VARIANCE COMPONENT ESTIMATION FOR REPRODUCTIVE TRAITS AND ANALYSES OF MYOFIBRILLAR PROTEINS AND AGE AT PUBERTY IN ANGUS BEEF CATTLE DIVERGENTLY SELECTED FOR BLOOD SERUM INSULIN-LIKE GROWTH FACTOR I CONCENTRATION

DISSERTATION

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By

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ABSTRACT

The first objective of this study was to obtain (co)variance component estimates for reproductive traits of beef cattle. Heritabilities for insulin-like growth factor I (IGF-I) concentration of three blood samples collected at d 28, 42, and 56 of the 140-d postweaning test, which are abbreviated as IGF28, IGF42, and IGF56, respectively, and mean IGF-I were 0.32, 0.34, 0.30, and 0.32, respectively. Heritabilities for scrotal circumference, percent sperm motility, percent normal sperm cells, age of heifers at first calving, and calving rate were 0.51, 0.09, 0.43, 0.26, and 0.13, respectively. The calving rate data were entered as either 1 (if conceived) or 100 (if not conceived). Additive genetic correlations of scrotal circumference, percent motile sperm cells, and calving rate with mean IGF-I concentration were 0.35, 0.43, and –0.41, respectively. These results suggest that selection for increased serum IGF-I concentration should increase scrotal circumference, percent motile sperm cells, and calving rate.

The second objective in this study was to examine effects of high or low blood serum IGF-I concentration on age of heifers at puberty. Results of a progesterone assay showed that age at puberty did not differ between heifers in the high and low
IGF-I selection lines. A cubic relationship existed between age at puberty and mean IGF-I concentration. Residual correlations of age at puberty with IGF-I measurements tended to be negative and nonsignificant.

The third objective of this study was to examine effects of high or low blood serum IGF-I concentration on expression of myofibrillar proteins. Troponin C levels were higher in the high IGF-I line bulls and myosin light chain 2 levels were higher in the low IGF-I line bulls. Although their levels did not differ between the high and low IGF-I lines, desmin and the 32 kDa protein had significant correlations with IGF-I concentration. Coefficients for the quadratic regression of density of desmin on IGF28, IGF42, and mean IGF-I were significant. Cubic relationships existed between density of 32 kDa protein and IGF56 and mean IGF-I.

Results from this study indicate that serum IGF-I concentrations are genetically correlated with reproductive traits, there are some IGF-I selection line effects on expression of myofibrillar proteins, and selection for blood serum IGF-I does not change age of heifers at puberty.
This work is dedicated to my advisor, Dr. Michael E. Davis, for being an exemplary advisor, and all other academic advisors alike, reliable and responsible
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The most important lesson I have learned from my six years of graduate school experience is that the advisor is the most important component of the success or failure of the graduate work. It is nearly impossible to complete a high-quality graduate work without good guidance from an advisor. The most important characteristics of a good advisor are reliability and responsibility, which have to be maintained throughout the work.

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CHAPTER 1

INTRODUCTION

Insulin-like growth factor I (IGF-I) is a hormone-like polypeptide related to several economically important traits including growth and reproduction in cattle (Spicer and Chamberlain, 1998). Recent experiments show that normal growth can be maintained even if circulating IGF-I levels are less than normal and that locally-produced IGF-I is more important in maintaining tissue growth and development (Yakar et al., 1999). However, these experiments do not rule out the importance of circulating IGF-I as a regulator of growth hormone concentration and its correlation with local IGF-I production in several tissues. IGF-I receptors have been detected throughout the body and IGF-I deficiency is associated with growth abnormalities in cattle (Kitagawa et al., 2001). Several studies have shown a close association between male reproductive traits and IGF-I measurements (Glander et al., 1996; Vickers et al., 1999). IGF-I protects cells in the ovary from apoptosis, increases sperm motility, maintains normal cell morphology (Baker et al., 1996), and is related to scrotal circumference (Yilmaz et al., 1999).
Selection for serum IGF-I concentration in our herd of beef cattle has been successful (Davis et al., 1995). It is important to determine sources of the variation in IGF-I concentration, as well as in reproductive traits, to develop effective breeding and selection strategies to improve productivity. For example, genetic correlations of scrotal circumference with IGF-I measurements provide an estimate of how much gain in scrotal circumference can be obtained in response to selection for IGF-I. In this study, therefore, we aimed to obtain genetic, phenotypic, and environmental correlations of male and female reproductive traits with IGF-I concentration.

Age at puberty influences lifetime productivity of the heifer and, therefore, is an important part of breeding programs designed to improve reproductive efficiency. Decreasing age at puberty is complicated by the contribution of several environmental factors, including nutrition and season. Previous research has shown increases in IGF-I concentration during puberty (Bishop, 1991; Jones et al., 1991). IGF-I mediates effects of luteinizing hormone-releasing hormone secretion, and, therefore, is a candidate for the regulation of age at puberty. In our herd, age at first calving is lower in the high IGF-I line females (unpublished data). It would be interesting to determine whether the change in age at first calving is reflected in age at puberty. Our second objective was, therefore, to determine the age of heifers at puberty in high and low IGF-I line heifers using a progesterone assay.

One of the ultimate goals of animal breeding is to produce meat with high quality and quantity. IGF-I causes hypertrophy in muscle cells (Huang et al., 2002) and induces changes in expression of the proteins that make up the myofibril, the functional unit of the muscle tissue. The relative amounts of the proteins that make
up muscle tissue may determine its biochemical and biophysical properties. Researchers interested in improving meat tenderness have used semi-quantitative SDS-PAGE analyses of muscle proteins as an important tool to determine quantity of protein molecules expressed in tender or tough meat samples (Fritz et al., 1993; Huff-Lonergan et al., 1995). In this study, our third objective was to perform SDS-PAGE analysis to examine possible changes that might have occurred in expression of myofibrillar proteins in response to high or low circulating IGF-I concentration.
CHAPTER 2

LITERATURE REVIEW

General information about insulin-like growth factors

Salmon and Daughaday (1957) isolated a “factor” from rat serum that stimulated sulphate incorporation into cartilage cells in vitro and named the substance “sulphation factor”. Later, it was discovered that the “sulphation factor” had insulin-like and stimulatory effects on cell proliferation and differentiation. After taking several names such as non-suppressible insulin-like activity and somatomedins, the factors were named by Daughaday and Rotwein (1989) as “insulin-like growth factors (IGFs)”, because of their stimulatory effects on cell growth and structural similarity to proinsulin.

IGF-I and IGF-II polypeptides are composed of 70 and 67 amino acids and have 7.6 and 7.4 kDa molecular weights, respectively. IGF polypeptides have five domains (A through E). Both of the growth factors share 70% homology with proinsulin in the amino acid sequence of their A and B domains (Zapf and Froesch,
IGF-I and IGF-II also share 70% homology in their amino acid sequences. The C domain, however, is distinct (Werner et al., 1994) and is essential for binding to the IGF receptor. Both ligands and receptors of IGF-I and insulin are similar, suggesting an evolutionary link between these molecules (Zapf and Froesch, 1986).

In porcine, the IGF-I polypeptide has a leader peptide of 25 amino acids and a C-terminal trailer of 35 amino acids in addition to the 70 amino acid IGF-I polypeptide (Tavakkol et al., 1988), suggesting that the polypeptide undergoes significant post-translational processing before reaching maturity. The mature IGF-I polypeptide has identical amino acid sequences in bovine, porcine, and human. Rat and mice IGF-I have a small number of different amino acids from the species mentioned above.

The IGF-I gene has five exons and is mapped to chromosome 12q23 in humans (Tricoli et al., 1984), and chromosome 5 in cattle (Miller et al., 1992). Exons 4 and 5 are alternatively expressed, resulting in multiple mRNAs. Due to the alternative splicing, several leader sequences have been reported in the rat liver, indicating the presence of IGF-I mRNA species with different 5’ends. The IGF-II gene has eight exons. Promoter sequences in the IGF-II gene ensure tissue and developmentally specific expression (Butler and LeRoith, 2001). IGF-II and its receptor are maternally imprinted (Lee et al., 2001), providing important insights into how cancer is inherited and priority of parents in determining the developmental regulation of the progeny.
IGF-I can act as an endocrine (in dwarfism), paracrine (in cartilage cells), or autocrine (in cancer cells) regulator of cell proliferation and differentiation. Although IGF-I is produced mainly in the liver, it can exert its effects on systems far from the liver. In addition, most cells produce IGF-I locally and have IGF-I receptors.

Both IGF-I and IGF-II exert their effects via their receptors. Insulin-like growth factor I receptor (IGF1R) is a dimer that has similarities to the insulin receptor. Insulin-like growth factor II receptor (IGF2R) is a monomer. IGF1R and IGF2R are also called type I and type II receptors, respectively. Both insulin and IGF-II can bind IGF1R, but insulin cannot bind IGF2R. IGF-I binds IGF2R only weakly. IGF-I, IGF-II, and their receptors have been found in almost all tissues examined, including muscle and adipocytes (Lee et al., 1993). IGF-II may play a role in regulating IGF-I action, because it can bind the IGF1R.

The IGFs in the circulation can be found free, but are mostly bound to insulin-like growth factor binding proteins (IGFBP) (Svoboda and Van Wyk, 1985). The half-life of IGF-I is longer than that of GH in the blood, because it is bound to carrier binding proteins that are synthesized in the liver under the control of growth hormone. Seven IGFBPs have been identified (Oh et al., 1996). The binding proteins can serve as storage reservoirs for the IGFs. They regulate access of the IGF-I polypeptide to its target tissues. Almost 80% of IGF-I in human circulation is found as a tertiary complex that includes IGF-I, IGFBP3, and an acid labile subunit (ALS). The acid labile subunit is a molecule that has high affinity for the complexes that the IGF-I molecule makes with IGFBP3 or IGFBP5. It protects the complexes from degradation. Blood IGF-I and IGFBP3 concentrations in mice with ALS knockout
decrease 60 and 90%, respectively, yet the mice exhibit normal growth (Boisclair et al., 2000). A truncated form of IGF-I binds IGFBPs less and is 10 times more potent than normal IGF-I in stimulating cell proliferation (Ballard et al., 1996). GH regulates both IGF-I and IGFBP3 concentration in the circulation.

The liver is the main site of IGF-I production in cattle. IGF-I, IGF-II, insulin, and growth hormone (GH) receptors, and IGFBP1, IGFBP2, and IGFBP3 mRNAs were detected in the liver of bulls (Cordano et al., 2000). Increases in serum IGF-I are associated with increases in IGF-I mRNA levels in the liver.

IGF-I regulates production of its own receptor. Almost no IGF1R mRNA production occurs in the liver, because IGF-I down regulates IGF1R production and IGF-I concentration is highest in the liver. IGF1R expression can be detected in almost all tissue and cell types during embryogenesis, suggesting the importance of IGF-I in early development.

Most, if not all, of the growth promoting effects of GH are mediated through IGF-I. Responses of cartilage, adipose tissue, and many other tissues to GH are largely enhanced by IGF-I.

A distinct difference between GH and IGF-I is that the IGF-I concentration is more stable in circulation and does not change under most of the conditions that change GH levels. For example, low blood sugar increases GH, but not IGF-I (Berne and Levy, 1993). Although IGF-I levels in blood are very sensitive to GH levels, IGF-II levels do not show the same degree of dependency, likely because of the structure of their receptor. Fasting and lower protein intake, as well as diabetes, increase GH, but decrease IGF-I, indicating that the dependency on GH is lost under
these conditions (Vandehaar et al., 1995). Fasting and glucose levels cause changes in free and unbound IGF-I without changing the total concentration of the IGFs in humans (Frystyk et al., 1994).

Tissue-specific gene targeting has enabled scientists to generate mice that have no hepatic IGF-I production, but maintain local IGF-I production in the kidney, muscle, and other tissues. No IGF-I mRNA expression was found in the liver in these mice and plasma IGF-I was 75% lower than in the controls. Surprisingly, growth of both male and female mice was not affected (Yakar et al., 1999), but GH levels were elevated. These results suggest that increased GH levels, 25% of circulating and the locally-produced IGF-I could replace functions of circulating IGF-I in growth. In these mice, IGFBP3 was also decreased and it was not certain whether the free IGF-I also decreased by 75%. This study helped, yet did not conclusively prove, that circulating IGF-I was not important in postnatal growth, because 25% of the IGF-I still remained in the circulation and there was a clear regulation of GH by circulating IGF-I.

Systemic effects of IGF-I observed in multiple systems in animals prompted researchers to inject IGF-I directly into the animal tissues and observe changes that may occur due to increased IGF-I concentration in the circulation. Cottam et al. (1992) injected male sheep with IGF-I for 8 wk. IGF-I concentration in the injected animals was two-fold higher than in the controls. The injection did not have any effect on food intake, wool production, bone length, carcass weight or dimensions, but increased plasma glucose, kidney and spleen weights up to 40% of the controls. The injection also decreased insulin, plasma urea, and creatinine. The authors
concluded that IGF-I injection in sheep did not have dramatic effects on somatic growth, but resulted in changes in kidney and spleen growth and glucose metabolism.

In humans (Breier et al., 1993) and cows (Buskirk et al., 1996), injection with human or bovine GH increases milk production, and the increase is associated with increases in IGF-I concentration. Injection with growth hormone-releasing factor also increases IGF-I concentration in cows (Abribat et al., 1990). The authors reported that age and stage of lactation were important factors in regulating IGF-I concentration.

**Effects of IGF-I concentration on growth of humans and mice**

IGF-I expression is essential for normal tissue growth and development (Yakar et al., 2002). Effects of IGF-I are more pronounced in postnatal life, whereas those of IGF-II are more important in fetal growth and development (Constancia et al., 2002). Effects of IGF-I on cell proliferation and differentiation are larger in fetal cells than in adult cells (Koike et al., 1995). IGF-I deficiency in the embryo results in severe growth abnormalities (Baker et al., 1993), indicating that IGF-I expression is essential for normal tissue growth and development. Recent evidence suggests that locally produced IGF-I may be more important than circulating IGF-I in regulating these essential functions (Yakar et al., 1999). Transgenic mice, in which IGF-I or IGF-II genes were knocked out, weighed only half of the control mice at birth (Baker et al., 1993). IGF1R knockout mice die at birth due to respiratory dysfunction, but
IGF2R knockout mice seem to be normal, although they exhibit growth retardation after birth. IGF-I may act almost exclusively through the IGF1R, because results obtained from IGF1R and IGF-I knockout mice are very similar.

Laron syndrome, a type of dwarfism in humans, is associated with a mutation in the GH receptor gene that inhibits IGF-I production (Brat et al., 1997). These individuals have almost no IGF-I or IGFBP3 in their circulation and exhibit retarded muscle growth. On the other hand, their GH concentration is elevated. It is interesting to note that experimentally generated mice with IGF-I concentration 75% lower than control mice and higher GH levels exhibit normal growth (Yakar et al., 1999), while a natural condition in humans with similar hormonal profile results in dwarfism. These studies show that effects of IGF-I on mouse and human growth may differ.

Circulating IGF-I may not be essential for postnatal growth, but it has a negative effect on GH concentration. It also is an indicator for local production of IGF-I in several organs including the ovary (Cushman et al., 2000).

**Effects of IGF-I concentration on performance traits of cattle**

Differences in feed conversion rates of cattle are associated with differences in IGF-I concentration (Bishop et al., 1989). The authors concluded that IGF-I concentration was related to growth and feed efficiency in cattle, because the coefficients for correlation of average daily gain and feed efficiency with IGF-I
concentration were 0.28 \( (P = 0.001) \) and 0.16 \( (P = 0.07) \), respectively. Lemal et al. (1989) injected heifers with bovine somatotropin. IGF-I levels increased and mean IGF-I was related to average daily gain. Cattle with lower IGF-I concentration have greater weights and weight gains (Davis and Simmen, 1997). The authors reported genetic correlations of weaning and postweaning weights and weight gains with serum IGF-I concentration that ranged from -0.21 to -0.54.

A recently reported pathologic condition characterized by larger birth weights and prolonged gestation in calves produced using somatic cell cloning may be related to a defective IGF-I system in these animals (Matsuzaki and Shiga, 2002). Cloned calves had high cortisol, IGF-I, and IGFBP2 levels. The authors concluded that the IGF system in cloned calves did not switch to the adult mode, and, therefore, was the reason for the observed abnormalities. In cloned calves, a change in IGF1R expression was also reported (Lazzari et al., 2002). In addition to changes in expression of heat shock proteins and glucose transporter genes, the change in IGF1R gene expression may be responsible for the large offspring syndrome observed in the cloned calves.
Effects of IGF-I concentration on reproductive functions

Blood serum IGF-I may be the link between nutritional status and reproductive performance in cattle, because it is related to ovarian production of gonadotropins and function of the corpus luteum (Zulu et al., 2002). The authors concluded that changes in plasma IGF-I concentration could be predictive of nutritional and reproductive status in cattle.

IGF-I is present in the ovary, uterus, oviduct, and blastocysts. IGFs are important in embryonic development, because the embryo interacts with the IGFs throughout the development (Watson et al., 1999). IGF-I and IGF-II expression can be detected in the blastocyst even before implantation. After implantation, the blastocyst again interacts with the IGF system either in the oviduct or uterus.

IGF-I is expressed in the endometrium and corpus luteum in cows. Treatment with GH does not change IGF-I mRNA levels in the bovine uterus, perhaps because GH decreases expression of the GH receptor in reproductive tissues (Kirby et al., 1996).

Uterine IGF-I expression was detected during the estrous cycle and early pregnancy, indicating that its expression may play a role in embryonic development and uterine function (Robinson et al., 2000). Expression of IGF-I, IGF-II, IGF1R, and IGFBPs differed in different regions of the uterus and at different stages of pregnancy, suggesting that these changes may have played a role in pregnancy and early development of the embryo. In rats, IGFBP3 expression was detected in the uterus, placenta, and fetus. IGFBP2 and IGFBP3 levels fluctuated as pregnancy
progressed (Cerro et al., 1997). IGFBP3 regulates availability of IGF-I, and, therefore, IGF-I may be important in the course of pregnancy in rats (Davenport et al., 1992). In pigs, IGF-I mRNA in the uterus was highest in early pregnancy (Tavakkol et al., 1988).

In the ovary, the two important cell types, theca and granulosa cells, secrete estrogen and are important in ovulation (Berne and Levy, 1993). After ovulation, debris removed from the ruptured follicle make a structure called the corpus luteum (CL). The CL mainly secretes progesterone that optimizes the implantation of the ovum and maintenance of the zygote if fertilization occurs (Millam, 1997). The CL consists of 80% granulosa and 20% theca cells. If fertilization and pregnancy do not occur, the CL regresses and converts to the corpus albicans, a scar tissue with no apparent function.

Immediately postpartum, IGF-I increases in cows with regular cycles, but not in cows with follicular cysts (Zulu et al., 2002). IGF-I is positively correlated with blood urea nitrogen, and, in animals with follicular cysts, estradiol concentration. IGF-I concentration is also negatively correlated with free fatty acids in cows. Therefore, serum IGF-I may be useful in predicting nutritional, as well as reproductive status, of cows.

In primates, IGF-I concentration increases before follicles enter the growth period. This does not seem to be the case in cows. However, before the late stages of follicular development, IGFBP3 concentrations decrease, resulting in increases in free
IGF-I. The free IGF-I increases sensitivity of the granulosa cells to follicle stimulating hormone in the ovary (Monget and Bondy, 2000). Therefore, IGF-I is important in the process of follicular development and ovulation in cattle.

Production of IGF-I, IGF-II, and IGFBPs in the ovary has been reported, suggesting local action of IGFs (Lucy, 2000). Growth promoting effects of circulating and locally-produced IGF-I on ovarian cell growth is synergistic. Therefore, effects of locally produced IGF-I in the ovary might be as important as those of circulating IGF-I. Ovine and bovine granulosa cells do not produce IGF-I in response to GH in vitro.

Direct injection of IGF-I into the ovary increased estradiol concentration in small follicles, but not in large follicles, in cattle (Spicer et al., 2000). The injection also did not change progesterone, IGFBP, or androstenedione levels. Similarly, GH injection in cattle changes only the number of small follicles (Lucy et al., 1999). Effects of GH on steroid hormone production by the ovary depends on the presence of IGF-I. In cultured bovine theca and granulosa cells, treatment with cortisol did not change progesterone and IGF-I synthesis, but decreased IGF1R levels in the ovary, suggesting that IGF1R mediates inhibitory effects of cortisol in ovarian function during stress (Spicer and Chamberlain, 1998). Atresia of the dominant follicle during bovine estrus induced by progesterone is associated with increases in IGFBPs and decreases in IGF-I and IGF-II in follicular fluid, suggesting that IGFs may be important in the atresia of the dominant follicle (Manikkam and Rajamahendran, 1997). Treatment with GH increases IGF-I and IGFBPs in the follicular fluid, but does not change plasma progesterone levels (Lucy et al., 1995).
IGF-I expression has been reported in the testis (Dombrowicz et al., 1992). IGF-I stimulates testicular development by inhibiting the aromatase enzyme activity, which results in decreased estrogen production in the testis (Rappaport and Smith, 1996). Injection of GH-deficient mice with IGF-I increases number of motile sperm cells, as well as number of sperm cells with normal morphology (Vickers et al., 1999). IGF-I has a non-linear relationship with scrotal circumference (Yilmaz et al., 1999). Bulls with very high or very low IGF-I concentration have smaller scrotal circumference than bulls with intermediate IGF-I.

Age of heifers at puberty: importance and hormonal regulation

Decreasing age at puberty is important, because it increases lifetime productivity of the heifer (Patterson et al., 1992). Age at puberty is defined as the age when estrus and ovulation first occur and the CL is maintained for a specific period of time. The first “quiet” ovulations usually occur without estrus. Colder climates delay puberty (Grass et al., 1982).

Breed influences age at puberty (Rodrigues et al., 2002). Heterosis influences age at puberty without interference from heterosis from weight (Martin et al., 1992). Photoperiod and nutrition have large influences on age at puberty in heifers (Schillo et al., 1992).
Circulating levels of gonadotropins increase at the onset of puberty. The increase in gonadotropin secretion is likely due to an increase in responsiveness of other hormones to Luteinizing Hormone-Releasing Hormone (LH-RH). The LH-RH regulates levels of gonadotropin secretion. In the cow, as long as there is an antral follicle in the ovary, increased gonadotropin levels cause increases in estrogen levels. The positive feedback of estrogen on its own release is first observed during puberty to ensure high levels of estrogen needed for ovulation. Before puberty, only the negative feedback of estrogen on the pituitary gland is observed. Administration of estrogen to prepubertal heifers increases circulating estrogen levels, indicating that the responsiveness to LH-RH is present before puberty. The number of receptors for 17β-estradiol in hypothalamus and hypophysis decrease before puberty in heifers, indicating the decreased sensitivity of the hypothalamus and hypophysis to 17β-estradiol (Day et al., 1987). The 17β-estradiol has an inhibitory effect on LH secretion. The LH concentration starts to increase, because the LH is now free of the inhibitory effects of the 17β-estradiol. LH starts the ovulation process by increasing follicle stimulating hormone levels. The other gonadotropins are very sensitive to the removal of the inhibitory effects of 17β-estradiol on LH pulses. Dietary restrictions prevent the preovulatory LH surge (Kurz et al., 1989).

Progesterone is released primarily by the corpus luteum, but is also released, in small amounts, by the adrenal glands and placenta. Progesterone is likely regulated by LH in domestic animals. Its actions are opposite to those of estrogen. It inhibits uterine contractions, increases the number of glandular cells in the uterus, and prepares the uterus for fetal development. It is important in the appearance of estrus
behavior. High levels of progesterone inhibit estrus behavior. Progesterone treatment inhibits the ovulatory LH surge and is used successfully in estrous synchronization in heifers (Funston et al., 2002) and in birth control in humans.

Melengesterol acetate is a synthetic progesterone that increases feed efficiency and growth by increasing production of estrogen in the ovary. Melengesterol acetate prevents the LH surge, and, therefore, ovulation, but it stimulates estrogen production by the ovaries, which results in increased growth of the heifer.

Garcia et al. (2002) measured leptin and IGF-I in heifers reaching puberty. Serum leptin concentration was second most predictive, after body weight, of onset of puberty. Mean serum IGF-I concentration during puberty was 21% higher than IGF-I concentration measured before reaching puberty, when IGF-I levels were grouped by week, because IGF-I concentration was too variable compared to leptin concentration. The authors reported large, but nonsignificant, coefficients for correlations of birth weight and leptin concentration with IGF-I concentration ($r = 0.55; P = 0.19$ in each case). Leptin concentration began to increase 17 wk before puberty and continued to increase linearly until puberty was attained. Simpson et al. (1991) concluded that IGF-I was one of the factors that initiated puberty in heifers. IGF-I mediates effects of LH on puberty (Srivastava et al., 1999) and inhibition of LH secretion by ethanol is mediated by IGF-I in rats (Hiney et al., 1998). IGF-I levels increase during puberty in humans (Hiney et al., 1991) and cattle (Jones et al., 1991; Bishop, 1991). In bulls, IGF-I and IGFBP3 levels increase and IGFBP2 levels decrease during puberty. Treatment with exogenous testosterone does not influence these changes (Renaville
et al., 1996). Body weight has an influence on age at puberty (Wolfe et al., 1990) and IGF-I concentrations are related to body weight (Davis and Simmen, 1997). In pigs, however, IGF-I concentration is not significantly correlated with age at puberty (Lamberson et al., 1995).

**Use of progesterone assay in the determination of age at puberty**

An increase in progesterone concentration during puberty occurs in females of many species, including humans and cattle. The increase is associated with physical changes that occur in females during puberty. In zoo animals, fecal progesterone concentration can be used for determination of age at puberty (Graham et al., 2002). In humans, as well as pigs and cows, progesterone concentration in the milk is an indicator of pregnancy (Pitcher et al., 1990).

Exogenous progesterone administration decreases concentration and pulse frequency of LH (Roberson et al., 1989) and induces attainment of puberty (Hall et al., 1997). Ability of progestins (i.e., synthetic progesterone) to induce puberty depends on nutritional status (Patterson et al., 1992) and age of the heifer. Hall et al. (1997) concluded that progestins induced puberty by accelerating natural cascades that lead to the onset of puberty. Although the mechanisms by which progesterone administration decrease LH concentration are not completely understood, it has been
suggested that neurotransmitters such as gamma-aminobutyric acid and opiates (Barb et al., 1986) may play a role. Roberson et al. (1989) reported that naloxone, an opiate antagonist, decreased LH secretion in pigs.

A heifer reaches puberty if the animal maintains an increase in blood serum progesterone concentration for a specific period of time (Honaramooz et al., 1999; Mejia et al., 1999). Collection of blood samples for progesterone assay can be weekly (Evans et al., 1992; Mejia et al., 1999) or bi-weekly (Bishop, 1991; Purvis et al., 1996; Lopez-Diaz et al., 1998; Wright et al., 2000; So et al., 2001). In blood samples collected weekly, puberty is attained when progesterone concentration exceeds 1 ng/mL in a single (Mejia et al., 1999) or two consecutive samples (Purvis et al., 1996). Several criteria, in addition to progesterone concentration, can be considered in determining age at puberty. Hall et al. (1995), Rekwot et al. (2000), and So et al. (2001) considered that a heifer attained puberty if she had a palpable corpus luteum, and showed estrus behavior, in addition to blood serum progesterone concentration higher than 1 ng/mL.

So et al. (2001) measured progesterone concentration in cattle fed either a restricted or standard diet. The authors reported delayed puberty in heifers fed the restricted diet. Progesterone assay was more sensitive, in determining onset of puberty, than monitoring of estrus behavior by an experienced herdsman. The age and weight at puberty were 344 d and 188 kg, respectively, when they used results from a progesterone assay to determine age at puberty. The age and weight were 433 d and 215 kg, respectively, when they used results from monitoring estrus behaviors, indicating that ovulation(s) had occurred without observable estrus behavior.
Another reason for the different results could have been that the estrus of some heifers was too short to detect. These results indicate that visual monitoring of estrus may be misleading, and that a progesterone assay is more reliable in determining age of heifers at puberty. The authors also used a progesterone assay to monitor luteal activity in postpartum cows. Eighty-two percent of standard-fed and 55% of restricted-fed cows had progesterone concentration greater than 1 ng/mL, whereas only 40% of cows in each group showed observable estrus behavior. The authors reported specific progesterone profiles associated with specific ovarian cycles, when they monitored ovaries and corpora lutea using ultrasonography. The authors concluded that plasma progesterone concentration and ultrasonography could be used to monitor ovarian activity and dysfunction.

Bishop (1991) determined age at puberty using a progesterone assay. The author measured progesterone concentration in serum samples collected bi-weekly from six paternal half-sib heifers from birth through 15 mo of age. With the exception of an increase of less than 1 ng/mL at 6 mo of age, progesterone concentration was less than 0.5 ng/mL and did not change until 13 mo of age, when a sharp increase was observed. Mean progesterone concentration exceeded 1 ng/mL by the age of 15 mo, but the increase was associated with a large standard error (i.e., > 2 ng/mL), indicating that, although the average progesterone concentration was greater or equal to 1 ng/mL, some of the heifers had high, whereas the others had low, progesterone concentration. The increase in progesterone concentration at 15 mo of age was associated with an increase in IGF-I concentration.
In lambs, progesterone concentration greater than 0.4 ng/mL was considered positive for attainment of puberty (Wright et al., 2002). The authors collected blood samples twice weekly and defined age at puberty as the age when progesterone concentration exceeded 0.4 ng/mL in two consecutive blood samples, followed by estrus behavior.

Lopez-Diaz et al. (1998) used ELISA and RIA to measure progesterone and estradiol-17β concentrations, respectively, in heifers infected with a parasite, F. Hepatica. They collected blood samples twice weekly, from 8 mo of age until estrus was observed two times. Serum estradiol concentration was higher, progesterone concentration was lower, and onset of puberty was delayed for 39 d in infected heifers. The authors concluded that infection with the parasite increased estrogen concentration, which decreased progesterone, and, consequently, caused a delay in reaching puberty.

Purvis et al. (1996) validated a commercially available, “Coat-A-Count” RIA kit (Diagnostic Products Corporation, Los Angeles, CA), to measure blood serum progesterone concentration in heifers treated with anthelmintics. Intra- and inter-assay coefficients of variation were 13.2 and 6.3%, respectively. The authors defined age at puberty as the age when progesterone concentration was greater than 1 ng/mL in two consecutive blood samples collected twice weekly. Heifers treated with anthelmintics reached puberty at a younger age.
Selection experiments involving IGF-I concentration

Divergent selection for blood serum IGF-I concentration has been successful in cattle (Davis and Simmen, 2000). The authors reported that Angus beef bulls with lower IGF-I concentrations had higher marbling scores and quality grades, but also had higher backfat thickness. The additive genetic correlation of marbling score with serum IGF-I concentration was -0.53. Direct heritability for mean IGF-I concentration was 0.42. Davis and Simmen (1997) reported genetic correlations of weaning and postweaning weights and weight gains with serum IGF-I concentration that ranged from -0.21 to -0.54, indicating that animals with lower IGF-I concentration also had greater weights and weight gains.

Mice selected for high blood plasma IGF-I concentration had greater liveweight (Blair et al., 1988). Siddiqui et al. (1992) reported that mice selected for high plasma IGF-I concentration had greater liveweight and weights of several tissues including the heart, brain, testes, pancreas, kidneys, lungs, and liver. Kroonsberg et al. (1989) reported heavier weights, but no difference in conception rate of female mice selected for high plasma IGF-I concentration. They reported greater weights and number of fetuses in high IGF-I line mice, but the difference could have been due to differences in maternal body weight and ability of the dam to provide nutrients for the fetus, rather than direct IGF-I selection line effects.

Blair et al. (2002) selected sheep for high or low blood plasma IGF-I concentration and reported larger birth weights in low IGF-I line sheep after five generations of selection. Medrano and Bradford (1991) measured IGF-I
concentration in sheep selected for high or low growth. Although the authors detected, on the average, higher IGF-I concentration in the high growth line, they did not recommend selection for plasma IGF-I concentration in sheep, because the concentrations were too variable compared to the other traits measured.

**MTDFREML methods in estimation of (co)variance components**

The variance of a sample is the amount of variation in a set of observations and equals sum of squared deviations from the mean divided by \( n - 1 \), where \( n \) is the number of observations. Additive variance is the variance of the breeding values. Breeding value is the sum of an individual’s genes that influence the trait of interest. The breeding value can also be defined as the value of the animal as a breeder. The breeding value of a dam is the deviation of the phenotypic values of her calves from the population mean multiplied by two, because the other half of the genes is transmitted through the sire. The breeding value is a random effect and the residual variance associated with its prediction increases as the difference between the observed and predicted values increases (Schaeffer, 1984). A bull can fertilize thousands of females if artificial insemination is used. Therefore, it is very important to estimate the breeding value of the bull as accurately as possible.

It is important to divide the phenotypic variance (i.e., the variance of the phenotypic values) into its components in order to identify the factors most responsible for the variation. The additive genetic portion of the phenotypic variation
is called heritability. Heritability can also be defined as the regression of the breeding values on total variance. There are two types of heritability: Broad sense heritability is the proportion of the phenotypic variance due to additive, dominance, and epistatic effects, whereas narrow sense heritability is defined as the proportion of the phenotypic variance exclusively due to the additive variance. Partitioning environmental (non-genetic) and genetic effects is rather difficult and may require use of highly inbred pure line progeny (Falconer, 1981). The additive variance is the most important of the variance components, because it indicates how much of the variation is due to resemblance among the relatives. Response of the population to selection is mostly determined by the additive variance (Falconer, 1981). Additive variance can be calculated using resemblance among the relatives. Additive variance is obtained by first calculating the squared deviations of the breeding values from the mean. The obtained value is then multiplied by genotypic frequencies and the values are summed across the generations. The term “additive” does not mean an additive effect and it does not imply anything about the mode of action of the genes. It only refers to the sum of the variance of the breeding values (Falconer, 1981).

The phenotypic variance is equal to the sum of the genetic and environmental variances and any interactions that may exist between the genotype and the environment. The environmental variance is also called residual (error) variance, because it decreases the precision of the estimates of the genetic variance. The manner in which carcasses are judged by eye is an example of an environmental type of variation. Age of the dam is also a cause of environmental variance, because an older dam may provide more milk to her progeny.
Twin studies can be employed to estimate variance components (Davis and Bishop, 1991) and heritability. Since identical twins are genetically identical, the resulting difference in performance is purely environmental, if the potential interactions between the genotype and environment are ignored. Subtracting environmental variance from the phenotypic variance gives the genetic variance and, thus, heritability can be obtained.

A mating scheme that involves full-sib matings can also be used to estimate broad sense heritability. First the phenotypic variance is calculated. Then a sib-mating scheme can be used to create a high degree of inbreeding within the population. The genetic effects on the differences in the performance of the individuals cancel each other out, because all animals have similar genetic material. The difference in phenotypic variance measured before and after inbreeding can be used to obtain the additive genetic variance. Dividing the additive genetic variance by the total variance gives the heritability estimate.

Heritabilities cannot be estimated easily and with great precision, because environmental effects such as season may differ from one population to another. Heritabilities may have large standard errors. Usually, the closer the relationship between the relatives, the greater is the accuracy of the heritability estimates. If the individuals are distantly related, the heritability estimate obtained must be multiplied by a number to obtain the true heritability. For example, if a sire model is used, then the resulting heritability is multiplied by 4 to obtain the true heritability, which decreases the accuracy.
Assortative matings and removing maternal effects may change heritability estimates. The inaccuracy of the heritability estimate due to assortative matings can be corrected using the phenotypic variance of the individuals. If the heritability is based on families, then the families can be given a weight depending on the family size.

Heritability can be computed using estimates of variance components, correlation, or regression methods (Van Vleck, 1995). Genetic, environmental, and phenotypic correlations may be obtained using covariances between parents and their offspring.

Log likelihood is a function of the variance. It is well accepted that restricted maximum likelihood (REML), a method that maximizes the log likelihood, is the method of choice to estimate (co)variance components in data sets that involve unbalanced data (Meyer, 1990). REML algorithms are iterative methods that create and modify mixed model equations.

Several models can be used to estimate heritability and (co)variance components, but the animal model is preferred over sire models. The animal model takes into account the breeding values of sire, dam, and calves, and, therefore, is more accurate than the other models. The animal model takes into account the animal’s own performance and the performance of relatives when calculating the breeding value of that animal. Thus, there is no need to wait to obtain performance values of the animal’s progeny. The animal’s breeding value can be obtained when the animal is still young. This increases the turnover in the herd, because younger sires can be used. The animal model also calculates the breeding value for the dam and adjusts
for assortative matings. Therefore, the contribution of dams and sires to a change in performance of the progeny can be calculated. Since the animal model can be used to calculate breeding values for all of the animals in the herd, it is possible to compare past and present performance of the same or different animals.

One way to estimate variance components is to use Analysis of Variance (ANOVA), but this method is not always easily applicable, because ANOVA estimation of variance components requires assumption of normality. The REML method, first described by Corbeil and Searle (1976), does not require assumption of normality. A difficulty associated with REML estimation is that a matrix with the size of $n$, $n$ being the number of observations, must be inverted. REML is based on estimating solutions for fixed effects using mixed models and then calculating the likelihood function of the $n$ residual contrasts, $n$ representing the error degrees of freedom for the fixed effects. The likelihood function of the residual contrasts is a function of the variance components (Steele and Torrie, 1997). The REML estimates for balanced data always equal $s^2$, where $s^2$ is the biased estimator of the population variance. Derivative-free REML involves finding the maximum likelihood without obtaining derivatives, expectations, or inverses (Graser et al., 1987).

Multiple-trait derivative-free restricted maximum likelihood (MTDFREML) is currently a method of choice for estimating variance components. MTDFREML is a statistical method with optimal properties. It consists of a set of computer programs used to obtain estimates of (co)variance components with derivative-free REML. The program can be used for analyses of single traits, multiple traits, and records that include traits with sex-limited expression (Boldman et al., 1995), such as scrotal
circumference for males and calving rate for females, in one data set using missing values for traits that cannot be measured. Breeding values, contrasts, expectations of solutions, and solutions for fixed effects can all be calculated using MTDFREML.

MTDFREML methodology has been used extensively in animal breeding. Using MTDFREML, Riley et al. (2002) concluded that sufficient variation existed in Brahman breeds to obtain genetic parameter estimates for carcass traits. Davis and Simmen (2000) used MTDFREML to obtain heritability estimates and estimates of correlations of IGF-I concentration with carcass traits. Heritability for mean IGF-I was 0.42. Moderate to high genetic correlations of carcass traits with IGF-I concentrations were obtained. Tonhati et al. (1999) used MTDFREML to obtain heritability and repeatability estimates for number of transferable embryos produced using superovulation. The heritability and repeatability estimates were 0.003 and 0.13, respectively. Van Tassell et al. (1996) compared mean estimates for genetic variances obtained using MTDFREML and a Bayesian-based Gibbs sampler for a multi-trait animal model. Very high correlation was found between the estimates (r = 0.99) obtained using the two methods. The authors argued that the likelihood approach of obtaining estimates of variance components was a more generalized Bayesian method of inference.
SDS-PAGE analysis of muscle proteins

The basic principle of electrophoresis is that charged molecules migrate to the electrode with the opposite charge. The particles migrate based on their size and charge and are separated into single fractions. Several factors influence the mobility of the charged molecules. These factors include the pK value of the charged groups on the molecule, the pH and type of the buffer used, and nature and concentration of the gel matrix, as well as the temperature. The gels can be used for quantitative purposes or to measure purity of the mixtures. Electrophoresis can be done for virtually any molecule with a charge. Such molecules include nucleic acids, proteins, whole cells, acids and bases, drugs, etc. When working with proteins, the pH of the buffer is especially important because proteins carry both positive and negative charges and the charges change depending on the pH of the buffer. In sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gels, the sample must be denatured and converted into micelles that can enter the gel matrix. The pH of the buffer generally is chosen to be high or low so that the sample migrates towards the positive or negative electrode. The buffer molecules also move from one pole to another as does the sample. The buffer must have minimal electrical ionic strength in order to generate as little heat as possible during electrophoresis, but at the same time, it must be in adequate volume so that the pH of the sample will not influence the pH of the system. Glycine, the simplest amino acid, is often used in running buffers.
N-N’ methylenebisacrylamide is added to polyacrylamide gels to minimize electroosmosis in the polyacrylamide gels. Sulphonate ions in the gel matrix become ionized if electric current is applied and try to migrate to the anode, but they cannot move, because they are stabilized in the gel matrix. This results in the flow of H³O⁺ ions towards the cathode that carries the soluble sample along with it. Although some techniques take advantage of this phenomenon, electroosmotic charges generally interfere with the separation of the protein bands and the gel matrix should not have this property.

N-N’ methylenebisacrylamide contains disulfide bonds that can be broken by treatment with thiol reagents, and, therefore, it is possible to solubilize the polyacrylamide gels after electrophoresis for protein purification purposes. Polyacrylamide gels can separate proteins that have molecular weights as low as 500 Da. Although the gels are generally used to separate proteins, they can also be used to separate nucleic acids for sequencing purposes. In several mutation detection methods, such as rapid amplification of polymorphic DNA (RAPD), single-stranded conformation polymorphism (SSCP), and denaturing gradient gel electrophoresis (DGGE), these gels are used. Use of polyacrylamide gels in mutation detection systems leads to much sharper bands (Bassam et al., 1991). However, migration of nucleic acids in polyacrylamide gels is partially influenced by the bases in the DNA, such that the AT-rich regions of the nucleic acids migrate more slowly. This effect is not observed in agarose gels, in which nucleic acids migrate based strictly on their size and not on their nucleotide composition.
SDS-PAGE gels are mostly of the discontinuous (DISC) type. DISC gels consist of two gels with different polyacrylamide concentration. The stacking (upper) gel is usually a 4% gel with a lower pH and the resolving (lower) gel is a 12% gel with similar composition except that it has a more alkaline pH. Proteins first migrate in the stacking gel based only on their charge, because the pores in the polyacrylamide gel are large. Proteins move quickly to the border of the resolving gel, which has a higher concentration of polyacrylamide and smaller pore size. Glycine, a molecule in the running buffer, which leads the proteins to the resolving gel, is not influenced by the pH changes nor the size of the pores in the resolving gel. The glycine molecules move quickly through the resolving gel ahead of the proteins. Because the resolving gel has a higher pH and smaller pores than the stacking gel, proteins start to move slowly and become charged as they enter the resolving gel. The charge and the slower speed of the protein molecules result in sharper protein bands.

In SDS-PAGE electrophoresis, the movement of the protein micelles is based strictly on molecular size. Charges of the proteins, as well as secondary and tertiary structures, are lost as the protein unfolds, because hydrogen bonds are broken due to the SDS content. A constant quantity of 1.4 g SDS binds 1 g of protein. Disulfide bonds are broken using reducing agents such as β-mercaptoethanol or dithiothreitol (DTT). Schagger and Von Jagow (1987) reported a method that resulted in improvement of conventional SDS-PAGE gels. Their gel detected proteins as small as 100 Da molecular weight. They used tricine rather than glycine
in the running buffer and increased molarity of the buffer. They also used a third gel as an additional resolving gel. Coomassie blue staining of SDS-PAGE gels is very sensitive and can detect peptides as short as 10 to 15 amino acids in length.

All of the micelles produced when running SDS-PAGE gels are negatively charged; the negative charge depends on the molecular size. Radius of the micelles depends on the molecular mass of the protein, resulting in separation strictly based on molecular weight. Boiling does not fragment the proteins, because they form micelles and are already reduced. It is not desirable, however, to heat the DTT, because heating may cause oxidation of the chemical.

**Basic structure and function of muscle proteins**

The sarcomere, the basic contractile unit of muscle tissue, has a complex structure involving many proteins. Myosin forms the thick filaments of skeletal muscle that slide over actin filaments. Actin and myosin are both contractile proteins. The head of the myosin molecule binds actin and the neck of the myosin can move, which causes the sarcomere to shrink and the muscle to contract. Myosin isoforms do not seem to play an important role during muscle hypertrophy in humans, but their ATPase activity differs (Mercadier et al., 1983).

Actin provides binding sites for the myosin heads to attach. Contraction is achieved by myosin heads that change conformation upon binding the actin filaments. Actin is resistant to degradation. Phillips et al. (2000) reported that actin and myosin were not digested by metalloproteases, enzymes that were used to tenderize meat. If
the enzyme digestion causes extensive myofibrillar damage, then the texture of the meat becomes undesirable. Metalloproteases are enzymes purified from bovine placenta. They specifically cleave collagen and gelatin without causing extensive damage to actin or myosin. Therefore, they can be used to tenderize beef.

Titin is a string-like molecule that extends from the thick filaments to the Z disc. It is thought to act like a spring to keep the myosin thick filaments centered in the sarcomere. Uytterhaegen et al. (1994) reported that more titin was observed in degrading myofibrils upon injection of serine proteases into the beef to increase meat tenderness, indicating that titins may play a role in beef tenderness.

Nebulin extends from one end of actin filaments to the other end. It acts as a "molecular ruler" that regulates the actin filament assembly and length of actin filaments during myogenesis. Nebulin is an elastic protein. Disappearance of nebulin on SDS-PAGE gels of longissimus muscle samples is generally associated with increased tenderness (Huff-Lonergan et al., 1995).

Tropomyosin is a negatively charged, rod-like protein (Kay et al., 1982). It is a regulatory protein that binds both actin and tropomyosin. Together with troponin, it mediates skeletal muscle contraction regulated by Ca++.

Troponin is a regulatory protein that binds both actin and tropomyosin. Troponin is the name of a protein complex with three subunits: troponin T, troponin C, and troponin I. Troponin T binds tropomyosin and is thought to play a role in positioning the complex in the thin filament. In the presence of troponin T and tropomyosin, troponin I binds actin and inhibits interactions between actin and myosin in the presence of Ca++ ions. Troponin C binds Ca++. It is closely related to
calmodulin, which mediates Ca\(^{++}\)-related signals in most cells. Grabarek et al. (1992) reported that troponin C in fast and slow twitch muscles had different isoforms. Troponin C has two sites that bind Ca\(^{++}\). Both of the sites must bind Ca\(^{++}\) for muscle contraction to occur.

The 32 kDa protein is a degradation product of troponin T (Ho et al., 1994) that appears in tender, but not in tough, meat samples (Huff-Lonergan et al., 1995). It is not clear why this band appears only in tender meat samples. Several explanations, including defects in the formation of actomyosin, interruption in calcium regulation, and migration of troponin T molecules into the Z-line, have been suggested to explain this phenomenon (Basu, 2000).

Myomesin and creatine kinase are proteins associated with the M-line of the thick filament. Myomesin is of two types: myomesin 1 and myomesin 2. The proteins have approximately 165 kDa molecular weight. Some of the human satellite cells that differentiate into myofibrils do not have myomesin (Van Der Ven and Furst, 1997), indicating that myomesin is not essential for myogenesis. Myomesin binds the head of titin and pushes it into the M line. Therefore, it plays an important role in the myofibrillar integrity of bovine muscle (Vinkemeier et al., 1993). The C-protein plays a role in binding myosin heavy chains to each other.

Several molecular models are used to study muscle structure and function. In the presence of ATP, actin and myosin bind each other and form a network called “superprecipitant”. Superprecipitation is a phenomenon utilized to study binding of myosin to actin in the presence of other muscle proteins such as troponin, tropomyosin, and alpha-actinin. The actual formation occurs when all ATP in the
environment is used up. Superprecipitation occurs with or without Ca\textsuperscript{++}. The speed of formation of the superprecipitant can be modulated by Ca\textsuperscript{++} concentration, if tropomyosin is present in the environment.

Formation of arrowhead structure is a phenomenon that can be utilized to study important events such as muscle contraction, formation of cross-bridges, and functions of myosin light chains, and to study sites at which actin and myosin contact each other. Arrowhead formation is observed when S1 portions of the myosin molecules interact with the actin filaments and the mixture is negatively stained. The S1 subunits of myosin always point away from the Z-line, resulting in the appearance of an arrowhead structure.

Contraction of skeletal muscle begins when extracellular Ca\textsuperscript{++} enters the cell, changing electrical charge of the membrane. The change in the charge of the membrane results in release of Ca\textsuperscript{++} stored in the sarcoplasmic reticulum into the cell. The Ca\textsuperscript{++} then binds troponin C, which then passes the information to tropomyosin. Tropomyosin undergoes conformational changes to allow myosin and actin to contact each other. Muscle contraction occurs when myosin molecules undergo conformational changes, which result in movement of the actin molecules. ATP is necessary for the contraction process. The rate of ATP hydrolysis in muscle is much higher during exercise than during the relaxed state, because more rapid cycling of cross-bridges consumes more ATP during exercise. Fast muscles have higher ATPase activity and faster shortening velocity than slow muscles.
ATP is stored in the form of creatine phosphate. If the creatine phosphate is used up, there will be no ATP available for hydrolysis to separate myosin heads attached to the actin molecules. The heads of the myosin molecules remain attached to the actin filament, making it harder to isolate muscle proteins. Adenosine triphosphate must be added during the isolation process to release myosin heads from the actin filaments. Thus, fresh meat should be used to purify muscle proteins.

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CHAPTER 3

(CO)VARIANCE COMPONENT ESTIMATION FOR REPRODUCTIVE TRAITS IN ANGUS BEEF CATTLE DIVERGENTLY SELECTED FOR BLOOD SERUM IGF-I CONCENTRATION

Introduction

Economic principles applied to livestock production show that reproductive traits are four times more important than production traits (Melton, 1995). Number of calves weaned per cow mated is of great economic importance in beef cattle herds. Therefore, it is desirable to increase calving rate in a herd. It is also desirable to reduce age of heifers at first calving, since this will increase lifetime productivity of the animal (Patterson et al., 1992). Male reproductive traits are also an important component of programs that aim to improve reproductive efficiency, because they have influence on both male and female reproductive traits. Heritability estimates that have been reported for reproductive traits in recent years are generally larger than ones reported previously. The increased heritability estimates could be due to improvement in the statistical procedures used to estimate heritability (Evans et al., 1999). Scrotal circumference is an easily measured characteristic and provides a rapid method of reproductive improvement in a herd (Keeton et al., 1996) due to its
effects on fertility of both male and female progeny. Christmas et al. (2001) reported that the genetic correlations of scrotal circumference with motility and percent abnormal sperm cells were 0.56 and -0.25, respectively. Meyer et al. (1991) reported a genetic correlation of -0.28 between scrotal circumference and days to calving in Angus cattle, indicating that progeny of bulls with larger scrotal circumference calved earlier. Evans et al. (1999) reported a favorable correlation between scrotal circumference and age of heifers at puberty.

Due to its influence on reproductive functions of animals, serum insulin-like growth factor I (IGF-I) concentration is an important candidate for selection to improve reproductive efficiency. IGF-I has been isolated from components of the male and female reproductive tract including the testis (Dombrowicz et al., 1992), ovary, uterus, amniotic fluid, and embryonic (Lee et al., 1990) and fetal (Hill et al., 1983) tissues. Expression of IGF-I in pig endometrium (Simmen et al., 1992) and of IGF-I receptor in cattle (Robinson et al., 2002) has been detected in the uterus during the estrous cycle and early pregnancy, indicating a role for IGF-I in the regulation of uterine function and fetal development. IGF-I has been suggested to play a role in fertilization (Roudebush et al., 2001), and in regulation of bovine (Woad et al., 2000) and sheep (Leeuwenberg et al., 1995) ovarian function. It has been postulated to mediate communication between fetal and maternal tissues and has been suggested to be involved in the initiation, development, and maintenance of pregnancy (Simmen et al., 1993).
A significant correlation exists between IGF-I concentration in seminal plasma and percentage of morphologically normal spermatozoa, sperm concentration, and maturation, in humans (Glander et al., 1996). Knights et al. (1984) reported low heritability estimates for semen traits that ranged from 0 to 0.24. The genetic and phenotypic correlations between scrotal circumference and semen traits were low and moderate, respectively. On the other hand, genetic and phenotypic correlations among semen traits were high and ranged from 0.49 to 1.11. Correlations between semen and growth traits were low (i.e., 0.08 or less).

Kroonsberg et al. (1989) found no difference in conception rates of mice divergently selected on the basis of blood plasma IGF-I concentration, although high line females produced significantly larger litters. Davis and Bishop (1991) detected earlier calving dates in identical twin heifers with high serum IGF-I concentration.

If the genetic correlation between IGF-I concentration and reproductive traits is high, then it may be possible to increase rate of genetic gain in reproductive traits as a correlated response to IGF-I selection. The objectives of this study were, therefore, to obtain heritabilities and genetic, environmental, and phenotypic correlations of IGF-I concentration with scrotal circumference, percentage of morphologically normal spermatozoa, percent motile sperm cells, calving rate, and age of heifers at first calving.
Materials and Methods

Selection Procedures. Divergent selection for blood serum IGF-I concentration was initiated in 1989 using 100 spring-calving (50 high line and 50 low line) and in 1990 using 100 fall-calving (50 high line and 50 low line) purebred Angus cows with unknown IGF-I levels located at the Eastern Ohio Resource Development Center (EORDC). Selection was based on the mean IGF-I concentration of three blood samples taken at d 28, 42, and 56 of the 140-d postweaning test, which are abbreviated as $\text{IGF28}$, $\text{IGF42}$, and $\text{IGF56}$, respectively. Detailed selection procedures are described elsewhere (Davis et al., 1995).

Management Procedures. The spring breeding season was approximately 60 d in length, whereas the fall breeding season was approximately 45 d in length prior to 1994. Beginning in 1994, the fall breeding season was extended to 60 d. Spring-born calves were reared by their dams until weaning at 7 mo of age. During an adjustment period of approximately 3 wk and a 140-d postweaning test period, bull calves were fed a corn-soybean meal based concentrate diet and, from 1989 through 1993, heifer calves were fed corn silage. Bulls were kept at EORDC throughout the study and, from 1989 through 1993, heifers were transferred to the North Appalachian Experimental Watershed, Coshocton, OH, for the postweaning test. Beginning in 1994, heifers remained at EORDC and were fed a corn-soybean meal concentrate diet formulated to yield gains of approximately 0.7 kg/d. Bulls and heifers were given ad libitum access to feed.
Fall-born calves were fed a growing diet that was designed to yield gains of 0.9 kg/d during a 112-d growing period in drylot after weaning at approximately 140 d of age. Following the growing period, bulls were kept at EORDC and, from 1989 through 1993, heifers were transferred to Coshocton, OH. Heifers were generally fed corn silage at Coshocton. However, due to unavailability of corn silage, a corn-soybean meal-based concentrate diet was used for feeding of fall 1990 heifers beginning between d 42 and 56 of the postweaning test and for feeding of fall 1992 heifers beginning between d 84 and 112. Heifers born in fall 1991 were fed a corn-soybean meal concentrate diet during the entire postweaning test period due to drought conditions and unavailability of corn silage. Beginning in 1994, fall-born heifers remained at EORDC following weaning and were fed the same diet as spring-born heifers.

Serum Samples. Approximately 25 mL of blood was collected into sterile glass tubes at d 28, 42, and 56 of the postweaning test, allowed to clot for 24 h at 4°C, and centrifuged. Serum was drawn off and frozen at –20°C until it was assayed.

RIA for IGF-I. The RIA for IGF-I was performed in R.C.M. Simmen's laboratory at the University of Florida using antiserum raised against human IGF-I in rabbits (UBK487), following previously described procedures (Bishop et al., 1989).

Breeding Soundness Examinations. Breeding soundness exams (BSE) were performed by Ohio Agricultural Research and Development Center (OARDC) veterinarians immediately following the postweaning test, on bulls that were approximately 12 to 14 mo old. Before 1995, breeding soundness exams were performed only on bulls that were saved for breeding purposes. The exams were
performed on all bulls born in or after 1995. The number of observations for each of the male reproductive traits differed, because the ejaculate collected from some of the bulls was not of sufficient quantity or quality to allow accurate measurement. Data in this study were collected from bulls born in 1990 through 2001.

The ejaculate was collected by inserting a lubricated electroejaculator probe into the rectum and pushing it down to stimulate the prostate gland and seminal vesicles. The semen was collected into conical tubes and placed in a warm bath immediately after ejaculation. A drop of the semen was placed on a glass slide and covered by a second slide. The number of forward swimming sperm cells out of 100 cells was counted under the microscope and recorded. The same procedure was followed to count percent normal sperm cells except that cells were stained using a mixture of eusin and nigrosin stains. A drop of semen was placed on a microscope slide. The drop was pulled using a second slide on the first slide to make a thin smear. The smear was then mixed with a drop of the stain and number of normal sperm cells out of 100 cells counted under the microscope. Sperm cells with tail or head defects, acrosomal abnormalities, abnormal head shape, or coiled, rudimentary or broken tails were considered abnormal. Scrotal circumference was measured by pulling both testes down into the scrotum with thumbs and fingers without separating the two testes. A special metal tape was used to measure the largest diameter of the testes.

Statistical Analyses. All data were analyzed using the General Linear Models Procedure (PROC GLM) of the Statistical Analysis System (SAS) (SAS Inst. Inc., Cary, NC) and a set of Multiple-Trait, Derivative-Free Restricted Maximum Likelihood (MTDFREML) programs written by Boldman et al. (1995). Traits
analyzed in this study included IGF28, IGF42, IGF56, and mean IGF-I, calving rate, age of heifers at first calving, scrotal circumference, and percent normal and motile sperm cells. First, the fixed effects and covariates were tested for significance using SAS and only effects with a significance level of less than 0.05 were included in the MTDFREML analysis. Fixed effects that were tested for significance included birth year, line, season of birth, age of dam, sex, and mating number. This approach has been reported in the literature in order to remove nonsignificant fixed effects from the MTDFREML analyses (Crews and Kemp, 2001; Devitt and Wilton, 2001). The variable “mating number” was created to track the sequence of matings and used only in the analysis of calving rate. The mating number was 1 for the first mating of a female, 2 for the second mating, etc. On-test age of calf was used as a linear covariate in the analysis of male reproductive data, age of heifers at first calving, and IGF-I concentration. Sex was deleted from the models when reproductive data were analyzed, because all of the traits were available only on either males or females.

Pedigrees of base population animals were traced back three generations to create the numerator relationship matrix. Total number of animals in the numerator relationship matrix, including base animals, was 2,864, of which 1,861 were inbred. The average inbreeding coefficient was 0.03. All of the traits were first analyzed using a full animal model that included direct genetic effects, maternal genetic effects, permanent environmental effect of the dam, and the covariance between direct and maternal genetic effects. The full animal model also included all of the significant fixed effects as determined in the SAS analysis. The analysis was
repeated using a reduced model with no maternal genetic or permanent environmental effects, because these effects contributed less than 21 percent of the total variance.

In a second analysis, IGF-I values, along with reproductive traits, were included in bivariate analyses to estimate direct genetic ($r_{A1A2}$), environmental ($r_{E1E2}$), and phenotypic ($r_{P1P2}$) correlations between IGF-I measurements and the reproductive traits.

In all analyses, it was assumed that the convergence criterion was attained if $-2 \log$ likelihood of the simplex algorithm was less than $10^{-9}$.

**Results and Discussion**

Simple statistics for the traits analyzed in this study are summarized in Table 3.1. Results from the PROC GLM analysis of fixed effects used in this study are shown in Table 3.2. Percent normal and motile sperm cells were not influenced by IGF-I selection line, season, or age of dam. Effect of IGF-I selection line on calving rate also was not significant. Season effects on calving rate were significant only after “mating number” was added to the model as a fixed effect. Effects of age of dam on scrotal circumference and IGF56 were significant. The remaining effects were not significant. Effects that had a significance level of less than 0.05 were included in the final models to obtain estimates shown in Tables 3.3, 3.4, and 3.5.
Presented in Table 3.3 are estimates of direct ($h_d^2$) and maternal ($h_m^2$) heritability, the proportion of phenotypic variance due to permanent environmental effect of the dam ($c^2$), and the correlation between direct and maternal genetic effects ($r_{am}$). Estimates of $h_m^2$ and $c^2$ were small (<0.21) for all of the traits, except that $h_m^2$ and $c^2$ for age of heifers at first calving were 0.77 and 0.28, respectively, when a full animal model was used. Davis and Simmen (2000) also reported $c^2$ and $h_m^2$ estimates that were less than 0.20 for IGF-I measurements in the same herd. In addition, the authors estimated direct heritability for IGF28, IGF42, IGF56, and mean IGF-I as 0.32, 0.59, 0.31, and 0.42, respectively. These results generally agree with our estimates. The observed differences in heritability estimates may be due to the larger sample size in the current study. Another reason could be that the authors did not exclude nonsignificant fixed effects from the models before performing MTDFREML analyses. Sample size and mean IGF-I concentration were 1,283 and 225.8 ng/mL in the study by Davis and Simmen (2000) compared to 1,848 and 238.8 ng/mL in our study. The minimum and maximum values, as well as maternal heritability and $c^2$ for IGF-I measurements, did not differ between the two studies. Proportion of phenotypic variance due to permanent environmental effect of the dam was zero in both studies for all of the measures of IGF-I.

In the current study, estimates of $h_d^2$, $h_m^2$, and $c^2$ for age of heifers at first calving were unusually large (i.e., 1.00, 0.77, and 0.28, respectively). The heritability of 1.00 for age at first calving was likely due to small sample size. The heritability of
age at first calving decreased from 1.00 to 0.26 when the reduced model was employed. The correlation between direct and maternal genetic effects was negative for all traits analyzed and ranged from -0.40 to -1.00.

Summarized in Table 3.4 are direct heritability estimates obtained using the reduced model (i.e., maternal genetic and maternal permanent environmental effects removed from the model), as well as comparison of our heritability estimates with estimates previously reported in the literature. The heritability estimates obtained in our study agree well with previous estimates. With the exception of the estimates for IGF-I measurements and age at first calving, using the reduced model in place of the full animal model resulted in only small differences in direct heritability estimates. Estimates for mean IGF-I decreased from 0.50 to 0.32 when the reduced model was employed. The reason for the decrease may be that maternal genetic effects for IGF-I measurements were relatively higher, with the exception of age at first calving, than was observed for the reproductive traits.

Genetic, phenotypic, and environmental correlations of IGF-I concentration with reproductive traits are shown in Table 3.9. Genetic correlations of percent normal sperm cells with IGF28 and IGF42, as well as environmental correlations of scrotal circumference with IGF42 and IGF56 were negative, but small. With the exception of environmental and phenotypic correlations with IGF28, all of the correlations of IGF-I measurements with age at first calving were negative. The remaining correlations were positive.
Promising correlations of IGF-I measurements with scrotal circumference, percent sperm motility, and age at first calving were obtained. Genetic correlations of IGF28, IGF42, IGF56, and mean IGF-I with scrotal circumference were 0.24, 0.32, 0.26, and 0.35, respectively. Moderate to high genetic correlations of IGF28, IGF42, IGF56, and mean IGF-I with percent sperm motility were obtained. Important negative genetic correlations of IGF-I measurements with age of heifers at first calving were also found. Therefore, the genetic influence on IGF-I concentrations would also have an “overlapping” genetic effect on these traits. With the exceptions of environmental and phenotypic correlations of IGF42 and IGF56 with age at first calving, all environmental and phenotypic correlations were small (<0.20). Thus, phenotypic changes in IGF-I concentrations are not associated with phenotypic changes in the other traits analyzed. Phenotypic correlations of IGF-I measurements with scrotal circumference and percent sperm motility were small, as expected, due to small environmental correlations. However, it is important to note that these small phenotypic correlations were also associated with moderate genetic correlations. Although IGF-I measurements did not have high phenotypic correlations with scrotal circumference and percent sperm motility, IGF-I actually shares a large common genetic source of variation with these two traits.

The correlations of IGF28, IGF42, IGF56, and mean IGF-I with a particular trait were generally similar, except that environmental correlations of IGF-I measurements with scrotal circumference did not seem to follow the same pattern
across the various measures of IGF-I. The environmental correlations of IGF-I measurements with scrotal circumference were 0.12, -0.03, -0.09, and 0.01, respectively.

Genetic, phenotypic, and environmental correlations of IGF-I concentration with reproductive traits included in this study have not been reported in the literature.

Presence of IGF-I secretion and action in both male and female reproductive tracts has been well documented. Significant correlations of seminal plasma IGF-I with sperm motility (Breier et al., 1996) and percent normal sperm cells (Glander et al., 1996) have been reported in rats and humans, respectively. Yilmaz et al. (1999) reported significant non-linear relationships between blood serum IGF-I concentration and scrotal circumference and semen characteristics in bulls. The authors concluded that once the blood serum IGF-I concentration of a bull is determined, the semen characteristics of that animal can be estimated. The current study provides further evidence that selection for increased blood serum IGF-I concentration results in improvement of reproductive traits in Angus beef cattle.

**Calving rate analysis**

Analysis of calving rate required creating new data sets so that a repeated measures approach could be used. The cow, rather than the calf, was treated as the “animal” in the animal models to create mixed model equations, because calving rate was a trait of the cow. The “mating number”, a sequential number created to track
the matings, was added to the model as a fixed effect to utilize the repeated measures approach. IGF-I concentration, on the other hand, was not a repeated measurement. For example, a cow could have only one IGF28 measurement, but more than one mating, in her lifetime.

First, data set A, in which identical IGF-I measurements were entered repeatedly as many times as an animal was mated, was created (Table 3.5). The heritability for calving rate, using this data set, was 0.11, which agreed well with the estimates reported in the literature. Evans et al. (1999) and Doyle et al. (1996) reported heritability estimates of 0.13 and 0.26, respectively, for heifer pregnancy rate. However, in our study, estimates obtained for genetic correlations of calving rate with IGF-I measurements converged to 1.00.

**Approaches used to find cause of genetic correlation of 1.00 obtained using data set A**

*Single trait analysis.* Heritability estimates for calving rate and IGF-I concentrations in single trait analyses, using a full model animal model, were 0.13 and 1.00, respectively, when data set A was used. Birth year, IGF-I selection line, age of dam, and sex of the animal were included as fixed effects in the analysis of IGF-I measurements. On-test age of calf was also added as a covariate. Age of dam and “mating number” were included as fixed effects for calving rate. Uncorrelated random effects, as well as maternal genetic and permanent environmental effects, were deleted from the model to remove possible confounding between direct and
maternal genetic effects. This “reduced” model resulted in a direct heritability estimate of 0.11 for calving rate and 1.00 for IGF-I measurements.

Two-trait analysis. A two-trait analysis, which included IGF-I measurements and calving rate as the dependent variables in a “reduced” model, was used to estimate the correlations between the two traits. All of the effects used in the single trait analysis were included in the two-trait analysis. The direct heritability was 0.05 and 1.00 for calving rate and all IGF-I measurements, respectively, when data set A was used. Genetic correlations of calving rate with IGF-I measurements converged to 1.00.

Standardization of the variance for calving rate. Likelihood estimates of correlations between the traits are based on variance of both traits (Van Vleck, 2002). The calving rate data were originally entered as either 1 (if conceived) or 2 (if not conceived). The standard deviations for IGF28 and calving rate were 142.6 ng/mL and 0.42%, respectively, when this approach was used. To make the variances more similar, 1 (if conceived) and 100 (if not conceived) were entered for calving rate. The standard deviation became 41.5 for calving rate after making this change. Neither single-trait nor two-trait analyses using standardized variances resulted in reasonable heritability estimates for IGF-I measurements.

Deleting years of data to remove the “undetermined” effects. Data were deleted one year at a time starting with the year 1989 to remove potential founder effects that might have existed in early years of the experiment. The data were analyzed using a single-trait model. The heritability of 1.00 for IGF-I remained unchanged even after deleting 8 yr of data (out of a total of 12 yr).
Deleting “Line” from the model and combining “Year” and “Sex” into one variable. In order to remove any confounding that might have existed between year and sex, these two variables were combined into one variable and the IGF-I line effect was dropped from the model. This approach again resulted in a heritability estimate of 1.00 for the IGF-I measurements.

Sire model. In an attempt to determine whether the problem was related to the sire of the animals, we used a two-trait-sire model to obtain genetic and phenotypic correlations. Zeros were inserted into the columns for sires and dams. The sire was treated as the “animal” in the model. This resulted in a sire model with no relationships between the sires. Number of sires was 348. The heritability for mean IGF-I and calving rate was 1.22 and 0.20, respectively. Although the heritability estimate for IGF28 concentration was not reasonable, a genetic correlation of -0.39 was obtained between IGF28 and calving rate.

Partitioning IGF-I values based on sex. To find out whether IGF-I concentrations were confounded with sex, male and female IGF-I concentrations were analyzed separately. In this analysis, the heritability for mean IGF-I was 0.32 for males, which agreed with the previous estimates for the heritability of IGF-I when both males and females were included in the data set. The heritability of IGF-I measurements of females, however, was 1.00.

Including only the first matings. To test whether repeated measures were the cause of unreasonable heritability estimates for IGF-I, only the matings that involved heifers were included in a revised data set. A heritability of 0.32 was obtained for IGF-I measurements in females.
It was concluded that entering identical IGF-I values many times for the same animal resulted in a heritability estimate of 1.00, because the heritability decreased to 0.32 when all but the first matings were deleted. Entering identical IGF-I concentration for the same animal many times could have resulted in an increase in repeatability, and, therefore, heritability for IGF-I in females.

**Other approaches used to obtain genetic correlations of calving rate with IGF-I concentration**

Data set B was created by entering an IGF-I measurement only for the first mating and missing values for the remaining matings (Table 3.6). IGF-I concentration was entered once per animal, because it was not a repeated measurement and entering identical IGF-I concentration many times for the same animal resulted in a heritability estimate of 1.00. In this data set, a cow had only one IGF-I measurement, but could have more than one mating. This model allowed estimation of genetic correlations, but it ignored environmental correlations for matings other than the first. Genetic correlations between IGF-I measurements and calving rate for matings other than the first could still be obtained using data set B, even though an animal would not necessarily have data for both IGF-I and calving rate. In matings other than the first, a cow had a breeding value for the IGF-I
measurement even though she had a missing value for that measurement. This is because the relationship matrix tied the two traits together through sires that had progeny with records for both of the traits.

Genetic correlation between two traits that cannot be measured on the same animal, such as scrotal circumference and age at first calving, can be obtained. A bull can have a breeding value for milk production, because the bull passes on half of his genes to his daughters. Although he cannot produce milk, he has a genetic influence on milk production of his daughters, and, therefore, an estimated breeding value for milk production. The mixed model equations in the MTDFREML approach are augmented such that mixed model equations are created for animals without records. Unlike genetic correlations, environmental correlations cannot be obtained if at least one of the traits has missing values. An explanation for this situation is that a bull would transmit half of his genes to his daughters; so, he can have an estimated breeding value for milk production. However, he cannot have an estimated value for the environmental covariance, because he cannot transmit any environmental effects to his progeny. Therefore, a mixed model equation cannot be created for a missing environmental value. Genetic correlations depend on what is being estimated, rather than on what is being measured. Environmental correlations, on the other hand, depend on what is being measured.

The minimum function values have a chi-square distribution (Van Tassell et al., 1996) and the difference between the values from two different runs can be used to compare models. The degrees of freedom is the difference in the number of variance components. Adding or deleting the environmental covariance would make
a difference of “1” in the number of variance components. Thus, a chi-square test
with a single degree of freedom was used to test whether the minimum function
values obtained from the two runs (i.e., one with zero and the other with a non-zero
starting value for environmental covariance) were significantly different from each
other. For example, using data set C, which was identical to data set B except that
matings other than the first were deleted, the difference in minimum function values
obtained from the analyses using zero and non-zero starting values for environmental
covariances was 23,807.67 – 23,803.16 = 4.51 (Table 3.8). This difference was
significant, because 4.51 exceeded the critical chi-square value of 3.84 at a 0.05
significance level with a single degree of freedom. Therefore, the minimum function
values obtained from these two analyses were different from each other.

Using data set B and a zero or non-zero starting value for the environmental
covariance in the first and second runs resulted in identical minimum function values
(Table 3.8), indicating that environmental correlations of calving rate with IGF-I
measurements were not important when all of the matings were included in the data
set. Using data set C, on the other hand, resulted in minimum function values that
were significantly different from each other. The reason for obtaining different
results when data sets B and C were used remains to be elucidated.

Moderate genetic correlations of calving rate with IGF-I measurements were
obtained when data set B was used (Table 3.9). The genetic correlations ranged from
-0.41 to -0.48, indicating that cows with high breeding values for IGF-I
measurements also had high breeding values for calving rate. Entering calving rate as
either 1 (if conceived) or 100 (if not conceived) resulted in a negative genetic
correlation between calving rate and IGF-I measurements, but the negative
correlation indicated that calving rate increased as IGF-I concentration increased.
The environmental and phenotypic correlations of calving rate with IGF-I
measurements were close to zero and ranged from 0.00 to –0.08. These results
indicate that, although observed values for IGF-I measurements are not
phenotypically correlated with calving rate, calving rate can be changed rapidly by
selecting cows for lower IGF-I concentration. This rapid increase is, however,
prevented by large environmental effects on both of the traits. The environmental
effects on IGF-I measurements and calving rate are not correlated.

It is interesting to note that the genetic correlation of calving rate with mean
IGF-I concentration was -0.40 when a sire model was used with data set A. The
animal model using data set B resulted in a genetic correlation -0.41. Similarity of
these results may indicate that using the sire model could have solved the problem
due to entering identical IGF-I measurements many times in the data set. In sire
models, a mixed model equation is created per sire rather than per animal.

Implications

Results from this study indicate that including IGF-I measurements in
selection programs that aim to improve reproductive traits may increase rate of
genetic gain. In addition, these results further support the findings that IGF-I
influences reproductive traits of both bulls and heifers.
<table>
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<th>Trait</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV,%</th>
<th>Minimum</th>
<th>Maximum</th>
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</thead>
<tbody>
<tr>
<td>IGF28, ng/mL</td>
<td>1,787</td>
<td>232.2</td>
<td>158.9</td>
<td>68.5</td>
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<td>1,718</td>
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<td>173.1</td>
<td>66.8</td>
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<td>IGF56, ng/mL</td>
<td>1,792</td>
<td>252.7</td>
<td>174.6</td>
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<td>Mean IGF-I, ng/mL</td>
<td>1,848</td>
<td>238.8</td>
<td>164.9</td>
<td>69.1</td>
<td>4.1</td>
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<td>Scrotal circumference, cm</td>
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<td>47</td>
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<td>Sperm motility, %</td>
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<td>18.2</td>
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<td>Normal sperm cells, %</td>
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<td>794</td>
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Table 3.1. Means, standard deviations (SD), coefficients of variation (CV), and minimum and maximum values for serum IGF-I concentration and reproductive traits
<table>
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<th>IGF28</th>
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<th>PNSC</th>
<th>PMSC</th>
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<td>0.0006</td>
<td>0.009</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.03</td>
<td>0.003</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) Effects that had a P value of less than 0.05 were considered significant and were included in the MTDFREML analysis.

\( ^b \) Abbreviations: SC: scrotal circumference, PNSC: percent normal sperm cells, PMSC: percent motile sperm cells, CR: calving rate, AFC: age at first calving

Table 3.2. Levels of significance for fixed effects used in the analysis of serum IGF-I concentration and reproductive data.
<table>
<thead>
<tr>
<th>Trait</th>
<th>$h_d^2$</th>
<th>$h_m^2$</th>
<th>$c^2$</th>
<th>$r_{am}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF28</td>
<td>0.43</td>
<td>0.17</td>
<td>0.00</td>
<td>-0.86</td>
</tr>
<tr>
<td>IGF42</td>
<td>0.51</td>
<td>0.20</td>
<td>0.00</td>
<td>-0.89</td>
</tr>
<tr>
<td>IGF56</td>
<td>0.41</td>
<td>0.06</td>
<td>0.00</td>
<td>-0.91</td>
</tr>
<tr>
<td>Mean IGF-I</td>
<td>0.50</td>
<td>0.17</td>
<td>0.00</td>
<td>-0.87</td>
</tr>
<tr>
<td>Scrotal circumference</td>
<td>0.51</td>
<td>0.06</td>
<td>0.00</td>
<td>-0.40</td>
</tr>
<tr>
<td>Percent sperm motility</td>
<td>0.08</td>
<td>0.17</td>
<td>0.00</td>
<td>-1.00</td>
</tr>
<tr>
<td>Percent normal sperm cells</td>
<td>0.47</td>
<td>0.05</td>
<td>0.00</td>
<td>-1.00</td>
</tr>
<tr>
<td>Age at first calving</td>
<td>1.00</td>
<td>0.77</td>
<td>0.28</td>
<td>-1.00</td>
</tr>
<tr>
<td>Calving rate</td>
<td>0.11</td>
<td>0.00</td>
<td>0.01</td>
<td>-0.94</td>
</tr>
</tbody>
</table>

$a$ $h_d^2$ = direct heritability, $h_m^2$ = maternal heritability, $c^2$ = proportion of phenotypic variance due to permanent environmental effect of dam, $r_{am}$ = correlation between direct and maternal effects

Table 3.3. Parameter estimates for blood serum IGF-I concentration and reproductive traits using full animal model $^a$
<table>
<thead>
<tr>
<th>Trait</th>
<th>$h_d^2$</th>
<th>Previously reported estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF28</td>
<td>0.32</td>
<td>0.19 $^{12}$</td>
</tr>
<tr>
<td>IGF42</td>
<td>0.34</td>
<td>0.32 $^{12}$</td>
</tr>
<tr>
<td>IGF56</td>
<td>0.30</td>
<td>0.18 $^{12}$</td>
</tr>
<tr>
<td>Mean IGF-I</td>
<td>0.32</td>
<td>0.25 $^{12}$</td>
</tr>
<tr>
<td>Scrotal circumference</td>
<td>0.51</td>
<td>0.57 $^3$, 0.53, 0.49 $^5$, 0.56 $^7$</td>
</tr>
<tr>
<td>Percent sperm motility</td>
<td>0.09</td>
<td>0.16 $^6$, 0.07 $^7$, 0.01 $^{11}$</td>
</tr>
<tr>
<td>Percent normal sperm cells</td>
<td>0.43</td>
<td>0.35 $^6$, 0.35 $^7$</td>
</tr>
<tr>
<td>Age at first calving</td>
<td>0.26</td>
<td>0.46 $^8$, 0.37 $^9$, 0.27 $^{10}$</td>
</tr>
<tr>
<td>Calving rate</td>
<td>0.13</td>
<td>0.13 $^1$, 0.21 $^2$</td>
</tr>
</tbody>
</table>


Table 3.4. Estimates of direct heritability ($h_d^2$) for reproductive traits using the reduced model (i.e., maternal genetic and permanent environmental effects deleted from the model) and comparison with previously reported estimates.
Table 3.5. Representative data from data set A used in the analysis of calving rate. Calving rate was entered as either 1 (i.e., conceived) or 100 (i.e., did not conceive).

<table>
<thead>
<tr>
<th>Calf ID</th>
<th>Mating number</th>
<th>IGF-I measurement</th>
<th>Calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>94357</td>
<td>1</td>
<td>140.6</td>
<td>1</td>
</tr>
<tr>
<td>94357</td>
<td>2</td>
<td>140.6</td>
<td>1</td>
</tr>
<tr>
<td>94357</td>
<td>3</td>
<td>140.6</td>
<td>1</td>
</tr>
<tr>
<td>94357</td>
<td>4</td>
<td>140.6</td>
<td>100</td>
</tr>
<tr>
<td>94357</td>
<td>5</td>
<td>140.6</td>
<td>100</td>
</tr>
<tr>
<td>94360</td>
<td>1</td>
<td>135.7</td>
<td>1</td>
</tr>
<tr>
<td>94362</td>
<td>1</td>
<td>145.7</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3.6. Representative data from data set B used in the analysis of calving rate. Data set B was identical to data set A except that IGF-I measurements for matings other than the first were deleted. A zero represents a missing data point.

<table>
<thead>
<tr>
<th>Calf ID</th>
<th>Mating number</th>
<th>IGF-I measurement</th>
<th>Calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>94357</td>
<td>1</td>
<td>140.6</td>
<td>1</td>
</tr>
<tr>
<td>94357</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>94357</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>94357</td>
<td>4</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>94357</td>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>94360</td>
<td>1</td>
<td>135.7</td>
<td>1</td>
</tr>
<tr>
<td>94362</td>
<td>1</td>
<td>145.7</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3.7. Representative data from data set C used in the analysis of calving rate. Data set C was identical to data set B except that matings other than the first were deleted.
<table>
<thead>
<tr>
<th>IGF-I Measurement</th>
<th>Data Set B (All matings)</th>
<th>Data Set C (First matings only)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC Zero</td>
<td>EC Non-Zero</td>
</tr>
<tr>
<td>IGF28</td>
<td>35,607.81</td>
<td>35,607.81</td>
</tr>
<tr>
<td></td>
<td>$P &gt; 0.10$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>IGF42</td>
<td>35,180.08</td>
<td>35,180.07</td>
</tr>
<tr>
<td></td>
<td>$P &gt; 0.10$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>IGF56</td>
<td>35,756.30</td>
<td>35,756.30</td>
</tr>
<tr>
<td></td>
<td>$P &gt; 0.10$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>Mean IGF-I</td>
<td>35,918.23</td>
<td>35,918.23</td>
</tr>
<tr>
<td></td>
<td>$P &gt; 0.10$</td>
<td>$P &lt; 0.05$</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviation used: EC: environmental covariance.

<sup>b</sup> $P$ is the level of significance for the difference in minimum function values for a chi-square test with 1 degree of freedom.

Table 3.8. Comparison of minimum function values from the MTDFREML analysis of calving rate using data set B or C with either zero or non-zero starting values for environmental covariance.
### Table 3.9. Genetic, environmental, and phenotypic correlations of IGF-I measurements with reproductive traits

<table>
<thead>
<tr>
<th>IGF-I measurement</th>
<th>Correlation (^b)</th>
<th>Trait (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r_{A1A2})</td>
<td>SC</td>
</tr>
<tr>
<td>IGF28</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>IGF42</td>
<td>0.32</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>-0.03</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>IGF56</td>
<td>0.26</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>-0.09</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean IGF-I</td>
<td>0.35</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: SC: scrotal circumference, PMSC: percent motile sperm cells, PNSC: percent normal sperm cells, AFC: age at first calving, CR: calving rate

\(^b\) \(r_{A1A2}\) = additive genetic correlation between traits 1 and 2, \(r_{E1E2}\) = environmental correlation between traits 1 and 2, and \(r_{P1P2}\) = phenotypic correlation between traits 1 and 2.
Literature Cited


Van Vleck, L. D. 2002. Professor, Animal Science Department, University of Nebraska, Lincoln, NE. Personal communication.


CHAPTER 4

DIVERGENT SELECTION FOR BLOOD SERUM INSULIN-LIKE GROWTH FACTOR I CONCENTRATION DOES NOT CHANGE AGE OF HEIFERS AT PUBERTY

Introduction

Typical definitions of age at puberty are the age when an animal begins to exhibit sexual behavior and the first time the ovary releases an egg that is ready to be fertilized. Increasing levels of sexual hormones initiate puberty. Most heifers reach puberty when they are approximately 1 yr old if given high quality nutrition, but doing so may not be cost-efficient. Age at puberty has negative genetic correlations with growth traits, including birth and 200-d weight in cattle, suggesting that animals that grow faster reach puberty earlier (Bennett and Gregory, 2001). It is desirable to decrease age at puberty, because it increases lifetime productivity of the heifer and is moderately heritable (Gregory et al., 1995).

Metabolic changes that lead to the attainment of puberty begin approximately 75 d before reaching puberty (Kinder et al., 1987). Several metabolites and metabolic hormones including fatty acids, insulin-like growth factor I (IGF-I), glucose, and insulin have been implicated in the initiation of puberty (Schillo et al., 1992). In
lambs and heifers, the primary event that initiates puberty is the release of increased pulses of luteinizing hormone (LH) due to decreased negative feedback of estradiol on LH (Kinder et al., 1995). LH induces changes in the ovary, which then starts to produce the estrogen that results in the appearance of sexual behaviors, as well as the prepubertal gonadotropin surge. Ovulation or luteinization of the follicles results in secretion of progesterone, but the secretion is usually for shorter periods than in adult animals. The secretion of progesterone is not associated with sexual behavior. There is no corpus luteum before puberty and, therefore, no progesterone is released from the ovary into the blood. The release of progesterone from transient luteal structures is an indicator of the start of pubertal activity of the animal. Transient luteal structures finally are removed and the corpus luteum develops after the first ovulation and attainment of puberty. Pregnancy rate at first estrus after attainment of puberty is low. Byerley et al. (1987) found higher pregnancy rates in animals mated at their third estrus than in heifers mated at first estrus after reaching puberty. The authors concluded that the increase in fertility was because animals at the third estrus were more mature and could conceive more readily.

Kinder et al. (1995) suggested that the season in which puberty is attained, genetics, treatment with hormones, gender, growth, and nutritional development of the animal influence age at puberty. Progesterone analysis, which is relatively difficult to perform, must be done to measure age at puberty accurately. Anderson et al. (1990) described an alternative method in which they estimated age at puberty by measuring size of the ovaries and uterine horns by rectal palpation. The measurements, which provided crude estimates of age at puberty, had a heritability of
0.32, which agreed with other heritability estimates for age at puberty reported in the literature. The authors also reported high genetic, environmental, and phenotypic correlations, which ranged from -0.37 to 0.94, of age at puberty with weaning and yearling weights. Animals that grow more rapidly reach puberty at an earlier age (Roberson et al., 1991). Heifers that reach puberty earlier also have higher pregnancy rates (Morris et al., 1999), and lower milk production in their first lactation (Lammers et al., 1999).

Season likely has an influence on age at puberty in heifers although recent data suggest that this may not be the case. Rodrigues et al. (2002) found no seasonal effects on age at puberty in B.indicus and B. taurus heifers fed adequate nutrition before and after puberty. On the other hand, season had a large influence on age at puberty in a study by Schillo et al. (1992). Fall-born heifers attained puberty at an earlier age, likely due to effects of photoperiod. Honaramooz et al. (1999) studied rise in gonadotropin release before puberty. Age at puberty was not influenced by season, but the sample size was only five heifers per season in their study. Spring-born heifers had a higher follicle stimulating hormone (FSH) surge before puberty and also exhibited an earlier surge in LH secretion. LH pulse and mean concentration were higher in spring-born heifers. Increased exposure to light decreased age of heifers at puberty, but did not cause a significant change in LH secretion in the study by Hansen et al. (1983). The authors also detected several ovarian changes that were influenced by light. Melatonin secretion is influenced by light and induces metabolic
changes that cause LH secretion (Misztal et al., 2002). Cattle are not seasonal breeders, but their puberty is influenced by season, because of influences of season on LH and prolactin secretion or growth (Schillo et al., 1983).

Nutrition has a large influence on age at puberty. Nutrition likely influences reproductive functions via neuroendocrine metabolism in the hypothalamus. Schillo et al. (1992) reported that a restricted energy diet delayed puberty by inhibiting pulsatile secretion of luteinizing hormone releasing hormone (LHRH) from the hypothalamus. There was not enough LH for ovarian development during the prepubertal stage. Among beef heifers fed at three levels of gain (1.36 kg/d; 0.68 kg/d; 0.23 kg/d), heifers that were fully fed reached puberty earlier and had more plasma IGF-I and insulin (Hopper et al., 1993). Hall et al. (1995) divided heifers into rapid growth large frame and average growth medium frame groups. They then fed heifers in each group high or low growth diets and measured IGF-I concentration before puberty. Body condition was not a predictor of age at puberty and IGF-I concentration was not different between the groups. The authors concluded that neither body condition nor levels of IGF-I were predictors of age at puberty. Greer et al. (1983), on the other hand, reported that body weight was useful for predicting age at puberty, but that weight itself did not cause the initiation of puberty. Secondary factors were associated with increased weight at puberty.

Age at puberty differs among breeds. Brahman-based Bos indicus breeds reach puberty at an older age than European breeds (Bos taurus). Heterosis, sire and dam effect, and genetic merit of the heifer are important factors in determining age at puberty (Byerley et al., 1987). Age at puberty in a herd can be changed through
selection or heterosis. An undesirable consequence of reducing age at puberty is calving difficulty due to a calf with a weight larger than pelvic measurements of the dam can accommodate (Brinks, 1990). Feeding the dam such that her body size will not be a limiting factor, reducing birth weight of the calf, or providing careful assistance to heifers during calving could prevent the calving difficulty associated with reducing age at puberty. Recent data, however, suggest that earlier age at puberty is associated with decreases in birth weight and calving difficulty (Bennett and Gregory, 2001).

Due to its influence on reproductive functions of animals, serum IGF-I concentration is an important candidate for selection to improve reproductive efficiency. Insulin-like growth factor I has been isolated from the female reproductive tract, and has been shown to play a role in regulation of bovine ovarian function (Woad et al., 2000). IGF-I and IGF-II are polypeptides with 70 and 67 amino acids and molecular weights of 7.6 and 7.4 kDa, respectively (Daughaday and Rotwein, 1989). Effects of IGFs are regulated, in part, through insulin-like growth factor binding proteins (IGFBP). The binding proteins usually block effects of the IGFs by preventing them from binding to their receptors, but in some cases they increase effects of the IGFs by increasing their half-life. The IGFs have strong anabolic effects on both protein and carbohydrate metabolism. They may exert their effects via interaction with a variety of other factors such as growth factors, sex or steroid hormones, and growth hormone (Jones et al., 1995). IGF-I has large effects on cell proliferation, differentiation, and apoptosis.
Renaville et al. (1996) measured IGF-I, IGFBP, and testosterone concentrations in control bulls and bulls treated with gonadotropin releasing hormone (GnRH) or testosterone propionate before reaching puberty. Control group bulls experienced increases in testosterone, IGF-I, and IGFBP-3 and a decrease in IGFBP-2 concentrations. GnRH or testosterone treatment did not influence the changes. The authors concluded that the changes in IGF-I, IGFBP-2, IGFBP-3, and testosterone were independent of each other. However, they only had six control bulls and three bulls per testosterone and GnRH treatment group.

IGF-I, along with other metabolites, has been implicated to play a role in initiation of puberty due to its large systemic influences. We began selecting Angus beef cattle for high or low blood serum IGF-I concentration in 1989. In this study, heifers in the high IGF-I line had significantly (50.0 ± 10.2 ng/mL; P < 0.004) higher mean IGF-I concentration than animals in the low line. The objectives of this study were to examine differences in age of heifers at puberty between these two lines of Angus cattle and to detect linear and non-linear relationships between age at puberty and IGF-I concentrations.

**Materials and Methods**

*Selection Procedures.* Divergent selection for blood serum IGF-I concentration was initiated in 1989 using 100 spring-calving (50 high line and 50 low line) and in 1990 using 100 fall-calving (50 high line and 50 low line) purebred
Angus cows with unknown IGF-I levels located at the Eastern Ohio Resource Development Center (EORDC). Selection was based on the mean IGF-I concentration of three blood samples taken at d 28, 42, and 56 of the 140-d postweaning test, which are abbreviated IGFS28, IGFS42, and IGFS56, respectively. Detailed selection procedures are described elsewhere (Davis et al., 1995).

Management Procedures. The spring breeding season was approximately 60 d in length, whereas the fall breeding season was approximately 45 d in length prior to 1994. Beginning in 1994, the fall breeding season was extended to 60 d. Spring-born calves were reared by their dams until weaning at 7 mo of age. During an adjustment period of approximately 2 wk and a 140-d postweaning test period, bull calves were fed a corn-soybean meal based concentrate diet and, from 1989 through 1993, heifer calves were fed corn silage. Bulls were kept at EORDC throughout the study and, from 1989 through 1993, heifers were transferred to the North Appalachian Experimental Watershed, Coshocton, OH, for the postweaning test. Beginning in 1994, heifers remained at EORDC and were fed a corn-soybean meal concentrate diet. Heifers were fed to gain approximately 0.7 kg/d.

Fall-born calves were fed a growing diet that was designed to yield gains of 0.9 kg/d during a 112 d-growing period in drylot after weaning at approximately 140 d of age. Following the growing period, bulls were kept at EORDC and, from 1989 through 1993, heifers were transferred to Coshocton, OH. Beginning in 1994, fall-born heifers remained at EORDC following weaning and were fed the same diet as spring-born heifers.
**Serum Samples.** Approximately 25 mL of blood were collected into sterile glass tubes at d 28, 42, and 56 of the postweaning test, allowed to clot for 24 h at 4°C, and centrifuged. Serum was drawn off and frozen at –20°C until it was assayed.

**RIA for IGF-I.** The RIA for IGF-I was performed in R.C.M. Simmen's laboratory at the University of Florida using antiserum raised against human IGF-I in rabbits (UBK487), following previously described procedures (Bishop et al., 1989).

**RIA for Progesterone and Determination of Age at Puberty.** Blood samples were collected via jugular puncture once weekly for 17 wk in spring- and fall-born heifers. All blood samples were collected in 1998 (Appendices A and B). Blood sampling began when heifers were approximately 9 mo of age. Progesterone concentration was determined using a “Coat-A-Count” RIA kit (Diagnostic Products Corporation, Los Angeles, CA), following the manufacturer’s instructions. Puberty was assumed attained if the progesterone concentration exceeded 2 ng/mL in a single or 1 ng/mL in two consecutive blood samples. Age at puberty was obtained by subtracting 7 d from the first date progesterone exceeded 1 or 2 ng/mL.

**Statistical Analyses.** All data were analyzed using the General Linear Models (PROC GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). Line and season were combined into one variable to obtain a unique identification for the nested effect of sire, since different sets of four bulls were used for breeding within each selection line each season. All models used in this study included line-season and the random effect of sire nested within line-season. Age of dam and on-test age of calf were also added to the models as a fixed effect and a covariate, respectively. The fixed effects and the covariate were first tested for
significance and only effects with a significance level of less than 0.10 were included in the subsequent analyses, except that year-season was always kept in the model. Residual correlations were obtained by including one of the IGF-I measurements and age at puberty simultaneously as dependent variables in a model in that included significant fixed effects and the covariate. A separate analysis was done for each of the IGF-I measurement and age at puberty combination. Linear, quadratic, and cubic terms for the respective IGF-I measurements, as well as significant fixed effects and covariates, were included in the models as independent variables to examine possible cubic relationships of age at puberty with IGF28, IGF42, IGF56, and mean IGF-I. Orthogonal linear contrasts were used to compare age at puberty and IGF-I concentrations of high vs low IGF-I line heifers. Sire nested within line-season was used as the error term to test line-season effects and to obtain significance levels for contrasts of high vs low line animals.

**Results and Discussion**

Simple statistics for age at puberty, on-test age, and IGF-I measurements are shown in Table 4.1. Ranges and standard deviations for IGF-I measurements were larger than those for age for puberty. Although blood samples were collected from 61 heifers in this study, only 51 of the animals attained puberty during the period blood samples were collected. A chi-square analysis was performed to determine if the number of heifers that did or did not attain puberty differed in the high and low selection lines. The analysis showed that attainment of puberty was independent of
selection line (Table 4.2; $P < 0.86$). Data from heifers that did not attain puberty were not included in the contrast, correlation, or regression analyses. Results from the PROC GLM analysis of fixed effects are shown in Table 4.3. Of the fixed effects tested, only on-test age effect on IGF56 and line-season effects on all IGF-I measurements were significant. Results of contrast analysis for IGF-I measurements are shown in Table 4.4.

**Selection Line and Season Effects on Age at Puberty.** Data used to analyze age at puberty included calves born in spring ($n = 28$) and fall 1997 ($n = 23$) calving seasons. Total number of sires of females used in the study was 15. The overall mean for age at puberty was 356 d. Age at puberty did not differ between high and low IGF-I line heifers ($P = 0.71$; Table 4.4). Season effects on age at puberty were not significant ($7.63 \pm 2.63$ d older in fall-born heifers; $P = 0.26$). Postweaning IGF-I concentrations of heifers that had data for age at puberty were significantly higher in high line heifers than in low line heifers. Mean IGF-I was $50.0 \pm 10.2$ ng/mL higher ($P = 0.004$) in the high line heifers. Therefore, the significantly higher IGF-I concentrations of high line heifers did not result in line differences for age of heifers at puberty.

Given the effects of IGF-I on the reproductive system, it is surprising that selection for IGF-I did not influence age of heifers at puberty. Heifers selected for weaning weight, final weight, and final weight plus muscling score reached puberty at a younger age than control line animals (Wolfe et al., 1990). Age at puberty is influenced by body weight and IGF-I has effects on body weight and average daily gain. Davis and Simmen (1997) reported genetic, environmental, and phenotypic
correlations of IGF-I measurements with weights measured at various times including birth, weaning, and postweaning in beef cattle. Genetic correlations of birth, weaning, on-test, and off-test weights and postweaning weight gain with mean IGF-I concentration were -0.75, -0.36, -0.45, -0.31, and -0.40, respectively. These large and negative genetic correlations indicate that selection for lower IGF-I concentration would result in rapid increases in the traits measured. Environmental and phenotypic correlations of mean IGF-I concentration with weights measured were moderate and small, respectively. These results agree with those of Blair et al. (2002) who reported that lambs selected for lower IGF-I concentration were 0.6 kg heavier at birth than lambs selected for high IGF-I concentration after five generations of selection. Effects of IGF-I on weaning weights of lambs, however, depended on sex, low line ewes and high line rams having larger weaning weights than their counterparts. The reason results for weaning weights were not consistent with those for birth weights could have been because of the IGF-I in the milk that the dams passed on to their progeny. Skeletal muscle, as well as bone growth, is mediated by IGF-I in rats (Ellis et al., 1981).

Luteinizing hormone releasing hormone is secreted from the hypothalamus, which causes LH secretion from the pituitary gland. The LH surge is the most important factor in initiation of puberty, because it causes ovulation. Exogenous IGF-I stimulates LH secretion. It has been suggested that endogenous IGF-I secretion induces LH secretion and mediates the effects of LH on puberty, possibly via nutritional regulation in sheep (Adam et al., 2000). Expression of IGF-I, IGFBP-2,
IGFBP–3, and IGF-I receptors has been detected in both the hypothalamus and pituitary gland, suggesting the presence of IGF-I activity in these organs. Ethanol inhibits secretion of LH by inhibiting the stimulatory effects of IGF-I at the hypothalamic level in rats (Hiney et al., 1998). Induction of LH secretion by IGF-I could, therefore, be a signal for initiation of puberty in female rats. IGF-I and insulin increase both basal levels and GnRH-stimulated LH secretion from male rat pituitary cells (Soldani et al., 1994).

IGF-I concentrations increase before puberty in female rodents (Handelsman et al., 1987), primates (Copeland et al., 1982), and humans (Hiney et al., 1991). In cattle, Jones et al. (1991) reported increased IGF-I concentrations before puberty. Bishop (1991) measured IGF-I concentration in blood samples obtained weekly from six paternal half-sib heifers. Small and nonsignificant increases in IGF-I concentration occurred until the age of 13 mo. IGF-I and progesterone concentrations increased rapidly at the age of 13 mo, indicating that IGF-I increased at the time of puberty. Granger et al. (1989) reported that decreased IGF-I concentrations were associated with delayed puberty in heifers. Jones et al. (1991) reported increases in frequency of LH release and concentrations of free fatty acids and IGF-I measured bi-weekly from d -56 to 0 from puberty in Angus heifers, but not in heifers from other breeds. Simpson et al. (1991) concluded that IGF-I and somatotropin concentration were important factors in the initiation of puberty in heifers. Animals immunized against bovine growth hormone experienced delayed puberty. Immunization also increased fat depth and lowered feed intake, ADG, and feed efficiency.
The difference in IGF-I concentration between high and low line animals in our study may not have been sufficient to observe the true effect of IGF-I on age at puberty. Circulating IGF-I concentrations are highly correlated with IGF-I concentration in the ovary (Pellegrini et al., 1995). Therefore, it would be expected that heifers in the high IGF-I line would have higher IGF-I concentrations in their ovaries and would experience changes in their ovarian activity.

Our results are consistent with those of Lamberson et al. (1995) who reported no correlation of age at puberty with IGF-I concentration in male and female pigs. Increased ovulation rate and litter size associated with higher IGF-I concentration has been observed in mice (Kroonsberg et al., 1989), but not in pigs (Lamberson et al., 1995).

**Residual Correlations of Age at Puberty with IGF-I Concentrations.**

Coefficients for residual correlations between IGF-I concentrations and age of heifers at puberty were negative, but nonsignificant. Correlations of age at puberty with IGF28, IGF42, IGF56, and mean IGF-I were -0.27 ($P = 0.10$), -0.20 ($P = 0.24$), -0.31 ($P = 0.06$), and -0.27 ($P = 0.10$), respectively.

**Cubic Relationships of Age of Heifers at Puberty with IGF-I Concentrations.**

Cubic relationships were observed between age of heifers at puberty and IGF-I concentrations ($P = 0.0009, 0.06, 0.08,$ and $0.003$ for the cubic regression of age of heifers at puberty on IGF28, IGF42, IGF56, and mean IGF-I, respectively; Figure 4.1). The significant cubic relationships indicate that the rate of decrease in age of heifers at puberty varied as IGF-I concentration increased.
<table>
<thead>
<tr>
<th>Trait</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-test age, d</td>
<td>51</td>
<td>262.3</td>
<td>12.3</td>
<td>229</td>
<td>283</td>
</tr>
<tr>
<td>Age at puberty, d</td>
<td>51</td>
<td>356.3</td>
<td>20.5</td>
<td>320</td>
<td>398</td>
</tr>
<tr>
<td>IGF28, ng/mL</td>
<td>51</td>
<td>135.2</td>
<td>60.4</td>
<td>51.3</td>
<td>278.4</td>
</tr>
<tr>
<td>IGF42, ng/mL</td>
<td>51</td>
<td>183.9</td>
<td>85.9</td>
<td>59.4</td>
<td>459.2</td>
</tr>
<tr>
<td>IGF56, ng/mL</td>
<td>51</td>
<td>148.4</td>
<td>70.3</td>
<td>42.7</td>
<td>307.6</td>
</tr>
<tr>
<td>Mean IGF-I, ng/mL</td>
<td>51</td>
<td>155.8</td>
<td>71.61</td>
<td>52.9</td>
<td>348.4</td>
</tr>
</tbody>
</table>

Table 4.1. Means, standard deviations (SD), and minimum and maximum values for serum IGF-I concentration, age at puberty, and on-test age

<table>
<thead>
<tr>
<th>IGF-I line</th>
<th>Puberty attained</th>
<th>Puberty not attained</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percent puberty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percent puberty</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF: 1; Chi-Square Test Statistic: 0.03; \( P < 0.86 \)

Table 4.2. Chi-square analysis of number of heifers that did or did not attain puberty
<table>
<thead>
<tr>
<th>Statistic b</th>
<th>IGF28</th>
<th>IGF42</th>
<th>IGF56</th>
<th>Mean IGF-I</th>
<th>Age at Puberty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple means (standard deviations)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>135(60)</td>
<td>184(86)</td>
<td>148(70)</td>
<td>156(72)</td>
<td>356(20)</td>
</tr>
<tr>
<td>Contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High minus low line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>43.9 ± 9.9</td>
<td>62.0 ± 14.9</td>
<td>41.2 ± 12.6</td>
<td>50.0 ± 10.2</td>
<td>-2.5 ± 6.2</td>
</tr>
</tbody>
</table>

a Effects that had a P value of less than 0.10 were considered significant and included in the subsequent analyses, except that line-season was always kept in the model regardless of the magnitude of the significance level.

Table 4.3. Levels of significance for fixed effects used in the analysis of blood serum IGF-I concentration and age at puberty a

<table>
<thead>
<tr>
<th>Statistic b</th>
<th>IGF28</th>
<th>IGF42</th>
<th>IGF56</th>
<th>Mean IGF-I</th>
<th>Age at Puberty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple means (standard deviations)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>135(60)</td>
<td>184(86)</td>
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<td>156(72)</td>
<td>356(20)</td>
</tr>
<tr>
<td>Contrast</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High minus low line</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>43.9 ± 9.9</td>
<td>62.0 ± 14.9</td>
<td>41.2 ± 12.6</td>
<td>50.0 ± 10.2</td>
<td>-2.5 ± 6.2</td>
</tr>
</tbody>
</table>

a Number of observations was 51 for IGF-I measurements and age at puberty.
b Unit of measurement for IGF-I concentrations was ng/mL and for age at puberty was days.
c P is the level of significance for the contrast of high vs low line using sire within line-season as the error term.

Table 4.4. Contrasts for IGF-I concentrations and age of heifers at puberty a
Figure 4.1. Cubic relationship between age of heifers at puberty and mean IGF-I concentration. P value for significance level of the cubic term and R² were 0.003 and 0.54, respectively.

Implications

Age of heifers at puberty did not differ between the high and low insulin-like growth factor I (IGF-I) selection lines. However, cubic relationships were found between serum IGF-I concentrations and age of heifers at puberty, and residual correlations of age at puberty with IGF-I measurements tended to be moderate. These results may be useful in attempts aimed at regulating age of heifers at puberty.
Literature Cited


CHAPTER 5

SDS-PAGE ANALYSIS OF MYOFIBRILLAR PROTEINS IN ANGUS BEEF BULLS WITH HIGH OR LOW BLOOD SERUM IGF-I CONCENTRATION

Introduction

The main goal of this study was to gain a better understanding of the variations in relative concentrations of proteins that make up the muscle tissue of beef cattle selected for high or low insulin-like growth factor I (IGF-I) concentration.

Insulin-like growth factor I is a candidate molecule to regulate growth and muscle quality in beef cattle due to its effects on multiple systems in the body and on cell proliferation and differentiation (Baserga et al., 1994; Yeh et al., 1997). IGF-I mediates effects of growth hormone (GH) and has both insulin-like and GH-like effects. Growth retardation due to lower IGF-I concentrations, despite higher or normal GH concentrations, has been reported in cattle (Kitagawa et al., 2001), indicating its important role in growth. Muscle cells are specific targets for several growth factors including fibroblast growth factor, epidermal growth factor, and IGF-I (Parker et al., 1990). Donath et al. (1994) reported that IGF-I was able to bind muscle cells a thousand times more strongly than growth hormone in vitro. Addition of IGF-I stimulates myofibril and sacromere development in rat cardiac cell culture. IGF-I
plays a role in hypertrophy of muscle via autocrine secretion (Russell-Jones et al.,
1993). During hypertrophy, the muscle cells begin to express fetal proteins and go
through fetal phases of development. IGF-I plays a role in expression of the fetal
proteins including actin and myosin heavy chain.

Production of high quality meat products at low prices is possible only by
producing animals with high feed conversion and growth rates, as well as acceptable
carcass composition. IGF-I has been extensively studied by scientists who aim to
produce such products, because IGF-I concentrations are related to growth, feed
conversion efficiency (Stick et al., 1998), and several carcass characteristics including
quality grade and marbling score (Davis and Simmen, 2000) in cattle, and weight and
growth rate (Owens et al., 1994), as well as carcass characteristics (Gatford et al.,
1996) in sheep. Holland et al. (1988) reported positive correlations of serum IGF-I
concentration with weaning weight, total weight gain from birth to weaning, and
average daily weight gain in cattle. Davis and Simmen (2000) reported moderate to
high additive genetic correlations that ranged from 0.19 to -0.53 between serum IGF-I
concentration and carcass traits including backfat thickness, Longissimus muscle area,
and carcass weight. Bulls with lower IGF-I concentrations had higher marbling
score, quality grade, backfat thickness and yield grade, indicating the important role
of IGF-I in determining body composition.

Myofibrils are fibers with white and dark bands that give muscle tissue its
typical striated structure under the microscope. The fundamental functional unit of
the myofibril is the sarcomere, consisting of thin (mainly actin) and thick (mainly
myosin) filaments. The sarcomere is the area between two Z lines that are in the
middle of the I-band. The Z-line mostly contains alpha-actinin. Several additional bands can be seen under the microscope due to the striated structure of the muscle. The A-band is dark and consists of myosin and actin. The I-band is a light band composed of actin. The H-band consists only of myosin. The M-line functions as a bridge between myosin filaments that make up the thick filaments and is located in the center of the A-band. The sarcomere also has a membrane system that consists of a cell membrane (sarcolemma), invaginations of the cell membrane (T-tubules) and a sarcomplasmic reticulum that stores calcium. T-tubules serve as amplifiers of the signals that result in Ca\(^{++}\) release from sarcoplasmic reticulum and contraction of the muscle. Proteins that make up the myofibril are (their approximate proportions in parentheses) myosin (40%), actin (20%), titin (10%), nebulin (5%), tropomyosin (2%), troponin (2%), alpha-actinin (2%), and C-protein (2%) (Yates and Greaser, 1983). Troponin and tropomyosin play regulatory roles, whereas actin and myosin play contractile roles in muscle. Actin has globular and fibrous forms and binds to myosin, troponin I, and tropomyosin. Troponin has three forms; troponin I that inhibits ATPase and binds actin, troponin C that binds Ca, and troponin T that binds tropomyosin. Titin is a giant molecule whose function is largely unknown, but is predicted to maintain cell integrity. Nebulin determines the length of actin (thin) filaments (Kruger et al., 1991). Relative proportions and biochemical characteristics of the proteins mentioned above that make up the myofibril may determine its biophysical properties. A correlation between the myofibrillar protein pattern and meat tenderness has been reported (Basu, 2000). The author reported a consistent protein profile that existed in tender, but not in tough, beef samples. Huff-Lonergan
et al. (1995) reported that the density of titin and nebulin bands observed on a sodium dodecyl sulphate polyacrylamide (SDS-PAGE) gel were related to meat tenderness and sensory analyses of beef steaks. Nebulin and titin are two giant proteins that may be important in the “elasticity” of the sarcomere.

We began selecting Angus beef cattle for high or low blood serum IGF-I concentration in 1989. In the current study, bulls in the high IGF-I line had significantly (53.8 ± 4.2 ng/mL; $P < 0.0001$) higher mean IGF-I concentration than bulls in the low line. Our hypothesis was that animals selected for high or low serum IGF-I concentration may have experienced changes in muscle structure that can be revealed upon separation of myofibrillar proteins on an SDS-PAGE gel. Changes in IGF-I concentration can either directly influence myofibrillar structure or can do so indirectly by influencing concentration of other growth factors or hormones. Our objective was, therefore, to examine differences in myofibrillar protein patterns of high and low IGF-I line animals using SDS-PAGE analysis.

**Materials and Methods**

*Animals.* Samples were collected from Angus bulls that were divergently selected on the basis of blood serum IGF-I concentration and located at the Eastern Ohio Resource Development Center. Bulls were slaughtered approximately 1 mo after the conclusion of the 140-d day postweaning test at a commercial abattoir located in Bristolville, OH. Age at slaughter ranged from 374 to 443 d with a mean
of 409 d and a standard deviation of 16 d. Fourteen *M. Occipitalis* (a muscle located on back of the head) muscle samples were removed from carcasses of six high and eight low line animals after slaughter. Twenty-nine *Longissimus* muscle samples were also removed from carcasses of 15 high and 14 low line animals after carcasses were stored at 4°C for 1 d following standard industry practices. *M. Occipitalis* samples were from bulls born in spring 2000 and *Longissimus* muscle samples were from bulls born in fall 2000 (16 bulls) and spring 2001 (13 bulls). Samples were collected from approximately the center of the muscle and perpendicular to the myofibers. Samples were stored at −80°C until used.

*Selection and management procedures, blood collection, and RIA for IGF-I.* See Chapter 1 for a detailed description of these procedures. Selection was based on the mean IGF-I concentration of three blood samples taken at d 28, 42, and 56 of the 140-d postweaning test, which are abbreviated as IGF28, IGF42, and IGF56, respectively.

*Myofibril preparation.* Myofibrils were prepared at 4°C using protocols described by Basu (2000) and Huff-Lonergan et al. (1996) and slightly modified for our purpose. Approximately 0.25 g of muscle sample was first finely minced, visible fat and connective tissue were removed, and the muscle mass was recorded. Samples were then homogenized in the presence of approximately 5 mL of freshly-prepared, ice-cold homogenization buffer (20 mM potassium phosphate, 75 mM potassium chloride, 2 mM EGTA, 1 mM magnesium chloride, 1 mM adenosine triphosphate, 1 mM sodium azide, and 1 mM dithiothreitol, pH 7.4) for 30 s. Some of the homogenate was saved for SDS-PAGE analysis and the rest was filtered through a
cheese cloth to further remove connective tissue and fat and centrifuged at 8,000 rpm for 2 min. After centrifugation, the tube was inverted to allow flow of excessive buffer and the supernatant was saved for SDS-PAGE analysis. The pellet was re-suspended in 5 mL of homogenization buffer, mixed thoroughly with a glass rod, and homogenized at a lower speed for 30 s to minimize damage to large proteins. The mixture was centrifuged again at 8,000 rpm for 2 min. The supernatant was discarded and the pellet was washed three times using the homogenization buffer following the homogenization and stirring steps. A portion of the pellet was removed, weighed, and mixed with distilled water for use in determination of myofibrillar protein concentration using a Bradford assay. One and a half mililiters of reducing buffer were added to the remaining pellet and mixed thoroughly using a glass rod. Samples were then stored at –20°C overnight. On the next day, samples were removed from the freezer and incubated at room temperature for 1 h by continuous stirring using a glass rod. Samples were then centrifuged using a micro centrifuge at the maximum speed of 12,000 rpm for 10 min. The small white pellet at the bottom of the tube was discarded, because it was likely DNA. Samples were then boiled in water for 5 min (100°C), stirred further, aliquoted, and stored at –80°C until further analysis.

Reducing buffer. The composition of the reducing buffer was identical to that of Fritz et al. (1989), except that dithiothreitol was used instead of β-mercaptoethanol. One-hundred-twenty grams of urea and 38 g of thiourea were added to approximately 70 mL of water and dissolved by gentle stirring and heating. Mixed bed resin (25 g) was added and stirred for 15 min. The solution was passed through a paper filter to remove the resin, and 2.89 g dithiothreitol, 1.51 g trizma
base, and 7.5 g SDS were added. The solution was brought to a volume of approximately 180 mL. The pH was adjusted to 6.8. Water was added to bring the volume to 250 mL. Drops of 0.05% (wt/vol) bromphenol blue were added until a dark blue color was obtained. The solution was then filtered through a filter paper, aliquoted, and stored at –20°C until used.

Running buffer. The same running buffer was used to fill both the upper and lower chambers of a Bio-Rad Protean II electrophoresis cell and consisted of 6.06 g tris, 1 g SDS, and 14.4 g glycine in 1 L double-distilled water.

Bradford assay. Total myofibrillar protein concentration was determined using a Bradford assay. All of the reagents were brought to room temperature. Two-hundred-fifty microliters of Bradford reagent were added (Sigma-Aldrich Chemical Company, St. Louis, MO) to each well of a 96-well plate using a multi-channel pipettor. Samples were serially diluted to obtain an approximate concentration of 0.1-1.4 mg/mL in distilled water. Five microliters of sample dilutions and the standard (bovine serum albumin, 2 mg/mL) were added in duplicate. The plate was agitated for 5 min using the shaker function of an ELISA reader. The plates were incubated in the dark for 5 min and read at 620 nm wavelength.

SDS-PAGE gel composition. Discontinuous slab gels that consisted of 4% upper and 12% lower polyacrylamide were run at 18°C and under 20 mA constant current per gel until the bromphenol blue dye in the sample buffer reached 1 mm from the bottom of the glass plates. The upper gel consisted of 6.7 mL water, 2.5 mL 4X upper tris buffer (.5 M tris; pH 6.8), 900 μL of 30% polyacrylamide:bis solution (acrylamide:bis ratio of 37.5:1; Bio-Rad, Hercules, CA), 20 μL of TEMED and 200
µL of 10% ammonium persulphate (wt/vol). The lower gel was a 12% polyacrylamide gel and consisted of 8.3 mL water, 6.2 mL tris buffer (3 M tris; pH 9.6), 10 mL 30% polyacrylamide (acrylamide:bis ratio of 200:1), 250 µL of 10% SDS (wt/vol), 30 µL of TEMED, and 300 µL of 10% ammonium persulphate (wt/vol).

**Staining and destaining.** Gels were stained overnight using 0.5% Coomassie brilliant blue R-250 (wt/vol), 7% glacial acetic acid (vol/vol), and 40% (vol/vol) methanol. Gels were de-stained for approximately 6 h using the same solution but without coomassie blue.

**Semi-quantitative determination of protein bands using a laser scanning densitometer.** Approximately 5 µg of myofibrillar protein were loaded in each lane of the gels. The protein bands in each lane were quantified using a laser scanning GS-800 densitometer (Bio-Rad, Hercules, CA). Scanning was repeated three times for each sample and densities were averaged. The scanning process was kept as uniform as possible for each gel and the bands were always scanned in the same direction, and at the same speed and settings.

**Statistical analysis.** Data were analyzed using the PROC GLM procedure of SAS (SAS Institute, Cary, NC). Year, line, and season were combined into one variable to obtain a unique identification for the nested effect of sire. All of the fixed effects and covariates were first tested for significance and only effects with a significance level of less than 0.10 were included in the subsequent analyses, except that year-line-season was always kept in the model regardless of the magnitude of the significance level. Effects that were tested for significance included year-line-season,
age of dam, age of calf, and density of the actin band. Density of the actin band was added to the model as a covariate to adjust for variations in amount of sample loaded. The samples had to be concentrated and loaded on the gel in small quantities in order to increase resolution of the protein bands. It was difficult to add the same amount of protein to each gel so that approximately equal readings would be obtained on the densitometer. Therefore, approximately 5 µg of myofibrillar proteins were loaded on the gel and the readings on the densitometer were normalized using the density of the actin band.

The 32 kDa protein is a troponin T degradation product that appears in tender, but not in tough, meat samples on an SDS-PAGE gel (Huff-Lonergan et al., 1995). Increased density of the 32 kDa band on an SDS-PAGE gel is considered a “marker” for tenderness of meat samples. Orthogonal linear contrasts were used to compare high vs low IGF-I line animals. Sire nested within year-line-season was used as the error term to test year-line-season effects and to obtain significance levels for the contrast of high vs low line animals. Pairs of dependent variables were analyzed using the above model in order to obtain residual correlations. In addition, IGF28, IGF42, IGF56, and mean IGF-I were included in the model as independent variables in a regression analysis. A separate regression analysis was done using each IGF-I measurement.
Results and Discussion

*Bradford assay.* Results of the Bradford assay are shown in Figure 5.1. The assay was very sensitive and detected minimal (i.e., 0.2 µg) increases in the quantity of the bovine serum albumin (Figure 5.1). However, protein quantification of myofibrils was not entirely successful. The amount of solubilized myofibrillar protein loaded on the gel was only approximate due to difficulties in solubilisation of the myofibrils and high sensitivity of the densitometer. Another reason was that only small volumes of the protein could be loaded on the gel, because loading large volumes caused appearance of wavy bands. Use of highly concentrated myofibrillar protein solution might have resulted in unavoidable pipetting errors. To overcome this difficulty, approximately 5 µg of myofibrillar protein solution were added to each lane and bands were normalized for the amount of actin in the sample. Actin is known to be resistant to degradation.
Figure 5.1. Bradford assay performed to measure concentration of myofibrillar proteins. Concentrations of myofibrillar protein samples were obtained by replacing X in the formula created for the trendline with their absorbance measured at 620 nm. Formula for the trendline and coefficient of determination for the above graph were Y = 0.5073 X + 0.2 and R² = 0.98, respectively. A new standard curve was generated for each run.

Myosin to actin ratio in myofibrils. Mean myosin to actin ratio was 2.31:1. Samples that had a myosin to actin ratio of either less than 1.9:1 or greater than 3.0:1 were discarded, because they had undergone protein degradation. Since myosin is more sensitive to degradation, the myosin to actin ratio could change over time. Extracted myofibrils were kept at -80°C and the same extraction protocol was applied.
to all of the samples. However, some of the steps used during sample preparation might not have been precisely standardized. Even a few seconds of over-homogenization might have caused some of the proteins to escape to the supernatant and resulted in an imperfect myosin to actin ratio.

Myosin to actin ratio in myofibrils is important for three reasons: First, relative amounts of proteins in the A- and I-bands of the sarcomere can be estimated using the myosin to actin ratio. Second, the ratio proves that the muscle follows the contractility model in which two myosin molecules react with one actin molecule. Third, it indicates the quality of myofibril preparation. In muscle tissue, myosin to actin ratio is approximately 2.4:1. Lower or higher ratios indicate that some of the myofibrillar proteins remained in the supernatant and were discarded by mistake.

The 2.4:1 ratio of myosin to actin remains the same regardless of muscle type or age of the animal. Myosin to actin ratios in the literature range from 1.65:1 (Mohen et al., 1985) to 2.65:1 (Kyoto et al., 1994). The variation is mainly due to problems associated with determining protein content of the myofibrils, as well as problems with quantification of proteins using a densitometer. Sixteen out of 59 samples in our study had myosin to actin ratios of either less than 1.9:1 or larger than 3.0:1 and were deleted from the analysis. Possible reasons for these large or small ratios include:

a) Age of the muscle samples: *M.Occipitalis* muscle samples used in this study were 17 mo old at the time of myofibril preparation. The *Longissimus* muscle samples collected from bulls born in fall 2000 and
spring 2001 were 12 and 5 mo old, respectively. Myosin is more sensitive to degradation than actin. Because actin is more resistant to degradation, the ratio becomes smaller as the muscle sample ages.

b) Length of the sarcomere: Yates and Greaser (1983) reported that myofibrils with different sarcomeric lengths migrated differently in a Percoll gradient, which in turn resulted in differences in myofibrillar protein migration and differences in myofibrillar protein profile.

c) The diffusion coefficient for each protein in an SDS-PAGE gel is roughly proportional to the inverse cube root of the molecular weight of the protein. Therefore, the diffusion coefficient for each protein differs, resulting in differences in migration on the gel.

*Analysis of muscle homogenate and supernatant.* Myofibrils, muscle homogenates, and supernatants were run on the same gel to observe which proteins were lost during myofibril preparation (Figure 5.2). Representative examples of purified myofibrils scanned using a densitometer are shown in Figure 5.3.
Figure 5.2. A representative SDS-PAGE gel loaded with supernatant, muscle homogenate, and myofibrils. S1, H1, and M1 represent supernatant, homogenate, and myofibrils from *M. Occipitalis* muscle and S2, H2, and M2 represent supernatant, homogenate, and myofibrils from *Longissimus* muscle, respectively. Large quantities of mitochondrial, lysosomal, and mitochondrial membrane fractions are seen in the supernatant. Very little or no sarcoplasmic proteins migrate with myosin or actin. Myosin to actin ratio in muscle homogenate and myofibrils did not differ significantly, suggesting that major loss of proteins did not occur during preparation and sarcoplasmic proteins were removed from the myofibrils.
Figure 5.3. A representative SDS-PAGE gel of myofibrils extracted from *M. Occipitalis* (M) and *Longissimus* (L) muscle samples. Approximately 5 µg of myofibril solution were loaded on each lane. Lane 1 contains a molecular weight marker.
Significance levels from the PROC GLM analysis of fixed effects used in this study are shown in Tables 5.1 and 5.2. Year-line-season effects on IGF42, IGF56, actin, and myosin light chains were significant. Effects of quantity of actin loaded on the gel on troponin T and tropomyosin, age of dam effect on myosin light chain 2, and the effect of muscle type on density of troponin T were significant. All of the fixed effects tested were significant for density of troponin C.

Results of contrast analysis for IGF-I measurements and density of myofibrillar proteins are shown in Table 5.3. IGF-I measurements did not differ between high or low IGF-I line animals. Although there were relatively large between-line differences in IGF28, IGF42, IGF56, and mean IGF-I, the differences were also associated with large standard errors, resulting in significance levels higher than expected.

Significant between-line differences were detected only in density of troponin C and myosin light chain 2. Troponin C density was $23.5 \pm 6.7$ units higher in high line than in low line bulls ($P < 0.05$; Table 5.3). Myosin light chain 2, on the other hand, was $25.8 \pm 13.3$ units higher in low line than in high line bulls ($P < 0.05$; Table 5.3).

Not much research involving myofibrillar proteins per se has been done at the molecular level in agricultural animals. Most research involving effects of IGF-I on myofibrillar protein structure, distribution, and expression has been done in laboratory animals and humans.

Analysis of myofibrillar proteins with lower molecular weights using SDS-PAGE has been successful (Ho et al., 1997). Using an 18% gel, the authors monitored effects of electrical stimulation, as well as postmortem storage, on
degradation of beef myofibrillar proteins, and therefore, meat tenderness. They reported decreased troponin T levels upon electrical stimulation. A band of 1.2 kDa was present at d 0 postmortem, but disappeared by d 7 in both electrically-stimulated and non-stimulated beef samples. Nebulin was also present from d 0 to 7, but disappeared by d 14 in both groups of samples. Desmin and troponin T almost completely disappeared from the gel by d 29 postmortem. There was a slight increase in the amount of 32 kDa protein, and, therefore, troponin T degradation in electrically-stimulated beef samples. Alpha-actinin levels were not affected by storage, regardless of electrical stimulation of the samples. Electron microscopy revealed a disruption in the Z-line that began immediately after slaughter. As the muscle aged, two to five gaps occurred in each Z-line region and edges of the Z-line lost their straight appearance. Disruption of the I band started at d 3 in electrically-stimulated and d 14 in non-stimulated samples, indicating that electrical stimulation disrupted the I band.

Giulian et al. (1983) described a method to quantify lower molecular weight proteins in silver-stained and dried SDS-PAGE gels. The gel was 0.75 mm thick and the sample well was 5 mm wide. They loaded commercially available protein standards, single muscle fibers, and heart muscle cell suspension on the gels and quantified lower molecular weight proteins using a densitometer. Their staining procedure optimized signal to noise ratio and resulted in very low background staining. They emphasized that inadequate washing could leave silver stain on the surface of the gel. The stain would not penetrate into the gel if the gel was not allowed to incubate in developing solution for a sufficient period of time. The mylar
and cellophane that they used to cover the gels had a much smaller contribution to the background than the gel matrix. They also emphasized that the containers used had to be very clean and gloves must be changed frequently and rinsed with distilled water. Another reason they obtained very low background was that they loaded the lowest amounts of protein solution possible on the gels, which prevented the appearance of spurious bands. The lowest amount of protein detected was 2 ng. The linear range was from 2 to 70 ng, but they indicated that a linear range at much higher loads could be obtained by reducing the developing time. They concluded that if a large quantity of sample was to be loaded on the gels, coomassie blue staining would be the more appropriate and convenient method. Silver staining was 10 to 100 times more sensitive than the coomassie blue staining. Myosin light chain 1 and 2, and troponin I levels showed a good linear response to the amount of muscle fibers and heart muscle cells loaded. Troponin C and myosin light chain 3 levels showed nonlinearity at fiber lengths of 0.75 cm or greater. Troponin C stained less than troponin I. Troponin C had low affinity to both silver and coomassie blue stains. Silver staining was repeatable only if all of the steps during staining were standardized, because the developing process was empirically determined by the operator. There was no operator bias in coomassie blue staining, because the stain saturated both the protein bands and the gel matrix. Destaining removed the stain from the gel matrix, but not from the protein.

Adding enzymes to the meat tissue is another method used to improve tenderness. Myofibrils incubated with bovine fetal proteases did not show much degradation (Phillips et al., 2000). Myosin heavy chain and actin did not degrade as a
result of incubation with the fetal enzymes, suggesting that these enzymes increase meat tenderness via digesting the carbohydrates in the connective tissue rather than in the myofibrils.

The absence of change in alpha-actinin and desmin levels shows that high and low line animals in our study did not experience dramatic changes in structure of their sarcomeres. Desmin is an indicator of the integrity of the Z-line. Absence of differences between high and low line animals may indicate that animals in the two lines have similar Z-line structures. Alpha-actinin has important functions in muscle cell motility. IGF-I receptor kinase acted together with alpha-actinin in breast cancer cells in the process of cell migration in breast cancer cell lines (Guvakova et al., 2002). Neuronal cells move through rapid arrangements of actin filaments into microspikes. The process is mediated by IGF-I receptors at cell-to-cell junctions (Fukushima et al., 2002). The polymerization and de-polymerization is mediated through changes in alpha-actinin.

Myosin light chain 1 mRNA expression is a marker for the number of muscle cells. Human and beef myosin light chains have a high degree of homology in their amino acid sequences (Klotz et al., 1982). Vann et al. (1998) reported no increase in myosin light chain 1 mRNA expression or number of satellite cells in finishing steers injected with bovine somatotropin (bST), although the animals exhibited increases in lean muscle growth. In our study, myosin light chain 1 levels did not differ between the high and low line bulls, suggesting that the effects of IGF-I on muscle growth are not through increases in cell number. Vann et al. (1998) reported that bST treatment did not change IGF-I levels, which could have been due to poor feeding conditions.
GH control over IGF-I is lost in starved animals (Breier et al., 1991). Vann et al (1998) concluded that bST treatment caused unexplained events that resulted in muscle growth, without affecting muscle cell number, as evidenced by myosin light chain 1 mRNA expression.

Troponin C is a regulatory protein in the thin filament of the sarcomere that is important in the process of muscle contraction. The complete amino acid sequence of troponin C has been reported (Ojima et al., 2001). Troponin C is one of the most highly conserved proteins. The amino acid sequence of the protein molecule is almost identical in most species. The protein molecule has a molecular weight of 17 kDa and consists of 147 amino acids. It contains four calcium binding loops, but only one of the loops has a completely identical sequence of loops that binds to calcium, indicating that troponin C may bind calcium via only one of the loops. Muscle contraction occurs when calcium ions originating from the sarcoplasmic reticulum bind to troponin C (Ashley et al., 1991). Calcium ions, as well as the troponin complex, along with tropomyosin, undergo structural changes that allow myosin heads to contact actin during the contraction process. Grabarek et al. (1992) reported that troponin C binds to four calcium ions, troponin T, and troponin I to make a complex that plays an important role in muscle contraction. Troponin C, then, undergoes conformational changes that result in binding of myosin to actin and finally formation of cross-bridges. The cross-bridges are strong formations that cycle and cause the sarcomere to shorten as long as a threshold amount of calcium is present. Missense mutations that prevent binding of the troponin C to either troponin I or calcium disrupts the contraction and muscle function (Terami et al., 1999). High
levels of troponin C in high line bulls in our study may, therefore, have implications about differences in the contraction process in muscle of high and low IGF-I line bulls. Loss of functional properties of cardiac and diaphragm muscles during hypoxia in mice is caused by denaturation of troponin I and troponin C (Brotto et al., 2001). Denaturation of troponin C and troponin I causes decreases in isometric contractile properties of the skinned single muscle fibers, calcium-activated muscle force, and calcium sensitivity of the muscle. De Paula Brotto et al. (2001) concluded that failure of muscle contraction under hypoxia, such as in heart failure or respiratory arrest, was mediated through inactivation of troponin I and troponin C, because the two proteins were involved in formation of cross-bridges during muscle contraction.

The density of the 32 kDa protein did not differ between muscle samples from high and low line bulls (33.8 ± 39.2 unit higher in the low line; \( P = 0.42 \)). The 32 kDa protein is known to be a “marker” for beef tenderness (Huff-Lonergan et al., 1995). Meat tenderization is a complex process with many unexplained components. Calpain, an endogenous proteolytic enzyme of the muscle and calpastatin, the inhibitor of calpain, have been shown to be important contributors to tenderization of meat samples stored at 0 to 14°C (Dransfield, 1993). Factors that cause meat tenderness are yet to be determined, but storage at refrigeration temperature is associated with increased tenderness, because structural elements that make up the myofibrils degrade over time (Robson et al., 1991). Most of the degradation of myofibrils during tenderization comes from the Z-line region, although it has been suggested that the I-band also breaks to contribute further to the degradation. Because titin, nebulin, and troponin T are all present in the I-band region of the
sarcomere, degradation of all of these proteins may contribute to disruption of the sarcomere, causing increased tenderness. In tender samples, bands in the troponin T region disappear and bands in the 32 kDa region increase in density (Koohmaraie et al., 1991). Using monoclonal antibodies, the 32 kDa protein product was shown to be a degradation product of troponin T (Ho et al., 1994). Thus, SDS-PAGE analysis of myofibrils may provide important clues regarding tenderness. Huff-Lonergan et al. (1995) reported consistently higher 32 kDa protein product in tender samples than in tough samples. Basu (2000) also reported that beef samples that were known to be tender had more dense bands at the 32 kDa region of the myofibrils. In addition, Huff-Lonergan et al. (1995) reported changes in migration of T1 and T2, degradation products of titin, named based on the migration of the two bands, in tough and tender samples. The T1 band was most dense in tough samples, whereas T2 was most dense in tender samples, indicating a role for titin in meat tenderness. However, this was not the case in a study by Fritz et al. (1993), who reported no correlation of beef tenderness with T1 and T2 bands. The different findings between the two studies could have occurred because Huff-Lonergan et al. (1995) heated the samples at 50°C as opposed to the 100°C used by Fritz et al. (1993). Heating at 100°C could have resulted in degradation of titin and disappearance of the bands. It is not known why the 32 kDa band appears only in tender samples. Some possible explanations are that troponin T might play a role at the point of contact between the myosin and actin heads. Degradation of troponin T, therefore, may influence occurrence of events required for contraction of muscle. Troponin T degradation may also result in initiation of other cascades that ultimately result in increased tenderness.
Breed of the animal may have a large influence on meat tenderness. *Bos indicus* cattle have higher calpastatin activity (Williams et al., 1994) and fewer I band fractures (Wheeler et al., 1990), causing tougher meat compared to *Bos taurus*. As the share of *Bos indicus* inheritance increases in crossbred animals of *Bos indicus* and other breeds, tenderness decreases. Electrical stimulation increases tenderness in meat samples from *Bos indicus* cattle by disrupting muscle structure (Crouse et al., 1987).

Levels of myosin light chain 3, a degradation product of myosin light chain 2, are markers for cardiac hypertrophy (Klotz et al., 1982). Levels of cardiac troponin I in serum are markers for cardiac injury (Kim et al., 2002). Troponin I levels are significantly associated, in a dose-response fashion, with mortality in patients undergoing vascular surgery. Patients with higher than 3 ng/mL cardiac troponin I levels have significantly higher death rates compared to patients with lower troponin I levels.

Myosin light chain 2 also plays an important role in the contraction process by stabilizing the neck of the myosin head. Myosin light chain 2 is called a “regulatory” light chain, because it regulates the ATPase activity of the myosin in muscle. Higher myosin light chain 2 density detected in low line animals in our study (*P* < 0.05) suggests the presence of changes in muscle contraction via a possible change in ATPase activity of the myosin molecule.

*Residual correlations of density of myofibrillar proteins with IGF-I concentrations.* Residual correlations of the density of myofibrillar proteins with IGF-I measurements are shown in Table 5.4. With the exception of a negative correlation
of density of troponin T with IGF28 (r = -0.44; P = 0.05), no significant correlations were found. The negative correlation between troponin T and IGF28 shows that as IGF28 levels increase, troponin T levels decrease and vice versa. Residual correlations of the density of the 32 kDa protein band with IGF28, IGF42, IGF56, and mean IGF-I were -0.07 (P = 0.73), -0.06 (P = 0.76), -0.07 (P = 0.75), and -0.02 (P = 0.91), respectively. Density of the 32 kDa protein band is considered to be a “marker” for troponin T degradation and meat tenderness. These small and nonsignificant correlations indicate a lack of association of serum IGF-I concentrations with rate of troponin T degradation, density of 32 kDa protein, and meat tenderness.

*Linear, quadratic, and cubic relationships between density of myofibrillar proteins and IGF-I concentrations.* Levels of significance for the linear, quadratic, and cubic regression of density of myofibrillar proteins on IGF-I measurements are shown in Table 5.5. Coefficients for the linear regression of densities on IGF-I measurements were all nonsignificant. Coefficients for the quadratic regression of density of desmin on IGF28, IGF42, and mean IGF-I were significant. Significant cubic relationships were detected between density of the 32 kDa protein and IGF56 and mean IGF-I. Myosin heavy chain had significant cubic relationships with IGF28, IGF56, and mean IGF-I. In addition, alpha actinin had cubic relationships with IGF28 and mean IGF-I. The remaining coefficients were nonsignificant. Residuals for the density of the 32 kDa band varied at low, medium, and high residuals for mean IGF-I concentration (Figure 5.4). Other proteins that had cubic relationships with IGF-I measurements also followed the same pattern.
Based on the fact that neither myosin nor actin differed between high and low line bulls and that no correlation was found between amount of the two proteins and IGF-I concentration, we conclude that the crude sarcomeric structure was intact in both high and low line bulls.

Alpha-actinin is known to link actin filaments to receptors for extracellular matrix molecules (Pavalko et al., 1991). Alpha-actinin did not differ between high and low line bulls, but had a cubic relationship with IGF28 and mean IGF-I, suggesting that there may have been changes in the extracellular matrix. Extracellular matrix is composed of glycoproteins and proteoglycans that provide cells with an environment in which to develop and proliferate. Jockusch et al. (1983) reported that complexes containing alpha-actinin and extracellular matrix were involved in the formation of membrane ruffling. Alpha-actinin has a high degree of homology with dystrophin, but it does not replace the function of the dystrophin gene product, which functions as a "shock absorber” during muscle contraction (Harper et al., 2002).

**Implications**

Our results suggest that there may have been some changes in muscle contraction between high and low IGF-I line animals, because troponin C and myosin light chain 2 levels differed between high and low line bulls. Of the residual
correlations tested, only the correlation of troponin T with IGF28 was significant. A non-linear (i.e., quadratic) relationship was observed between mean IGF-I and desmin levels. Cubic relationships of density of the 32 kDa protein, myosin heavy chain, and alpha-actinin with mean IGF-I concentration were found. Contrast analysis showed that a 35.2 ± 32.1 ng/mL difference in mean IGF-I concentration between high and low line animals did not result in line differences in density of most of the myofibrillar proteins or the 32 kDa protein band, and, therefore, meat tenderness and integrity of the sarcomere. We base these conclusions on previous findings that showed a clear link between levels of the 32 kDa protein band and meat tenderness. Meat tenderness was not directly measured in the current study. Our conclusions are not definite due to lack of a statistically significant difference in IGF-I concentration between animals in the high and low lines. Our results do not exclude the possibility of changes in expression of protein isoforms in response to IGF-I. Perhaps SDS-PAGE analysis is not sensitive enough and a larger study with more precise techniques is needed to reveal the changes in protein isoforms in animals with high or low IGF-I concentration.
<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>IGF28</th>
<th>IGF42</th>
<th>IGF56</th>
<th>Mean IGF-I</th>
<th>32 kDa protein</th>
<th>MHC</th>
<th>AACT</th>
<th>DES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year-line-season</td>
<td>0.43</td>
<td>0.02</td>
<td>0.02</td>
<td>0.06</td>
<td>0.31</td>
<td>0.87</td>
<td>0.56</td>
<td>0.43</td>
</tr>
<tr>
<td>Sire (YRLS)</td>
<td>0.66</td>
<td>0.54</td>
<td>0.21</td>
<td>0.46</td>
<td>0.17</td>
<td>0.79</td>
<td>0.75</td>
<td>0.59</td>
</tr>
<tr>
<td>Actin added</td>
<td>0.09</td>
<td>0.19</td>
<td>0.88</td>
<td>0.14</td>
<td>0.75</td>
<td>0.59</td>
<td>0.59</td>
<td>0.59</td>
</tr>
<tr>
<td>On-test age of calf</td>
<td>0.46</td>
<td>0.30</td>
<td>0.19</td>
<td>0.28</td>
<td>0.80</td>
<td>0.74</td>
<td>0.09</td>
<td>0.46</td>
</tr>
<tr>
<td>Age of dam</td>
<td>0.27</td>
<td>0.18</td>
<td>0.33</td>
<td>0.22</td>
<td>0.70</td>
<td>0.66</td>
<td>0.66</td>
<td>0.96</td>
</tr>
<tr>
<td>Muscle type</td>
<td>0.17</td>
<td>0.77</td>
<td>0.75</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Effects that had a P value of less than 0.10 were considered significant and included in the subsequent analyses, except that year-line-season was always kept in the model regardless of the magnitude of the significance level.*

*Sire nested within year-line-season was used as the error term to test year-line-season effects.*

*Muscle type was either *M. Occipitalis* or *Longissimus* muscle.*

*Abbreviations used: MHC: Myosin Heavy Chain, AACT: Alpha-actinin; DES: Desmin.*

Table 5.1. Levels of significance for fixed effects used in the analysis of serum IGF-I concentration and myofibrillar proteins
<table>
<thead>
<tr>
<th>Fixed effect (^a)</th>
<th>ACT</th>
<th>TrpT</th>
<th>TRPM</th>
<th>LC1</th>
<th>TrpI</th>
<th>TrpC</th>
<th>LC2</th>
<th>LC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year-line-season</td>
<td>0.03</td>
<td>0.59</td>
<td>0.93</td>
<td>0.008</td>
<td>0.67</td>
<td>0.007</td>
<td>0.001</td>
<td>0.0003</td>
</tr>
<tr>
<td>Sire (YRLS) (^b)</td>
<td>0.60</td>
<td>0.44</td>
<td>0.38</td>
<td>0.93</td>
<td>0.87</td>
<td>0.04</td>
<td>0.55</td>
<td>0.76</td>
</tr>
<tr>
<td>Actin added</td>
<td>0.03</td>
<td>0.004</td>
<td>0.48</td>
<td>0.01</td>
<td>0.0005</td>
<td>0.16</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>On-test age of calf</td>
<td>0.15</td>
<td>0.55</td>
<td>0.15</td>
<td>0.61</td>
<td>0.28</td>
<td>0.03</td>
<td>0.19</td>
<td>0.73</td>
</tr>
<tr>
<td>Age of dam</td>
<td>0.33</td>
<td>0.13</td>
<td>0.44</td>
<td>0.69</td>
<td>0.79</td>
<td>0.005</td>
<td>0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>Muscle type (^c)</td>
<td>0.25</td>
<td>0.07</td>
<td>0.88</td>
<td>0.41</td>
<td>0.88</td>
<td>0.02</td>
<td>0.23</td>
<td>0.70</td>
</tr>
</tbody>
</table>

\(^a\) Effects that had a P value of less than 0.10 were considered significant and included in the subsequent analyses, except that year-line-season was always kept in the model regardless of the magnitude of the significance level.

\(^b\) Sire nested within year-line-season was used as the error term to test year-line-season effects.

\(^c\) Muscle type was either *M. Occipitalis* or *Longissimus* muscle.

\(^d\) Abbreviations used: ACT: Actin; TrpT: Troponin T; TRPM: Tropomyosin; LC1: Myosin Light Chain 1; Trp I: Troponin I; Trp C: Troponin C; LC2: Myosin Light Chain 2; LC3: Myosin Light Chain 3.

Table 5.2. Levels of significance for fixed effects used in analysis of density of myofibrillar proteins.
Table 5.3. Contrasts for IGF-I concentrations and density of myofibrillar proteins

<table>
<thead>
<tr>
<th>Contrast b</th>
<th>Trait c, d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IGF28</td>
</tr>
<tr>
<td>High minus low line</td>
<td>P &lt; 0.44 b</td>
</tr>
<tr>
<td></td>
<td>24.1 ± 34.6</td>
</tr>
<tr>
<td>High minus low line</td>
<td>P &lt; 0.14</td>
</tr>
<tr>
<td></td>
<td>-29.1 ± 24.3</td>
</tr>
</tbody>
</table>

* Number of observations was 43.
* P is the level of significance for the contrast of high vs low line using sire within year-line-season as the error term.
* Unit of measurement for IGF-I concentrations was ng/mL and for density of myofibrillar proteins was optical density units.
* Abbreviations used: MHC: Myosin Heavy Chain, AACT: Alpha-actinin; DES: Desmin; ACT: Actin; TrpT: Troponin T; TRPM: Tropomyosin; LC1: Myosin Light Chain 1; Trp I: Troponin I; Trp C: Troponin C; LC2: Myosin Light Chain 2; LC3: Myosin Light Chain 3.
<table>
<thead>
<tr>
<th>Myofibrillar protein</th>
<th>IGF28</th>
<th>IGF42</th>
<th>IGF56</th>
<th>Mean IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 kDa protein</td>
<td>-0.07 ($P = 0.73$)</td>
<td>-0.06 ($P = 0.76$)</td>
<td>-0.07 ($P = 0.75$)</td>
<td>-0.02 ($P = 0.91$)</td>
</tr>
<tr>
<td>MHC</td>
<td>0.42 ($P = 0.42$)</td>
<td>0.12 ($P = 0.58$)</td>
<td>0.21 ($P = 0.36$)</td>
<td>0.18 ($P = 0.43$)</td>
</tr>
<tr>
<td>AACT</td>
<td>0.07 ($P = 0.76$)</td>
<td>0.04 ($P = 0.85$)</td>
<td>0.04 ($P = 0.85$)</td>
<td>0.05 ($P = 0.81$)</td>
</tr>
<tr>
<td>Desmin</td>
<td>-0.02 ($P = 0.37$)</td>
<td>0.09 ($P = 0.74$)</td>
<td>-0.15 ($P = 0.56$)</td>
<td>-0.16 ($P = 0.52$)</td>
</tr>
<tr>
<td>Actin</td>
<td>-0.07 ($P = 0.75$)</td>
<td>0.02 ($P = 0.99$)</td>
<td>0.02 ($P = 0.99$)</td>
<td>-0.02 ($P = 0.96$)</td>
</tr>
<tr>
<td>Troponin T</td>
<td>-0.44 ($P = 0.05$)</td>
<td>-0.25 ($P = 0.30$)</td>
<td>-0.19 ($P = 0.42$)</td>
<td>-0.31 ($P = 0.19$)</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>0.01 ($P = 0.95$)</td>
<td>0.14 ($P = 0.54$)</td>
<td>0.001 ($P = 0.99$)</td>
<td>0.05 ($P = 0.81$)</td>
</tr>
<tr>
<td>Troponin I</td>
<td>0.08 ($P = 0.70$)</td>
<td>0.15 ($P = 0.53$)</td>
<td>0.16 ($P = 0.50$)</td>
<td>0.14 ($P = 0.56$)</td>
</tr>
<tr>
<td>Troponin C</td>
<td>0.18 ($P = 0.53$)</td>
<td>0.17 ($P = 0.57$)</td>
<td>0.27 ($P = 0.36$)</td>
<td>0.22 ($P = 0.46$)</td>
</tr>
<tr>
<td>LC1</td>
<td>-0.21 ($P = 0.36$)</td>
<td>-0.26 ($P = 0.24$)</td>
<td>-0.09 ($P = 0.69$)</td>
<td>-0.20 ($P = 0.39$)</td>
</tr>
<tr>
<td>LC2</td>
<td>-0.12 ($P = 0.65$)</td>
<td>-0.15 ($P = 0.67$)</td>
<td>-0.17 ($P = 0.50$)</td>
<td>0.16 ($P = 0.55$)</td>
</tr>
<tr>
<td>LC3</td>
<td>-0.15 ($P = 0.56$)</td>
<td>0.01 ($P = 0.96$)</td>
<td>-0.17 ($P = 0.50$)</td>
<td>-0.12 ($P = 0.66$)</td>
</tr>
</tbody>
</table>

*a Abbreviations used: MHC: Myosin Heavy Chain, AACT: Alpha-actinin; LC: Myosin light chain.

Table 5.4. Residual correlations of density of myofibrillar proteins with IGF-I measurements
<table>
<thead>
<tr>
<th>Type of regression</th>
<th>IGF-I measurement</th>
<th>Trait&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>32 kDa P</td>
</tr>
<tr>
<td>Linear</td>
<td>IGF28</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>IGF42</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>IGF56</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Mean IGF-I</td>
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</tr>
<tr>
<td>Quadratic</td>
<td>IGF28</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>IGF42</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>IGF56</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Mean IGF-I</td>
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<sup>a</sup> Abbreviations used: 32 kDa P: 32 kDa protein; MHC: Myosin heavy chain; AACT: Alpha-actinin.

Table 5.5. Levels of significance for linear, quadratic, and cubic regression of density of myofibrillar proteins on IGF-I concentrations.
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$^a$ Abbreviation used: LC: Myosin light chain

Table 5.5 Levels of significance for linear, quadratic, and cubic regression of density of myofibrillar proteins on IGF-I concentrations (continued).
Figure 5.4. Cubic relationship between mean IGF-I concentration and density of the 32 kDa band. P value for significance level of the cubic term and $R^2$ were 0.001 and 0.74, respectively.
Literature Cited


Basu, L. 2000. Myofibrillar Protein Patterns: As Predictors of Post Mortem Tenderness. M. S. Thesis. The Ohio State University, Columbus, OH.


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Appendix A. Progesterone concentration (ng/mL) and week puberty was attained (in bold) in spring-born heifers. *a
**Appendix A. Progesterone concentration (ng/mL) and week puberty was attained (in bold) in spring-born heifers a (continued)**

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| 97864     | 0.34 | 0.19 | 0.27 | 0.27 | 0.73 | 1.7 | 0.05 | **0.94** | 2.73 | 0.04 | 1.3 | 3.04 | 0 |
| 97865     | 0.14 | 0.07 | 0 | 0.04 | 0.1 | 0.04 | 0 | 0 | 0.21 | 0.18 | 0.46 | 0.51 | 0.44 |
| 97872     | 0 | 0 | 0.01 | 0.01 | 0.02 | 0 | 0 | 0 | 0 | 0 | 0 | 1.89 | 3.5 | 0.02 |
| 97873     | 0.13 | 0.12 | 0.14 | 0 | 0.09 | 0 | 0.59 | **0.37** | 2.56 | 0.06 | 2.73 | 1.59 | 0.84 |
| 97874     | 0.13 | 0.03 | 0 | 0 | 0.5 | **0.39** | 1.66 | 0 | 1.42 | 0.59 | 0.05 | 1.13 | 0.06 |
| 97877     | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0|
| 97878     | 0.56 | 0 | 0.05 | 0 | 0 | 0.03 | 0 | 0 | 0.09 | **0.13** | 1.1 | 2.72 | 0.14 |
| 97882     | 0 | 0 | 0 | 0 | 0.21 | 0.06 | 1.6 | 0 | **0.82** | 2.48 | 0 | 0.99 | 3.23 |
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Appendix B. Progesterone concentration (ng/mL) and week puberty was attained (in bold) in fall-born heifers a
### Appendix B. Progesterone concentration (ng/mL) and week puberty was attained (in bold) in fall-born heifers

*a* Dates blood samples collected [week(day/month)]: 1(6/2), 2(6/9), 3(6/16), 4(6/23), 5(6/30), 6(7/7), 7(7/14), 8(7/21), 9(7/28), 10(8/4), 11(8/11), 12(8/25), 13(9/1), 14(9/8), 15(9/15), 16(9/21), 17(9/29). All samples were collected in 1998.

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