DEVELOPMENTAL PROGRAMMING IN BEEF CATTLE: THE EFFECTS OF CIRCULATING MATERNAL IGF-1 ON CONCEPTUS DEVELOPMENT, UTERINE HEMODYNAMICS AND SUBSEQUENT OFFSPRING GROWTH

By

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To my husband and family
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<tr>
<td>ADG</td>
<td>Average daily gain</td>
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<tr>
<td>AI</td>
<td>Artificial insemination</td>
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<tr>
<td>ALS</td>
<td>Acid labile subunit</td>
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</tr>
<tr>
<td>BC</td>
<td>Before Christ</td>
<td></td>
</tr>
<tr>
<td>BCS</td>
<td>Body condition score</td>
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<td>BF</td>
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<td>BNC</td>
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<td>bST</td>
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<td>BST</td>
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<td></td>
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<tr>
<td>bw</td>
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<td>BW</td>
<td>Body weight</td>
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<tr>
<td>CIDR</td>
<td>Controlled internal drug release</td>
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<tr>
<td>CL</td>
<td>Corpus luteum</td>
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<tr>
<td>CNL</td>
<td>Crown-to-nose length</td>
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<td>CONT</td>
<td>Control group of heifers</td>
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<tr>
<td>CRL</td>
<td>Crown-to-rump length</td>
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<tr>
<td>CTL</td>
<td>Control group of cows</td>
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<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
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<td>Cyp1</td>
<td>Cyclophilin-1</td>
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<td>E2</td>
<td>Estradiol</td>
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<td>ETOH</td>
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<td>FDA</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<td>GnRH</td>
<td>Gonadotropin releasing hormone</td>
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ha  Hectare
HG  Heart girth
IFNT Interferon-tau
IGF  Insulin-like growth factor system
IGF-1 Insulin-like growth factor 1
IGF1 Insulin-like growth factor 1 gene
IGF2 Insulin-like growth factor 2 gene
IGFBP Insulin-like growth factor binding proteins
IGFBP3 Insulin-like growth factor binding protein 3 gene
IGFR1 Insulin-like growth factor receptor 1
IGFR2 Insulin-like growth factor receptor 2
KPO4 Potassium phosphate
NFREC North Florida Research and Education Center
P4 Progesterone
PAG Pregnancy associated glycoproteins
P4 Progesterone
PBS Phosphate buffered saline
PGE Prostaglandin E2
PGF Prostaglandin F2α
PI Pulsatility index
RI Resistance index
RPS9 Ribosomal protein S9
SEM Standard error of means
TAI Fixed-time artificial insemination
TBS Tris buffered saline
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<td>Uridine diphosphogluconic acid</td>
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<td>US</td>
<td>United States</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<tr>
<td>$V_{\text{max}}$</td>
<td>Maximal systolic velocity</td>
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<tr>
<td>WW</td>
<td>Weaning weight</td>
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DEVELOPMENTAL PROGRAMMING IN BEEF CATTLE: THE EFFECTS OF CIRCULATING MATERNAL IGF-1 ON CONCEPTUS DEVELOPMENT, UTERINE HEMODYNAMICS AND SUBSEQUENT OFFSPRING GROWTH

By

Carla Dean Sanford

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Major: Animal Sciences

The effects of growth hormone (GH) and the insulin-like growth factor (IGF) system on developmental programming in beef cattle was investigated by two studies. In the first study, the effects of recombinant bovine somatotropin (bST) administration on conceptus development was investigated by either administering 500 mg of bST (BST) biweekly until 57 days of gestation, or no bST (CONT) to Angus heifers immediately prior to fixed-time artificial insemination (TAI; d 0). Blood samples were collected for analysis of concentrations of plasma insulin-like growth factor 1 (IGF-1). A subset of pregnant heifers was retained for harvest of liver tissue and assessment of conceptus characteristics by gravid reproductive tract collection. Mean concentrations of IGF-1 were greater in BST than CONT. Mean placental weight, fetal membrane weight, uterine weight, as well as ovarian and corpus luteum weights did not differ between treatments. Fetal crown-to-rump length, fetal weight, heart girth, and liver weight did not differ between treatments. Extraembryonic samples from BST tracts resulted in greater quantities of fetal fluid compared to CONT. There was a tendency for BST heifer tracts to have fewer placentomes and greater
umbilical diameter than CONT. Administration of bST to gestating heifers increased concentrations of IGF-1 but failed to alter fetal development. In the second study, the effects of bST administration on uterine hemodynamics and subsequent development programming of beef calves was investigated. Crossbred beef cows were assigned to receive either bi-weekly injections of bST at TAI (TAI) until d 97 or no bST (CTL). Mean plasma concentrations of IGF-1 were greater in BST than CTL cows. Mean heart girth diameter, crown-to-rump length, and calf BW did not differ between treatments. Uterine hemodynamic parameters were assessed via Color Doppler ultrasonography on d 97 and 233 of gestation. There was a treatment by day interaction for maximal systolic velocity on d 233 to be greater for BST than CTL. Therefore, bi-weekly administration of bST increased plasma concentrations of IGF-1 in beef heifers and cows but failed to alter fetal growth or uterine blood flow. In conclusion, bST administration is not a viable option for stimulating intrauterine development in beef heifers or cows.
CHAPTER 1
INTRODUCTION

Fetal growth success, as well as placental growth and development, are closely interdependent and affected by maternal nutrient supply even at the earliest stage of gestation (Reynolds and Redmer, 1995; 2001). Inadequate maternal nutrient supply leads to poor placental development, resulting in compromised fetal growth (McLean et al., 2017). Offspring performance in livestock has been historically considered to be primarily impacted by genetically determined potential. However, more recent research indicates that events occurring during the intrauterine phase lays the foundation for performance, health and disease. Adverse maternal environment can affect early development by inducing permanent changes in physiology and metabolism in humans (Barker, 2007). Fetal programming is the concept that alterations in the concentration of nutrients and hormones occurring during critical periods during intrauterine development impact fetal growth and cause lifelong modifications of the endocrine and vasoregulative systems (Nüsken et al., 2011). The concept of fetal programming has led to a new area of research in livestock as the consideration that the fetus will rarely express its full genetic potential for growth due to constraints imposed by the maternal environment (Gluckman and Liggins, 1984) would greatly impact modern livestock production. Fetal growth and development influence lifelong performance, health, and disease and are commensurate with placental function (Fowden, 2008; Burton, 2016). More importantly, research in animal models indicate that impaired gestation leads to long-term effects on the offspring in decreased health and productivity (Wu et al., 2006; Caton and Hess, 2010; Funston et al., 2010). Recent fetal programming research has focused on examining how placental function stimulation, or even realignment, can lead to a decrease in morbidity and mortality as well as suboptimal offspring growth performance (Vonnahme and Lemley, 2012). Exponential growth of the fetus and
placenta occurs during the second and third trimester of gestation. However, it has been reported that these pregnancy outcomes are dependent on the growth of the uteroplacental vascular beds that occur in the first half of the pregnancy (Meschia, 1983; Reynolds and Redmer, 1995). More research is needed to better understand the critical timepoints of fetal and placental susceptibility to developmental programming and how food animal producers can utilize this information. Thus, increased knowledge of the effects can lead to strategies of mitigation.
CHAPTER 2
LITERATURE REVIEW

**Beef Production**

Beef production around the world varies by breed, or even species produced, as well as management, nutrition, and reproduction strategies employed to reach the end goal of producing a safe and nutritious product for the consumer. In 2017, there were 32.5 million beef cows in production and 11.4 billion kg of beef produced in the United States (US; USDA, 2018). Furthermore, the US cattle production sector contributed $78.2 billion in cash receipts to the economy in 2016 which represented 21% of the $377 billion generated from agricultural commodities (USDA, 2016). While the US has only 10% of the total global cattle inventory, it is both the largest producer and importer of beef (USDA, 2016). Surprisingly, the number of US citizens directly employed in production agriculture is only 2% (International Labour Organization, 2017). Through continued scientific advancements and adoption of improved management practices, the relatively small subset of the population that produces beef does so yielding more pounds of beef with less inputs, land, and resources for the population than ever before.

The beef industry includes sectors of breeding, feeding, and marketing cattle with an end goal of harvesting, processing, and retail products. The US beef industry can be broken into 3 different sectors, cow-calf, stocker/backgrounder, and feedlot operations (Galyean et al., 2011). The largest sector of the beef industry is cow-calf production (McBride and Mathews, 2011). The seedstock producers, also known as purebred or registered breeders, and commercial cow-calf producers comprise the cow-calf sector with the vast majority of operations selling their calves at weaning while others retain ownership of their calves after weaning (NAHMS, 2017). Commercial cattle primarily raised for consumption make up over 70 percent of all herds in the
US (NAHMS, 2009). It takes approximately 2 to 3 years from the time of breeding a heifer or cow until a finished beef product can be obtained and made available to the consumer. While the cattle production practices, operating environment, market prices, and costs of maintaining a beef operation vary substantially by region, the common goal is to be profitable, and a major determinant of a success in cow-calf operations is reproductive efficiency. Considering the costs associated with developing replacement heifers and maintaining adequate body condition on mature cows, the loss of revenue due to non-pregnant females can be significant.

The selection of genetic traits used for breeding cattle is critical, and should complement both the environment and expected production, despite reproduction being a trait that is not highly heritable. The objective of a cow-calf operation should be for each female to produce a healthy and viable calf annually, and multiple tools are available to assist producers in assessing pregnancy status, such as transrectal palpation, ultrasonography, and milk or blood tests, with estimated costs ranging from $5 to $10 per head. Blood collection or milk sampling can be used from d 28 of pregnancy to detect pregnancy associated glycoproteins (PAG) produced by the placenta and then secreted into the maternal circulation. Additionally, research has indicated that the use of PAGs may be beneficial as a means of predicting late embryonic success in both beef and dairy cattle (Pohler et al., 2013 and 2016; Breukelman et al., 2012; Thompson et al., 2010).

In 2011, it was estimated that it cost the US producer as much as $6.25 for every cow exposed for every 1% decrease in the pregnancy rate, with a national projected gross loss of $2.8 billion (Lamb et al., 2011). The average pregnancy rate for U.S. cow-calf operation is …Producers should strive to have a 95% calf crop during a 45-d calving season with a 600 pound average weaning weight (Stewart and Dyer, 2011). Traditionally, producers have marketed non-pregnant females after confirmation from pregnancy testing; however, more recently, producers have
elected to identify young, non-pregnant females post breeding, expose them to rebreeding, and sell them pregnant to increase their value (da Silva et. al, 2016). This has been a viable option for many producers when pregnancy rates for rebreeding are at least 50% and bred cattle prices and feed costs are manageable (da Silva et. al, 2016).

Assisted reproductive technologies (ART), such as AI, protocols for synchronization of estrus and ovulation, and fixed-time artificial insemination (TAI) have revolutionized cattle production by reducing the generation time needed to improve genetic traits of interest in a herd, increasing the number of females that are pregnant earlier in the breeding season and, consequently, shortening the resulting calving season (Rodgers et al., 2012; Steichen et al, 2013). Decreasing and defining seasons of production results in a more uniform calf crop, which enables producers to better manage their operations by focusing their efforts and inputs into the cyclic phases of production. Furthermore, production profitability and sustainability are dependent upon the longevity of each female in the operation and the necessity of each to produce a healthy and viable calf every year (Damiran et al., 2018). It is also important to take calving time into consideration, it has been reported that average lifetime calf weight produced is highest in those females which calved in the first 21 d of the season (Sprott, 2000). One way to assist in having more calves born in the beginning of the calving season is to utilize ART. Despite the use of ART, not all females will respond to a protocol, conceive, or escape the occurring issue of embryonic loss. Deducing the cause of a nonpregnant female can be difficult as a failure to conceive could result from various problems regarding ovulation or fertilization, early embryonic loss, as well as fetal loss. The use of improved genetics and a strategic nutrition and health management plan continues to be key for a successful pregnancy and productive calf for either beef production or breeding purposes. Additionally, producers must be deliberate in
mitigating environmental stresses that can be detrimental to production such as climate change, heat stress, and drought as environmental stressors can negatively impact the biological, physiological, and immunological systems of cattle. Despite the many advancements in ART used to benefit beef cattle production, there is a lack of knowledge in how environmental stressors, nutrition, and management impact gestating beef females. Further research in the area of reproduction, such as fetal and placental development, reproductive management, and nutrition could be advantageous in understanding how to mitigate these factors and developing therapeutic options to improve global beef production.

**The Bovine Estrous Cycle**

The estrous cycle is the period between the initiation of one estrus to the beginning of the next. In cattle, the estrous cycle is 21 days on average but can vary in length from 17 to 24 days, with activity being modulated by the hypothalamus, pituitary gland, ovary, and uterus. Cattle are continuously polyestrous; thus, a pubertal female will display reoccurring estrous cycle intervals throughout adulthood unless interrupted by pregnancy, postpartum anestrus, or lactation. Anestrus can also occur due to nutritional status, environmental conditions, and pathological conditions of the reproductive system (Senger, 2005).

The estrous cycle is regulated by gonadotropin releasing hormone from the hypothalamus, follicle stimulating (FSH) and luteinizing (LH) hormones from the anterior pituitary, estradiol (E2) and progesterone (P4) from the ovary, and prostaglandin F2α (PGF) from the uterus. As chemical messengers, these hormones travel via the bloodstream to specific target tissues containing hormone-specific receptors, which regulate the phases of the estrous cycle. The bovine estrous cycle is maintained by the follicular and luteal phases in a recurring and sequential manner giving rise to estrus and ovulation. The follicular phase consists of estrus and proestrus stages while the luteal phase consists of metestrus and diestrus. Typically, cattle have 2
to 3 follicular waves per cycle that initiate with the recruitment of a small antral follicle followed by the development of a dominant follicle being selected and eventually becoming either atretic or ovulated (Sirois and Fortune, 1988). The first stage of the estrous cycle initiates when the female exhibits sexual receptivity, estrus, or standing heat, which may be visually observed when a female stands to be mounted by a bull or another female. This behavior is brought about by an increase in the circulating concentrations of E2, resulting from a developing dominant follicle on the ovary, that acts upon several tissues including the uterus (Fortune et al., 1988). Until the development of TAI, labor intensive heat detection was required to observe estrus behavior, and the AM/PM breeding rule whereby females were artificially inseminated 12 hours after the first sign of standing estrus (Trimberger and Davis, 1943). Metestrus occurs with follicular maturation and, finally, ovulation, coupled with the formation of the early corpus luteum (CL) and its ability to secrete P4 (Stevenson, 2007), which is critical for pregnancy maintenance and preventing the return to estrus. Ovulation, the release of the oocyte from the follicle into the oviduct, typically occurs between 24 and 32 hours after the initiation of estrus. As concentrations of P4 increase, the luteal phase, or diestrus, commences until CL regression at the onset of luteolysis, with PGF, produced by the uterus, being responsible for CL regression. Production of P4 decreases concurrent with the regression of the CL when conception does not occur, followed by the initiation of another follicular phase, resulting in a subsequent selection and rapid growth of a new ovulatory follicle (Stevenson, 2007).

**Pregnancy**

Pregnancy success in beef cattle is important from an evolutionary, agronomical, and economical standpoint, and can only occur with successful fertilization, establishment, and maintenance of gestation. When a mammalian female is exposed to breeding, a resulting failure in conception can be due to a variety of factors, such as size or maturity of the ovulatory follicle,
endocrine insufficiency, age, body weight, and body condition or adiposity (Lamb et al., 2001; Vasconcelos et al., 2001; Perry et al., 2005; Pelican et al., 2010; Wen et al., 2010; Var et al., 2011; Scheffer et al., 1999; Souter et al., 2011). A successful breeding, however, will result in significant events occurring over the course of 280 days in the reproductive tract from the fusion of the male and female pronuclei, which will form the zygote that will develop into an embryo and ultimately a calf will be expelled through parturition. Gestational length can vary due to the influence of fetal sex, number of fetuses, breed, genotype of the sire, dam, or fetus, plane of nutrition, and environmental condition; however, undernutrition and heat stress can decrease the length of gestation, negatively impact fetal growth, and yield weak calves (Garverick et al., 1993; Stevenson, 2007).

Following fertilization, the CL remains present on the ovary secreting elevated levels of P4, which suppresses gonadotropin releasing hormone (GnRH) and, consequently, the production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) resulting in decreased levels of E2 production, and prevention of a subsequent estrous cycle (Thatcher et al., 1986). The establishment and maintenance of pregnancy in ruminants is dependent upon interactions between the conceptus, the fetus/embryo and the entirety of the extraembryonic membranes, uterus, as well as the ovarian CL (Bazer et al., 1997). Maternal recognition of pregnancy occurs between d 15 and 17 of gestation in cattle and is a necessary biochemical signaling process blocking luteal regression, and therefore maintaining the lifespan of the CL secreting P4 (Thatcher et al., 1986; Lemley et al., 2015). Progesterone secretion must continue at greater levels to ensure embryogenesis and attachment of the conceptus can occur (Senger, 2012). In cattle, maternal recognition of pregnancy is dependent upon signaling from what was first identified as bovine trophoblast protein 1 and now as interferon-tau (IFNT) (Roberts, 2007;
Helmer et al., 1989). Interferon-tau is secreted by embryonic trophoblast cells with peak secretion at d17 of gestation in cattle before rapidly declining after d 21, which is also the time when the trophectoderm attaches to the uterus (Demmers et al., 2001; Ealy and Yang, 2009). However, IFNT is observed up to day 28 of pregnancy (Lemley et al., 2015). This secretion further stimulates both luteotropic, stimulation of luteal secretion of P4, and antiluteolytic signals, blockage of luteolysis by inhibiting endogenous luteolytic signals (Lemley et al., 2015). By inhibiting the release of PGF within the endometrium and promoting of pre-embryonic protein secretions the uterine environment is receptive to pregnancy (Bazer, 1992; Cheng et al., 2007). Thus, pregnancy failure may be the result of insufficient production and action of P4 and prostaglandin E2 (PGE) due to inadequate response of the endometrium to IFNT (Lemley et al., 2015). Cattle have an elongated conceptus hence, there is an extensive trophectoderm mass that is critical for placental formation as well as attachment and IFNT production. In the absence of a conceptus, oxytocin receptors (OTR) will be expressed, which yields oxytocin (OT) by the CL with an induction of COX-2 and increased synthesis of P4 intermediates with resulting interactions resulting in an embryo-toxic uterine environment (Bazer, 1992; Cheng et al., 2007).

The Bovine Placenta

The placenta is a temporary organ occurring during pregnancy that is an essential endocrine organ that mediates and/or modulates the maternal environment and is responsible for the metabolic exchange between the maternal and fetal systems including the transportation of nutrients, respiratory gases, and wastes (Owens, 1991; Bauer et al., 1998, Reynolds et al., 2006). The placenta type of ruminants is synepitheliochorial in its histological classification based upon the presence of the feto-maternal syncytium, formed by trophoblast binucleated cells that fuse with the uterine epithelial cells as well as the number of cell layers that separate the bloodstreams.
between the maternal circulation and the conceptus circulation (Roberts et al., 2016; Wooding, 1992).

The bovine placenta is complex in its structure with epithelium, connective tissue, and capillaries being present in both the maternal and conceptus layers (Woody, 2008). The placenta that develops in cattle is cotyledonary with 100 to 140 cotyledon-caruncle complexes or placentomes formed from fetal cotyledonary tissue interdigitating into the caruncular crypts of the maternal endometrium (Haeger, 2016; Mossman, 1987). Within these defined placental caruncular crypts, trophoblast cells blanket the chorionic villi, and connective tissue form the villous core and serves as support for the fetal vasculature (King et al., 1979; Chavatte-Palmer and Tarrade, 2016; Ockleford and Wakely, 1982).

Placentomes in cattle are flat and convex with the number of formations increasing with placental growth and development but remains constant from mid-gestation through parturition (Laven and Peters, 2001). Placentome number and vascularity increases during the last two trimesters of gestation in association with increased growth (Borowicz et al., 2007). While it would be assumed that the weight of the placenta alone would elude to the functionality of the organ, research has indicated that weight alone is not sufficient in explaining the changes in placental efficiency and functional capacity and furthermore cannot provide a complete understanding of the effects of factors such as nutrition on placental development (Reynolds et al., 2005). Over the course of gestation, the placental structures alter both in gross morphology and histology to support the continuous growth and development of the fetus as it grows and needs change (Burton et al., 2016). Just as gestational length, birth weight, and estrous cycle length is species specific, so are placental morphometric measurements and are highly dependent upon maternal breed and parity (Bienson et al., 1999; Ford et al., 2002). Exponential growth of
the fetus occurs during the last half of gestation requiring an increase in transplacental function which depends primarily on growth of the placenta during early pregnancy and then further development and reorganization of the uteroplacental vascular function of the placenta later in gestation (Meschia, 1983; Reynolds and Redmer, 1995).

What is particularly astounding is that the placenta is a dynamic temporary organ that has autocrine, paracrine, and endocrine actions which synthesize a range of steroids and peptide hormones that are critical in regulating conceptus development and altering the physiology of the maternal system to support the pregnancy (Gootwine, 2004). Such hormones include estrogens and progesterone, lactogen and placental growth hormone (Murphy et al., 2006). Research has indicated a direct role of estrogens and progesterone concentration in fetal growth regulation, specifically on birth weight and placental weight (Mucci et al, 2003; Mucci et al, 2004). Placental lactogen hormone functions in promoting early embryonic growth and is considered to impact the fetus by stimulating production of other hormones such as IGF-I and insulin (Karabulut et al, 2001; Handwerger & Freemark, 2000).

**Uterine Blood Perfusion**

Growth and development of the fetus, and ultimately neonatal survival, is influenced by vascular development and function of the placenta (Assheton, 1905; Trudinger et al., 1985; Roberts et al., 1992; North et al., 1994; Meyer et al., 1995; Reynolds and Redmer, 1995; Harrington et al., 1997; Spencer et al., 2007; Vonnahme et al., 2007; Grazul-Bilska et al., 2010; Reynolds et al., 2010; Grazul-Bilska et al., 2011; 2013). Blood flow occurs systemically though the body via arteries to serve tissues where it is perfused through capillary beds and is responsible for nutrient and waste exchange (Gannon et al., 1997). Placental blood flow and vascularity requirements on the uterus increase over the duration of gestation to meet the metabolic demands of the developing conceptus (Meschia, 1983). Interestingly, placental
function has been that of interest in the scientific community since the time of Aristotle, who artistically described placental flow in his work ‘On the Generation of Animals’ by stating that

The vessels join on the uterus like the roots of plants and through them the embryo receives its nourishment. Aristotle. On the Generation of Animals. (ca. 340 B.C.)

A negative correlation between P4 and uterine blood flow has been indicated by past research (Ford and Chenault, 1981). Conversely, blood flow to the uterus and endometrium is increased by E2 in cattle and swine (Ford and Chenault, 1981; Ford, 1982; Gannon et al., 1997). Normal fetal growth and development are important to ensure optimum health of offspring throughout their subsequent lifetime and uterine blood perfusion is essential to intrauterine development (Reynolds et al., 2006). In humans, increased blood flow and perfusion has been indicative of endometrial receptiveness and the cause of embryo implantation failure associated with poor uterine perfusion (Gannon et al., 1997; Yang et al., 1999; Sarnik et al., 2007; Barad et al., 2014). In cattle, blood flow of the uterine artery increases by 250% and heart rate by 118% from days 180 to 262 of gestation (Brockus et al., 2016). It is known that exponential growth of the conceptus occurs during the last half of gestation in mammals. In contrast, the utero-placental growth rate declines or ceases (Reynolds and Redmer, 1995). In numerous sheep models of compromised pregnancy, in which fetal and/or placental growth, were impaired, utero-placental blood flows were reduced. In the models that have been evaluated, placental vascular development has also been altered. Recent studies have indicated that treatments designed to increase placental blood flow can ‘rescue’ fetal growth that was restricted due to low maternal dietary intake. Placental blood flow and vascular development are thus potential therapeutic targets in compromised pregnancies (Reynolds et al., 2006).
**Ultrasonography**

In recent years the use of vascular imaging applications using Color Doppler ultrasonography has been used as a method to investigate uterine perfusion blood flow in human, horse, cattle, and small ruminant pregnancies (Elmetwally et al., 2016). Doppler ultrasonography was first used as a tool to measure blood flow in uterine vessels during pregnancy in humans during the 1980s (Campbell, 2013). The principle of the Doppler Effect relies on the measurement of the blood flow through a particular vessel. The technique is particularly improved in comparison to other uteroplacental perfusion measurement methodologies as it noninvasive. Furthermore, past methods were impractical due to the required incorporation of radioactive material such as the infusion of gamma-labeled microspheres that would perfuse the vascular bed. However, at the time, the method was instrumental in determining the location of perfusion in uterine horns, regardless of the fact that the animals had to be sacrificed for data collection. Thus, the use of Doppler ultrasonography allows for noninvasive and repeated measurements to be taken and uses waveform analysis of uterine arteries.

**Intrauterine Programming**

Animal science studies have indicated that uterine environment influences birth weight and size and that milk and carcass characteristics are directly related to birth weight (Walton and Hammond, 1938; Allen et al., 2004; Jenkinson et al., 2007; Greenwood et al., 1998; Blair et al., 2010). However, intrauterine growth retardation can result in onset of various morphometric signs and might have long-term effects without any change in birthweight (Barker, 1998; Zhu et al., 2006; Vehaskari et al., 2001; Oliver et al., 2005). In addition, there are numerous factors that have been researched that influence fetal growth or birth weight including: nutritional status, parity, fecundity of the dam, genotype, or breed of sire, dam, and/or recipient female, environmental temperature, and gender (Ferrell, 1990). Maternal uterine environment indications
on fetal growth as well as subsequent offspring growth has been indicated in sheep, cattle, swine, rabbit, and horses (Hunter, 1956; Joubert and Hammond, 1958; Venge, 1950; Walton and Hammond, 1938). Furthermore, adverse maternal environment can affect early development by inducing permanent changes in physiology and metabolism in humans (Barker, 2007). This has led to additional research as the consideration that the fetus will rarely express its full genetic potential for growth due to constraints imposed by the maternal environment (Gluckman and Liggins, 1984). Thus, increased knowledge of the effects can lead to strategies of mitigation.

Fetal growth and development influence lifelong performance, health, and disease and are commensurate with placental function (Fowden, 2008; Burton, 2016). Additionally, research has indicated that embryonic loss during early pregnancy is associated with impaired placental vascularization and development (Reynolds et al., 2014).

Fetal growth success, as well as placental growth and development, are closely interdependent and are affected by maternal nutrient supply even at the earliest stage of gestation (Reynolds and Redmer, 1995; 2001). Inadequate maternal nutrient supply leads to poor placental development, resulting in compromised fetal growth (McLean et al., 2017). In ewes, nutrient restriction resulted in the alteration of placentome formation with an increase in cotyledon and caruncle morphological changes occurring earlier in gestation (Vonnahme et al., 2006). More importantly, research indicates that impaired gestation leads to long-term effects on the offspring in decreased health and productivity (Wu et al., 2006; Caton and Hess, 2010; Funston et al., 2010). Additionally, there has been research conducted examining how placental function stimulation or even realignment can lead to a decrease in morbidity and mortality as well as suboptimal offspring growth performance (Vonnahme and Lemley, 2012). Exponential growth of the fetus and placenta occurs during the second and third trimester of gestation. However, it
has been reported that these pregnancy outcomes are dependent on the growth of the uteroplacental vascular beds that occur in the first half of the pregnancy (Meschia, 1983; Reynolds and Redmer, 1995). More research is needed to better understand the critical timepoints of fetal and placental susceptibility to developmental programming and how food animal producers can utilize this information.

Maternal nutrient intake and body condition during gestation have been reported to alter fetal growth trajectory with altered calf birth weight concluding (Bellows and Short, 1978; Boyd et al., 1987; Café et al., 2006; Frety et al., 2000; Tudor, 1972; Warrington et al., 1988). Furthermore, reports have been made that fetal growth in cattle may be affected by maternal nutrient intake as early as d 39 of gestation; however, following the insult, fetuses expressed compensatory growth dependent upon maternal nutrition (Micke et al., 2010). Interestingly, it was also reported that calf birth weight was reduced by maternal under-nutrition of protein during the second trimester of gestation, despite the maintenance of positive maternal liveweight gain throughout this period (Mick et al., 2010). Therefore, protein supplementation to mediate the negative consequences of limited nutrient availability to the developing fetus may have merit.

**The Insulin-like Growth Factor Axis**

The insulin-like growth factor (IGF) system is so named as it has similarities in structure to insulin and its growth promoting effects (Velazquez et al., 2009). Ligands insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2), receptors insulin-like growth factor receptor 1 (IGFR1) and insulin-like growth factor receptor 2 (IGFR2), and six high affinity insulin-like growth factor binding proteins (IGFBP 1-6), and binding proteases collectively make up the IGF superfamily (Giudice, 1995; Hwa et al., 1999; Spicer, 2004). The insulin-like growth factor axis is a critical regulator for many aspects of placental development and fetal growth with research indicating that insulin-like growth factors, IGF-1 and IGF-2, mediate this effect by the
promotion of optimum placental development and functionality (Forbes and Westwood, 2008). These single chain insulin-like polypeptides, IGF-1 and IGF-2 are also important for postnatal growth in addition to regulation of prenatal growth in animals, and have a similar sequence to insulin, but bind to several distinct classes of membrane receptor (El-Shewy et al., 2007). These growth factors also have mitogenic properties, including that of somatic cell growth and proliferation (Zapf et al., 1978; Ashton and Spencer, 1983). During pregnancy, these peptides can influence the transport of glucose and amino acids across the placenta. While there are similarities in function with IGF-1 and IGF-2 being important for embryonic development, during postnatal development it is IGF-I that predominantly regulates growth.

In humans, birth weight is positively associated with the concentration of IGF-1 in umbilical cord blood and circulating concentration of fetal IGF-1 is lower in intrauterine growth restriction pregnancies (Wali et al., 2012). Clinical attempts to improve the intrauterine environment by maternal supplementation of protein (Kramer and Kakuma, 2003). However, more recently, when gestating ewes received once-weekly intra-amniotic injections of a low dose of IGF-1 there was an increase in growth in ovine fetuses with growth-restriction secondary to placental insufficiency providing a potential approach to intrauterine treatment of fetal growth restriction (Wali et al., 2012).

**Bovine Somatotropin**

Somatotropin, or GH, is a naturally occurring protein hormone in humans and animals that is secreted by the anterior pituitary into blood circulation. The primary location for GH receptors is the liver, where GH binding increases synthesis and secretion of IGF-I inducing cell proliferation and differentiation as well as controlling cell metabolism (Le Roith, 2001). Additionally, GH can either cause a direct response or mediates a response through the induction of IGF-1 to regulate body growth through the effects IGF-1 on bone, adipose and muscle.
Receptors for GH as well as IGF-1 are also present on the bovine uterus as well as the embryo and conceptus with endocrine, paracrine, and autocrine actions of both hormones during establishment of pregnancy (Kolle et al., 1997, 2001; Rhoads et al., 2008; Robinson et al., 2000; Yaseen et al., 2001). Past in vitro and in vivo research in several species has indicated a positive effect of GH and the IGF system in stimulating uterine function and conceptus development at the pre- and peri-implantation phase (Wathes et al., 1998; Spencer et al., 1999; Kolle et al., 2002; Moreira et al., 2002; Markham et al., 2003; Bilby et al., 2006).

The action of growth factors is mediated by binding to the Type-I IGF receptor (IGFR1) although IGF-2, and to a lesser extent, IGF-1 can also bind to the type-II IGF/mannose-6-phosphate receptor (IGFR2), or the insulin receptor; ligand access to these receptors is regulated by a family of binding proteins termed IGF-binding proteins (IGFBPs) 1-6. The IGFBPs serve many functions, such as prolonging the half-life of IGF and providing a circulating storage reservoir of IGF (Forbes and Westwood, 2008). Additionally, IGFBP play a major role in the availability of IGF-1 and its actions on the ovary as well as other critical reproductive tissues (Schams et al., 2002). The IGFBP can inhibit the effects of IGF-1 by sequestering extracellular IGF-1 and limiting binding to cell surface receptors; yet, can also potentiate IGF-1 action by protecting it from degradation, acting as a reservoir to sustain controlled delivery to target cells, and facilitate transport from the peripheral circulation to target tissues (Clemmons, 1998; Baxter, 2000; Firth and Baxter, 2002). Most of the IGF in the circulation exists in a 150-kDa ternary complex consisting of IGFBP3 and the acid labile subunit (ALS); when this complex dissociates, the IGFs form smaller, binary complexes with the other IGFBPs, which then transport IGF across the endothelium to target tissues (Le Roith et al., 2001). IGFBP proteases alter the bioavailability of IGF-1 by degradation of its binding proteins (Velazquez et al., 2009) There is currently a lack
of complete understanding of how the complex IGF axis regulates growth and development of other tissues and organs.

A recombinant form of growth hormone, bovine somatotropin (bST), became commercially available after the approval of the U.S. Food and Drug administration (FDA) in 1994 as a slow release injectable product (Posilac, Elanco). The DNA that is responsible for producing bovine growth hormone in cattle is combined with a plasmid vector from E. coli bacteria then allowed to reproduce giving rise to an increase in the quantity of the bST (Bauman et al., 1985; Elvinger et al., 1988; Monsanto Company, 2003). The recombinant protein is labeled for use in dairy cattle and is administered every two weeks to increase milk yield by its antagonizing action effects of insulin and shifting nutrient partitioning to yield more pounds of milk produced per lactation period (Bauman, 1999). Recombinant bovine somatotropin was discovered in the 1970s by Cornell University scientists that realized that the injectable hormone had the ability to increase the milk production of a cow without yielding any adverse effects. In addition to increasing more pounds of milk per cow per lactation, bST also allows feed resources to be more efficiently utilized with less animal waste and a reduced carbon footprint (Collier and Bauman, 2014). Since the discovery of bST and approval of use, researchers have extensively studied the hormone, the milk produced, and the effects on the animals receiving the injections regarding safety, efficacy, animal welfare, and implications to the knowledge base of physiology and endocrinology. However, in recent years due to consumer scrutiny the use of bST in U.S. dairies has significantly declined.
CHAPTER 3
EFFECTS OF BIWEEKLY ADMINISTRATION OF RECOMBINANT BOVINE
SOMATOTROPIN DURING THE FIRST THIRD OF GESTATION ON CONCEPTUS
DEVELOPMENT AS WELL AS STEROID AND EICOSANOID METABOLIZING
ENZYMES IN REPLACEMENT HEIFERS

A major determinant in the profitability of livestock production systems is offspring
survival and performance. The intrauterine phase of fetal development is a critical period
as nutrient availability may have lasting impacts on offspring performance (Barker and Osmond,
1986; Barker et al., 1993; Ford and Long, 2012). The dynamic growth of the fetus is supported
by the placenta which secretes hormones, including estrogens, progesterone, lactogen, and
placental growth hormone, and growth factors that alter intrauterine development (Gootwine,
2004). The GH/IGF system is primarily responsible for regulating growth in all vertebrates.
Growth hormone receptors, primary located in the liver, and GH binding causes an increase of
synthesis and secretion of IGF-1 (Le Roith et al., 2001). The insulin-like growth factors IGF-1
and IGF-2 also promote growth, both before and after birth of the offspring (Sferruzzi-Perri et
al., 2011). Uterine blood flow is linked to fetal growth, development, and survival of the
offspring (Reynolds and Redmer, 1995; Lang et al., 2003; Vonnahme and Lemley, 2012).
Research has indicated that chronic restrictions in uterine BF causes a placental and fetal
response in that the form of growth adaptation to the reduced availability of oxygen and nutrient
supply to the conceptus (Lang et al., 2003). Supplementation of steroid hormones, particularly
estradiol-17β, has been observed to increase blood flow to various uterine tissues (Rosenfeld et
al., 1973; Ford and Chenault, 1981). Progesterone is the primary steroid hormone produced
during pregnancy and is primarily metabolized in the liver but has been noted to be metabolized
in extra-hepatic tissues (Iwano et al., 2001; Darwish et al., 2010; Girolami et al., 2016).
Therefore, local extra-hepatic metabolism of steroids and eicosanoids could potentiate placental
nutrient transport and uteroplacental BF. Recently, uteroplacental secretion of steroids was
associated with alterations in placental hormone metabolizing activity in an ovine model of nutrient restriction (Lemley et al., 2018).

This study was designed to evaluate the administration of bST and its effects on conceptus development, binucleate cells (BNC) as well as steroid and eicosanoid metabolizing enzyme activity when administered during the first-third of gestation in beef heifers. The objective was to determine the interplay of components of the somatotropic axis and how they may stimulate conceptus development and steroid and eicosanoid metabolizing enzyme activity. We hypothesized that bST administration concurrent with TAI would initially increase concentrations of IGF-1 in blood plasma, and that continued biweekly bST administration would sustain increased plasma concentrations of IGF-1, potentially resulting in an increase in BNC numbers formed and altering fetal and placental development.

**Materials and Methods**

All procedures for the study conducted at the North Florida Research and Education Center in Marianna, FL were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee (number 201609600).

**Experimental Design**

An initial group of Angus-based, crossbred beef heifers \((n = 97)\) that ranged from 15 to 18 months of age were enrolled in a completely randomized design study and housed at the North Florida Research and Education Center in Marianna, FL. Heifers were maintained according to standard operating procedures of the facility with heifers being housed in 7.7 ha bahiagrass \((Paspalum notatum)\) pastures, offered natural shade from trees, supplemented with a finishing diet, and *ad libitum* water. The finishing diet that was fed ad libitum and consisted of 10% fiber pellets, 41.2% corn gluten, 40.8% soy hull pellets, 5% vitamin supplement, and 3% whole soybeans. Additionally, heifers were offered Tifton 85 bermudagrass \((Cynodon dactylon)\)
hay. All heifers were exposed to the 7-day CO-Synch + controlled internal drug release (CIDR) estrus synchronization control protocol followed by fixed-time artificial insemination (TAI; d 0; Figure 1). Heifers were then randomly assigned to receive one of two treatments: single subcutaneous injections containing 500 mg of bST (Posilac, sometribove zinc, Elanco Animal Health, Greenfield, IN) in the neck at TAI and biweekly until day 57 of the experiment (BST); or to receive no bST treatment to serve as untreated controls (CONT).

**Blood Sampling Analysis**

Blood samples were collected on d 0, 22, 50, and 64 relative to TAI to determine the concentrations of plasma IGF-1 via jugular venipuncture using EDTA-treated Vacutainers (BD Diagnostics, Franklin Lakes, NJ). Blood samples were placed on ice until centrifugation (1,500 x g at 4°C for 15 min). Plasma was collected post centrifugation, aliquoted, and then stored at -20°C until analysis. Concentrations of plasma IGF-1 were determined with an immunoassay system (Immulite® 1000 Version 5.22; Siemens Healthcare Diagnostics, Malvern, PA).

**Bodyweight and Pregnancy Status**

Heifer BW was recorded on d -9, -3, 0, 15, 22, 29, 43, 50, 57, 64, and 77. Initial BW at the time of study enrollment was 430 ± 21.30 kg. Pregnancy status was determined via transrectal ultrasonography (Ibex portable ultrasound, 5.0-MHz linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO) on d 29 and 64 after TAI. Confirmation of pregnancy status was determined by the presence or absence of uterine fluid as well as embryonic membranes on d 29 and the direct observation of the fetus on d 64.

**Post-Harvest Collection**

On d 84, all heifers were transported to a commercial abattoir for harvest. At time of harvest (d 85) a subset of the heifers (BST, n = 7; CONT, n = 5) were selected and used for assessment of fetal and placental characteristics. The complete gravid reproductive tracts and
dam liver was collected immediately following harvest. Following collection of the tracts and liver tissue, fetal measurements collected were: brain weight, crown-to-nose length (CNL), crown-to-rump length (CRL), heart girth (HG), fetal BW, eviscerated BW, and liver weight. Extraembryonic measurements collected included: umbilical cord diameter, fetal fluid weight and volume, fetal membrane weight, placentome weights, and total placentome number. Fetal fluid weight, fetal membrane weight, and fetal weight were measured utilizing a scale while umbilical cord diameter was obtained utilizing a digital caliper. One placentome near the umbilicus was selected per tract, trimmed to a 1 cm cube, and fixed in formalin for histological analysis. Placentomes were stored in a 70% ethanol (ETOH) solution prior to being embedded in paraffin. In addition, maternal reproductive tract parameters of gravid uterine weight, empty uterine weight, ovarian weight, and CL weight were recorded. Approximately 1 g of maternal liver, fetal liver, caruncle, cotyledon, and CL were collected and placed in pre-labelled 1.5 mL cryovials (Wheaton, DWK Life Sciences, Millville, NJ) and dropped into liquid nitrogen to snap freeze. All tissue samples were collected within 2 hours of maternal death. Caruncle and cotyledon tissues were collected adjacent to the umbilicus and the CL were collected ipsilateral to the fetus. After all measurements and tissues were collected, placentomes were counted. On day 89, carcass quality grade (as determined by USDA Standards), carcass yield, and carcass weight data were obtained from harvested heifers.

**Tissue Processing**

Approximately 200 mg of tissue was placed into a polypropylene tube with 1000 µL of potassium phosphate (KPO₄) buffer (400 mM, pH = 7.4). Samples were then mechanically homogenized using the Tissue Tearor (Biospec Products, Bartlesville, OK) following the manufacturer’s protocol. Tissue homogenate samples were placed into microcentrifuge tubes and then centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was stored at −80°C until
enzyme assays were conducted. The protein concentration of the supernatant was determined by a Coomassie Plus (Bradford) protein assay following the manufacturer’s protocol (Thermo Scientific, Rockford, IL). Following protein analysis, samples were diluted to a similar concentration, 4000 µg/mL.

**CYP Enzymes**

Assay kits for CYP1A, CYP3A, and CYP2C and NADPH regeneration solution were purchased from Promega Corporation (Madison, WI) and assays conducted according to Hart et al. (2014) with minor adaptations. Briefly, reconstitution buffer was added to luciferin detection reagent. Luciferin CEE (CYP1A), luciferin IPA (CYP3A), and luciferin H (CYP2C) were diluted in KPO4 buffer. Tissue homogenates (28 µg of protein per well) and enzyme-specific luciferin substrate was added to 96-well plates in duplicate. Plates were then preincubated for 10 min (CYP1A), 30 min (CYP3A), or 90 min (CYP2C) at 37°C. Following the incubation, NADPH regeneration solution was added to each well and plates were incubated for 30 min (CYP1A and CYP2C) at 37°C or 10 min (CYP3A) at room temperature. After the incubation, luciferin detection reagent was added to each well and plates were protected from light and incubated for an additional 20 min at room temperature. Plates were then placed into a Promega Multi-Plus plate reader and luminescence was measured.

**UGT**

The UGT assay kit was purchased from Promega Corporation and the assay performed according to Hart et al. (2014) with minor adaptations. Briefly, uridine diphosphoglucoronic acid (UDPGA) was added to half the plates to act as reaction wells, and distilled water was added to the other half as control wells. The UGT reaction mixture containing UGT multienzyme substrate was then combined with tissue homogenates (28 µg of tissue protein per well), and the plates were preincubated for 90 min at 37°C. After incubation, detection reagent was added to
each of the wells followed by an incubation period of 20 min at room temperature while protected from light. The plates were then analyzed using a Promega Multi-Plus plate reader with luminescence detection mode.

**Luteal Tissue Progesterone Assay**

Concentrations of luteal tissue P4 were determined using radioimmunoassay procedures based on manufacturer recommendations (MP Biomedicals, Costa Mesa, CA, 92626). Approximately 50 mg of luteal tissue was placed into a 15-mL conical tube with 5 mL of phosphate buffered saline (PBS). Samples were then mechanically homogenized using the Tissue Tearor (Biospec Products, Bartlesville, OK). Tissue homogenate samples were centrifuged at 1500 × g for 15 min at 4°C, then the supernatant was placed into 5-mL polypropylene tube. The supernatant was vortexed and diluted (1:20) in PBS, which was used to analyze for luteal progesterone concentrations. The inter- and intra-assay coefficients of variation were 4.6% and 5.8%. Concentrations of P4 are reported as µg P4 per gram of CL as well as total P4 content of CL, which was calculated by multiplying the P4 concentration (µg of P4 per gram of CL) by the CL weight at harvest.

**Immunofluorescence Staining**

Placentome samples were trimmed to a width of 0.50 cm and embedded in paraffin to be utilized for placentome sectioning (3 µm thickness). Placentome sections were deparaffinized, rehydrated in descending concentrations of ETOH (100%, 95%, 70%, and 50% ETOH), and rinsed in distilled water. Heat induced antigen retrieval was performed utilizing 10mM sodium citrate buffer (pH = 6.0) and were exposed to 121°C for 20 min under pressure to expose target proteins (2100 Antigen Retriever; Aptum Biologies; Southampton, UK). Tissues were blocked in 10% normal goat serum (NGS; Vector Labs; Burlingame, CA, USA) for 60 min at 25°C. Placentome sections were incubated with 20µg/ml biotinylated lectin Dolichos Biflorus
Agglutinin (DBA; Vector Labs; Burlingame, CA, USA) at similar conditions. Texas red-avidin (20μg/ml; Vector Labs; Burlingame, CA, USA) was added to tissues and tissues at 25°C for 60 mins in a dark room under similar conditions. This was completed to allow for fluorescent staining of BNCs. Next, 20μg/ml Fluorescein labeled Griffonia (Bandeiraea) Simplicifolia lectin I (BS1-FICT; Vector Labs; Burlingame, CA, USA) was added to tissues and with tissues being held at similar condition as before. This was completed to allow for fluorescent staining of cotyledon area. All reagents were diluted in a mixture of 1x tris buffered saline (TBS) and 1% NGS and prepared at least 15 min prior to addition to the slides.

**Imaging and Image Analysis**

Five representative images of each slide were acquired with ZEN 2 pro software using a Zeiss Inverted AxioObserver.Z1 Microscope equipped with Plan-Apochromat 20x objective and Axiocam 506 monochrome camera (Carl Zeiss; Thornwood, NY, USA). Image analyses were performed using Image-Pro Premier 9.1 software (Media Cybernetics; Rockville, MD, USA) to obtain BNC number and cotyledon area from each slide. Analysis was conducted on each image per slide and the average being calculated. The average cotyledon and BNCs number per heifer were used to calculate percentage BNCs per cotyledon area.

**Statistical Analysis**

Data were analyzed as a completely randomized design and heifer was considered the experimental unit. The SAS (version 9.4; SAS/STAT, SAS Inst. Inc., Cary, NC) statistical package was used for all statistical analyses. All continuous data were analyzed by PROC MIXED. In addition, heifer BW and plasma concentrations of IGF-1 were analyzed as repeated measures. The model included the fixed effects of treatment, day, and treatment x day interaction. Activities were expressed per mg of protein, and data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), using the Wilcoxon rank sum test.
Enzyme activities were expressed relative to mg of protein. Data, respective to each tissue, enzyme activity, and CL parameters (CL weight (wt), µg P4/ gram of CL, and total luteal P4) were analyzed using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), using the Wilcoxon rank sum test, and the model statement included maternal treatment. Pearson correlation coefficients were determined using the CORR procedure of SAS to analyze the relationships between CL wt, µg P4/ gram of CL, total CL P4, and enzyme activity with respect to each tissue. Treatment means were separated using the PDIFF options of the LSMEANS statement. Two tissues were removed from the study for the BNC analysis as one tissue was biologically inconsistent, and the other tissues collected was not placental tissue. Binucleate cells and conceptus parameters were analyzed using CORR procedure of SAS. Least square means and standard error are reported. Statistical significance was declared at $P \leq 0.05$, and tendencies discussed when $0.05 < P \leq 0.10$.

Results and Discussion

Somatotropin, or GH, is a protein hormone produced in animals by the anterior pituitary. This protein can either cause a direct response or mediate a response through the induction of IGF-1 to regulate body growth through the effects IGF-1 on bone, adipose and muscle. Until recent years a recombinant form of the hormone, bST was commonly used in the US dairy industry to extend the persistence of lactation in healthy cows. Administration of bST resulted in an increase in milk yield for each lactation used with the effects proposed by antagonizing actions of insulin and the shifting of nutrient partitioning to yield more pounds of milk produced per lactation period (Bauman, 1999). Research evaluating the safety and impacts of bST administration have been numerous since its first used almost 50 years ago. When bST was administered in studies involving cyclic lactating dairy cows there was a tendency to improve fertility (Santos et al., 2004; Starbuck et al., 2006). In beef cattle the potential use of bST prior to
AI, as well as concurrently with AI, in beef cows and heifers for the improvement of pregnancy risk has been studied (Mercadante et al., 2016; Oosthuizen et al., 2018). However, there have been no studies investigating the effect of fetal and placental development in gestating beef heifers. Thus, this study was conducted to evaluate the effects of bST administration during the first third of pregnancy on fetal and placental development in beef heifers. Mean change in BW (71.9 ± 7.40 kg) and ADG (0.95 ± 0.11 kg; Table 3-1) of the heifers from TAI to d 77 of gestation did not differ between treatments (P ≥ 0.05). As expected, mean concentrations of IGF-1 were greater (P < 0.001) in heifers that received bST treatment (346.50 ± 27.7 ng/mL) when compared to CONT (134.70 ± 32.8 ng/mL; Figure 2). This data concurs with reports that demonstrated a significant increase in IGF-1 in maternal circulation but no effect on BW when heifers received a diet high in protein supplementation (Perry et al., 2002). In addition, no differences were noted between treatments with regards to carcass quality grade, yield, or weight (P = 0.88, 0.35 and 0.99 respectively; Table 3-1). In contrast, the results of this study differ from previous reports indicating that bST use improves performance in finishing cattle. When Simmental beef heifers were fed a finishing diet of concentrate and corn silage and received either 320 or 640 mg of bST every other week until time of slaughter, heifers receiving bST tended to have an increase in ADG with a reduction in intramuscular fat and marbling scores of longissimus muscle (Schwarz et al., 1993). In addition, Angus based heifers receiving bST every other week for 196 days had similar ADG but reduced fat thickness (Cooke et al., 2013). Interestingly, preweaning treatment of bST in Bos indicus influenced beef heifers resulted in greater ADG from d 0 to 42 but less ADG from d 42 to 127 and similar BW at weaning and postweaning (Piccolo et al., 2018).
Mean placentome weight (66.46 g ± 6.85 g), fetal membrane weight (0.26 ± 0.14 kg), uterine weight (1.42 ± 0.08 kg), as well as ovarian and CL weights (15.1 ± 1.7 g and 4.8 ± 0.4 g, respectively) did not differ (P ≥ 0.05) between treatments (Table 3-2). In addition, µg P4/gram of CL and total luteal P4 (40.7 ± 12.3 µg/g and 192.9 ± 64.4 µg, respectively) did not differ (P ≥ 0.05) between treatments. These results are contrary to those previously reported (Spicer et al., 1993), indicating that P4 production of bovine granulosa cells increased after exposure to increased IGF-1. However, those indications were concluded from short-term in vitro work with culture medium having 0 to 200 ng/mL of IGF-1 present. Thus, this represents an acute dose and not a continuous elevated, or chronic, concentration of IGF-1 as carried out in the current study.

Activity of CYP1A was not different between treatments within maternal liver (P = 0.19), fetal liver (P = 0.53), caruncle (P = 0.32), cotyledon (P = 0.63), or CL (P = 0.76; Figure 3). Activity of CYP2C was greater (P = 0.01) in the maternal liver of BST vs. CONT heifers; however, activity was not different between treatments in caruncle (P = 0.91) or CL (P = 0.97; Figure 3). Increased CYP2C may indicate greater P4 metabolism in the dam’s liver, but no differences were observed in CL P4 parameters. Concentrations of serum P4 did not differ in ovariectomized, non-lactating dairy cattle supplemented with P4 in the form of a CIDR in addition to bST supplementation on d 0, 9, and 18 of the estrous cycle (Aboin and colleagues, 2013). Concentrations of serum P4 at d 19 were lesser in pregnant ewes supplemented with bST beginning on d 0 of the estrous cycle (Fukui et al., 2000).

Activity of UGT tended to be greater (P = 0.09) in CL samples from BST compared to CONT; however, activity was not different between treatments in maternal liver (P = 0.49) or fetal liver (P = 0.95) and was not detected in the caruncle or cotyledon (Figure 3-3). The UGT enzymes have 2× the activity when exposed to P4 metabolites compared to estrogen metabolites.
in monkey tissues (Albert et al., 2000), which may result in a lack of differences between treatments.

Fetal CRL, weight, HG, and liver weight did not differ between treatments ($P \geq 0.05$). These results are in contrast to those in dairy cattle where those females receiving bST had fetuses that were larger at d 40 of gestation (Ribeiro et al., 2014) and longer concepti at d 19 (Bilby et al., 2004). Activity of CYP2C was not detected in the fetal liver or cotyledon. Activity of CYP3A was only observed in maternal liver and did not differ between treatments ($P = 0.82$). Experimental treatment had no impact on BNC number ($P = 0.13$), BNC size ($P = 0.19$), or percentage BNC per cotyledon area ($P = 0.25$). However, extraembryonic samples collected from heifers receiving bST (521.6 ± 22.9 g) resulted in greater ($P = 0.03$) quantities of fetal fluid compared to CONT heifers (429.6 ± 27.14 g). There was also a tendency for BST heifer reproductive tracts to have fewer placentomes ($P = 0.08$) and greater umbilical diameter ($P = 0.09$) than CONT. It should also be noted that Costine et al. (2005) reported no difference in early (d 25) pregnancy chorioallantoic weight due to GH administration at time of breeding in well fed sheep, but they did observe cotyledonary differences by mid gestation (d 80). Perhaps an observation difference in placentome number would have been noted by mid pregnancy. There is the potential that bST treatment during early pregnancy could enhance BNC numbers and perhaps placentome growth to aid in nutrient delivery to the calf. The observed tendency for an increase in umbilical cord diameter due to bST treatment suggests that nutrient delivery may increase as gestation continues. An increased number of BNCs are expected to increase PL secretion and therefore fetal weight (Schoknecht et al., 1991; Hossner et al., 1997). The present study indicated that there is no change in fetal weight during early pregnancy, which contradicts previous findings (Wallace et al., 2014). These differences could be due to species differences or
the time of pregnancy these observations were recorded. An increase in fetal fluid volume with no difference in fetal membrane weight and fetal weight due to GH has also been observed in over nourished sheep (Wallace et al., 2014). Reduced amniotic fluid volume has been reported in pregnancies with small-for-gestational-age human fetuses (Roberts et al., 2018). Perhaps compensatory function of individual placentomes allowed for similar transplacental function in comparison to the lesser number of placentomes observed in the CONT group. Therefore, although concentrations of IGF-1 were increased in heifers that received biweekly administration of bST from TAI to day 57 of gestation, overall fetal development parameters did not differ between treatments.

Results indicate that the administration of 500 mg of slow-release recombinant bST from TAI to day 57 of gestation did not alter overall fetal growth; however, based on these data the extraembryonic parameters may have resulted in a potential benefit to placental and luteal function. As a result of increased IGF-1, there was greater fetal fluid volume, a tendency for increased umbilical diameter, and decreased placentome number in the BST heifers. Further research is needed further understand the possible placental function benefits of an increase in GH by IGF-1 activity.

**Conclusion**

In the present study, administration of 500 mg of bST every other week through early gestation was successful in increasing circulating IGF-1 in gestating beef heifers. There was no effect on BW, ADG, carcass quality grade, yield, or weight between bST treated heifers and the CONT heifers. Likewise, the treatment failed to alter measured fetal parameters, ovarian weight or uteroplacental weights measured. There was a tendency whereby the extraembryonic samples from BST tracts contained greater quantities of fetal fluid yet fewer placentomes and greater umbilical diameter. Activity of CYP1A was not different between treatments within maternal
liver, fetal liver, caruncle, cotyledon, or CL, while activity of CYP2C was greater in the maternal liver of BST heifers, and activity of UGT tended to be greater in CL samples from BST.
Table 3-1. Growth performance and carcass parameters for gestating beef heifers treated with recombinant bovine somatotropin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment(^1)</th>
<th>P-value</th>
<th>SEM(^2)</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean change in BW, kg</td>
<td>CONT</td>
<td>BST</td>
<td>18.476</td>
<td>0.88</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>162.40</td>
<td>158.50</td>
<td>0.098</td>
<td>0.88</td>
</tr>
<tr>
<td>Carcass quality grade(^3)</td>
<td>2</td>
<td>3</td>
<td>0.25</td>
<td>0.88</td>
</tr>
<tr>
<td>Carcass yield grade</td>
<td>2</td>
<td>3</td>
<td>0.25</td>
<td>0.35</td>
</tr>
<tr>
<td>Carcass weight, kg</td>
<td>147.71</td>
<td>147.73</td>
<td>3.03</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\(^1\)BST: Angus-based crossbred heifers receiving biweekly subcutaneous injections of 500 mg of recombinant bovine somatotropin through d 57; CON: Angus-based crossbred heifers receiving no injections. All heifers were exposed to the 7-day CO-Synch + CIDR estrus synchronization protocol and artificial insemination.

\(^2\)Pooled standard error of treatment means. BST: \(n = 7\); CON: \(n = 5\).

\(^3\)Heifers were harvested and carcass quality was measured by USDA Standards.
Table 3-2. Effects of experimental treatment on extraembryonic, maternal, and fetal development measurements in gestating beef heifers treated with recombinant bovine somatotropin.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (^1)</th>
<th>P-value (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>CON</td>
<td>BST</td>
</tr>
<tr>
<td>Fetal membrane weight, kg</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>No. of placentomes</td>
<td>73</td>
<td>54</td>
</tr>
<tr>
<td>Placentome weight, g</td>
<td>73.61</td>
<td>61.30</td>
</tr>
<tr>
<td>Fetal fluid, mL</td>
<td>430</td>
<td>522</td>
</tr>
<tr>
<td>Gravid uterine weight, kg</td>
<td>1.31</td>
<td>1.49</td>
</tr>
<tr>
<td>Empty uterine weight, kg</td>
<td>0.64</td>
<td>0.53</td>
</tr>
<tr>
<td>Ovarian weight, g</td>
<td>15.86</td>
<td>14.34</td>
</tr>
<tr>
<td>Corpus luteum weight, g</td>
<td>4.94</td>
<td>4.62</td>
</tr>
<tr>
<td>Brain weight, g</td>
<td>2.72</td>
<td>2.34</td>
</tr>
<tr>
<td>Crown-nose length, cm</td>
<td>3.84</td>
<td>4.08</td>
</tr>
<tr>
<td>Crown-rump length, cm</td>
<td>13.18</td>
<td>13.28</td>
</tr>
<tr>
<td>Fetal BW, g</td>
<td>60.08</td>
<td>59.40</td>
</tr>
<tr>
<td>Heart girth, cm</td>
<td>9.20</td>
<td>9.22</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>3.27</td>
<td>3.01</td>
</tr>
<tr>
<td>Umbilical cord diameter, mm</td>
<td>6.66</td>
<td>8.90</td>
</tr>
<tr>
<td>*BNC number</td>
<td>98.20</td>
<td>112.57</td>
</tr>
<tr>
<td>*BNC size, µm</td>
<td>441.14</td>
<td>524.46</td>
</tr>
<tr>
<td>*%BNC per cotyledon area</td>
<td>9.43</td>
<td>11.45</td>
</tr>
</tbody>
</table>

\(^1\) Angus-based crossbred heifers either receiving biweekly subcutaneous injections of 500 mg of bST through d 57 (BST; \(n = 7\)); or no bST injections (CON; \(n = 5\)). All heifers were exposed to the 7-day CO-Synch + CIDR estrus synchronization protocol and artificial insemination.

\(^2\) Statistical significance was declared at \(P \leq 0.05\), while a statistical tendency was declared at \(0.05 < P \leq 0.10\).

\(^3\) Pooled standard error of treatment means.

*Nelson et al., 2017.*
Figure 3-1. Schematic of treatments. All heifers received an injection of gonadotropin-releasing hormone (GnRH), and a controlled internal drug release (CIDR; 1.38 g of progesterone) insert on d -9; an injection of prostaglandin F2α (PG) at CIDR removal on d -3; and a second injection of GnRH concurrent with fixed-time AI (TAI) on d 0. Heifers were randomly assigned to receive one of two treatments: 500-mg of bovine somatotropin (bST) by a single subcutaneous injection in the neck at TAI and then biweekly to d 57 (BST); or an untreated control (CONT). Blood samples (Blood) were collected on d 0, 22, 50, and 64. Pregnancy status was determined by transrectal ultrasonography (US) on d 29 and 64 after TAI. Heifers were transported to a commercial abattoir and a subset of pregnant heifers (n = 7 BST and n = 5 CONT) were retained to evaluate fetal and placental characteristics. At time of harvest on d 85 complete gravid reproductive tracts and liver tissue were collected for analysis.
Figure 3-2. Results for plasma concentrations of insulin-like growth factor 1 (IGF-1) from blood analysis of gestating beef heifers. Heifers in the BST treatment group received 500-mg of bovine somatotropin (bST) biweekly from fixed-time AI (TAI) to d 57 while the CONT heifers did not. * Effect of treatment (P < 0.001); treatment × day interaction (P < 0.001).
Figure 3-3. Results for enzymatic activity of steroid and eicosanoid metabolizing enzymes from Angus heifers. The BST treatment group (n = 7) received 500-mg of bovine somatotropin (bST) biweekly from fixed-time AI (TAI) to d 57 while the CONT heifers (n = 5) did not. A) Enzyme activity of Cytochrome P450 1A (CYP1A) in tissue samples collected post-harvest. B) Enzyme activity of Cytochrome P450 2C (CYP2C) in tissue samples collected post-harvest. C) Enzyme activity of uridine 5'-diphospho-glucuronosyltransferase (UGT) in tissue samples collected post-harvest. Means with an asterisk (*) represent a difference (P ≤ 0.05).
CHAPTER 4
EFFECTS OF BIWEEKLY ADMINISTRATION OF RECOMBINANT BOVINE SOMATOTROPIN DURING THE FIRST TRIMESTER OF PREGNANCY FAILED TO ALTER UTERINE HEMODYNAMICS IN SUCKLED BEEF COWS

Gestation is a dynamic process that relies on extensive adaptation by the maternal system to partition nutrient and oxygen availability to the developing fetus. Livestock production systems typical focus on genetic selection and nutrition to achieve optimal growth and performance of offspring. However, researchers are now evaluating how the hormonal, nutritional, and metabolic environment during the intrauterine phase can have a lasting impact on livestock offspring based on the concept of fetal, or developmental programming (Godfrey, 1998). Alterations in the concentration of nutrients and hormones can impact fetal growth in particular and cause lifelong modifications of the endocrine and vasoregulative systems (Nüsken et al., 2011).

Nutrient delivery to the growing fetus is dependent upon numerous factors including placental growth and development, uteroplacental blood flow, nutrient availability, and placental metabolism and transport capacity (Dunlap et al., 2015). Maternal nutrition may affect development and function of the placenta (Funston et al., 2010). Maternal and fetal nutrient availability is particularly important as in events of suboptimal nutrition the fetus may never achieve its maximal genetic potential (Gluckman et al., 2013). Efficiency of placental nutrient transport is directly related to uteroplacental blood flow, which is critical for fetal growth (Ferrell, 1991; Reynolds and Redmer, 1995; Vonnahme and Lemley, 2012). Fetal demand on the uteroplacenta requires adaptation and the production of growth hormones in addition to an increase in placental and fetal blood flow (Nüsken et al., 2011; Gluckman et al., 2007; Murphy et al., 2006). Insulin-like growth factor-1 (IGF-1), regulated by growth hormone (GH), is important for both prenatal and postnatal growth (Lawrence et al., 2012).
Fetal growth, development, and subsequent performance are critical to livestock producers, but few studies have addressed the impact of the maternal IGF system on intrauterine programming in beef cattle. The objective of this experiment was to determine the effects of recombinant bovine somatotropin (bST) administration on uterine hemodynamics in suckled cows and subsequent calf growth. It was hypothesized that the bST-induced increase in concentration of IGF-1 in maternal circulation from the time of breeding through the first trimester of gestation would benefit conceptus development and subsequent calf performance due to improved uterine hemodynamics.

Materials and Methods

All procedures for the study conducted at the North Florida Research and Education Center in Marianna, FL were reviewed and approved by the University of Florida institutional Animal Care and Use Committee (number 201609600).

Experimental Design

A total of 152 primiparous \( (n = 29) \) and multiparous \( (n = 123) \) suckled beef cows composed of Angus \( (n = 78) \), Brangus \( (n = 56) \), and SimAngus \( (n = 18) \) were enrolled in the experiment. Cow-calf pairs were maintained together according to standard operating procedures of the facility and housed on Pensacola bahiagrass \( (Paspalum notatum) \) pasture from birth to weaning with ad libitum access to bahiagrass hay, bahiagrass pasture, water, and provided natural shade from trees. On d -22 and -10, body condition score (BCS) were assigned and blood samples were collected via jugular venipuncture to determine estrous cyclity status for all cows. Body condition score was individually assigned using a scale of 1 to 9 (Whitman, 1975). Cows were then stratified by breed, days postpartum, parity, estrous cyclicity status, and BCS before being randomly assigned to either receive bi-weekly injections of recombinant bST (BST; 500 mg/14 d; Posilac; Elanco Animal Health, Greenville, IN) at fixed-time artificial insemination
(TAI; d 0) through d 97 or to receive no bST treatment to serve as untreated controls (CTL). On d -10, cows were exposed to the 7-d CO-Synch + controlled internal drug release (CIDR) estrus synchronization protocol. In brief, all cows received a 100-μg injection of GnRH (2 mL Factrel; Zoetis Animal Health, Parsippany, NJ) concurrent with the insertion of a CIDR (1.38 g Progesterone (P4); Zoetis Animal Health), followed by a 25-mg injection of PGF (5 mL Lutalyse; Zoetis Animal Health) at the time of CIDR removal, and 66 h later concluding with an injection of 100-μg GnRH and TAI (Figure 4-1). Following TAI (d 0), individual BW was recorded and BCS was assigned on d 13, 27, 41, 55, 69, 83, 97, 173, and 233. The administration of bST to BST cows was achieved by subcutaneous injection in the neck every other week through the first trimester of gestation. Following parturition, calf BW and gender were recorded and at 7±5 d of age liver tissue was collected and calf morphometrics of heart girth (HG) and crown-to-rump length (CRL) were measured and recorded. Weaning weight of all calves was measured using a scale (TRU-TEST, Mineral Wells, TX).

**Blood Collection and Analysis**

Blood samples from all cows were collected (d -22, -10, 0, 13, 27, 41, 55, 69, 83, and 97 relative to TAI) via jugular venipuncture into 10-mL evacuated tubes spray-coated with 143 IU of Na heparin (BD Diagnostics, Franklin Lakes, NJ) immediately placed on ice, and later centrifugated at 1,500 x g, at 4°C for 15 min. Plasma from each blood sample was then aliquoted into polypropylene vials and stored at -20°C until analysis. Samples collected on d -22 and -10 were used to determine the concentrations of progesterone in circulation of each cow with the results then being used to assess cyclicity status prior to estrus synchronization. When one or both samples had P4 concentration of ≥ 1 ng/ml, the cow was determined to have a functional corpus luteum and considered to be cyclic and no longer in a state of anestrous. Blood samples were also collected bi-weekly from 0 to 97 d to determine the concentrations of IGF-1 in
circulation of all cows. Concentrations of plasma P4 and total IGF-1 were determined with an immunoassay system (Immulite 1000 Version 5.22; Siemens Healthcare Diagnostics, Malvern, PA).

**Ultrasonography Evaluation**

Pregnancy status was determined via transrectal ultrasonography (Ibex Pro portable ultrasound 5.0-MHz linear multi-frequency transducer, E.I. Medical Imaging, Loveland, CO) on d 41 and 173 postinsemination. Uteroplacental hemodynamic measurements were determined using Color Doppler ultrasonography (MicroMaxx, Sonosite, Inc., Bothell, WA) and a transrectal probe (Linear Endorectal L52x probe, Sonosite, Inc.) Uterine perfusion measurements were obtained on d 97, while uterine artery hemodynamics, ipsilateral and contralateral to the conceptus, were measured on d 233 (BST, n = 24; CTL, n = 28) of gestation following the techniques described by Brockus et al. (2016). The uterine artery was located transrectally by following the abdominal aorta toward the origin of the external iliac artery (Figure 4-4). The probe was moved caudally to locate the internal iliac artery. The left and right uterine arteries were palpated and were identified as a major branch of the iliac arteries. On d 233 the left and right uterine arteries noticeably differed post-palpation to assure pliability and pulsatility. Hemodynamic examinations were approximately 30 min per cow. Cardiac cycle waveforms from 2 independent ultrasound scans were used to calculate systolic velocity (s; cm/s), diastolic velocity (d; cm/s), s:d ratio, pulsatility index (PI), and resistance index (RI) using preset functions on the Doppler ultrasound device (Figure 4-5). Mean velocity (MnV) was calculated using the equation: \((s – d)/PI\). Uterine BF was calculated using the following equation: \((MnV \times \text{vessel area} \times 60 \text{ s})\). Total uterine artery blood flow was calculated as the summation of both the right and left uterine arteries. Ipsilateral and contralateral uterine artery blood flow is presented as the vessels on the same and opposite side of the fetus, respectively.
Tissue Collection

Liver tissue samples were harvested from calves at 7±5 days of age. Samples were collected via needle biopsy, following the techniques described by Arthington and Corah (1995). Immediately following collection, 100-mg of wet liver tissue was placed into 1 mL of RNA stabilization solution (RNAlater, Ambion Inc., Austin, TX) placed on ice for 6 h and stored at either -20 or -80°C until analysis.

RNA Extraction and Quantitative RT-PCR

Approximately 50 mg of liver tissue from each biopsy was homogenized with the addition of 1 mL of TRIzol (Ambion Inc., Austin, TX) a pestle and mechanical homogenizer, followed by phase separation with 0.2 mL of chloroform. Precipitation of the nucleic acids was achieved using isopropanol and pellet formation using centrifugation (12,000 x g for 15 min at 4°C). The RNA pellet for each sample was isolated using ethanol and centrifugation, then resuspended in molecular grade RNase-free water. Extracted total RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). All samples resulted in 260 nm/280 nm ratios between 1.9 and 2.0 and deemed acceptable for downstream procedures. Complementary DNA was achieved using the Verso cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA) and then amplified in duplicate using iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). The cDNA and SYBR based master mix were pipetted in duplicate for each sample into a 96-well plate and used for quantitative real-time reverse transcription PCR (CFX Connect, Bio-Rad Laboratories). The genes of interest for the investigation were: insulin-like growth factor 1 (IGF1), insulin like growth factor 2 (IGF2), insulin-like growth factor 1 receptor (IGFR1), and insulin-like growth factor 3 (IGFBP3). Cyclophilin-1 (CYP1) and ribosomal protein S9 (RPS9) served as reference genes. Assays for the genes of interest were validated for efficiency and specificity prior to real-time qPCR.
Efficiency of primers was assessed and deemed to be within normal limits. The geometric mean of the two reference genes (CYP1 and RPS9) was calculated and relative expression for the established genes of interest were calculated using the $2^{-\Delta Ct}$ method relative to the housekeeping genes.

**Statistical Analysis**

Cows were stratified by breed, days postpartum, parity, estrous cyclicity status, and BCS before being randomly assigned to one of two treatments. All data was analyzed as a completely randomized block design with either cow or calf considered the experimental unit using the SAS statistical package (version 9.4; SAS/STAT, SAS Inst. Inc., Cary, NC). All continuous data, concentrations of IGF-1, P4, cow bodyweight, and body condition score were analyzed as repeated measures using the MIXED procedure. The model included the effects of parity, treatment, day, and the interactions of each with the covariance structure of AR (1). In addition, the random effect of treatment, day, and parity were included in the model. Uterine blood flow was analyzed using the MIXED procedure. The model included treatment, day of gestation, day by treatment interaction, parity, cyclicity, and cow breed. Bodyweight and weaning weight of the suckled 2017 calf was analyzed using the MIXED procedure. Days postpartum, gestational length, 2018 calf birthweight, heart girth and crown-to-rump length at 9± 3 days of age were analyzed using the MIXED procedure. The model included the effects of parity, treatment, and the interaction with the random effect of treatment and parity being included in the model. The model included the effects of treatment, parity, calf gender and its interaction. Relative mRNA expression was analyzed using the MIXED procedure with Kenward–Roger’s adjusted degrees of freedom. Statistical significance was declared at $P \leq 0.05$, and tendencies discussed when $0.05 < P \leq 0.10$. 
Results and Discussion

This study focused on examining the effects of bST administration during the first trimester of gestation on uterine hemodynamics and subsequent calf performance in beef cattle. The somatotropic axis components of GH and the IGF system are essential for controlling growth and reproduction (Lucy et al., 1995; LeRoith et al., 2001). The IGF system is expressed throughout the body, including the reproductive tract (Rhoads et al., 2008) with both IGF-1 and IGF-2 having regulatory actions on preimplantation and placental development (Wathes et al., 1997). Uterine BF for optimal fetal growth and development, as well as postnatal performance, of the calf is critical to the beef industry, but few studies have been conducted to investigate the impact of the maternal IGF system on intrauterine programming in beef cattle.

At TAI (d 0), prior to the initial bST treatment, plasma concentrations of IGF-1 were similar between treatments (98.59 ± 1.47 ng/mL; \( P = 0.936 \); Figure 4-2). However, the subcutaneous injections of 500-mg of bST in the neck administered every other week from TAI until d 97 of gestation increased plasma concentration of IGF-1 (\( P < 0.001 \); Figure 4-2) in beef cows. While past reports have noted a dose response of bST, this data agrees with research conducted in beef cows receiving 325 mg of bST either at TAI, two weeks post TAI, or at TAI and two weeks post TAI (Mercadante et al., 2016). Furthermore, research in dairy cows receiving 325 mg of bST at TAI and then two weeks post TAI noted an increase in plasma concentrations of IGF-1 (Ribeiro et al., 2013). While bST administration concluded on d 97 in this study, IGF-1 in circulation will remain elevated for greater than 14 days after the last injection. Research in dairy cows reported that concentration of IGF-1 peaks on d 7 post injection and can persist for 31 d when consecutive injections are given biweekly (Riberio et al., 2013). However, other data indicate that IGF-1 stimulation by the effects of bST administration
continue for approximately 14 d in beef and dairy females (Bilby et al., 1999; Bilby et al., 2004; Cooke et al., 2013; Mercadante et al., 2016; Riberio et al., 2013).

No differences ($P > 0.10$) were detected for Doppler ultrasonography parameters. A treatment $\times$ d interaction ($P = 0.007$) was detected for maximal systolic velocity ($V_{\text{max}}$), where no differences were observed on d 97; however, on d 233, $V_{\text{max}}$ was greater ($P < 0.001$) for BST-treated cows. While $V_{\text{max}}$ was greater in BST cows this parameter does not have a biological significance alone. Therefore, increased plasma concentrations of IGF-1 did not appear to have an impact on uterine hemodynamics. Past reports in cattle have indicated that bST can stimulate embryonic development directly (Moriera et al., 2000) or in vitro through its actions on IGF-1 (Palma et al., 1997). Maternal plasma IGFs play a role in maternal tissue growth and metabolism resulting in the modulating nutrient availability for conceptus growth and have been positively correlated with fetal growth and bw in several livestock species (Sferruzzi-Perri et al., 2011). Maternal plasma IGF-1 could potentially affect fetal growth indirectly and is itself altered by both body condition score and the gestation diet (Osgerby et al. 2003a and 2003b; Osgerby et al. 2002).

While the bi-weekly bST administration increased plasma concentration of IGF-1 in maternal circulation in the present study there were no differences detected in gestational length, calf bw, mean HG diameter, or CRL length (Figure 4-2; $P > 0.10$) between calves when assessed at 7±5 days of age. Additionally, cow body weight and body condition score were not different ($P < 0.10$; Figure 4-3) between treatments. On d -10 suckled beef cows weighed 528.7 ± 15.2 kg and had a BCS of 6 then on d 233 weighed 522.8 ± 15.1 kg and had a BCS of 5. The administration of bST during the periconception period in sheep has resulted in improved postnatal offspring performance (Costine et al., 2005; Koch et al., 2010). When calves were
assessed at the time of weaning for performance WW of calves was not affected by treatment. This data concurs with work by Starbuck and colleagues (2005) who detected no differences in bw or adjusted WW in beef calves born to cows that received a single injection of bST (500 mg; Posilac, Elanco Animal Health) at the time of AI ($n = 137$ inseminations) compared to those not receiving the bST ($n = 130$ inseminations). These data also concur with others reporting that injections of 325-mg bST either at TAI, two weeks prior to TAI, or both at TAI and two weeks prior had no effect on BW of subsequent beef calves when assessed at monthly from until 150 days of age (Mercadante et al., 2016).

Samples of liver tissue from each calf was collected for gene expression analysis. Analysis of mRNA expression of target IGF system ligands, IGF-1 and IGF-2, IGFR1, and the IGF binding protein IGFBP3 were conducted (Figure 4-6, 4-7, 4-8, and 4-9). There was no difference in relative gene expression of hepatic IGF-1 across treatments ($P = 0.99$). There was a treatment x gender interaction in BST heifers having an increased mRNA expression of IGFR1 than BST bulls ($P = 0.03$). However, research has indicated that GH stimulates liver expression of IGF1 (Kim et al., 2006; Jiang et al., 2007). While the current study did not measure mRNA expression in the maternal system, research has indicated that administration of 500 mg of bST increases mRNA expression of IGF1 in cows fed concentrates as well as cows only supplemented with and hay offered ad libitum (Wu et al., 2010).

**Conclusion**

In conclusion, beef cows that received bi-weekly injection of 500 mg of bST from the time of breeding through early gestation had increased plasma concentrations of IGF-1. However, increased maternal circulating concentration of IGF-1 did not alter uterine hemodynamics. Heifers born to cows treated with bST had an increase in gene expression of IGFR1 in comparison to bull calves generated from bST-treated cows. Furthermore, bST
administration failed to alter subsequent calf performance assessed from birth to time of weaning. Further investigations are needed on the effects of inducing the IGF system on uterine hemodynamics and postnatal gender specific calf growth performance of beef cattle.
Figure 4-1. Experimental outline and schematic of treatments. All cows were estrous synchronized using the 7-d CO-Synch + CIDR protocol where cows received an injection of GnRH on d -10 and CIDR was inserted, followed by injection of prostaglandin F$_{2\alpha}$ (PGF) and CIDR removal on d -3, followed by injection of GnRH and fixed-timed AI (TAI) on d 0. Blood samples were collected on d -20, -10, 0, 13, 27, 41, 55, 69, 83, and 97. Pregnancy was assessed on d 41 and 173 using transrectal ultrasonography. Uterine hemodynamics were recorded by Color Doppler ultrasonography on d 97 and 233. Treatments: CTL, no bST injected; BST, injection of 500 mg of bST on d 0, 13, 27, 41, 55, 69, 83, and 97.
Figure 4-2. Concentrations of insulin-like growth factor 1 (IGF-1) of beef cows on day of gestation by treatment. Treatments: CTL, no bST injected; BST, injection of 500 mg of bST biweekly early to mid-gestation. *P < 0.001.
Figure 4-3. Body weight of beef cows on day of gestation by treatment. Treatments: CTL, no bST injected; BST, injection of 500 mg of bST biweekly early to mid-gestation. $P = 0.999$
Table 4-1. Uterine hemodynamics evaluated by Doppler ultrasonography.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>SEM³</th>
<th>P-value TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$, cm/s</td>
<td>193.8</td>
<td>258.1</td>
<td>16.18</td>
<td>0.01</td>
</tr>
<tr>
<td>PI</td>
<td>1.0</td>
<td>1.0</td>
<td>0.06</td>
<td>0.92</td>
</tr>
<tr>
<td>RI</td>
<td>0.6</td>
<td>0.6</td>
<td>0.02</td>
<td>0.57</td>
</tr>
<tr>
<td>D, cm</td>
<td>0.7</td>
<td>0.7</td>
<td>0.05</td>
<td>0.56</td>
</tr>
<tr>
<td>BF, mL/min</td>
<td>3759</td>
<td>4355</td>
<td>763.9</td>
<td>0.58</td>
</tr>
<tr>
<td>BF MEAN, mL/min</td>
<td>2423</td>
<td>2800</td>
<td>463.1</td>
<td>0.57</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>68.1</td>
<td>74.0</td>
<td>2.55</td>
<td>0.12</td>
</tr>
</tbody>
</table>

¹BST: Angus-based crossbred cows receiving biweekly subcutaneous injections of 500 mg of recombinant bovine somatotropin through d 97; CTL: Angus-based crossbred cows receiving no injections. All cows were exposed to the 7-day CO-Synch + CIDR estrus synchronization protocol and fixed-time artificial insemination.

²$V_{\text{max}}$ = maximum velocity, PI = pulsatility index, RI = resistance index, D = vessel diameter, BF = blood flow of the uterine artery ipsilateral to the conceptus, BF MEAN = mean blood flow of the uterine arteries ipsilateral and contralateral to the conceptus.

³Pooled standard error of treatment means; BST: $n = 24$; CTL: $n = 28$. 
Figure 4-4. Representative image of the uterine artery vessel adjacent to the External Iliac artery and External Iliac vein in pregnant multiparous Angus cow on d 233 of gestation using Color Doppler ultrasonography.
Figure 4-5. Representative waveforms of blood flow in uterine artery vessel in pregnant multiparous Angus cow on d 233 of gestation using Color Doppler ultrasonography.
Table 4-2. Gestation length and morphometric measurement of calves with body weight being measured within 24 hours of birth and heart girth and crown rump length being measured at time of liver biopsy 7 ± 5 days of age from cows treated with bST (BST; \( n = 24 \)) or without (CTL; \( n = 28 \)). SEM is reported as the pooled standard error of treatment means.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation length, d</td>
<td>CTL 279</td>
<td>BST 277</td>
<td>1.5</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>30.01</td>
<td>30.59</td>
<td>4.326</td>
</tr>
<tr>
<td>Heart girth, cm</td>
<td>77.65</td>
<td>78.46</td>
<td>0.571</td>
</tr>
<tr>
<td>Crown rump length, cm</td>
<td>84.94</td>
<td>86.79</td>
<td>0.692</td>
</tr>
</tbody>
</table>
Table 4-3. Nucleotide sequence of bovine-specific primers used for real-time quantitative reverse transcription PCR to determine the hepatic expression of target genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene name</th>
<th>Primer</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp1</td>
<td>Cyclophilin 1</td>
<td>Forward</td>
<td>5’ – GCCATGGAGCGCTTTGG – 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>5’ – CCACAGTCAGCAATGGTGATCT – 3’</td>
</tr>
<tr>
<td>RPS9</td>
<td>Ribosomal protein S9</td>
<td>Forward</td>
<td>5’ – GCTTAGGCGACGAGGGCA – 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>5’ – AGCTCTTTGCTGACGGGGGA – 3’</td>
</tr>
<tr>
<td>IGF1</td>
<td>Insulin-like growth factor 1</td>
<td>Forward</td>
<td>5’ – CTCCTCGCATCTCTCTATCT – 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>5’ – ACTCATCCACGATTCTGTCT – 3’</td>
</tr>
<tr>
<td>IGF2</td>
<td>Insulin-like growth factor 2</td>
<td>Forward</td>
<td>5’ GACCGCGGCTCTACTTCAG – 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>5’ -AAGAACCTTGGCCACGGGAT -3’</td>
</tr>
<tr>
<td>IGFR1</td>
<td>Insulin-like growth factor 1 receptor</td>
<td>Forward</td>
<td>5’ – CAACGGCAACCTGAGCTACT – 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>5’ – TTCCTGATGGGAATTGTTGT – 3’</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>Insulin-like factor binding protein 3</td>
<td>Forward</td>
<td>5’ – AATGGCAGGTTCTGGCCATT – 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>5’ – AAGTTCTGGGTCTGTGCT – 3’</td>
</tr>
</tbody>
</table>
Figure 4-6. Relative gene expression of hepatic insulin-like growth factor 1 (IGF-1) mRNA of heifer and bull calves that were either exposed to or the absence of the treatment during intrauterine development. Treatments: CTL, no bovine somatotropin administration (bST); BST the cow received an injection of 500 mg of bST biweekly early to mid-gestation.
Figure 4-7. Relative gene expression of hepatic insulin-like growth factor 2 (IGF-2) mRNA of heifer and bull calves that were either exposed to or the absence of the treatment during intrauterine development. Treatments: CTL, no bovine somatotropin administration (bST); BST, the cow received an injection of 500 mg of bST biweekly early to mid-gestation.
Figure 4-8. Relative gene expression of hepatic insulin-like binding protein 3 (IGFBP3) mRNA of heifer and bull calves that were either exposed to or the absence of the treatment during intrauterine development. Treatments: CTL, no bovine somatotropin administration (bST); BST, the cow received an injection of 500 mg of bST biweekly early to mid-gestation.
Figure 4-9. Relative gene expression of hepatic insulin-like growth factor 1 (IGFR1) mRNA of heifer and bull calves that were either exposed to or the absence of the treatment during intrauterine development. Treatments: CTL, no bovine somatotropin administration (bST); BST, the cow received an injection of 500 mg of bST biweekly early to mid-gestation. *Effect of treatment x gender ($P = 0.03$) interaction.
CHAPTER 5
SUMMARY AND FUTURE RECOMMENDATIONS

Somatotropins, or GH, are naturally occurring proteins secreted by the anterior pituitary gland that stimulate growth, cell regeneration and reproduction in mammals. These proteins have anabolic and growth promoting effects, mediated through IGF-1, on bone, adipose and muscle. Insulin-like growth factors mediate various biological process including growth, lactation, reproduction, and health (McGuire et al., 1992). Research has indicated that an increase in blood bovine GH upregulated the synthesis of IGF-1, as measured in both milk and blood (De Morais et al., 2016). A recombinant form of bovine growth hormone, bST, is produced by recombinant DNA techniques. Until recent years the slow release recombinant formulation of the was commonly used in the U.S. dairy industry to extend the persistence of lactation in healthy dairy cows. Milk yield increase due to the use of bST occurs by its antagonizing actions of insulin and shifting nutrient partitioning thus resulting in more pounds of milk produced per lactation period (Bauman, 1999).

The goal of this research was to investigate what mechanisms exist that control fetal programming in the beef fetus and if an increase in IGF-1 would enhance the potential of the uteroplacental system resulting in an altered offspring. Uterine hemodynamics was of particular interest in address this need for knowledge as the more recent and noninvasive technique of Color Doppler Ultrasonography gives the ability to monitor hemodynamics pregnancies. While the goal of this study was to investigate the nutrient portioning abilities of bST administration neither the heifers nor the cows were subjected to nutrient restriction. The first experimental study included heifers that were supplemented compared to cows on a forage based diets. Differing results elicit the thought that differences due to age, heifer vs. cow, as well as potential
differences in diet and/or dry matter intake. Therefore, additional studies in the future are important investigating fetal undernutrition as it frequently occurs during livestock production.

The mean concentrations of IGF-1 were greater in heifers and cows that received bST treatment when compared to those that did not. This research data concludes that biweekly administration of 500 mg of bST from TAI through early gestation increases plasma concentration of IGF-1 in nulliparous heifers, primiparous cows, and multiparous beef cows. There was no difference detected in BW in either the heifer or cow study. This data concurs with reports that demonstrated a significant increase in IGF-1 in maternal circulation but no effect on BW when heifers received a diet high in protein supplementation (Perry et al., 2002).

Research is limited in cattle examining the impacts of altering uterine hemodynamics compared to other areas of mammalian research. Additional research is needed to better understand how management strategies and therapeutic applications affect uterine hemodynamics and its impact on fetal programming in cattle.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

A fifth-generation agriculturalists, Carla Dean Sanford was raised on a row crop, produce, and commercial beef cattle farm in Southwest Georgia. Even as a young child, Carla was passionate about learning and working with livestock thus, when she had the opportunity to exhibit beef cattle in grade school, it only solidified her interest in animal science and agriculture. This yearning to learn led her to Abraham Baldwin Agriculture College (ABAC), where she obtained her Associate of Science degree, before attending the University of Georgia, where she earned her Bachelor of Animal Science degree. The opportunity to conduct equine reproduction research and obtain her Master of Science degree from Texas Tech University (TTU) came about after completing an undergraduate internship at the 6666 Ranch in Guthrie, Texas. Post-graduation from TTU, Carla returned to ABAC where she was a lecturer teaching biological sciences lectures and laboratory courses as well as a Physiology of Reproduction course. This allowed her to acquire a great deal of experience in teaching and mentorship. In an attempt to continue her education, so that she may better serve her students and learn more about animal agriculture, Carla joined Dr. G.C. Lamb’s research team at the North Florida Research and Education Center at Marianna, Florida as a Graduate Research Assistant while completing her Ph.D. program. Under Dr. Lamb’s and Dr. Nicolas DiLorenzo’s mentorship, Carla conducted research focusing on the role of the IGF system in inducing fetal programming and the improvement of postnatal performance in beef cattle. Upon completion of her Doctor of Philosophy degree in animal sciences, Carla will join the faculty at the Department of Animal and Range Sciences at Montana State University as an Assistant Professor in Beef Cattle Extension.