EFFECTS OF SELECTION FOR BLOOD SERUM INSULIN-LIKE GROWTH FACTOR I CONCENTRATION ON REPRODUCTIVE PERFORMANCE OF FEMALE ANGUS BEEF CATTLE

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Mater of Science in the Graduate School of The Ohio State University

By

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Graduate Program in Animal Science

The Ohio State University

2012

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ABSTRACT

Reproductive performance of animals affects lifetime productivity. However, improvement of reproductive traits via direct selection is generally slow due to low heritability. Therefore, identification of indicator traits for reproductive performance may enhance genetic response. Previous studies showed that serum insulin-like growth factor I (IGF-I) concentration is a candidate indicator for growth and reproductive traits. The objectives of our study were to estimate the (co)variances for IGF-I concentration and reproductive, body composition, and growth traits. Data were collected from a divergent selection experiment for serum IGF-I concentration at the Eastern Agricultural Research Station owned by The Ohio State University. The study included a total of 2,662 calves in the 1989 to 2005 calf crops. (Co)variance components were estimated for genetic effects, maternal environment effects, environment effects, and phenotypic effects using an animal model in multiple-trait, derivative-free, restricted maximum likelihood for and ASReml computer programs. The results of direct additive genetic correlation suggest that selection for greater IGF-I concentration (heritability = 0.50 ± 0.07) could lead to increased conception (heritability = 0.11± 0.06, r = 0.32, P < 0.001) and calving rate (heritability = 0.13 ± 0.06, r = 0.43, P < 0.001), increased 140 postweaning ribeye area (heritability = 0.63 ± 0.08, r = 0.38, P < 0.001) and weight (heritability = 0.62 ± 0.08, r = 0.15, P < 0.001),
and decreased age at first calving in heifers (heritability = 0.35 ± 0.20, r = -0.40, P < 0.001).
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.
ACKNOWLEDGEMENTS

First and foremost, I am grateful to my advisor Dr. Michael E. Davis, for the excellent guidance, everlasting patience and unreserved sacrifice, that leading me all the way through my M.S. program. He created a free and active academic atmosphere with his gentle personality for me, a student pursuing her first academic program in USA.

Also, thank my committee members Dr. Joseph S. Ottobre, for his rigorous attitude on academic work of physiological reproduction but merciful style on students; and Dr. Steve J. Moeller for his penetrating insight on errors, proper guidance to students, and acute sense on science.

In addition, thank Dr. Chad Dechow from Penn State University for the help on ASReml, Dr. Robert Tempelman from Michigan State University for the help on my research and presentation, and Dr. Van Vleck for the help on MTDFREML while he was taking care of his wife in hospital.

Thank all graduate students in Animal Science department of The Ohio State University that instilled, taught, and supported me. Thank my parents and friends in China, USA and Australia standing firmly behind my back.

I would like to express my greatest appreciation to all of the people who have assisted and supported me in the past two years. They are the reason I am who I am today.
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CHAPTER 1: Introduction

1.1. IGF-I Background and its function

Since insulin-like growth factors (IGFs) were first identified in 1957 (Salmon and Daughaday, 1957), scientists have found that they affect growth, development, cell differentiation, metabolism and numerous biological functions of animals. IGF-I and IGF-II gene knock-out mice showed growth retardation compared with normal mice (Liu et al., 1993). The IGFs also promote differentiation of myoblastic or oesteoblastic tissues into muscle and bone (Schmid et al., 1983).

The IGF-I’s function of regulating growth and muscle quality provides a potential method to improve feed efficiency (Lancaster et al., 2008), decrease the length of the growing period, and improve product quality in domestic animals (Baserga et al., 1994). Based on this fact, selection experiments involving IGF-I have been conducted. Results demonstrated that circulating IGF-I concentration is related to growth (Davis and Simmen, 2006), age at first calving (Yilmaz et al., 2004), maturing rate (Qin et al., 2010), and body composition (Davis et al., 2000).

However, effects of IGF-I may vary between males and females. Whereas weaning serum IGF-I concentration showed positive correlation with residual feed intake (RFI) in bulls, it expressed a negative relationship in heifers (Lancaster et al., 2008). In
addition, its effects on growth and body composition are more significant in males. Bulls had improved (P < 0.05) feed conversion ratio compared with heifers (9.12 vs. 11.77 ± 0.47 kg dry matter intake:gain), and bulls gained (P < 0.01) more longissimus muscle area (25.25 vs. 18.52 ± 1.81 cm²) during the study than females (Davis et al., 2008). Reproductive functions are also influenced by IGF-I, which leads us to consider if there are sex-related differences in IGF-I regulation of growth.

Studies on cattle, sheep, goats, and horses have shown that IGF-I may regulate ovarian follicular development. The pathway of follicular development regulation involves molecules such as insulin-like growth factor I binding proteins (IGFBPs) and protease pregnancy-associated plasma protein-A (PAPP-A) (Silva et al., 2009). Yilmaz et al. (1999) demonstrated that high serum IGF-I concentration is associated with an increase in the percentage of motile sperm cells and scrotal circumference in bulls (P < 0.05).

In summary, serum IGF-I concentration is a good candidate hormone to regulate growth and body composition, as well as reproductive functions such as ovulation and sperm production in livestock.

We began selecting Angus beef cattle for high or low blood serum IGF-I concentration in 1989. Calves in the high IGF-I line had 75.04 ng/mL more serum IGF-I than calves in the low IGF-I line in the year 2004 (P <0.01), indicating that selection for IGF-I was successful. Our goal is to assess differences between the high and low IGF-I line animals that have resulted from selection for serum IGF-I
using a variety of statistical and genetic tools.

1.2. Hypothesis

Our research hypothesis is that animals selected for high or low serum IGF-I concentrations have experienced changes in reproductive traits.

1.3. Objectives

The specific aims of the proposed research project are to:

1. Compute heritability and genetic, environmental, and phenotypic correlations between blood serum IGF-I concentrations, conception rate (CR), calving rate (CAR), age at first calving (AFC), twinning rate (TW), and days to calving from calving of the first female in the same calving season (DC) using multiple-trait, derivative-free restricted maximum likelihood (MTDFREML) computer software.

2. Compute correlations between serum IGF-I concentration, reproductive traits, d 140 postweaning weight (WT), backfat thickness (BF), and ribeye area (REA) using ASReml computer software.

3. Compare line differences in means for these traits using the PROC MIXED
procedure and Chi-square analysis in the Statistical Analysis System (SAS) program.

### 1.4. Importance of this Project

Although in vitro trials offer evidence concerning the functions of IGF-I, tests on animals must be performed to study the intricate pathways and interactions of IGF-I with other hormones and molecules. Long-term selection of cattle for ovulation rate and twinning rate by Echternkamp et al. (2004) showed enhanced follicular development and that circulating IGF-I concentrations were two-fold greater in cows selected for twinning than in control cows. They also demonstrated positive correlations between IGF-I and twinning rate ($P < 0.01$). Therefore, the authors suggested that IGF-I may have supported an increase in the sensitivity of the ovary to gonadotropins. Yilmaz et al. (2004) demonstrated a correlation between serum IGF-I concentration and AFC ($r_A = -0.14 \sim -0.23, P = 0.02$), but found no relationship with calving rate ($P = 0.12$). Therefore, the in vivo effects of field-level measures of IGF-I need further exploration.

The current study updated Ahmet Yilmaz’s analysis (2004 and 2006) by inserting 1,157 new pregnancy records from years 2001 to 2003 and confirmed his results on the relationship between IGF-I and AFC. Furthermore, I also analyzed the effects of IGF-I selection on CR, CAR, TW, and DC using contrast and Chi-square tests to
determine the line differences within seasons. The work described in this thesis will contribute significantly to our understanding of the effects of IGF-I on reproduction in cattle and may also apply to other species, because the IGF-I polypeptide shares a high degree of homology among cattle, pigs, humans, and other livestock species. According to Yilmaz’s studies (2004), the CR did not change dramatically as a result of selection on IGF-I, which is in contrast with several other studies that showed strong evidence of change. It is obvious that complex mechanisms are involved in carrying out the effects of IGF-I on cells and to elucidate these mechanisms is our biggest challenge. Therefore, based on discussion with my committee members in the proposal presentation, I included body composition traits and growth traits in my study to see how IGF-I affects all of these traits synchronously and separately, and how these traits interact with each other while affecting the impact of IGF-I. Results of this study will contribute significantly to our current understanding of the physiological roles of IGF-I in cattle, as well as in other species.
CHARPT 2: Literature Review

2.1. General Information about IGFs and IGFBPs

2.1.1. Molecular Structure

IGF-I is a single chain polypeptide composed of 70 amino acid residues cross-linked by three disulfide bridges (Rinderknecht et al., 1978). Its structure displays obvious homology to proinsulin and insulin, which explains the derivation of the name (Guo et al., 2008). Nucleotide sequences of IGF-I among horses, cattle, sheep, pigs, dogs, humans, and rats are homologous; the amino acid sequences and DNA sequences are even more similar (Nixon et al., 1999).

2.1.2. Expression and Function

IGF-I is universally generated by liver (Froesch et al., 1985), muscles, kidney, cartilage (Nixon et al., 1999), ovary (Echternkamp et al., 2004), and perhaps other organs. Growth hormone (GH) and insulin are the main hormones regulating IGF-I expression. Chase Jr. et al. (2011) indicated that subcutaneous injection treatment of GH could increase plasma IGF-I concentration (P < 0.01) in miniature Brahman cattle. Also, GH and insulin together increased plasma IGF-I (P < 0.01). GH acts on the
liver to increase IGF-I production, and IGF-I acts on bone and skeletal muscle to stimulate growth and development (Maki, 2010). In addition, IGF-I can play roles in activating cell differentiation, controlling apoptosis (Ma et al., 2009), and acting as a combined regulator of other functions such as reproduction (Wandji et al., 1992). Its effect is widely detected on adipose tissue, muscles, and bones. Therefore, IGF-I is crucial for the growth and development of animals, mainly postnatal growth (Tricoli et al., 1984). Although IGF-I can have some of the same actions as insulin, the potency of IGF-I, in that regard, is only 1% ~ 2% of that of insulin. IGF-I binds to specific cellular receptors, which transduce its action on the cell (Maki, 2010). IGF binding proteins also play roles in IGF action (Froesch et al., 1985). Nutritional and other environmental changes can also dramatically alter its effects (Davis et al., 1995).

2.1.3. Regulation

The IGFs and insulin act primarily through tyrosine-kinase-linked receptors, the Type 1 receptor (IGF-IR) and insulin receptor (IR). IGF-IR and IR are highly structurally and functionally homologous, and both can bind to IGFs and insulin, stimulating growth or metabolism. The Type 2 receptor (IGF-IIR) is a scavenger receptor and does not participate in growth or metabolic effects (LeRoith and Yakar, 2007). The IGF-IR is necessary for the survival of mice. The IGF-IR<sup>−/−</sup> mice died at birth in the experiment of Liu et al. (1993) and were approximately 55% smaller than their littermates. Binding of IGF-IR also plays a role in tumorigenesis (Tomizawa et al.,
Among all of the molecules, IGF-I and its interaction with IGFR is principally modulated by insulin-like growth factor binding proteins (IGFBPs) in the serum. The predominant binding protein, IGFBP3, is believed to play a role in mediating apoptosis in both normal and disease processes, which relates to cell growth and cancer (Grimberg and Cohen, 2000). IGFBP3 is positively regulated by both GH and IGF-I (Silva et al., 2009). Also, the IGFBPs have IGF independent ability to regulate growth, modulated by proteases via cell surface proteins or receptors. A recent study shows that IGF-I and -II can stimulate granulosa cell proliferation and progesterone production, indicating a possible ability to regulate ovarian follicular development. This whole pathway of IGF-I and -II in follicular development is complicated, but involves IGFBP2, IGFBP4, IGFBP5, and protease pregnancy-associated plasma protein-A (Silva et al., 2009).

2.2. Effects of IGFs on Female Reproductive Functions

2.2.1. Folliculogenesis and Ovarian Function

The IGF system as an intraovarian regulator of folliculogenesis has been intensively studied in various species. The ovary has long been known as a site of IGF-I expression and binding (Adashi, 1998).

In bovine, follicular growth initiates around d 180 of gestation and until d 220 to 230
fetal ovaries mainly consist of preantral follicles (Russe, 1983). The number of antral follicles are few before birth (d 270), but increase drastically in neonatal calves (Erickson, 1966). The initiation of follicular growth in bovine fetuses is associated with an increase in ovarian adenylate cyclase activity. However, no gonadotropin (FSH and LH) stimulated adenosine 3’, 5’-monophosphate (cAMP) production is detectable before birth, which is due to low IGF-I binding activity of preantral follicles (Wandji et al., 1992). Chun et al. (1996) found that IGF-I could inhibit apoptosis in ovarian follicular cells. Armstrong et al. (2002) reported that IGF-IR, IGFBP2, and IGFBP3 were expressed in granulosa cells and in the oocytes of bovine preantral follicles. The expression of IGF-IR increases during the period when the preantral follicle develops into the antral follicle stage in bovine fetuses and neonatal calves.

The IGF-I action may be restricted to granulosa cells in the bovine ovary. Other species such as porcine have high specific binding sites for IGF-I in theca cells from large antral follicles (Caubo et al., 1989). Schams et al. (2002) showed high expression of IGF-I in the theca interna at the beginning of follicle development, whereas a growing expression of mRNA in granulosa cells occurred as follicles developed. Silva et al. (2009) detected IGF-I and IGF-II in both oocytes and somatic cells of mammals, which were regulated by attaching of IGFBPs, and the latter are degraded and released by proteases, thus stimulating the secondary follicles to grow. IGF-IR mRNA is present in the theca interna and granulosa cells during the final growth of antral follicles in bovine (Schams et al., 2002). IGF-IIR was manifested
in bovine granulosa and theca cells from antral follicles (Spicer et al., 2007).

Although IGF-I can be secreted locally by the ovary, oviduct (Pushpakumara, et al., 2002) and endometrium (Robinson, et al., 2000) in bovine, the main source of IGF-I is the liver, the exocrine secretion of which brings IGF-I to other parts of the body through blood circulation (Funston et al., 1996). Concentrations of IGF-I in serum and follicular fluid were correlated positively ($r = 0.69, P < 0.01$; Echternkamp et al., 1990). However, recently some scientists have begun to transfer their focus from circulating IGF-I to paracrine IGF-I. Clapper and Taylor (2011) discovered similar increases among mRNA expression of IGF-I, IGF-IR, LHβ subunit, IGFBP2, and IGFBP5 in the anterior pituitary on d 19 of the estrous cycle ($P < 0.05$), but not in serum IGF-I in vivo. They claimed the estradiol induced IGFBP2 and IGFBP5 that occurred near the time of ovulation in pigs might enhance IGF activity and cause a direct release of LH, eventually leading to final follicular development and subsequent ovulation.

2.2.2. Steroidogenesis

IGF-I can promote FSH-supported progestin and inhibin biosynthesis, estrogen production, and LH receptor induction. IGF-I has also been shown to promote both the basal and FSH-supported production of proteoglycans (Adashi, 1998). Mani et al. (2010) used human IGF-I to treat bovine granulosa cell cultures and found that the method for IGF-I to up-regulate estrogen production is by promoting the cholesterol
side chain cleavage enzyme, 3β-HSD and aromatase transcription. The most effective concentration of IGF-I for doing so was 50 ng/mL.

2.2.3. Twinning Rate

A fine mapping study for twinning rate in cattle (Kim et al., 2009) found that an SNP in intron 2 of the IGF-I gene was significantly associated with twinning rate (P < 0.03), confirming that the IGF-I gene is a positional candidate gene for twinning rate in cattle. However twinning rate in cattle is not generally preferred, as it increases the dystocia occurrence.

2.2.4. Embryo Transplantation

IGF-I also contributes to transferred embryo survivability. *In vitro* treatment of bovine embryos with human IGF-I at 50 ng/mL (P < 0.05) increased blastocyst cell number (Sirisathien et al., 2003) and at 100 ng/mL (P < 0.05) reduced apoptosis (Byrne et al., 2002). IGF-I protected *in vitro* bovine embryos from heat shock at 100 ng/mL, thus the increasing the pregnancy rate (d 53, P < 0.05) and calving rate (P < 0.07) for the recipients conceiving via embryo transfer (Block J et al., 2003). However, no *in vivo* study has been conducted so far on the effect of IGF-I on the survivability of transferred embryos, probably due to the difficulty in measuring embryo IGF-I concentration.
2.2.5. **Dose-Dependent Apoptosis in Female Reproductive Function**

Although IGF-I acts mainly in cell proliferation, differentiation and apoptosis inhibition, its role is not always the same. Mani et al. (2010) found that adding 50 ng/mL of human IGF-I to cultured bovine granulosa cells initiated apoptosis through the AKT pathway, and this was not related to the down-regulation of IGF-IR. Velazquez et al. (2011) found that supraphysiological IGF-I concentration at 1,000 ng/mL did not improve blastocyst formation in in vitro bovine embryos but induced higher levels of apoptosis. However, no studies involving the dose between 100 ng/mL and 1,000 ng/mL were found.

2.3. **Effects of IGF-I on Growth and Body Composition**

As early as 1993, gene knock-out experiments showed that IGF-I−/− or IGF-II−/− mice were approximately 40% smaller than littermates, though they were viable; IGF-I-IGF-II doubly deficient mice were 70% smaller than littermates (Liu et al., 1993). IGF-I and other IGF family members are crucial to the growth of animals. Recent studies have validated the effects of IGF-I on growth rate, feed conversion efficiency, and meat quality (Davis et al., 2003; Estany et al., 2007; Lancaster et al., 2008).

Ge et al. (2003) found a single nucleotide polymorphism in the promoter region of the
growth hormone receptor gene that was associated with postweaning serum IGF-I concentration. Mullen et al. (2011) identified an association (P < 0.05) of the IGF-I gene promoter region with increased cow carcass weight, and of the 3’ region with increased functional survival and decreased chest width. Ge et al. (2001) identified a genotype specific association (P < 0.05) of exon region of IGF-I gene at 5’ site with increasing weight gain during the first 20 d after weaning. Estany et al. (2008) demonstrated that intronic polymorphic cytosine-adenosine (CA) repeats played a role in IGF-I expression, and that increased circulating IGF-I concentration at 160 d of age was associated with early fat thickness in Landrace boars (\(-0.57 \pm 0.20\) mm per CA; \(P = 0.005\)) and lean content (\(7.52 \pm 3.00\) g/kg per CA at 105 kg; \(P = 0.013\)) adjusted for carcass weight in Duroc barrows.

Some scientists are interested in the local effects of IGF-I. Villafuerte et al. (2000) found strong correlations (0.53 ~ 0.75, \(P < 0.001\)) between IGF-I and leptin mRNA expression in rat epididymal, retroperitoneal, mesenteric, and subcutaneous inguinal white adipose tissue. However, the adipose expression of IGF-I is not consistent with the plasma concentration (\(P = 0.16\)). The strong correlation (\(r > 0.51, P < 0.0001\)) of IGF-I and leptin with fat-cell size confirmed their relationship to adipose development, indicating the potential importance of studying region-specific IGF-I regulation.

2.4. Reproduction Traits and Related Body Development for Cattle and Other Ruminants
The age at which heifers reach puberty is approximately 350 d ~ 389 d. Purebred Angus heifers reached puberty at 372.2 ± 10 d in the previous century (Laster et al., 1972), but approximately 15 d earlier currently due to improvement in nutrition and management improvement. Fertility of heifers bred at the pubertal estrus was 21% lower than for those bred on their third estrus, which means heifers need to be bred 2 to 3 mo after reaching puberty (Patterson et al., 1992). The average gestation length is 273 d for Aberdeen Angus, and up to 19 d longer for other cattle, which varies by the breed and species and is affected by body weight, environmental factors such as season, and management methods (Andersen and Plum, 1965). Some studies showed that a faster preweaning growth rate, but a slower postweaning growth rate were related to earlier age at puberty. Also, a delayed decrease in lean body weight:fat ratio occurred in later-maturing breeds of cattle (Patterson et al., 1992). Age at puberty could be decreased by selection for younger puberty age (Morris et al., 2011).

As age at puberty define the earliest date for mating, similar physical situations that affect rate of maturing also affect the mating age and calving age of cattle. However, age at first calving or mating is restricted mostly by management considerations rather than by the physiological condition of cattle themselves. Because most calves are weaned simultaneously at a particular time point for the sake of convenience, calves born late in the calving season are usually lighter than those born early, which tends to decrease the total lifetime productivity of their dams. In addition, cows that calve late in one year tend to calve late or not to calve at all in the next year (Patterson et al.,
Therefore early calving is preferred. Twenty four months is the most common age at first calving for the beef industry in the U.S. Calving at 24 mo of age is also optimal for dairy cattle. For the dairy cattle industry, age at first calving could affect milk yield, fat yield, fat percentage, and lifetime and productive life. Earlier age at first calving of Holstein cows is slightly related to decreased milk yield, fat yield, and productive life, but increased lifetime and fat percentage (phenotypic correlations are -0.089, -0.034, -0.093, 0.052 and 0.055, respectively, P < 0.001, Nilforooshan and Edriss, 2004). A study in pigs (Saito et al., 2010) also showed that gilts in high performance herds had earlier age at first mating, which was related to fewer pigs born alive, but a lower culling risk (P < 0.05).

For a long period of time in the beef industry, mating was mainly conducted by natural service. This method is still used today, though AI is becoming more popular. In our experiment, although AI was used in the later years, the total number of AI matings was only 2 per breeding season for each female, which is also the most common situation for the whole U.S. beef industry. Therefore, the method for calculating conception or pregnancy rate is simply the total number of pregnant females divided by total number of mated females, unlike that for dairy cattle, which is for each female, 1 for success or 0 for failure, divided by the number of AI services. According to a study in Angus heifers (Bormann et al., 2006), examination of each AI matings is unnecessary, as the heritability for first service conception rate is only 0.03 ± 0.03, compared to the estimate of 0.13 ± 0.07 for overall pregnancy rate.
Interestingly, Warnick and Hansen (2009) found in their experiment involving British beef cattle and Braham crossbred beef cattle that infertility was not related to reduced ovulation or fertilization rates (P > 0.1), but rather was related to embryonic mortality (P < 0.001). This may attribute the difference in pregnancy rate and calving rate to the embryo survival, and, also, implies an important apoptotic role of IGF-I in embryonic development.

### 2.5. Selection Experiments involving Serum IGF-I Concentration

Postweaning serum IGF-I concentration is reported to be moderately to highly heritable with a heritability ranging from 0.26 to 0.52 (Davis and Simmon, 1997; Davis and Simmen, 2000; Davis et al., 2003; Yilmaz et al., 2004; Davis and Simmen, 2006).

Divergent selection for blood serum IGF-I concentration has been successful in a variety of animals. Blair et al. (1988) demonstrated a difference in mature body weights of mice after 7 generations in high and low selected IGF-I lines, and observed a positive correlation (high vs. low difference = 1.7 g = 12%) between IGF-I and 6-wk live weight (Blair et al., 1989). Siddiqui et al. (1990) found mice from the high IGF-I line had significantly greater body weight (P < 0.001) during the 28 to 105 d period than those from the low and control lines in the same experiment. Davis et al. (1997) demonstrated in beef cattle genetic correlations of serum IGF-I concentration
with weaning and postweaning weight and postweaning weight gain of -0.21, -0.38, and -0.54, respectively, indicating that low IGF-I line animals had greater weights and weight gains. Huang et al. (2011) in the same experiment found the selection responses of birth weight and postweaning body weight gain in high IGF-I line cattle were negative (-0.05 kg/yr and -0.46 kg/yr, respectively, P < 0.001), but in low line cattle were positive (0.09 kg/yr and 0.22 kg/yr, respectively, P < 0.05), implying that selection for high serum IGF-I concentration leads to smaller body weight and fewer postweaning weight gain.

Davis et al. (2003) reported moderate positive (0.23) genetic correlations of serum IGF-I with backfat thickness and longissimus muscle area at 140-d postweaning, whereas in bulls (2000), lower IGF-I concentrations were related to higher marbling scores (-0.53), quality grades (-0.45), backfat thickness (-0.26), and yield grade (-0.27), indicating a possible role of IGF-I in determining body composition. Afolayan et al. (2008) reported that the genetic correlation of IGF-I was positive for ultrasound eye muscle depth (0.15), and negative for ultrasound fat depth (-0.12) in mature ewes. The genetic correlation between IGF-I and the average number of lambs born per ewe mated was negative (-0.18), whereas that for the average number of lambs weaned per ewe mated was positive (0.10), although the relationship was not strong.

Lancaster et al. (2008) established a regression relationship between IGF-I and residual feed intake (RFI), and found that the regression coefficients tended (P = 0.15)
to be positive for bulls (5.38 g/d) and negative for heifers (-9.49 g/d). They also reported a sex and IGF-I line interaction for the regression between serum IGF-I concentration and RFI at weaning. IGF-I had positive effects on RFI in heifers but negative effects in bulls (coefficients: 5.16 ± 2.04 g/d for bulls, P < 0.05; -3.14 ± 3.37 g/d for heifers, P < 0.05).

High circulating IGF-I concentration is associated with earlier calving in Angus heifers (Davis and Bishop, 1991). Yilmaz et al. (2004) reported that heifers in the high IGF-I line calved 4.02 ± 2.18 d earlier (correlation (r_A) = -0.23 ~ -0.14, P = 0.07) than did the low IGF-I line heifers. Genetic correlations of IGF-I measurements with calving rate were moderate (r_A = 0.41~ 0.48), indicating a tendency toward an increase in calving rate with increased IGF-I concentration in cows. Yilmaz et al. (1999) also reported increased scrotal circumference and percentage of normal sperm cells in bulls selected for increased serum IGF-I concentration (P < 0.05).

2.6. Selection Experiment involving Female Reproductive Traits

A selection experiment based on twin births (Echternkamp et al., 1990) indicated higher (P < 0.05) IGF-I concentration in both serum and follicular fluid of twin-producing purebred and crossbred Simmental and Charolais cows than in monotocous cows. Moreover, the ratio of percentage of concentration of follicular fluid IGF-I to that of serum was lower (P < 0.05) in large follicles of twin-producing
cattle than in monotocous cattle. On the other hand, the concentration of estradiol and progesterone in follicular fluid of small or large follicles did not differ between the two groups (P > 0.05). Results of Echternkamp et al. (1990) imply that high natural twinning rate is associated with high IGF-I concentration in both blood and follicular fluid.

2.7. Genetic Parameters and Correlations for Female Reproductive, Growth, and Body Composition Traits

Reproductive traits usually have low heritabilities. Therefore, direct selection for reproductive traits has generally resulted in slow progress. Boligon and Albuquerque (2011) showed that heifer pregnancy rate at 16 mo of age has a heritability of 0.45 ± 0.02, which is greater than the estimate of 0.10 ± 0.01 for age at first calving. Genetic correlations between heifer pregnancy at 16 mo of age and weight gain from birth to weaning at 240 d of age, yearling weight, and mature weight were 0.19 ± 0.04, 0.25 ± 0.06 and 0.14 ± 0.05, respectively (P < 0.001). Genetic correlations between age at first calving and the other 3 traits listed above were -0.18 ± 0.06, -0.22 ± 0.05, and -0.12 ± 0.05, respectively (P < 0.001). However Yimaz et al. (2004) obtained a higher heritability (0.26 ± 0.28) for age at first calving and a lower heritability (0.11 ± 0.05) for conception rate across years in a selection experiment for serum IGF-I concentration of Angus beef cattle. Forni and Albuquerque (2005) demonstrated that the correlation between days to calving and
age at first calving was 0.76, between days to calving and weight at 550 d was 0.07, and between days to first calving and age at first calving was 0.94, but between days to first calving and weight at 550 d was -0.02 (P < 0.05) (I checked the article it is true. The sample size is big). Segura-Correa et al. (2012) also found a very small direct genetic correlation (-0.02) between age at first calving and weaning weight. These results indicate that earlier age at first calving is related to higher pregnancy rate, greater body weight and weight gain of offspring, and thus earlier age available for mating or slaughter, as well as shorter generation interval. Sufficient pedigree information and potential major effect candidate genes such as the IGF-I gene could help accelerate the rate of improvement due to selection for these reproductive traits.

2.8. General Description of MTDFREML

Multiple-trait derivative-free restricted maximum likelihood (MTDFREML) is a statistical procedure with optimal properties employed in a set of computer programs used to obtain estimates of (co)variance components using derivative-free REML (Boldman, 1993). It can be used to analyze single traits, multiple traits and sex-limited traits, such as conception rate for females in one data set using missing values for traits that cannot be measured. After running, the breeding values, contrasts, expectations of solutions, and solutions for fixed effects can be calculated. MTDFREML consists of 3 main programs. MTDFNRM computes the inverse of the numerator relationship matrix (\(A^{-1}\)). MTDFPREP prepares the data and formulates
the mixed model equations by converting the data from MTDFNRM to file format, recoding levels of fixed and random effects and coding continuous variables as deviations from the mean. MTDFRUN produces solutions for covariates, fixed and random effects, (co)variance components and expectations of solutions, and searches for (co)variances to maximize the log likelihood function. MTDFRUN uses an iterative process that replaces the worst point estimate during each round until convergence is achieved. Estimation of variance components using MTDFREML consists of 2 independent steps. The first step involves updating of the data using the simple method such that the log likelihood for the data is maximized. The second step involves usage of the derivative-free method to minimize the F VALUE. The F VALUE is the corresponding function value for -2 log likelihood function of the parameter vector to be estimated. The point with the largest F VALUE represents the worst point estimate.

2.9. General Description of ASReml

ASReml is used to fit linear mixed models to large data sets with complex variance models, which is often the case for biological experimental data. It also uses the REML method like MTDFREML, and uses the average information algorithm as well as sparse matrix methods in the analysis (Gilmour et al., 2009).

Compared to MTDFREML, ASReml has several advances in application: it can be used to analyze (un)balanced longitudinal data, repeated measurements with spline
type models, multivariate analysis with more than 2 variates, (un)balanced designed experiments, multi-environment trials and meta analysis, with regular or irregular spatial data.

In my experiment, ASReml was used for multivariate analysis of IGF-I, reproductive traits, growth traits, and body composition traits.
CHAPTER 3: Materials and Methods

3.1. Description of Traits

Serum IGF-I concentration measurements at d 28, 42, and 56 of postweaning period are abbreviated as IGF28, IGF42, and IGF56, respectively. The average of these 3 measurements is abbreviated as MEANIGF.

Age at first calving (AFC) was the age in days when a heifer gave birth to her first calf. Twinning rate (TR) was the number of twin sets born, given the number of pregnant females. Conception rate (CR) corresponded to whether a palpable fetus was present or a calf was born, given the cow or heifer was mated. The CR was calculated using the total number of palpable fetuses or calves born divided by the total number of matings for the heifers and cows. All born or unborn calves in each mating were created and assigned an ID, with a “conception” column as either 1 for palpable or 0 for open (i.e., not palpable). In the correlation and heritability analysis in MTDFREML, estimates of conception rate were approximate, given the conception rate was treated as a continuous variable (Yilmaz et al., 2004). The same situation was true for calving rate, which was defined as whether a calf was born, given the cow or heifer was mated. Days to calving (DC) was the difference in days from the earliest calving date in the herd to when a given female calved.
3.2. Experimental Design

3.2.1. Selection Procedures

Divergent selection for blood serum IGF-I concentration began at the Eastern Agricultural Research Station (EARS) 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 concentration. Cows from the initial base population were randomly assigned to the 2 IGF-I selection lines in both seasons. Each year, cows from each of the 4 groups (spring high IGF-I line, spring low IGF-I line, fall high IGF-I line, fall low IGF-I line) were mated only within their groups. Four bulls with highest IGF-I concentrations and 4 bulls with lowest concentrations were selected for breeding as yearlings and were then sold following the breeding season. The male: female ratio was approximately 1:13 for each breeding season, with the fluctuation due to death or animal deficiency at that moment. Approximately 8 cows from each line were culled each year (based on physical unsoundness, failure to conceive in 2 consecutive years, and oldest age) and replaced with approximately 8 pregnant heifers with the highest or lowest residuals (adjusted for age of calf and age of dam) for serum IGF-I concentration. Selection was based on the mean IGF-I concentration of 3 blood samples taken at d 28, 42, and 56 of the 140-d postweaning test (Davis et al., 1995; Davis and Simmen, 1997; Yilmaz et al.,
Mating of relatives such as brothers and sisters, parents and offspring, and offspring and their grandparents were avoided to decrease inbreeding. The average inbreeding coefficient among all animals born from 1989 to 2003 was 0.04.

Mating was by natural service except that some artificial insemination was used from the spring 1991 to fall 1994 breeding seasons to create links between the EARS herd and other herds contributing to North Central Regional Project NC-196, “The Genetics of Body Composition in Beef Cattle”. Approximately 10 cows per selection line were randomly chosen and artificially inseminated in each breeding season using semen from an Angus reference sire. Starting from year 2002 all matings were by AI using semen collected from bulls within the selection line instead of by natural service.

In 1990, excess heifers were available at the end of the spring breeding season and additional heifers were needed for the fall breeding season in the high and low IGF-I selection lines. Thus, pregnant heifers were aborted and transferred from the spring- to fall-breeding herds without changing their IGF-I selection lines. The number of heifers transferred from the spring to fall herds in the high and low IGF-I lines were 10 and 7, respectively. These heifers that were transferred from the spring- to the fall-calving herd were approximately 2.5 yr old, and, hence, were excluded from the age at first calving analysis, but kept for the conception rate and calving rate analyses.

All information presented here concerning the selection procedures was taken from 2004).
3.2.2. Management Procedures

Spring-born calves were reared by their dams without creep feed until weaning at approximately 7 mo of age. After weaning, bull calves were given ad libitum access to a corn-soybean meal based diet and grass hay. Heifers born before 1994 were given ad libitum access to nonprotein nitrogen (feed grade urea)-treated corn silage and grass hay at the North Appalachian Experimental Watershed (NAEW). Heifers born from 1994 onward were fed a corn-soybean meal diet at EARS in order to obtain postweaning gains of 0.75 kg/d. After an approximately 3 wk adjustment period following weaning, the postweaning test began, and the first day of test was considered as d 0 or on-test date.

Fall-born calves were weaned at a mean age of 140 d and then fed a corn-soybean meal diet and grass hay in drylot for 112 d. The postweaning test began thereafter. Bull calves remained at EARS for the postweaning test with the same management as spring-born bulls. Heifers born in fall 1993 or earlier were transported to the NAEW and managed in the same manner as spring-born heifers, whereas heifers born after 1993 stayed at EARS (Bishop et al., 1991; Davis et al., 1995; Davis and Simmen, 1997).

The first mating of heifers was supposed to occur at a date after all heifers reached puberty, so that the age of puberty would be excluded from being a confounding

Davis et al. (1995) and Davis and Simmen (1997).
factor. According to a progesterone test conducted in 1997 (Yilmaz, 2003), the age at puberty for heifers ranged from 320 to 398 d, with a mean of 356 d. The age at first mating across all years was about 450 d of age. Therefore, most of the heifers should have reached puberty before they were mated.

3.3. Data Collection

Data from years 1989 to 2003 were used in this study. Tracking information of conception and calving rate in 2004 and 2005 was used for females born in 2002 and 2003.

3.3.1. Serum Samples and Radio Immunoassay (RIA) for IGF-I

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. 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). The number of observations for IGF28, IGF42, IGF56, and mean IGF-I differed because IGF42 was not measured in calves born in 1989 and a few of the blood samples were lost during the experimental procedures. In addition, the IGF-I measurements for d 28, 42, and 56 were
unavailable for heifers born in spring 1990 owing to a freezer malfunction. Heifers born in the spring 1990 calving season were resampled on d 84, 98, and 112 of the postweaning test. However, these samples were deleted from the analysis because they were collected at different ages from those used in the other years of the study.

3.3.2. Pregnancy Diagnosis

Pregnancy diagnosis check was conducted by either ultrasound or rectal palpation. Palpation via the rectum was performed approximately 60 d after the end of the breeding season. Conception rate was based on the pregnancy diagnosis records. Females conceiving to the clean-up bulls that were used after the conclusion of the AI breeding season were considered to be non-pregnant for purpose of the data analysis because the clean-up bulls were of a different breed and were not selected for IGF-I concentration. Pregnancy records for females that were mated to AI reference sires in 1991 to 1994 were deleted from the analysis.

3.4. Statistical Analysis

3.4.1. Test for Significance Levels of Factors and Test for Block Comparisons

The Statistical Analysis System 9.2 (SAS 9.2) (SAS Inst. Inc., Cary, NC) was used for tests of significance of fixed effects and covariates in the single-variate model.
PROC MIXED was used for the normally distributed traits IGF28, IGF42, IGF56, MEANIGF, BF140, REA140, WT140, AFC, and DC. PROC GLIMMIX was used for the binomially distributed traits CR, CAR, and TW. Although in SAS 9.3 PROC HPMIX is able to create A^{-1} for pedigree analysis, in SAS 9.2 this function is not complete, thus, only simpler functions were used. Based on the large number of observation (more than 2,000 observations), the LSMEANS analysis without pedigrees could be similar to that with pedigree information (reference?). A P-value of 0.05 was used as the criterion for significance. Except for IGF-I line and season of birth, all other effects, including birth year, on-test age of calves, age of dams, sex, and mating number that were nonsignificant were removed from the models. The variable “mating number” was created to track the sequence of matings for a female. On-test age of calf was used as a linear covariate for all traits except conception rate, calving rate, and days to calving, which were repeated traits. Age of dam was grouped into 6 categories: 2-yr-old, 3-yr-old, 4-yr-old, 5- to 9-yr-old, 10-yr-old and 11-yr old and greater, according to the Beef Improvement Federation Guidelines (Cundiff et al., 2010). Sex was deleted from the models when female reproductive data were analyzed.

3.4.2. Single- and Bi-Variate Analysis for (Co)variance within and between IGF-I and Reproductive, Growth, and Body Composition Traits

The MTDFREML programs (Boldman et al., 1995) were used for single- and
bi-variate analyses to estimate variance and covariance components. All of the traits were first analyzed using full animal models with significant fixed effects and random effects including additive and maternal genetic, environment, permanent environment, and phenotypic components, but were simplified for bi-variate analyses if models without permanent environment effects for either dam or female calves had smaller logL or bigger -2logL in likelihood ratio tests. Final results also include heritabilities of all traits addressed in the above section, genetic, environmental and phenotypic correlations between traits, and correlations between direct and maternal genetic effects within traits.

Note that although CR and CAR are binary variables, MTDFREML can only treat them as continuous variables. When analyzing the correlations between them and other non-repeated traits, the non-repeated traits only kept the records in the 1st mating, and left the other records of the same female as -99.

Pedigrees of base population animals were traced back 3 generations to create the numerator relationship matrix. The total number of animals in the numerator relationship matrix, including base animals, was 4,142, of which 1,757 were inbred. The average inbreeding coefficient was 0.04.

In all analyses, it was assumed that convergence was attained if the variance of the simplex algorithm was less than $10^{-9}$, and -2log likelihood did not change between the last 2 computer runs at the second decimal digit.
### 3.4.3. Multi-Variate Analysis for All Traits

ASReml software (Gilmour et al., 2009) was used for the multi-variate analysis among all traits, to obtain the additive genetic correlations among them to desire the additive genetic, environmental and phenotypic correlations. The first step was to do a single analysis for each trait to obtain the variances, which were used as initial values in the multi-variate model. Then traits were added into the model one by one to finally form a full model. For the reason that ASReml can only include 1 binomially distributed variate in the model, CR and CAR were analyzed separately with all other traits except DC. The DC is a variable that only come with successful calving records, therefore it was analyzed with all other traits except CR and CAR.

The factors used for all traits were the same as in MTDFREML. The differences between the ASReml and MTDFREML analyses were 1) the models in ASReml were multi-variate models; therefore the variance of each trait was smaller than those in bi-variate or single-variate analysis in MTDFREML. 2) CR and CAR were treated as binary variables in ASReml with a distribution of

$$\Theta(x_i) = \log \left[ \frac{\mu(x_i)}{1 - \mu(x_i)} \right].$$

The default convergence criterion of the ASReml software is that the variance of the simplex algorithm is less than $10^{-8}$ and the log likelihood does not change between the last 2 computer runs at the third decimal digit.
CHAPTER 4: Results and Discussion

4.1. Factor and LSMeans Analysis in SAS

4.1.1. Statistical Summary of Data

Simple statistics for the traits analyzed in this study are summarized in Table 1. The total number of live animals in the raw data was 2,662, including animals in the pedigree without records the total number of animals was 4,142. Mean values for IGF28, IGF42, IGF56 and MEANIGF are slightly higher than those of Davis and Simmen (2010) using data from the same study, due to the deletion of the records in 1990. The AFC of 733 d agrees well with the value of 731.8 d in Yilmaz (2004). In order to count the conception and calving rate, unborn or dead-after-birth calves were created for the calculation. Therefore, the numbers of calves for conception and calving rate were more than 2,662. The conception and calving rates were lower than the typical value of 85% found in the beef industry (Smith et al., 2012), because clean-up bulls and reference sires were not members of the selection lines and were excluded from the analysis. The mean percentage for the calving rate of 69.2% was lower than in Yilmaz (2004) based on data collected up to year 2001 (77.3%). Twinning rate across all years was only 0.41%, because only 11 twin sets were found in the data. There were additional grafted twins, however the parents of these twins could not be traced.
4.1.2. Data Analysis for Normally Distributed Traits

All continuous variates were approximately normally distributed based on normal-quantile plots, and therefore, did not require transformation.

Effects of line on IGF28, IGF42, IGF56, and MEANIGF in Table 2 were significant, indicating the selection was successful. The high vs. low line difference for mean values of IGF28, IGF42, IGF56 and MEANIGF were 61.83, 62.84, 56.05, and 50.03, respectively (ng/mL, P < 0.01) across years (Table 3). The significant line difference in the last year of selection also proved the success of selection (Table 4). Only IGF28 was affected by season, but IGF28, IGF42, and MEANIGF demonstrated a line × season interaction. The levels of significance for season, age of dam, and on-test ages of calves were not all the same as in the previous study of Yilmaz (2004), due to the larger sample size resulting from the addition of data from 2002 and 2003.

In the initial year of 1989, all traits except conception and calving rate were similar in the 2 lines (Table 3). The reason for the relatively higher conception and calving rates in the high line than in the low line is unclear. In 2003, only IGF-I concentrations and ribeye area showed significant differences between lines (Table 3). However, line differences across years (Table 4) showed that high line calves had 7.13 kg lower WT140, 5 fewer twin sets, 4.02 d earlier AFC, 2.35 d shorter DC (P < 0.01), 1.62 cm² larger ribeye area, and 0.05 cm thicker BF140 (Table 3, 4 and 5).
Birth year was a significant source of variance for all traits except TW, and sex was significant for all IGF-I concentration measurements, body composition traits, and growth traits (Table 5 and 6). All traits except MEANIGF, BF140, and REA140 were related to the on-test age.

### 4.1.3. Binomial Analysis of Conception and Calving Rate

Under the PROC GLIMMIX procedure of SAS, significant fixed effects for conception rate of all females were only birth year and age of dam group (Table 6). Line and line × season interaction were not significant. Cows had greater conception (cow vs. heifer = 76.07% vs. 71.2%, P < 0.05) and calving rates (cow vs. heifer = 79.34% vs. 56.8%, P < 0.0001) than heifers, which might have been due to greater body weights, greater hip height and pelvic size, and more mature hormone regulation. The cows that did not calve for 2 consecutive years were culled for economic reasons, which might have introduced bias; therefore, data from heifers and cows were separated. Line and season had significant effects on conception rate of heifers but not cows (Table 6). A frequency test, however, indicated that high line females had 2.67% higher conception rate than low line females under Fisher’s exact sided test (P = 0.05, Table 7), although under a Chi-square test the P-value was 0.08. The difference between the Chi-square test and Fisher’s exact sided test is that the former requires the frequency in each cell be at least 5, and its P-value is usually larger than the latter. This small P-value compared to mixed model probably was
due to differences between the methods of analysis, considering there is no fixed or covariate effect in the Chi-square test. Also, this difference can be attributed to the line difference in the spring, but not fall, because no significant line difference in the fall was detected (Fisher’s exact sided test P = 0.4). Heifers had higher conception rates in the high line than in the low line in the spring herd (line difference = 1.67%, P < 0.0001), and also across seasons (line difference = 6.02%, \( \chi^2 \) P = 0.05, and Fisher’s exact sided test P < 0.03). In the fall herd this difference was nonsignificant. The pattern for cows was complicated. In the spring herd, cows in the high line had 5.43% greater conception rate than low line cows (\( \chi^2 \) P = 0.03; Fisher’s exact sided test P = 0.02). However, in the fall, low line cows had 3.74% greater conception rate (Fisher’s exact sided test P = 0.05).

Selection line affected calving rate of all females (P=0.06; Table 6). No difference was found between spring and fall, but a line × season interaction occurred (P=0.09; Table 6). Both season and line affected heifer calving rate. Line did not affect cows’ calving rate, but a line × season interaction was observed. Mating number influenced cows’ calving rate. Age of dam affected conception and calving rate of all females but not of cows, implying a maternal effect in the early age of females, which was reduced as the females aged.

The Chi-square test results were in consistent with the PROC GLIMMIX results in that high line females had a 4.04% higher calving rate than low line females (Table 7). The line difference in heifers was 7.23%, and in both seasons high line heifers showed
higher calving rate than low line heifers. Spring-born heifers also had a higher calving rate than fall-born heifers. However, the seasonal pattern in cows was opposite to that of heifers in that spring-born cows had a 3.83% lower calving rate, and in the fall the high line cows also had a 2.98% lower calving rate. This pattern is in accordance with the results for conception rate. This conflicting pattern for cows in fall and spring is hard to explain. Santolaria et al. (2012) found that Holstein-Friesian dairy cows inseminated in a cool season had higher calving rate than those inseminated in a warm season (likelihood ratio difference = 0.49, P < 0.001), providing evidence for the influence of season on the fertility of cows. King and Macleod (1983) also indicated that fall-calving Hereford and Hereford×Holstein cows had better reproductive performance (P < 0.01). In our study, fall-calving cows also had a higher calving rate than spring-calving cows (spring vs. fall = 70.45% vs. 74.27%, sided test P = 0.03). This result may have been caused by lower temperature in winter than in summer and the nutritional difference in grass and hay at mating and during the gestation period.

4.2. Analysis of Calving Distribution by 21-d Period of Calving Season

The calving distribution has a certain pattern by date of the calving season. Considering the date of calving is related to date of mating, and the latter is affected by estrus cycle, which is 21 d in length in beef cattle, the calving season was divided
into 5 21-d periods. The calving rate in high vs. low line females differed in the first 21-d periods (37.26% vs. 33.1%, P < 0.001). In later periods the number of calves born in both lines decreased rapidly. The number of calves born to high line females decreased more rapidly than in low line females, but this difference was nonsignificant (Figure 1). In the spring herd, the first 21-d period had a significant line difference in calving rate (Figure 2, high vs. low line = 38.22% vs. 29.63%, P < 0.001). In the fall herd, the line difference across periods was significant (Fisher’s exact sided test P = 0.04), but in single 21-d periods was not. The first 21-d period showed an opposite pattern in that high line females calved at a 2.07% lower rate than low line females, although the difference was not significant.

Cows and heifers also had different calving patterns. Cows had a higher calving rate than heifers (72.08% vs. 60.54%, P < 0.0001). Dystocia of heifers due to small size may be one of the reasons for this result (Patterson et al., 1992). Heifers in the spring herd had a significant line difference in the first 21-d period, but not in other periods (Table 3). In the fall herd a difference occurred in the second 21-d period. Cows in the spring herd showed significant line differences in the first and second 21-d periods, but in fall no periods had significant line differences. The first 21-d period even showed a higher calving rate in the low line (high vs. low line = 38.90% vs. 42.41%, P > 0.05, Table 8), but the difference was not significant. High line calving rate after the second period in both seasons of heifers and cows dropped more drastically than in the low line.
4.3. (Co)variance Analysis using Single-Variate Model in MTDFREML

Presented in Tables 9 and 10 are the estimates of direct \( \left( h_d^2 \right) \) and maternal \( \left( h_m^2 \right) \) heritability, the proportion of phenotypic variance due to permanent environmental effect of the dam \( \left( c^2 \right) \) and female calves \( \left( c'^2 \right) \), and the correlation between direct and maternal genetic effects \( \left( r_{am} \right) \). The sample size for TW was very small (11 twin sets out of 2,662 calvings). Therefore, TW was not analyzed further. Estimates of \( h_m^2 \) were small \( \left( \leq 0.21 \right) \) for all traits. Estimates of \( c^2 \) and \( c'^2 \) were close to 0 \( \left( < 0.01 \right) \) for all traits except that \( c'^2 \) for AFC was 0.46. These results agree well with those of Yilmaz et al. (2004) except that \( c^2 \) for AFC in their study was 0.28. The very small \( c^2 \) and \( c'^2 \) may have been due to the small selection proportion of approximately 0.2, and dams that were only in the pedigree files and did not have records in this experiment, so that insufficient offspring \( \left( < 2 \right) \) per dam did not allow the separation of \( c^2 \) and \( c'^2 \) from the environmental effects.

For IGF28, IGF42, IGF56, and MEANIGF, \( h_d^2 \) ranged from 0.43 to 0.50, slightly larger than the estimates of 0.41 ~ 0.46 in Davis and Simmen (2010) and 0.35 ~ 0.48 in Huang et al. (2011). The estimates of \( h_m^2, c^2, \) and \( r_{am} \) for IGF28, IGF42, IGF56, and MEANIGF also agree well with their results of 0.09 ~ 0.16 and 0.15 ~ 0.17 for \( h_m^2 \), 0 for \( c^2 \), and -0.92 ~ -0.87 and -0.91 ~ -0.87 for \( r_{am} \).

For AFC, the \( h_d^2 \) of 0.35 is larger than Forni and Albuquerque’s (2005) estimate of
0.06 in Nelore cattle, Segura-Correa et al.’s (2011) estimate of 0.08 for Brown Swiss cattle, and Boligon et al.’s (2010) estimate of 0.17 ± 0.01. In addition my estimate is also different from the estimate of 1 by Yilmaz et al. (2004), because in addition to the line-transfer heifers in 1990 that were mentioned in chapter 3, I also deleted AFC values larger than 951 d, which were outliers. The reason for defining the outliers is that some heifers failed to conceive in the first year that they were mated, but conceived in the second year, and these data were used to determine their AFC. The distribution of AFC before and after deleting the outliers is shown in Figure 5 and 6. The age of 951 d was derived from farm records. Heifers in the spring herd were born between March 11th and May 10th, and were mated between approximately June 1st and July 30th of the next year. Heifers in the fall herd were born between August 8th and October 9th, and were mated between approximately November 1st and December 30th of next year. Therefore, the AFC should have ranged from 586 to 708 d for spring-born heifers and from 586 to 709 d for fall-born heifers. If they were mated the second year, the AFC ranged from 951 to 1,074 d. The dataset contained extremely large values greater than 1,000 d, and no records for AFC between 800 and 1,000. Therefore, AFC records larger than 1,000 d were deleted. Before deleting the outliers, the $h^2$ was 0.03, still different from the value of 1 reportedly by Yilmaz et al. (2004). The estimate of $c^2$ was smaller than 0.01, different from the estimate of 0.77 in Yilmaz et al. (2004), but $c^2$ equaled 0.46. The large $c^2$ for AFC occurred because all heifers in the model were used, and the average number of offsprings (> 2) per heifer throughout the years allowed the separation of
c² from the residual (environment variance).

For DC, the small $h^2_\text{D}$ of 0.06 ± 0.05 agrees with the results of Forni and Albuquerque (2005) of 0.04 ~ 0.06, although their DC was days from mating to calving. This low heritability is probably due to 2 reasons. First, the variation in DC was very small, ranging from 1 to 92 (the true values range from 0 to 91, but because 0 is an invalid value in MTDFREML and ASReml, 1 was added to each value). The second reason is that days from mating to calving is a more meaningful trait than DC with fewer confounding factors. However, in this study most of the matings were by natural service; therefore, accurate records for date of mating were unavailable. The variation in gestation length was unaccounted for and only contributed to the residual variance (environmental effects) in the model, leaving very little genetic variation for DC. The small $h^2_\text{D}$ of 0.06 may imply that DC is not a good approximation of gestation length.

Estimates of $h^2_\text{D}$ of BF140, REA140, and WT140, were 0.53 ± 0.08, 0.63 ± 0.08, and 0.62 ± 0.08, respectively. Estimates of $h^2_\text{D}$ for BF140 and REA140 were much larger than those of 0.17 ± 0.01 and 0.31 ± 0.13 in the study of Davis et al. (2003), and the estimate of $h^2_\text{D}$ for WT140 was larger than the estimate of 0.46 ± 0.08 by Davis and Simmen (2008), and 0.58 ± 0.07 in the study of Splan et al. (2002), probably due to new records in later selection years.

Estimates of $h^2_\text{D}$ for CR and CAR were small, ranging from 0.04 to 0.23 (Table 9). The estimate for calving rate of 0.13 ± 0.06 agrees well with that of 0.11 in Yilmaz et
al. (2004). The $h_d^2$ for heifers CR was the largest of all estimates for CR and CAR, and the estimate of $h_d^2$ for cows was less than 0.1. The difference in cow vs. heifer estimates may be because some cows were culled after 2 consecutive failures for conception, which could have caused errors, and decreased the genetic variance. Also, cows may be affected more by environmental effects such as nutrition, disease and management than heifers. Estimates of $h_m^2$ and $c^2$ were smaller than 0.1. The $h_m^2$ of cows was even smaller than for heifers, indicating that the maternal genetic effects decreased when heifers grew to adulthood. This could also be explained by the exclusion of age of dam in the model for CR and CAR of cows. The $r_{am}$ close to -1 for all females and for cows, indicating that the separation of maternal genetic variances from direct genetic variances was probably incomplete. However Yilmaz et al. (2004) also reported very large estimates of $r_{am}$ (-0.94) for CR. The estimates of $c^2$ for all females and for cows were small, but were moderate for heifers.

In this study, body composition, growth traits, and MEANIGF were highly heritable, and IGF28, IGF42, IGF56, and AFC were moderately heritable. Heritabilities of DC, CR, and CAR were low.

Direct genetic ($r_{AIA2}$), maternal genetic ($r_{MIM2}$), phenotypic ($r_{P1P2}$), and environmental ($r_{E1E2}$) correlations of IGF-I concentration with reproductive, body composition, and growth traits are shown in Table 10 and 11. The estimate of $r_{AIA2}$ for AFC was -0.4 in the full model, smaller than the estimate of reported by Yilmaz e al. (2004), who used a reduced model without maternal variances, indicating that selection on serum
IGF-I concentration could lead to earlier AFC. The estimate of $r_{P1P2}$ was smaller than $r_{A1A2}$, probably due to the negative $r_{E1E2}$, as a negative environmental correlation always implies a smaller phenotypic correlation than genetic correlation (Searle, 1961). The estimate of $r_{A1A2}$ for DC and MEANIGF was -0.08. Considering that the heritability of DC was also low, selection on IGF-I may be affected if the goal is to improve DC.

The estimate of $r_{A1A2}$ for BF140 and MEANIGF was 0.03. This value is surprisingly smaller than the value $0.19 \pm 0.2$ reported in the study of Davis et al. (2003), and the value of 0.18 for the correlation between weaning IGF-I and backfat thickness found in the study of Lancaster et al. (2008). In our study, the small direct genetic correlation of 0.03 is in accordance with the nonsignificant line difference (Table 4).

However, Davis and Simmen (2000) displayed a negative genetic correlation (-0.28) between MEANIGF and BF140. Olausson et al. (2010) found that women with a lower body fat content before pregnancy had greater increases in serum IGFBP-1 during pregnancy than women with a higher prepregnant body fat content, and IGFBP-1 is positively related to IGF-I in serum. These results implied that the effect of IGF-I on fat deposition traits may be contrary in males and females.

The estimate of $r_{A1A2}$ for REA140 and MEANIGF was 0.38, larger than the value of $0.20 \pm 0.22$ in Davis et al. (2003), 0.17 in Davis and Simmen (2000), and 0.15 between weaning IGF-I and longissimus muscle area in Lancaster et al. (2008) check if has std.
The estimate of $r_{A1A2}$ for WT140 and MEANIGF was 0.15, interestingly, opposite to the pattern of negative difference for the high vs. low line ($P < 0.01$, Table 4). If the correlation is correct, then the difference in WT140 in the high vs. low lines must have been affected by other factors that were not in the model. This result is also opposite to the estimates of -0.54 ~ -0.21 for $r_{A1A2}$ between postweaning serum IGF-I concentration and weaning weight, postweaning weight, and postweaning weight gain in the study of Davis et al. (1997), but in accordance with the estimate of 0.28 ± 0.11 between MEANIGF and d 140 postweaning weight in the study of Davis and Simmen (2006). Huang et al. (2011), using the same data, reported negative genetic trends for postweaning weight gain over years in the high line, and positive genetic trends in the low line ($P < 0.05$). Olausson et al. (2010) published positive correlations between serum IGF-I concentration and maternal body weight ($r = 0.47 ~ 0.56$) in human. Their results may imply that, although selection on IGF-I has a positive effect on postweaning body weight of Angus cattle, the effect may decrease as year of selection increases.

Estimates of $r_{A1A2}$ for IGF-I with CR and CAR were moderate, ranging from 0.28 to 0.43. The $r_{A1A2}$ of 0.43 between CAR and IGF-I of all females agrees with the value of -0.41 in Yilmaz et al. (2004). The negative value from his study was due to the fact that conception was coded as 1 and open was coded 100. In the current study I the signs of correlations were converted to make the values accord with practical meaning. The estimate for $r_{A1A2}$ for CR and MEANIGF was smaller than that for CAR and MEANIGF, probably due to the impacts of IGF-I on embryo development,
such as cell proliferation, differentiation, and apoptosis. The $r_{A1A2}$ for heifers was larger than for cows. The difference may be because 1) IGF-I concentration was only measured during postweaning periods, when soon afterwards the heifers were mated. However, mating for cows was a repeated measurement by years, and may be related more to the serum IGF-I concentration at mating and during gestation than to postweaning IGF-I. Swangchan-Uthai et al. (2011) demonstrated that circulating IGF-I is lower in cows than in heifers in Holstein-Friesian cattle ($P < 0.05$); 2) cows may be affected more by environmental effects than heifers; 3) changes in hormones and other physiological factors in cows could have different patterns from changes in IGF-I with age.

Estimates of $r_{M1M2}$ were somewhat larger than estimates of $r_{A1A2}$, indicating that CR and CAR are strongly affected by maternal effects. The estimates of $r_{E1E2}$ were close to 0 except between CR of cows and MEANIGF, implying common environmental effects on both serum IGF-I concentration and calving rate of cows. The $r_{P1P2}$ were generally smaller than the genetic correlations. Searle (1961) demonstrated that a phenotypic correlation less than its genetic counterpart, together with a small positive environmental correlation, will occur where the genes governing 2 traits are similar, but where the environments pertaining to the expression of these traits have a low correlation. Therefore serum IGF-I concentration may shares genes with CR and CAR. These genes could be hormone or hormone regulation genes.
4.4. (Co)variance Analysis using Multi-variate Model in ASReml

The additive genetic correlations from multi-variate ASReml analysis are shown from Tables 11 to 14. Table 15 provides a comparison of the results of MTDFREML and ASReml using the representatives of the binary variable CR and the continuous variable MEANIGF. For both variables, ASReml gave smaller $h_d^2$ estimates than MTDFREML. For CR, ASReml gave larger $h_m^2$ than MTDFREML, and the $h_m^2$ estimate from the binary model was larger than the $h_d^2$ estimate.

ASReml can only include 1 binary variable in a model; therefore CR and CAR were analyzed separately. The correlations with other variables in the 2 models were slightly different, due to 2 reasons: 1) CR and CAR had missing values in different records; therefore the samples and sample sizes in the 2 models were different; 2) The total variance and proportion of each variance for each trait in the 2 models were different, considering variables, samples, and sample sizes were different.

The estimated correlations of MEANIGF with CR and CAR were slightly smaller using the bivariate model in ASReml in MTDFREML (0.12 vs. 0.32 and 0.24 vs. 0.43, respectively). Also, correlations of MEANIGF with BF140, REA140, WT140, and AFC were different in the bivariate analysis than in MTDFREML (-0.09 and -0.15 vs. 0.03, 0.63 and 0.29 vs. 0.38, -0.56 and -0.15 vs. 0.15, and -0.14 and -0.15 vs. -0.40, respectively). In addition to the difference in software, the multi-variate model had different total variance, and different proportions of variances for the model and for
the residual from the bi-variate model. Both models scale the independent variables
together by the covariates, and then do the same thing for the dependent variables,
eventually finding the first canonical correlation for the independent and dependent
variables. However, in the multi-variate model, each variable is scaled more closely
to the average internal size variable than in the bi-variate model, and appears
isometric as a consequence (Jungers and German, 1981). Some variables are scaled
largely while some are not, and some are positively scaled, whereas others are
negatively done, to keep the squares of the coefficients average equal to 1.0. For
example, variables with large variations, each as MEANIGF, are more likely to shrink,
but variables such as BF140 with small variations are more likely to expand.

The AFC was negatively associated with CR and CAR, but the correlation was
nonsignificant. It is understandable that heifers that initiate calving early also
sexually mature early. Perry (2012) reported in his research on South Dakota cattle
that heifers that calved with their first calf during the first 21 d period of the calving
season had increased longevity compared to heifers that calved in the second 21 d
period (5.1 ± 0.1yr and 3.9 ± 0.1yr, respectively). Better longevity could relate to
higher calving and conception rates, but no evidence of this relationship has been
reported so far.

The \( r_{A1A2} \) between WT140 and AFC ranged from -0.41 to -0.31 (P < 0.05). Boligon
et al. (2010) reported a range of -0.26 to -0.14 for genetic correlations of AFC with
weaning to yearling weight gain, yearling to after yearling weight gain, weight at
weaning, yearling weight, after yearling weight and weight, from 2 to 5 yr of age. Segura-Correa et al. (2011) reported a -0.02 direct genetic correlation and -0.29 phenotypic correlations between AFC and weaning weight of Brown Swiss cattle. These results indicate that early age at first calving is correlated with increased body weight. It also relates to heavier weaning weight of the offspring (Ferry, 2012).

\( r_{A1A2} \) of WT140 with CR and CAR are nonsignificant (-0.02 and -0.00, respectively, \( P > 0.05 \)). It is obviously that CR and CAR are more likely to be influenced by body size, hormone and lipo distribution, which are not necessarily linearly proportional to postweaning body weight.

BF140 has small negative additive genetic correlations with CR and CAR, but is moderately to highly and positively related to AFC (-0.12, -0.00, and 0.22 and 0.71, respectively). Patterson et al. (1992) reported that a delayed decrease in lean body weight:fat ratio occurred in later-maturing breeds of cattle (Patterson et al., 1992), implying that increased fat percentage is related to decreased age at puberty. Brethour (2004) reported that backfat thickness was an important predictor of the percentage of empty body fat in steers (percent empty body gain as fat = 35.69 + 2.152 (±0.279) initial backfat, \( r^2 = 0.807, P < 0.001 \)). His results are contrary to those of the current study.

The REA140 was not correlated with AFC or CAR (\( r_{A1A2} = 0.0001 \) and 0.0002, and 0.03, respectively \( P > 0.3 \)) but was positively correlated with CR (\( r_{A1A2} = 0.12, P < 0.001 \)).
The model with DC, MEANIGF, BF140, REA140, WT140, and AFC yielded very small correlations \((10^{-9} \sim 10^{-7})\) and went to 0 at convergence; therefore those results were excluded from the Results and Discussion. It was difficult to fit the correct variance matrix for the model, possibly because DC is not an accurate indicator of the progesterone length. A similar study by Forni and Albuquerque (2005) reported that the additive genetic correlation between DC and AFC was 0.76 and between DC and body weight adjusted to d 550 of age was -0.02. From a management point of view, heifers that calve early at first calving have longer post-partum intervals and are more likely to breed back as 2-yr-olds and continue to calve early in the calving season (Perry, 2012). As a result, AFC is positively related to DC. However, the correlation of body weight in their study was small, as body weight is less likely to change as cows mature.

Single variate analysis results in ASReml were not fully recorded in this study. However a comparison of SAS and ASReml results using the data from this study can be subject of future research.

4.5. Prediction Function for Reproductive Traits

The best LS fitted regression equation with all possible factors used to predict AFC even had an \(r^2\) equal to 0.05, although coefficient analysis for MEANIGF and CALFAGE were significantly different from 0, and model variance was significantly
greater than 0 (P < 0.05). WT140 was also a significant covariate for AFC, but the $r^2$ of the model was only 0.03. For models of CR and CAR, in which MEANIGF was not a repeated measurement, the regression equation could not be formed.

4.6. Implications

Results from this study indicate that including serum IGF-I concentration in selection programs that aim to improve female reproductive traits may increase rate of genetic gain. However, selection based on a single hormone is not an ideal method to obtain large genetic improvement. The effect of IGF-I on postweaning weight and backfat thickness are complicated, and these 2 traits are correlated with female reproductive traits. Gene mapping and genome wide selection may have greater impact on selection response for reproduction traits, as these methods can identify desirable combinations of multiple genes that benefit to favored economically important traits.
Tables and Figures

Table 1. Unadjusted means, standard deviations, coefficients of variation, and minimum and maximum values for serum IGF-I concentration, reproductive, body composition, and growth traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>No.</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF28, ng/mL</td>
<td>1,978</td>
<td>249.03</td>
<td>166.51</td>
<td>66.86</td>
<td>15.10</td>
<td>1025.80</td>
</tr>
<tr>
<td>IGF42, ng/mL</td>
<td>1,975</td>
<td>266.23</td>
<td>176.97</td>
<td>66.47</td>
<td>15.38</td>
<td>1020.80</td>
</tr>
<tr>
<td>IGF56, ng/mL</td>
<td>1,973</td>
<td>266.74</td>
<td>176.30</td>
<td>66.09</td>
<td>9.52</td>
<td>974.70</td>
</tr>
<tr>
<td>MEANIGF, ng/mL</td>
<td>1,977</td>
<td>260.84</td>
<td>164.33</td>
<td>63.00</td>
<td>14.21</td>
<td>913.27</td>
</tr>
<tr>
<td>AFC, d</td>
<td>403</td>
<td>732.76</td>
<td>21.91</td>
<td>2.99</td>
<td>677</td>
<td>798</td>
</tr>
<tr>
<td>CAR, %</td>
<td>3,913</td>
<td>69.20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR, %</td>
<td>3,686</td>
<td>74.66%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR, %</td>
<td>2,656</td>
<td>0.41%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC, d</td>
<td>2,003</td>
<td>27.59</td>
<td>18.01</td>
<td>65.28</td>
<td>1</td>
<td>92</td>
</tr>
<tr>
<td>BF140, cm</td>
<td>1,755</td>
<td>0.75</td>
<td>0.33</td>
<td>43.80</td>
<td>0.03</td>
<td>2.03</td>
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<tr>
<td>REA140, cm²</td>
<td>1,717</td>
<td>64.35</td>
<td>20.24</td>
<td>31.45</td>
<td>5.29</td>
<td>133.48</td>
</tr>
<tr>
<td>WT140, kg</td>
<td>2,050</td>
<td>387.55</td>
<td>82.96</td>
<td>21.41</td>
<td>147.42</td>
<td>607.81</td>
</tr>
</tbody>
</table>

Acronyms: IGF28, IGF42, and IGF56 = serum IGF-I concentration at d 28, 42, and 56, respectively, of the 140-d postweaning test; MEANIGF = the average of 3 IGF-I measurements; CR = conception rate; CAR = calving rate; AFC = age at first calving; DC = days to calving from calving of the first female in the same calving season; BF140 = d 140 postweaning backfat thickness; REA140 = d 140 postweaning ribeye area; WT140 = d 140 postweaning body weight.

Calving rate was entered as either 1 (if calved) or 100 (if did not calve). In this study, 69.20% of the matings resulted in a calf. Similarly, conception rate was entered as either 1 (if conceived) or 100 (if did not conceive), and in this study 74.66% of the matings resulted in a palpable fetus. Missing values were excluded from the study.
Table 2. Levels of significance for fixed effects used in the analysis of serum IGF-I concentration

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>Trait^b</th>
<th>IGF28</th>
<th>IGF42</th>
<th>IGF56</th>
<th>MEANIGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth year</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IGF-I line</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td>0.005</td>
<td>0.4</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Line × season</td>
<td></td>
<td>0.005</td>
<td>0.001</td>
<td>0.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Age of dam</td>
<td></td>
<td>0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>On-test age</td>
<td></td>
<td>0.005</td>
<td>0.01</td>
<td>0.01</td>
<td>0.06</td>
</tr>
</tbody>
</table>

^aEffects that had a P-value of less than 0.10 (in bold) were considered significant and were included in the MTDFREML analysis.

^bAcronyms: IGF28, IGF42, and IGF56 = serum IGF-I concentration at d 28, 42, and 56, respectively, of the 140-d postweaning test; MEANIGF = the average of 3 IGF-I measurements.
Table 3. LS means for serum IGF-I concentration, reproductive, body composition, and growth traits in initial year (1989) and end year (2003) of the experiment

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Line</td>
<td>Low line</td>
<td>High Line</td>
<td>Low line</td>
</tr>
<tr>
<td>IGF28, ng/mL</td>
<td>36.94 ± 1.77</td>
<td>38.08 ± 2.12</td>
<td>463.06 ± 30.52</td>
<td>284.41 ± 30.91</td>
</tr>
<tr>
<td>IGF42, ng/mL</td>
<td>122.56 ± 7.11</td>
<td>120.53 ± 8.05</td>
<td>487.91 ± 28.69</td>
<td>318.93 ± 29.05</td>
</tr>
<tr>
<td>IGF56, ng/mL</td>
<td>61.62 ± 3.01</td>
<td>63.90 ± 3.77</td>
<td>388.24 ± 24.63</td>
<td>267.99 ± 22.05</td>
</tr>
<tr>
<td>MEANIGF, ng/mL</td>
<td>115.11 ± 9.81</td>
<td>111.19 ± 10.76</td>
<td>412.94 ± 21.92</td>
<td>289.02 ± 25.79</td>
</tr>
<tr>
<td>CR, %</td>
<td>88.1%</td>
<td>77.55%</td>
<td>71.43%</td>
<td>40.00%</td>
</tr>
<tr>
<td>CAR, %</td>
<td>83.76%</td>
<td>72.34%</td>
<td>71.43%</td>
<td>40.00%</td>
</tr>
<tr>
<td>DC, d</td>
<td>28.30 ± 1.90</td>
<td>28.98 ± 2.04</td>
<td>19.24 ± 9.93</td>
<td>46.46 ± 12.10</td>
</tr>
<tr>
<td>AFC, d</td>
<td>788.61 ± 19.05</td>
<td>788.23 ± 21.30</td>
<td>745.11 ± 5.78</td>
<td>742.19 ± 5.65</td>
</tr>
<tr>
<td>TW</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BKFT140, cm</td>
<td>0.44 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.68 ± 0.05</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>REA140, cm</td>
<td>61.91 ± 1.87</td>
<td>64.48 ± 1.83</td>
<td>65.91 ± 3.82</td>
<td>56.64 ± 4.35</td>
</tr>
<tr>
<td>WT140, kg</td>
<td>384.99 ± 5.82</td>
<td>397.12 ± 6.15</td>
<td>352.57 ± 14.05</td>
<td>353.23 ± 16.53</td>
</tr>
</tbody>
</table>

*Acronyms: IGF28, IGF42, and IGF56 = serum IGF-I concentration at d 28, 42, and 56, respectively, of the 140-d postweaning test; MEANIGF = the average of 3 IGF-I measurements; CR = conception rate; CAR = calving rate; AFC = age at first calving; DC = days to calving from calving of the first female in the same calving season; BF140 = d 140 postweaning backfat thickness; REA140 = d 140 postweaning ribeye area; WT140 = d 140 postweaning body weight.

*bIGF42, BF140, and REA140 did not have records in 1989; therefore, the 1990 data were used for the initial year.

cCR and CAR only contain heifers of that year.

Significant difference between each pair of means (P < 0.05).
Table 4. Line and season LS means for IGF-I, body composition and growth traits across years

<table>
<thead>
<tr>
<th>Trait</th>
<th>Line</th>
<th>Season</th>
<th>High</th>
<th>Low</th>
<th>Spring</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF28, ng/mL</td>
<td>305.28 ± 18.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>243.41 ± 18.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>265.57 ± 18.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>283.12 ± 18.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IGF42, ng/mL</td>
<td>328.41 ± 19.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>265.57 ± 19.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>298.32 ± 19.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>295.66 ± 19.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IGF56, ng/mL</td>
<td>284.13 ± 18.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>228.09 ± 18.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>259.50 ± 18.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>252.72 ± 18.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MEANIGF, ng/mL</td>
<td>292.15 ± 17.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>233.12 ± 17.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>261.67 ± 17.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263.60 ± 17.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DC, d</td>
<td>26.82 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>29.17 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.49 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.37 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>AFC, d</td>
<td>734.37 ± 1.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>738.39 ± 2.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>742.35 ± 1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>730.41 ± 2.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Twin</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>BKFT140, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.75 ± 0.02</td>
<td></td>
<td>0.74 ± 0.02</td>
<td>0.77 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>REA140, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>64.62 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>62.90 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.82 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.71 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>WT140, kg</td>
<td>375.44 ± 2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>382.57 ± 2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>386.47 ± 2.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>371.54 ± 2.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Acronyms: IGF28, IGF42, and IGF56 = serum IGF-I concentration at d 28, 42, and 56, respectively, of the 140-d postweaning test; MEANIGF = the average of 3 IGF-I measurements; CR = conception rate; CAR = calving rate; AFC = age at first calving; DC = days to calving from calving of the first female in the same calving season; BF140 = d 140 postweaning backfat thickness; REA140 = d 140 postweaning ribeye area; WT140 = d 140 postweaning body weight.

<sup>x,y</sup> Significant difference between each pair of means (P < 0.05).

Table 5. Levels of significance for fixed effects used in the analysis of reproductive, body composition, and growth data<sup>a</sup>

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>Trait&lt;sup&gt;b&lt;/sup&gt;</th>
<th>AFC</th>
<th>DC</th>
<th>TW</th>
<th>BF140</th>
<th>REA140</th>
<th>WT140</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth year</td>
<td>0.0003</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>IGF-I line</td>
<td>0.02</td>
<td>0.005</td>
<td>0.03</td>
<td>0.9</td>
<td>0.2</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>&lt; 0.0001</td>
<td>0.2</td>
<td>0.6</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Line × season</td>
<td>0.2</td>
<td>0.05</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Age of dam</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.008</td>
<td>0.03</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>On-test age</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.9</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Mating no.</td>
<td>-</td>
<td>0.2</td>
<td><strong>0.05</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Effects that had a P-value of less than 0.10 (in bold) were considered significant and were included in the MTDFREML analysis.

<sup>b</sup>Acronyms: CR = conception rate; CAR = calving rate; AFC = age at first calving; DC = days to calving from calving of the first female in the same calving season; BF140 = d 140 postweaning backfat thickness; REA140 = d 140 postweaning ribeye area; WT140 = d 140 postweaning body weight.
Table 6. Levels of significance for fixed effects used in the analysis of conception rate and calving rate data

<table>
<thead>
<tr>
<th>Trait</th>
<th>Fixed effects</th>
<th>Birth year</th>
<th>IGF-I line</th>
<th>Season</th>
<th>Line × season</th>
<th>Age of dam</th>
<th>Mating no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>&lt; 0.0001</td>
<td>0.2</td>
<td>0.7</td>
<td>0.1</td>
<td>0.05</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>CR Heifer</td>
<td>&lt; 0.0001</td>
<td>0.02</td>
<td>&lt; 0.0001</td>
<td>0.4</td>
<td>0.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>&lt; 0.0001</td>
<td>0.9</td>
<td>0.1</td>
<td>0.005</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>&lt; 0.0001</td>
<td>0.06</td>
<td>0.6</td>
<td>0.09</td>
<td>0.03</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>CAR Heifer</td>
<td>&lt; 0.0001</td>
<td>0.03</td>
<td>0.0003</td>
<td>0.7</td>
<td>0.06</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>&lt; 0.0001</td>
<td>0.6</td>
<td>0.05</td>
<td>0.02</td>
<td>0.3</td>
<td>0.0002</td>
<td></td>
</tr>
</tbody>
</table>

aEffects that had a P-value of less than 0.10 (in bold) were considered significant and were included in the MTDFREML analysis.

bAcronyms: CR = conception rate; CAR = calving rate.

Table 7. Chi-square and Fisher’s exact sided test for line and season differences in conception rate (%) across years

<table>
<thead>
<tr>
<th></th>
<th>χ²</th>
<th>1</th>
<th>2</th>
<th>χ² P-value</th>
<th>Fisher’s P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High vs. Low</td>
<td>75.67x</td>
<td>73.00y</td>
<td>0.09</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Spring vs. Fall</td>
<td>75.13</td>
<td>73.40</td>
<td>0.3</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Spring × Line</td>
<td>77.73x</td>
<td>72.53y</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Fall × Line</td>
<td>73.13</td>
<td>73.72</td>
<td>0.8</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Heifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High vs. Low</td>
<td>73.48x</td>
<td>67.46y</td>
<td>0.05</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Spring vs. Fall</td>
<td>75.75x</td>
<td>63.85y</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Spring × Line</td>
<td>78.09</td>
<td>73.39</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Fall × Line</td>
<td>67.94x</td>
<td>58.82y</td>
<td>0.07</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High vs. Low</td>
<td>76.62</td>
<td>75.30</td>
<td>0.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Spring vs. Fall</td>
<td>74.90</td>
<td>77.49</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Spring × Line</td>
<td>77.61x</td>
<td>72.18y</td>
<td>0.03</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Fall × Line</td>
<td>76.36x</td>
<td>80.10y</td>
<td>0.1</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

x,y Significant difference between each pair of means (P < 0.05).
### Table 8. Chi-square and Fisher's exact sided test for line and season differences in calving rate (%) across years

<table>
<thead>
<tr>
<th></th>
<th>$\chi^2$</th>
<th>1</th>
<th>2</th>
<th>$\chi^2$ P-value</th>
<th>Fisher's P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High vs. Low</td>
<td>71.14$^a$</td>
<td>67.10$^y$</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Spring vs. Fall</td>
<td>69.37</td>
<td>68.97</td>
<td>0.8</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Spring × Line</td>
<td>72.77$^a$</td>
<td>65.97$^y$</td>
<td>0.04</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Fall × Line</td>
<td>69.16</td>
<td>68.75</td>
<td>0.9</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Heifer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High vs. Low</td>
<td>64.24$^a$</td>
<td>57.01$^y$</td>
<td>0.05</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Spring vs. Fall</td>
<td>66.05$^a$</td>
<td>53.97$^y$</td>
<td>0.001</td>
<td>$&lt; 0.0001$</td>
<td></td>
</tr>
<tr>
<td>Spring × Line</td>
<td>69.68$^a$</td>
<td>57.69$^y$</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Fall × Line</td>
<td>62.43$^a$</td>
<td>50.00$^y$</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><strong>Cow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High vs. Low</td>
<td>73.40</td>
<td>70.60</td>
<td>0.2</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Spring vs. Fall</td>
<td>70.45$^a$</td>
<td>74.27$^y$</td>
<td>0.06</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Spring × Line</td>
<td>73.78$^a$</td>
<td>67.13$^y$</td>
<td>0.01</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Fall × Line</td>
<td>72.94</td>
<td>75.92</td>
<td>0.4</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

$^a,^y$ Significant difference between each pair of means (P < 0.05).

### Table 9. Parameter estimates from single-trait analyses of blood serum IGF-I concentration, reproductive, body composition, and growth traits

<table>
<thead>
<tr>
<th></th>
<th>IGF28$^a$</th>
<th>IGF42</th>
<th>IGF56</th>
<th>MEAN IGF</th>
<th>AFC</th>
<th>DC</th>
<th>BF140</th>
<th>REA140</th>
<th>WT140</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h_d^2$</td>
<td>0.46</td>
<td>0.43</td>
<td>0.35</td>
<td>0.50</td>
<td>0.35</td>
<td>0.06</td>
<td>0.53</td>
<td>0.63</td>
<td>0.62</td>
</tr>
<tr>
<td>$h_m^2$</td>
<td>± 0.07</td>
<td>± 0.07</td>
<td>± 0.07</td>
<td>± 0.07</td>
<td>± 0.20</td>
<td>± 0.05</td>
<td>± 0.08</td>
<td>± 0.08</td>
<td>± 0.08</td>
</tr>
<tr>
<td>$r_{am}$</td>
<td>0.21</td>
<td>0.17</td>
<td>0.11</td>
<td>0.21</td>
<td>0.10</td>
<td>0.01</td>
<td>0.13</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>$c^2$</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.1</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$c'$</td>
<td>-</td>
<td>-</td>
<td></td>
<td>&lt; 0.01</td>
<td></td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Acronyms: IGF28, IGF42, and IGF56 = serum IGF-I concentration at d 28, 42, and 56, respectively, of the 140-d postweaning test; MEANIGF = the average of 3 IGF-I measurements; AFC = age at first calving; DC = days to calving from calving of the first female in the same calving season; BF140 = d 140 postweaning backfat thickness; REA140 = d 140 postweaning ribeye area; WT140 = d 140 postweaning body weight.

$^b$h$_d^2$ = direct heritability; h$_m^2$ = maternal heritability; c$^2$ = proportion of phenotypic variance due to permanent environmental effect of dam; c' = proportion of phenotypic variance due to permanent environmental effect of female calf; r$_{am}$ = correlation between direct and maternal genetic effects.

For items ≥ 0.01, P < 0.004.
Table 10. Parameter estimates from single-trait analyses of conception rate and calving rate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CR</th>
<th>CAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h_d^2$</td>
<td>0.11 ± 0.06</td>
<td>0.23 ± 0.12</td>
</tr>
<tr>
<td>$h_m^2$</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>$r_{am}$</td>
<td>-0.88</td>
<td>-0.74</td>
</tr>
<tr>
<td>$c^2$</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>$c_2^2$</td>
<td>0.09</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Acronyms: CR = conception rate; CAR = calving rate. $h_d^2$ = direct heritability; $h_m^2$ = maternal heritability; $c^2$ = proportion of phenotypic variance due to permanent environmental effect of dam; $c_2^2$ = proportion of phenotypic variance due to permanent environmental effect of female calf; $r_{am}$ = correlation between direct and maternal genetic effects.

For items ≥ 0.01, $P < 0.05$.

Table 11. Additive genetic, maternal genetic, environmental, and phenotypic correlations of mean IGF-I concentration with reproductive, body composition, and growth traits

<table>
<thead>
<tr>
<th>Correlation</th>
<th>AFC</th>
<th>DC</th>
<th>BF140</th>
<th>REA140</th>
<th>WT140</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{A1A2}$</td>
<td>-0.40</td>
<td>-0.08</td>
<td>0.03</td>
<td>0.38</td>
<td>0.15</td>
</tr>
<tr>
<td>$r_{M1M2}$</td>
<td>0.33</td>
<td>-0.24</td>
<td>0</td>
<td>0.37</td>
<td>-0.11</td>
</tr>
<tr>
<td>$r_{E1E2}$</td>
<td>-0.10</td>
<td>0.13</td>
<td>0.22</td>
<td>0.55</td>
<td>0.40</td>
</tr>
<tr>
<td>$r_{P1P2}$</td>
<td>-0.53</td>
<td>0.08</td>
<td>0.04</td>
<td>0.32</td>
<td>0.31</td>
</tr>
</tbody>
</table>

All standard errors were zero when rounded to 2 decimal places.

For any correlation ≠ 0, $P ≤ 0.0001$.

MTDFREML was used for the analysis.

Table 12. Additive genetic, maternal genetic, environmental, and phenotypic correlations of mean IGF-I concentration with CR and CAR

<table>
<thead>
<tr>
<th>Correlation</th>
<th>CR</th>
<th>CAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{A1A2}$</td>
<td>0.32</td>
<td>0.43</td>
</tr>
<tr>
<td>$r_{M1M2}$</td>
<td>0.37</td>
<td>0.51</td>
</tr>
<tr>
<td>$r_{E1E2}$</td>
<td>0</td>
<td>-0.01</td>
</tr>
<tr>
<td>$r_{P1P2}$</td>
<td>0.07</td>
<td>0.11</td>
</tr>
</tbody>
</table>

All standard errors were zero when rounded to 2 decimal places.

For any correlation > 0, $P ≤ 0.0001$. 

56
MTDFREML was used for the analysis.

Table 13. Additive genetic correlations among conception rate, age at first calving, body composition traits, and growth traits

<table>
<thead>
<tr>
<th></th>
<th>CR</th>
<th>MEANIGF</th>
<th>BF140</th>
<th>REA140</th>
<th>WT140</th>
<th>AFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>-</td>
<td>0.12</td>
<td>-0.12</td>
<td>0.12</td>
<td>-0.02</td>
<td>-0.00</td>
</tr>
<tr>
<td>MEANIGF</td>
<td>-</td>
<td>-</td>
<td>-0.09</td>
<td>0.63</td>
<td>-0.56</td>
<td>-0.14</td>
</tr>
<tr>
<td>BF140</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.45</td>
<td>-0.39</td>
<td>0.22</td>
</tr>
<tr>
<td>REA140</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.00</td>
<td>-</td>
<td>0.00</td>
</tr>
<tr>
<td>WT140</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.41</td>
</tr>
<tr>
<td>AFC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

2,977 records in total.

For any term > 0.03, P < 0.05.

ASReml was used for the analysis.

Table 14. Additive genetic correlations among calving rate, age at first calving, body composition traits, and growth traits

<table>
<thead>
<tr>
<th></th>
<th>CAR</th>
<th>MEANIGF</th>
<th>BF140</th>
<th>REA140</th>
<th>WT140</th>
<th>AFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR</td>
<td>-</td>
<td>0.24</td>
<td>-0.00</td>
<td>0.03</td>
<td>-0.00</td>
<td>-0.00</td>
</tr>
<tr>
<td>MEANIGF</td>
<td>-</td>
<td>-</td>
<td>-0.15</td>
<td>0.29</td>
<td>-0.15</td>
<td>-0.15</td>
</tr>
<tr>
<td>BF140</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.29</td>
<td>0.29</td>
<td>0.71</td>
</tr>
<tr>
<td>REA140</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>WT140</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.31</td>
</tr>
<tr>
<td>AFC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

2,982 records in total.

For any term > 0.03, P < 0.05.

ASReml was used for the analysis.

Table 15. Comparison of variance terms for CR and MEANIGF estimated using MTDFREML and ASReml

<table>
<thead>
<tr>
<th>Method</th>
<th>CR</th>
<th>MEANIGF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$h_d^2$</td>
<td>$h_m^2$</td>
</tr>
<tr>
<td>MTDFREML</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>ASReml (continuous)</td>
<td>0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>ASReml (binary)</td>
<td>0.02</td>
<td>0.24</td>
</tr>
</tbody>
</table>

$h_d^2$ = direct heritability; $h_m^2$ = maternal heritability

P < 0.05
Figure 1. Percentage of calves born in 21-d intervals across seasons and years

High vs. low line calving rate was 71.14% vs. 67.05% (P = 0.02). Percentage of calves born in 5 intervals: high line: 37.26%, 25.63%, 7.18%, 1.15%, 0.07%; low line: 33.10%, 22.74%, 9.65%, 1.55%, 0.46%.

x,y Significant difference between each item (Chi-sq test P < 0.05).
Figure 2. Percentage of calves born in 21-d intervals across years in spring and fall herds

a. High vs. low line calving rate in spring was 72.77% vs. 65.97% (P = 0.02). Calving rate in 5 intervals: high line: 38.22%, 27.09%, 6.68%, 1.83%, 0; low line: 29.63%, 23.6%, 10.3%, 2.61%, 1%.

b. High vs. low line calving rate in fall was 69.16% vs. 68.75% (P = 0.04). Calving rate in 5 intervals: high line: 36.09%, 25.76%, 7.79%, 1.43%, 0.16%; low line: 38.14%, 22.35%, 8.71%, 0.76%, 0.19%.

x,y Significant difference between each pair of percentages (P < 0.001)
Figure 3. Percentage of calves born to heifers by 21-d intervals across years in spring and fall herds

High vs. low line calving rate was 64.24% vs. 56.76% (P = 0.05)
a. High vs. low line calving rate in spring was 66.05% vs. 53.97% (P = 0.03). Calving rate in 5 intervals: high line: 39.89%, 21.81%, 5.85%, 2.13%, 0%; low line: 27.13%, 22.34%, 9.04%, 2.66%, 1.06%.

b. High vs. low line calving rate in fall was 62.43% vs. 50.00% (P = 0.02). Calving rate in 5 intervals: high line: 26.28%, 22%, 7.05%, 1.92%, 0.68%; low line: 26.03%, 15.75%, 7.53%, 0.68%, 0%.

*xy* significant difference between each pair of percentages (P < 0.05).
Figure 4. Percentages of calves born to cows in 21-d intervals across years in spring and fall herds

High vs. low line calving rate was 73.39% vs. 71.03% (P = 0.3)

a. High vs. low line calving rate was 73.72% vs. 67.04% (P = 0.03). Calving rate in 5 intervals:
   high line: 37.67%, 27.95%, 6.77%, 1.39%, 0%; low line: 30.45%, 24.05%, 10.21%, 1.90%, 0.52%.

b. High vs. low line calving rate was 72.99% vs. 77.00% (P = 0.2). Calving rate in 5 intervals:
   high line: 38.90%, 25.79%, 7.61%, 0.63%, 0%; low line: 42.41%, 23.82%, 8.64%, 0.79%, 0.34%.

*xy* significant difference between each pair of percentages (P < 0.05).
Normal probability plot shows short-tailed pattern with extreme values on the upper right side; standardized residual vs. fitted value plot shows nonsymmetrical pattern with large value outliers; histogram of standardized residual is left skewed; and standardized residual vs. order plot is nonsymmetrical with extremely large values.
Normal probability plot shows linear pattern; standardized residual vs. fitted value plot shows symmetrical irregular pattern; histogram of standardized residual is symmetrical unimodal distributed; and standardized residual vs. order plot does not have extremely large values, although it may indicate a time-series pattern.
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