13% of the IMMUNO carcasses did. Most of the BS2 (57.1%) and DS (70.0%) carcasses received a Canada A grade, while the BS1 carcass grades were more evenly distributed between Canada AA and A.

2.3.3 *Behavior*

There was no significant (P>0.05) castration treatment by breed-type interaction for either sexual or aggressive behavior. Neither was behavior influenced by breed-type. Compared to the IMMUNOs, BULLs showed significantly (P<0.05) greater interest in estrus cows at d 219 (9.5 to 10.5 mo of age), however, at d 289 (11.5 to 12.5 mo of age) their behavior was not significantly different (Table 2.5). STEERs showed significantly (P<0.05) less interest in the cows than the BULLs or IMMUNOs at both ages. Mounting behavior of IMMUNOs (6.5 acts 15 $min.^{-1}$) at 9.5 to 10.5 mo of age was intermediate to that of the BULLs (7.5 acts 15 $min.^{-1}$) and STEERs (2.1 acts 15 $min.^{-1}$). At 11.5 to 12.5 mo of age the IMMUNOs and STEERs mounted significantly less (P<0.01) than BULLs. At the younger age (9.5 to 10.5 mo of age), IMMUNOs showed less (P<0.05) fighting behavior than BULLs (IMMUNO= 0.09 aggressive acts 15 $min.^{-1}$, BULL= 0.64 acts 15 $min.^{-1}$). At 11.5 to 12.5 mo, STEERs fought significantly less (P<0.01) than the BULLs, but IMMUNOs were not different from BULLs.

2.4 DISCUSSION

2.4.1 *Antibody Binding and Testicular Traits*

Adams et al. (1996) considered an antibody response to immunization to be 'good' if 10% or more was bound at serum dilutions of 1:1000. Teague et al. (1992) reported overall antibody binding values of 42% at a dilution of 1:320 for

immunocastrates vaccinated at 10 months and boosted at 11 and 12 mo, and the present study found antibody binding values of 25.6% at 12 mo of age (d 290) at a dilution of 1:1000. These findings are similar to those of Adams et al. (1996) who reported 12 mo antibody binding values of 24.7%, 27.2% and 31.2% at a 1:1000 dilution for bulls immunized at 1.5, four and seven mo of age respectively.

The first booster vaccine given at d 100 of the present study created a high immune response within seven days, with 71% of GnRH being bound to antibody at 1:1000 dilution by that time (Fig. 2.1). A second booster administered on d 194 in response to falling testosterone levels (Fig. 2.3), increased antibody binding capacity to a level one third higher than it had been on d 162, but the titre regressed to 20% binding by d 290. The anti-GnRH titres of the IMMUNOs (mean after the first booster of 47.5% binding and at slaughter of 19.5% binding at 1:1000 dilution) were generally higher than those found by Adams et al. (1993) and Jago et al. (1997), and could still be described as a 'good' immune response using the definition of Adams et al. (1996).

Prior to slaughter at approximately 12 mo of age, GnRH antibodies were detectable in all of the IMMUNO cattle at a dilution of 1:100, but could not be detected in 12 out of 29 cattle at a dilution of 1:1000. Lobley et al. (1992) showed that when antibody titres are detectable at a dilution of 1:1000, serum testosterone concentrations remain low; below that titre, testosterone levels return rapidly to normal. Other studies have shown that hormone concentrations and other biological variables returned to control levels, even while significant antibody titres existed (Finnerty et al.1994; Adams

and Adams 1992; Robertson et al. 1982; Robertson et al. 1984). Jago et al. (1997) reported that although antibody titre remained significantly elevated until slaughter, biological response began to wane in some animals as early as two months after the final booster immunization, so that at slaughter 86% had testosterone concentrations similar to those of intact bulls. The decline in biological response may be due to the actual change or decline in antibody titres (Finnerty et al. 1996), rather than the presence of low titres. The animals in this study whose antibody titres were undetectable at 1:1000 dilution followed this trend.

According to Lobley et al. (1992) an animal can be considered effectively immunosuppressed if its serum testosterone level is below 0.3 $\mu g\ L^{-1}$ (30 $ng\ dL^{-1}$). On this basis, even though significant GnRH-antibody titres were present, any effects of immunization wore off in the 96 days after their previous booster, so that none of the immunocastrates in this study were effectively suppressed prior to slaughter. The individual variation among these cattle is consistent with observations in other studies on GnRH immunization of cattle (Jago et al. 1997; Jeffcoate et al. 1982; Gonzalez et al. 1990; Lobley et al. 1992) and is probably a reflection of the natural variation in immune response that is typical among animals (Hunter 1989).

Significantly lower testosterone levels and scrotal circumferences relative to intact males have been reported in response to active immunization against GnRH in cattle (Teague et al. 1992; Adams et al. 1996), as well as reduced testosterone production and

testes mass (Jago et al. 1997). Immunocastrates in this study had a lower level of testosterone and a significantly lower scrotal circumference on d 290 than intact males (Table 2.2). Conversely, Adams et al. (1992) reported no significant difference in testosterone concentration prior to slaughter at 15.2 - 15.7 mo, between bulls (2.04 ng mL^{-1}) and immunocastrates (2.32 ng mL^{-1}) which were vaccinated at 10.5 to 11 mo of age. Scrotal circumference has been shown to be inversely related ($P<0.001$) to anti-GnRH titre (Adams et al. 1996), and in this study a negative correlation was found between testosterone concentration and percent binding. The IMMUNOs scrotal circumference was significantly ($P<0.01$) smaller at all measured times after the first booster vaccination (Fig. 2.4), and averaged 5.4% less than that of BULLs overall, and 4.9% less at 11.5 to 12.5 mo of age. These differences are less than those reported by Adams et al. (1993), who found that immunocastrates had 15.5% smaller scrotal circumferences than bulls at 15.2 to15.7 mo of age. In addition, Adams et al. (1996) reported differences in scrotal circumference of 12%, 13.6% and 7.6% between immunocastrates and bulls when the immunocastrates were immunized with a single injection at four, seven and 12 mo of age respectively.

2.4.2 *Production and Carcass Traits*

The IMMUNO and BULL groups were similar to each other and different ($P<0.05$) from STEERs for all production and carcass traits reported, except for estimated

cutability. These results are similar to those of Adams and Adams (1992), Finnerty et al. (1994) and Gonzalez (1990) who found no differences between bulls and immunocastrates for slaughter weight, ADG, hot carcass weight, dressing percentage, rib-eye area and yield grade. However, other studies have found immunocastrates to be intermediate to bulls and steers for feedlot gain and rib-eye area (Adams et al. 1993) and for ADG and yield grade (Teague et al. 1992). The similarity between BULLs and IMMUNOs in this study suggests that GnRH, and subsequently testosterone production, was only partially suppressed at the time of slaughter.

The interaction between castration treatment and breed-type for REA shows that STEERs of all three breed-types have smaller REAs, while DS IMMUNOs and BS1 BULLs have the largest REAs. This also indicates anti-GnRH immunization affects the REA of dairy synthetics differently than beef synthetics. The BS2 group had smaller scrotal circumferences, greater fat cover and lower cutability than the BS1 and DS groups. This agrees with other studies using these breed-types (Price et al. 1984; Makarechian et al. 1985; Mwansa et al. 1990).

2.4.3 *Behavior*

The IMMUNO cattle in this study varied significantly from bulls in expression of sexual behavior, especially mounting, but they behaved similarly to bulls for aggressive behavior. The exception to this was that IMMUNOs fought significantly less ($P<0.05$) than BULLs (IMMUNO= 0.09 aggressive acts. 15 $min.^{-1}$, BULL= 0.64 acts. 15 $min.^{-1}$), at

the younger age (9.5 to 10.5 mo of age). At 11.5 to 12.5 mo, STEERs fought significantly less than BULLs and IMMUNOs, which were not different ($P>0.05$) from each other. Jago et al. (1996) also found that although altering GnRH function during the prepubertal period changed development of sexual and social behavior of bulls, generally these effects did not persist, and by slaughter behavior of intact and immunized bulls was similar. It should be noted that in this study, the first test (10 mo of age) took place only 25 days after a booster vaccination, whereas by the second test (12 mo of age) it had been 95 days since the last vaccination and the effects of the vaccine would have lessened over time.

Teague et al. (1992) have reported a lower incidence ($P<0.01$) of mounts in immunocastrates vaccinated at 10 mo of age compared to bulls. This agrees with the present study in which the IMMUNOs mounted significantly ($P<0.01$) less than the BULLs on both occasions.

The pattern and type of behavior expressed by the BULLs and IMMUNOs varied with time. At a younger age they exhibited more sexual activity whereas at the older age they were more aggressive. A previous study also recorded a higher incidence of sexual (mounting) than aggressive behavior in pre-pubertal bulls and steers (Reinhardt et al. 1978; Appleby 1986). Finnerty et al. (1996) observed more general sexual than general aggressive behavior in bulls between the ages of 8 and 13 mo, while at 22 mo of age, most of the observed behavior was aggressive. The aggressive character of bulls

markedly increases during the peri-pubertal period (8 to 16 mo of age) (Baker and Gonyou 1986; Price and Wallach 1991), and is likely correlated with the increased secretion of testosterone that is evident during this stage of development (McCarthey et al. 1979; Staigmiller 1985).

A higher incidence of dark cutting among bull carcasses compared to steers has been reported (Tarrant 1981), particularly if they interact with strange bulls (Price and Tennessen 1981). The Canada B4 grade, given to a dark cutting carcass, reflects pre-slaughter stress, which could result from differences in breed-type and castration treatment. Using the number of carcasses that graded Canada B4 from each castration treatment as an index of stress susceptibility, this study suggested that IMMUNOs were similar to BULLs, and higher than STEERs for stress susceptibility (Table 2.4).

2.5 CONCLUSIONS

In general, there was little difference between breed-types for most traits measured, except that Beef Synthetic 2 cattle were fatter, had smaller rib-eye areas, and consequently had a lower cutability. Although surgical castration lowered acts of sexual and aggressive behavior, it negatively affected growth and feed conversion efficiency, lowered slaughter and carcass weight, dressing percentage, rib-eye area and cutability. This study demonstrated that the anti-GnRH vaccine created a high immune response in feedlot bulls. Pre-pubertal immunization against GnRH impaired testis function, but had little effect on production and carcass traits. As the cattle were fairly young at slaughter,

one year of age, most of the observed behavior was sexual rather than aggressive. For sexual behaviors, immunocastrates behaved intermediately to bulls and surgically castrated steers. Immunocastration shows potential as an alternative castration method, however repeated booster immunizations are needed to maintain the response. Therefore, this method of immunocastration would not work with a 90 day pre-slaughter withdrawal, since effects of the vaccine wear off in less than 90 days, allowing immunocastrates to return to 'normal' bull status. The next experiment also looked at the effects of immunocastration and was initiated on the assumption that the 90 day pre-slaughter withdrawal would be reduced.

Table 2.1: Composition of feedlot diet (per kg).

Ingredient	
Rolled barley (g)	638
Rolled oats (g)	212
Sun cured alfalfa pellets (g)	100
Premix * (g)	50

Calculated Nutrient Content (dry matter basis)	
Net Energy for Maintenance (kJ)	7074
Net Energy for Gain (kJ)	4688
Crude Protein (g)	122
Calcium (g)	5
Phosphorus (g)	4
Vitamin A (IU)	11200
Vitamin E (IU)	11

* 72.2% canola meal, 12.9% limestone, 6.5% phosphorus (as biophos), 3.2% salt, 2.5% barley grain, 2.1% molasses and 0.65% vitamins A, D and E.

Table 2.2: Least squares means of castration treatment and breed-type by period on anti-GnRH titres (1:1000 dilution), serum testosterone concentration and scrotal circumference (boosters given on d 100 and d 194) in young male cattle.

	Castration treatment			Breed-type				
Traits / Period	**BULL**	**IMMUNO**	**P**	**BS1**	**BS2**	**DS**	**P**	**SEM**
Number of animals	28	30		20	18	20		
Anti-GnRH Titres (% binding)			<0.01				0.35	3.1
Day 100	0	6.5		5.7	2.2	11.5		
Day 107	0	70.9		62.8	71.0	78.9		
Day 114	0	62.6		54.7	62.0	71.1		
Day 128	0	62.6		54.8	61.3	71.8		
Day 162	0	30.2		20.2	31.3	39.1		
Day 219	0	39.4		33.8	44.3	40.1		
Day 290	0	19.5		10.8	20.3	27.5		
Testosterone (ng dL^{-1})			<0.01				0.22	1.3
Day 100	98.1	109.6		124.0	75.0	115.4		
Day 128	80.8	95.2		121.1	66.3	75.2		
Day 162	80.8	109.6		132.7	109.6	46.1		
Day 219	46.1	89.4		101.0	37.5	66.3		
Day 290	796.1 [a]	530.7 [b]		654.7	522.1	813.4		
Scrotal circumference (cm)			<0.01				<0.01	0.25
Day 100	25.5	25.2		25.1	25.0	25.9		
Day 128	25.3 [a]	23.8 [b]		24.6	23.7	25.3		
Day 162	28.0 [a]	25.7 [b]		27.1	25.8	27.7		
Day 219	29.7 [a]	28.0 [b]		28.9	27.6	30.0		
Day 290	30.8 [a]	29.3 [b]		29.4	29.2	31.5		

a, b...means with different superscripts in rows are significant for castration treatment (P<0.01)

Table 2.3: Least squares means of castration treatment and breed-type on growth, efficiency and carcass traits in young male cattle.

Traits	Castration treatment				Breed-type				
	BULL	IMMUNO	STEER	P	BS1	BS2	DS	P	SEM [z]
Number of animals	28	30	30		30	28	30		
Day 0 wt. (kg)	150.4	149.0	148.6	0.15	152.4	148.7	146.9	0.11	4.67
Slaughter wt. (kg)	566.3 a	571.8 a	516.8 b	<0.01	567.4	531.1	556.4	0.06	9.4
Slaughter age (d)	396	395	394	0.90	390 a	389 a	406 b	0.02	3.7
ADG (kg d^{-1})	1.7 a	1.6 a	1.4 b	<0.01	1.7	1.6	1.6	0.14	0.05
Feed intake (kg d^{-1})	11.7	10.9	10.6	0.18	11.2	11.1	11.0	0.95	0.40
Gain:feed	0.15 a	0.15 a	0.13 b	0.03	0.15	0.14	0.14	0.23	0.004
Hip height (cm d^{-1}) [x]	0.11	0.10	0.10	0.06	0.11 a	0.10 b	0.11 a	0.02	0.002
Warm carcass wt. (kg)	304.4 a	308.1a	269.1b	<0.01	298.6	285.8	297.9	0.27	5.0
Dressing (%)	53.9 a	54.0 a	52.3 b	<0.01	52.7 a	53.8 b	53.6 b	0.03	0.27
Average fat (mm)	9.6	10.3	11.1	0.29	9.4 a	12.5 b	9.0 a	<0.01	0.63
REA (cm^2)	82.1 a	81.8 a	73.8 b	<0.01	81.8 a	75.9 b	0.1 a	0.02	0.2
Marbling score [y]	8.6	8.3	8.1	0.12	8.2	8.4	8.5	0.55	0.15
Estimated cutability (%)	60.5 a	59.8 ab	58.0 b	0.03	60.6 a	57.3 b	60.5 a	<0.01	0.57

[x] the change in hip height from day 0 to slaughter
[y] Marbling score: smaller number indicates more marbling;
[z] pooled standard error of the mean
a,b.... means with different subscripts in rows are significant (P<0.05).

Table 2.4: Number (percentage) of carcasses in each grade by castration treatment and breed-type in young male cattle.

	Castration treatment			Breed-type		
Carcass Grade*	**BULL**	**IMMUNO**	**STEER**	**BS1**	**BS2**	**DS**
Number of animals	28	30	30	30	28	30
Canada AAA	1 (3.6)	0	0	0	1 (3.6)	0
Canada AA	7 (25.0)	7 (23.3)	16 (53.3)	14 (46.7)	9 (32.1)	7 (23.3)
Canada A	15 (53.6)	19 (63.3)	14 (46.7)	11 (36.7)	16 (57.1)	21 (70.0)
Canada B4	5 (17.9)	4 (13.3)	0	5 (16.7)	2 (7.1)	2 (6.7)

*** Description of grades:**

Canada AAA	youthful carcasses with small or more marbling
Canada AA	youthful carcasses with slight to small marbling
Canada A	youthful carcasses with traces to slight marbling
Canada B4	youthful carcasses with dark-coloured fleshing

Table 2.5: Least squares means of castration treatment and breed-type at two different ages on behavior traits in young male cattle expressed as acts 15 min.$^{-1}$ on d 219 and acts 10 min.$^{-1}$ on d 289.

		Castration treatment				Breed-type				
Behavior	Day	BULL	IMMUNO	STEER	P	BS1	BS2	DS	P	SEM [z]
Sexual										
Interest [x]	219 [y]	7.5 a	6.5 b	2.1 c	<0.01	5.3	5.1	5.7	0.55	0.25
	289 [y]	5.8 a	6.1 a	2.1 b	<0.01	4.4	4.1	5.5	0.06	0.27
Mounting [x]	219	3.2 a	2.2 b	1.3 c	<0.01	1.8	2.2	2.6	0.20	0.20
	289	2.3 a	1.5 b	0.9 b	<0.01	1.6	1.4	1.7	0.84	0.17
Aggressive										
Bunting [x]	219	0.3	0.4	0.2	0.76	0.4	0.3	0.2	0.70	0.09
	289	0.08	0.08	0.07	0.99	0.03	0.15	0.03	0.17	0.03
Fighting [x]	219	0.6 a	0.1 b	0.4 a	0.04	0.2	0.4	0.5	0.38	0.09
	289	1.03 a	0.99 a	0.07 b	<0.01	0.93	0.75	0.40	0.12	0.11

[x] higher numbers indicate greater activity; see text for full description
[y] d 219 (Feb. 28; 9.5 to 10.5 mo of age)
d 289 (May 10; 11.5 to 12.5 mo of age)
[z] pooled standard error of the mean
a,b..... means with different subscripts are significant (P<0.05).

Fig. 2.1: GnRH antibody titres in immunocastrates (1:1000 dilution).

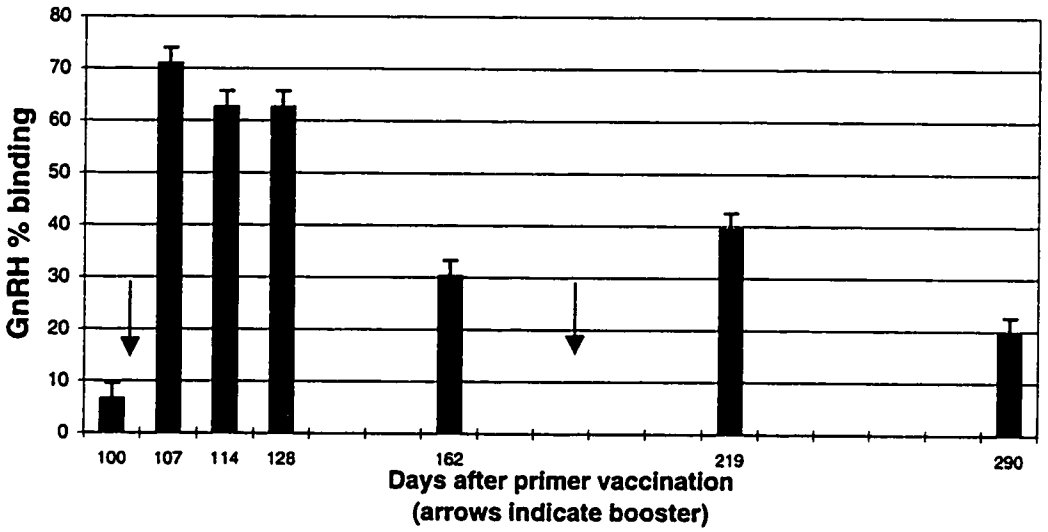


Fig. 2.2: Least squares means for the interaction between castration treatment and breed-type on ribeye area.

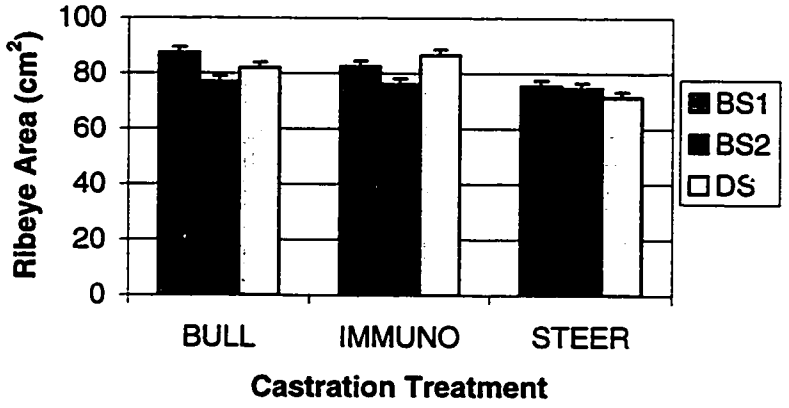


Fig. 2.3: Changes in serum testosterone concentration in bulls and immunocastrates.

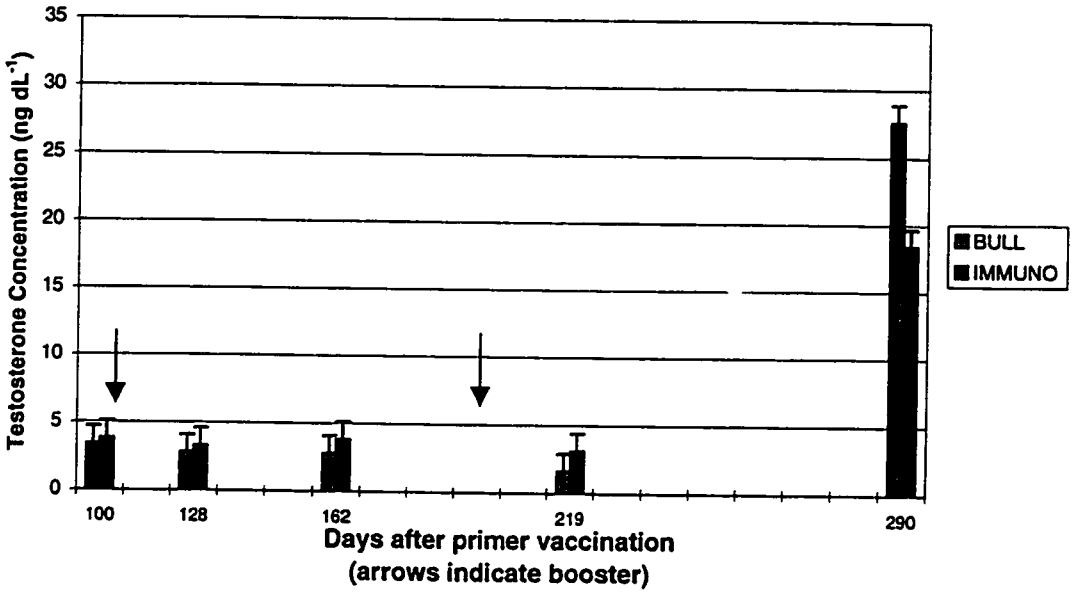
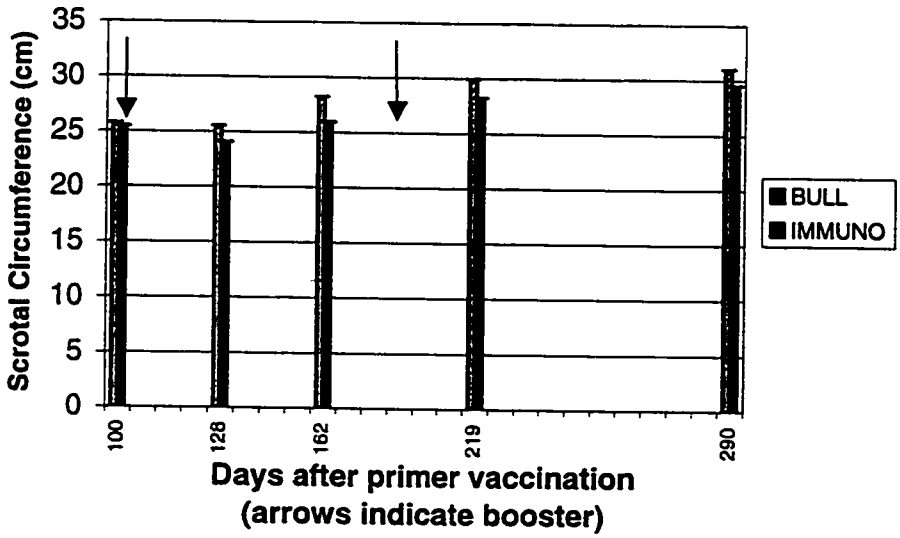


Fig. 2.4: Changes in scrotal circumference of bulls and immunocastrates.



2.6 REFERENCES

Adams, T. E. and Adams, B. M. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. **70**:1691-1698.

Adams, T. E., Daley, C.A., Adams, B. M., and Sakursi, H. 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. **71**:811-817.

Adams, T. E., Daley, C. A., Adams, B. M., and Sakursi, H. 1996. Testes function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: Effect of age at immunization. J. Anim. Sci. **74**:950-954.

Agriculture and Agri-Food Canada. Graders Manual - Beef, Amended May 1992, Chapter V, Section 5.18.8, p. v-27.

Amann, R. P., Wise, M. E., Glass, J. D. and Nett, T. M. 1986. Prepubertal changes in the hypothalamic-pituitary axis of holstein bulls. Biol. Reprod. **34**:71-80.

Appleby, M. C. 1986. Development of sexual and agonistic behaviour in bulls and steers. Appl. Anim. Behav. Sci. **15:**190 (abstr.).

Baker, A. M. and Gonyou, H. W. 1986. Effects of zeranol implantation and late castration on sexual, agonistic and handling behavior in male feedlot cattle. J. Anim. Sci. **62:**1224-1232.

Berg, R. T., Goonewardene, L. A. and Makarechian, M. 1986. The University beef breeding project - 1986 update. 65th Annual Feeders' Day Report University of Alberta. p.3-6.

Canadian Council of Animal Care. 1993. Guide to the care and use of experimental animals. Vol.2 CCAC, Ottawa, ON.

Finnerty, M., Enright, W. J., Morrison, C. A. and Roche, J. F. 1994. Immunization of bull calves with a GnRH analogue-human serum albumin conjugate: effect of conjugate dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. J. Reprod. Fert. **101**:333-343.

Finnerty, M., Enright, W. J., Prendiville, D. J., Spicer, L. J. and Roche, J. F. 1996.

The effect of different levels of gonadotropin-releasing hormone antibody titres on plasma hormone concentrations, sexual an aggressive behaviour, testis size and performance of bulls. Anim. Sci. **63**:51-63.

Gonzalez, A., Goubau, S., Allen, A. F., Mapletoft, R. J., Cohen, R., and Murphy, B. D. 1990. Livestock Reproduction in Latin America (IAEA Panel Proceedings Series STI/PUB 833). pp.269-284.

Hage-van Noort, M., Puijk, W. C., Schaaper, W. M. M., Kuperus, D., Beekman, N. J. C. M., Plasman, H. H., Lankof, H., Wensing, C. J. G., and Meloen, R. H. 1992. Immunomodulation of reproductive systems. J. Anim. Reprod. Sci. **28**:187-193.

Hand, R. K. and Goonewardene, L. A. 1989. The effects of dehorning and/or castrating feedlot cattle. Proc. West. Sec. Amer. Soc. Anim. Sci. West. Can. Soc. Anim. Sci. **40**:88-90.

Hunter, A. G. 1989. Immunology and fertility in the bovine. J. Dairy Sci. **72**:3353-3362.

Jago J. G., Cox, N. R., Bass, J. J. and Matthews, L. R. 1997. The effect of prepubertal immunization against gonadotropin-releasing hormone on the development of sexual and social behavior of bulls. J. Anim. Sci. **75**:2609-2619.

Jeffcoate, I. A., Lucas, J. M. S. and Crighton, D. B. 1982. Effects of active immunisation of ram lambs and bull calves against synthetic luteinizing hormone-releasing hormone. Theriogenology **18**:413-416.

Lobley, G. E., Connell, A., Morris, B., Anderson, J., Clayton, P. E., Williams, V. and Nevison, I. M. 1992. The effect of active immunization against gonadotropin-hormone-releasing-hormone on growth performance and sample joint composition of bulls. Anim. Prod. **55**:193-202.

Makarechian, M., Farid, A. and Berg, R. T. 1985. Scrotal circumference, semen characteristics, growth parameters and their relationships in young beef bulls. Can. J. Anim. Sci. **65**:789-798.

McCarthy, M. S., Hafs, H. D. and Convey, E. M. 1979. Serum hormone patterns associated with growth and sexual development in bulls. J. Anim. Sci. **49**:1012-1020.

Mwansa, P., Makarechian, M. and Arthur, P. F. 1990. The effect of level of concentrate in diet on the ranking of young bulls for postweaning gain. 69th Annual Feeders' Day Report. The University of Alberta.

Price, E. O. and Wallach, S. J. R. 1991. Development of sexual and aggressive behaviours in Hereford bulls. J. Anim. Sci. **69**:1019-1027.

Price, M. A., Jones, S. D. M. and Berg, R. T. 1977. The effect of breed and slaughter weight on feedlot performance and carcass composition in bulls. Agriculture and Forestry Bulletin, University of Alberta, Special Issue; Annual Feeders' Day Report. pp.59-61.

Price, M. A., Jones, S. D. M., Mathison, G. W. and Berg, R. T. 1980. The effects of increasing dietary roughage level and slaughter weight on the feedlot performance and carcass characteristics of bulls and steers. Can. J. Anim. Sci. **60**:345-358.

Price, M. A. and Tennessen, T. 1981. Preslaughter management and dark-cutting in the carcasses of young bulls. Can. J. Anim. Sci. **61**:205-208.

Robertson, I. S., Fraser, H. M., Innes, G. M. and Jones, A. S. 1982. Effect of immunological castration on sexual and production characteristics in male cattle. Vet. Rec. **111**:529-531.

Robertson, I. S., Wilson, J. C., Fraser, H. M., Innes, G. M. and Jones, A. S. 1984. Immunological castration of young bulls for beef production. Current Topics in Vet. Med. Anim. Sci. **26**:137-145.

SAS Institute, Inc. 1990. SAS. User's guide, Statistics, Version 5 Edition, SAS, Cary, N.C.

Staigmiller, R. B., Brownson, R. M. Kartchner, R. J. and Williams, J. H. 1985. Sexual development in beef bulls following zeranol implants. J. Anim. Sci. **60**:342-351.

Tarrant, P.V. 1981. The occurrence, causes and economic consequences of dark-cutting in beef – a survey of current information. In: D.E. Hood and P.V. Tarrant (Editors), The Problems of Dark – Cutting in Beef. Current Topics in Veterinary Medicine, Vol. 10, Martinus Nijhoff, The Hague, pp.3-36.

Teague, G. L, Boyd, G. W., Smith, G. C., Finnerty, M., Rollin, B. E, Field, T. G., Grandin, T., Kniffen, D. M., Taylor, R. E. and Enright, W. J. 1992. Bovine carcass characteristics and overall lean meat production efficiency of early castrates (EC), late castrates (LC), immunocastrates (IC) and intact males (IM). Environ. Liv. Prod. (abstr.) pp.103.

VanKessel, Andrew. 1998. Personal Communication. Department of Animal Science, University of Saskatchewan. Saskatoon Saskatchewan.

3. EFFECTS OF SURGICAL, IMMUNO- AND NON-CASTRATION ON FEEDLOT PERFORMANCE IN TWO BREED-TYPES OF BULL CALVES IMPLANTED OR NOT WITH A GROWTH PROMOTOR

3.1 INTRODUCTION

Testosterone induces aggressive and sexual behavior in males (Balthazart 1990), making them more difficult to manage. Due to increased handling difficulties associated with bulls and their higher incidence of dark cutting when slaughtered (Price and Tennessen 1981), it is common practice in North America to castrate bulls intended for the feedlot. However, this practice has drawbacks. Gonadal steroids play a critical role in mammalian growth and development (Adams and Adams 1992). Entire bulls show higher growth rates than steers, produce more lean meat with less fat and convert feed more efficiently (Price et al. 1980). Animals castrated upon feedlot entry show significant reductions in final live weight, average daily gain and hot carcass weight when compared to intact contemporaries (Adams and Adams 1992; Gonzalez et al. 1990).

Growth promotion implants such as Estradiol-17β, can be administered to steers to minimize production losses from castration, but they do not fully compensate for the reduction in testosterone production. In addition to the loss of growth potential and some animal welfare concerns about surgical castration, there is an associated risk of infection that may lead to morbidity or mortality, as well as labor and equipment costs. These costs increase as animal age at castration increases.

Testicular function in bulls is primarily regulated by the secretion of LH and

FSH, both of which are regulated by the secretion of GnRH from the hypothalamus (Amann et al. 1986). GnRH is the key hypothalamic hormone regulating the pituitary-testicular axis, and consequently regulates aggressive and sexual behavior in bulls. A practical research goal is to disrupt this hormonal flow by neutralizing GnRH activity. This would reduce testosterone concentrations to levels where sexual and aggressive behavior are reduced, but some anabolic advantages of the intact male are maintained (Finnerty et al. 1996). The primary objective of the present study was to follow up the previous experiment by further examining the effects of immunization with an anti-GnRH peptide on production, efficiency, behavior and carcass traits in two breed groups of bull calves. A second objective was to combine immunocastration with an estrogenic growth promoter to determine whether a synergistic effect could occur, leading to suppression of aggressive and sexual behavior while maintaining high growth rates, and thereby creating a less stressful feedlot management system for bulls.

3.2 METHODS AND MATERIALS

This study, which was conducted in accordance with Canadian Council on Animal Care (1993) guidelines, used 96 male calves born at the University of Alberta Beef Research Ranch at Kinsella. The design was a factoral with three castration treatments, two breed-types, two implant treatments, four calves per pen and two replications. The three castration treatments were non-castration (BULL), immunocastration (IMMUNO)

and surgical castration (STEER). The two breed-types (Berg et al. 1986) were Beef Synthetic (BS), a hybrid of Angus, Hereford, Galloway and Charolais and Dairy Synthetic (DS), a hybrid dominated by Holstein, Brown Swiss and Simmental. Implant treatment was either the presence (IMP) or absence (NOIMP) of an estradiol-17β growth promoting implant (Compudose®). Thirty-two calves were randomly allocated at d 0 (August 10, 1995; aged 3 to 4 mo) to each of the three castration treatments. Immediately after assignment, the three castration treatment groups were vaccinated with either an anti-GnRH vaccine or a placebo and the STEERs castrated in the same manner as the previous year (Chapter 2, Section 2.2). All calves were weaned on d 53 (October 2) and the IMMUNO group received booster vaccinations on d 71 (October 20; aged 5.5 to 6.5 mo) and d 152 (January 10; aged 8 to 9 mo). No further boosters were given to comply with the Bureau of Veterinary Drugs' determination of a 90 day withdrawal of this product prior to commercial slaughter. On d 83 half of the calves in each treatment by breed combination were implanted with 25.7 mg of estradiol-17β (Compudose®, Elanco Animal Health, Eli Lilly Canada Inc., Calgary, AB) and were allocated to six by eight metre feedlot pens where the feed ration (Table 3.1) started at 10 kg/pen/day and was increased gradually until they were on ad libitum intake by d 100. Feed intake per pen was recorded daily. Between weaning and slaughter, live weight was measured on days 71, 83, 97, 125, 152, 181, 208, 215, 235 and 250 and hip height (Chapter 2, Section 2.2) was measured on days 71, 83, 125, 181, 235 and 250. Blood was collected by jugular

puncture (18 gauge vaccutianer® needles) into 10 ml silicone coated vaccutainer® tubes (Becton Dickinson) on days 71 (immediately prior to the second immunization), 83, 97, 125, 152, 208 and 250. A minimum of sixty minutes after collection, the blood was centrifuged at 1650 g for 50 minutes and the serum was pipetted using disposable pasteur pipettes (Borosilicate glass; Allied Fisher Scientific) into two sterile vials. Samples were stored at –23 °C until they were used to determine serum testosterone concentration using a Coat-A-Count® kit (Diagnostic Products Corporation, Los Angeles, California) and percent binding of GnRH (VanKessel, 1998).

3.2.1 *Testicular Measurements*

Scrotal circumference was measured on days 71, 125, 181, 208 and 250, to the nearest 0.5 cm using a plasticized cloth tape pulled snugly around the widest part of the scrotum. During scrotal circumference measurements on days 250, 268 and 275, the testicles of the BULLs and IMMUNOs were manually palpated for firmness. Scrotums were also visually assessed for shape (Fig. 3.1) at these times. They were categorized as either 'normal' (bottle-shaped), 'straight-sided' or 'wedge-shaped' (Cates 1975).

Testicular consistency, scrotal ultrasonography and infrared thermography were measured on all BULLs and IMMUNOs on d 216 of the experiment. Testicular consistency of the left and right testicles was measured with a tonometer (Hahn et al. 1969; Lane Manufacturing Inc., Denver, CO) and the mean was recorded. Scrotal

ultrasonographic evaluations were performed with a Dynamic Imaging Ultrasound Scanner (Model 220C; Dynamic Imaging Co. Ltd., Livingstone, Scotland, UK) equipped with a 7.5 MHz transducer. The face of the transducer was coated with scanning gel, placed on the posterior surface of the scrotum, and pressed firmly to produce an acceptable image. Each testis was imaged separately. Image analysis was performed with specialized software (Image; National Institutes of Health) to determine pixel intensity (testicular echotexture; mean and range). Scrotal surface temperature was measured using infrared thermography in the manner previously described by Cook et al. (1994).

Semen was collected by electroejaculation (Bonded Electro, San Diego, CA) on d 241 (10 to 11 mo of age) from the BULLs and IMMUNOs. The cattle were restrained in a squeeze; feces were evacuated from the rectum and an electroejaculation probe, approximately seven centimetres in diameter with three ventral electrodes, was inserted into the rectum. Electrical stimulation with a rythmic on and off sequence and gradual increase in peak stimulation was provided, and adjusted according to the response of the bull. The ejaculate was collected into a rubber cone. The stimulation period lasted for a maximum of one minute (less if ejaculation occurred sooner). If no sample was collected during the first collection period or if the sample was unsatisfactory, collection of a second sample was attempted after a few minutes using the same procedure. The percentage of progressively motile spermatozoa was estimated in increments of 5% and the semen was evaluated on a percentage basis for normal sperm, as well as head, mid-

piece, tail and cytoplasmic droplet defects (Cook et al. 1994).

3.2.2 *Behavior*

Behavior was assessed on two occasions: d 236 (9.5 to10.5 mo of age) and d 253 (10.5 to 11.5 mo of age). Interactions among four calves (two each from two different pens) from within the same castration treatment by implant treatment by breed-type, were observed in a six by eight metre pen. Total observation time per group was five minutes and during each 30-second interval, behavior was recorded as having occurred or not. Recorded behaviors were flehmen, sheath sniffing, mounting or attempted mounting, bunting (one animal bunting another anywhere on the body) and fighting (prolonged head to head pushing).

3.2.3 *Slaughter*

Cattle were slaughtered on a per pen basis when the majority were estimated to have reached 8 mm of backfat, assessed by a combination of ultrasonic and visual appraisal. A total of three lots were shipped, on days 255, 270 and 277. On the day before slaughter, cattle were fed in the morning as usual and continually had water available. The day of slaughter, cattle were loaded by pen onto a truck so pens were not mixed until they were loaded. They left the ranch at approximately six am, were trucked directly to a commercial abattoir 300 km away, arrived in 3.25 to 3.5 hours and were

slaughtered immediately upon arrival. Testicles were collected during the slaughter process. One testicle from each bull (alternating between the left and right), as well as the epididymis removed from the testis, were weighed approximately one to three hours after collection. Warm carcass weight, fat cover, estimated yield, and carcass grade were evaluated by Canadian Beef Grading Agency graders at the abattoir on the morning following slaughter. Cutability was calculated as: 57.96 - (0.027 x warm wt.) + (0.202 x REA) - (0.703 x avg. fat) (Agriculture and Agri-Food Canada 1992). The size of the erector muscle in each carcass was visually scored on a scale from zero to five. Numbers correspond to the following descriptions; 0 means no muscle is present, 1 means the muscle is very small, 2 means the muscle is smaller than a normal steer, 3 is the score for a normal steer, 4 means the muscle is somewhat larger than a normal steer, and 5 means the muscle is large enough that the carcass would grade as a bull.

3.2.4 *Statistical Analysis*

The main effects in the statistical model were castration treatment (BULL, IMMUNO and STEER), breed-type (BS and DS), implant treatment (IMP and NOIMP), pen (castration treatment x breed-type x implant treatment), period (one to seven) and interactions. Data were analyzed using the fixed linear model:

$$Y_{ijklmn} = \mu + C_i + B_j + I_k + CB_{ij} + CI_{ik} + BI_{jk} + CBI_{ijk} + PN_{l(ijk)} + PE_m + PEC_{im} + PEB_{jm} + PEI_{km} + PECB_{ijm} + PECI_{jkm} + PEBI_{jkm} + PECBI_{ijkm} + e_{ijklmn},$$

where Y_{ijklmn} = animal observation, μ=overall mean, C_i = castration treatment (i=IMMUNO, BULL, STEER), B_j = breed-type (j=BS, DS), I_k = implant treatment (k=IMP, NOIMP), CB_{ij} = interaction between castration treatment and breed-type, CI_{ik} = interaction between castration treatment and implant treatment, BI_{jk} = interaction between breed-type and implant treatment, CBI_{ijk} = interaction between castration treatment, breed-type and implant treatment, $PN_{l(ijk)}$ = pen with castration treatment, breed-type and implant treatment nested, PE_m = period (l=1 to 7), PEC_{im} = interaction between period and castration treatment, PEB_{jm} = interaction between period and breed-type, PEI_{km} = interaction between period and implant treatment, $PECB_{ijm}$ = interaction between period, castration treatment and breed-type, $PECI_{jkm}$ = interaction between period, castration treatment and implant treatment, $PEBI_{jkm}$ = interaction between period, breed-type and implant treatment, $PECBI_{ijkm}$ = interaction between period, castration treatment, breed-type and implant treatment and e_{ijklmn} = random error. The dependent variables which were repeated over time were: serum testosterone concentration, percent binding of GnRH at a dilution of 1:1000, live weight, average daily gain, hip height and scrotal circumference. These were analysed as a split plot in time (repeated measures) using the General Linear Model (GLM) procedure of SAS (1990). Scrotal circumference was adjusted for differences in live weight. Feed intake and gain:feed were analysed on a pen basis using the same model as above. Day 0 weight, slaughter age, testicular measures, semen, carcass traits and behavior were analysed by GLM. The main effects were castration treatment, breed-type, implant treatment, pen (castration treatment by breed-

type by implant treatment) and interactions. Least square means were separated using the PDIFF option procedure when there was a significant treatment effect at the 0.05 probability level (SAS 1990). A regression analysis was computed for ADG and scrotal circumference for both castration treatment and breed-type. Slaughter weight was determined by linear regressions of age on weight. Different observers were used for the behavior studies and observer was tested as a main effect in SAS. As there was no significant difference between observers, it was removed from the model.

3.3 RESULTS

Four calves (two BS, STEER, NOIMP; one BS, BULL, IMP and one BS, IMMUNO, IMP) died during the study. The first two STEERs and the BULL died as a result of illness and because their data was not representative of a normal animal, it was not used in the statistical analyses.

3.3.1 *Anti-Gonadatropin Releasing Hormone Antibody Titres*

There were no significant (P>0.05) interactions among castration treatment, breed-type, implant treatment and period for GnRH antibody binding (Table 3.2). Following the booster vaccination given on d 71, the IMMUNO cattle showed a large increase in the amount of GnRH bound, with titre levels increasing from 8.6% binding on d 71 to 77.3% binding on d 83 at 1:1000 dilution (Table 3.2). From d 83 to when the second booster was given on d 152, the titre levels declined by 65%. After the d 152

booster the titre levels again increased, but had decreased by d 250. A further booster was not possible because of the 90 day pre-slaughter withdrawal requirement. At days 83, 97, 125, 152, 208 and 250, 81%, 63%, 28%, 9%, 13% and 13% of IMMUNO animals respectively showed 70% or higher binding of GnRH at a dilution of 1:1000, while 53%, 41%, 9%, 3%, 0% and 6% of calves showed 80% or higher binding at that dilution. The binding levels pooled over time for the two breed-types and implant treatments in the IMMUNO group were BS=46.7% ± 2.5, DS=37.8 ± 2.4, (P=0.06) and implant=45.8 ± 2.5, non-implant=38.6 ± 2.4, (P=0.1). On days 125 and 250 the BS cattle had significantly (P<0.05) higher antibody titres than the DS (Table 3.2).

3.3.2 *Serum Testosterone Concentration*

There were no significant (P>0.05) interactions among castration treatment, breed-type, implanting and period for testosterone concentration, except for a significant castration treatment by period interaction (Table 3.2). Serum testosterone concentration (TC) was significantly (P<0.05) lower in the IMMUNOs than in the BULLs at days 97, 125 and 208 but not on days 71, 83, 152 or 250. The largest observed difference in TC appeared on d 125, almost two months after the first booster was given, when the TC in the IMMUNOs was less than one third of that in the BULLs. During the period between the first and second boosters (d 83 to d 152), antibody titres declined by 65% (from 77.3 to 27.1% bound) in IMMUNOs, as TC increased by 35,995% (from 2.1 to 755.9 ng dL^{-1}). Correlations of testosterone concentrations and GnRH antibody titres are shown in

Appendix 1.

The TC of the DS was significantly higher than that of the BS on d 125, but lower ($P<0.05$) on d 152. There was no significant ($P<0.05$) difference between implant treatments at any of the measured times (Table 3.2).

3.3.3 *Testicular Measurements*

There were no significant ($P>0.05$) interactions among castration treatment, breed and implant treatment for testicular measurements, except for ultrasound mean which was significantly ($P<0.05$) influenced by the interaction of breed-type and castration treatment, and ultrasound maximum which was significantly ($P<0.05$) influenced by the interaction of castration treatment and implant treatment. Beef synthetic BULLs had lower ultrasound mean values than dairy synthetic (Fig. 3.2) while beef synthetic IMMUNOs had similar values to dairy synthetic. The ultrasound maximum values of implanted BULLs were lower than non-implanted BULLs with the opposite effect occurring in IMMUNOs (Fig. 3.3). There was also a significant ($P<0.05$) interaction between castration treatment and implant treatment, and between breed-type and implant treatment ($P<0.01$) for scrotal circumference. Implanting decreased the scrotal circumference of the IMMUNOs, but had little effect on BULLs. Anti-GnRH immunization decreased scrotal circumference, but immunization combined with implanting showed a greater decrease in scrotal circumference (Fig. 3.4) as the scrotal

circumference of implanted IMMUNOs was two centimetres smaller than IMMUNOs not implanted. Implanted BS cattle (scrotal circumference = 28.1 cm) had reduced scrotal circumferences when compared to implanted DS (scrotal circumference = 31.1 cm) and non-implanted BS (scrotal circumference = 30.6 cm) cattle (Fig. 3.5).

BULLs and IMMUNOs were not significantly ($P>0.05$) different for testicle or epididymis weights, scrotal surface temperatures, testicular ultrasound measures, testicular consistency or semen evaluations (Tables 3.3 and 3.4). By manual testicle palpation, it was observed that eight animals from the IMMUNO group had markedly 'mushy' (soft and pulpy) testicles. No animals from the BULL group had 'mushy' testes. When their scrotums were visually assessed for shape (Fig. 3.6), the majority of the BULLs had normal shaped scrotums (65%), 27% had straight-sided scrotums and 8% had wedge-shaped. Conversely, the IMMUNO scrotal shapes were more evenly distributed among the three descriptions with 32% having normal shaped scrotums, 40% had straight-sided and 27% had wedge-shaped. All the carcasses received a score of two, three or four for erector muscle size. The IMMUNOs and STEERs were similar with most of them scoring two or three. The scores of the BULLs were more evenly distributed between two, three and four (Table 3.5).

Breed-type and implanting did not have a significant effect ($P<0.05$) on any of these measures, except that Dairy Synthetic cattle had significantly ($P<0.05$) more sperm with head defects than Beef Synthetic.

3.3.4 *Behavior*

There were no significant (P>0.05) interactions between breed-type and castration treatment, or between breed-type and implant treatment for any of the behaviors measured. The castration treatment by implanting interaction was significant (P<0.05) for fighting at 9.5 to 10.5 mo of age (d 236), and for flehmen, sheath sniffing and fighting at 10.5 to 11.5 mo of age (d 253), but was not significant (P>0.05) for other traits. On d 236, implanting increased fighting in the IMMUNOs and STEERs but decreased it in BULLs (Fig. 3.7). On day 253, implanting decreased flehmen in IMMUNOs and slightly in STEERs, but increased it in BULLs (Fig. 3.8). Implanting also decreased sheath sniffing in IMMUNOs and STEERs, while increasing it in BULLs (Fig. 3.9). Similar to d 236, implanted IMMUNOs and STEERs on day 253 fought more than non-implanted, but the implanted BULLs fought less (Fig. 3.10).

IMMUNOs were intermediate (P<0.01) to BULLs and STEERs for flehmen and sheath sniffing on d 236 (Table 3.6). STEERs mounted significantly less (P<0.01) than BULLs and IMMUNOs on d 236, but on d 253 there were no significant differences among the three castration treatments for mounting. There was no significant (P>0.05) effect of castration treatment for bunting on either day. The DS cattle exhibited flehmen more often (P<0.05) than the BS on d 236 (Table 3.6). Except for interactions with castration treatment, none of the behaviors were influenced by implanting.

3.3.5 *Production and Carcass Traits*

There were no significant (P>0.05) interactions among castration treatment, breed-type, implant treatment and period for any of the production or carcass traits measured with two exceptions. Warm carcass weight was significantly (P<0.05) influenced by the interaction between castration treatment and breed-type (Fig. 3.11) as the DS BULLs and IMMUNOs had higher carcass weights than the BS. However, the DS STEERs had a lower carcass weight than the BS STEERs. Warm carcass weight was also significantly (P<0.01) influenced by the interaction between castration treatment and implanting (Fig. 3.12) with the carcass weight of the BULLs increasing in response to implanting and the carcass weight of the IMMUNOs decreasing.

Castration significantly (P<0.05) affected slaughter weight, ADG and gain:feed, with BULLs and IMMUNOs being similar to each other, but having significantly greater (P<0.05) values than STEERs for all these traits. Sixty percent of the STEER carcasses received Canada AAA grades whereas more of the BULL (67.7%) and IMMUNO (76.7%) carcasses were graded Canada AA (Table 3.7).

The DS were significantly (P<0.01) heavier than the BS at d 0 of the experiment (Table 3.7). For all of the other traits measured there was no significant breed-type effect, though the difference between DS and BS for grade fat approached significance (P<0.09). The distribution of quality grades among for BS and DS were similar.

Implanted cattle consumed significantly (P<0.01) more feed than non-implanted cattle, but showed no significant difference in feed efficiency. Implanting also

significantly ($P<0.05$) improved growth, as measured by hip height. For all of the other traits measured, there was no significant implant treatment effect (Table 3.7).

3.4 DISCUSSION

3.4.1 *Anti-Gonadatropin-Releasing Hormone Titres*

The first booster vaccine, given on d 71 of the present study, created a high response within twelve days in the IMMUNOs, with an average of 77% of GnRH being bound by antibodies at 1:1000 dilution (Fig. 3.13). A second booster administered on d 152, in response to rising TC, increased the antibody binding capacity to one third higher by d 208 (36% binding) than it had been on d 152, but titres regressed to 28% binding by d 250. Thus, TC of IMMUNOs was approaching the same concentrations as that of BULLs by slaughter. These results are similar to those found in the first experiment (Chapter 2, Section 2.3.1) and to findings by Finnerty et al. (1996) where GnRH-immunized bulls reached control concentrations before the end of the experiment.

Using the definition of Adams et al. (1996), the anti-GnRH titres reported in this study at each time measured could be classified as 'good' (>10% [^{125}I] GnRH bound by a 1:1000 serum dilution). Titres of the IMMUNOs (mean 47.8% after the first booster vaccination and 28.4% binding prior to slaughter in 1:1000 dilution) were generally higher than those reported by Jeffcoate et al. (1982), Adams and Adams (1992), Adams et al. (1993), Gonzalez et al. (1990) and Jago et al. (1997).

Finnerty et al. (1996) reported overall antibody binding values of 20% at a

dilution of 1:2560 for immunocastrates vaccinated at two to two and half months of age, while the present study found average antibody binding values of 28% (1:1000 dilution) at 10.5 to 11.5 mo of age. These findings are similar to those of Adams et al. (1996) who reported 12 mo antibody binding values of 24.7%, 27.2% and 31.2% at a 1:1000 dilution for bulls given a primary immunization at 1.5, 4 and 7 mo of age respectively. There is no obvious reason why there was a significant ($P<0.05$) breed-type difference for antibody titres on days 125 and 250. Implanted cattle had higher anti-GnRH titres than non-implanted at all measured times, but the differences were not significant (Fig. 3.14).

3.4.2 *Serum Testosterone Concentrations*

The serum testosterone concentration of the BULLs began to rise when the cattle were approximately six to seven months of age and peaked when they were seven to eight months old. After this time, TC declined slightly (Fig. 3.15). This pattern of testosterone concentrations rising during puberty and then declining has been demonstrated before. Finnerty et al. (1996) reported a rise in TC of bulls between three and five months of age followed by a decline in TC from approximately 10 to 14 mo of age. Similarly, Rawlings et al. (1972) and Swanson et al. (1971) reported a decrease in TC in bulls at 11 to 12 mo of age.

The first booster vaccination given to the IMMUNOs on d 71, resulted in high GnRH antibody titres, which inhibited TC to a very low level. As the effects of the vaccine lessened, testosterone levels increased again. However, it appears that

testosterone was lower and the peak at puberty occurred later in IMMUNOs than in BULLs. Finnerty et al. (1996) also reported a two month delay in the pubertal rise of testosterone in immunocastrates.

Just prior to slaughter, at 10.5 to 11.5 mo of age (d 250), GnRH antibodies were detectable in all but one of the IMMUNOs at a dilution of 1:1000. Lobley et al. (1992) reported that when antibody titres are detectable at a dilution of 1:1000, serum testosterone concentrations remain low. Below that titre, testosterone levels return rapidly to normal. The animals in this study that had low antibody titres at 1:1000 dilution followed this trend. The degree to which GnRH antibodies were bound just prior to slaughter, (28%), was proportional to the degree to which testosterone production was suppressed (35%) in the IMMUNOs.

An animal can be considered effectively immunosuppressed if its serum testosterone level is below 0.3 $\mu g\ L^{-1}$ (30.0 $ng\ dL^{-1}$) (Lobley et al. 1992). On this basis, none of the IMMUNOs in this study were effectively suppressed immediately prior to slaughter. The greatest difference between BULLs and IMMUNOs for TC appeared on d 125, almost two months after the first booster was given, when TC in IMMUNOs was less than one third of that in the BULLs. This difference occurred approximately six weeks after antibody titres in the IMMUNOs peaked, suggesting a delay in testosterone reaction to the change in GnRH (Figs. 3.13 and 3.15). Of the days on which a measurement was taken, only days 97, 125 and 208 showed a significant difference in TC between BULLs and IMMUNOs. The time interval from the previous booster to these

days was approximately four weeks (26 days) for d 97, and eight weeks (54 and 56 days) for days 125 and 208 respectively. This suggests that the duration of effectiveness for this vaccine is less than 80 days. Due to the 90 day withdrawal period these cattle were slaughtered between 100 and 122 days after they were last vaccinated, when there may have been little remaining effect from the vaccine. This is supported by the fact that only two calves showed antibody titres of 80% or higher on d 250 (98 days after the last booster).

Significantly lower testosterone levels and scrotal circumferences relative to intact males have been reported in response to active immunization against GnRH in bulls (Teague et al. 1992; Adams et al. 1996; Finnerty et al. 1996). Prior to slaughter (d 250), IMMUNOs in this study had a lower level of testosterone and smaller scrotal circumference than BULLs (Fig. 3.16). Adams et al. (1992) reported no significant difference in TC prior to slaughter at 15.2 to 15.7 mo of age, between bulls (2.04 ng mL^{-1}) and immunocastrates (2.32 ng mL^{-1}) which were vaccinated at 10.5 to 11 mo of age. Scrotal circumference has been shown to be inversely related ($P<0.001$) to anti-GnRH titre (Adams et al. 1996) and in the present study TC and GnRH percent binding had a negative relationship.

3.4.3 *Testicular Measurements*

IMMUNOs scrotal circumference averaged 6.8% less than BULLs over the experimental period and 10% less just prior to slaughter at 10.5 to 11.5 mo of age (Fig.

3.16). These differences are less than those reported by Adams et al. (1993), who found that immunocastrates had 15.5% smaller scrotal circumferences than bulls at 15.2 to15.7 mo of age. In addition, Adams et al. (1996) reported differences in scrotal circumferences of 12%, 13.6% and 7.6% between immunocastrates and intact males when the immunocastrates were immunized with a single vaccination at four, seven and 12 mo of age respectively.

Testicle weights of the BULLs and IMMUNOs in this study were not significantly different, whereas others (Finnerty et al. 1996; Adams et al. 1993; Jago et al. 1997; Huxsoll et al. 1998) have found testes of GnRH-immunized bulls to be significantly smaller than testes of control bulls. Brown et al. (1994) reported that GnRH-immunized rams with smaller testes than control rams produced testosterone and normal ejaculate with motile sperm. This suggests that even though a temporary suppression of testes growth occurs during the peri-pubertal period, affecting subsequent testes development, the testes of a GnRH-immunized bull can compensate for the reduction in size and produce normal concentrations of testosterone (Finnerty et al. 1996).

This study did not find a significant difference between BULLs and IMMUNOs for testicular consistency, ultrasound measures, scrotal surface temperature or semen quality. With the exception of testes weight and scrotal circumference, very little has been published on the effects of immunocastration on testicular function. However, Cook et al. (1998) reported an immunocastration effect ($P<0.01$) on daily sperm production and epididymal sperm reserves, with immunocastrates having average values of 40% to 15%

respectively, of control bulls. Robertson et al. (1979) also found that immunocastration had an effect on sperm and semen production, and Adams et al. (1993) reported that a significant ($P<0.01$) reduction in sperm concentration in testicular tissue collected at slaughter was due to the effects of immunocastration.

Scrotal shape has an influence on testicular development and function (Cates 1975). Bulls having a scrotum with a distinct neck (Fig. 3.1) are considered normal, while scrotums that are 'straight-sided' or 'wedge-shaped' are associated with lower fertility (Cates 1975). A majority of BULLs in this study had 'normal' shaped scrotums while the IMMUNOs were more evenly distributed among the three descriptions, indicating the testicular physiology of some of the IMMUNOs was likely affected by vaccination. This is supported by the fact that 12 out of 31 IMMUNOs had mushy testes while none of the BULLs did.

Active immunization against GnRH is one way of attenuating the effect of endogenous GnRH secretion, by establishing a humoral barrier between the hypothalamus and pituitary gland. Another way is through the use of gonadal steroids, which act at the hypothalamic loci to suppress GnRH secretion (Schanbacher 1984). The suppressive effect of estrogen-containing implants on testicular function in bulls is well documented (Staigmiller et al. 1985; Calkins et al. 1986; Silcox et al. 1986). Early and continued estrogenic stimulation is required to significantly retard testicular development of bulls (Silcox et al. 1986). Although testosterone secretion is reduced in implanted calves (Staigmiller et al. 1985; Silcox et al. 1986), the same effect does not seem to occur in

more mature animals. It has been shown that serum TC does not vary with implant status in bulls 12 mo of age or older (Staigmiller et al. 1985; Adams et al. 1993; Huxoll et al. 1998). It could be hypothesized that the use of steroid-containing implants in young calves may suppress testicular growth and development, and have a synergistic affect with anti-GnRH immunization. Implanting did not significantly influence most reproductive measures in this study. However, even though it had little effect on BULLs, implanting combined with anti-GnRH immunization, reduced the scrotal circumference of IMMUNOs (Fig. 3.4). This study suggests that implanting had a complementary effect with immunization on affecting sexual development in bulls.

3.4.4 *Behavior*

The pattern and type of behavior expressed by bulls appears to vary with time. Finnerty et al. (1996) reported more sexual than aggressive behavior with eight to 13.4 mo old bulls on pasture, but found that prior to slaughter at 22 mo of age most of the observed behavior was aggressive. Others (Robertson and Lowman 1977; Hinch et al. 1982; Appleby et al. 1996) have noticed a similar pattern in bull behavior. In this study, on d 236, at 9.5 to 10.5 mo of age, there was a significant difference ($P<0.01$) between BULLs and IMMUNOs for flehmen and sheath sniffing, but no difference ($P>0.01$) between BULLs, IMMUNOs or STEERs for aggressive behavior. Previous studies (Chapter 2, Section 2.3.3, Teague et al. 1992) reported a lower incidence ($P<0.01$) of

mounts in immunocastrates compared to bulls. Possibly the cattle in this study were too young to exhibit much aggressive behavior. The fact the IMMUNOs displayed sexual behavior less often than the BULLs on d 236 and not on d 253, may be due to the effects of the vaccine wearing off, since by d 253 it was 101 days from their last vaccination. Similarly, Jago et al. (1996) found that although altering GnRH function during the prepubertal period changed the development of sexual and social behavior of bulls, generally these effects did not persist so that by slaughter the behavior of intact bulls and immunized bulls was similar. Huxsoll et al. (1998) found that the frequency of spars and butts initiated did not differ ($P>0.05$) between nonimplanted steers and anti-GnRH immunized bulls and was less ($P<0.05$) that the same measures of aggressive behavior in control bulls at 16 mo of age. Similarly, Finnerty et al. (1996) observed that control bulls were more active than immunocastrated bulls (age of primary immunization was 1.5 to 2.5 mo), both at pasture (8 to 10.8 mo of age) and one week prior to slaughter which occurred when the cattle were approximately 22 mo of age.

Implanting appeared to have a synergistic effect with anti-GnRH immunization for behavior. The castration treatment by implanting interaction was significant ($P<0.05$) for fighting at 9.5 to 10.5 mo of age (d 236), and for flehmen, sheath sniffing and fighting at 10.5 to 11.5 mo of age (d 253). For all these interactions, implanting elicited a similar response in IMMUNOs and STEERs, and the opposite response in BULLs (Figs. 3.7, 3.8, 3.9, 3.10). In the IMMUNOs and STEERs, implanting decreased flehmen and sheath

sniffing, but increased fighting. Possibly the additional anabolic influence elevated the behavior of the IMMUNOs and STEERs to be more like that of mature bulls, causing them to exhibit behavior more often, and for the behavior to be more aggressive than sexual. Conversely, implanting reduced aggressive behavior in BULLs. This is similar to findings by Baker and Gonyou (1986) who reported increased butting of castrated males implanted with zeranol, and decreased butting of implanted, intact males. McKenzie (1984) also reported that implanting with zeranol before three months of age effectively controlled sexual behavior in bulls. However, Price et al. (1983) found no significant effect on behavior of bulls implanted with zeranol.

3.4.5 *Production and Carcass Traits*

The IMMUNOs and BULLs were similar to each other and different ($P<0.05$) from STEERs for all of the production and carcass traits reported (Table 3.7). These results are similar to those found in previous work (Chapter 2, Section 2.3.2) and those of Adams and Adams (1992), Finnerty et al. (1994) and Gonzalez (1990) who found no differences between bulls and immunocastrates for slaughter weight, ADG, hot carcass weight, dressing percentage, rib-eye area or yield grade. However, other studies have found immunocastrates to be intermediate to bulls and steers for feedlot gain and rib-eye area (Adams et al. 1993) and for ADG and feed efficiency (Teague et al. 1992). Huxsoll et al. (1998) found that immunocastration increased marbling and decreased masculinity, carcass weight and REA. Similarly, Finnerty et al. (1996) reported that during a period of

suppressed testosterone concentration, bulls with a high response to vaccination had a lower ADG and were lighter than control bulls, and Lobley et al. (1992) found that carcass composition was similar ($P<0.05$) to that of steers in high responding immunocastrates. The similarity between BULLs and IMMUNOs for these traits in the present study suggests that GnRH, and subsequent testosterone production, was only partially suppressed. It has been suggested that the maintenance of a high rate of growth in immunized bulls may be due to residual serum concentrations of testosterone noted in immunized cattle (Huxsoll et al. 1998).

Price et al. (1983) found that implanting with zeranol had no effect on carcass traits, including carcass weight in bulls. Other reports agree that zeranol, trenbolone and progesterone and estradiol benzoate did not improve feedlot performance or carcass traits of bulls (Silcox et al. 1986; Doornenbal et al. 1987; Jones et al. 1991; Adams and Adams 1992; Adams et al. 1993). This indicates that bulls have sufficient endogenous anabolic steroids for maximum growth (Lee et al. 1990) and administration of supplemental steroid hormones does not markedly improve the anabolic effects (Adams and Adams 1992). This study found that although implanting increased the warm carcass weight of BULLs, it decreased the carcass weight in IMMUNOs (Fig. 3.12). Considering the BULLs and IMMUNOs did not differ for any other production or carcass traits, this is surprising, but indicates that implanting combined with anti-GnRH immunization in this study, had a synergistic effect on reducing carcass weight. However, the results from this study overall, indicate that the addition of estradiol-17β did not significantly enhance the

immunocastration effect for production or carcass traits. Similarly, Huxsoll et al. (1998) who implanted one month old calves with combined progesterone and estradiol benzoate, found no additional benefit in terms of production and carcass traits or suppression of testicular development with a treatment regime that combined anti-GnRH immunization and early implantation.

The use of growth promoting implants improves growth rates and carcass weights in steers (Apple et al. 1991; Adams et al. 1993; Foutz et al. 1997), and exhibits favorable effects on feed efficiency and carcass composition (Foutz et al. 1997). The implant treatment by castration treatment interaction for ADG in this study is approaching significance ($P<0.08$) with the IMP STEERs gaining 13% more than the NOIMP STEERs. The IMP cattle grew significantly ($P<0.05$) taller, and consumed significantly ($P<0.01$) more feed than the NOIMP cattle but showed no significant difference in feed efficiency (Table 3.7).

The DS cattle were significantly ($P<0.01$) heavier at the start of the experiment than the BS, but both breed-types reached a similar weight at slaughter. The heavier initial weight of the DS is indicative of the greater milking ability of their dams (Butson and Berg 1984), while the BS calves experienced compensatory gain in the feedlot.

3.4.6 *Comparison of High and Low Response Groups to Immunocastration*

3.4.6.1 *Introduction*

There was a wide range of anti-GnRH titre levels (from 0.5% binding to 82%

binding at d 250) among individual animals in the IMMUNO group, indicating that the vaccine was affecting some cattle more than others. Other studies have also found a variable degree of response to anti-GnRH vaccination among individual animals due to individual variations in the immune response system (Robertson et al. 1982; Finnerty et al. 1996; Jago et al. 1997). To further investigate this, IMMUNO cattle were separated into two groups based on their anti-GnRH titre levels, and were considered as either high or low responders to immunization. They were separated on the basis of their GnRH titre levels on d 208, 56 days after the second booster vaccination was administered. The objective was to see if there were differences in ADG, testosterone concentration, testicle weight, epididymis weight and carcass traits between the two response groups.

3.4.6.2 *Materials and Methods*

The group considered to be high responders had anti-GnRH titre levels of 34.9% binding or higher (1:1000 dilution), while the low responders had titre levels of less than 34.9% binding (1:1000 dilution) at d 208. These groups were determined by calculating the mean, with the high responders being above the mean and the low responders below the mean. There were 18 cattle in the low responding group and 14 cattle in the high responding group. The main effects in the statistical model for testosterone concentration, testis weight, epididymis weight, carcass weight, carcass fat and yield, were response group, breed-type, implant treatment, pen (response group by breed-type by implant treatment) and interactions. The main effects in the statistical model for ADG

were response group, breed-type, implant treatment, period and interactions. These data were analyzed as a split plot analysis of variance using the GLM procedure of SAS (1990).

3.4.6.3 *Results and Discussion*

On d 83 there was a significant ($P<0.05$) titre group by implant treatment interaction for testosterone concentration (Fig. 3.17), with the low responding-NOIMP cattle having much higher testosterone concentrations than cattle that were implanted or responded highly to immunization. Since there was no significant ($P>0.05$) implant effect, it appears that the low titre-NOIMP group experienced little counteractive effects from either the vaccine or the estradiol implant. This agrees with other findings from this study that immunization, and immunization combined with implanting, adversely affects sexual development.

On d 97, 14 days after implantation, there was a significant ($P<0.01$) implant effect among the IMMUNOs, with the NOIMP group having a higher ($P<0.01$) testosterone concentration (IMP=3.3 $ng.dL^{-1}$; NOIMP=64.6 $ng.dL^{-1}$). Administration of steroid implants decreases testosterone secretion, perhaps through a feedback mechanism (Staigmiller et al. 1985; Lee et al. 1990). Other studies found that this reduction in testosterone secretion appears to lead to suppression of testicular development and function in implanted bulls, as was noted in bulls implanted with estradiol-17β (Schanbacher 1984; Calkins et al. 1986), zeranol (Silcox et al. 1986) and Synovex®

(Adams et al. 1993). Suppression of testicular growth and development in cattle with implants containing anabolic steroids is most likely the result of implant-induced attenuation of episodic secretion of GnRH and associated reduction in gonadotrophin secretion (Schanbacher 1984). In support of this, the frequency and amplitude of secretory episodes of LH are reduced and testicular growth attenuated in bull calves implanted with estradiol (Schanbacher 1984; Deaver et al. 1988).

There was a significant ($P<0.01$) difference between the high and low titre groups for TC on d 125, with the high titre cattle having a lower TC. This again suggests there is a delay from when the cattle produce anti-GnRH titres to when their bodies respond to these titres by secreting less testosterone.

There was no significant ($P>0.05$) difference between the high and low titre groups for TC on d 152. By this time, effects of the immunocastration vaccine were 'wearing off' as evidenced by declining anti-GnRH titres in all IMMUNOs. As a consequence, the TC of these cattle increased to where they were more similar to BULLs and there was no longer a difference between the high and low responding groups for TC. It was on this day that the IMMUNOs were given the second booster vaccination.

On d 208, 56 days after the second booster, there was again a significant ($P<0.05$) difference between the high and low titre groups for TC. This was a direct consequence of the booster which increased titre levels and decreased TC in the high response group more than it did in the low response group. By d 250, the effects of the vaccine had again diminished enough that TC in both groups was similar.

There was a significant ($P<0.05$) implant and titre response group interaction for carcass yield, with the interaction for grade fat approaching significance ($P=0.06$). Implanting appeared to make these high responding cattle fatter (high response-IMP group=11 mm; high response-NOIMP group=5 mm), therefore the difference in yield is probably due to increased fat rather than decreased muscling. Although these cattle were selected for slaughter when they appeared to be at the same fat level, some variation would have occurred. This implant effect on carcass yield can be considered a decrease in carcass masculinity, as surgical castration has a similar effect on carcasses. Similarly, Adams et al. (1993) has shown that along with serum testosterone concentrations, carcass masculinity in bulls is reduced by implantation.

There was a significant difference between the high and low titre response groups for ADG (high=1.7 kg d^{-1}, low=2.0 kg d^{-1}; $P< 0.01$), warm carcass weight (high=667.4 kg, low=752.1 kg; $P<0.01$), testis weight (high=205.5 g, low=333.5 g; $P<0.01$) and epididymis weight (high = 18.0 g, low=23.6 g; $P<0.05$). Robertson et al. (1982) considered anti-GnRH immunized cattle as either high or low responders and reported reduced testosterone concentration and testis volume for the high responders compared to the low for about six month, but this effect wore off by slaughter. This is in contrast to results found by Finnerty et al. (1996) who, based on anti-GnRH titre levels, considered immunocastrates to be either medium or high responders to vaccination and reported no significant difference between the high and medium response groups for either carcass weight or testes weight, although testes weight was significantly lower than control bulls.

Upon manual palpation, twelve IMMUNOs were considered to have 'mushy' testicles. Out of these calves, eight were high responders to vaccination and four were low responders, further indicating that high responders were more affected biologically to immunization.

3.5 CONCLUSIONS

In general, there were few breed-type differences for any measured traits. Although surgical castration of bulls increased marbling and reduced acts of sexual behavior, it negatively affected growth and feed conversion efficiency, and lowered slaughter and carcass weight. Implanting with Estradiol-17β improved growth and feed conversion efficiency in steers, but not to the same level exhibited by bulls. This study demonstrated that the anti-GnRH vaccine created a high immune response in feedlot bulls. Pre-pubertal immunization against GnRH impaired testis function and affected behavioral development of young bulls. This was likely due to the reduction of testosterone from the general circulatory system. Furthermore, immunization had little effect on growth, feed conversion efficiency or carcass traits, as immunocastrates were similar to bulls. There was, however a high degree of variability among individual animal responses to immunization. The cattle that maintained very high anti-GnRH titres exhibited more severe reductions in testicular function, growth and carcass traits compared to low responding cattle. One of the key elements of successful immunization

may be the ability of the vaccine to highly affect all cattle. Implanting with Estradiol-17β may complement the effects of anti-GnRH immunization as they interacted significantly together, for scrotal circumference and warm carcass weight. Implanting also elicited a similar behavior response in immunocastrates and steers, which was opposite to the response in bulls.

In conclusion, immunocastration shows potential as an alternative way of castrating bulls. However, before this method could have any commercial relevance, all animals must respond highly to vaccination. It would also be necessary to repeat booster immunizations to maintain the response. Therefore, this method of immunocastration would not work with a 90 day pre-slaughter withdrawal, as the effects of the vaccine wear off in less than 90 days, allowing the immunocastrates to return to their 'normal' bull status.

Table 3.1: Composition of feedlot diet (per kg).

Ingredient	
Rolled barley (g)	638
Rolled oats (g)	212
Sun cured alfalfa pellets (g)	100
Premix [1] (g)	50

Calculated Nutrient Content (dry matter basis)	
Net Energy for Maintenance (kJ)	7074
Net Energy for Gain (kJ)	4689
Crude Protein (g)	122
Calcium (g)	5
Phosphorus (g)	4
Vitamin A (IU)	11200
Vitamin E (IU)	11

[1] 72.2% canola meal, 12.9% limestone, 6.5% phosphorus (as biophos), 3.2% salt, 2.5% barley grain, 2.1% molasses and 0.65% vitamins A, D and E.

Table 3.2: Least squares means of castration treatment, breed-type and implant treatment by period on GnRH antibody titres and serum testosterone concentration in young male cattle.

	Castration treatment			Breed-type			Implant treatment			
Traits / Period	**BULL**	**IMMUNO**	**P**	**BS**	**DS**	**P**	**IMP**	**NOIMP**	**P**	**SEM**
Number of animals	31	31		28	32		30	30		
Anti-GnRH titres (1:1000)			<0.01			0.76			1.00	
Day 71	0	8.6		10.3	7.0	0.65	11.5	5.7	0.42	3.58
Day 83	0	77.3		77.9	76.7	0.87	79.3	75.3	0.58	3.64
Day 97	0	69.5		72.7	66.3	0.37	73.2	65.8	0.30	3.58
Day 125	0	49.0		56.5	41.4	0.04	54.4	43.5	0.13	3.58
Day 152	0	27.1		31.9	22.4	0.18	31.5	22.8	0.22	3.58
Day 208	0	35.7		41.7	29.6	0.09	38.8	32.5	0.38	3.58
Day 250	0	28.4		35.6	21.2	0.05	31.9	24.9	0.33	3.58
Testosterone (ng dL^{-1})			<0.01			0.41			0.50	
Day 71	101.7	34.6	0.32	69.6	66.6	0.98	55.9	80.3	0.82	51.8
Day 83	35.6	2.1	0.67	27.6	10.0	0.83	8.7	29.0	0.85	51.8
Day 97	151.4	33.1	0.04	108.1	76.5	0.69	35.9	148.6	0.29	51.8
Day 125	1061.6	316.5	<0.01	608.3	769.8	0.03	712.6	665.5	0.51	52.7
Day 152	842.0	755.9	0.13	891.8	706.1	0.02	876.1	721.8	0.15	51.8
Day 208	807.8	549.4	<0.01	643.0	714.2	0.21	755.5	601.8	0.16	52.2
Day 250	633.4	546.1	0.11	587.0	592.5	0.96	621.6	557.9	0.55	52.2

Table 3.3: Least squares means of castration treatment, breed-type and implant treatment for testicular measures and sperm characteristics of young male cattle.

	Castration treatment			Breed-type			Implant treatment			
Testicular Traits	**BULL**	**IMMUNO**	**P**	**BS**	**DS**	**P**	**IMP**	**NOIMP**	**P**	**SEM**
Number of animals	31	31		28	32		30	30		
Testicle weight (g)	321.6	284.1	0.29	276.6	329.0	0.15	287.6	318.1	0.38	23.3
Epididymis weight (g)	23.4	21.5	0.35	20.9	24.0	0.15	21.1	23.8	0.20	1.33
Testicle consistency (mm)	20.7	21.1	0.53	21.2	20.5	0.29	21.4	20.4	0.16	0.44
Top temperature (°C)	24.9	24.0	0.43	25.0	23.9	0.35	25.0	24.0	0.38	0.74
Bottom temperature (°C)	21.8	21.4	0.76	22.0	21.2	0.54	22.3	20.9	0.28	0.85
Average temperature (°C)	23.4	22.7	0.55	23.5	22.6	0.40	23.6	22.5	0.32	0.75
Ultrasound minimum	19.6	19.5	0.18	19.6	19.5	0.56	19.5	19.6	0.79	0.08
Ultrasound maximum	35.9	34.0	0.24	36.0	33.9	0.22	35.9	33.9	0.23	1.07
Ultrasound mean	23.6	23.4	0.35	23.7	23.4	0.27	23.5	23.5	0.91	0.17

Table 3.4: Means of castration treatment, breed-type and implant treatment for sperm characteristics of young male cattle expressed as a percentage.

	Castration treatment		Breed-type		Implant treatment	
Sperm Traits	**BULL**	**IMMUNO**	**BS**	**DS**	**IMP**	**NOIMP**
Number of animals	31	31	28	32	30	30
Animals ejaculating	88	72	69	88	72	88
Sperm motility	36.8	32.9	37.0	33.4	34.6	35.3
Normal sperm	68.5	72.0	72.4	68.5	70.0	70.2
Head defects	5.6	4.2	3.1	6.3	5.2	4.8
Mid-piece defects	13.0	13.4	13.6	13.1	12.1	14.4
Tail defects	1.7	1.6	2.3	1.2	1.6	1.6
Cytoplasmic droplet	10.6	8.8	8.7	10.6	11.4	8.5

Table 3.5: Means of castration treatment, breed-type and implant treatment for distribution of erector muscle scores of young male cattle, expressed as a percentage.

	Castration treatment			Breed-type		Implant treatment	
Erector Score[x]	**BULL**	**IMMUNO**	**STEER**	**BS**	**DS**	**IMP**	**NOIMP**
0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
2	38	27	29	34	27	34	26
3	31	59	67	52	57	59	48
4	31	14	5	14	17	6	26
5	0	0	0	0	0	0	0

[x] See description Section 3.2.3

Table 3.6: Least squares means of castration treatment, breed-type and implant treatment at two different ages on behavior traits of young male cattle, expressed as acts 5 min.$^{-1}$.

		Castration treatment				Breed-type			Implant treatment			
Behavior	**Day**	**BULL**	**IMMUNO**	**STEER**	**P**	**BS**	**DS**	**P**	**IMP**	**NOIMP**	**P**	**SEM**
Flehmen [y]	236 [x]	2.1 a	2.0 b	0.3 c	<0.01	1.1	1.8	0.02	1.4	1.5	0.56	0.17
	253 [x]	1.7 a	2.0 a	0.1 b	<0.01	1.1	1.4	0.23	1.2	1.4	0.53	0.19
Sheath sniffing [y]	236	1.4 a	1.0 b	0.1 c	<0.01	0.7	1.0	0.56	0.69	1.0	0.37	0.21
	253	2.1 a	2.2 a	0.1 b	<0.01	1.2	1.7	0.27	1.2	1.7	0.23	0.24
Mounting [y]	236	1.6 a	1.8 a	0.3 b	<0.01	1.5	1.0	0.17	1.4	1.2	0.58	0.25
	253	1.0	1.2	0.3	0.10	0.9	0.8	0.65	0.9	0.9	0.96	0.22
Bunting [y]	236	1.1	1.1	0.9	0.71	1.1	1.0	0.71	1.0	1.0	0.75	0.15
	253	1.6	1.0	1.0	0.36	1.5	0.9	0.11	1.1	1.2	0.70	0.24
Fighting [y]	236	1.9	2.3	1.8	0.61	2.0	2.0	0.83	2.0	2.0	0.64	0.29
	253	2.9	2.0	2.3	0.16	2.6	2.1	0.11	2.6	2.1	0.28	0.23

[y] higher numbers indicate greater activity; see text for full description

[x] d 236 (Apr. 5; 9.5 - 10.5 mo of age)
d 253 (Apr. 22; 10.5 - 11.5 mo of age)

a,b.... means in rows with different subscripts are significant (P<0.05).

Table 3.7: Least squares means of castration treatment, breed-type and implant treatment on growth, efficiency and carcass traits in young male cattle.

Traits	Castration treatment				Breed-type			Implant treatment			SEM
	BULL	IMMUNO	STEER	P	BS	DS	P	IMP	NOIM P	P	
Number of animals	31	31	30		44	48		46	46		
Day 0 wt. (kg)	164.1	161.5	161.9	0.8	154.9	170.1	<0.01	161.1	163.9	0.39	2.4
Slaughter wt. (kg)	560.7 a	565.7 a	508.8 b	<0.01	541.1	549.3	0.38	544.5	544.9	0.96	6.25
Slaughter age (d)	369.3	364.8	359.5	0.26	362.5	366.5	0.39	364.2	364.8	0.90	3.68
ADG (kg d^{-1})	1.9 a	1.9 a	1.6 b	<0.01	1.8	1.8	0.50	1.8	1.7	0.11	0.04
Hip height (cm d^{-1}) [x]	0.11	0.11	0.11	0.49	0.11	0.11	0.48	0.115	0.108	<0.05	0.002
Feed intake (kg d^{-1})	9.8	9.8	9.6	0.13	0.20	0.19	0.18	9.9	9.6	<0.01	0.08
Gain:feed	0.20 a	0.20 a	0.18 b	<0.01	0.20	0.19	0.18	0.19	0.19	0.28	0.005
Warm carcass wt. (kg)	361.4 a	356.2 a	314.2 b	<0.01	342.3	345.6	0.65	307.7	309.9	0.63	11.2
Grade fat (mm)	10.0	7.8	9.6	0.41	8.1	9.9	0.09	9.31	8.80	0.78	0.99
Estimated cutability (%)	58.8	59.6	57.6	0.29	59.3	58.1	0.28	58.5	58.8	0.77	0.84
Grade [y]: number (%)											
Canada AAA	2 (6.5)	3 (10)	18 (60.0)		10 (22.7)	13 (27.7)		11 (23.9)	12 (26.7)		
Canada AA	21 (67.7)	23 (76.7)	12 (40.0)		28 (63.6)	28 (59.6)		28 (60.9)	28 (62.2)		
Canada A	8 (25.8)	4 (13.3)	0		6 (13.6)	6 (12.8)		7 (15.9)	5 (11.1)		

[x] the change in hip height from day 0 to slaughter; a,b...means with different subscripts in rows are significant for castration treatment.

[y] **Description of grades:**

Canada AAA Young carcasses with small or more marbling
Canada AA Young carcasses with slight to small marbling
Canada A Young carcasses with traces to slight marbling

Fig. 3.1: Three scrotal shapes commonly seen in beef bulls. A=straight sided scrotum, B= normal scrotum, and C= wedge-shaped scrotum. (From Cates, 1975)

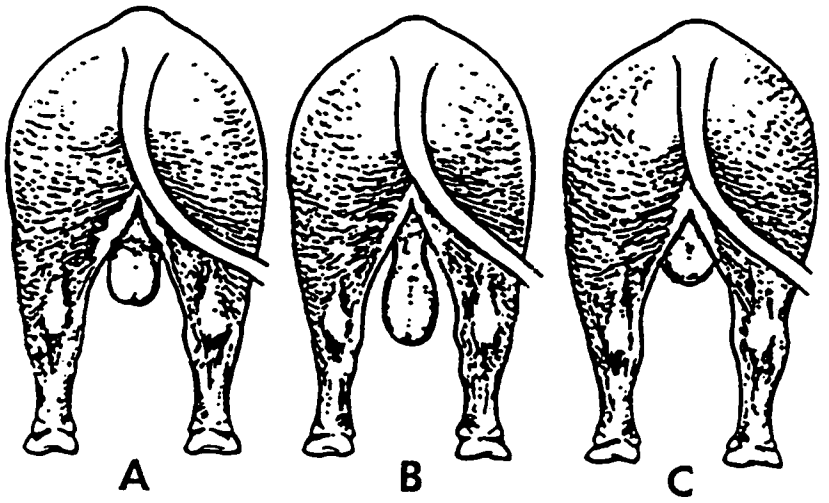


Fig. 3.2: Least square means for the interaction between castration treatment and breed-type for ultrasound mean in bull calves.

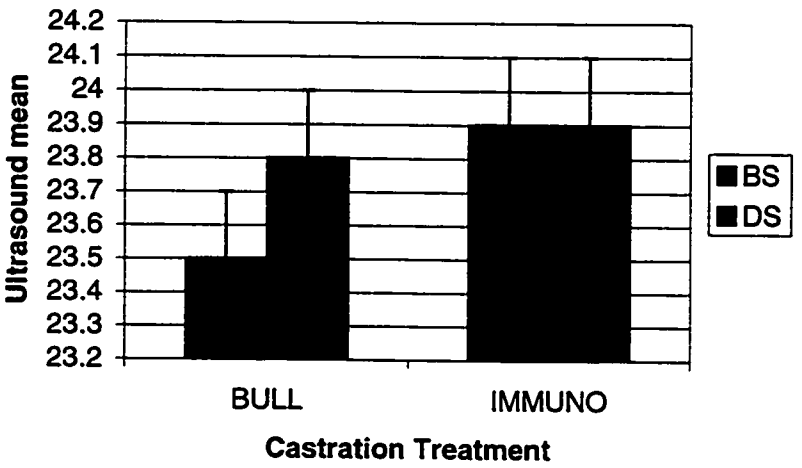


Fig. 3.3: Least squares means for the interaction between castration and implant treatment for ultrasound maximum in bull calves.

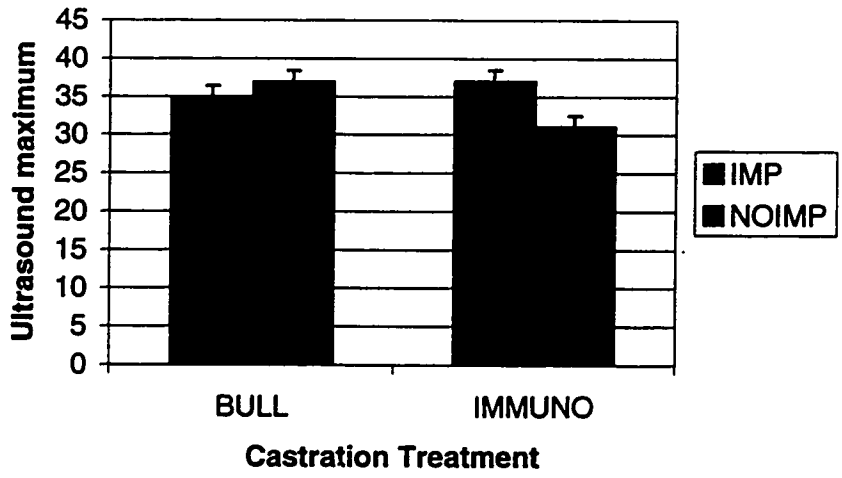


Fig. 3.4: Least squares means for the interaction between castration method and implant treatment for scrotal circumference in bull calves.

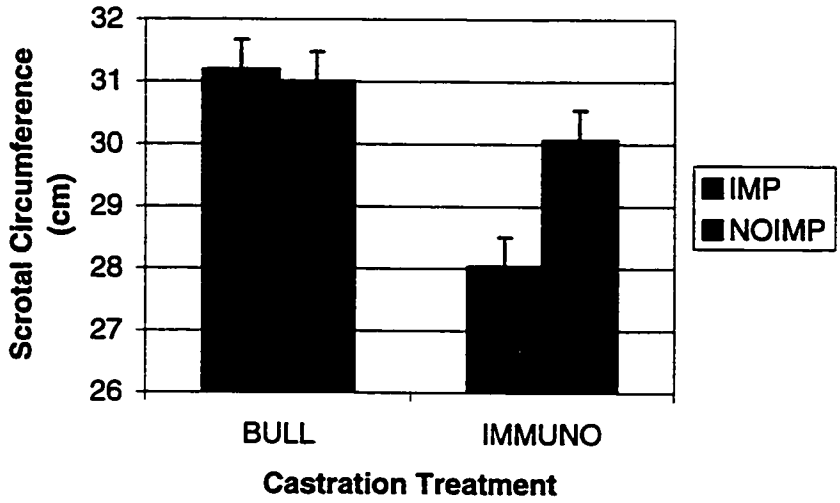


Fig. 3.5: Least squares means for the interaction between breed-type and implant treatment for scrotal circumference in bull calves.

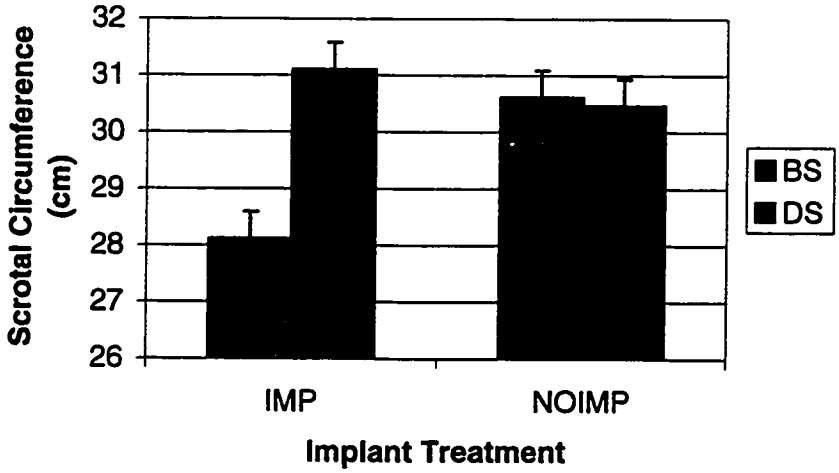


Fig. 3.6: Percentage of scrotal shapes among bulls and immunocastrates.

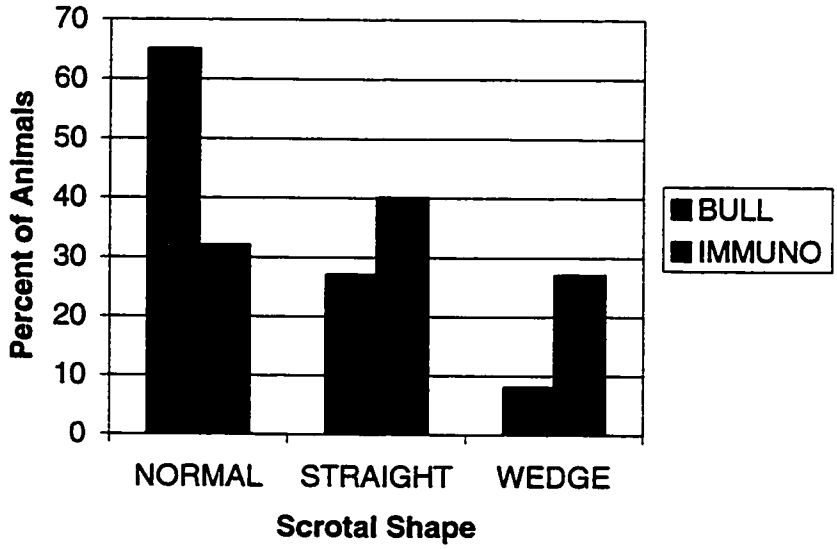


Fig. 3.7: Least squares means for the interaction between castration and implant treatment for fighting in bull calves on d 236.

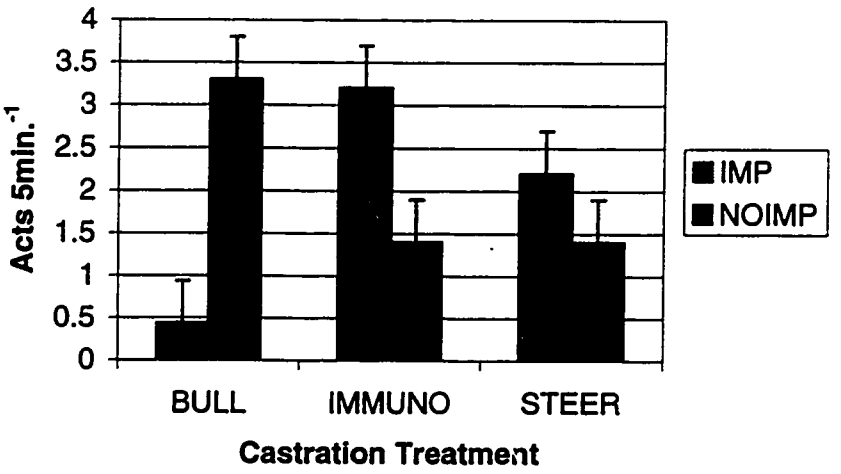


Fig. 3.8: Least squares means for the interaction between castration and implant treatment for flehmen in bull calves on d 253.

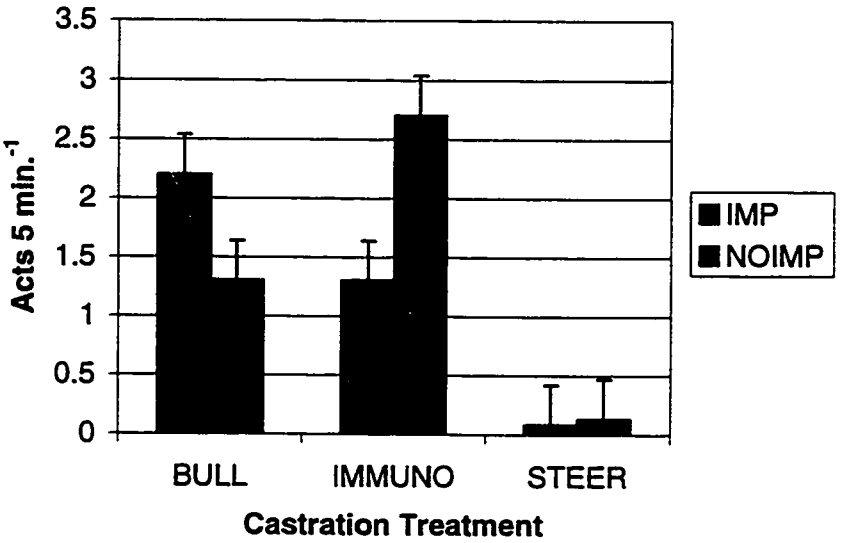


Fig. 3.9: Least squares means for the interaction between castration and implant treatment for sheath sniffing in bull calves on d 253.

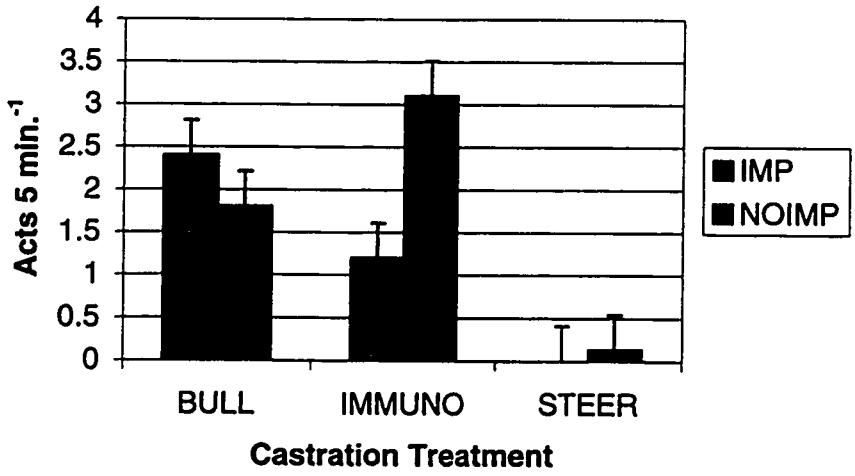


Fig. 3.10: Least squares means for the interaction between castration and implant treatment for fighting in bull calves on d 253.

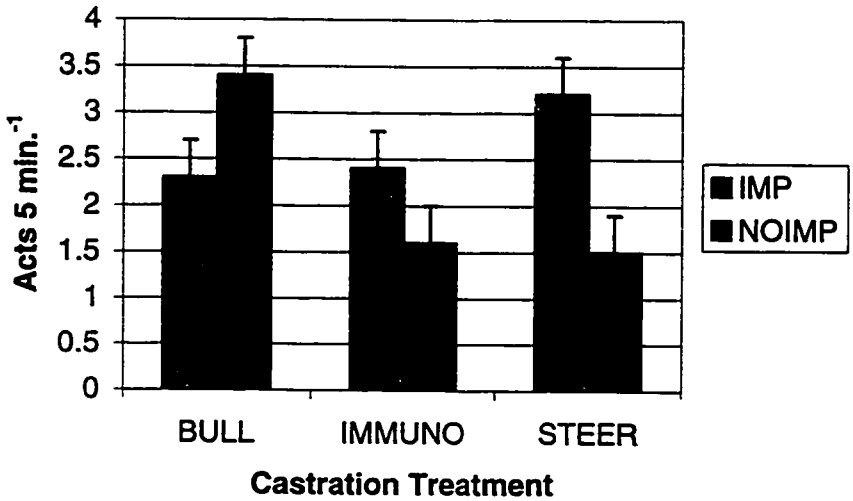


Fig. 3.11: Least squares means for the interaction between castration treatment and breed-type for warm carcass weight in bull calves.

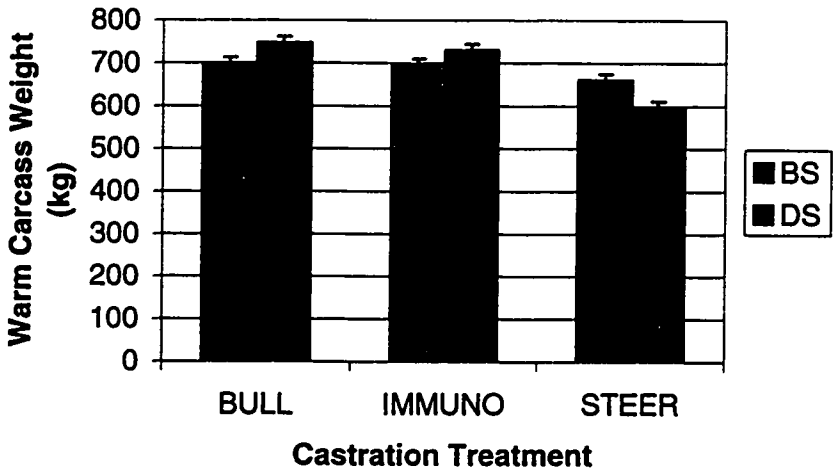


Fig. 3.12: Least squares means for the interaction between castration treatment and implant treatment for warm carcass weight in bull calves.

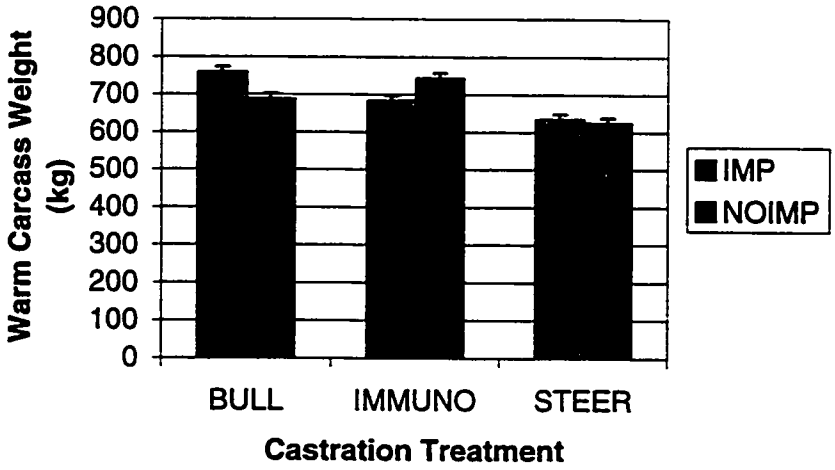


Fig. 3.13: GnRH antibody titres in immunocastrates (1:1000 dilution).

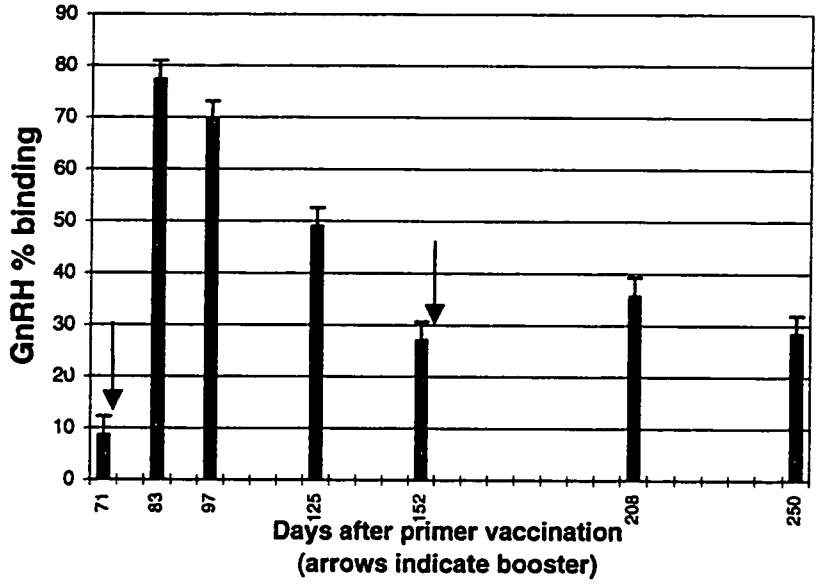


Fig. 3.14: GnRH antibody titres of immunocastrates by implant treatment (1:1000 dilution).

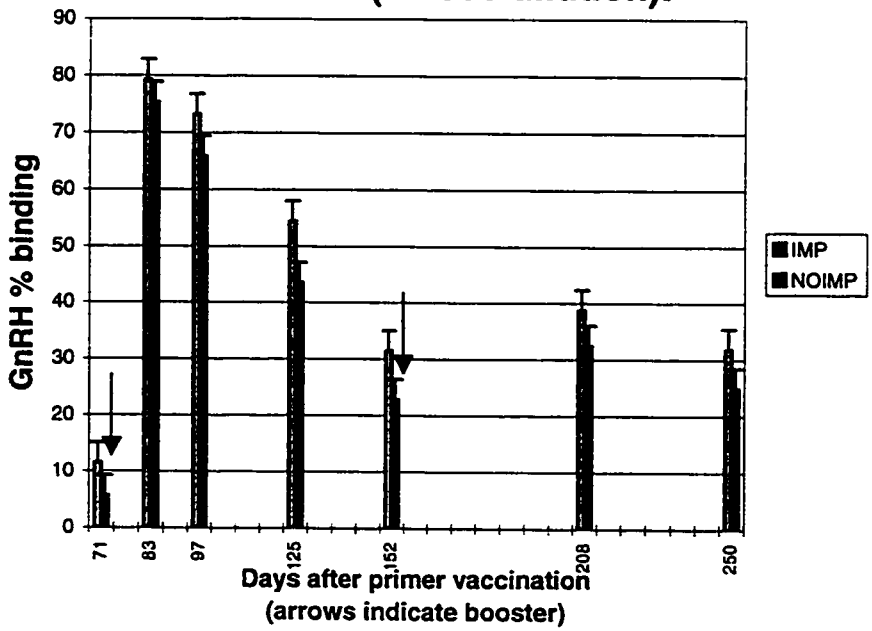


Fig. 3.15: Changes in serum testosterone concentration of bulls and immunocastrates.

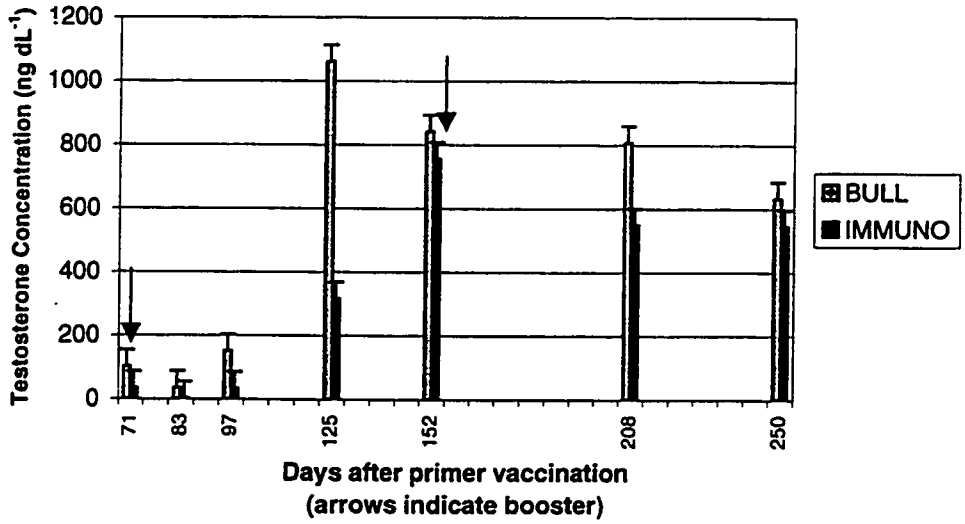


Fig. 3.16: Changes in scrotal circumference of bulls and immunocastrates.

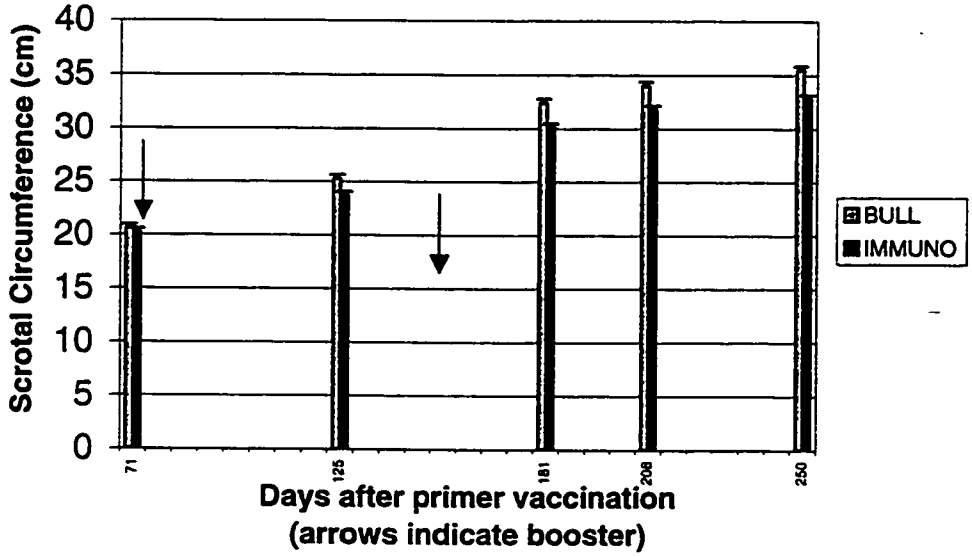
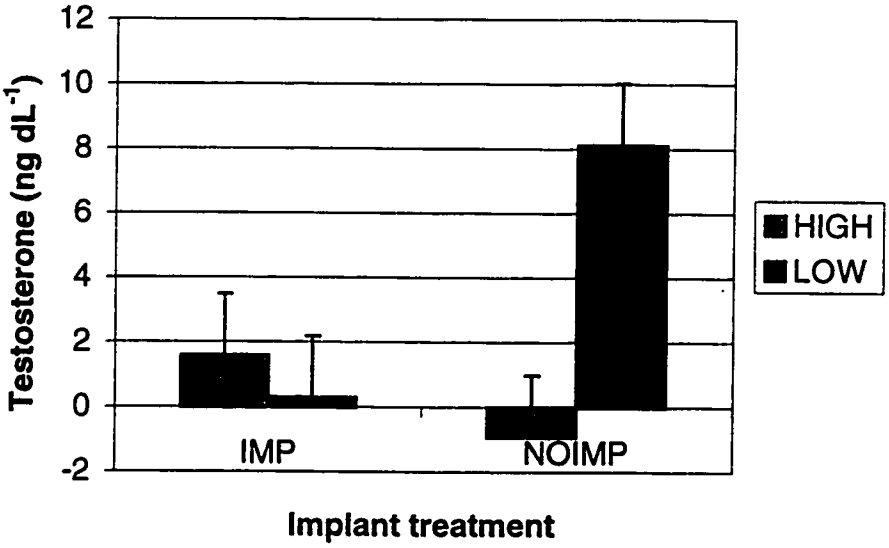


Fig. 3.17: Least squares means for the interaction between titre group and implant treatment in bull calves.



3.6 REFERENCES

Adams, T. E. and Adams, B. M. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. **70**:1691-1698.

Adams, T. E., Daley, C. A., Adams, B. M. and Sakursi, H. 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. **71**:811-817.

Adams, T. E., Daley, C. A., Adams, B. M., and Sakursi, H. 1996. Testes function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of age at immunization. J. Anim. Sci. **74**:950-954.

Agriculture and Agri-Food Canada. 1992. Graders Manual - Beef, Amended May 1992, Chapter V, Section 5.18.8, pp. v-27.

Amann, R. P., Wise, M. E., Glass, J. D. and Nett, T. M. 1986. Prepubertal changes in the hypothalamic-pituitary axis of holstein bulls. Biol. Reprod. **34**:71-80.

Apple, J. K., Dikeman, M. E., Simms, D. D. and Kuhl, G. 1991. Effects of synthetic hormone implants, singularly or in combination, on performance, carcass traits, and longissimus muscle palatability of holstein steers. J. Anim. Sci. **69**:4437-4448.

Appleby, M. C. 1986. Development of sexual and agonistic behavior in bulls and steers. Appl. Anim. Behav. Sci. **15**:190 (abstr.).

Balthazart, J. 1990. Brain aromatization of testosterone regulates male reproductive behavior in birds. Progress of Comparative Endocrinology. Wiley-Liss Inc. Chichester. P. 92-98.

Berg, R. T., Goonewardene, L. A. and Makarechian, M. 1986. The university beef breeding project - 1986 update. 65th Annual Feeders' Day Report, University of Alberta. pp.3-6.

Baker, A. M. and Gonyou, H. W. 1986. Effects of zeranol implantation and late castration of sexual, agonistic and handling behavior in male feedlot cattle. J. Anim. Sci. **62**: 1224-1232.

Brown, B. W., Mattner, P. E., Carroll, P. A., Holland, E. J., Paull, D. R., Hoskinson, R. M. and Rigby, R. D. F. 1994. Immunisation of sheep against GnRH early in life: effects on reproductive function and hormones in rams. J. Reprod. Fert. **101**:15-21.

Butson, S. and Berg, R. T. 1984. Factors influencing lactation performance of range beef and dairy-beef cows. Can. J. Anim. Sci. **64(2)**:267-277.

Calkins, C. R., Clanton, D. C., Berg, T. J. and Kinder, J. E. 1986. Growth, carcass and palatability traits of intact males and steers implanted with zeranol or estradiol early and throughout life. J. Anim. Sci. **62**:625-631.

Canadian Council of Animal Care. 1984. Guide to the care and use of experimental animals. Vol.2. CCAC, Ottawa, ON.

Cates, W. F. 1975. Observations on scrotal circumference and its relationship to classification of bulls. Proc. Ann. Meeting of Theriogenology. Cheyenne, Wyoming.

Cook, R. B., Coulter, G. H. and Kastelic, J. P. 1994. The testicular vascular cone, scrotal thermoregulation, and their relationship to sperm production and seminal quality in beef bulls. Theriogenology **41**:653-671.

Cook, B., Popp, J., Kastelic, J., Robbins, S. and Harland, R. 1998. Active immunization of bulls against GnRH: effects on testes, feedlot performance and carcass characteristics. J. Anim. Sci. **76(Suppl.)**:277 (Abstr.).

Deaver, D. R., Glass, J. D., Grieger, D. M. and Reeves, J. J. 1988. Effects of estradiol on secretion of LH, hypothalamic function and testicular development in bull calves. Domest. Anim. Endocrinol. **5**:307.

Doornenbal, H., Tong, A. K. W., Newman, J. A., Murray, N. L. and Mears, G. J. 1987. Blood and serum components and organ weights in steers, bulls and zeranol-implanted bulls. J. Anim. Sci. **64**:489.

Finnerty, M., Enright, W. J., Morrison, C. A. and Roche, J. F. 1994. Immunization of bull calves with a GnRH analogue-human serum albumin conjugate: effect of conjugate dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. J. Reprod. Fert. **101**:333-343.

Finnerty, M., Enright, W. J., Prendiville, D. J., Spicer, L. J. and Roche, J. F. 1996. The effect of different levels of gonadotropin-releasing hormone antibody titres on plasma hormone concentrations, sexual and aggressive behaviour, testis size and performance of

bulls. Anim. Sci. **63**:51-63.

Foutz, C. P., Dolezal, H. G., Gardner, T. L., Gill, D. R., Hennsley, J. L. and Morgan, J. B. 1997. Anabolic implant effects on steer performance, carcass traits, subprimal yields, and longissimus muscle properties. J. Anim. Sci. **75**:1256-1265.

Gonzalez, A., Goubau, S., Allen, A. F., Mapletoft, R. J., Cohen, R. and Murphy, B. D. 1990. Livestock Reproduction in Latin America (IAEA Panel Proceedings Series STI/PUB 833). pp.269-284.

Hahn, J., Foote, T. H. and Cranch, E. T. 1969. Tonometer for measuring testicular consistency of bulls to predict semen quality. J. Anim.Sci. **29**:483-289.

Hinch, G. N., Lynch, J. J. and Thwaites, C. J. 1982/83. Patterns and frequency of social interactions in young grazing bulls and steers. Appl. Anim. Behav. Sci. **9**:15-30.

Huxsoll, C. C., Price, E. O. and Adams, T. E. 1998. Testis function, carcass traits and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. **76**: 1760-1766.

Jago, J. G., Cox, N. R., Bass, J. J. and Matthews, L. R. 1997. The effect of prepubertal immunization against Gonadatropin-Releasing Hormone on the development of sexual and social behavior of bulls. J. Anim. Sci. **75**:2609-2619.

Jeffcoate, I. A., Lucas, J. M. S. and Crighton, D. B. 1982. Effects of active immunization of ram lambs and bull calves against synthetic luteinizing hormone releasing hormone. Theriogenology **18(1)**: 65-77.

Jones, S. J., Johnson, R. D., Calkins, C. R. and Dikeman, M. E. 1991. Effects of trenbolone acetate on carcass characteristics and serum testosterone and cortisol concentrations in bulls and steers on different management and implant schemes. J. Anim. Sci. **69:**1363.

Lee, C. Y., Henricks, D. M., Skelley, G. C. and Grimes, L. W. 1990. Growth and hormonal response of intact and castrate male cattle to trenbolone acetate and estradiol. J. Anim. Sci. **68**:2682-2689.

Lobley, G. E., Connell, A., Morris, B., Anderson, J., Clayton, P. E., Williams, V. and Nevison, I. M. 1992. The effect of active immunization against gonadotropin-hormone-releasing-hormone on growth performance and sample joint composition of bulls. Anim. Prod. **55**:193-202.

McKenzie, J. R. 1984. Effects of different zeranol implanting programmes on behaviour and liveweight gain in grazing bull. J. Anim. Sci. **59(Suppl. 1)**:147.

Price, M. A., Jones, S. D. M., Mathison, G. W. and Berg, R. T. 1980. The effects of increasing dietary roughage level and slaughter weight on the feedlot performance and carcass characteristics of bulls and steers. Can. J. Anim. Sci. **60**:345-358.

Price, M. A., Makarechian, M., Tennessen, T. and Mathison, G. W. 1983. The effects of zeranol on the feedlot performance of beef bulls. Can. J. Anim. Sci. **63**:803-809.

Rawlings, N. C., Hafs, H. D. and Swanson, L. V. 1972. Testicular and blood plasma androgens in holstein bulls from birth through puberty. J. Anim. Sci. **34**:435-440.

Robertson, I. S. and Lowman, B. G. 1977. Bull beef production from suckler herds of traditional breeds. Anim. Prod. **24**:144(abstr.).

Robertson, I. S., Wilson, J. C. and Fraser, H. M. 1979. Immunological castration of male cattle. Vet. Rec. **105**:556-557.

Robertson, I. S., Fraser, H. M., Innes, G. M. and Jones, A. S. 1982. Effect of immunological castration on sexual and production characteristics in male cattle. Vet. Rec. **111**:529-531.

SAS Institute, Inc. 1990. SAS. User's guide, Statistics. Version 5 Edition, SAS. Cary, N.C.

Schanbacher, B. D. 1984. Active immunization against LH-RH in the male. Immunological Aspects of Reproduction in Mammals. Butterworths. England.

Silcox, R. W., Keeton, J. T. and Johnson, B. H. 1986. Effects of zeranol and trenbolone acetate on testis function, live weight gain and carcass traits of bulls. J. Anim. Sci. **63**:358-368.

Staigmiller, R. B., Brownson, R. M., Kartchner, R. J. and Williams, J. H. 1985. Sexual development in beef bulls following zeranol implants. J. Anim. Sci. **60(2)**:342-351.

Swanson, L. V., Wettemann, R. P., Rawlings, N. C., Hafs, H. D. and Magee, W. T. 1971. Pubertal relationships of some endocrine and reproductive criteria in hereford bulls. J. Anim. Sci. **33**:823-828.

Teague, G. L., Boyd, G. W., Smith, G. C., Finnerty, M., Rollin, B. E, Field, T. G., Grandin, T., Kniffen, D. M., Taylor, R. E. and Enright, W. J. 1992. Bovine carcass characteristics and overall lean meat production efficiency of early castrates (EC), late castrates (LC), immunocastrates (IC) and intact males (IM). Environ. Liv. Prod. (abstr.) pp.103

VanKessel, Andrew. 1998. Personal Communication. Department of Animal Science, University of Saskatchewan. Saskatoon Saskatchewan.

4. GENERAL DISCUSSION AND CONCLUSIONS

The research reported in this thesis examined the effects of anti-GnRH immunization on reproductive physiology, production, carcass and behavior traits of feedlot bulls, and compared them to intact bulls and surgically castrated bulls. Surgical castration is routinely performed on feedlot bulls because it lowers the incidence of sexual and aggressive behavior, even though it also reduces growth rate and feed conversion efficiency. The purpose of the research reported here was to investigate the potential use of immunocastration as an alternative to surgical castration. If immunocastration is to be acceptable, it must cause immunized bulls to behave more like steers. At a minimum, it must reduce the behavior problems associated with feeding bulls. Effective immunocastration is expected to reduce growth and feed efficiency, but any retained anabolic advantages of immunocastrated bulls over steers would be beneficial. In feedlots there is no practical need to inhibit reproductive function however, studying the effects of immunocastration on reproductive function provides important information on how bulls react biologically to immunization.

The first experiment compared surgical, immuno- and non-castration on feedlot performance of bull calves. GnRH antibody binding, serum testosterone concentrations, change in scrotal circumference, growth, feed efficiency, carcass traits and behavior were examined. The results reconfirmed that surgical castration of bulls inhibits growth and feed efficiency, and lowers slaughter and carcass weight at a fixed fat level. It also

increases marbling and reduces typical bull behavior. There was little difference between breed-types for most traits measured, except that Beef Synthetic 2 cattle were fatter, had smaller rib-eye areas, and consequently had lower cutabilities than Beef Synthetic 1 and Dairy Synthetic cattle. Anti-GnRH immunization led to a high immune response with mean antibody titres rising to over 70% bound at 1:1000 dilution. This was associated with a reduction in scrotal circumference throughout the experiment and of testosterone concentration at slaughter. However, it showed little relationship with growth, feed efficiency or carcass traits. Similar to reports in the literature of cattle this age, feedlot cattle in this experiment exhibited more sexual behavior than aggressive. For sexual behaviors, immunocastrates behaved intermediately to control and surgically castrated bulls.

The second experiment looked at the effects of surgical, immuno- and non-castration on feedlot performance of bull calves implanted with a Estradiol-17β. Anti-GnRH titres, serum testosterone concentrations, change in scrotal circumference, testicular measurements, behavior, growth, feed efficiency and carcass traits were compared. Effects of surgical castration were similar to those found in the first experiment. The use of a growth promoting implant only partially compensated for anabolic losses due to castration. As with the first experiment, the cattle responded strongly to immunization with GnRH antibody binding of almost 80%, one week after boosting. Unlike the first experiment, this led to severe reductions in testosterone concentration of the immunocastrates. The pubertal testosterone peak normally seen in

bulls was delayed in the immunocastrates and testosterone never appeared to reach the same level seen in control bulls. As with the first experiment, immunization had little effect on growth, feed conversion efficiency or carcass traits. Implanting with Estradiol-17β appeared to complement the effects of immunization as they interacted significantly for scrotal circumference and warm carcass weight. Implanting also elicited a similar behavior response in immunocastrates and steers, but had an opposite response in bulls. While implanting decreased sexual behavior of the immunocastrates and steers, it increased their aggressive behavior.

In summary, immunocastration affected dairy and beef synthetic breed-types similarly in terms of performance. Overall, the beef synthetics matured earlier and had lower cutabilities than the dairy synthetics. The anti-GnRH vaccine elicited an immediate and high immune response in bulls. This was followed by a large reduction in serum testosterone concentration and in turn, inhibited sexual development and behavior in immunocastrates, but had little effect on production and carcass traits. In these traits immunocastrates more closely resembled bulls. The lack of effect on production and carcass traits may be partially attributed to the 90-day withdrawal period of the vaccine. Within approximately two months of each vaccination, anti-GnRH titres, and consequently biological effects of immunization had effectively 'worn off'. As a result, in the month prior to slaughter the immunocastrates would have reverted back to the hormonal status of entire bulls.

It was demonstrated that there was a high degree of variation among individual

cattle in their response to immunization. Cattle that were high responders exhibited more severe reductions in sexual function, growth and carcass traits, and as a result were more similar to surgical castrates than the low responders. The low responding group was more similar to intact bulls.

Immunocastration has potential for altering management of feedlot bulls, but work needs to be done to ensure all cattle respond highly to vaccination and that immunocastrated bulls behave similar to steers. Implanting may complement the immunization effect.

Immunization of pre-pubertal bulls against GnRH may be a useful and practical alternative to surgical castration. However, duration of active anti-GnRH titres in the circulatory system and variable animal response to immunization will need to be addressed in future research.

APPENDIX 1

Correlations of GnRH antibody titres, testosterone concentrations and testis weights in young male cattle.

Bleed day	Testosterone concentration and GnRH antibody titres			Testosterone concentration and testis weight			GnRH antibody titres and testis weight		
	Correlation coefficient	P	Number of animals	Correlation coefficient	P	Number of animals	Correlation coefficient	P	Number of animals
71	-0.05	0.77	32	0.28	0.14	30	-0.53	<0.01	30
83	-0.56	<0.01	31	0.28	0.13	30	-0.43	0.02	29
97	-0.52	<0.01	32	0.29	0.13	30	-0.42	0.02	30
125	-0.50	<0.01	31	0.53	<0.01	29	-0.53	<0.01	30
152	-0.42	0.02	32	0.59	<0 01	30	-0.70	<0.01	30
208	-0.44	0.01	32	0.39	0.03	30	-0.70	<0.01	30
250	-0.24	0.19	32	0.35	0.06	30	-0.71	<0.01	30