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Nutritional Effects on Scrotal Circumference, Motility and Morphology of Spermatozoa and Cytokine Concentrations within Seminal Plasma of Beef Cattle

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I am submitting herewith a thesis written by Taylor D. Harrison entitled "Nutritional Effects on Scrotal Circumference, Motility and Morphology of Spermatozoa and Cytokine Concentrations within Seminal Plasma of Beef Cattle." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Nutritional Effects on Scrotal Circumference, Motility and Morphology of Spermatozoa and Cytokine Concentrations within Seminal Plasma of Beef Cattle

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"You've got what it takes, but it will take everything you've got."

ABSTRACT

Sire fertility is impacted by a wide variety of environmental and management conditions, potentially causing critical losses within beef operations. Nutrition is a main influence on the ejaculate due to bulls undergoing multiple planes of nutrition yearly. By identifying the effects of nutritional planes on the ejaculate composition, reproductive efficiency could be maximized at pregnancy establishment. We hypothesized periods of differing nutritional planes and body condition score (**BCS**) would affect bull fertility and cytokine concentrations within the ejaculate of bulls. Mature Angus bulls (n = 11) were individually housed and randomly assigned to one of two treatments: 1) over-fed (**OVER**, n = 5) targeting a BCS of 8, or 2) restricted (**RES**, n = 6) targeting a BCS of 4. Bulls were fed the same ration at different intake volumes to achieve desired effects with different nutritional periods: gain, loss, ideal body condition steady-state (**ISS**) at a BCS of 6, and abnormal body condition steady-state (**ABS**), at a BCS of 8 or 4 as per treatment design. Body weight (**BW**) and BCS were taken every two weeks to monitor bull weight and adipose changes. Ejaculates were collected every 84 d to determine bull fertility and cytokine profiles within seminal plasma (**SP**). A completely randomized design was implemented and data analyzed with mixed model ANOVAs via PROC GLIMMIX (SAS 9.4, Cary, NC) to determine if nutrition period, treatment and the interaction influenced bull fertility. Progressive forward motility tended to be greater (P=0.10) during the ISS period regardless of treatment. Morphological head and total abnormalities of spermatozoa were influenced by an interaction (P<0.005) with increases in abnormalities occurring when deviated from basal BCS. Pro- and anti-inflammatory cytokines (P<0.05) decreased from initial concentrations over the gain, ABS and loss

periods with a return to similar concentrations as the initial. Pro-inflammatory cytokines had reduced cytokine concentrations compared to anti-inflammatory cytokines. The cytokines, MIP-1α, TNF-α, and IL-1β had the greatest impact on cytokine profiles within SP during nutritional periods. In conclusion, cytokine concentrations, motility, and morphology were influenced by different nutritional levels which could hinder the ability to establish a successful pregnancy.

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CHAPTER ONE
INTRODUCTION

In order to accommodate the rising population by 2050, a 70 percent increase in global food production is required, including 200 million tons of meat as countries become more developed (FAO, 2009). By increasing reproductive efficiency and maximizing genetic potential of beef cattle, the required increase in meat production can be achieved. Bull fertility is highly influential in reproductive efficiency of cow-calf production. Bull management for maximum fertility includes selective breeding, health management, and proper nutrition to produce bulls that fit industry and consumer standards (Kastelic, 2013). Whereas, injuries and infections can decrease semen quality by decreasing progressive forward motility and increasing morphological abnormalities of spermatozoa (Azenabor et al., 2015). Correct nutritional management of bulls is critical due to one sire producing multiple offspring in a single breeding season.

Optimal reproductive performance throughout the breeding season is key for successful bull performance. Nutrition is a major factor that impacts the success of any bull heading into the breeding season (Short and Adams, 1988). The breeding season is extremely demanding as the bull's focus shifts from eating to mating. Therefore, bulls should begin the breeding season at a body condition score (**BCS**) of 7 due to the imminent loss of 45 to 135 kg, or 1 to 2 BCS (Farney, 2016), from increased physical activity and decreased feed consumption. Low energy and protein diets have also been found to decrease seminal volume, libido as well as the progressive motility of spermatozoa (Singh et al., 2018) all of which will impact reproductive performance during the breeding season. Restricted feed intake can also inhibit spermatogenesis (NRC, 2000). Whereas high energy and protein diets, can induce a hormonal imbalance, increase

spermatozoa abnormalities, and induce inflammatory cytokines within SP (Tremellen et al., 1998; Selvaraju et al., 2012; Singh et al., 2018). The cytokines within SP are crucial for the successful establishment of pregnancy through fetal tolerance and uterine tissue reconstruction in beef cattle (Robertson et al., 2009). Further demonstrating that improper nutritional management can hinder herd reproductive success due to influences from the sire.

Inadequate bull fertility has traditionally been attributed to sperm quality (i.e. motility and morphology) while the contribution of bovine SP to fertility has been largely ignored (Nongbua et al., 2018). Seminal plasma is composed of hormones, sugars, lipids, peptides, proteins, and cytokines for immunoregulatory functions and motility. All of these components influence the spermatozoa and the immune system of both the male and female, playing a critical role in fertilization and the establishment of pregnancy. Cytokines, specifically, within the female reproductive tract to either induce a immuno-suppressive environment for the establishment of the pregnancy or to recruit immune cells to the endometrium (Bromfield, 2014). Concentrations and types of inflammatory cytokines are known to be greatly influenced by nutrition (Caroleo et al., 2019). Therefore, nutrition may maintain balance to promote the necessary changes within the uterus for pregnancy (Klasing, 1988).

Cytokines and chemokines within SP are essential for the establishment of pregnancy. These cytokines initiate and control uterine inflammation including the tissue reconstruction necessary for early pregnancy (Bromfield, 2014). The inflammation initiated after coitus influences the survival of the semi-allogenic conceptus during the establishment of pregnancy (Robertson et al., 2018). Studies in human, ovine and murine models have demonstrated the importance of the uterine inflammatory response in order to establish pregnancy (Lovell and

Getty, 1968; Robertson et al., 1997; Sharkey et al., 2012). The maternal inflammatory reaction occurs within the cervix and uterus to recruit leukocytes (Bromfield, 2014), and produce additional inflammatory cytokines (Chatterjee et al., 2014). The creation of an immuno-tolerant environment allows development of the semi-allogenic fetus and to prevent rejection by the maternal system (Aluvihare et al., 2004; Robertson et al., 2018). Cytokine profiles differ according to stage of pregnancy (Dutta and Sengupta, 2017) with the uterine environment being mostly pro-inflammatory during implantation and then shifts to anti-inflammatory during the second and third trimester in mice and humans (Zhang et al., 2017). Another shift occurs with an final influx of pro-inflammatory macrophages and cytokines during parturition (Zhang et al., 2017). However, the cytokines in the uterus of cattle have been explored only during early pregnancy (Leung et al., 2000). Research is required to determine the cytokine associations and interactions between SP and the uterine environment. Therefore, the objectives of the current thesis were to quantify the cytokines within bovine SP on different nutritional planes and at differing BCS which influence the uterine response to mating and affect reproductive potential of a sire during the breeding season.

CHAPTER TWO
LITERATURE REVIEW

BULL MANAGMENT

Industry Standards for Sires

Proper bull management, including nutrition, health programs and animal selection, is critical to the production of spermatozoa in order to maximize reproductive success. Improper sire management can compromise the fertility of bulls. External and environmental factors such as nutrition, weather, injury, disease and stress can impact semen quality and cause a loss in production value through decreased pregnancy rates (NRC, 2000; Suriyasomboon et al., 2005; Singh et al., 2018). Failure to accommodate the bull and protect the testis, via bedding or housing, during inclement weather can decrease semen quality through decreased motility and increased morphological abnormalities of the spermatozoa (Koivisto et al., 2009; Thomas, 2009). Another management impact on fertility is breeding pressure by exceeding the recommended bull-to-cow ratio. The recommended bull-to-cow ratio is the number of females is about equal to the bull age in months (Greiner, 2010), potentially resulting in decreased pregnancy numbers. Sire libido and stamina may decrease when pushed to cover more land and breed more females (Sprott et al., 2003); however, libido is influenced by age, exercise and nutrition (Singh et al., 2018). The stress from exceeding the bull-to-cow ratio will drastically increase cortisol levels in bulls causing an increase in ROS which can impair sperm (Borg et al., 1991). Therefore, bulls used for natural service are exposed to a wide range of management and environmental conditions that should be adjusted constantly to ensure optimal fertility.

In order for a bull to service as many females possible, bulls need to be managed so nutritional requirements are adequate for the time of year, age, and body condition score (**BCS**). A yearling bull will have increased nutrient requirements, to support growth and development,

compared to a mature bull who need to just support body maintenance (National Academies of Sciences and Medicine, 2016). The onset of puberty greatly impacts reproductive performance of young bulls. Puberty has been defined as the point of time when a bull is able to first produce an ejaculate containing at least 50 million spermatozoa with a minimum of 10% motility (Lunstra et al., 1978). The age of pubertal onset varies across breeds and depends on body weight; however, puberty is usually around 9 to 11 months of age (Lunstra et al., 1978). Increased yearling scrotal circumference has been positively related to increased sperm motility and decreased abnormal sperm morphology (Sprott et al., 2003). Scrotal circumference is a valued indicator of male fertility and is highly correlated with paired testes weight which corresponds to daily sperm production and semen quality (Youngquist and Threlfall, 2006; Latif et al., 2009). A sire with increased scrotal volume will also sire daughters that reach puberty at a younger age (Martin et al., 1992) which is important due to heifers who reach puberty earlier will maximize lifetime profits and reproductive efficiency. Semen characteristics are influenced by the age of the bull with mature sires having greater volume and quality ejaculate than younger bulls due to the physiological changes such as an increase in body and testicular mass (Brito et al., 2002) as well as increased semen production from simultaneous development of the testis and accessory sex glands (Almquist, 1978). Therefore, adequate nutrition is necessary for the onset of puberty, sexual maturation, and overall optimal semen production.

The nutritional plane of a sire will decrease during the breeding season via reduced feed intake (Bryne, 2020). Thus, it is important that bulls are kept at a proper BCS prior to the breeding season in order to successfully cover the land and mate with all females while maintaining optimal fertility (NRC, 2000). Bull management between breeding seasons is critical

since issues such as inactivity and excess weight and fat deposition from overfeeding could lead to potential injuries and lameness (Sprott et al., 2003). During the time when bulls are not breeding, bulls need exercise to ensure stamina is maintained to decrease the incidences of injuries (Thomas, 2009). Minimizing diseases of limbs and joints and physical injuries to the penis or testicles, legs, or hooves can increase bull performance levels and fertility (Sprott et al., 2003). Proper sire management is required to maximize bull fertility while minimizing stress, injury, infection and environment stress.

Breeding Soundness Exam

A vital component of beef herd management to ensure sire fertility prior to the breeding season is a complete systematic evaluation known as a breeding soundness exam (**BSE**). A BSE is indicative of current fertility of a bull and should be done no less than 60 days prior to the breeding season. There are four components of a BSE: physical exam, scrotal circumference, sperm motility and sperm morphology. Failure to perform a BSE could negatively impact herd efficiency due to decreases in the number of females that become pregnant from poor semen quality (Wiltbank and Parish, 1986). Spermatogenesis is vulnerable to a variety of internal and external factors including age, temperature and season, injury, nutrition, infections or other stressors (Parkinson, 1987; Kastelic, 2013; Murphy et al., 2018). Therefore, a BSE cannot ensure that bulls will remain fertile throughout the breeding season but can ensure that bulls with high quality semen are used to start the breeding season. Once evaluated, bulls will be placed into three classifications including: satisfactory potential breeder (fertile), unsatisfactory potential breeder (subfertile or infertile) or deferred (Hopkins and Spitzer, 1997). To be considered a satisfactory fertile breeder, the bull has to pass the physical exam, meet or surpass the minimum

thresholds for scrotal circumference, sperm motility and sperm morphology as well as show no genetic, infectious or other detrimental problems that could impair the bull's ability to copulate and conceive a pregnancy (Hopkins and Spitzer, 1997). An unsatisfactory breeder is classified as any sire who has failed to meet the set standards in one or more of the above categories with an unlikely chance of improvement (Hopkins and Spitzer, 1997). A deferred classification occurs when the bull does not fit into the first two categories, but has the potential of another test resulting in satisfactory fertility (Hopkins and Spitzer, 1997).

The physical exam of a BSE evaluates conformation and external appearance including feet and legs structure, BCS (1 = emaciated and 9 = obese, (Wagner et al., 1988)), vision, and hearing (Sprott et al., 2003). An external reproductive examination is performed to visually evaluate and palpate the scrotum, penile sheath, and glans penis to ensure there are no abnormalities which may hinder copulation and semen quality (Karayat et al., 2016). Scrotal circumference must meet minimum thresholds set for bulls of a given age. According to the Society for Theriogenology, bulls ≤ 15 months should have a minimum of 30 cm, 15 ≤ 18 months = 31 cm, > 18 ≤ 21 months = 32 cm, > 21 ≤ 24 months = 33 cm and > 24 months should have a scrotal circumference of at least 34 cm (Chenoweth, 2015). The internal reproductive examination is conducted via rectal palpation to determine the vesicular glands size, prostate and inguinal rings status. Infections can occur within seminal vesicles known as seminal vesiculitis which can cause an enlargement of the seminal vesicles and lead to poor semen quality (Sprott et al., 2003). After collection of the ejaculate, a drop of semen is evaluated under a microscope for motility and morphology of the spermatozoa. The semen is placed on a warmed slide and diluted with a few drops of warm saline to efficiently evaluate individual motility under the microscope

at medium power. The threshold for a satisfactory breeder is ≥ 30% progressively forward, motile sperm (Youngquist and Threlfall, 2006; Chenoweth, 2015). Morphology is then evaluated under oil immersion after staining the slide and counting 100 sperm cells. Spermatozoa abnormalities such as detached heads, midpiece reflects and coiled tails (Senger, 2012), are classified by location of the defect including head, midpiece or tail, with satisfactory breeders producing ≥ 70% morphologically normal sperm cells (Barth and Oko, 1989; Chenoweth, 2015). The importance of sperm morphology is well documented as high levels of morphologically abnormal sperm can reduce rates of fertilization and embryonic development (Menon et al., 2011). Morphological abnormalities can occur from temporary conditions such as injury, disease or an extended amount of time without ejaculation (Almquist, 1982; Singh et al., 2018). Bulls with temporary conditions of infertility that can likely resolve are classified as deferred. Breeding soundness exam results are essential to identify bulls that are grossly abnormal and could negatively impact calving and pregnancy rates.

Impacts of Artificial Insemination and Cryopreservation

Artificial insemination (**AI**) and cryopreservation allows access to genetics from across the world as well as overall increased performance and value of progeny from superior sires. The success of AI is dependent on many factors, including the fertility of the bull which can be impacted by collection interval and frequency, extender and cryopreservation process (Almquist, 1982; Raheja et al., 2018; Peris-Frau et al., 2020). Cryopreservation is a consecutive process of reduction in temperature, dehydration of the cell, freezing, and storage within liquid nitrogen containers. One of the main concerns of cryopreservation is the irreversible effects of cold shock, the rapid cooling of semen, reducing the quality of the sperm during freezing and thawing.

Therefore, sperm integrity is influenced by alterations to the membrane structure and function as well as cell metabolism due to the detrimental aspects of cryopreservation (Ugur et al., 2019). The challenges facing cryopreservation include membrane changes, reactive oxygen species (**ROS**) and overall molecular issues. During the process of cooling, the cells are being exposed to a series of harmful effects including cellular acidosis, deprivation of energy, destabilization of the cytoskeleton and production of ROS (Baust et al., 2009). During the freezing process, sperm are predisposed to detrimental effects of ice crystal formation, hyper-osmolarity, alterations in the cell volume, and protein denaturation (Baust et al., 2009). While sperm cells are more durable than any other cell in the body for cryopreservation due to their low water content (Ugur et al., 2019), there are still many gaps and issues in the process of perfecting cryopreservation to maximize the success of AI for the highest genetic influence.

Semen extenders were created to lessen cell damage from cryopreservation and improve post thaw viability and overall quality as well as increase the efficiency when used in ART. To aid cryopreservation, semen extenders have been developed to lessen cryodamage and improve the overall quality and post-thaw viability. The most common semen extender used in bovine sperm is based on 20% egg yolk and glycerol throughout cryopreservation to prevent cell damage (Wall and Foote, 1999). However, heated whole milk or pasteurized skim milk was traditionally proven to also be used as practical diluting fluid for semen (Almquist et al., 1954). Combinations of anti-microbial drugs such as penicillin and streptomycin have also been used in semen extenders to control bacteria growth and increase fertility (Almquist, 1951). However, the presence of substances in egg yolk-based extenders such as high-density lipoproteins and minerals in yolk granules can inhibit respiration of sperm cells and reduce the motility of

spermatozoa (Moussa et al., 2002). Yet, it has been found that low density lipoproteins of egg yolk can protect the sperm cells from damage by covering the sperm membrane during the freezing and thawing process (Amirat et al., 2004). Almquist and others demonstrated how the thawing rate of semen straws when performing AI, is extremely important for pregnancy success with higher pregnancy rates occurring with a 40 second thaw compared to 9 seconds (Almquist et al., 1982). Furthermore, extenders and other techniques during cryopreservation are used in order to reduce negative effects on the overall fertility of spermatozoa.

SPERMATOGENESIS AND EJACULATE COMPONENTS

Spermatogenesis

The intricate process of male gamete development within the seminiferous tubules of the testis is known as spermatogenesis. In order for spermatogenesis to occur with minimal morphological defects, the testis must be 4 to 6 ℃ cooler than the normal body temperature. In the course of spermatogenesis, germ cells undergo three phases: 1) proliferation, 2) meiotic, and 3) differentiation, during the transformation from stem cells to mature spermatozoa (Senger, 2012). Spermatogenesis occurs over 61 days in the bull; however, this duration varies by species. Throughout spermatogenesis, a somatic cell type known as Sertoli cells, nurtures the developing germ cells by providing nutrients, hormones, and proteins that regulate development. Beginning with spermatogonia, the testicular stem cells, are located on the basal compartment of the seminiferous tubules. Then move toward the lumen as they mature and proliferate through a series of mitotic divisions. The divisions begin with spermatogonia A_1 to A_4 and continues on to spermatogonia I and then B spermatogonia completes the proliferation phase of spermatogenesis. The meiotic phase begins with the cell division from B spermatogonia to primary spermatocyte.

Then meiotic divisions take the primary spermatocytes to secondary spermatocytes and on to round spermatids. After meiosis, the spermatids undergo morphological changes to form cells that are capable of fertilization including the condensing of the nucleus, acrosome formation and tail elongation (Senger, 2012). The differentiation phase, also known as spermiogenesis, consist of four stages: 1) the Golgi phase, 2) the cap phase, 3) the acrosomal phase, and 4) the maturation phase (Johnson et al., 2010). The Golgi phase is characterized by the acrosomic vesicle formation from the fusion of proacrosomic granules as well as the migration of centrioles. The cap phase occurs as the acrosome development continues and flattens to form a distinct cap over the nucleus of the spermatozoon. The formation of the flagellum that extends away from the nucleus and into the lumen of the seminiferous tubules completes the cap phase (Senger, 2012). The acrosomal phase consists of cytoplasmic and nuclear elongation with the final morphological changes occurring in the maturation phases. The fully development spermatozoon will have a head, neck, middle piece and principal piece, a self-powered flagellum known as the tail. The spermatozoa are released into the lumen of the seminiferous tubules continuously as a spermatogenic wave. Once released, the mature sperm will travel to the rete testis located in the hilum of the testicle that will carry the infertile spermatozoa to the efferent ducts and into the caput epididymis (Amann and Schanbacher, 1983). The efferent ducts and caput of the epididymis are involved in fluid and solute resorption (Amann and Schanbacher, 1983). The corpus and cauda epididymis are critical for final maturation membrane changes of spermatozoa with quiescent factors until combined with SP from the accessory sex glands. The continuous smooth muscle contractions and spermatogenic waves are essential to allow a collection of mature sperm to be located in the cauda epididymis and stored until ejaculation

occurs. However, once the ejaculate is released into the female numerous membrane modifications will continue to occur to the spermatozoa to allow them to be fertile. These changes occur within the female as the spermatozoa travel through the cervix. When spermatozoa are mixed with SP, they become coated with specific proteins and once inside the female reproductive tract, these proteins are stripped away (Senger, 2012). Decapacitation of spermatozoa can happen if capacitated spermatozoa are removed from the female tract and returned to SP causing them to once again be infertile from the various proteins (Senger, 2012).

Semen

Semen is composed of a cellular component (the spermatozoa) and a liquid component (the SP). Semen quality can be affected by a wide range of genetic and environmental factors including age, nutrition, collection frequency, trauma and season (Almquist, 1982; Senger, 2012; Kastelic, 2013; Murphy et al., 2018; Tank and Monke, 2020). There can be a two to four week delay before effects from external factors such as fever, stress, injury or environmental temperature can be observed within the ejaculate (Senger, 2012). Studies have also reported factors including age to have an effect on semen such as age with mature bulls having a greater semen quality and volume compared with younger bulls (Mathevon et al., 1998; Fuerst-Waltl et al., 2006; Murphy et al., 2018). Diseases and fevers have been known to hinder the ejaculate quality since spermatogenesis can be affected from the increased body temperatures and influx of immune responses. Seasonal variation also seems to have an effect on semen characteristics potentially due to changes in scrotal thermoregulation (Menegassi et al., 2015). Scrotal thermoregulation is completed via multiple mechanisms including the countercurrent heat exchange through the pampiniform plexus, the tunica dartos and the cremaster muscle control the

distance of the testes from the body, and evaporative heat transfer via scrotal sweat glands (Senger, 2012). Hinderance of these processes will cause a decrease in semen quality and can be a cause of reproductive failure. Spermatogenesis can be hindered as well through the influence of photoperiod when analyzing luteinizing hormone and testosterone concentrations (Godfrey et al., 1990). The sire's ability to adapt to new climates and conditions may have semen quality consequences and account for the failure in their reproductive capacity.

Semen quality can be reduced from the nutritional impacts of low energy and protein on male fertility by decreasing libido and spermatozoa concentration, increasing morphological abnormalities and reducing progressive forward motility of the spermatozoa (Meacham et al., 1963; Mwansa and Makarechian, 1991; Kastelic, 2013; Singh et al., 2018). Prolonged deficiencies of nutrition have also been found to impact the interstitial and Sertoli cell populations as well as Leydig cells and, thus, testicular steroidogenesis and spermatogenesis (NRC, 2000; Bollwein et al., 2017). More specifically, it has been found that low energy and protein diets in young bulls can nutritionally impact SP by decreasing the amount of seminal volume produced with each ejaculate (Hiroe K, 1964; Van Demark and Mauger, 1964). In contrast, a high energy concentrate diet in mature bulls can increase morphological abnormalities, reduce epididymal sperm reserves and sperm production possibly due to the adipose deposition altering scrotal thermoregulation (Coulter and Kozub, 1984; Coulter et al., 1999). Stress induced hormonal imbalance, decreased libido and impaired testicular development can also occur due to increased levels of energy and protein within the sire diet. However, it has been found that high energy and protein an increase motility percentage of spermatozoa, sperm concentration and the volume of the ejaculate (Perkovic S, 2001). Furthermore, in adult rams,

level to high energy fed groups compared to low every groups had an increase in the production of Insulin-like Growth Factor (IGF)-1 within SP (Selvaraju et al., 2012). Moreover, there are many nutritional effects that can hinder the overall quality of semen which would decrease reproductive capacity and success as a whole.

Seminal Plasma Composition

Seminal plasma is known as a nutritive-protective fluid that is comprised of secretions from the accessory sex glands within the male reproductive tract (Senger, 2012). The majority of SP production comes from the seminal vesicles (Juyena and Stelletta, 2012). This complex medium functions to protect, immune modulate, transport, and nourish the spermatozoa after ejaculation until fertilization (Nongbua et al., 2020). Cytokines from the SP play a critical role in the signaling of the uterus to prepare for the semi-allogenic fetus (Robertson et al., 2009). Inflammatory cytokines and prostaglandins synthesized in the male accessory sex glands are transferred to the female through the active components of the SP (Schjenken and Robertson, 2020). The SP cytokines stimulate the endometrial epithelial cells to synthesize additional cytokines which in turn facilitate uterine tolerance and cause inflammation needed to support the expansion and implantation of the fetus within rodents (Robertson, 2005). This immune response has been found in mice and cattle where cytokines initiate the cascade of inflammatory responses in the endometrium during conception to prepare for pregnancy and the fetus (Tremellen et al., 1998; Bromfield, 2014). This response activates changes in gene expression leading to the modification of structure and function of the local female reproductive tissues through the recruitment of leukocytes, macrophages and dendritic cells due to molecular signaling from cytokines (Robertson, 2005). Another prominent feature of the immune response is the induction

of acute phase proteins by cytokines in order to maintain homeostasis (Gulhar et al., 2021). Through uncontrolled and prolonged action of cytokines there could be harmful effects; therefore, a balance of pro- and anti-inflammatory cytokines depending on environment and physical status (Cicchese et al., 2018). Mediated cytokine receptors and receptor antagonists are in place to achieve this desired balance of inflammatory cytokines (Moshage, 1997). Therefore, there is a required balance of inflammatory cytokines and their biological effects in order to achieve maximum fertility and successful pregnancy establishments.

Besides the immunological moieties in SP, this non-cellular fluid is composed of proteins, amino acids, hormones, antioxidants, lipids, ions, sugars and more to protect and transport spermatozoa. Proteins within epididymal fluid have many benefits through sequential interactions including the enhancement of sperm penetration into oocytes, motility, and capacitation (Thérien et al., 1998). Additionally, the balance within SP of pro-oxidant and antioxidant activity is paramount to the success of spermatozoa maturation and zygote formation. Oxidative stress is an important factor related to poor semen quality due to the increased rate of cellular damage induced by ROS. The two main sources of ROS production in semen is immature spermatozoa and leukocytes which initiates the process of lipid peroxidation. Interestingly, ROS can play an important role in sperm capacitation and viability, the acrosome reaction, and the stabilization of the mitochondrial capsule in the midpiece in bovine (Gonçalves et al., 2010). Another component within SP that contributes to sperm viability is fructose, a known essential sugar present within ruminant SP. Fructose is a major saccharide that is synthesized by seminal vesicles from blood glucose stimulated by testosterone concentrations and increased in concentration during the breeding season of rams (Matsuoka et al., 2006).

Fructose is essential due to its role in sperm motility and metabolism for spermatozoa to utilize the ATP available (Sanchez-Partida, 1999). In conclusion, each component of SP is essential for reproductive success whether through the maintenance of function of ions to spermatozoa transport with hormones, each component provides a motile fertile spermatozoon.

BULL NUTRITION

Breeding Season

The efficiency of a beef cattle enterprise is highly dependent on optimal reproductive performance throughout the breeding season. One of many factors that influence reproductive success of a mature bull heading into the breeding season, is nutrition (Short and Adams, 1988; Singh et al., 2018). The breeding season is highly demanding for a bull due to decreased feed intake and increased physical activity. However, if bulls are performing to expectations, they will mentally put themselves on a nutrient restricted diet by not eating in order to breed as many females as possible during the breeding season. During the breeding season, nutritional opportunities and management are limited due to bulls having access to nutrients on the same plane of nutrition as females on pasture. Therefore, bulls should begin a breeding season with a BCS of 5.5 to 6.5 to account of the upcoming loss of 45 to 135 kgs that will occur as focus shifts from eating to mating. However, a bull that is over-conditioned or under-conditioned needs to have BCS adjusted accordingly at least 60 days before the breeding season, in order to reduce metabolic stress which could possibly decrease semen quality. Bulls transitioning from a higher-concentrate feed ration to support the gain in BCS required prior to the breeding season, to forage-based diets to maintain bulls on pastures during the breeding season can cause potential fertility problems. This demonstrates how essential it is to start bulls at a healthy or even slightly

heavier BW well before the breeding season. Replacing bulls during the breeding season can minimize stress due to nutrient restriction which can lead to decreased libido and overall semen quality (NRC, 2000). Another mechanism to minimize bull stress, is multiple-sire breeding groups to ensure the female to male ratio is small enough to maximize reproductive success (Sprott et al., 2003). However, aggression and fighting for social dominance are potential issues of a multiple-sire group. Multi-sire breeding groups can be less efficient if the dominant bull has poor semen quality which will decrease conception rates (Sprott et al., 2003). Therefore, bull breeding soundness exams should be performed to prevent the loss of a future herd. Nutritional management of bulls in between breeding seasons is critical to maintain a healthy basal level BCS for proper homeostasis and reproductive function (NRC, 2000). The major goal for bulls directly after the breeding season is regain the BW lost (Mullenix and Elmore, 2020). The nutrients supplied to bulls during the intermediate time period must be critically monitored since this timeframe generally occurs when forage quality is lower. Ultimately, preparing bulls for the breeding season is critical to maximize fertility; however, bull fertility and performance could change quickly depending on many factors such as time of year, age, nutrition, female to male ratio and more.

Nutrients and BCS

Nutritional management and diet quality are essential for metabolic functions as well as maximal reproductive outputs to increase pregnancy rates. Body energy reserves and fat deposition can be assessed through the management tool known as body condition scoring (BCS; 1 = emaciated and 9 = obese, (Wagner et al., 1988)) of cattle. With adipose synthesis, leptin, a protein hormone, is synthesized by white adipocytes to play a role in immune, neuroendocrine

and reproductive function (Wang et al., 2018). The main role within the immune system is through modulation of immune cell proliferation and activity through regulation of innate and adaptative response (Francisco et al., 2018) whereas in the neuroendocrine axis, leptin communicates the body's energy reserves to the brain to maintain function (Blüher and Mantzoros, 2004). Furthermore, in mice and humans', exogenous leptin was proposed to decrease male fertility due to increased sperm ROS production through the blood-testis barrier (**BTB**) (Hofny et al., 2010; Abbasihormozi et al., 2013). High amounts of leptin has shown to inhibit semen quality through testicular apoptosis and suppress testicular steroidogenesis (Wang et al., 2018). This past research shows the fluctuations of leptin due to various levels of adipose deposition from sire BCS needs to be managed due to the negative impacts of nutrition on male fertility.

The production of good quality semen within breeding bulls can be achieved through proper feeding management since there is a constant need for the supply of dietary nutrient categories such as energy and protein for general tissue maintenance, thermoregulation and bodily movement to maintain homeostasis. Once maintenance requirements have been met, the remaining nutrients can be allocated to reproductive functions (Brown, 1994). The nutrient requirements for bulls prior to a breeding season aim to maximize fertility and account for the fact that nutritional requirements will not be met during the breeding season (NRC, 2000). Diets with low crude protein rations showed a decrease in epididymis and seminal glands thickness as well as the overall weight of the testes, and seminiferous epithelium thickness and tubules diameter reduced (Meacham et al., 1964). In contrast, high protein diets have resulted in larger scrotal

circumference, BCS, high semen volume, sperm motility, and semen concentration compared with low protein diets (Rekwot, 1988).

Carbohydrates and fats are the primary sources of energy within the diet and are crucial for reproductive functions. Excess energy will be deposited as fat which become energy reserves measured by BCS, to be utilized during the nutrient restrictive breeding season (NRC, 2000). Failure to supply the energy needed during development will result in delayed puberty, decreased testicular growth, permanent sperm cell damage (Bratton, 1959), decreased endocrine function of the testicles, diminished libido, and less secretions from the accessory sex glands (Hurley and Doane, 1989; NRC, 2000). Severe malnutrition can also decrease or even halt spermatogenesis (NRC, 2000). Reduction in testis size due to restricted diets may be due to atrophy of the interstitial and Sertoli cell populations (NRC, 2000; Bollwein et al., 2017). Conversely, sires that are provided excess energy from overfeeding have been found to also have diminished libido due to hormonal imbalances (Flipse and Almquist, 1961). Level to high energy have been reported to improve sperm motility and velocity as well as mitochondrial membrane potential and integrity (Selvaraju et al., 2012). Therefore, energy and protein are key nutrient categories that can deter reproductive function and overall semen quality through improper sire diet.

Proper mineral and vitamin supplementation are key to prevent production loss through reproductive issues (Hurley and Doane, 1989). Trace minerals such as: copper, manganese, selenium, and zinc and vitamin A and E all play crucial roles for maintenance of reproductive function as antioxidants. Oxidative stress occurs from an imbalance between ROS and antioxidants. Oxidative stress can potentially hinder sperm quality through deterred acrosome reaction, hyperactivation, motility and capacitation (Agarwal et al., 2004; Pal et al., 2017).

Antioxidants, such as vitamin E and selenium, are nutrients that protect cells against the effects of free radicals from oxygen during cellular metabolism, like the reactive signaling molecules known as ROS (Baskaran et al., 2021). Selenium is required for normal spermatogenesis as it can remove dead or damaged spermatozoa, reduce oxidative stress by protecting spermatozoa from ROS, increase spermatozoa motility, and decrease spermatozoa abnormalities (Watanabe and Endo, 1991; Marai et al., 2009; Ghafarizadeh et al., 2018). Inadequate levels of the trace mineral manganese may result in abnormal sperm production due to altered cholesterol synthesis resulting in insufficient testosterone production (Kappel and Zidenburg, 1999). Deficiency in copper, leads to decreased libido, lower semen quality, and induces severe damage to testicular tissue due to high levels of ROS and downregulation of anti-oxidant activity (Deb et al., 2014). Testosterone synthesis is dependent on adequate dietary levels of zinc; therefore, deficiency of zinc will directly cause testicular atrophy which leads to poor semen quality, sperm production, and overall libido (Arthington et al., 2002).

While vitamins account for a small proportion of daily dry matter intake in beef cattle diets, they are critical within a nutritional program for proper animal maintenance and function and should not be overlooked. Vitamins such as A and E, play an essential role in oxidative balance as antioxidants, since oxidative stress can disrupt spermatogenesis as well as decrease overall libido in bulls (Rode et al., 1995). Bulls usually graze during the breeding season, allowing them to receive increased amounts of vitamin A in the form of β-carotene. However, vitamin A deficiency is known to be associated with delayed puberty, reduced libido, and degeneration of testicular germinal epithelium, causing a halt in spermatogenesis (Hurley and Doane, 1989). Vitamin E is known as a fat-soluble vitamin required for normal testicular

function and a deficiency of this vitamin has harmful effects on germ cell proliferation and differentiation (Cooper, 1987). Furthermore, the essential balance of all nutrients including vitamins and minerals is for fertilization and maximized reproductive efficiency within mature and developing bulls.

EJACULATE WITHIN THE FEMALE

Paternal Contribution

The paternal contribution to the embryo has classically been recognized as half of the genetic DNA. While spermatozoa function has been widely explored (Rodriguez-Martinez, 2001; Selvaraju et al., 2018; Avidor-Reiss et al., 2020), the functions of SP still remains to be completely elucidated not that the role has been reported to go beyond nutritive support and protection of the spermatozoa (Bromfield, 2014). The inflammatory response initiated within the female reproductive tract by the components of SP in humans and mice, can potentially impact the success of embryonic implantation (Robertson et al., 1996; Sharkey et al., 2012; Bromfield, 2014). However, four steps must occur before embryo attachment can be achieved: development within the zona pellucida, hatching of the blastocyst, formation of extraembryonic membranes, and maternal recognition of pregnancy (Senger, 2012). These initial steps are critical in order to establish a successful pregnancy.

Embryo development begins with syngamy, the fusion of the oocyte and spermatozoa to form the zygote. Through mitotic cleavage divisions yielding a two-celled embryo, also known as blastomeres, will continue to divide producing totipotent daughter cells until they form the morula. Further development of the inner cell mass and the blastocoele cavity continues, as the embryo transitions to the early blastocyst stage. The blastocyst hatches from the zona pellucida

as it continues to rapidly grow and float freely within the uterus before attachment occurs (Senger, 2012). In order for the blastocyst to survive, there must be a correct identification of location and invade the uterine epithelium during the window of implantation, known as the short period of time in which the endometrium is receptive to embryo attachment. To begin, a loose connection of the embryo within the uterine cavity during the window of attachment must occur. Along with directional changed adhesion occurs through the upregulation of integrins and bonded glycoproteins to promote attachment to the uterine wall (Dominguez et al., 2005; Salamonsen et al., 2016). Finally, invasion of the blastocyst into the endometrial tissues for humans and mice occurs in order for remodeling of tissues and increased vascularity to take place (Salamonsen et al., 2016). Whereas in cattle on day 25, the chorion initiates attachment to the caruncles of the uterus and attachment is well established by day 40. The growth of placentomes provides enough surface area for the exchange of maternal nutrients and metabolic waste from the fetus (Senger, 2012). The steps needed to prepare the uterus for embryo development is vital for a successful pregnancy.

One key player in promoting the correct uterine environment for embryonic development, is a member of the IL-6 cytokine family, Leukemia Inhibitory Factor (**LIF**). This pro-inflammatory cytokine triggers multiple processes associated with inflammation, angiogenesis, and tissue remodeling (Nicola and Babon, 2015). However, multiple cytokines including pro- and anti-inflammatory, are involved in the processes of immune-tolerance and tissue remodeling, to balance the uterine inflammatory status during early pregnancy (Yang et al., 2014). Another key immune cell during implantation and immuno-tolerance, are regulatory T (**Treg**) cells. Regulatory T cells are required to sustain tissue homeostasis and establish immuno-tolerance of

the semi-allogenic fetus (Sakaguchi et al., 1995). The maternal immune response to paternal antigens within the ejaculate stimulates vital immune pathways for implantation to facilitate early development in mice (Robertson et al., 1997) and paternal antigen specific Treg cells can expand locally and systemically during pregnancy in murine models (Robertson et al., 2009; Mao et al., 2010) . Therefore, the fetus is protected against maternal immunological attack from the epitheliochorial uterus due to Treg populations (Aluvihare et al., 2004). After coitus, SP induces cytokines and chemokines, TNF-α, IL-8, IL-6, IL-1, MIP-1α and MIP-1β, that induce the accumulation of paternal antigen-specific Treg cells from the maternal system and the differentiation of dendritic cells (Robertson et al., 1997). The role of dendritic cells is to take paternal antigens within SP and then process, present, and activate these antigens to Treg cells to promote tolerance of the fetus. This results in an abundance of Treg cells in the uterine lymph nodes to prevent the maternal immune system from rejecting the semi-allogenic fetus (Saito et al., 2016). Furthermore, studies have shown that the lack of Treg cell populations and activation can lead to the resorption of embryos as well as greater inflammation and fibrosis in mice (Samstein et al., 2012). However, further studies are required to evaluated what specific cytokines during early pregnancy could lead to the prevention of the necessary Treg proliferation and differentiation for successful pregnancy.

Role of Seminal Plasma in Pregnancy

Seminal plasma is known mainly for its role in spermatozoa transport within the female reproductive tract. However, this non-cellular fluid has been reported to be vital for the successful establishment of pregnancy (Robertson et al., 2009). This uterine inflammatory response, initiated at insemination or mating, influences the survival of the semi-allogenic

conceptus and is vital to establish pregnancy in human, ovine and murine models (Lovell and Getty, 1968; Robertson et al., 1997; Sharkey et al., 2012). Disruptions to the inflammatory processes for embryo attachment and development are detrimental for pregnancy outcomes (Robertson et al., 2018). The inflammatory reaction during pregnancy occurs within the cervix and uterine endometrium to recruit leukocytes such as neutrophils, dendritic cells and macrophages in mice, humans and cattle (Robertson, 2005; Bromfield, 2014). The role of leukocytes within the female reproductive tract includes clearance of dead spermatozoa and debris after coitus. Leukocytes also cause the induction of immuno-tolerance to the semi-allogenic fetus in order to prevent rejection by the maternal immune system (Aluvihare et al., 2004). Immune cells such as macrophages and lymphocytes produce cytokines that act as intracellular mediators of immunological functions (Chen et al., 2018). Cytokines are known to be pleiotropic and synergistic; acting in cascading pathways to create a strong biological effect in a given tissue (Kany et al., 2019). The biological effects within the uterus of cytokines will be one of the main determining factors for pregnancy establishment and reproductive success.

The effects and processes of cytokines and respective receptors can be both redundant and pleiotropic properties as signaling molecules. A wide array of cytokines is expressed within the uterus in a range of species including porcine, murine, cattle and humans (Robertson et al., 2009; Sharkey et al., 2012; Geisert et al., 2014; Fair, 2015). Uterine cytokine sources include uterine epithelial and decidual cells, the fetal trophoblast cells, as well as the resident leukocytes (Dimitriadis et al., 2005). There is also variation in cytokine expression throughout the estrous cycle which may suggest that steroid hormones could be an influencing profiles in reproductive tissues (Care et al., 2014). Cytokines, in general, function to control the balance of immuno-

regulatory functions within the body. Many cytokines including Interferon (IFN)-γ and Interleukin (IL)-6 have both pro- and anti-inflammatory functions dependent on tissue and physiological event (Mühl and Pfeilschifter, 2003; Scheller et al., 2011). Interleukin-6 (Gabay, 2006), Tumor Necrosis Factor (TNF)-α, and the IL-1 family are considered acute phase cytokines which work to cause a large number of systemic and metabolic changes as the body's initial response to infection and tissue damage. (Burger and Dayer, 2002). Angiogenic cytokines like Vascular Endothelial Growth Factor (VEGF)-A, can induce endothelial cell activation and proliferation for angiogenesis, the creation of new blood vessels from precursor cells such as angioblasts (Ucuzian et al., 2010). The role of VEGF-A is critical during every step of placental growth and vascular formation to provide blood required to the growing fetus (Chen and Zheng, 2014). Cytokines and their functions can switch to accommodate physiological events such as pregnancy or stress from environmental factors in an attempt to maintain homeostasis.

Anti-inflammatory cytokines inhibit the synthesis of pro-inflammatory cytokines to initiate an immuno-suppressive response (Zhang and An, 2007). In humans, the concentrations of IL-10 are greater in healthy individuals after coitus which potentially demonstrates a mechanism for sperm survival in the hostile female reproductive tract due to IL-10's immuno-tolerant functions (Camejo, 2003). These functions are to prevent the rejection of the semi-allogenic fetus (Chatterjee et al., 2014) and promote conceptus attachment to the uterine endometrium in dairy and beef cattle (Odhiambo et al., 2009). Interleukin-36RA is a part of the IL-36 family a subset of the IL-1 super family. However, the antagonistic effects of IL-36RA inhibits inflammation by the intracellular portion containing the Toll-like domain involved in the signaling generated when IL-36 agonists bind to IL-36R (Yi et al., 2016). The antagonistic

effects from IL-36RA impede the signals between the toll/interleukin-1 receptor domain which inhibits the NF-κB signaling cascade (Murrieta-Coxca et al., 2019). Without the signals to stimulate inflammation there would be no production of pro-inflammatory cytokines and chemokines (Murrieta-Coxca et al., 2019).

Pro-inflammatory cytokines are predominantly produced by activated macrophages and monocytes to up-regulate systemic inflammation and culminate in an influx of leukocytes in humans and rodents (Robertson et al., 1996; Sharkey et al., 2012). The IL-1 family, including α and β, are pro-inflammatory cytokines that act as paracrine and autocrine factors to stimulate signaling pathways (Mantovani, 1998). Interleukin-1α is known as a dual-function cytokine as it functions to increase gene expression and can also increase IL-8 levels (Werman et al., 2004). Furthermore, IL-1β has been documented to modulate the maternal immune system and coordinate the communication between the embryo and uterus to establish pregnancy in bovine (Correia-Álvarez et al., 2015) . Concentrations of IL-1β increased in the bovine endometrium in response to the embryo for 3 days within the uterus, which suggests that embryos may stimulate endometrial receptivity during the first days of pregnancy in cattle (Correia-Álvarez et al., 2015). Similar to the IL-1 family, the immuno-stimulatory properties of TNF-α are essential for early pregnancy and pregnancy establishment (Toder et al., 2003). Yet the overproduction of TNF-α could cause early embryonic loss or implantation failure in humans (Saito et al., 2010; Alijotas-Reig et al., 2017). Tumor Necrosis Factor-α can also increase bovine IL-8 concentrations to incite inflammation (Sohn et al., 2007). Not only does TNF-α have pro-inflammatory properties, but may also have important anti-inflammatory functions which could potentially protect sperm cells from the female reproductive tract and immune system (Kelly, 1995). Additionally, TNF-α,

IL-10 and IL-1β have been found to be regulated by IL-17A (Jovanovic et al., 1998). Interleukin-17A within the uterus has been known to be produced majorly by γδ T cells, which express a unique T cell receptor and are found in low abundance within the body such as the gut, lungs and uterus that are involved in the initiation of immune responses (Ribot et al., 2021). The γδ T cells can be influenced by SP in order for immunostimulatory effects to occur to prepare the uterus for the fetus (Song et al., 2016). While the balance of pro- and anti-inflammatory levels through cytokines and chemokines is necessary, more research is needed to determine how different levels of uterine inflammation influences attachment and implantation.

Natural Mating vs. Artificial Insemination

Enhancing genetic and production efficiency of cattle can be achieved by incorporating assisted reproductive technologies (**ART**) into management practices. The most widely recognized ART is artificial insemination (**AI**) and fixed-time AI throughout both dairy and beef industries. Other ART include estrous synchronization, cryopreservation, and in vitro fertilization to improve genetic potential in cattle industries. Depending on the type of operation, one type of breeding technique may be more efficient than the other in terms of natural mating or AI. Natural mating requires less labor, cost and risk for most operations; however, AI provides more opportunities for a genetically superior calf-crop (DeJarnette et al., 2004). When considering natural mating, costs rely on medical, feed to maintain the sire, labor, and equipment whereas AI costs focus on equipment, supplies, and labor. Yet even with natural mating resulting an overall higher pregnancy rate of 90% (Lunstra and Laster, 1982) compared to AI 60% rate (Dorsey et al., 2004; Bormann et al., 2006), bulls naturally can only service 25 to 30 cows during a breeding season. Whereas with the correct amount of labor, three times as many cows can be

serviced within an hour via AI. This allows for more days postpartum before the breeding season begins, increased calf-crop uniformity (Crites et al., 2018). Heifers born early in the calving season are more likely to conceive early in their first breeding season, they are able to wean heavier calves, and be more profitable over their earlier lifetime (Damiran et al., 2018). On another note, early embryonic mortality occurring prior to d 27 during AI, is the highest (20 to 44%) percentage of embryonic mortality reported in beef cattle (Humblot, 2001). This increased mortality from AI could be due to a number of factors including genetic, nutritional and environmental (DeJarnette et al., 2004).

Cryopreservation is an ART process that allows straws of spermatozoa to be frozen and preserved as it is distributed globally to advance genetics of cattle. However, this process uses diluted semen with semen extender for spermatozoa preservation meaning that cows are not being exposed to similar semen components or volumes as a natural mating. Another factor to potentially influence pregnancy success is seminal placement, since in natural mating the semen is deposited in the fornix vagina and has to travel through the cervix whereas in AI the semen is deposited within the uterine body. The location placement in AI is crucial as an AI technician in order to increase the chances of successful pregnancies. Furthermore, within semen, SP has cytokines and chemokines that cause immuno-stimulatory and immuno-suppressive effects required for the embryo to properly establish itself within the endometrium of cattle. Therefore, diluting these cytokines within the SP could potentially cause issues with the establishment of pregnancy via alteration in cellular mechanism pathways within the uterus. Odhiambo et al., (2009) found that administration of TFG-β increased pregnancy rates; however, no difference in fertility was reported when inserting whole SP into females at the time of insemination. In

contrast, another study using AI, observed improved pregnancy rates in cows when adding a bull with a surgically diverted penis to include more SP volume (Pfeiffer et al., 2012). More research is necessary for the elucidation of the complete impacts of SP pregnancy outcomes, specifically during AI and other ART techniques on.

SUMMARY

Proper bull management is critical to promote and maintain sire fertility in order to maximize reproductive efficiency. Nutritional level and components critically impact male fertility and is a crucial factor in bull management which could hinder the reproductive success of the herd. Furthermore, nutritional management should be constantly adjusted according to age and time of year (NRC, 2000). Time of year has the greatest influence on mature bull nutrition, specifically prior to the breeding season, since bulls are known to lose up to 135 kg due to reduced feed intake when with females and an increased activity from mating. The influence of malnutrition on the ejaculate has been demonstrated to have severe reproductive consequences. For example, low energy and protein diets can increase spermatozoa abnormalities, impair testicular morphology, reduce overall ejaculate and specifically seminal volume, and decrease overall fertility in bulls (Meacham et al., 1964; Lindsay et al., 1984; Mwansa and Makarechian, 1991). Sire research have traditionally focused on sperm quality; however, SP data have increased exponentially from a protective nutrient medium for spermatozoa to a vital role for pregnancy establishment (Bromfield, 2014). The endometrial tissue reconstruction needed for a successful pregnancy, is performed by both pro- and anti-inflammatory cytokines and chemokines within SP to initiate maternal immune processes required for pregnancy (Robertson, 2005). The pleiotropic roles of cytokines include recruiting leukocytes to the endometrium for

uterine inflammation for successful embryo establishment or causing an immunosuppressive environment to prevent the rejection of the semi-allogenic embryo (Robertson et al., 2009; Bromfield, 2014). Furthermore, data have demonstrated that concentrations and types of cytokines can be influenced greatly by nutrition (Caroleo et al., 2019). Further studies are required to determine the complete influences of the sire diet on the ejaculate and the effects within the uterine environment during the establishment of pregnancy.

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CHAPTER THREE

THE EFFECTS OF DIFFERING NUTRITION LEVELS AND BODY CONDITION SCORE ON SCROTAL CIRCUMFERENCE, MOTILITY, AND MORPHOLOGY OF BOVINE SPERMATOZOA

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ABSTRACT

Bulls often experience various levels of nutrition throughout the year. Nutritional management is a critical factor to fertility, ejaculate composition, and the ability to get females pregnant. We hypothesized that differing nutritional levels and body condition score (**BCS**) would affect reproductive fertility parameters in bulls. Mature Angus bulls (n = 11) were individually housed and randomly assigned to one of two treatments: 1) over-fed (**OVER**, n = 5) targeting a BCS of 8, or 2) restricted (**RES**, n = 6) targeting a BCS of 4. Bulls were fed the same ration at different volumes to achieve desired effects with different nutritional periods: gain, loss, ideal steady state (**ISS**) at a BCS of 6, and abnormal steady state (**ABS**) at a BCS of 8 or 4 as per treatment design. Body weight (**BW**) and BCS were taken every two weeks to monitor bull weight and adipose changes. Scrotal circumference was measured every 28 d. Body fat and spermatozoa motility and morphology were evaluated every 84 d. Statistical analyses were conducted with PROC GLIMMIX of SAS to determine if diet levels and adiposity influenced BW, BCS, scrotal circumference, motility, morphology, and adipose thickness. Scrotal circumference ($P < 0.05$) had the greatest change for both treatments during the gain period and decreased during the loss period. Spermatozoa morphology was impacted ($P < 0.05$) by a treatment by nutritional period interaction for both head and total defects. Increased head abnormalities occurred during the loss period (37.60 ± 8.90) for the OVER treatment, whereas in the RES treatment, there was a decrease in head abnormalities from the initial during the ABS (-4.50 ± 8.11). Total defects during the OVER treatment increased during the loss period (43.80 ± 9.71) but the greatest change for the RES treatment occurred during the ISS (34.13 ± 8.85). Motility tended ($P = 0.10$) to be influenced by nutritional period with the greatest change

occurring after the loss (-10.58 ± 4.97) and gain periods (-10.10 ± 4.97). In conclusion, male fertility was impacted when deviated from a BCS of 6 which could be detrimental to reproductive and beef production efficiency.

INTRODUCTION

There is a critical need for efficient beef production due to the predicted exponential growth of the world's human population (Reynolds et al., 2015). As countries become more developed, diets change from a larger proportion of starches to more protein, causing an estimated needed increase of 200 million tons of meat by 2050 (FAO, 2009). Efforts to increase cattle production have largely been focused on the female due to the long-term interaction and influence on offspring (Funston et al., 2012; Endecott et al., 2013; Diskin and Kenny, 2014). However, the paternal contribution to production efficiency may be greater than previously anticipated due to a sire having multiple offspring per breeding season. Bull fertility can be impacted by many factors including the environment and nutrition (Parkinson, 1987; NRC, 2000; Thomas, 2009). Sire nutrition is a major limiting factor for male reproductive performance (Short and Adams, 1988) and can affect libido expression, testicular function, endocrinology, and overall fertility (Singh et al., 2018). Prolonged and severe malnutrition can cause morphological defects and decreased sperm motility (Coulter et al., 1999; Singh et al., 2018), potentially decreasing pregnancy rates. Bulls can lose a considerable amount of weight (45 to 135 kg) during the breeding season due to mating and reduced feed intake (NRC, 2000; Barth, 2018). Therefore, evaluating the interaction between diet and semen quality is necessary to maximize reproductive efficiency.

To aid in the production of high quality semen, proper nutritional management should be adjusted depending on bull age and time of year (Leathem, 1975; NRC, 2000). Nutrient restriction can impact male fertility via poor testicular development, diminished libido, reduced progressive forward motility, and increased morphological defects (Mwansa and Makarechian,

1991; Singh et al., 2018). Prolonged nutritional restriction has also been found to impact the interstitial, Sertoli, and Leydig cell populations which will impact testicular steroidogenesis and spermatogenesis (NRC, 2000; Bollwein et al., 2017). Furthermore, high intake levels can impact ejaculate volume, sperm concentration, motility, and morphology of the spermatozoa (Coulter et al., 1997; Perkovic S, 2001) as well as create a hormonal imbalance, impair testicular development, and decrease libido (Tremellen et al., 1998; Selvaraju et al., 2012; Singh et al., 2018), all of which can impact male fertility. Bulls receiving high concentrate levels (37% concentrate) had increased GnRH-stimulated testosterone production compared to bulls on a diet without concentrate (Barth et al., 2008). Increasing energy levels by 20% has been reported to improve sperm velocity and motility as well as mitochondrial membrane potential and integrity in rams (Selvaraju et al., 2012). Thus, nutritional management of the sire prior to the breeding season is a critical factor for reproductive efficiency. Therefore, the hypothesis of the current study is altering nutritional period and body condition score (**BCS**) will impact fertility measurements of mature bulls.

MATERIALS AND METHODS

All experimental procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee.

Experimental Design

Mature Angus bulls (n=12; Body Weight (**BW**)= 738 kg; BCS= 6; Age= 4 years) were purchased from Jorgensen Land & Cattle (Ideal, SD). One bull developed bovine leukemia virus and was required to be euthanized during the study; thus, was excluded from all analyses. All bulls were individually housed in a 2.44-meter by 12.19-meter paddock with ad-libitum access to

water and a 100 g mineral supplement daily (CO-OP Supreme Cattle Mineral; Tennessee Farmers Cooperative; Lavergne, TN). Bulls were provided feed to target intakes to meet BW and BCS goals as per experimental design. The diet consisted of 35% ground hay, 35% cracked corn, 20% dried distillers' grain and 10% soybean meal. Following a 21 d adaptation period, each bull was randomly assigned to one of two treatment pathways consisting of either restricted (**RES**) or over-fed (**OVER**) diets (**Figure 1**). The RES treatment bulls began with an average initial scrotal circumference of 38.47 cm prior to treatment, whereas fertility parameters such as motility was 42.5%, and abnormal initial morphology was done for head (20), midpiece (10), tail (0.5), and total defects (63). The OVER treatment bulls began with average initial scrotal circumference of 38.58 cm and fertility parameters prior to treatment with motility at 42% and abnormal morphology as head (20), midpiece (3), tail (1) and total defects (28). On d 21, RES bulls began treatment diets targeting a decrease of 2 BCS over 84 d. On d 105, bulls began adaptation to diet intake adequate for maintenance at an abnormal condition steady state over 10 d. On d 189 bulls began re-alimentation back to initial basal BW and BCS with intakes targeting a BCS increase of 2. Bulls assigned to the OVER treatment were subjected to inverse dietary changes of the RES treatment. Following the 21 d adaptation, bulls on the OVER treatment received an intake volume to support ~1.25 kg/d to increase BCS by 2 in 84 d. On d 105, OVER bulls were acclimated to ABS intakes adequate for maintenance at the new BW and remained on this nutritional period for 84 d. On d 189, OVER bulls began the return to basal BW and BCS by targeting a 2 BCS decrease in 84 d. All bulls received ideal condition (BCS = 6) steady state (**ISS**) intake levels for 84 d to complete the objectives.

Semen Collection and Analysis

Individual non-shrunk BW and BCS (1 = emaciated and 9 = obese, (Wagner et al., 1988)) was taken every other week to monitor changes and ensure treatments met experimental goals. Scrotal circumference was measured every 28 d with the Reliabull scrotal tape (Lane Manufacturing Inc., Denver, CO.). Each nutritional period has a d 28, 56 and 84 for scrotal circumference, corresponding as sample A, B, and C, respectively. Spermatozoa, temperature, hip height, back fat, rump fat and scrotal fat was collected and quantified every 84 d. Temperature was only taken to monitor general health of all bulls (Data not reported). Body fat was measured by a single, CUPP lab certified ultrasound technician via ultrasonography with an IBEX EVO II. Rectal palpation was conducted for each sampling date prior to electro-ejaculation to ensure normal internal tract morphology. To collect the ejaculate, a semen collection handle with a saline bag was connected to a disposable cone and vial. The attached saline bag was kept in a heated water bath (~37 °C) to keep the ejaculate at a constant temperature during and after collection to eliminate environmental temperature effects on the spermatozoa. The progressively forward motile spermatozoa were evaluated with a Fischer Scientific Light microscope under 40 × magnification after dilution with a drop of warm saline. A sample of each ejaculate was stained with Eosin Nigrosin morphology stain (Lane Manufacturing Inc., Denver, CO.) to assess spermatozoa morphology. Percentage of abnormal spermatozoa was quantified by a Diplomate of American College of Theriogenologist, by counting individual sperm with defects out of a total of 100 spermatozoa. The same bottle of morphology stain was used throughout the entire study to avoid variation in morphological measurements due to osmolality issues between bottles.

Statistical Analyses

A complete randomized design was implemented to assign treatments to each bull completely at random in GLIMMIX SAS 9.4 (SAS Institute, Cary, NC) to determine the effects of treatment, nutritional period, and the interaction on motility, morphology, scrotal circumference, back fat, rump fat and scrotal fat. The experimental unit was each individual bull and repeated measures included sampling date. Experimental model confirmation was done by evaluating BW and BCS to assess if predicted BW and BCS were reached through dietary volume adjustments for each designated treatment. This statistical model included date, treatment, nutritional period, and the 3-way interaction for BW, BCS and scrotal circumference. Random effects included bull within treatment. A covariate of hip height was included when evaluating BW and BCS. Scrotal circumference, motility, and morphology were normalized to the initial sample prior to the onset of treatments, allowing each bull to serve as their own respective control. Means were determined to be different when $P \leq 0.05$ and a tendency when $P \leq 0.10$.

RESULTS

Body Weight and BCS

The covariate, hip height, had no effect on BCS; however, there was a significant ($P < 0.001$) effect of hip height on BW. Both BW and BCS were influenced ($P < 0.001$) by a treatment × date interaction (**Table 1**). Body weight and BCS followed the predicted experimental design model with an increase and decrease in BW and BCS during the respective gain and loss periods according to treatment pathway. Yet, BW tended to differ between the OVER and RES treatments at the beginning of the study even after the 21 d acclimation diet

prior to treatments (**Table 1**). The two maintenance periods, ABS and ISS, kept bulls at a steady BW and BCS as desired by the model. Bulls on RES achieved the targeted decrease of 2 BCS with a decrease of 67.27 kg over 84 d. On d 105, bulls were held at a BW of 693.86 kgs and a BCS of 4 for 84 days during the ABS period at an abnormal BCS. Bulls on the RES treatment began re-alimentation during the gain period with a projected increase of 2, yet only achieved a BCS change of 1.2 and an increase of 118.03 kg to achieve the basal BW of 832.50 kgs and a BCS of 6.10. Bulls assigned to the OVER treatment had a projected increase in BCS of 2; however, only increased in BCS by 0.95 and gain 169.82 kg during the initial gain period over 84 days. The OVER bulls when acclimated to the ABS period over 84 days as maintenance, reached the new BW of 885.40 kg and a BCS of 6.85. Over the loss period, the bulls returned to a basal BW of 813.58 kg and a BCS of 5.20 with a targeted decrease in BCS of 2. However, the actual decreased change in BCS was only 0.80 and lost only 43.09 kg over the 84 d loss period.

Scrotal Circumference and Body Fat

Scrotal circumference was impacted ($P < 0.05$) by a treatment pathway × nutritional period × date interaction (**Figure 2a and 2b**). The OVER treatment, had the greatest increase in scrotal circumference (3.90 ± 0.74) during the gain period, with similar measurements for the ABS (3.80 ± 0.74). All scrotal circumference changes were positive for the OVER bulls when compared back to the initial scrotal circumference (**Figure 2a**). Bulls on the OVER treatment scrotal circumference never returned to the initial scrotal circumference even during the ISS period. Similarly, the RES diet treatment had the greatest scrotal circumference measurement on sample C of the gain period (3.45 ± 0.67) when returning to basal BCS. The RES bulls had the greatest decrease in scrotal circumference after sample A on the loss period (-0.55 ± 0.67;

Figure 2b; $P < 0.03$). In contrast to scrotal circumference measurements, the scrotal fat measurements via ultrasound had no significant treatment, nutritional period or interaction effects ($P > 0.05$). Back fat thickness was impacted by nutritional period ($P < 0.05$; **Figure 3**) and rump fat was influenced by a treatment × nutritional period effect ($P < 0.005$; **Figure 4a and 4b**). Similar to BW and BCS, back had the greatest increase fat ($P < 0.05$; **Figure 3**) in adipose deposition during the gain period (0.71 ± 0.50) and the least during the ISS (0.50 ± 0.50), regardless of treatment pathway. Rump fat for the OVER treatment pathway ($P < 0.005$; **Figure 4a**) was greatest during the gain period (1.13 ± 0.17) and the most reduced during the ISS (0.45 ± 0.17). The RES bulls had rump fat thickness ($P < 0.005$; **Figure 4b**) that was thicker during the ISS (1.13 ± 0.16) and remained thinner during the ABS (0.27 ± 0.16).

Motility and Morphology

Forward progressive motility tended ($P = 0.10$) to decrease more during the loss period (-10.58 ± 4.97) and gain period (-10.10 ± 4.97), compared with the ISS (0.75 ± 4.97) and ABS (-6.92 ± 4.97) was intermediate regardless of treatment (RES or OVER; **Table 2**). Morphological head abnormalities of spermatozoa were influenced ($P < 0.05$; **Table 2**) by a treatment × nutritional period interaction. The OVER bulls had the greatest increase in head abnormalities change during the loss period (37.60 ± 8.90) compared to the gain (0.25 ± 8.90), ABS (1.00 ± 8.90) and ISS (1.80 ± 8.90; **Table 2)**. In contrast, the nutritional periods for the RES treatment were not different from each other. When evaluating the change in total spermatozoa abnormalities ($P < 0.001$; **Table 2**) from the initial sample, similar trends occurred as the head abnormalities for both treatment pathways. The OVER bulls had the greatest increase from the initial sample during the loss period (43.80 ± 9.71) whereas the least amount of change occurred

during the gain period (2.61 ± 9.71), with the ABS (17.20 ± 9.71) and ISS (14.60 ± 9.71) being intermediate in total defects. The greatest defects occurred during the ISS (34.13 ± 8.85) for RES bulls, potentially due to the change in maintenance requirements with BW and BCS data for RES bulls demonstrating an continued increase during the ISS. However, in contrast to head defects the lowest amount of total defect change compared to the initial sample, occurred during the loss period (2.53 ± 8.85).

DISCUSSION

The role of the sire is often underestimated, since females are responsible for large nutrient investments and long-term interactions with the calf. The bull does not provide any nutrients to the offspring but is responsible for half the genetic information and a majority of postnatal performance in numerous calves per breeding season. Semen quality can be hindered through many external and environmental factors such as age, season, nutrition, collection frequency, and injuries (Almquist, 1982; Senger, 2012; Kastelic, 2013; Murphy et al., 2018; Tank and Monke, 2020). Specifically, factors causing temperature changes within the scrotum such as injuries, diseases, season and nutrition will decrease semen quality in response to elevated body temperature (Vogler et al., 1993). Bulls often experience various nutritional planes throughout the year due to a short and extreme period of high activity, with most of the year spent with relatively low activity levels. Nutrition is generally accepted as the limiting factor of sire fertility; thus, any fluctuations can be detrimental to reproductive herd efficiency (Short and Adams, 1988). Since diet has been shown to impact fertility parameters within the ejaculate, we evaluated the motility and morphology of bulls undergoing different levels of nutrition. Both BW and BCS were different, confirming the success of the nutritional treatment and experimental

design. However, BCS during the ISS was increased during the RES treatment compared to the OVER, potentially due to changes in maintenance requirements after undergoing restricted treatment (NRC, 2000). Changes in nutrient requirements after different planes of nutrition should be considered so that overcompensation does not occur; specifically following the breeding season in bulls.

Scrotal circumference is a heritable trait that is an essential fertility marker due to increased testicular size causing an increase in sperm output, and also carries over into heifer fertility by reducing age at the first breeding season (Martin et al., 1992; Youngquist and Threlfall, 2006; Latif et al., 2009). Scrotal circumference is a valued and important trait on bull fertility that can be influenced by nutrition (Barth et al., 2008) and corresponds with the results of the current dataset. High energy and protein diets have been reported to increase scrotal circumference (Mwansa and Makarechian, 1991; Coulter et al., 1997; Coulter et al., 1999; Barth et al., 2008). The speculated increase in adipose deposition from overfeeding can have negative effects (Coulter et al., 1997; Coulter et al., 1999) or no effect (Schrick, 1998; Lemaster, 1999) on spermatogenesis. Interestingly, scrotal fat in our study was not influenced by treatment or nutritional period even though scrotal circumference measurements was influenced by the 3-way interaction.

The decrease in motility and increase in morphological abnormalities associated with overfeeding is speculated to be related to adipose deposition within the scrotum (Coulter and Kozub, 1984). The current data show both motility and morphology were influenced during the gain period; however, no scrotal fat differences were observed based on ultrasound. In contrast,

prolonged nutritional deficiencies can hinder spermatogenesis and decrease testes size (Hurley and Doane, 1989). Which also agrees with this current study, where decreases in scrotal circumference were observed during the loss periods. Moreover, data within demonstrate that deviating from and back to the ideal BCS of 6 caused a decrease in motility; however, when diets meet maintenance requirements even at a non-ideal BCS (ABS period), the motility percentage recovers slightly. This has also been demonstrated in other bull studies that undergo different nutritional periods (Flipse and Almquist, 1961; Pakenas, 1966). Sperm motility can be enhanced by seminal plasma (**SP**) (Graham, 1994); however, certain components of SP including inflammatory cytokines like Tumor Necrosis Factor-α, have decreased the motility of human spermatozoa during malnutrition (Eisermann et al., 1989). Increases in cytokines could have potentially occurred during abnormal nutritional periods due to response to inflammation and stress (Eckel and Ametaj, 2016). Thus, a possible association between nutritional stress and motility may exist in bulls (Flipse and Almquist, 1961), boars (Stevermer et al., 1961), rodents (Ghanayem et al., 2010), and humans (Skoracka et al., 2020).

Even though forward progressive motility is vital for spermatozoa to reach the egg, morphological defects are one of the greatest causes for pregnancy failures (Thundathil et al., 2002; Walters et al., 2005). As the sperm head contains the genetic material and key effectors of fertilization, most abnormalities of the head are associated with fertility impairment (Wilmington, 1981). The fact that head defects were greatest during the loss period for OVER bulls, may indicate that returning back to basal BCS after being overfed due to the respective treatment, is more detrimental to morphology than when losing BW at a BCS of 6. The current dataset demonstrated increased abnormal spermatozoa during overfed nutritional periods;

conjointly, other research found bulls decreased from high nutritional periods, continued abnormal productions trends potentially due to affected heat exchange mechanisms (Coulter and Kozub, 1984). Furthermore, the total spermatozoa defects increased during the gain and ISS for the RES pathway potentially due to the acquired fat deposition from nutritional stress of overfeeding (Skinner, 1981).

In conclusion, spermatozoa motility and morphology fluctuated throughout nutritional periods. Nutritionally preparing bulls for the breeding season is vital to ensure optimal fertility by increasing or maintaining slightly elevated BCS. Within the current study, the ABS through the loss period in OVER bulls and the onset through the ABS periods for the RES bulls resembles the nutritional levels and potentially the morphological and motility effects that would occur during the breeding season. Overall, nutritional periods and treatments influenced semen quality by undergoing different adiposity levels and stress that potentially resembles the various BCS that bulls undergo in normal production scenarios. Further studies are need in order to fully understand the long-term impacts on the ejaculate due to sire diet and reproductive efficiency.

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APPENDIX

Nutritional Period	Sample, d	OVER BW, kgs	RES BW, kgs	OVER BCS	RES BCS
Initial	21	715.58[w,1]	761.13[x]	5.90[a]	6.10[a]
Period 1[2]	49	799.22[a,3]	740.83[b]	6.05[a]	4.96[b]
	77	851.58[a]	721.74[b]	6.50[a]	4.67[b]
	105	885.40[a]	693.86[b]	6.85[a]	4.09[b]
Period 2[4]	133	852.49[a]	719.47[b]	6.30[a]	4.75[b]
	161	853.95[a]	717.19[b]	6.05[a]	4.96[b]
	189	852.86[a]	714.46[b]	6.05[a]	4.92[b]
Period 3[5]	217	837.58[w,5]	790.07[x]	5.80[a]	5.50[a]
	245	821.40[a]	808.86[a]	5.50[a]	5.75[a]
	273	809.77[a]	832.49[a]	5.25[a]	6.09[b]
Period 4[4]	301	804.31[a]	835.37[a]	5.25[a]	6.05[b]
	329	807.04[a]	831.74[a]	5.45[y]	5.88[z]
	357	813.58[a]	852.95[a]	5.20[y]	5.63[z]

[w x,y,z] Within a row, means without a common letter differ for BW and BCS ($P < 0.10$)

[2] Period 1: OVER (Over-fed) treatment= gain period; RES (Restricted) treatment= loss period

[3 a,b] Within a row, means without a common letter differ for BW and BCS ($P < 0.05$)

[4] Both treatment pathways- Period 2: ABS; Period 4: ISS

[5] Period 3: OVER treatment= loss period; RES treatment= gain period

Table 1. Body weight (kgs) and BCS for each randomly generated treatment pathway per individual sampling date through the designated nutritional planes.

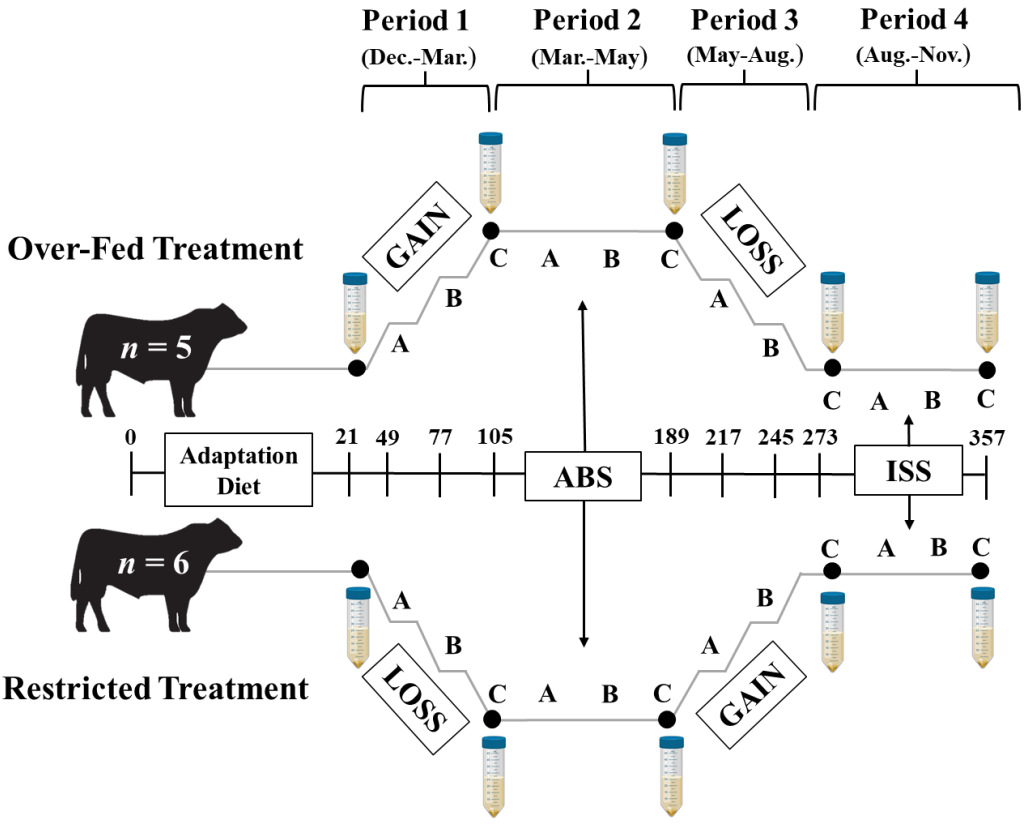
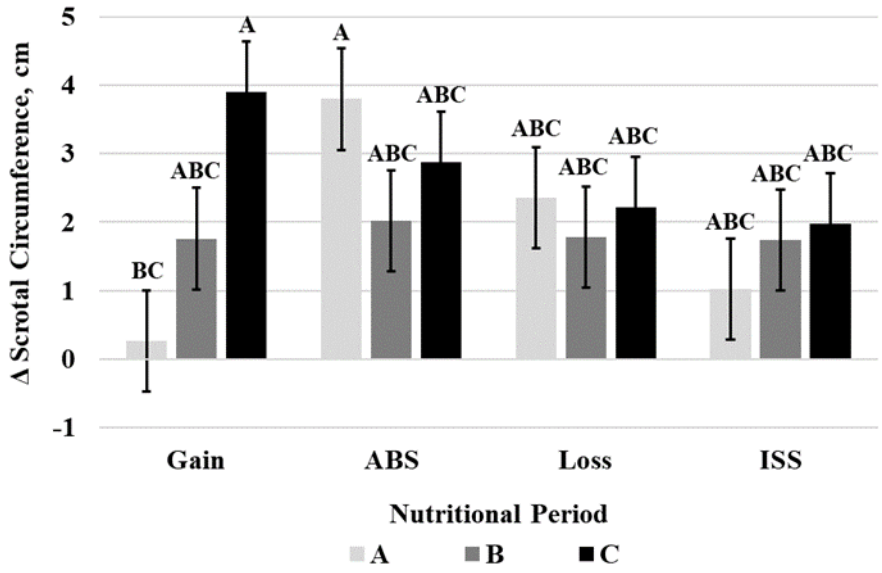
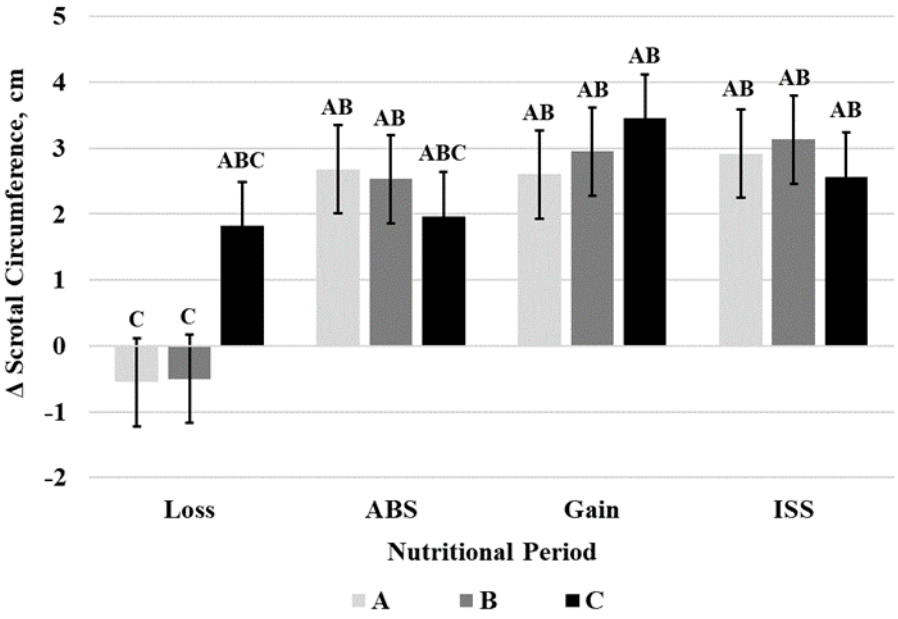


Figure 1. Fertility project timeline with two treatment pathways: OVER and RES, with four respective nutritional planes. Sample collections followed after the 21 d maintenance diet prior to treatment pathways. Including: semen collection for morphology and motility every 84 d (large falcon tubes), diet changes (*) and scrotal circumference measurements (*).



A



B

Figure 2. Scrotal circumference measurements (Normalized Mean ± SEM; $P < 0.03$) for the OVER treatment (a) and RES treatment (b) per exposure day (A= 28 d, B= 56 d, C= 84 d during each nutritional period) compared to the initial scrotal circumference prior to treatment, for each nutritional period.

[ABC]Bars (arithmetic means ± SEM) that do not share a letter denotes differences at $P \leq 0.05$.

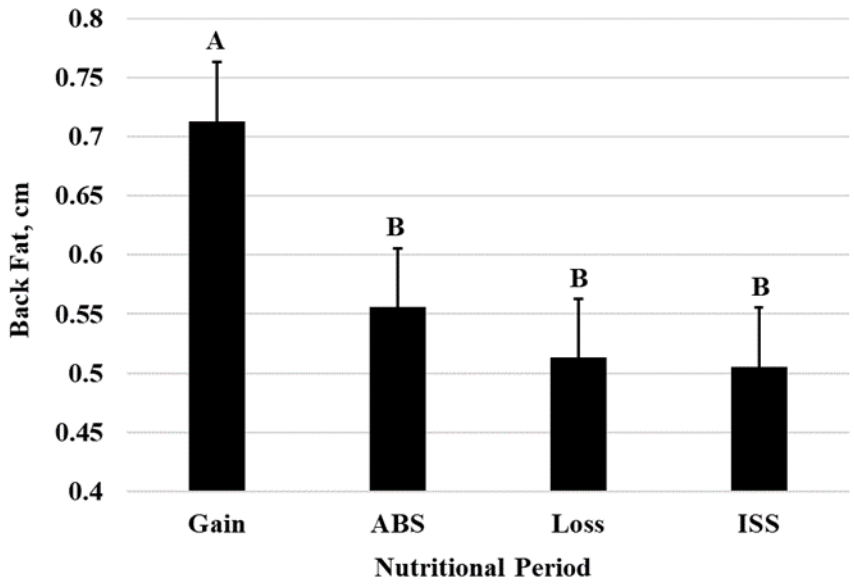
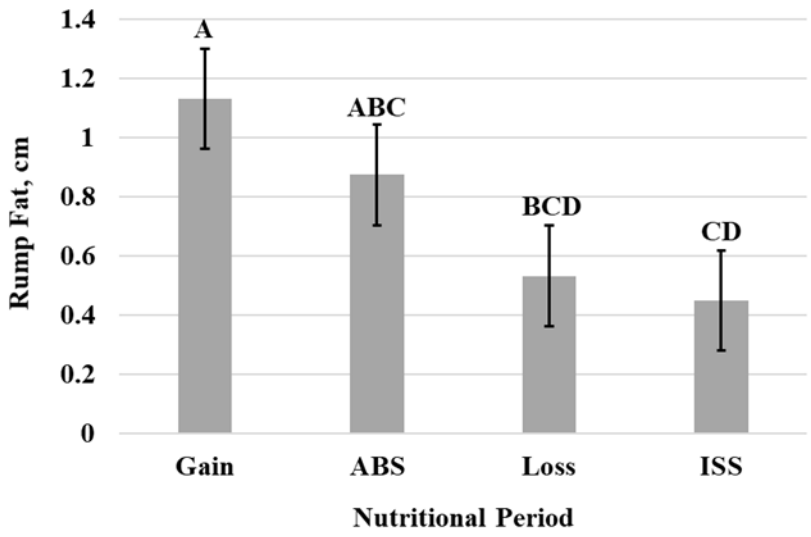
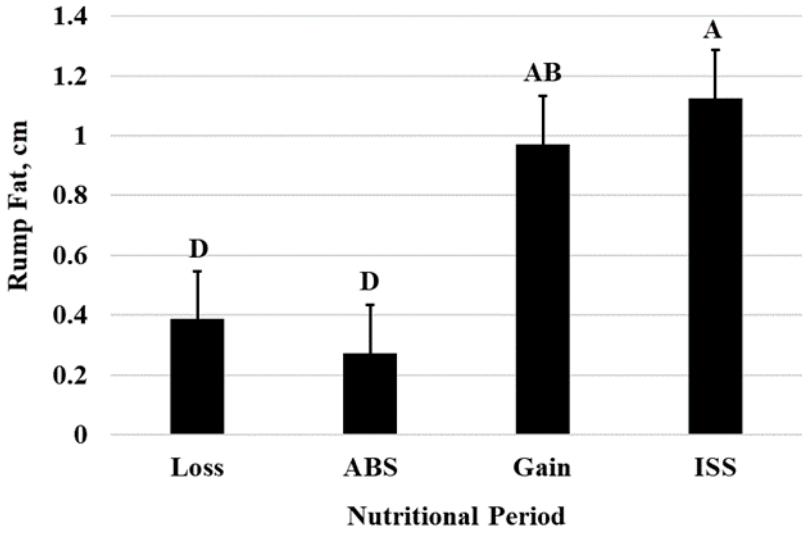


Figure 3. Back fat measurements (Raw Mean ± SEM; *P* = 0.02) for each nutritional period included: the gain (0.71 ± 0.50), ABS (0.56 ± 0.50), loss (0.51 ± 0.50) and ISS period (0.51 ± 0.50). ^{AB}Bars (arithmetic means ± SEM) that do not share a letter denotes differences at $P \leq 0.05$.



A



B

Figure 4. Rump fat measurements (Raw Mean ± SEM; P < 0.005) for the OVER treatment pathway (a) included: the gain (1.13 ± 0.17), ABS (0.88 ± 0.17), loss (0.53 ± 0.17) and ISS period (0.45 ± 0.17). The RES pathway (b) included: the loss (0.39 ± 0.16), ABS (0.27 ± 0.16), gain (0.97 ± 0.16) and ISS (1.13 ± 0.16). [ABCD]Bars (arithmetic means ± SEM) that do not share a letter denotes differences at $P \leq 0.05$.

Table 2. Change in motility (%) and morphology (#/100) from the initial collection prior to treatment for the designated effects for treatment pathways and nutritional periods[1].

		Nutritional Period[2]					
	Treatment	**Gain**	**ABS**	**Loss**	**ISS**	**Pooled SE**	**P-Value**
Motility, %	**-**	-10.10^{b}	-6.92^{ab}	-10.58^{b}	0.75^{a}	4.97	**0.10**
Head Defects	**OVER**	0.25^{b}	1.00^{b}	37.60^{a}	1.80^{b}	8.90	**< 0.005**
	RES	10.33^{b}	-4.50^{b}	-2.61^{b}	10.77^{b}	8.11	
Total Defects	**OVER**	2.61^{cd}	17.20^{bcd}	43.80^{a}	14.60^{bcd}	9.71	**< 0.001**
	RES	28.34^{abc}	11.67^{cd}	2.53^{d}	34.13^{ab}	8.85	

^{a,b,c,d} Within a row, means without a common letter differ for motility and morphology ($P < 0.05$)
[2] ABS: Abnormal Steady State, ISS: Ideal Steady State

CHAPTER FOUR

THE EFFECTS OF DIFFERING PERIODS OF NUTRITION AND BODY CONDITION SCORE ON THE CYTOKINES AND CHEMOKINES OF BOVINE SEMINAL PLASMA IN BEEF CATTLE

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ABSTRACT

Beef cattle production is highly influenced by the sires' ability to produce quality semen and successfully impregnate females. Nutrition and environmental factors impact the components within the ejaculate, specifically inflammatory cytokines that are essential for pregnancy establishment and fetal tolerance. We hypothesized that differing nutritional periods and body condition scores (**BCS)** would affect cytokine concentrations in the seminal plasma (**SP**) of bulls. Mature Angus bulls (n = 11) were individually housed and randomly assigned to one of two treatments: 1) over-fed (**OVER**, n = 5), or 2) restricted (**RES**, n = 6). Bulls were fed the same ration (35% ground hay, 35% cracked corn, 20% distillers' grain and 10% soybean meal) at differing volumes to achieve desired treatment effects with different nutritional periods: gain, loss, and 2 periods of maintenance: ideal condition (BCS of 6) steady state (**ISS**) and abnormal condition (BCS of 8 or 4) steady state (**ABS**). Body weight (**BW**) and BCS was taken every two weeks to monitor and manage intake volume. Ejaculates were collected every 84 d to determine cytokine profiles within SP. A complete randomized design with mixed model ANOVAs were used to evaluate SP initially and on d 84 of each nutritional period to via PROC GLIMMIX (SAS 9.4, Cary, NC) and MetaboAnalyst 5.0. Initial cytokine concentrations prior to treatment were included as a covariate. All cytokines returned to similar concentrations as the initial value during the ISS period. Nutritional period affected ($P < 0.05$) IFN-γ, IL-8, MIP-1α, MIP-1β, TNF-α, IL-1β, and IL-10. Treatment by nutritional period influenced ($P<0.05$) IL-36RA and VEGF-A. Cytokines with the greatest impact during nutritional periods included: MIP-1α, TNF-α and IL-1β, occurring the greatest during the ISS period and had similar reduced concentrations during the gain, ABS and loss period regardless of treatment ($P<0.05$). In

conclusion, different levels of nutrition altered cytokine concentrations within SP which could potentially impact the cytokine balance and immune responses needed for pregnancy establishment.

INTRODUCTION

A cow-calf producer's income is highly dependent on bull fertility since profits are based on the number of calves born each year (Taylor and Field, 1995). Monitoring bull fertility is critical in order to maximize reproductive efficiency in beef production (Butler et al., 2020). One infertile bull is more detrimental than one infertile cow due to the number of offspring that bulls should produce in a breeding season (Kastelic, 2013). Bull management for maximum fertility includes nutrition management to produce bulls that fit industry and consumer standards. However, bull fertility and reproductive success can become limiting when issues such as inadequate nutrition, injuries or infections are present (Kastelic, 2013). Correct nutritional management of bulls is critical due to one sire serving multiple females while experiencing variable periods of nutrition throughout the year. Therefore, understanding the complete impacts of nutrition on seminal plasma (**SP**) and male fertility could help increase reproductive efficiency.

Seminal plasma is the non-cellular portion of the ejaculate composed of cytokines, amino acids, enzymes, hormones, ions, sugars, lipids, antioxidants and proteins that act as a nutritive-protective and transport medium for spermatozoa as it travels through the hostile female reproductive tract (Juyena and Stelletta, 2012). Moreover, SP moieties, can be impacted and influenced by many factors including nutrition (Eckel and Ametaj, 2016). Restricted diets can impact SP by decreasing the amount volume produced for each ejaculate (Singh et al., 2018). Whereas with overfeeding, there was an increase in the production of Insulin-like Growth Factor-1, potentially causing negative fertility effects within rams (Selvaraju et al., 2012) as well as pro-inflammatory cytokines which are influenced by high energy diets (Eckel and Ametaj,

2016). Therefore, nutrition may be influential in maximizing bull fertility and establishing a pregnancy. Recently, SP was found to target the female tissues in many species such as humans, mice and cattle, to illicit an immunological response for pregnancy establishment (Robertson et al., 2009; Bromfield, 2014). This response activates structural modifications and overall functions of female tissues through the recruitment of leukocytes, macrophages and dendritic cells due to molecular signaling from cytokines (Robertson et al., 1997). Cytokines are a diverse group of signaling proteins involved in a multitude of immunological functions (Chen et al., 2018). Cytokines are known to be pleiotropic and synergistic; acting in cascading pathways to create a strong biological effect in a given tissue (Kany et al., 2019). The cytokines within the SP cause cascading signals to the endometrium to promote the inflammatory immune response that will in turn facilitate pregnancy, embryo tolerance and development in humans, mice, and cattle (Tremellen et al., 1998; Robertson, 2005). Furthermore, complex cytokine networks have important roles in a wide range of reproductive processes such as maternal-fetal interaction by Interferon-τ, uterine expansion by IL-1β and IL-8 (Orsi and Tribe, 2008) and cervical remodeling by IL-10 to prepare for parturition (Van Engelen et al., 2009). However, at the time of copulation, SP stimulates the release of IL-10 in the female reproductive tract, suggesting is the need to maintain an immunological balance to avoid rejection of the spermatozoa and subsequent embryo (Denison et al., 1999). The balance of pro- and anti-inflammatory cytokines is crucial for the survival of the spermatozoa in the female tract and the tolerance of the fetus.

Since nutrition can affect the overall inflammatory state of SP and impact immune responses within the female after intercourse, it is crucial to understand how different diets affect cytokines within the SP. Therefore, the hypothesis of the current study is that differing levels of

nutrition and body condition score (**BCS**) will alter cytokine concentrations within the SP of beef bulls.

MATERIALS AND METHODS

All experimental procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee.

Experimental Design

Mature Angus bulls (n=12; body weight (**BW**) = 738 kg; BCS = 6; Age = 4 years) were purchased from Jorgensen Land & Cattle (Ideal, SD). One bull developed leukemia virus and was required to be euthanized; thus, was excluded from all analyses. All bulls were individually housed in a 2.44 m × 12.19 m paddock with ad-libitum access to water and a 100 g mineral supplement daily (CO-OP Supreme Cattle Mineral; Tennessee Farmers Cooperative; Lavergne TN). Bulls were provided targeted intakes to meet BW and BCS goals as per experimental design. The diet consisted of 35% ground hay, 35% cracked corn, 20% dried distillers' grain and 10% soybean meal. Following a 21 d adaptation period, each bull was randomly assigned to one of two treatment pathways consisting of either restricted (**RES**) or over-fed (**OVER**) diets (**Fig. 1**). On d 21, RES bulls began treatment diets targeting a decrease of 2 BCS (actual BCS Δ = -2; BW Δ = -67.27 kg) over 84 d. On d 105, bulls began adaptation to diet intake adequate for maintenance at an abnormal condition steady state (**ABS**; BW= 693.86 kg; BCS = 4) over 10 d. On d 189 bulls began re-alimentation back to initial basal BW and BCS (832.495 kg and ~6.1, respectively) with intakes targeting a BCS increase of 2 (actual BCS Δ = 1.2; BW Δ = 118.03 kg). Bulls assigned to the OVER treatment were subjected to inverse dietary changes of the RES treatment. Following the 21 d adaptation, bulls on the OVER treatment will receive an intake volume to support ~1.25 kg/d to increase BCS by 2 (actual BCS Δ = 0.95; BW Δ = 169.82 kg) in

84 d. On d 105, OVER bulls were acclimated to ABS intakes adequate for maintenance at the new BW (885.40 kg; BCS = 6.85) and remained on this nutritional period for 84 d. On d 189, OVER bulls began the return to basal BW and BCS (813.58 kg and ~5.20, respectively) by targeting a 2 BCS decrease (actual BCS Δ = -0.80; BW Δ = -43.09 kg) in 84 d. All bulls received ideal condition (BCS = 6) steady state (**ISS**) intake levels for 84 d (actual BCS = 5.20; actual BW= 813.58 kg) to complete the objectives.

Semen Collection

Individual non-shrunk BW and BCS (1 = emaciated and 9 = obese, (Wagner et al., 1988)) was taken every other week to monitor changes and ensure treatments met experimental goals. Intake volumes were adjusted on weigh days if adequate gain or loss were not achieved. Semen was collected every 28 d for fertility evaluation and further cytokine concentrations. From the ejaculate, laboratory analysis of the cytokine composition of SP was conducted. Temperature was only taken to monitor illness and wellbeing of the all bulls (Data not reported) and was how, in addition to decreased intake, the bull with bovine leukemia virus was identified and removed. Rectal palpation was conducted for each sampling date prior to electro-ejaculation to ensure normal internal tract morphology. To collect the ejaculate, a semen collection handle with a saline bag was connected to a disposable cone and vial. The attached saline bag kept in a heated water bath (~37 °C) to keep the ejaculate at a constant temperature during and after collection to eliminate environmental temperature effects on the spermatozoa. The ejaculate was centrifuged in the lab for 5 min at 2500 × G to pellet spermatozoa at the bottom of the tube, SP was then aliquoted out into 2 mL microcentrifuge tubes, and stored at -80 until cytokine analyses could be conducted. All samples were stored at -80°C until cytokine analyses could be performed.

Cytokine Quantification

Cytokine concentrations of Interleukin (**IL**)-1α, IL-1β, Tumor Necrosis Factor (**TNF**)-α, Interferon (**IFN**)-γ, IL-4, IL-6, IL-10, IL-17A/Cytotoxic T-Lymphocyte-associated Antigen 8 (**CTLA8**), IL-36 Receptor Antagonist (**RA**)/Interleukin 1 Family Member 5 (**IL-1F5**), IL-8/C-X-C motif Ligand 8 (**CXCL-8**), Monocyte Chemoattractant Protein (**MCP**)-1/C–C motif chemokine Ligand (**CCL**)-2, Macrophage Inflammatory Protein (**MIP**)-1α/CCL-3, MIP-1β/CCL-4, and Vascular Endothelial Growth Factor (**VEGF**)-A were quantified within SP using the MILLIPLEX ® MAP Bovine Cytokine/Chemokine Magnetic Bead Panel (MilliporeSigma, Burlington, MA, USA) according to the manufacturer protocol, and analyzed on the Luminex 200 system (Luminex, Austin, TX, USA) at the University of Tennessee Institute of Agriculture Genomics Hub. This system allows for up to 100 analytes to be detected through a multiplex bead-based immunoassay to determine the bead region and corresponding assigned analytes.

Statistical Analyses

A complete randomized design was implemented to assign treatments to each bull completely at random in GLIMMIX SAS 9.4 (SAS Institute, Cary, NC) to determine the effects of treatment, nutritional period, and the interaction on cytokine concentrations. The experimental unit was each individual bull. Random effects included bull within treatment. The initial cytokine concentrations prior to the onset of treatments, after the 21 d acclimation diet, were included as a covariate for all cytokine analysis. Normality of cytokine concentrations was determined by the Shapiro-Wilk statistic > 0.8 and the Kolmogorov-Smirnov test > 2.0. Cytokine concentrations that were not normally distributed were log transformed to achieve normality. Interleukins 4 and 6, IP-10 and MCP-1 were not able to achieve normality with or without

transformation due to the concentrations being under the detectable limit of the assay and were removed from further analysis. Means were determined to be different when $P \leq 0.05$ and a tendency when $P \leq 0.10$. Log transformed cytokine concentrations were also analyzed using MetaboAnalyst 5.0 to identify any differences in cytokine profiles. The chemometrics analysis using partial least squares discriminant analysis (**PLS-DA**) was utilized to evaluate treatment, nutritional period and the interaction. Variable importance in projects (**VIP**) was also performed on cytokines within PLS-DA to determine which cytokines were most influential in the resulting profile.

RESULTS

Cytokine Concentrations

The initial sample influenced ($P < 0.05$) IFN-γ and TNF-α but did not influence ($P > 0.30$) any other cytokine and, thus, the covariate was removed from those analyses. The initial cytokine concentrations prior to treatment, and after the 21 d acclimation diet, were compared to the ISS ($P < 0.035$) resulting in the cytokines: MIP-1α, TNF-α, IL-36RA and VEGF-A, to not have statistically similar concentrations. The main effect of treatment pathway did not influence ($P > 0.30$) any cytokines; therefore, the second main effect of nutritional period will be the only main effect discussed from here on. A treatment × nutritional period effect ($P < 0.047$) occurred for IL-36RA (**Figure 2**) and VEGF-A (**Figure 3**). Interleukin-36RA concentrations were not different in the OVER bulls at any time (**Figure 2a**); however, the RES bulls were lowest ($P = 0.05$) during the loss and ABS period and greatest during the gain and ISS period (**Figure 2b**). In the OVER bulls, VEGF-A, there was a decrease ($P = 0.02$) during the gain period before increasing during the ABS, loss and ISS (**Figure 3a**). Whereas, the RES bulls decreased ($P =$

0.02) during the loss period were intermediate after the ABS and gain periods and increased after the ISS period (**Figure 3b**).

Nutritional period affected INF-γ, IL-8, MIP-1α, MIP-1β, TNF-α, IL-1β and IL-10. Concentrations of IFN-γ were lower ($P = 0.01$) after the ISS period but greater during the loss period regardless of treatment (**Figure 4a**). Nutritional period also affected ($P < 0.0001$) IL-8 (**Figure 4b**) and MIP-1α (**Figure 4c**), with ISS having the greatest ($P < 0.01$) concentrations and the lowest concentrations at the end of the gain period. Macrophage Inflammatory Protein-1α remained decreased during the ABS and loss period as well. (**Figure 4c**). Concentrations of MIP-1β were greatest ($P < 0.01$) during the ISS with the lower concentrations during the loss period (**Figure 4d**). The pro-inflammatory cytokines, TNF-α (**Figure 5a**) and IL-1β (**Figure 5b**), followed similar trends with the greatest ($P < 0.01$) concentrations occurring after the ISS period and the lower concentrations occurring during the gain, ABS and loss period ($P < 0.01$). The anti-inflammatory cytokine, IL-10, had greater ($P = 0.04$) concentrations during the ISS and ABS periods and reduced concentrations during the gain and loss periods (**Figure 5c**).

Cytokine Profiles

To establish cytokine profiles for each the nutritional periods, a PLS-DA was created within MetaboAnalyst 5.0. Cytokine profiles for treatments, OVER and RES, and the interaction of treatment × nutritional period was not found to overlap. Only nutritional periods appeared to be impactful with a distinct separation between some groups (**Figure 6a**). Specifically, there was a distinct overlap of the gain, loss and ABS periods which were completely separate from the initial and ISS periods (**Figure 6a**). Partial overlap did occur between the initial and ISS periods. Variable importance in the projection scores were generated to determine the cytokines that

contributed to these profiles during the nutritional periods. All of the cytokines were included within the PLS-DA but the cytokines with < 0.5 VIP score included: MCP-1, IP-10, IL-17α, IL-4, IL-10, MIP-1β and VEGF-A, were deemed relatively insignificant in the overall profile of the ejaculate (**Figure 6b**). Cytokines identified as moderately significant (VIP score = > 0.5 and < 1.0) included: IL-6, IFN-γ, IL-8, IL-36RA and IL-1α (**Figure 6b**). Cytokines (IL-1β, TNF-α and MIP-1α) with a VIP score > 1, were highly influential to the cytokine profiles of each nutritional period (**Figure 6b**). Macrophage inflammatory protein-1α had the greatest impact (VIP score > 2) on the ejaculate among all nutritional periods and was greatest during the ISS period and the lowest during the gain period (**Figure 6b**). Tumor Necrosis Factor-α and IL-1β followed a similar prevalence pattern with greatest impacts (VIP score > 2 and > 1.5, respectively) during the initial period and the most reduced during the ABS period; however, TNF-α had a greater overall impact (**Figure 6b**).

DISCUSSION

Sire management, through the control of nutrition and other environmental factors, is imperative in order to ensure optimal fertility and advance overall herd genetics. Nutritional effects have been shown to impact the ejaculate in boars, rams and bulls (Brown, 1994). Nutrition has also played a role in influencing the components of SP, specifically cytokines (Eckel and Ametaj, 2016). Cytokines rarely act individually, more so as a network of highly influential, cascading protein molecules to cause each cytokine and chemokine perform biological functions to stimulate local and systemic inflammation (Dinarello, 1989). The majority of cytokines and chemokines, IFN-γ, IL-8, MIP-1α, MIP-1β, TNF-α, IL-1β and IL-10, quantified in the current study were impacted by nutritional periods. Additionally, IL-36RA and

VEGF-A were influenced by an interaction between adiposity and nutritional level. Therefore, the presence of cytokines and chemokines within the SP from sire nutrition may impact reproductive success.

Interferon-γ is known as a pleiotropic cytokine that can have both pro- and anti-inflammatory effects, and has been found in the uterus during early pregnancy (Murphy et al., 2009). Within the maternal endometrium, IFN-γ is abundantly produced by uterine natural killer cells as well as trophoblasts to initiate endometrial vasculature remodeling, angiogenesis and the maintenance of the placenta in mice (Murphy et al., 2009). Interestingly, IFN-γ in SP have been linked to female infertility in humans outside of sperm and male parameters (Robertson et al., 2003). Concentrations of IFN-γ in the current dataset were greatest during periods of nutritional perturbation. This may be indicative of the nutritional stress influencing IFN-γ concentrations in bovine SP which could impact the ability to produce viable embryos and establish a pregnancy. In support of these results, the immune-regulatory functions of IFN-γ in humans have generally been detected at low levels within SP; however, was substantially elevated when a disease or infection was present (Leutscher et al., 2005; Vanpouille et al., 2016). Indicating the fluctuations of cytokine levels when external and internal factors occur in order for the body to fight to return back to homeostasis.

Tumor necrosis factor-α is an acute phase cytokine responsible for mediating acute inflammatory reactions to diseases or infection sites (Burger and Dayer, 2002). The pro-inflammatory properties of TNF-α are known to hinder sperm motility and functional capacity by increasing chemotactic activity and inducing the immuno-cascade effects of neutrophils (Hill et al., 1989). In contrast, TNF-α has been found to be essential for early pregnancy establishment

(Toder et al., 2003). Yet the overproduction of TNF-α could cause early embryonic loss or implantation failure in humans (Saito et al., 2010; Alijotas-Reig et al., 2017). Bovine TNF-α also incites inflammation by promoting neutrophil recruitment when induced by lipopolysaccharides (Sohn et al., 2007). Interleukin-1β has similar effects of TNF-α on semen quality and establish pregnancy (Gruschwitz et al., 1996). Interluekin-1β is also responsible for activating the innate immune response as well as mediating recruitment and activation of macrophages and neutrophils (Ott et al., 2007). The highly correlated pro-inflammatory cytokines, TNF-α and IL-1β (Eggert-Kruse et al., 2007), had similar cytokine concentration trends in the current study but were also found to have a significant impact on the cytokine profiles. These closely associated and impactful pro-inflammatory cytokines demonstrate their potential needed inflammatory roles in pregnancy establishment.

Chemokines are a family of small cytokines, mostly known for their role in chemotaxis (Hughes and Nibbs, 2018), inflammation, immune surveillance, and angiogenesis (Dimberg, 2010). Many chemokines are known to be pro-inflammatory while others are thought to control cell migration for normal tissue growth and maintenance (Hughes and Nibbs, 2018). Interleukin-8 is a potent pro-inflammatory chemokine involved in leukocyte migration and cell activation for events associated with inflammation (Nederlof et al., 2017). Interleukin-8 decreased substantially during the gain period in comparison to the greatest concentration following the ISS period. In humans, IL-8 concentrations have been shown to be present in high concentrations within SP of healthy fertile men (Politch et al., 2007). In contrast, IL-8 has also been known to dramatically increase in response to bacterial and viral infections which effected spermatozoa to cause poor sperm motility within the ejaculate (Eggert-Kruse et al., 2001). Therefore, increased levels of

this immunostimulatory chemokine from nutritional stress could result in potential pregnancy failures and diminished herd outcomes.

The pro-inflammatory chemokines, MIP-1α and MIP-1β, are known as chemoattractant and activators of monocytes and macrophages which contributes to the regulation of uterine macrophage populations of mice (Robertson et al., 1998). However, MIP-1α and MIP-1β has not been extensively researched within bovine. The current study identified MIP-1α to have the greatest impact, VIP score, on cytokine profiles within bull SP during different nutritional periods. Previous research has shown increases in MIP-1α and MIP-1β concentrations are indicators of infection or disease (Garzino-Demo et al., 1999; O'Grady et al., 1999; Chaisavaneeyakorn et al., 2003). However, humans with HIV expressed lower concentrations of MIP-1α and MIP-1β released by cytotoxic T cells (Cocchi et al., 2000). Thus, concentrations of MIP-1α and MIP-1β may increase acutely but long-term stress from disease, like HIV (Cocchi et al., 2000), or nutritional stress (current dataset) results in lower concentrations altering the impacts of these chemokines on physiological functions.

Immuno-suppressive cytokines, IL-10 and IL-36RA, play important roles in inhibiting the synthesis of pro-inflammatory cytokines (Zhang and An, 2007). More specifically to reproduction, anti-inflammatory cytokines create an overall immunosuppressive state in the mucosal environment to account for sperm survival within the oviduct (Torres-Poveda et al., 2014). Evidence for this has been shown in humans, where the concentrations of IL-10 are greater in healthy individuals after coitus to demonstrate the needed immune-tolerant environment sperm survival in the hostile female reproductive tract (Camejo, 2003). The immunosuppressive functions of IL-10 re also to prevent the rejection of the semi-allogenic fetus

(Chatterjee et al., 2014) and promote conceptus attachment to the uterine endometrium in dairy and beef cattle (Odhiambo et al., 2009). Similarly, our study demonstrated IL-10 to be similar to the initial with the greatest concentrations occurring during the ISS. Indicating the desired immune-tolerant levels for sperm survival in healthy individuals as bulls returned to ideal BCS. The antagonistic effects of IL-36RA, a member of the IL-1 super family, inhibits inflammation by inhibiting IL-36 from binding to IL-36R (Yi et al., 2016). The antagonistic effects from IL-36RA impede the signals between the toll/interleukin-1 receptor domain which inhibits NF-κB signaling cascade (Murrieta-Coxca et al., 2019). The roles of the IL-36 cytokine family remains mostly unknown in pregnancy; however, the immunosuppressive effects of IL-36RA could promote a balanced uterine environment for successful pregnancy establishment. Our findings quantified IL-36RA at a greater concentration than IL-10; as well as, IL-36RA having a moderate impact compared to the low impact of IL-10 on cytokine profiles.

Angiogenic cytokines can induce endothelial cell activation and proliferation for angiogenesis, the creation of new blood vessels from precursor cells such as angioblasts (Ucuzian et al., 2010). Proper function of VEGF-A is critical during every step of placental growth and vascular formation to provide blood required to the growing fetus (Chen and Zheng, 2014). Thus, VEGF-A being present in the greatest concentrations within SP of bulls is not entirely surprising. Furthermore, research has demonstrated a decrease of VEGF-A in the SP of obese humans causing a negative effect of semen quality (Han et al., 2017). Our study portrays these results since VEGF-A had the lowest VIP impact of all they cytokines presented, more specifically during the gain period. Therefore, even though VEGF-A can be different in cytokine concentrations, this angiogenic cytokine did not play a significant role in the cytokine profile.

This could potentially be an indicator that other cytokines within SP are needed to promote the stimulation of maternal VEGF-A rather than the paternal VEGF-A for embryo establishment and development.

In conclusion, cytokines and chemokines fluctuated between nutritional periods, potentially in response to the nutritional stress. The cytokines, MIP-1α, TNF-α, and IL-1β, had the greatest impact on the overall profile of SP from nutritional periods. Moreover, this dataset demonstrated a similarity between cytokine profiles when the animal gains or loses BW or is maintained at an abnormal BCS. The cytokines: IFN-γ, MIP-1β, IL-1β, IL-8 and IL-10, during the ISS period, returned back to the initial concentrations and profiles. Therefore, varying nutritional levels can influence the immunological substrates within SP of mature bulls which could potentially affect the sire's ability to successfully establish pregnancy. However, these changes are not permanent and will return under correct feeding. Further studies are still required in order to fully understand the long-term impacts and influences on SP as well as the impacts on the uterine environment to maximize reproductive efficiency and success.

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APPENDIX

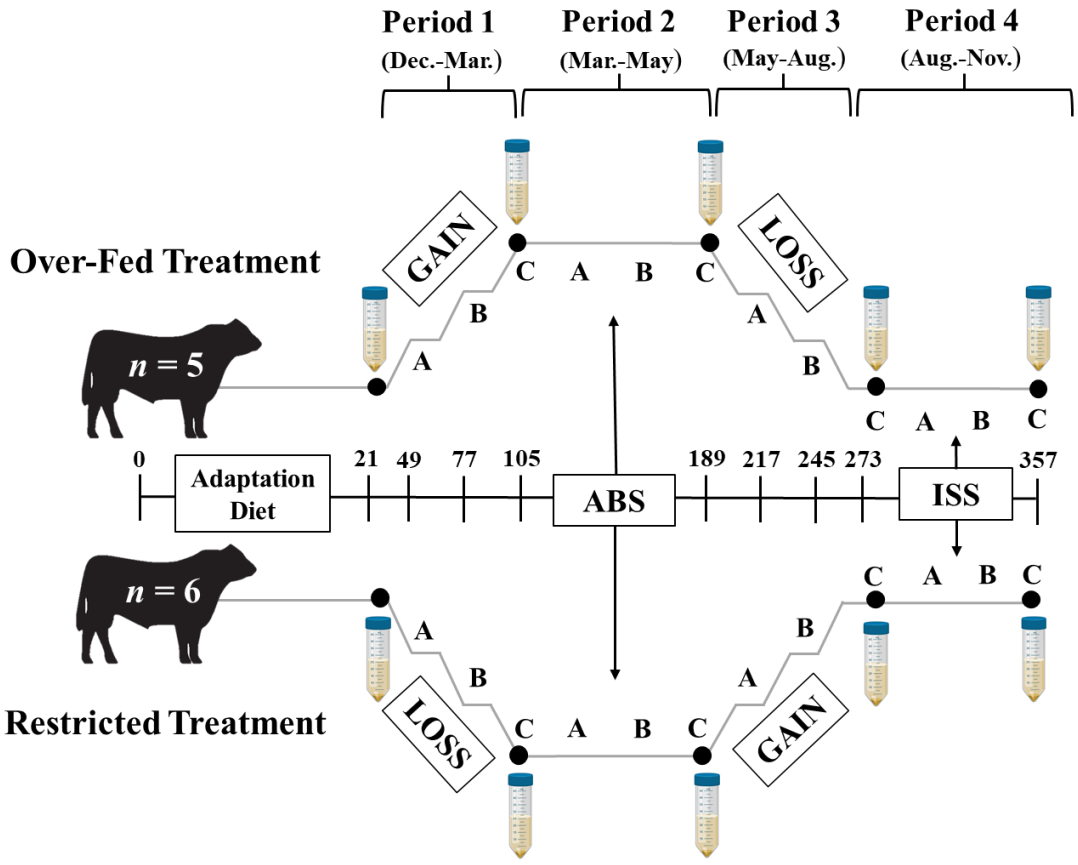
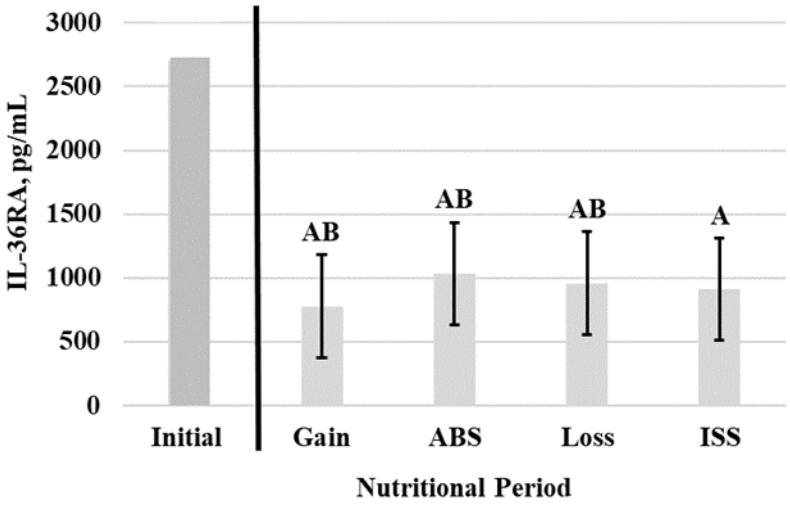
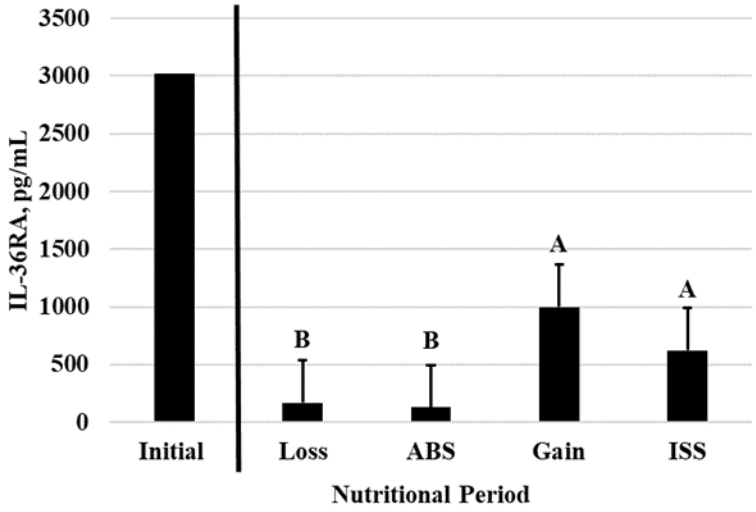


Figure 5. Cytokine project timeline with two treatment pathways: OVER and RES, with four respective nutritional periods: gain, loss, ABS and ISS. Sample collections followed after the 21 d maintenance diet prior to treatment pathways. Including: semen collection for cytokine analysis for initial and every 84 d (large falcon tubes) and diet changes (28 d= A, 56 d= B and 84 d= C).

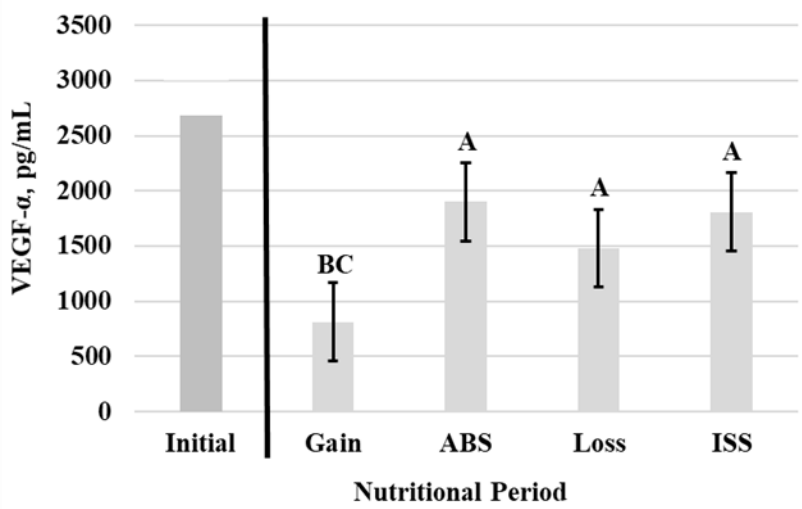


A

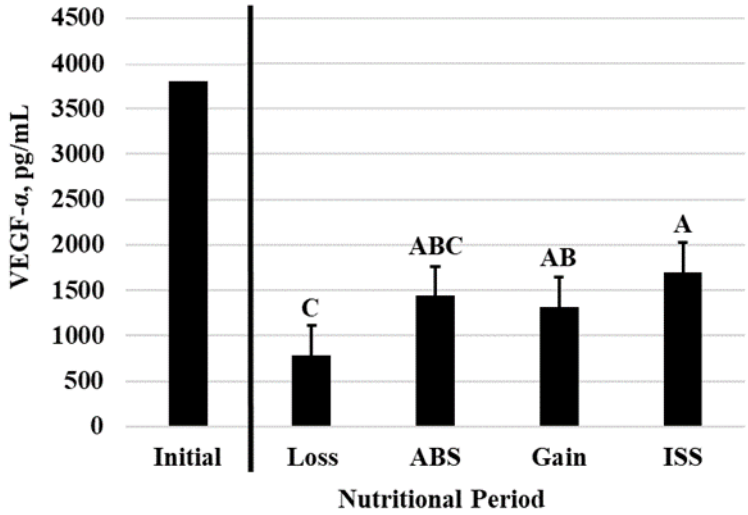


B

Figure 6. Interleukin-36RA concentrations ($P < 0.05$) differed according to treatment by nutritional period effects; the two graphs can be compared as an interaction. The OVER treatment (a) included: the gain (777.69 ± 403.11), ABS (1,032.83 ± 403.11), loss (956.92 ± 403.11) and ISS (911.96 ± 403.11) with the initial concentration at 2,698.46 pg/mL. The RES treatment (b) included: the loss (167.06 ± 367.93), ABS (128.73 ± 367.93), gain (999.91 ± 367.93) and ISS (911.96 ± 367.93) with the initial concentration at 3,030.10 pg/mL. ^{AB}Bars (arithmetic means ± SEM) that do not share a letter denotes differences at $P \leq 0.05$.



A



B

Figure 7. Vascular Endothelial Growth Factor-A concentrations ($P < 0.02$) differed according to treatment by period effects; the two graphs can be compared as an interaction. The OVER treatment (a) included: the gain (813.48 ± 353.69), ABS (1,900.25 ± 353.69), loss (1,478.30 ± 353.69) and ISS (1,809.56 ± 353.69) with the initial concentrations at 2,678.20 pg/mL. The RES treatment (b) included: the loss (788.64 ± 321.21), ABS (1,441.33 ± 321.21), gain (1,321.26 ± 321.21) and ISS (1,702.39 ± 321.21) with the initial concentrations at 3,809 pg/mL. ^{ABC}Bars (arithmetic means ± SEM) that do not share a letter denotes differences at $P \leq 0.05$

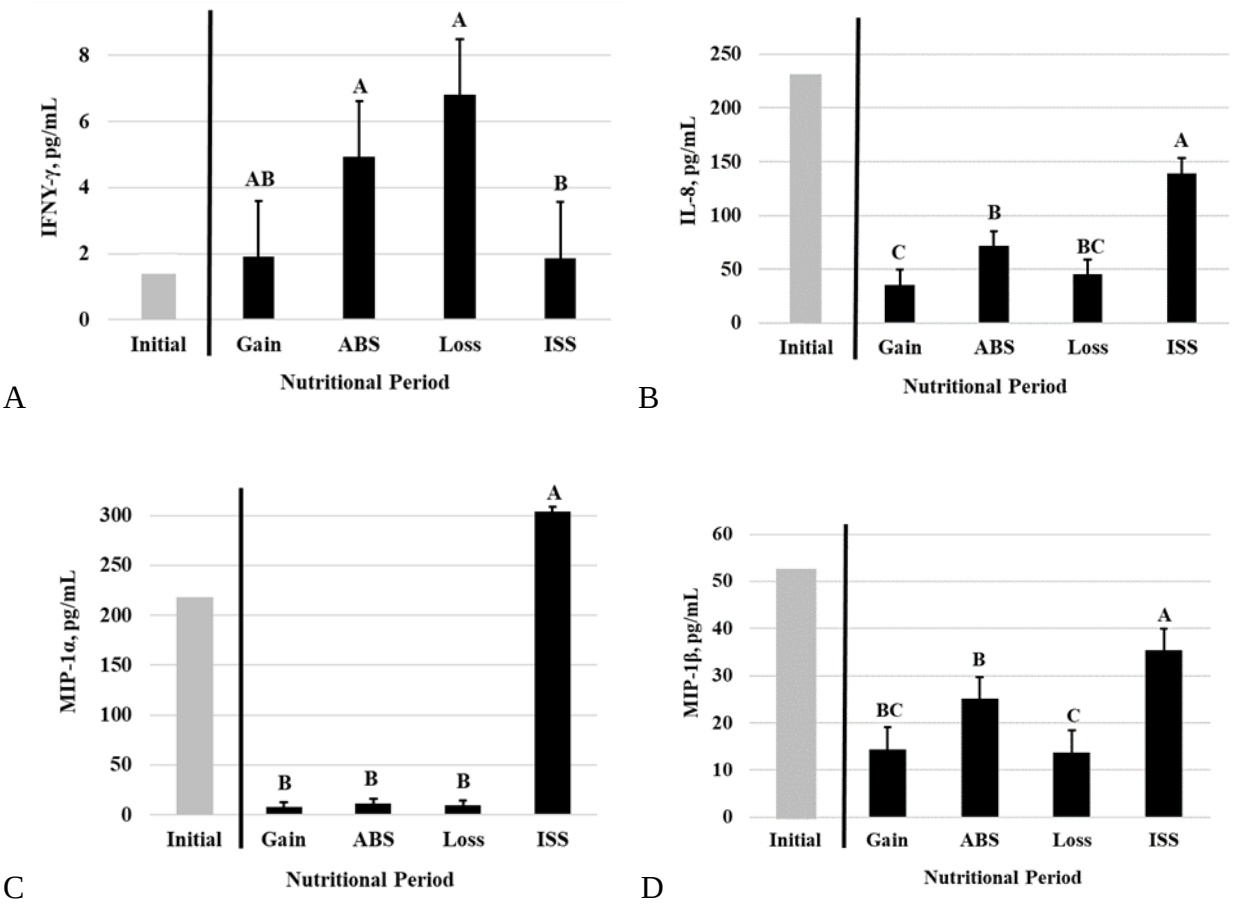


Figure 8. Concentrations of IFN-γ (a), IL-8 (b), MIP-1α (c) and MIP-1β (d) within seminal plasma were affected by nutritional period. [ABC]Bars (arithmetic means ± SEM) that do not share a letter denotes differences at $P \leq 0.05$.

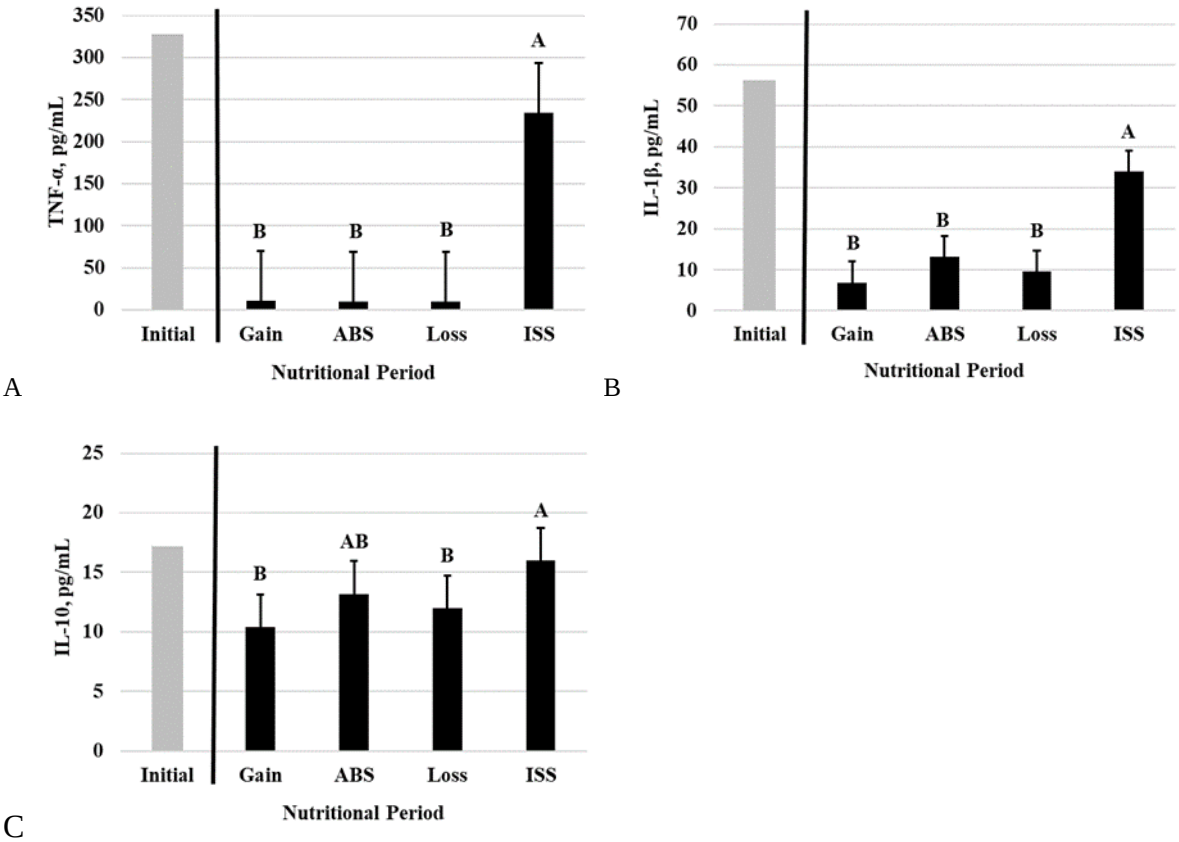
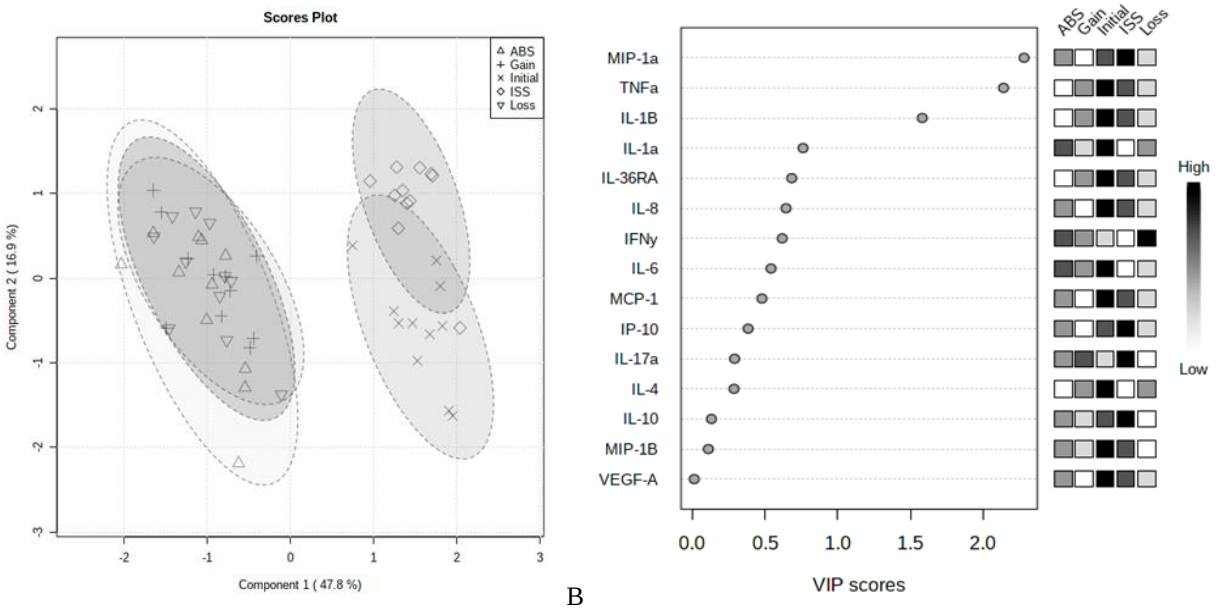


Figure 9. Concentrations of TNF-α (a), IL-1β (b) and IL-10 (c) were affected by nutritional period. [AB]Bars (arithmetic means ± SEM) that do not share a letter denotes differences at P ≤ 0.05.



A B

Figure 10. Analysis of cytokine profiles with partial least squares discriminant analysis (a) visualizing differences in inflammatory cytokines during differing nutritional periods: gain (plus sign), ABS (triangle), loss (upside down triangle) and ISS (diamond). Ellipse represents a 95% confidence interval. Variable importance in the projection (b) plot indicates MIP-1α to have the greatest influence on the differences in cytokine concentrations between all nutritional periods.

CHAPTER FIVE
CONCLUSIONS

Proper management and nutrition of bulls remains critical within the cattle industry since bulls have the ability to increase profitability of producers as well as advance herd genetics. While nutritional management of bulls can be costly and challenging, semen quality can be influenced with constant evaluation and changes of the sire diet to maximize reproductive success. The reproductive impacts of nutrition on bulls and other species has been well documented (Brown, 1994; Singh et al., 2018); however, the nutritional influences on the bovine cytokines and chemokines of SP has not. The cascading signal effects of cytokines have been found to be influenced by varying levels of nutrition (Eckel and Ametaj, 2016). Furthermore, cytokines have critical pro- and anti-inflammatory factors that are essential for the establishment of pregnancy through the reconstruction of uterine tissues and to prevent rejection of the semi-allogenic fetus from the maternal immune system (Bromfield, 2014). Therefore, the sire diet can potentially influence the components of SP and possibly hinder reproductive outcomes due to a decrease in semen quality. Our study provides varying levels of nutrition on different treatment pathways to beef bulls to show the potential influence of nutritional stress on cytokines and chemokines within SP. Further research is required to determine the optimal amount of adiposity within bulls to maximize fertility parameters and to ensure success throughout an entire breeding season. As well as how dietary components affect spermatozoa quality and function. This research should also expand into searching for the dietary effects of SP within the uterine environment for pregnancy establishment. By understanding the dietary mechanisms that impact SP, we can further increase our understanding of how the sire diet can improve reproductive efficiency within beef bulls to advance herd success.

VITA

Taylor Dawn Harrison was born and raised in Las Cruces, New Mexico on February 22nd, 1997 to Chris and Sherri Harrison. Taylor became interested in veterinary medicine and animal science at a young age that only increased her passion for this field throughout high school. These early interests and passions led her to pursue her Bachelor of Science in Agriculture degree in Animal Science with a minor in Chemistry from New Mexico State University. While studying there, she realized her passion for animal reproductive physiology and implementing this knowledge to improve herd advancement and potential within the commercial beef industry. Taylor then chose to pursue a Master of Science degree in Reproductive Physiology with a concentration in sire beef cattle from the University of Tennessee.