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Assessment of Management Factors Prior to Breeding and their Impact on Bovine Fertility

Kathryn Erin Pfeiffer

ASSESSMENT OF MANAGEMENT FACTORS PRIOR TO BREEDING AND THEIR IMPACT ON BOVINE FERILITY

By

Kathryn Erin Pfeiffer

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By

Kathryn Erin Pfeiffer

Approved:

Jamie Larson Assistant Professor of Animal and Dairy Sciences (Director of Thesis)	Jane Parish Associate Professor of Animal and Dairy Sciences (Committee Member)
Trent Smith Assistant Professor of Animal and Dairy Science (Committee Member)	
Peter Ryan Associate Provost for Academic Affairs Professor of Animal and Dairy Sciences (Graduate Coordinator)	George Hopper Dean of College of Agriculture and Life Sciences

Name: Kathryn Erin Pfeiffer

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Institution: Mississippi State University

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Major Professor: Jamie Larson

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Candidate for Degree of Master of Science

Management of female infertility is a primary determinant of economic efficiency in the cattle industry. Management factors involved in impacting fertility include identification of females with suboptimal fertility and reducing the period of anestrus, prior to pubescence and after parturition. The use of anti-Müllerian hormone in the identification of females with suboptimal follicular populations allows for selection of females with optimal follicular populations and could reduce infertility resulting from a decrease in the quantity of follicles. A reduction in the period of anestrus also impacts fertility and management strategies that induce an ovulatory response in anestrous females improves fertility. Biostimulation has advanced pubescence in heifers and reduced the length of postpartum anestrus in cows. Advancing the understanding of anti-Müllerian hormone and the biostimulatory effect allows for further assessment of these management factors and their impact on infertility. Improved management of female infertility increases profitability of cattle production.

DEDICATION

I would like to dedicate this thesis to my family, both north and south. This would not have been possible without you. Specifically, I would like to thank my mother for her guidance and instilling the value of education in me.

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CHAPTER I

INTRODUCTION

Reproduction is the primary factor influencing the efficiency of beef cattle production (Short et al., 1990) and reproductive failure is considered the main source of economic loss in the cattle industry (Trenkle and Willham, 1977). The failure to reproduce is the primary reason for a female to be removed from production in a cow-calf operation (Erickson et al., 1976; Martinez et al., 2004). The interaction of many factors affect reproductive function but management of female infertility is a primary determinant of economic efficiency in the cattle industry.

Management factors impacting fertility include identification of females with suboptimal fertility and reducing the period of anestrus, prior to pubescence and after parturition. Identification of indicators associated with infertility has been difficult to quantify. The development of methods that utilize phenotypic differences to identify females with suboptimal fertility would allow for removal of these females from production, potentially increasing reproductive efficiency in a cow-calf operation.

Identification of fertility indicators involves quantification of the ovarian reserve. The ovarian reserve has been defined as the number of morphologically healthy oocytes and follicles within the ovaries (Ireland et al., 2009). In females, the ovarian reserve is established prenatally and inherently variable with females exhibiting optimal and suboptimal follicular populations (Ireland et al., 2008). During the reproductive lifespan of the female, follicular development causes atresia of oocytes and a continuous depletion

of the ovarian reserve. As reproductive senescence occurs, the reduction in quantity and quality of oocytes affects fertility. Measurement of the ovarian reserve allows for identification of females with differing follicular populations. Selection of females exhibiting optimal follicular populations could reduce the infertility associated with a reduction in the ovarian reserve.

Measurement of the ovarian reserve involves ovariectomy to establish the population of follicles. This procedure reduces the applicability of utilizing the ovarian reserve as an indicator of fertility. The introduction of antral follicle count (AFC) has allowed for the indirect measurement of the ovarian reserve using ultrasonography to quantify the number of follicles on the ovary. Antral follicle count is considered the maximum number of follicles, greater than or equal to 3 mm in diameter per pair of ovaries, measured during a follicular wave (Jimenez-Krassel et al., 2009). Although this count is highly correlated to the ovarian reserve, the measurement of follicles using ultrasonography requires a more intensive management system than applicable in a common production setting.

The recent discovery of the correlation of AFC with anti-Müllerian hormone (AMH) has furthered the applicability of utilizing the ovarian reserve as an indicator of fertility. Establishing circulating concentrations of AMH provides a method for quantification of the population of morphologically healthy follicles on the ovary (Ireland et al., 2008). Variations in the concentrations of AMH are independent of the follicular development occurring during the bovine estrous cycle, allowing for determination of concentrations of AMH through the collection of a single blood sample (Rico et al., 2009). Changes in concentrations of AMH have recently been evaluated during the natural bovine estrous cycle of cows but not during an estrous cycle synchronized using

exogenous hormones. Additional research is necessary to establish the significance of gonadotropins in secretion of AMH (Rico et al., 2011) in addition to other hormones utilized in an estrus synchronization protocol. Understanding the mechanisms involved in regulation of AMH would allow for the utilization of this hormone in assessment of female fertility and further elucidate the impact of nutrition, disease, and stress on reproduction (Ireland et al., 2011).

A reduction in the period of anestrus also increases reproductive efficiency. Management of factors that have an effect on the establishment or reestablishment of cyclicity could improve profitability in the cow-calf industry. In the development of heifers, the age at which puberty is attained influences lifetime production. Heifers exhibiting estrus early in the breeding season will be more likely to calve as 2-yr-olds, increasing their lifetime production capability as they will be more likely to rebreed as first calf heifers and continue to conceive early in the breeding season. Therefore, management of replacement heifers should focus on the physiological process that promotes pubescence (Patterson et al., 1992).

In the management of cows, the most important factor to increase reproductive efficiency is the early resumption of cyclicity after parturition (Hornbuckle et al., 1995). Prolonged postpartum anestrus is a major cause of failure to rebreed or to conceive late in the breeding season (Short et al., 1994). Management strategies, including biostimulation, that induce an ovulatory response in anestrous females could improve fertility.

Biostimulation is considered the stimulatory effect of males on estrus and ovulatory activity in females (Chenoweth, 1983). The use of biostimulation to advance pubescence has produced inconsistent results (Rekwot et al., 2001) but bull exposure has stimulated an increase in the occurrence of estrus and enhanced the efficacy of timed AI

in pubescent heifers (Small et al., 2000). Exposure to bull urine also increased the percentage of heifers reaching puberty by 35% (Izard and Vandenbergh, 1982) with heifers exposed to a vasectomized bull attaining puberty 3.3 months earlier than non-exposed heifers (Rekwot et al., 2001).

In addition, a multitude of research has demonstrated the significance of bull exposure on decreasing the length of postpartum anestrus (Macmillan et al., 1979; Zalesky et al., 1984; Berardinelli et al., 1987; Custer et al., 1990; Peres-Hernandez et al., 2002; Landaeta-Hernandez et al., 2004; 2006). Primiparous females exposed to bulls or the excretory products of bulls after calving resume luteal function sooner than cows not exposed to bulls or the excretory products of bulls (Custer et al., 1990; Berardinelli and Joshi, 2005). Conception rates after a 21-day AI breeding season were greater for cows exposed to bulls than for cows not exposed (Berardinelli, 1987; Fernandez et al., 1993). Timed AI pregnancy rate and overall AI pregnancy rate of primiparous cows were improved by exposing the cows to bulls before, during, and after an estrus synchronization protocol (Berardinelli et al., 2007). Although the mechanisms involved in the biostimulatory effect have not been fully elucidated, biostimulation has been hypothesized to elicit a response through genital stimulation, priming pheromones, or other undefined external cues (Chenoweth, 1983).

Understanding factors associated with female infertility allows for advances in the management of reproduction. Selection of females based on classification of the ovarian reserve increases efficiency in cow-calf production. In addition, development of management factors to reduce the period of anestrus prior to puberty or after parturition increases profitability in the cow-calf industry. Further assessment of management factors and their impact on infertility are necessary to advance cattle production.

CHAPTER II

DETERMINATION OF CONCENTRATIONS OF ANTI-MÜLLERIAN HORMONE AT ESTRUS DURING A SYNCHRONIZED AND A NATURAL BOVINE ESTROUS CYCLE

Introduction

Reproduction has been established as the primary factor influencing efficiency in the cattle industry (Dickerson, 1970; Dziuk and Bellows, 1983; Koch and Algeo, 1983) with failure to become pregnant as the primary reason for removal from production (Erickson et al., 1976; Martinez et al., 2004). The reduced production attributed to infertility has the greatest economic impact on reproduction (Bellows and Short, 1994). Economic losses associated with infertility have been estimated annually at 441 to 502 million dollars in the beef industry and 473 to 484 million dollars in the dairy industry, as of 2002 (Bellows et al., 2002). These losses represent a significant problem in cattle production.

Although it has been established that reproductive failure leads to substantial economic losses, identification of indicators associated with suboptimal fertility have been difficult to quantify. This is due to the complexity of interacting factors affecting reproduction. The development of methods that utilize phenotypic differences to identify cattle with suboptimal fertility would greatly increase production efficiency and profitability in the cattle industry.

The ovarian dynamics of cattle are becoming the focus of research to identify these fertility indicators. The ovarian reserve has been defined as the number of morphologically healthy follicles contained in the ovary (Ireland et al., 2009) and is associated with fertility in cattle (Jimenez-Krassel et al., 2009). A decrease in the ovarian reserve leads to a reduction in reproductive efficiency through pregnancy failure (Ireland et al., 2009). Development of methods to quantify this reserve has the potential to influence the efficiency of cattle production. The direct correlation of the ovarian reserve with fertility remains unknown due to the complexity in measurement of these factors.

Measurement of the ovarian reserve requires terminal histological procedures to count the number of follicles within the ovaries. With the utilization of transrectal ultrasonography, antral follicle count (AFC) can be determined by measuring the total number of follicles greater than or equal to 3 mm in diameter per pair of ovaries during a follicular wave (Jimenez-Krassel et al., 2009). Antral follicle count has been utilized as a phenotypic indicator of the size of the ovarian reserve and is positively associated with the total quantity of morphologically healthy follicles in the ovary (Ireland et al., 2008). The use of AFC as an indicator of ovarian reserve has provided a beneficial foundation for further research but requires a more intensive management system than applicable in a common production setting. Assessment of fertility using AFC is also problematic and requires a large number of animals because of the binary distribution of fertility data. In addition, suboptimal fertility is affected by many factors and determined by number of inseminations required for pregnancy.

The recent discovery of the correlation of AFC with anti-Müllerian hormone (AMH) has furthered the applicability of ovarian reserve as an indicator of fertility. This hormone is produced by the granulosa cells and provides a reliable method for

determination of the population of morphologically healthy follicles classified by an intact basal membrane, organized granulosal cell layers, and an intact oocyte and nucleus (Ireland et al., 2008). Advancing the quantification of the ovarian reserve would demonstrate the effect of follicle variation on altering ovarian function. This would allow for the development of diagnostic methods to predict the population follicles in breeding females, the determination of the impact of environmental factors on the ovarian reserve, and ultimately the improvement in methods to enhance fertility in cattle (Ireland et al., 2011).

The establishment of a correlation between AFC and pregnancy in beef heifers (Cushman et al., 2009) and dairy cows (Mossa et al., 2010) signifies the requirement for additional research in reproductive performance and response to synchronization (Perry, 2011). Changes in concentrations of AMH in plasma have not been evaluated at estrus in either a natural bovine estrous cycle or during an estrous cycle synchronized using hormones. Additional research is necessary to establish the significance of gonadotropins in secretion of AMH (Rico et al., 2011). An increased understanding of AMH would allow for the utilization of this hormone to identify females with suboptimal fertility and assess the impact of nutrition, disease, and stress on reproduction (Ireland et al., 2011).

Review of Literature

Estrous Cycle

In cattle, development of ovarian follicles is commonly characterized by 2 or 3 follicular waves that occur at 7 to 10 day intervals during each 21-day estrous cycle. The emergence of a new follicular wave usually occurs on days 2 and 11 in females with 2 follicular waves. In females with 3 follicular waves, emergence usually occurs on days 2,

9, and 16 (Savio et al., 1988). During the initiation of each follicular wave, a synchronous growth of a cohort of 3 to 5 mm follicles is induced by follicle stimulating hormone (FSH). As progression occurs during the estrous cycle, the selection and development of a single dominant follicle will cause inhibition and atresia of the remaining subordinate follicles. As a dominant follicle reaches ovulatory size of 15 to 20 mm, the follicle will either ovulate in the absence of progesterone or will undergo regression if luteal tissue producing progesterone is present (Burns et al., 2005). After ovulation or atresia of the dominant follicle the development of a new follicular wave will occur and cyclicity is established. The prevalence of this cyclicity will determine the reproductive life of the female.

Follicular Development

Regulation of follicular development is dependent on mechanisms of feedback. The ovarian steroid, progesterone, exhibits a negative feedback on the hypothalamus, reducing the production of gonadotropin releasing hormone (GnRH). Gonadotropin releasing hormone controls the secretion of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), from the anterior pituitary (Kinder et al., 1996). The frequency of pulses of GnRH subsequently determines the frequency of secretion of the gonadotropins. Follicle stimulating hormone is involved in the initial recruitment of follicles for development and the increased frequency of pulses of LH stimulates continued growth of the dominant follicle (Taft et al., 1996). As the follicle continues to develop, the increased secretion of estradiol-17β will occur. A decrease in the frequency of pulses of LH will not support the continued follicular growth and results in atresia of the follicle subsequently decreasing the secretion of estradiol-17β. The

interactions of progesterone, estradiol-17β, and frequency of pulses of LH have been analyzed to further quantify the relationship of these hormones (Inskeep, 2002). Concentrations of progesterone accounted for 37% of the variation in frequency of pulses of LH and 38% of the variation in concentrations of estradiol-17β. In addition, the frequency of pulses of LH accounted for 50% of the variation in concentrations of estradiol-17β (Inskeep, 2002).

Regulation of the follicular process is dependent on progesterone and decreased concentrations of progesterone during preovulatory follicular development can result in the development of persistent follicles on the ovary (Inskeep, 2004). The relationship of persistent large follicles and fertility has been evaluated. Cows with larger preovulatory follicles 5 days before the surge of LH had greater preovulatory concentrations of estradiol-17β and decreased conception rates (36%) compared to those with smaller follicles (91%; Breuel et al., 1993). Decreased concentrations of progesterone associated with persistent follicles results in greater secretions of estradiol-17β and the inadequate development of the zygote leading to early embryonic death occurring before the 16 cell stage of development (Ahmad et al., 1995).

Development of the Corpus Luteum

During development of the corpus luteum (CL), granulosa cells of the follicle develop into large luteal cells and the total number of granulosa cells determines the number of large luteal cells within the CL. Luteal function may be regulated by the number of granulosa cells prior to ovulation, with the functional capability of the CL to produce progesterone (P4) also dependant on the quantity of angiogenic factors present in the follicular tissue (Senger, 2003). The capacities of luteinized granulosa cells from

dominant follicles and of luteal cells from the CL to produce P4 were reduced in cattle with a low versus a high AFC (Jimenez-Krassel et al., 2009).

Progesterone has been established as a factor in the preparation of the uterus for maternal recognition of pregnancy (Vincent et al., 1986). Concentrations of P4 before estrus have been associated with altered endometrial morphology during the subsequent estrous cycles and decreased concentrations of P4 prior to estrus increased subsequent secretion of prostaglandin in response to oxytocin (Shaham-Albalancy et al., 2001). Increased prostaglandin between days 4 through 9 of the estrous cycle has been associated with increased luteolysis but also embryotoxic affects during the morula-to-blastocyst transition (Inskeep, 2004). Progesterone also has a positive physical role in uterine function through a reduction of myometrial tone and stimulates the production and secretion of endometrial proteins that effect embryonic growth (Geisert et al., 1991).

The bovine embryo inhibits the development of the luteolytic cascade through secretion of interferon-τ and maintains the secretion of P4 by the CL (Mann and Lamming, 1999). Mated cows that subsequently undergo luteolysis have significantly decreased plasma concentrations of P4 in comparison to cows that maintained pregnancy. Decreased concentrations of P4 have also been shown to increase the strength of the luteolytic signal (Mann and Lamming, 1999). Maternal P4 stimulates endometrial function, embryo development, and the secretion of interferon-τ signifying its importance in embryo survival but the relationship between the systemic concentration of P4 and pregnancy rate requires further establishment. A positive linear association occurs between the concentrations of P4 on the day of prostaglandin induced luteolysis and subsequent survival rate of the embryo. In addition, a positive linear relationship between

concentrations of P4 between days 4 and 7 and embryo survival rate has been demonstrated (Diskin and Morris, 2008).

Progesterone is the principal hormone responsible for the maintenance of pregnancy. Differences in concentrations of P4 between pregnant and nonpregnant animals occur in the early, mid-luteal, or late luteal phase and decreased concentrations of P4 after ovulation or a delay in the increase of P4 have been associated with reduced embryo development or survival (Starbuck et al., 1999). Luteal secretion of progesterone is essential in a successful gestation, for ovulation of a healthy oocyte, maintenance of uterine quiescence, nourishment and survival of the embryo/fetus, and normal parturition (Ulberg et al., 1951) but the establishment of a threshold concentration of progesterone in blood of cattle remains unknown. The optimal circulating concentration of progesterone necessary for pregnancy establishment and maintenance is in the range of 2.0 to 5.0 ng/mL (Spell et al., 2001). In contrast, females with concentrations of P4 outside this range and successfully producing a calf have been reported.

Even short periods of deprivation of P4 can decrease embryo survival during the time of the maternal recognition period. There is evidence for an association between embryonic loss and an excessive secretion of estrogen during maternal recognition of pregnancy in beef cows and this appears to limit successful maintenance of pregnancy (Bridges et al., 2000). Concentrations of P4 and estradiol-17β have also been studied as predictors of maintenance of pregnancy in dairy cows. Pregnancy loss before day 45 was increased in cows with the lowest 25% of serum concentrations of P4 at the 28 to 37th day of gestation compared to cows in the middle 50% or upper 25%, each of which had only 8% loss. Embryonic mortality after maternal recognition of pregnancy and during placentation is also a significant problem in the dairy cow. It is associated with decreased

P4 during days 28 to 37. Decreased concentrations of P4 could be due to reduced secretion by the CL, or to greater metabolism of P4 experienced by dairy cattle (Starbuck et al., 2004). Due to variability in concentrations of progesterone it is not a valuable predictive marker of reproductive function and the development of additional hormonal measurements are necessary.

Ovarian Reserve

Identification of factors to increase reproduction has recently focused on dynamics within the ovary. The primary purposes of the ovary include the production of competent oocytes and secretion of hormones necessary for reproductive function (Knight and Glister, 2006). The concept of ovarian reserve has been established to both quantify and qualify the female gonad similar to the establishment of scrotal circumference and production of spermatozoa in bulls (Ireland et al., 2011).

The ovarian reserve has been defined as the number of morphologically healthy oocytes and follicles within the ovaries (Ireland et al., 2009). In females, the ovarian reserve is established prenatally and is inherently variable with numbers of oocytes ranging from 10,000 to 350,000 in calves (Erickson, 1966). Prior to puberty, up to 80% of oocytes are lost through atresia and due to the follicular dynamics of the estrous cycle additional oocytes are depleted during the females' reproductive lifespan (Ireland et al., 2008). As reproductive senescence occurs, the reduction in the quantity and quality of oocytes affects fertility.

Although it has been established that an increased parity results in a decreased pregnancy rate (Erickson et al., 1976; Martinez et al., 2004), the direct correlation of the ovarian reserve with fertility remains unknown. Advances in the quantification of the

ovarian reserve would demonstrate the effect of oocyte variation on altering ovarian function. This would allow for the development of diagnostic methods to predict the ovarian reserve of breeding females, assess the impact of environmental factors on the ovarian reserve, and ultimately improve methods to enhance fertility in cattle (Ireland et al., 2011).

Antral Follicle Count

The delayed advancement and implementation of the ovarian reserve as a fertility indicator has been due to the difficulty in quantification of this reserve. Prior to ultrasonography, animals were ovariectomized to establish the number of follicles. With the introduction of ultrasonography, AFC could be used as a measurement of the follicular population on the ovary and correlated to the overall ovarian reserve.

Antral follicle count is considered the maximum number of follicles greater than or equal to 3 mm in diameter per pair of ovaries and is measured during a follicular wave (Jimenez-Krassel et al., 2009). The number of follicles growing during each day of a follicular wave can vary from 200 to 400% but the maximum number of follicles measured by AFC varies 10 to 30% (Burns et al., 2005; Ireland et al., 2007). This count is a reliable phenotypic indicator of the size of the ovarian reserve with a high repeatability of .85 to .95 independent of variables including breed, age, and stage of lactation (Ireland et al., 2011). Although within animal variance is low, this measurement is more variable among animals with ranges of 8 to 56 follicles recorded per wave (Ireland et al., 2008) creating a 7-fold variation measured in cattle populations (Burns et al., 2005). This allows for cattle to be phenotyped based on AFC.

Classification of AFC occurs through synchronization of ovulation and subsequent ultrasonography as the ovary progresses though an estrous cycle (Burns et al., 2005; Ireland et al., 2008; Jimenez-Krassel et al., 2009). Follicle counts are categorized as either: low, corresponding to ≤ 15 follicles of ≥ 3 mm in diameter, or high, with ≤ 25 follicles of ≥ 3 mm in diameter per follicular wave (Burns et al., 2005). Cattle with low AFC had 80% fewer morphologically healthy follicles classified by an intact basal membrane, organized granulosal cell layers, and an intact oocyte and nucleus (Ireland et al., 2008). In addition, cattle with a low AFC had 60% smaller ovaries (height and length) as compared to cattle with a high AFC. Antral follicle count has also been positively associated with the total number of follicles in the ovaries. Cattle with ≤ 15 follicles per wave have 80 to 90% fewer total number of follicles (Ireland et al., 2008). Reductions in follicle count have an effect on ovarian function through changes in the concentrations of FSH, LH, and P4 (Ireland et al., 2011).

Follicle stimulating hormone regulates follicular development and LH is the primary gonadotropin involved in ovulation of the follicle and differentiation of the CL. Chronically increased secretions of FSH and LH are indicative of follicle and oocyte quality (Ireland et al., 2011). It is hypothesized that increased secretion of FSH and LH in females with low AFC results in the desensitization of gonadotropin receptors from their respective signaling system in granulosa and thecal cells as the number of FSH and LH receptors does not differ between animals in regard to AFC (Conti et al., 1976; Amsterdam et al., 2002). A low follicle count corresponds with a 15 to 50% greater circulating concentration of FSH when compared to a high AFC (Burns et al., 2005; Ireland et al., 2007; Mossa et al., 2010). This inverse relationship is also expressed in a 2-

fold increase in basal concentration of LH (Jimenez-Krassel et al., 2009). Based on these findings low AFC correspond to a deleterious increased secretion of the gonadotropins.

Progesterone, produced by the CL, can be a physiological marker of CL function. Progesterone is involved in fertility, uterine function, and embryo development with decreased circulating concentrations of P4 associated with increased rates of embryonic mortality (Mann and Lamming, 1999; Inskeep, 2004; Stronge et al., 2005; McNeill et al., 2006; Diskin and Morris, 2008). A low AFC corresponds with a 30 to 50% decrease in the concentration of P4 during the luteal phase. The variation in ovarian reserve, measured by AFC, may have a negative impact on differentiation and function of the CL, potentially contributing to suboptimal luteal function. Gaining a greater understanding into the mechanisms that contribute to decreased luteal function and subsequent production of P4 will allow for development of methods to improve fertility in cattle (Jimenez-Krassel et al., 2009).

Although reduced AFC are associated with decreased concentrations of P4, the size of the CL did not differ between cattle designated as low and high AFC. The size of the ovulatory follicles was also unaffected by variation in AFC and concentrations of estradiol-17β did not differ between low and high AFC cattle. The differences in circulating concentrations of P4 were not considered dependent on amount of luteal tissue, as difference in the size of the CL was not significant, but the capacity of luteal cells to produce P4 was reduced by 50% between cattle with a low compared to a high AFC. Similarly, the capacity of luteinized granulosa cells to produce P4 was decreased by 80% between the cattle with a low AFC compared to cattle with a high AFC (Jimenez-Krassel et al., 2009).

The use of AFC has provided a basis for exploration of the ovarian reserve as an indicator of fertility but a greater number of animals are necessary to statistically determine factors affecting suboptimal fertility. The increased management and expertise required determine follicle counts with serial ultrasonography is not optimal in a production setting. The positive association of concentrations of anti-Müllerian hormone (AMH) with AFC allows for further development and quantification of the ovarian reserve (Jimenez-Krassel et al., 2009).

Anti-Müllerian Hormone

Anti-Müllerian hormone was originally identified for its role in male fetal sex differentiation during embryonic development and is produced in the sertoli cells of the testicles. The sertoli cells are postulated to share a similar embryonic origin with granulosa cells of the ovary. The granulosa cells produce and contain receptors for AMH (Ireland et al., 2011). Anti-Müllerian hormone is a dimeric glycoprotein and a member of the transforming growth factor β family (La Marca and Volpe, 2006) and was first detected in the follicular fluid of mature bovine ovaries by radioimmunoassay in 1986 (Takahashi et al., 1986).

Regulation of AMH occurs differentially within the granulosa cells, follicles, and ovaries. Within the granulosa cells, AMH expression is upregulated by the factor bone morphogenic protein 6 (Elvin et al., 2000). An inverse relationship occurs between AMH FSH with FSH inhibiting the production of AMH (Rico et al., 2011). Concentrations of AMH in serum are inversely correlated with concentrations of FSH in serum of heifers (Ireland et al., 2008).

Conversely, modulation of the capacity of granulosa cells to produce AMH in response to FSH is dependent on the concentrations of FSH. Both increased and decreased concentrations of FSH produce variation in the production of AMH in granulosa cells. Increased concentrations of FSH have been correlated with decreased production of AMH, as previously stated. The decline in production of AMH can possibly be attributed to the luteinization of the granulosa cells (Ireland et al., 2008; 2009). Decreased concentrations of FSH have been correlated with an increased production of AMH concomitant with an increased production of estradiol (Burns et al., 2005; Ireland et al., 2007).

Regulation of AMH by androgens has also been assessed within granulosa cells. As granulosa cells develop, variance occurs in expression of androgen receptors (Yang and Fortune, 2006) and AMH (Monniaux et al., 2008). Increased concentrations of androgens could down regulate the production of AMH (Crisosto et al., 2009). Specifically, testosterone has advanced the growth of bovine follicles (*in vitro*; Yang and Fortune, 2006). The possible mechanism in which this occurs involves a reduction in the production of AMH in granulosa cells (Crisosto et al., 2009). Anti-Müllerian hormone inhibits the recruitment of primordial follicles and decreases the responsiveness of developing follicles to FSH (Rico et al., 2011). It has been hypothesized that androgens synthesized in the ovary produce a reduction in the production of AMH in granulosa cells during the initial stages of development causing a subsequent decrease in the threshold for follicular recruitment and allowing more follicles to enter the gonadotropin independent stage of development (Crisosto et al., 2009).

Anti-Müllerian hormone gene expression is altered in granulosa cells during maturation (Ireland et al., 2009) with expression dependant on the phase of follicular

development (Rico et al., 2011). Although changes in plasma concentrations of AMH have not been established during the estrous cycle (Rico et al., 2011), AMH is hypothesized to be involved in the regulation of early follicular growth. Secretion of AMH is increased in granulosa cells of small antral follicles (Rico et al., 2009). Production of AMH decreases during terminal follicular growth and atresia, independent of the follicular wave during the estrous cycle (Rico et al., 2011). The variation in concentrations of AMH, specifically during the emergence and regression of follicular waves requires further establishment (Rico et al., 2011).

The bovine ovary is monovulatory and has initial follicular development independent of gonadotropins. This phase of development associated with 3 to 4 mm follicles corresponds to the maximum production of AMH (Monniaux et al., 2008). As the follicle reaches this size, FSH is necessary for the continued growth and development. Between 2 and 3 surges of FSH are involved in the recruitment of follicles with the development of a dominant follicle leading to ovulation after the surge of LH (Adams et al., 2008). As follicles become dependent on gonadotropins a reduction in the production of AMH occurs (Monniaux et al., 2008).

Regulation of the follicular development is dependant of gonadotropins. Interactions of FSH and LH with AMH impact reproductive performance. Administration of GnRH to ovariectomized cows produced an increase in concentrations of gonadotropins, independent of the number of follicles present on the ovary (Mossa et al., 2010). The variation in hormone secretion relative to the follicular population is possibly mediated through a feedback mechanism independent of the pituitary with similar expression of gonadotropins occurring regardless of follicle counts (Mossa et al., 2010). One method through which antral follicles may impact gonadotropin secretion is through

the production of AMH. Anti-Müllerian hormone is produced by granulosa cells and negatively regulates the sensitivity of follicles to FSH (La Marca and Volpe, 2006). Circulating concentrations of AMH have been correlated with the number of antral follicles in cattle (Ireland et al., 2008). In cattle with a reduced number of developing follicles, increased physiological concentrations of FSH may diminish the capacity of granulosa cells to produce AMH in response to FSH and inhibit FSH action in granulosa cells. The reduced follicle count corresponds to a reduction in the ovarian reserve and a diminished capacity of granulosal cells to produce AMH in response to FSH. A decreased concentration of AMH could increase the recruitment of follicles and result in a depletion of the ovarian reserve. Therefore, the measurement of AMH to establish follicle counts and predict reproductive performance, response to estrus synchronization, and lifetime productivity needs to be further investigated.

Anti-Müllerian hormone is produced in the granulosa cells of healthy growing follicles and concentrations are positively associated with the total number of follicles and oocytes contained in the ovary (Ireland et al., 2011). Circulating concentrations of AMH are increased by 2 to 6 times for cattle with intermediate or high AFC when compared to cattle with low follicle counts. The average concentration of AMH also positively correlates ($r = 0.88$) with average peak AFC (Ireland et al., 2008). Anti-Müllerian hormone has been proven a reliable endocrine marker of the population of follicles in bovine ovaries (Ireland et al., 2011).

The major source of variability in the success of superovulation is the status of ovarian follicles at the time of initiation of treatment (Rico et al., 2009). As an indicator of fertility in the embryo transfer industry, AMH is considered the best predictive marker of ovarian response to stimulatory treatment because of the high correlation ($r = 0.79$) of

the hormone with numbers of follicles targeted for superovulatory response (Rico et al., 2009). The 3 to 7 mm follicles are superovulated by FSH and a decrease in these follicles creates a decrease in ovulatory response reducing embryo production. Identification of animals with greater numbers of follicles at this stage of development increases the probability of a successful embryo collection. Anti-Müllerian hormone measured in the plasma before superovulation was found to be indicative of the number of gonadotropin-responsive follicles available to develop to the preovulatory stage. A positive correlation between concentrations of AMH in the plasma of donor females and the average and maximal number of embryos collected has been established (Rico et al., 2009). Establishment of the use of AMH in identification of follicular populations and responsiveness to gonadotropins is of continued interest in current research, as the use of estrus synchronization and embryo transfer increases in the industry.

Abstract

Concentrations of Anti-Müllerian hormone (AMH) have been correlated to AFC, which are indicators of fertility. The effects of exogenous hormones on AMH have not been evaluated. Therefore, the objective of this experiment was to determine if concentrations of AMH at estrus differ in a synchronized compared to a natural estrous cycle. Nulliparous heifers (11 to 15 mo; n = 68) consisting of Angus (n = 19), Charolais (n = 5), Holstein (n = 34) and Jersey (n = 10) breeds were synchronized using the Select Synch + CIDR protocol (GnRH+CIDR-7 d-CIDR removal+$PGF_{2\alpha}$). Heifers were observed for expression of synchronized estrus every 6 h until 84 h after the injection of $PGF_{2\alpha}$. Ovarian structures were evaluated by transrectal ultrasonography performed on heifers detected in standing estrus or with an activated heatmount detector and secondary

signs of estrus. Blood samples were collected at estrus via venipuncture of the coccygeal vein of the tail for analysis of concentrations of AMH during the synchronized and natural estrous cycles. Visual detection of the subsequent estrus, considered natural estrus, occurred every 6 h from d 16 to 24 after synchronized estrus. The number of days between synchronized and natural estrus was 20.05 ± 1.60 (mean ± SD). Concentrations of AMH were determined using the Beckman-Coulter AMH Gen II ELISA kit. The GLM and CORR procedures of SAS were used to analyze data. Concentrations of AMH between natural and synchronized estrus were highly correlated ($r = 0.67$, $P < 0.001$). The mean concentration of AMH did not differ ($P > 0.05$) between the natural (0.0543 ± 0.0076 ng/mL) or synchronized (0.0428 ± 0.0076 ng/mL) estrous cycles. In conclusion, concentrations of AMH were similar between a natural and a synchronized estrous cycle. Concentrations of AMH in a natural and a synchronized estrous cycle were highly correlated within individual heifer.

Keywords: Anti-Müllerian hormone, bovine, estrus synchronization

Introduction

Variability of a female to produce an oocyte and successfully sustain an embryo is the primary factor reducing the efficiency of cow-calf production. Development of a prognostic method, utilizing phenotypic factors, to identify females with suboptimal fertility could advance production in the cattle industry. However, establishment of these factors has been difficult due to the complexity of interactions affecting fertility and inability to quantify the female gonad.

The ovarian dynamics of cattle are becoming increasingly relevant as indicators of fertility. The concept of the ovarian reserve has been established to both quantify and

qualify the female gonad similar to the relationship of scrotal circumference and production of spermatozoa in the bull (Ireland et al., 2011). The ovarian reserve has been defined as the number of morphologically healthy follicles contained in the ovary (Ireland et al., 2009) and is associated with fertility in cattle (Jimenez-Krassel et al., 2009). In females, the ovarian reserve is established prenatally and is inherently variable with numbers of oocytes ranging from 10,000 to 350,000 (Erickson, 1966). Prior to puberty, up to 80% of oocytes are lost through atresia and due to follicular dynamics additional oocytes are depleted as the female undergoes normal reproductive cycles (Ireland et al., 2008). Advancing the quantification of the ovarian reserve would demonstrate the effect of follicle variation on altering ovarian function allowing for diagnostic methods to be developed to predict the follicular population of breeding females, determine the impact of environmental factors on the ovarian reserve, and ultimately improve methods to enhance fertility in cattle (Ireland et al., 2011).

Difficulty in quantification has delayed the advancement and implementation of the ovarian reserve as an indicator of fertility. With the introduction of ultrasonography, it was possible to measure the follicular population on the ovary and determine AFC. Antral follicle count could be utilized as a phenotypic indicator of the size of the ovarian reserve (Ireland et al., 2008). The AFC is considered the total number of follicles greater than or equal to 3 mm in diameter per pair of ovaries (Jimenez-Krassel et al., 2009) and is positively correlated to the overall ovarian reserve. Reductions in follicle counts have an effect on ovarian function through changes in the concentrations of FSH, LH and P4 (Ireland et al., 2011) ultimately affecting pregnancy. The establishment of a correlation between AFC and pregnancy in beef heifers (Cushman et al., 2009) and dairy cows

(Mossa et al., 2010) signifies the need for additional research in reproductive performance and response to estrus synchronization (Perry, 2011).

The use of AFC has provided a basis for exploration of the ovarian reserve as an indicator of fertility but the determination of factors affecting suboptimal fertility requires a greater number of animals to detect statistically significant differences. The increased management and expertise required to determine follicle counts with serial ultrasonography is not optimal in a production setting and recent discovery of the positive association of concentrations of Anti-Müllerian hormone with AFC allows for further development and quantification of the ovarian reserve (Jimenez-Krassel et al., 2009).

Anti-Müllerian hormone (AMH) is a glycoprotein produced exclusively in the granulosa cells of developing follicles and concentrations are positively associated with the total number of follicles and oocytes contained in the ovary (Ireland et al., 2011). Anti-Müllerian hormone has recently been established as an endocrine indicator of the small antral follicles responsive to gonadotropins (Rico et al., 2009). Anti-Müllerian hormone has been identified as a factor influencing folliculogenesis by inhibiting the recruitment of primordial follicles and decreasing the efficacy of FSH in developing follicles (di Clemente et al., 1994; Durlinger et al., 2001). Although regulation of secretion of AMH has not been established, concentrations are increased in granulosa cells of small antral follicles and decrease during terminal follicular growth and atresia (Rico et al., 2011). Variations in the concentration of AMH are independent of follicular dynamics occurring in the bovine estrous cycle allowing for determination through the collection of a single blood sample and are indicative of hormone production over a continuing period (Rico et al., 2009). Changes in concentrations of AMH in plasma have not been evaluated between a natural bovine estrous cycle and an estrous cycle

synchronized using hormones. Additional research is necessary to establish the significance of gonadotropins in secretion of AMH (Rico et al., 2011) in addition to the other hormones utilized in an estrus synchronization protocol. The objective of this experiment was to determine if differences in concentrations of AMH occur in a synchronized estrous cycle compared to a natural estrous cycle. An increased understanding of AMH would allow for the utilization of this hormone to identify females with suboptimal fertility and assess the impact of nutrition, disease, and stress on reproduction (Ireland et al., 2011).

Materials and Methods

Animal care, handling, and protocols used in this study were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Animals

This experiment was conducted prior to the fall breeding season (October to December) of 2011 utilizing nulliparous beef and dairy heifers. Beef heifers (n = 24), consisting of Angus (n = 19) and Charolais (n = 5) breeds, were managed at the Mississippi Agricultural and Forestry Experiment Station's Leveck Animal Research Center in Mississippi State, MS. Beef heifers averaged 385.1 ± 24.5 (mean ± SD) with a range of 330 to 426 d of age at the initiation of estrus synchronization. Body weight was determined at the initial blood sampling and averaged 340.7 ± 30.4 with a range of 301.2 to 422.8 kg. The average weight of Angus heifers was 330.4 ± 21.0 ranging from 301.2 to 388.3 kg and the average weight of Charolais heifers was 374.5 ± 32.4 ranging from 332.9 to 442.8 kg. Reproductive tract scores (scale of 1 to 5; 1 = immature < 20 mm diameter, no tone and no palpable follicles and 5 = > 30 mm diameter, good tone, erect

and > 10 mm follicles, corpus luteum present; Anderson, 1991) were determined at the time of the initial blood sample by the veterinarian at either the beef or dairy research center. The average RTS of the beef heifers was 4.6 ± 0.5 with a range of 4 to 5.

Dairy heifers (n = 44), consisting of Holstein (n = 34) and Jersey (n = 10) breeds, were managed at the Joe Bearden Dairy Research Center in Mississippi State, MS. Dairy heifers averaged 423.9 ± 25.7 with a range of 378 to 463 d of age at the initiation of estrus synchronization. Body weight was determined at the initial blood sampling and averaged 347.2 ± 53.0 with a range of 236.8 to 447.2 kg. The average weight of Holstein heifers was 365.1 ± 38.4 ranging from 297.6 to 447.2 kg and the average weight of Jersey heifers was 266.8 ± 29.1 ranging from 236.8 to 324.8 kg. Reproductive tract scores (scale of 1 to 5; Anderson, 1991) were determined at the time of the initial blood sample and averaged 1.1 ± 0.3 (mean ± SD) with a range of 1 to 3 in dairy heifers.

Experimental Protocol

Selection of heifers for inclusion in this experiment was based on the establishment of cyclicity. Two blood samples were obtained and analyzed for concentrations of P4 prior to the initiation of the estrus synchronization protocol. Heifers with at least 1 blood sample containing a concentration of P4 ≥ 1 ng/mL were considered to be cycling (Perry et al., 1991) and were included.

Synchronization of estrus was accomplished by the Select Synch + CIDR protocol. Heifers received a controlled internal drug release (CIDR; Pfizer Animal Health, New York, NY) vaginal insert containing 1.38 g of P4 and an injection of GnRH (100 μg, i.m.; Fertagyl, Intervet Inc., Millsboro, DE) on d -9. Seven d later, on d -2, the insert was removed and heifers received an injection of $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse;

Pfizer Animal Health). Heifers were observed for expression of synchronized estrus every 6 h until 84 h after the injection of $PGF_{2\alpha}$.

Concentrations of hormones and ovarian structures were evaluated on heifers detected in standing estrus or with an activated heatmount detector (Estrotect Heat Detector, Spring Valley, WI) affixed midline to the rump between the tailhead and the tuber coxae, and secondary signs of estrus. Blood samples were collected at estrus (± 6 h) via venipuncture of the coccygeal vein of the tail and were analyzed for determination of AMH during synchronized estrus and transrectal ultrasonography (10.0 to 5.0-MHz linear-array transducer, MicroMaxx, SonoSite, Inc., Bothell, WA) was utilized to measure ovarian structures. Fourteen days after the occurrence of the synchronized estrus, additional blood samples were collected via venipuncture of the coccygeal vein of the tail and were analyzed for determination of P4 during the luteal phase of the synchronized estrous cycle.

Visual detection of the subsequent estrus, considered natural estrus, occurred every 6 h from d 16 to 24 after synchronized estrus. Blood samples were collected at estrus (± 6 h) via venipuncture of the coccygeal vein of the tail and were analyzed for determination of concentrations of AMH during natural estrus and transrectal ultrasonography (10.0 to 5.0-MHz linear-array transducer, MicroMaxx, SonoSite, Inc.) was utilized to measure ovarian structures. Fourteen days after the occurrence of the natural estrus additional blood samples were collected via venipuncture of the coccygeal vein of the tail for determination of P4 during the luteal phase of the natural estrous cycle.

Blood Collection and Hormonal Analysis

The establishment of cyclicity was determined by collecting 2 blood samples in 10 mL evacuated tubes containing the anti-coagulent K_2 EDTA (BD Worldwide, Franklin Lakes, NJ) on d -20 and -10, relative to the expression of synchronized estrous. When at least 1 blood sample contained a concentration of P4 ≥ 1 ng/mL, the heifer was considered to be cycling at the initiation of synchronization (Perry et al., 1991). Concentration of P4 during the luteal phase, considered d 14 of the estrous cycles, was established during the synchronized estrous and natural estrous cycle by the collection of 1 blood sample in a 10 mL evacuated tube containing the anti-coagulent K_2 EDTA (BD Worldwide). Blood was centrifuged at 3,000 x *g* for 20 min at 4°C and plasma was recovered and stored at −20°C until analysis. Blood plasma was analyzed for concentrations of P4 using a commercial RIA (Coat-A-Count; Siemens Medical Solutions Diagnostics, Los Angeles, CA). The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 µL. Assay tubes for the standard curve contained 0.01, 0.025, 0.05, 0.2, 0.5, 1, 2, and 4 ng/tube. Assay sensitivity was 0.1 ng/mL. The intra- and inter-assay coefficients of variance (CV) were 2.2% and 4.8%, respectively.

Concentrations of AMH were established in 1 blood sample collected in 10 mL evacuated tubes not containing coagulant (BD Worldwide) at synchronized estrus (d 0) and natural estrus (d 21). Blood was centrifuged at 3,000 x *g* for 20 min at 4°C and serum was recovered and stored at −20°C until analysis. Blood serum was analyzed for concentrations of AMH by the AMH GEN II ELISA (Beckman Coulter, Inc., Webster, TX). The sensitivity of the assay was 0.002 ng/mL. The intra- and inter-assay CV were 2.7% and 4.4%, respectively. Concentrations of AMH in bovine serum from high AFC

(.061 ± 0.05 ng/mL) cows also served as controls for 4 assays and the CV was 16.3%. Concentrations of AMH at estrus during the estrous cycles were determined and analyzed for correlation between cycles and with progesterone.

Statistical Analysis

The GLM and CORR procedures of SAS (SAS Inst. Inc., Cary, NC) were used to analyze data. Least squares means (LSMeans) were analyzed and separated when a protected F test of $P \leq 0.10$ was detected. Differences were determined to be significant when $P \leq 0.05$ value and tendencies was reported at values of $P > 0.05$ and $P \leq 0.10$. Non-significant variables were removed from models in a step-wise fashion. Results are presented as LSMeans ± standard errors of means (SEM). Beef and dairy heifers were separated for analysis of concentrations of AMH, P4 and follicle diameter. The model assessing differences in LSMean concentrations of AMH, P4 and follicle diameter included the variables of breed, RTS, age, weight, and days between the estrus cycles. The variables of age and weight were further classified. The model assessing differences in LSMean concentrations of AMH in the estrous cycles included the variables of cycle (natural or synchronized), follicle diameter, and concentration of progesterone. Correlations were also determined between AMH, P4, and follicle diameter and the aforementioned variables.

Results

The concentration of AMH of heifers in this experiment was 0.04855 ± 0.06 (mean ± SD). Beef heifers had an increased ($P < 0.05$) concentration of AMH compared to dairy heifers (LSMeans ± SEM; 0.0638 ± 0.01 and 0.0402 ± 0.01 ng/mL, respectively; Table 1). Concentrations of progesterone were similar ($P > 0.05$) between

beef and dairy heifers (5.56 ± 0.40 and 5.92 ± 0.30 ng/mL, respectively; Table 1). The follicle diameter was smaller ($P < 0.001$) in the beef (10.99 ± 0.43 mm) compared to the dairy (13.08 ± 0.32 mm) heifers (Table 1).

Concentrations of AMH in Beef Heifers

The concentrations of AMH did not differ ($P > 0.05$) between Angus and Charolais heifers (0.0703 ± 0.01 and 0.0406 ± 0.02 ng/mL, respectively; Table 2). Heifers with a RTS of 4 tended ($P = 0.06$) to have increased concentrations of AMH compared to heifers with a RTS of 5 (0.0868 ± 0.01 and 0.0462 ± 0.01 ng/mL, respectively; Table 2). The concentrations of AMH did not differ ($P > 0.05$) among the age categories of heifers, with heifers < 12, 12 to 13, and > 13 mo having similar concentrations of AMH (0.0677 ± 0.02, 0.0800 ± 0.02, and 0.0228 ± 0.03 ng/mL, respectively; Table 2). The concentrations of AMH did not differ ($P > 0.05$) among the weight categories of heifers (0.0312 ± 0.03, 0.0718 ± 0.01, and 0.0684 ± 0.02 ng/mL for < 310, 310 to 350, and > 350 kg, respectively; Table 2). Concentrations of AMH, at estrus, differed ($P < 0.05$) among the number of days between the synchronized and natural estrus (0.0421 ± 0.02, 0.0890 ± 0.01, and 0.0203 ± 0.02 ng/mL for < 19, 19 to 21, and > 21 days, respectively; Table 2).

Concentrations of AMH in Dairy Heifers

The concentrations of AMH did not differ ($P > 0.05$) between Holstein and Jersey heifers (0.0382 ± 0.01 and 0.0464 ± 0.01 ng/mL, respectively; Table 3). An increase in mean RTS tended ($P = 0.07$) to result in an increase in concentrations of AMH (0.0045 ± 0.03, 0.0372 ± 0.01, 0.0351 ± 0.01, 0.0441 ± 0.02, and 0.0758 ± 0.02 ng/mL for 1, 1.5, 2, 2.5, and 3, respectively; Table 3). In addition, a positive correlation ($r = 0.31$, $P < 0.05$;

Table 4) was established between mean RTS and mean concentration of AMH. The concentrations of AMH did not differ (P > 0.05) among the age categories of heifers at the initiation of synchronized estrus, with heifers < 13.00, 13.00 to 14.75, and > 14.75 mo having similar concentrations of AMH (0.0531 ± 0.01, 0.0356 ± 0.01, and 0.0426 ± 0.01 ng/mL, respectively; Table 3). The concentrations of AMH did not differ (P > 0.05) among the heifers in differing weight categories (0.0374 ± 0.01, 0.0412 ± 0.01, 0.0373± 0.01 ng/mL for < 300, 300 to 400, and > 400 kg, respectively; Table 3). The concentrations of AMH, at estrus, differed (P < 0.05) among number of days between the synchronized and natural estrus (0.0376 ± 0.02, 0.0342 ± 0.01, and 0.0813 ± 0.02 ng/mL, for < 18, 18 to 22, and > 22 days, respectively; Table 3).

Concentrations of P4 and Follicle Diameter in Heifers

In beef heifers, concentrations of P4 and follicle diameter did not differ ($P > 0.10$) among the variables of breed, RTS, age classification, weight classification, or days between the estrous cycles. In addition, no correlation ($P > 0.10$) between the aforementioned variables was established with either concentrations of P4 or follicle diameter in the beef heifers. Concentrations of P4 differed ($P < 0.05$) between breed of dairy heifers, with Holstein heifers having an increased concentration of progesterone compared to Jersey heifers (6.36 ± 0.42 and 4.53 ± 0.76 ng/mL, respectively). The follicle diameter of dairy heifers differed ($P < 0.05$) among weight classification (12.41 ± 0.56, 13.78 ± 0.33, 14.49 ± 0.62 mm, for < 300, 300 to 400, and > 400 kg, respectively. In addition, a correlation ($r = 0.38$, $P < 0.05$) was established between follicle diameter and weight of dairy heifers. Dairy heifers with an increased number of days between the synchronized and natural estruses also had an increase in follicle diameter ($P < 0.05$;

13.63 ± 0.66, 12.65 ± 0.28, and 14.41 ± 0.66 mm, for < 18, 18 to 22, and > 22 days, respectively).

Concentrations of AMH in Natural and Synchronized Estrous Cycles

The mean concentration of AMH did not differ (P > 0.05) between the natural (0.0543 ± 0.01 ng/mL) or synchronized (0.0428 ± 0.01 ng/mL) estrous cycles (Table 5). Concentrations of AMH between natural and synchronized estrus were positively correlated (r = 0.67, P < 0.001; Figure 1). The mean follicle diameter did not differ (P > 0.05) between the natural (11.97 ± 0.38 mm) or synchronized (12.72 ± 0.38 mm) estrous cycles (Table 5). The mean concentration of P4 did not differ (P > 0.05) between the natural (5.74 ± 0.34 ng/mL) or synchronized (5.84 ± 0.34 ng/mL) estrous cycles (Table 5). Concentrations of P4 between natural and synchronized estrus were positively correlated (r = 0.31, P < 0.05; Figure 2). No correlations (P > 0.05) existed between concentration of AMH and concentration of P4, concentration of AMH and follicle diameter, or concentration of P4 and follicle diameter in either the natural or synchronized estrus.

Changes in Concentrations of AMH in Natural and Synchronized Estrous Cycles

The concentration of AMH was determined at estrus in the natural and synchronized estrus. Between the natural and synchronized estrus 49.23% of heifers demonstrated a decrease, 24.62% demonstrated no change, and 26.15% demonstrated an increase in the concentration of AMH. A change in the concentration of AMH between the natural and synchronized estrus did not have an effect ($P > 0.05$) on the concentration of P4, with heifers that experienced a decrease in AMH and heifers that experienced an increase in AMH having similar concentrations of P4 (5.55 ± 0.38 and 6.56 ± 0.52

ng/mL, respectively). A change in the concentration of AMH between the natural and synchronized estrus did not have an effect ($P > 0.05$) on the follicle diameter with heifers that experienced a decrease in AMH and heifers that experienced an increase in AMH having similar follicle diameters (12.45 ± 0.41 and 12.56 ± 0.56 mm, respectively).

Discussion

The ovarian dynamics of cattle are becoming the focus of research to phenotypically identify females with suboptimal fertility. The ovarian reserve has been defined as the number of morphologically healthy follicles contained in the ovary (Ireland et al., 2009) and is associated with fertility in cattle (Jimenez-Krassel et al., 2009). A decrease in the ovarian reserve leads to a reduction in reproductive efficiency through pregnancy failure (Ireland et al., 2009). However, the direct correlation of the ovarian reserve with fertility remains unknown due to the complexity in measurement of these factors. The discovery of a correlation between AMH, AFC, and the ovarian reserve allows for advancement of research in bovine reproduction.

Changes in concentrations of AMH in plasma have recently been evaluated during the natural bovine estrous cycle in cows but have not been evaluated during an estrous cycle synchronized using exogenous hormones in either cows or heifers. Additional research is necessary to establish the significance of gonadotropins in secretion of AMH (Rico et al., 2011) in addition to the other hormones utilized in an estrus synchronization protocol. Therefore, the objective of this experiment was to determine if differences in concentrations of AMH occur in a synchronized estrous cycle compared to a natural estrous cycle. An increased understanding of AMH could allow for

the utilization of this hormone to identify females with suboptimal fertility and assess the impact of nutrition, disease, and stress on reproduction (Ireland et al., 2011).

Concentrations of AMH did not differ between the natural or synchronized estrus in this experiment and were highly correlated. Although concentrations of AMH have not been established after estrous synchronization, changes in AMH have been evaluated during a natural estrous cycle in cows. Concentrations of AMH were similar between estrus and the subsequent estrus in Holstein cows classified as high AFC (0.123 ± 0.01 and 0.1301 ± 0.02 ng/mL, respectively) and low AFC (0.0438 ± 0.02 and 0.0426 ± 0.01 ng/mL, respectively; Rico et al., 2011). There was no change in concentrations of AMH from 6 to 9 d proceeding ovulation in Hereford × Charolais × Angus heifers (Ireland et al., 2008). In addition, a high correlation (r^2 = 0.97) has been reported between the concentration of AMH from a single blood sample obtained regardless of the day during the estrous cycle and the overall mean of 4 to 8 blood samples from beef heifers (Ireland et al., 2011). Beef heifers in the current experiment had similar concentrations of AMH between the natural and synchronized estrous cycles. A high correlation (r^2 = 0.97) has also been reported between the concentration of AMH from a single blood sample obtained regardless of the day of the estrous cycle and the overall mean for 3 blood samples from dairy heifers (Ireland et al., 2011). Dairy heifers in the current experiment also had similar concentrations of AMH at estrus between the natural and synchronized estrous cycles.

Concentrations of AMH have been evaluated in Holstein cows during a superovulatory treatment consisting of a subcutaneous P4 implant, 32 mg FSH and 22.5 mg $PFG_{2\alpha}$ (Rico et al., 2009). Variation in concentrations of AMH among cows were decreased ranging from 0.025 to 0.228 ng/mL, compared to heifers in this experiment,

which ranged from 0.002 to 0.218 ng/mL. However, concentrations of AMH in the current experiment were decreased compared to an experiment utilizing Holstein heifers, which ranged from 0.006 to 0.433 ng/mL (Ireland et al., 2011). During the aforementioned experiment in the cows, a significant increase in concentrations of AMH occurred between the administration of the superovulatory treatment and estrus with a significant decrease in the concentrations of AMH occurring between estrus and the subsequent luteal phase, d 7. Concentrations of AMH at the administration of the superovulatory treatment were highly correlated with concentrations of AMH at estrus and during the luteal phase ($r = 0.87$ and $r = 0.93$, respectively). In addition, concentrations of AMH at administration of superovulatory treatment were highly correlated with numbers of follicles at estrus and CL at day 7 ($r = 0.83$ and $r = 0.64$, respectively; Rico et al., 2009). A change in the concentration of AMH of heifers in the current experiment did not have an effect on the follicle diameter at estrus or concentrations of P4 on day 14. In addition, no correlations between AMH and follicle diameter or AMH and concentration of P4 on day 14 were present. Differences in results could be attributed to the use of FSH to superovulate cows in the aforementioned experiment. An increased recruitment of 3 to 7 mm follicles in response to FSH could produce the increased concentrations of AMH and correlates to the number of follicles ovulated and subsequent development of the CL.

Changes in the concentrations of AMH have been reported to occur independently of follicular waves during the estrous cycle (Rico et al., 2009). The bovine ovary is monovulatory and initial follicular development occurs independent of gonadotropins. This phase of development, associated with 3 to 4 mm follicles, corresponds to the maximum production of AMH (Monniaux et al., 2008). As the follicle reaches this size,

FSH is necessary for the continued growth and development. Between 2 to 3 surges of FSH are involved in the recruitment of follicles, with the development of a dominant follicle leading to ovulation after the surge of LH (Adams et al., 2008). As follicles become dependent on gonadotropins, a reduction in AMH expression occurs (Monniaux et al., 2008). Production of AMH decreases during terminal follicular growth and atresia, independent of the follicular wave during the estrous cycle (Rico et al., 2011). In the current experiment beef heifers had an increased concentration of AMH and decreased follicle diameter compared to the dairy heifers. Variations in the concentrations of AMH, specifically during the emergence and regression of follicular waves requires further establishment (Rico et al., 2011), but this relationship may translate to differences in fertility.

After estrus, the concentration of AMH decreases, with minimal concentrations of AMH occurring between days 4 and 8 of the estrous cycle. It has been hypothesized that the decreased concentration of AMH during this period results from inhibition by FSH and is not associated with a decreased population of follicles (Rico et al., 2011). The number of FSH and LH receptors does not differ between animals in regard to AFC (Conti et al., 1976; Amsterdam et al., 2002). A low follicle count corresponds with a 15 to 50% greater circulating concentration of FSH when compared to a high AFC (Burns et al., 2005; Ireland et al., 2007; Mossa et al., 2010). Concentrations of AMH have been inversely correlated with concentrations of FSH in heifers (Ireland et al., 2008). This inverse relationship is also expressed in a 2-fold increase in basal concentration of LH (Jimenez-Krassel et al., 2009). Based on these findings, low AFC correspond to a deleterious increased secretion of gonadotropins.

Conversely, modulation of the capacity of granulosa cells to produce AMH in response to FSH is dependent on concentrations of FSH. Increased and decreased concentrations of FSH produce variation in the production of AMH by granulosa cells. Increased concentrations of FSH have been correlated with decreased production of AMH, as previously stated. The decline in production of AMH could possibly be attributed to the luteinization of the granulosa cells (Ireland et al., 2008; 2009). The effects of gonadotropins on regulation of AMH require further elucidation (Rico et al., 2011).

The variation in hormone secretion relative to the follicular population is possibly mediated through a feedback mechanism independent of the pituitary with similar expression of gonadotropins occurring regardless of follicle counts (Mossa et al., 2010). One method through which the AFC may impact gonadotropin secretion is through the production of AMH. Anti-Müllerian hormone is produced by granulosa cells and negatively regulates the sensitivity of follicles to FSH (La Marca and Volpe, 2006). Circulating concentrations of AMH have been correlated with the number of antral follicles in cattle (Ireland et al., 2008). Chronically increased concentrations of FSH in cattle with a low compared to a high number of follicles growing during a follicular wave may diminish capacity of granulosa cells in growing follicles to produce AMH in response to FSH and inhibit the action of FSH in granulosa cells. The reduced follicle count corresponds to a reduction in the ovarian reserve and a diminished capacity of granulosal cells to produce AMH in response to FSH. The possible increased follicular recruitment associated with a decreased concentration of AMH could increase the rate of depletion of the ovarian reserve. Therefore, the use of AMH to estimate follicle counts to

predict reproductive performance, response to estrus synchronization, and lifetime productivity needs to be further investigated.

Decreased concentrations of FSH have been correlated with an increased production of AMH concomitant with an increased production of estradiol (Burns et al., 2005; Ireland et al., 2007). In contrast, an inverse relationship between AMH, during the first and last follicular wave of the estrous cycle and estradiol has also been reported. Anti-Müllerian hormone has been negatively (r = -0.94) then positively (r = 0.94) correlated with concentrations of estradiol during the first and last wave of the estrous cycle. The dominant follicle during both waves was associated with an increased concentration of estradiol (Rico et al., 2011). The mean follicle diameter in this experiment did not differ between the natural or synchronized estrous cycles and no correlation between diameter and concentration of AMH was established. The variation in concentrations of AMH specifically during the emergence and regression of follicular waves requires further establishment (Rico et al., 2011).

A low AFC corresponds with a 30 to 50% decrease in the concentration of P4 during the luteal phase (Jimenez-Krassel et al., 2009). Although reduced AFC are associated with decreased concentrations of P4, the size of the CL did not differ between cattle designated as low and high AFC in the experiment. The size of the ovulatory follicles was also unaffected by variation in AFC and concentrations of estradiol did not differ between low and high AFC cattle. The differences in circulating concentrations of P4 were not considered dependent on amount of luteal tissue, as difference in the size of the CL was not significant, but the capacity of luteal cells to produce P4 was reduced by 50% between cattle with a low and high AFC (Jimenez-Krassel et al., 2009). The capacity of luteinized granulosa cells to produce P4 was decreased by 80% between the

low AFC cattle and the high AFC cattle (Jimenez-Krassel et al., 2009). Further research is necessary for the establishment of associations of AMH and P4 with fertility.

Conclusion

In conclusion, the concentrations of AMH at estrus were similar between a natural and a synchronized estrous cycle but differed between beef and dairy heifers. Concentrations of AMH at estrus in a natural and a synchronized estrous cycle were highly correlated within individual heifer and variable among heifers in this experiment. The results indicate that the use of this estrous synchronization protocol did not have an effect on the concentration of AMH. This allows for further applicability of AMH in future fertility trials in which estrus synchronization can be utilized, allowing for the further assessment of females with differing follicular populations.

Table 2.1 Anti-Müllerian hormone (AMH), progesterone (P4) and follicle diameter between beef and dairy heifers[1]

	Heifers		
Parameter	Beef	Dairy	*P*-Value
AMH, ng/mL	0.06 ± 0.01	0.04 ± 0.01	0.0355
P4, ng/mL	5.56 ± 0.40	5.92 ± 0.30	0.4646
Follicle Diameter, mm	10.99 ± 0.43	13.08 ± 0.32	0.0002

[1]Data are presented as LSMeans ± SEM

Table 2.2 Concentrations of anti-Müllerian hormone (AMH) at natural or synchronized estrus among characteristics of beef heifers[1]

	Estrus					
Parameter	Natural	*P*-Value	Synchronized	*P*-Value	Mean[2]	*P*-Value
Breed		0.5621		0.2934		0.2551
Angus	0.0830 ± 0.02		0.0576 ± 0.02		0.0703 ± 0.01	
Charolais	0.0600 ± 0.03		0.0211± 0.02		0.0406 ± 0.02	
RTS[3]		0.0915		0.3486		0.0585
4	0.1086 ± 0.02		0.0650 ± 0.02		0.0868 ± 0.01	
5	0.0545 ± 0.02		0.0378 ± 0.01		0.0462 ± 0.01	
Age, mo		0.2034		0.3258		0.1127
< 12	0.0994 ± 0.03		0.0360 ± 0.03		0.0677 ± 0.02	
12-13	0.0887 ± 0.02		0.0713 ± 0.02		0.0800 ± 0.02	
> 13	0.0245 ± 0.03		0.0211 ± 0.03		0.0228 ± 0.02	
Weight, kg		0.7895		0.3382		0.3792
< 310	0.0555 ± 0.04		0.0069 ± 0.03		0.0312 ± 0.03	
310-350	0.0792 ± 0.02		0.0644 ± 0.02		0.0718 ± 0.01	
> 350	0.0905 ± 0.03		0.0463 ± 0.03		0.0684 ± 0.02	
Days[4]		0.0833		0.2123		0.0183
< 19	0.0608 ± 0.03		0.0234 ± 0.03		0.0421[a] ± 0.02	
19-21	0.1065 ± 0.02		0.0715± 0.02		0.0890[b] ± 0.01	
> 21	0.0213 ± 0.03		0.0192 ± 0.03		0.0203[a] ± 0.02	

[1]Data are presented as LSMeans ± SEM

[2]Mean concentration of AMH between the natural and synchronized estruses

[3]RTS = reproductive tract score

[4]Days between synchronized and natural estrus

Table 2.3 Concentrations of anti-Müllerian hormone (AMH) at natural or synchronized estrus among characteristics of dairy heifers[1]

	Estrus					
Parameter	Natural	*P*-Value	Synchronized	*P*-Value	Mean[2]	*P*-Value
Breed		0.9725		0.4085		0.5525
Holstein	0.0411 ± 0.01		0.0354 ± 0.01		0.0382 ± 0.01	
Jersey	0.0418 ± 0.02		0.0510 ± 0.02		0.0464 ± 0.01	
RTS[3]		0.4490		0.2896		0.0703
1	0.0020 ± 0.03		0.0070 ± 0.03		0.0045 ± 0.02	
1.5	0.0363 ± 0.02		0.0382 ± 0.01		0.0372 ± 0.01	
2	0.0378 ± 0.01		0.0324 ± 0.01		0.0351 ± 0.01	
2.5	0.0530 ± 0.03		0.0353 ± 0.03		0.0441 ± 0.02	
3	0.0730 ± 0.02		0.0787 ± 0.02		0.0758 ± 0.01	
Age, mo		0.3794		0.6505		0.5468
< 13	0.0543 ± 0.02		0.0512 ± 0.02		0.0531 ± 0.01	
13-14.75	0.0313 ± 0.01		0.0399 ± 0.01		0.0356 ± 0.01	
> 14.75	0.0570 ± 0.02		0.0281 ± 0.02		0.0426 ± 0.01	
Weight, kg		0.6743		0.5379		0.9440
< 300	0.0284 ± 0.02		0.0464 ± 0.02		0.0374 ± 0.01	
300-400	0.0410 ± 0.01		0.0422 ± 0.01		0.0416 ± 0.01	
> 400	0.0553 ± 0.02		0.0193 ± 0.02		0.0373 ± 0.01	
Days[4]		0.0235		0.4143		0.0312
< 18	0.0194[a] ± 0.02		0.0558 ± 0.02		0.0376[a] ± 0.02	
18-22	0.0352[a] ± 0.01		0.0332 ± 0.01		0.0342[a] ± 0.01	
> 22	0.1022[b] ± 0.02		0.0604 ± 0.02		0.0813[b] ± 0.02	

[1]Data are presented as LSMeans ± SEM

[2]Mean concentration of AMH between the natural and synchronized estruses

[3]RTS = reproductive tract score

[4]Days between synchronized and natural estrus

Table 2.4 Correlation of anti-Müllerian hormone (AMH) at natural or synchronized estrus among the parameters of RTS, age, weight and days between beef and dairy heifers[1]

Parameter	AMH			
	Beef		Dairy	
	r	*P*-Value	*r*	*P*-Value
RTS[2]	-0.31	0.1535	0.31	0.0439
Age, mo	-0.23	0.2844	-0.08	0.6345
Weight, kg	0.06	0.7694	0.01	0.9610
Days[3]	-0.10	0.6589	0.20	0.2105

[1]Data are presented as LSMeans ± SEM
[2]RTS = reproductive tract score
[3]Days between synchronized and natural estrus

Table 2.5 Anti-Müllerian hormone (AMH) and progesterone (P4) and follicle diameter between natural and synchronized estrus[1]

	Estrus				
Parameter	Natural	Synchronized	*P*-Value	*r*	*P*-Value
AMH, ng/mL	0.0543 ± 0.01	0.0428 ± 0.01	0.2908	0.67	< 0.001
P4, ng/mL	5.74 ± 0.34	5.84 ± 0.34	0.8328	0.31	0.0114
Follicle Diameter, mm	11.97 ± 0.38	12.72 ± 0.38	0.1657	0.11	0.3671

[1]Data are presented as LSMeans ± SEM

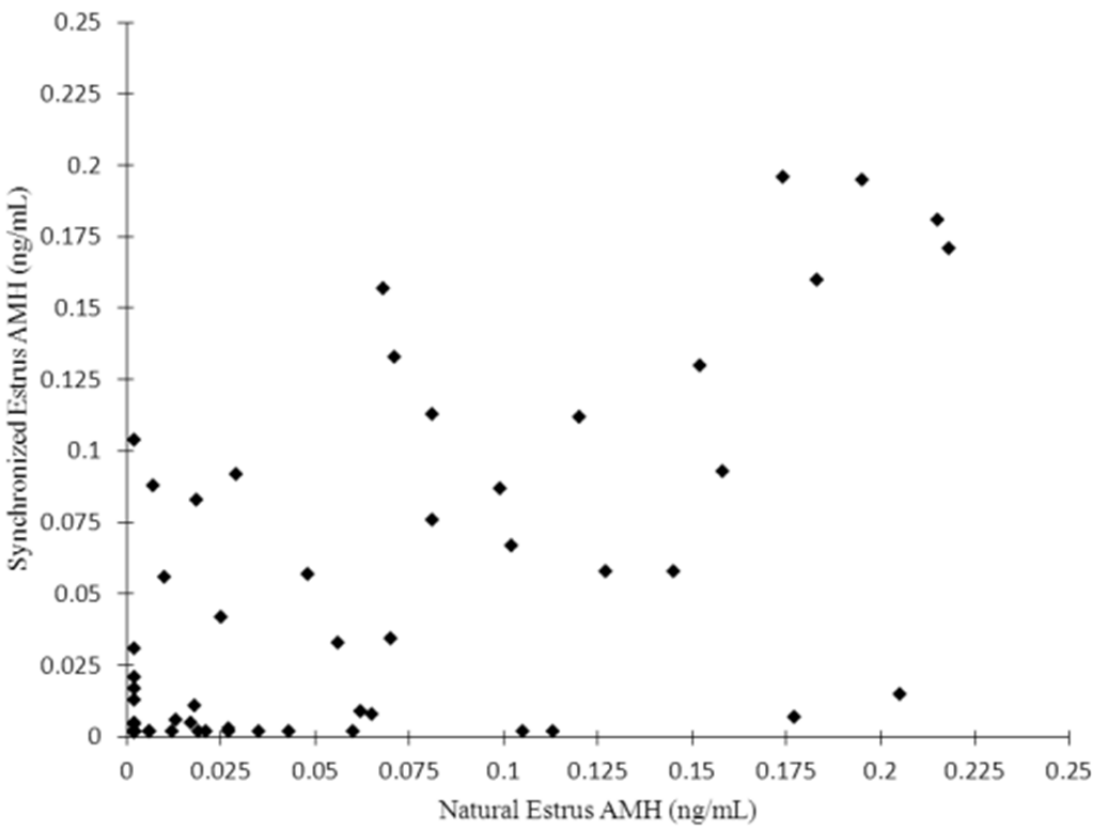

Figure 2.1 Correlation of anti-Müllerian hormone (AMH) between a natural and a synchronized estrus

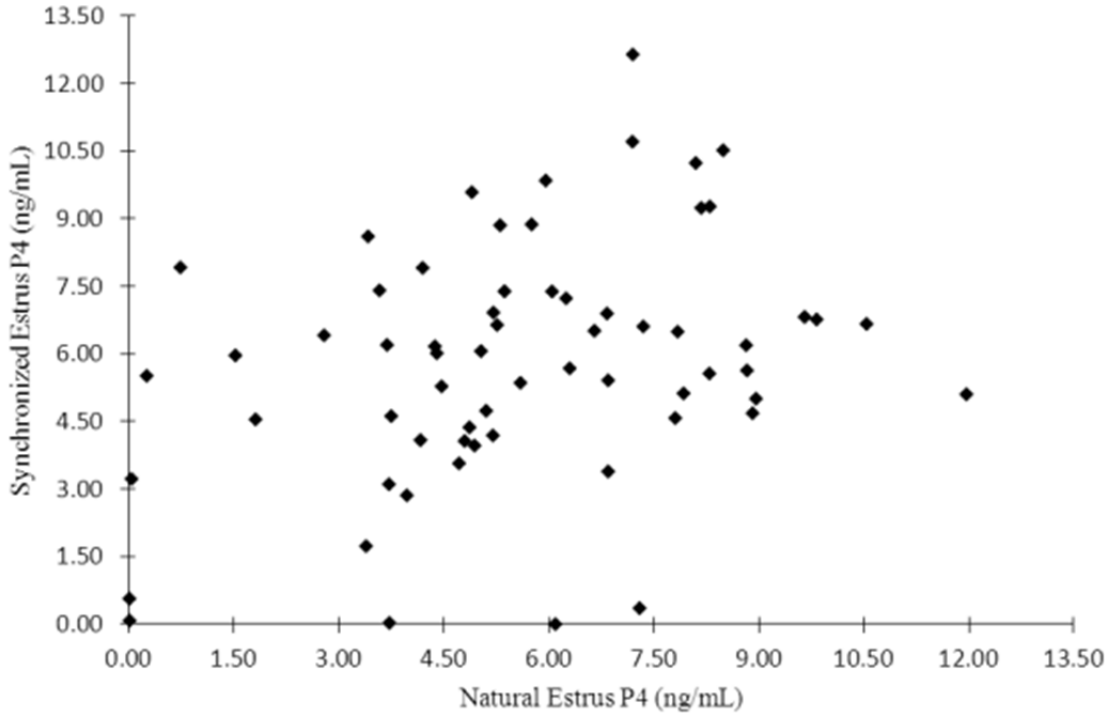

Figure 2.2 Correlation of progesterone (P4) between a natural and a synchronized estrus

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CHAPTER III

EXPOSURE OF BEEF FEMALES TO THE BIOSTIMULATORY EFFECTS OF BULLS WITH OR WITHOUT DEPOSITION OF SEMINAL PLASMA TO AI

Introduction

Reproduction is the primary factor influencing the efficiency of beef cattle production (Short et al., 1990) and reproductive failure is considered the main source of economic loss in the cattle industry (Trenkle and Willham, 1977). The failure of reproduction associated with the period of anestrus has the most significant impact on the productivity and profitability of a cow-calf operation. The interaction of many factors affect reproductive function in acyclic females but management of the duration of anestrus is the primary determinant of reproductive efficiency in the cattle industry.

In the development of heifers, age at the onset of first ovulation influences lifetime production efficiency. Heifers attaining puberty prior to the initiation of the breeding season demonstrate an increase in fertility due to the establishment of cyclicity (Patterson et al., 1992). In addition, a correlation has been established between the number of heifers that exhibit estrus early in the breeding season and the number that become pregnant (Short and Bellows, 1971). These heifers will be more likely to calve as 2-yr-olds, increasing their lifetime production as they will be more likely to rebreed as first calf heifers and continue to breed early in the breeding season producing more calves in their lifetime than latter calving contemporaries (Patterson et al., 1992).

In the management of cows, the most important factor to increase reproductive efficiency is the early resumption of cyclicity after parturition (Hornbuckle et al., 1995). Prolonged postpartum anestrus is a major cause of failure to rebreed or to conceive late in the breeding season (Short et al., 1994). Postpartum intervals ranging from 46 to 168 days cause management challenges (Dunn and Kaltenbach, 1980). Overcoming an extended postpartum interval allows for the achievement of optimum pregnancy rates through the use of estrus synchronization and incorporation of artificial insemination (AI; Larson et al., 2006).

The use of AI improves traits of major economic importance in beef cattle production by providing an economically efficient method to introduce desired genetics into a herd. Estrus synchronization provides an effective method to integrate AI into a production system (Larson et al., 2006) and is advantageous by increasing the proportion of females that become pregnant early in the breeding season. This results in a shorter calving season and the production of an older, more uniform calf crop that is heavier at weaning (Dziuk and Bellows, 1983). Management strategies, including biostimulation, that increase the success of estrus synchronization by inducing an ovulatory response in anestrous females improve fertility and enhance genetic progress.

Although estrus synchronization and AI remain the most important and widely applicable reproductive biotechnologies available for cattle (Seidel, 1995), the use of these technologies has been less employed in the beef industry in comparison to industries of other livestock species. The perception of AI not producing desired results was the primary reason producers stated for not utilizing this technology (NAHMS, 1997). While initial synchronization protocols failed to address anestrus, the acceptance of currently adapted protocols, which address anestrus, remains reduced. Increasing the

success of estrus synchronization through the use of biostimulation possibly allows for increased utilization of this technology in the cattle industry.

In heifers, the biostimulatory effect on puberty and fertility has been inconsistent (Rekwot et al., 2001) but bull exposure has stimulated an increase in the occurrence of estrus and enhanced the efficacy of timed AI in pubescent heifers (Small et al., 2000). Exposure to bull urine also increased the percentage of heifers reaching puberty by 35% (Izard and Vandenbergh, 1982). Heifers exposed to a vasectomized bull also attained puberty 3.3 months earlier than non-exposed heifers (Rekwot et al., 2001).

In cows, the presence of bulls decreased the postpartum interval to estrus and increased the number of primiparous females cycling before the beginning of the breeding season (Custer et al., 1990; Fernandez et al., 1993; Fike et al., 1996). Primiparous females exposed to bulls or bull excretory products after calving resume luteal function sooner than cows not exposed to bulls or the excretory products of bulls (Custer et al., 1990; Berardinelli and Joshi, 2005). Conception rates after a 21-day AI breeding season were greater for cows exposed to bulls than for cows not exposed (Berardinelli, 1987; Fernandez et al., 1993) and timed AI pregnancy rate and overall AI pregnancy rate of primiparous cows were improved by exposing the cows to bulls before, during, and after an estrus synchronization protocol (Berardinelli et al., 2007).

Mechanisms involved in the biostimulatory effect have not been fully elucidated. A male has been hypothesized to elicit a response in the female through genital stimulation, priming pheromones, or other undefined external cues (Chenoweth, 1983). Clitoral stimulation subsequent to AI increased conception rates in heifers (Randel et al., 1973) and cows (Short et al., 1979). In addition, the introduction of seminal plasma into the uterine lumen induces a cascade of cellular events (Robertson et al., 1996; O'leary et

al., 2004) postulated to promote conception (Robertson, 2007). The function of priming pheromones in bovine reproduction is less established in comparison to other species (Rekwot et al., 2001). Exposure of heifers to bull urine, hypothesized to contain pheromones, reduced the age at which puberty was attained (Izard and Vandenberg, 1982). To further understand these mechanisms, the objective of this experiment was to evaluate the biostimulatory effect of bull exposure, either with or without the deposition of seminal plasma, on the expression of estrus and pregnancy rate to AI in cattle.

Review of Literature

Puberty

Definition and Significance

The attainment of puberty is defined as the interval in development when the expression of behavioral estrus occurs in conjunction with ovulation (Short, 1984). Age at the onset of puberty ranges from 6 (Glencross, 1984) to 24 months (Robinson, 1977) and is influenced by a multitude of factors including weight, body size, breed and season (Moran et al., 1989). Physiological development, dependent on gains in body weight from birth to pubescence, correlates with the onset of puberty (Plasse et al., 1968; Arije and Wiltbank, 1971) with the reproductive system the final major organ system to develop in mammals (Ramaley, 1979). As heifers reach physiological maturity, a cascade of endocrine events occur leading to expression of estrus and subsequent ovulation necessary to establish cyclicity.

In the development of heifers, the age at which puberty is attained influences lifetime production efficiency. Heifers exhibiting estrus early in the breeding season will be more likely to calve as 2-yr-olds, increasing their lifetime production capability as

they will be more likely to rebreed as first calf heifers and continue to conceive early in the breeding season. Heifers exhibiting the ability to calve early in the calving season generally demonstrate an increase in lifetime calf production compared to later calving contemporaries (Lesmeister et al., 1973). Therefore, management of replacement heifers should focus on the physiological process that promotes pubescence (Patterson et al., 1992).

Endocrine Regulation of Puberty

The hypothalamic-pituitary-gonadal axis is considered the physiological regulatory mechanism of reproduction, with the hypothalamus specifically regulating the onset of puberty in heifers (Nakada et al., 2002). Heifers have a functionally mature hypothalamic-pituitary axis by 1 month of age as indicated by their ability to respond to gonadotropin releasing hormone (GnRH) with the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH; Nakada et al., 2002). Although the expression of estrus is evident in these females, the increased sensitivity of the hypothalamus to the negative feedback of low concentrations of estradiol regulates the release of GnRH and subsequent production of gonadotropins (Schillo et al., 1992).

At the onset of puberty, the sensitivity of the hypothalamus to the feedback mechanism of estradiol is reduced and a decrease in the number of unbound estradiol receptors is evident in the hypothalamus and anterior pituitary (Kinder et al., 1987). Estradiol increases the synthesis and insertion of GnRH receptors in the anterior pituitary, increasing the sensitivity to GnRH, and also stimulates a continual production of GnRH. The increased release of GnRH from the hypothalamus subsequently changes the release

pattern of LH from a decreased to an increased pulse frequency necessary for ovulation (Schillo et al., 1992).

However, ovulation is not considered synonymous with puberty (Moran et al., 1989), as the expression of behavioral estrus is necessary for reproduction to occur. Reproductive cyclicity is dependent on the interaction of endocrine events coordinating estrus with ovulation. Estradiol and progesterone are the primary hormones of significance in the synchronous development of these processes (Moran, 1989).

Augmentation of gonadotropin secretion in prepuberal and peripuberal heifers causes ovarian follicular development and increases the production of estradiol. As pubescence occurs, a change in the sensitivity of the hypothalamus to estradiol causes increased secretion of GnRH, subsequent modification of the release of LH, the occurrence of ovulation and consequent production of progesterone from the development of luteal tissue. However, this ovulation is generally not preceded by the expression of behavioral estrus.

Although the mechanism has not been elucidated, it has been postulated that progesterone sensitizes the hypothalamus to steroid hormones and is considered to be necessary for the expression of behavioral estrus in response to secretion of estradiol (Kieborz-Loos et al., 2003). Pretreatment of the hypothalamus with progesterone allows the subsequent ovulation to be preceded by expression of behavioral estrus followed by a normal luteal phase (Moran et al., 1989). This is required for the establishment of cyclicity associated with the attainment of puberty (Patterson et al., 1992).

Postpartum Anestrus

Definition and Significance

Postpartum anestrus is defined as the interval from parturition to first estrus (Short et al., 1990) during which the female is acyclic. A multitude of factors affect this interval including suckling by a calf, nutrition, season, breed, age or parity, dystocia, and presence of a bull (Short et al., 1990). Of these factors, suckling and nutrition are the most significant but interactions of factors create management challenges during the postpartum interval (Short et al., 1990). Developing an understanding of these interactions allows for the establishment of improved management practices to decrease the length of postpartum anestrus.

Success in the cow-calf industry is dependent on the females' ability for annual production of a calf. The most significant decrease in potential calf production occurs when cows fail to become pregnant during the designated breeding season (Bellows and Short, 1994). Prolonged postpartum anestrus is the primary cause of failure to rebreed or breed late in the breeding season (Short et al., 1994) and consequently reduces reproductive efficiency. The early onset of estrus after calving is an important factor to increase female productivity (Hornbuckle et al., 1995) and a reduction in the postpartum interval increases longevity in the cowherd.

Endocrine Regulation of Postpartum Anestrus

The hypothalamic-pituitary-gonadal axis is considered the physiological regulatory mechanism of reproduction and is involved in the reestablishment of cyclicity after parturition. During the postpartum interval, the hypothalamus has an increased sensitivity to the negative feedback mechanism of the decreased concentrations of

estradiol (Acosta et al., 1983) as a result of the reduction in follicular development during anestrus. In the acyclic female the infrequent release of GnRH from hypothalamic neurons subsequently decreases gonadotropin secretion. As progression occurs during the postpartum interval, a decrease in the sensitivity of the hypothalamus to secretion of steroids changes the release pattern of GnRH and subsequent change in the production of FSH and LH from the folliculotropes and luteotropes of the pituitary. The ability of the pituitary to produce gonadotropins in response to GnRH is reestablished by day 20 of the postpartum interval (Jadav et al., 2010).

Changes in concentrations of FSH begin to occur between 5 and 10 days after parturition. The increased production of FSH causes the resumption of follicular development and the establishment of a dominant follicle by day 11 postpartum (Crowe et al., 1998). The failure of the follicle to ovulate during this interval prevents the recommencement of cyclicity. Consequently, early postpartum follicular development produced by FSH is not considered the primary factor affecting the length of the postpartum interval.

Failure of ovulation is considered the primary endocrinological cause of postpartum anestrus and is characterized by the release pattern of LH during the postpartum period. Concentrations of LH in the anterior pituitary are reduced at parturition (Nett, 1987) and require 2 to 3 weeks to become reestablished (Silveria et al., 1993; Griffith and Williams, 1996). Synthesis of LH in the pituitary occurs in response to GnRH and factors affecting the pulsatility of GnRH will subsequently affect the availability of LH.

LH is involved in maturation, development, and ovulation of the follicle causing advancement from 7 to 9 mm in diameter to greater than 9 mm in diameter (Fortune et

al., 1991; Savio et al., 1993; Rhodes et al., 1995). During postpartum anestrus, the decreased frequency and increased amplitude of LH does not progress follicular development beyond 7 to 9 mm in diameter (Gong et al., 1995; 1996) and prevents the resumption of cyclicity. As development occurs after parturition, an increase in pulse frequency, amplitude and concentration of LH is required before the resumption of ovarian cycling activity (Walters et al., 1982; Humphrey et al., 1983; Peters and Lamming, 1990).

The reestablishment of the estrous cycle is dependent on follicular development involving the increased production of steroid hormones. After parturition, follicular waves begin to develop between day 5 and 11 (Crowe et al., 1998). Concentrations of estradiol remain decreased because the sizes of the follicles are reduced (Arije et al., 1974; Humphrey et al., 1983; Crowe et al., 1998). Concentrations of estradiol continue to be decreased until the development of a dominant follicle occurs in response to LH.

The continued development of the dominant follicle increases the concentrations of estradiol and when the threshold for this steroid is attained, stimulation of the preovulatory release of LH occurs causing ovulation and subsequent development of the corpus luteum (CL). A small increase in the concentration of progesterone develops 3 to 7 days preceding the initial resumption of cyclicity during the postpartum interval (Arije et al., 1974; Stevenson and Britt, 1979; Humphrey et al., 1983). After the occurrence of this increase, concentrations of progesterone return to basal amounts before the development of the first postpartum estrus. The decreased duration of the luteal phase, associated with the development of this CL and production of progesterone, occurs due to the premature release of PGF2α (Dailey et al., 1992). It has been hypothesized that the decreased concentrations of estradiol during anestrus cause retention of oxytocin

receptors and the subsequent increase in PGF2α that prematurely regresses the CL (Mann and Lamming, 2000). The production of progesterone is decreased after parturition and remains depleted throughout the postpartum anestrous period until development of the CL occurs. The increased expression of progesterone from the CL is necessary preceding ovulation to sensitize the hypothalamus to estrogen in order for the occurrence of behavioral estrus (Kieborz-Loos et al., 2003) and the development of cyclicity.

Biostimulation

Biostimulation is regarded as the stimulatory effect of males on estrus and ovulatory activity in females (Chenoweth, 1983). The use of biostimulation to advance pubescence has produced inconsistent results (Roberson et al., 1987; 1991; Small et al., 2000; Fiol et al., 2010). Conversely, a multitude of research has demonstrated the significance of bull exposure on decreasing the length of postpartum anestrus (Macmillan et al., 1979; Zalesky et al., 1984; Berardinelli et al., 1987; Custer et al., 1990; Peres-Hernandez et al., 2002; Landaeta-Hernandez et al., 2004; 2006). Although the mechanisms involved in the biostimulatory effect have not been fully elucidated, a male in the presence of a female has been hypothesized to elicit a response through genital stimulation, priming pheromones, or other undefined external cues (Chenoweth, 1983).

Genital Stimulation

The introduction of bulls has been reported to alter the associations of estrus and ovulation (Rodriguez and Rivera, 1999) by influencing the neural pathway from the female reproductive tract to the hypothalamic-pituitary axis (Randel et al., 1973). This advances the timing of the preovulatory LH surge and subsequent ovulation. The interval between expression of estrus and the maximum release of LH in cows, which copulated

with a sterile bull, was decreased compared to cows not exposed to a sterile bull (4.3 and 5.4 hours, respectively), decreasing the interval between estrus and ovulation by 3.3 hours (Randel et al., 1973). The difference in time between estrus and ovulation was also evident in heifers exposed to a vasectomized bull. Biostimulated heifers demonstrated a decreased interval compared to heifers not exposed to a bull (7.7 and 9.9 hours, respectively), decreasing the estrual period by 2.9 hours (Marion et al., 1950).

In contrast, exposure to a bull prior to copulation did not have an effect on the timing of the preovulatory LH surge in heifers (Zalesky et al., 1984) suggesting the possibility of another mechanism in which biostimulation elicits a response to alter the timing between estrus and ovulation. Clitoral stimulation produces a release of oxytocin from the posterior pituitary (Randel et al., 1975). Changes in uterine contractility and cervical dilation in response to oxytocin possibly have a stimulatory effect independent of the neural component of LH release.

Clitoral stimulation has produced an increase in conception rates of heifers (Segura and Rodriguez, 1994) and cows inseminated artificially (Short et al., 1979). Heifers and cows that received a sterile mount at estrus had an increased conception rate (60%) compared to females not receiving biostimulation (35.6%; Rodriguez and Rivera, 1999). In addition, the biostimulatory effect associated with multiple matings increased pregnancy rate (Lunstra and Laster, 1982). Although the exact mechanism involved in the biostimulatory effect has not been elucidated, these results indicate the significance of biostimulation particularly when conception rates are suboptimal.

Priming Pheromones

Pheromones are chemical substances released from urine, feces, or cutaneous glands that are sensed by the olfactory or respiratory systems causing behavioral and endocrine responses (Rekwot et al., 2001). The involvement of pheromones in bovine reproduction has not been established as completely as in other species (Roberson et al., 1991). It has been postulated that the biostimulatory effect could be mediated through pheromones specifically expressed in the excretory products of bulls.

The mechanism has been hypothesized to impact the hypothalamo-pituitary-gonadal axis (Small et al., 2000), increasing the concentration of gonadotropins and subsequent production of ovarian steroids including progesterone (Izard and Vandenbergh, 1982). Progesterone sensitizes the hypothalamus to estradiol and affects the LH profile of females in the transitory state to puberty (Gonzalez-Padilla et al., 1975). Exposure of heifers to bull urine has been reported to advance puberty with 67% of heifers exposed to urine attaining puberty compared to 32% of heifers exposed to water (Izard and Vandenbergh, 1982).

It has also been established that administration of bull excretory products during the postpartum period can decrease the interval from parturition to reestablishment of the estrous cycle. The pheromones present in bull urine have been postulated to impact neurons of the vomeronasal organ, initiating the neuroendocrine-endocrine events required for the development of cyclicity (Tauck, 2005). Specifically, the resumption of ovulatory activity is preceded by an increase in LH pulse frequency (Peters and Lamming, 1990). The change in the LH profile, similar to the expression occurring at puberty, is considered necessary for cyclicity to occur.

Oronasal administration of bull urine to dairy cows 7 days after parturition produced an increase in the concentrations of FSH and LH (Baruah and Kanchev, 1993). The 12 hour exposure of beef cows to the area in which bulls had been the previous 12 hours also reduced the postpartum anestrous interval (Berardinelli and Joshi, 2005). Contrastingly, the continuous exposure of postpartum cows to bull urine did not alter the interval to the resumption of estrus (Tauck et al., 2006). The method of exposure to this pheromone may explain some of the inconsistencies in the literature with a threshold necessary to initiate a response. Intermediate exposure to bulls (2 hours every 3 days) during the postpartum period did not produce a biostimulatory effect (Fernandez et al., 1996) and continuous exposure to bull urine (24 hours/day) also did not decrease the period of postpartum anestrus indicating the possibility of overexposure (Tauck et al., 2006). Further development of methodology could be beneficial to gain a greater understanding of the phermonal component of biostimulation in bovine reproduction.

Biostimulatory Effect on Heifers

The use of biostimulation to advance pubescence has produced inconsistent results in the literature (Roberson et al., 1987; 1991; Small et al., 2000; Fiol et al., 2010). A variation in the measured response to biostimulation can be attributed to the interaction of the biostimulatory effect with the numerous factors affecting puberty. Peripubescent heifers have responded to biostimulation with advancement in the onset of puberty, whereas prepubescent heifers have experienced a delay in puberty with the introduction of bulls. This response was further expressed enhancing or reducing the efficacy of timed AI in this experiment (Small et al., 2000).

The differing response of heifers is dependent on the pubertal status at exposure and can be detrimental or beneficial to the establishment cyclicity and subsequent pregnancy (Small et al., 2000). Exposure of heifers to bulls at 8 months of age produced a 7% reduction in the attainment of puberty by 14 months of age (Roberson et al., 1987). Contrastingly, exposure of yearling heifers increased the proportion of heifers that conceived early in the breeding season (Makarechian et al., 1985; Roberson et al., 1991). It has been hypothesized that exposure to bulls produces an initial increase in the concentration of progesterone. Progesterone has been established in the involvement of LH secretion of heifers transitioning from pre-puberty to puberty (Gonzalez-Padilla et al., 1975). Pubescent heifers have the ability to respond to the increase in concentrations of progesterone by initiating cyclicity while prepubescent heifers do not respond.

Additionally, the administration of bull urine has been postulated to augment the concentrations of gonadotropins and subsequent secretion of ovarian steroids (Izard and Vandenbergh, 1982) ultimately affecting the hypothalamo-pituitary-gonadal axis. The asynchrony of pubertal estrous cycles associated with development of this axis could be affected by biostimulation. Alteration of the ovarian response to the feedback of uterine hormones including $PGF_{2\alpha}$ may be involved in the advancement of pubescence (Small et al., 2000).

Responsiveness of prepubertal heifers to pheromones produced by bulls could be partially dependant on body weight (Izard and Vandenbergh, 1982). Body weight during the period of bull exposure has affected reproductive response, signifying the existence of a threshold necessary for reaction to biostimulation. The initial body weight of heifers can be considered the critical factor affecting reproductive response with an increased

ovulatory response of biostimulated heifers classified as a heavy body weight at 237 to 302 kg (Fiol et al., 2010).

In addition, the impact of nutrition on attainment of body weight in developing heifers interacts with biostimulation and comingled heifers reach puberty earlier than heifers not exposed to males (Rekwot et al., 2001). An increased proportion of heifers attained pubescence by 14 months of age when comingled (Tauck and Berardinelli, 2007), with a greater response occurring in heifers of increased growth rate compared to a moderate growth rate. Contrastingly, heifers fed to gain at a moderate or increased growth rate and exposed to bulls attained puberty at younger ages than heifers not exposed in either growth rate group. The magnitude of pubertal response is directly related to rate of gain with minute changes in growth rate altering stimulatory effects of bull exposure on heifers (Roberson et al., 1991).

An increase in the proportion of cyclic heifers exposed to biostimulation during a 21-day period occurred compared to heifers not biostimulated (Fiol et al., 2010). It has also been reported that bull exposure was not effective in altering the age or body weight at which heifers attained puberty (Berardinelli et al., 1987; Roberson et al., 1987). Discrepancies between results can be partially attributed to the type of exposure provided. Although the methodology of bull exposure differed between experiments, it can be hypothesized that a positive response to biostimulation is a function of the concentration of stimulus or an interaction of these factors rather than a function of the duration of bull exposure (Fernandez et al., 1996). In comparison of the aforementioned studies, the age of heifers was unknown and the percentage of females reaching puberty in the no bull treatment was 9% (1/11) compared to 0% (0/11) in the bull treatment (Berardinelli et al., 1987). The bull to heifer ratio was 1:26 and inadequate delivery of

pheromones may not provide exposure that can elicit a measureable response in females (Roberson et al., 1987). In previous research, more biostimulated heifers conceived earlier in the breeding season, advancing the calving date by 5.5 days and shortening the calving season by 2 weeks (Makarechian et al., 1985).

Biostimulatory Effect on Cows

A decreased postpartum interval in cows is necessary to maintain reproductive viability and can be affected by biostimulation. The primary challenge to efficient cattle production is the failure to rebreed within the designated breeding season due to the length of postpartum anestrus. Biostimulation has been advantageous in decreasing the interval between parturition and estrus resulting in an increased percentage of postpartum cows cycling before the onset of the breeding season (Zalesky et. al., 1984; Berardinelli et. al., 1987; Custer et. al., 1990; Stumpf et. al., 1992; Hornbuckle et. al., 1995; Fernandez et al., 1996; Fike et al., 1996; Perez-Hernandez et al., 2002).

The mechanism of the biostimulatory effect on postpartum anestrus has been hypothesized to involve the hypothalamo-pituitary-gonadal axis (Olson, 2009). Prior to the resumption of cyclicity, an increase in the pulse frequency of LH has been reported in anestrus cows (Peters and Lamming, 1990), signifying the requirement for alteration of the gonadotropin profile. The elucidation of a correlation between biostimulation and changes in concentrations of LH has been the subject of previous research but produced inconsistencies. One experiment reported no differences in mean concentration, pulse duration, pulse amplitude, or pulse frequency of LH between cows exposed or not exposed to bulls (Custer et al., 1990). Contrastingly, it has also been reported that cows exposed to bulls intermittently (2 hours every 3 days) or continuously during the

postpartum period produced increased mean concentrations and produced greater frequencies of LH pulses compared to cows that were not exposed to bulls (Fernandez et al., 1996). The differing results can be attributed to sampling interval for determination of LH. The initial experiment obtained a blood sample once weekly to establish concentrations of LH and this was likely insufficient in recognizing changes occurring in the gonadotropin profile. The subsequent experiment obtained blood samples in 10 minute intervals for a duration of 4 hours every 3 days, demonstrating a more complete profile of LH secretion. Changing the LH profile by increasing LH pulse frequency accelerates follicle maturation and ovulation subsequently reducing postpartum anestrus.

Bull exposure during the postpartum period potentially increases the sensitivity of the hypothalamus to estrogen, overriding the inhibitory effects of decreased concentrations of estrogen present after parturition (Acosta et al., 1983), producing increases in GnRH and subsequent release of LH. Biostimulation may also alter the sensitivity of ovaries to LH by increasing the number of LH receptors, resulting in an increase in progesterone corresponding to differences in the interval to estrus between cows exposed or not exposed to bulls (Custer et al., 1990). Concentrations of progesterone were also increased in cows exposed to bulls during the postpartum period (Hornbuckle et al., 1995). Postpartum cows exposed to biostimulation have expressed an increased cyclic activity (Hornbuckle et al., 1995).

The effect of biostimulation on postpartum anestrus has been directly related to the intensity of stimuli (Fike et al., 1996). Fence-line exposure of cows to bulls has produced inconsistent results dependent on duration of interaction. Dairy cows exposed to bulls, at a distance of 6 to 8 meters, 3 times per day demonstrated an equal or longer interval from parturition to resumption of estrus than cows not exposed to bulls (Shipka

and Ellis, 1998; 1999). Exposure of primiparous beef cows to direct fence-line contact of bulls for a duration of 24 hours/day produced a reduction the length of postpartum anestrus (Fike et al., 1996), signifying the requirement of a threshold of stimulation to be achieved for a response to be measured.

Another significant component affecting the response to biostimulation is the timing and duration of exposure, with cows responding differently to bulls during the postpartum interval. Cows exposed to bulls at 15, 35, and 55 days postpartum responded with decreased postpartum anestrous intervals compared to cows not exposed to bulls. However, an increased proportion of cows exposed to biostimulation at 55 days postpartum responded by initiating cyclity within 20 days of bull exposure compared to cows exposed earlier in the postpartum period, indicating a greater biostimulatory effect as the postpartum period progresses (Joshi, 2002). In addition, exposure of cows at 3 or 30 days postpartum did not produce a difference in the interval from parturition to resumption of cyclicity (Fernandez et al., 1993). A subsequent experiment also demonstrated continuous exposure to bulls beginning at 3 or 30 days after parturition resulted in a reduction in the postpartum interval to estrus by 15 days compared to cows not exposed (Fernandez et al., 1996).

Duration of bull exposure is significant in producing a biostimulatory effect. Research has been conducted on long-term or short-term exposure of females to bulls with 60 days determining the exposure term. Long-term exposure (greater than 60 days) has produced a decreased interval from parturition to the resumption of cyclicity (Berardinelli et al., 1987; Custer et al., 1990; Stumpf et al., 1992; Hornbuckle et al., 1995; Fernandez et al., 1996). After 60 days postpartum, biostimulation did not affect the percentage of cows in estrus (Burns and Spitzer, 1992) indicating this effect occurs

within this interval. Short-term exposure (less than 60 days) has also produced similar results in reducing postpartum interval to estrus (Tauck and Barardinelli, 2007).

The biostimulatory effect of bulls interacts with other factors known to influence the length of postpartum anestrus (Stumpf et al., 1992; Hornbuckle et al., 1995; Rekwot et al., 2001). The two most significant factors involved in the resumption of cyclicity include presence of a calf and nutrition. Minimal research has been conducted specifically on the biostimulatory effect and presence of a calf because most experiments stratify this effect across treatments. The interval to resumption of cyclicity was not different between cows suckled for 2 hours a day or continuously suckled and exposed or not exposued to bulls (Joshi, 2002). Delayed suckling, eight hours after milking, did not interact with bull exposure to reduce postpartum anestrus (Perez-Hernandez et al., 2002). The interaction of the biostimulatory effect with the presence of a calf has a negligible influence on postpartum anestrus.

The impact of nutrition on resumption of cyclicity in the postpartum interval has been well documented with many studies demonstrating the significance of nutrient intake on reproduction (Wiltbank, 1970; Bellows and Short, 1978; Wright et al., 1992). Cows in greater body condition have expressed a shorter postpartum interval compared to cows with less body condition (Richards et al., 1986). The interaction of the biostimulatory effect and nutrition has produced variable results based on body condition. Cows maintained on a low plane of nutrition have a reduced ovulatory response to biostimulation (Monje et al., 1982) and cows maintained on a high plane of nutrition have an increased response to biostimulation with a decreased interval to ovulation (MacMillan et al., 1979; Zalesky et al., 1984; Hornbuckle et al., 1995). This is contradictory to research that concluded cows in a lower body condition responded

greater to biostimulation with a reduced interval to estrus than cows in a greater body condition (Stumpf et al., 1992). Nutrition influences the release of LH during the postpartum interval (Randel, 1990) and the biostimulatory effect of bull exposure could interact with body condition to alter this release (Stumpf et al., 1992). In cows with a lesser amount of body condition, the inhibition of LH would prevent resumption of cyclicity and decrease the response to biostimulation. In cows with a greater amount of condition, the extent to which LH is inhibited is a less significant factor affecting cyclicity regardless of bull exposure. The contrasting results of the interaction of body condition and biostimulation on postpartum anestrus indicate that response is dependent on the ability of the cow to cycle. At a body condition where this is not occurring biostimulation does not impact interval to resumption of cyclicity but if the animal is in a body condition where this resumption is possible, cows with a greater body condition have a greater response than cows in a lesser body condition.

Seminal Plasma

The deposition of seminal plasma has been postulated to interact with the female reproductive tract through the introduction of ovulation-inducing factor (OIF; Ratto et al., 2006) and initiation of a sequence of immunological reactions involving transforming growth factor (TGF) β-1 (Tremellen et al., 1998). Ovulation-inducing factor, identified in bull seminal plasma, produced an increased induction of ovulation in llamas, an induced-ovulating species (Ratto et al., 2006). The mechanism of action of OIF has not been elucidated in cattle but may enhance the secretion of LH (Ratto et al., 2006). Seminal plasma also contains TGF- β-1, an inflammatory-inducing component produced in the seminal vesicle gland with immunosuppressive properties (Tremellen et al., 1998). It is

involved in induction of maternal immune tolerance to the conceptus and has been hypothesized to promote attachment of the conceptus to the uterine endometrium (Odhiambo et al., 2009).

Abstract

The objective of this experiment was to evaluate the biostimulatory effect of bull exposure, with or without the deposition of seminal plasma, on expression of estrus and pregnancy rate to AI in cattle. Beef heifers (n = 86) and cows (n = 193) were allocated to one of three treatments: 1) no bull exposure (**CON**; n = 95), 2) exposure to a bull with a surgically-deviated penis for 21 d prior to AI (**DB**; n = 88), or 3) exposure to a vasectomized bull for 21 d prior to AI (**VB**; n = 96). The DB treatment provided the physical presence of a bull but prevented intromission, whereas the VB treatment allowed for intromission and deposition of seminal plasma but not spermatozoa. The estrous cycles of all females were synchronized using the Select Synch+CIDR protocol (GnRH+CIDR-7 d-CIDR removal+$PGF_{2\alpha}$, detection of estrus+AI 12 h later for 84 h-clean-up TAI+GnRH). Pregnancy was detected via transrectal ultrasonography on d 35 post-AI. At the onset of the experiment, 75.7% of heifers and 86.1% of cows were cycling. The percentages of females that displayed estrus were similar ($P > 0.05$) among treatments (71.4, 76.8, and 74.4% for CON, DB, and VB, respectively). Pregnancy rates tended to be greater ($P = 0.06$) in females in the DB treatment (60.5%) compared to females in the VB treatment (42.2%), with the control group intermediate (49.5%). In conclusion, biostimulation did not affect the expression of estrus but females exposed to the DB treatment tended to have an increased pregnancy rate.

Keywords: beef cattle, biostimulation, seminal plasma

Introduction

Reproduction is the primary factor influencing efficiency of the beef cattle industry (Short et al., 1990) and is considered the most significant economic trait in cattle production (Trenkle and Willham, 1977). The failure of reproduction associated with anestrus has a significant impact on the productivity and profitability of a cow-calf operation. In the development of heifers, age at puberty influences lifetime production efficiency. In the management of cows, the most important factor to increase reproductive efficiency is the early resumption of cyclicity after calving (Hornbuckle et al., 1995). Prolonged postpartum anestrus is a major cause of failure to rebreed in a breeding season (Short et al., 1994) and overcoming an extended postpartum interval allows for the achievement of optimum pregnancy rates through the use of estrus synchronization and incorporation of AI (Larson et al., 2006).

Estrus synchronization provides a labor-efficient method to integrate AI into a production system (Larson et al., 2006), increasing the proportion of females that become pregnant early in the breeding season. This results in a shorter calving season and the production of an older, more uniform calf crop that is heavier at weaning (Dziuk and Bellows, 1983; Larson et al., 2006). Although estrus synchronization and AI remain the most important and applicable reproductive technologies available for cattle (Seidel, 1995), the use of these technologies has been less employed in the beef industry in comparison to other livestock species. The perception of AI not producing desired results was the primary reason producers stated for not utilizing this technology (NAHMS, 1997). While initial synchronization protocols failed to address anestrus, acceptance of the recently developed protocols which address anestrus remains reduced. Management strategies, including biostimulation that could improve the rate of synchronization by

inducing an ovulatory response in anestrous females will improve fertility of AI, enhancing genetic progress. Increasing the success of estrus synchronization through the use of biostimulation provides an incentive for increased utilization of this technology in the cattle industry.

Biostimulation, in the form of presence of bulls, has increased the occurrence of estrus and enhanced the efficacy of timed AI in pubescent heifers (Small et al., 2000). In cows, the presence of bulls has decreased the postpartum interval to estrus and increased the number of primiparous females cycling before the initiation of the breeding season (Custer et al., 1990; Fernandez et al., 1993; Fike et al., 1996). Conception rates after a 21 d AI breeding season were also greater for cows exposed to bulls before the breeding season than for those not exposed to bulls (Fernandez et al., 1993) and overall pregnancy rate to AI of primiparous cows were improved by exposure of bulls before, during, and after an estrus synchronization protocol (Berardinelli et al., 2007).

The mechanism of biostimulation has not been fully elucidated but the introduction of bulls has been reported to alter the associations of estrus and ovulation (Rodriguez and Rivera, 1999) by influencing the neural pathway from the female reproductive tract to the hypothalamic-pituitary axis (Randel et al., 1973). Biostimulation induces a response via genital stimulation, priming pheromones, or other undefined external cues (Chenoweth, 1983). Clitoral stimulation subsequent to AI has increased conception rates in both heifers (Randel et al., 1973) and cows (Short et al., 1979). In addition, the introduction of seminal plasma into the uterine lumen induces a cascade of cellular events (Robertson et al., 1996; O'leary et al., 2004) postulated to promote conception (Robertson et al., 2007). The function of priming pheromones in bovine reproduction is less established in comparison to other species (Rekwot et al., 2001); but

exposure of heifers to bull urine, hypothesized to contain pheromones, reduced the age at which puberty was attained (Izard and Vandenberg, 1982). In addition, the 12 hr exposure of cows to the area in which bulls had been the previous 12 hr reduced the postpartum anestrous interval (Berardinelli and Joshi, 2005). Although the mechanisms of biostimulation are not fully understood, it may be postulated that an additional improvement in reproductive response could occur in females by combining the multiple factors of bull exposure including genital stimulation, pheromones, and deposition of seminal plasma.

Therefore, the objective of this experiment was to evaluate the biostimulatory effect of bull exposure, either with or without the deposition of seminal plasma, on the expression of estrus and pregnancy rate to AI in cattle. The hypothesis was that deposition of seminal plasma along with exposure to a bull may promote an increased incidence of ovulation during the exposure period, increasing the successful response to estrus synchronization and conception to AI in those females, compared to females exposed to a bull only.

Materials and Methods

Animal care, handling, and protocols used in this study were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Animals

During the fall breeding seasons (November 15 to January 15) of 2009 and 2010, beef cattle included in this experiment were managed at the Mississippi Agricultural and Forestry Experiment Station Leveck Animal Research Center, in Mississippi State, MS. A total of 86 nulliparous and 193 multiparous, suckled females consisting of Angus (n =

171), Charolais (n = 72), Hereford (n = 36) and crossbred (n = 5) animals were utilized. Mean days postpartum at the onset of the breeding season were 93 d with a range of 54 to 139 d. Average parity of the multiparous animals was 3.48 ± 1.49 (mean ± SD) with a range of 2 to 9. Body condition scores (scale of 1 to 9; Whitman, 1975) were determined on multiparous animals in Year 2 by an experienced individual on d 0, the initiation of treatments. Mean BCS was 5.8 ± 0.5 (mean ± SD) and ranged from 5 to 7. The mean weight of heifers on d 0 in Year 1 and 2 and did not significantly differ ($P > 0.05$), averaging 375.1 ± 53.1 (mean ± SD) kg. However, the variance was significantly different ($P \leq 0.05$) with the range in weights being 250 to 497.7 kg in Year 1 and 328.2 to 436.4 kg in Year 2.

Treatments

Animals were sorted into either a primiparous or a multiparous group, stratified by breed and randomly allocated to 1 of 3 treatments: 1) no bull exposure (CON; n = 95), 2) exposure to a bull with a surgically deviated penis for 21 d prior to AI (DB; n = 88), or 3) exposure to a vasectomized bull for 21 d prior to AI (VB; n = 96). Heifers completed the experiment 4 d prior to cows. Within the treatments, heifers were managed in one group of 22 to 24 heifers per bull and cows were managed in two groups of 16 to 19 cows per bull (except the CON group had none). Bulls in the DB and VB treatments were affixed with chin-ball markers and expression of estrus was detected during the treatment period (21 d) preceding AI. Synchronization of estrus was accomplished with the Select Synch + CIDR protocol (Larson et al., 2006). Animals received a controlled internal drug release (CIDR; Pfizer Animal Health, New York, NY) vaginal insert containing 1.38 g of progesterone and an injection of GnRH (100 μg, i.m.; Cystorelin; Merial Limited,

Duluth, GA) on d 12. Seven d later, on d 19, the insert was removed and animals received an injection of $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse; Pfizer Animal Health), followed by visual detection of estrus 3 times daily with the aid of heatmount detectors (Estrotect Heat Detector, Spring Valley, WI) affixed midline to the rump of each cow between the tailhead and the tuber coxae. Animals observed in standing estrus, or with an activated detector and secondary signs of estrus, were inseminated approximately 12 h later. Animals not observed in estrus by 84 h post-$PGF_{2\alpha}$ received a second injection of GnRH and AI. Eight AI technicians and 24 sires were used in this experiment and were stratified across treatments. Pregnancy was diagnosed by an experienced technician using transrectal ultrasonography (5-MHz intrarectal transducer, Aloka 500V, Corometrics, Wallingford, CT) on d 35 post-AI to determine the presence of a viable embryo. The experimental timeline is depicted in Figure 1.

Blood Collection and RIA

Two blood samples were collected in 10 mL evacuated tubes containing the anti-coagulent K_2 EDTA (BD Worldwide, Franklin Lakes, NJ) via venipuncture of the median coccygeal vein on d -10 and 0 relative to the initiation of treatments. Blood was centrifuged at 3,000 x *g* for 20 min at 4°C and plasma was recovered and stored at −20°C until analysis. After the conclusion of the experiment, blood plasma was analyzed for concentrations of progesterone by commercial RIA (Coat-A-Count; Siemens Medical Solutions Diagnostics, Los Angeles, CA). The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 µL. Assay tubes for the standard curve contained 0.01, 0.025, 0.05, 0.2, 0.5, 1, 2, and 4 ng/tube. Assay sensitivity was 0.1 ng/mL. The intra- and inter-assay CV were 2.8% and 3.7%, respectively. When at least 1

blood sample contained a concentration of progesterone ≥ 1 ng/mL, the animal was considered to be cycling at the initiation of treatment (Perry et al., 1991).

Statistical Analysis

Procedure GLIMMIX of SAS (SAS Inst. Inc., Cary, NC) was used to analyze binomial data including cyclicity status, expression of estrus, and pregnancy rate. Least squares means were analyzed and separated when a protected F test of $P \leq 0.05$ was detected. Insignificant variables were removed from models in a step-wise fashion. The model for determination of the proportion of animals cycling at the onset of treatment included the variables of year, weight (heifers),body condition score (cows), days postpartum, breed and treatment. Expression of estrus included the variables of year, treatment, parity, breed and cyclicity in the model. The model used to analyze pregnancy rates to AI included year, cyclicity, parity, breed, technician, sire, treatment and expression of estrus.

Results

Cyclicity

There were no significant differences ($P > 0.05$) in the percentage of females cycling at the initiation of the experiment between Year 1 (80.0%) and Year 2 (86.6%), therefore data were combined. At the onset of the experiment, 75.7% of heifers had reached puberty and were cycling and 86.1% of cows had resumed cyclicity after calving.

Mean weights of heifers were similar between years ($P > 0.05$; 370.1 and 380.8 kg). Among heifers, cyclicity differed ($P < 0.05$) by body weight with animals weighing < 318.2 kg less likely to be cycling compared to animals weighing 318.2 to 408.6 kg or > 418.6 kg (Figure 2; 36.4, 80.6, and 90.0%, respectively). Body condition scores were

recorded for cows in Year 2 of the experiment and did not affect cyclicity status. Cows with BCS 5 (n = 33; 78.8%), 6 (n = 74; 89.2%) or 7 (n = 5; 100%) expressed similar cyclicity ($P > 0.05$) at the initiation of treatments. Cyclicity differed ($P < 0.05$) between cows with either a relatively shorter or longer period after calving, with those < 70 d postpartum at AI less likely to be cycling compared to cows between 70 and 100 d postpartum at AI (64.0 and 92.9%, respectively; Figure 3). A similar ($P > 0.05$) percentage of females were cycling among breed type (83.9, 83.6, 80.0% and 100% for Angus, Charolais, Hereford and crossbreds; respectively).

Cyclicity status did not differ ($P > 0.05$) among treatment groups (85.7, 87.2, and 77.8% for CON, DB, and VB, respectively; Table 2). In heifers, the percentage of females cycling was increased ($P < 0.05$) in the DB group compared to the VB treatment group (92.3 and 59.1%, respectively), with heifers in the CON group intermediate (78.1%). However, these results did not differ ($P > 0.05$) among treatment groups in cows (Table 2). Cyclicity did not have an effect ($P > 0.05$) on the subsequent expression of estrus after synchronization. Non-cycling females had a similar expression of estrus as cycling females (77.3 and 73.5%, respectively). Similarly, cyclicity on d 0 did not affect ($P > 0.05$) pregnancy rate in non-cycling or cycling (47.7 and 51.1%, respectively) females.

Expression of Synchronized Estrus

The overall percentage of females exhibiting a synchronized estrus increased ($P < 0.01$) between Year 1 and Year 2 (65.6% and 81.7, respectively). However, the percentage of heifers that displayed estrus between the injection of $PGF_{2\alpha}$ and 84 h later was increased ($P < 0.05$) in Year 1 (86.8%) compared to Year 2 (66.7%). In contrast to

the heifers, expression of estrus during the same interval in cows was decreased ($P < 0.001$) in Year 1 (59.1%) compared to Year 2 (84.2%).

Although expression of synchronized estrus did not differ ($P > 0.05$) among treatment groups (71.4, 76.8, and 74.4% for CON, DB, and VB, respectively; Table 2), an increased ($P < 0.05$) percentage of heifers in the DB group displayed estrus compared to those in the VB or CON group (Table 2; 96.3, 73.8, and 69.7%, respectively). Pregnancy rates were increased ($P < 0.05$) in females expressing estrus (56.4%) compared to females that were inseminated at 84 h (30.7%). Expression of synchronized estrus did not differ ($P > 0.05$) among nulliparous, primiparous, and multiparous females (80.0, 72.0, and 71.5%, respectively). A similar ($P > 0.05$) percentage of females among breeds were detected in estrus (70.2, 83.6, 77.1, and 40.0% for Angus, Charolais, Hereford and crossbred females, respectively) at the time of insemination.

Pregnancy Rates

There were no significant differences in pregnancy rates to AI between Year 1 and Year 2, therefore data were combined. Pregnancy rates were similar between females which were cyclic or acyclic at the initiation of the experiment (51.1% and 47.7%, respectively). Pregnancy rates did not differ ($P > 0.05$) among nulliparous, primiparous, and multiparous females (57.5, 48.0, and 47.4%, respectively) or among breeds (47.2, 58.2, 48.6, 75.0% for Angus, Charolais, Hereford and crossbred females; respectively). Technician and AI sire also did not affect ($P > 0.05$) pregnancy rates in this experiment.

The DB treatment tended ($P = 0.06$; Table 2) to increase pregnancy rate (60.5%) compared to VB (42.2%) or CON (49.5%). Heifer pregnancy rate was increased ($P < 0.05$) in the DB group compared to VB or CON groups (77.8, 46.2 and 45.5%;

respectively; Table 2). Additionally, 96.3% of heifers in the DB treatment were inseminated after being detected in estrus compared to 73.1% and 69.7% for the VB treatment and CON (Figure 4).

Discussion

Cyclicity

Attainment of puberty in heifers involves an integral cascade of events that ultimately determines the lifetime productivity of that female. Early onset of puberty is advantageous. Heifers that have established cyclicity prior to breeding have an increased probability of conceiving and calving earlier, allowing for a longer period of time to reestablish cyclicity before the subsequent breeding season (Izard and Vandenbergh, 1982). The use of biostimulation to advance pubescence has produced inconsistent results (Fiol et al., 2010; Roberson et al., 1987; Roberson et al., 1991; Small et al., 2000) and there is an incomplete understanding of the development of cyclicity in heifers exposed to bulls.

Peripubescent heifers have responded to biostimulation with advancement in the onset of puberty, whereas prepubescent heifers have experienced a delay in puberty with the introduction of bulls (Small et al., 2000). Responsiveness of prepubertal heifers to pheromonal signals from bulls may be partially dependent on body weight (Izard and Vandenbergh, 1982). Body weight during the period of bull exposure has affected reproductive response, signifying the existence of a threshold necessary for reaction to biostimulation (Fiol et al., 2010).

The threshold for attainment of cyclicity in this experiment appeared to occur at approximately 318 kg, with heifers below this weight approximately half as likely to be

cycling as heifers above this weight. While the number of animals below this threshold did not differ among treatments, fewer heifers weighing more than 318 kg were cycling in the VB treatment compared to the DB treatment. With the inclusion of British and Continental breeds, inherent growth differences likely occurred affecting attainment of puberty as larger mature body size (e.g., Charolais) tends to increase the age and weight at puberty (Martin et al., 1992). This inherent difference in growth rate and attainment of puberty among breeds could possibly explain some of the differences in the current experiment between weight and attainment of puberty.

Bull exposure has been reported to interact with growth rates of heifers to alter the age at puberty, with a greater effect occurring as growth rates increase (Roberson et al., 1991). The magnitude of pubertal response is directly related to rate of gain with minute changes in growth rate altering stimulatory effects of bull exposure on heifers (Roberson et al., 1991). Although the current experiment did not analyze cyclicity after the 21 d treatment period, exposure of heifers to bulls for an increased duration during growth could be more beneficial in triggering the reproductive performance of heifers. The 30 d exposure of beef heifers to androgenized steers did not produce an increase in the proportion of cyclic heifers that were provided a quality nutritional status during the experiment period (Fiol and Ungerfeld, 2011). This signifies the necessity for an increased duration of biostimulatory exposure in females that are not under nutritional stress during growth.

The achievement of a decreased postpartum interval in cows is necessary to maintain reproductive viability and can be affected by biostimulation. The failure to rebreed within the designated breeding season, due to postpartum anestrus, represents the primary challenge to efficient production in the cattle industry. Biostimulation has been

advantageous in decreasing the interval between parturition and estrus in postpartum cows exposed to bulls resulting in an increased percentage of primiparous females cycling before the onset of the breeding season (Custer et al., 1990; Fernandez et al., 1993; Fike et al., 1996). Exposing primiparous females to bulls has demonstrated a reduction in the postpartum interval to estrus by 15 d (Fernandez et al., 1996), which is significant when considering the 82 d breeding season needed to achieve a ≤ 12 mo calving interval. In addition, the exposure of females to bulls or the excretory products of bulls has increased the percentage of females cycling at the initiation of estrus synchronization and resulted in improved conception rates to timed AI (Berardinelli et al., 2007; Tauck and Berardinelli, 2007).

At the onset of this experiment, only 13.9% of cows were anestrous. The proportion of anestrous cows at the beginning of the breeding season was decreased in the current study compared to previously reported results, which ranged from 54.4% (Larson et al., 2009) to 64% (Stevenson et al., 2003). Cyclicity was dependent on d postpartum with females < 70 d postpartum at the time of AI less likely to be cycling when compared to animals between 70 and 100 d postpartum at AI. While exposure to bulls later (> 30 d) in the postpartum interval has increased the proportion of cows responding to biostimulation, as indicated by an earlier resumption of cycling activity (Berardinelli and Joshi, 2005), it has also been demonstrated that biostimulation had no effect on the percentage of cows in estrus after 60 d postpartum (Burns and Spitzer, 1992). Applying these concepts to the current study, biostimulation may have a decreased response than in other experiments because the majority of these cows were beyond 60 d postpartum. In addition, exposure of cows to biostimulation for 60 d before the initiation of estrus synchronization resulted in an increased proportion of cyclic cows (Berardinelli

et al., 2007) suggesting that the duration of exposure during the postpartum period is also significant in the production of a reproductive response.

Expression of Synchronized Estrus

The mechanism of biostimulation's effect on reproduction has been hypothesized to increase the sensitivity of the LH release center to estrogen, overriding inhibitory effects of decreased concentrations of estrogen on the hypothalamus (Acosta et al., 1983). Biostimulation may also alter the sensitivity of ovaries to LH by increasing the number of LH receptors resulting in an increase in progesterone corresponding to differences in the interval to estrus between cows exposed or not exposed to bulls (Custer et al., 1990). This could partially explain the lack of differences among treatments in this experiment, specifically in expression of estrus, because subsequent estrous cycles would be necessary to realize the positive effect of biostimulation on fertility.

Although 75.7% of all heifers were cycling at initiation of the experiment (significantly fewer in the VB treatment compared to DB and CON groups), 79.1% of heifers were detected in estrus after synchronization. Interestingly, 84.9% of heifers exposed to bulls displayed synchronized estrus, whereas only 69.5% of heifers that were not exposed displayed synchronized estrus. These results are in agreement with previous literature that reported an increase in the proportion of cyclic heifers which were exposed to biostimulation during a 21 d period compared to heifers not biostimulated (Fiol et al., 2010). Contrary to previous results, it has also been reported that bull exposure was not effective in altering the age or body weight at which heifers attain puberty (Berardinelli et al., 1987; Roberson et al., 1987). Discrepancies between experimental results can be partially attributed to the type of exposure provided. Although the methodology of bull

exposure differed between experiments, it can be hypothesized that a positive response to biostimulation is a function of the concentration of stimulus or an interaction of these factors rather than a function of the duration of bull exposure (Fernandez et al., 1996) in heifers. In comparison of the aforementioned studies, the age of heifers was unknown and the percentage of females reaching puberty in the no bull treatment was 9% (1/11) compared to 0% (0/11) in the bull treatment (Berardinelli et al., 1987). The bull to heifer ratio was also reduced in the current experiment (Year 1, 1:15.5 and Year 2, 1:11) compared to 1:26 (Roberson et al., 1987) and inadequate delivery of pheromones may not provide exposure that can elicit a measureable response in females. The exposure of females to androgenized steers also did not improve reproductive performance in a progesterone-based fixed-timed AI program (Ungerfeld, 2010). This could possibly be attributed to the reduced female to male density compared to previous experiments.

With the majority of cows (95.6%) greater than 60 d postpartum in this experiment, it is probable that this stimulus was inadequate in the timing and duration applied. Effect of biostimulation on postpartum anestrus has been directly related to the intensity of stimuli (Fike et al., 1996). Bulls used in the current experiment were exposed to heifers for 21 d prior to the 21 d exposure period to the cows. This could have possibly decreased the intensity of stimulation provided with bulls experiencing altered libido. The cows had similar expressions of estrus leading to similar pregnancy rates among treatments, concurring with results indicating biostimulatory effects did not alter the percentage of cows exhibiting estrus before TAI (Makarechian et al., 1985). In addition, the use of a progesterone-based synchronization protocol could have produced the maximum reproductive response of these females possibly negating the effects of biostimulation. No differences in fertility of females exposed to androgenized steers

before the initiation of a progesterone-based fixed timed AI were observed in comparison to females isolated from males (Ungerfeld, 2010).

Pregnancy Rate

The similar pregnancy rates between acyclic and cycling females in this experiment were consistent with other research where the estrous cycles of females were synchronized and AI was utilized (Lamb et al., 2001; Stevenson et al., 2000). Inclusion of progesterone in the synchronization protocol has been demonstrated to improve pregnancy rates in noncycling cows (Lamb et al., 2001), altering the expression of estrus in this subset of females. This resulted in an increased pregnancy rate among animals in estrus at the time of insemination.

In this experiment, the DB treatment tended to produce an increased pregnancy rate. The percentage of heifers inseminated to TAI decreased in the DB treatment because an increased percentage of females exhibited estrus after synchronization. Heifers receiving TAI (n = 18) had a pregnancy rate of 11.1% compared to 67.7% for heifers inseminated after expression of estrus (n = 68). Bull exposure has stimulated an increase in the occurrence of pubescent estrus and enhanced efficiency of AI in previous experiments (Small et al., 2000). The pregnancy rate of heifers within the DB treatment was increased compared to the VB or CON treatments. Although heifers in the current experiment responded to biostimulation, this contrasts several experiments reporting no difference in pregnancy rates between heifers exposed or not exposed to biostimulation (Izard and Vangenbergh, 1982; Makarechian et al., 1985). In previous research, more biostimulated heifers conceived earlier in the breeding season, advancing the calving date by 5.5 d and shortening the calving season by 2 wk (Makarechian et al., 1985). These

heifers were exposed to a longer breeding season than the heifers in the current experiment and were inseminated by bulls rather than AI.

Pregnancy rates of cows did not differ between biostimulated females and the control animals. This is in contrast to results demonstrating an increase in proportions of biostimulated cows that conceived to AI (Tauck and Berardinelli, 2007). In the aforementioned experiment, increased pregnancies could be attributed to an increase in the pregnancy rate of cows inseminated in estrus. In the current experiment, both the number of cows inseminated after expression of estrus and the percentage of females pregnant after insemination did not differ between treatments. Nutrition, recorded as BCS, is another factor possibly decreasing the efficacy of biostimulation in the current experiment. Cows in this experiment maintained adequate to high BCS (range of 5 to 7). Research has indicated that cows in moderate condition are more responsive to biostimulation than cows in a high body condition, as experienced in this experiment (Stumpf et al., 1992). The interaction of BCS with biostimulation represents an unknown variable and could have affected the response of cows in the current experiment. Overall, biostimulation of cows did not enhance expression of estrus or subsequent pregnancy rate to AI.

Biostimulation

The treatments in this experiment incorporated 2 different types of biostimulation. The DB treatment provided the physical presence of a bull but prevented intromission. The VB treatment provided the physical presence of a bull but did allow intromission and deposition of seminal plasma but not spermatozoa. The deposition of seminal plasma has been postulated to interact with the female reproductive tract through the introduction of

ovulation-inducing factor (OIF; Ratto et al., 2006) and initiation of a sequence of immunological reactions involving transforming growth factor (TGF) β-1 (Tremellen et al., 1998). Ovulation-inducing factor, identified in bull seminal plasma, produced an increased induction of ovulation in llamas, an induced-ovulating species (Ratto et al., 2006). The mechanism of action of OIF has not been elucidated in cattle but may enhance the secretion of LH (Ratto et al., 2006).

Seminal plasma also contains TGF- β-1, an inflammatory-inducing component produced in the seminal vesicle gland with immunosuppressive properties (Tremellen et al., 1998). It is involved in induction of maternal immune tolerance to the conceptus and has been hypothesized to promote attachment of the conceptus to the uterine endometrium (Odhiambo et al., 2009). However, seminal plasma deposited at the time of insemination produced a decreased pregnancy rate in heifers, whereas no difference was observed in pregnancy rates between treatments in cows. A possible explanation for differences between experiments involves the methodology. When seminal plasma was deposited at the time of AI there was no genital stimulation (Odhiambo et al., 2009) whereas in the current experiment, seminal plasma was deposited by a vasectomized bull, providing stimulation of the genitalia.

The introduction of bulls has been reported to alter estrus and ovulation associations (Rodriguez and Rivera, 1999) by influencing the neural pathway from the female reproductive tract to the hypothalamic-pituitary axis (Randel et al., 1973). This advances the timing of the preovulatory LH surge and subsequent ovulation. The interval between estrus and peak release of LH in cows which copulated with a sterile bull was decreased compared to cows not exposed to a sterile bull (4.3 and 5.4 h, respectively), decreasing the interval between estrus and ovulation by 3.3 h (Randel et al., 1973). The

difference in time between estrus and ovulation was also evident in heifers exposed to a vasectomized bull having a decreased interval compared to heifers not exposed to a bull (7.7 and 9.9 h, respectively) and decreasing the estrual period by 2.9 h (Marion et al., 1950). In the current experiment, intromission and deposition of seminal plasma provided by the VB treatment may have created an asynchrony between ovulation and insemination, leading to the decreased pregnancy rates achieved by this treatment.

Conclusion

The failure of reproduction associated with anestrus negatively impacts productivity of the beef industry. The potential for improvements in reproductive parameters demonstrated by biostimulatory effects on females has supported the need for a greater understanding of the mechanisms involved. Response to biostimulation in this experiment was more evident in heifers compared to cows, as heifers in the DB group had increased rates of expression of estrus and pregnancy compared to other groups. Further studies in heifers may produce results supporting the use of biostimulation as a management practice and elucidate the most efficient method for incorporating bull exposure into a breeding system.

Table 3.1 Summary of biostimulatory effects on reproductive parameters

Experiment	Parity[1]	Method of biostimulation	Duration of biostimulation	Reproductive Parameter	
				Control	Biostimulated
				Puberty, %	
Izard and Vandenbergh, 1982	N	Bull Urine	49 d	67.0[e]	32.0[f]
Roberson et al., 1987	N	Mature Bull	152 d	83.3	88.0
Roberson et al., 1991	N	Epididectomized bull	76 d	29.8[e]	60.3[f]
				Cycling, %	
Hornbuckle et al., 1995	M	Epididectomized bull	76 d	83.0	91.0
Landaeta-Hernandez et al., 2003	M	Epididectomized bull	30 d	26.6[c]	53.0[d]
Berardinelli et al., 2007	P	Epididectomized bull	> 60 d	31.3[a]	85.1[b]
				Expressing Estrus, %	
Fernandez et al., 1996	P	Epididectomized bull	71 d	58.0[e]	80.0[f]
Berardinelli and Joshi, 2005	P	Epididectomized bull	70 d	50.0[e]	86.7[f]
				Postpartum Interval to Estrus, d	
Custer et al., 1990	P	Penile-blocked bull	108 d	73.3[e]	56.6[f]
	P	Epididectomized bull	60 d	64.7[e]	46.5[f]

Table 3.1 (continued)

Fernandez et al., 1993					
Fernandez et al., 1996	P	Epididectomized bull	71 d	78.5[e]	62.6[f]
Fike et al., 1996	P	Fenceline contact	78 d	92.0[e]	78.0[f]
Fike et al., 1996	M	Fenceline contact	78 d	72.0	64.0
Shipka and Ellis, 1998	M	Fenceline contact	120 d	57.8	61.9
Tauck et al., 2006	P	Bull Urine	~ 64 d	55.8	62.5
				Conception Rate, %	
Shipka and Ellis, 1999	M	Fenceline contact	120 d	46.0	40.0
Berardinelli et al., 2007	P	Epididectomized bull	> 60 d	51.5[e]	66.3[f]
Fiol and Ungerfeld, 2011	N	Androgenized steer	30 d	83.0	92.0
				Pregnancy Rate, %	
Makarechian et al., 1985	N	Epididectomized bull	42 d	75.0	79.0
Fernandez et al., 1993	P	Epididectomized bull	81 d	83.0	94.0
Fike et al., 1996	P	Fenceline contact	109 d	38.0	53.0
Fike et al., 1996	M	Fenceline contact	109 d	53.0	62.0
	N, P, M	Vasectomized bull	At estrus	35.6[c]	60.0[d]

Table 3.1 (continued)

Rodriguez and Rivera, 1999					
Small et al., 2000	N	Epididectomized bull	~ 450 d	50.0	53.8
Tauck and Berardinelli, 2007	P	Epididectomized bull	35 d	60.0[e]	84.6[f]
Tauck and Berardinelli, 2007	P	Fenceline contact	42 d	77.0	57.7
Tauck and Berardinelli, 2007	P	Bull urine	64 d	55.0[e]	89.5[f]
Ungerfeld, 2010	N	Androgenized steer	20 d	54.3	54.8
Fiol and Ungerfeld, 2011	N	Androgenized steer	30 d	86.5	83.0

[1]Parity classification: Nulliparous (N), Primiparous (P), Multiparous (M)
[a,b] Within a row, means without a common superscript differ ($P < 0.001$)
[c,d] Within a row, means without a common superscript differ ($P < 0.01$)
[e,f] Within a row, means without a common superscript differ ($P < 0.05$)

Table 3.2 Rates of cyclicity, expression of estrus, and pregnancy in heifers and cows among treatments

Item	Treatment		
	CON[a]	SB[b]	VB[c]
Cyclicity, %	85.7	87.2	77.8
Heifers	78.1[de]	92.3[d]	59.1[e]
Cows	89.8	85.0	83.8
Estrus Expression, %	71.4	76.8	74.4
Heifers	69.7[e]	96.3[d]	73.8[e]
Cows	71.0	68.9	72.9
Pregnancy rates, %	49.5[g]	60.5[f]	42.2[g]
Heifers	45.5[e]	77.8[d]	46.2[e]
Cows	51.6	50.8	36.8

[a] CON = females in the control group not exposed to a bull.
[b] SB = females exposed to a bull with a deviated penis.
[c] VB = females exposed to a bull which was vasectomized.
[d,e] Within a row, means without a common superscript differ ($P < 0.05$).
[f,g] Within a row, means without a common superscript differ ($P = 0.06$).

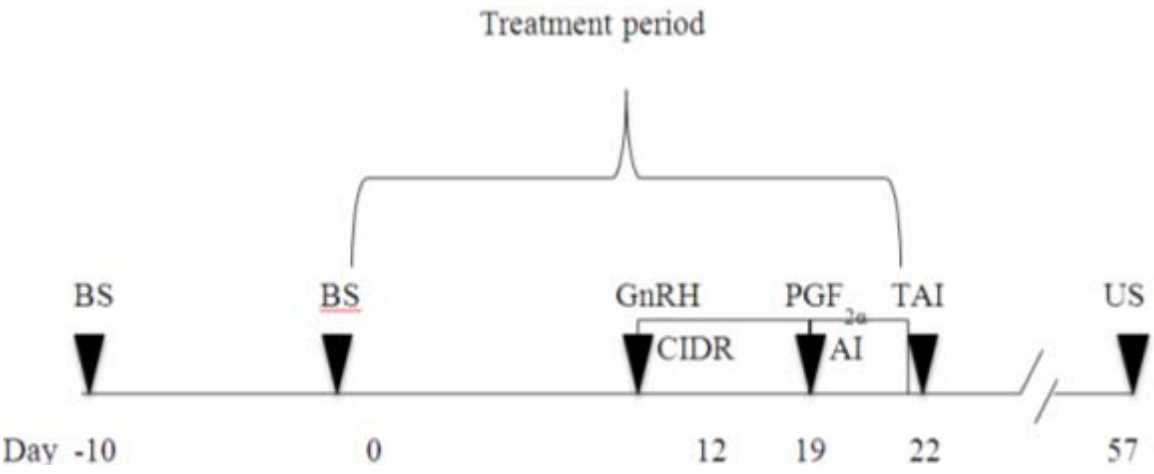

Figure 3.1 Experimental protocol including sampling and synchronization schedule

BS = blood sample; GnRH = Gonadotropin releasing hormone; CIDR = Controlled internal drug release device; $PGF_{2\alpha}$ = Prostaglandin $F_{2\alpha}$; TAI = timed artificial insemination; US = ultrasonography for pregnancy diagnosis

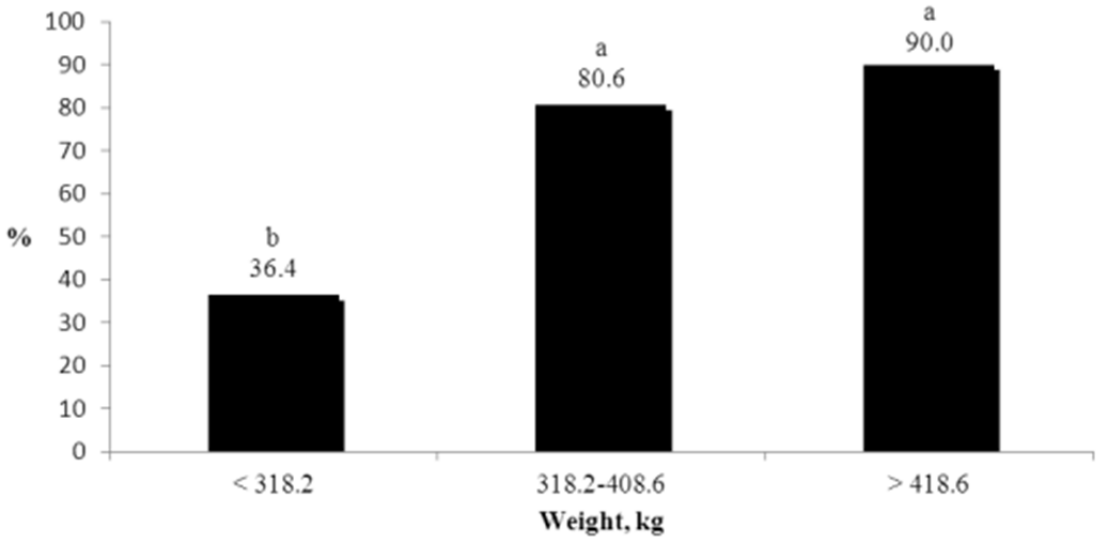

Figure 3.2 Percentage of heifers cycling, classified by weight. [a,b] Means without a common superscript differ ($P < 0.05$)

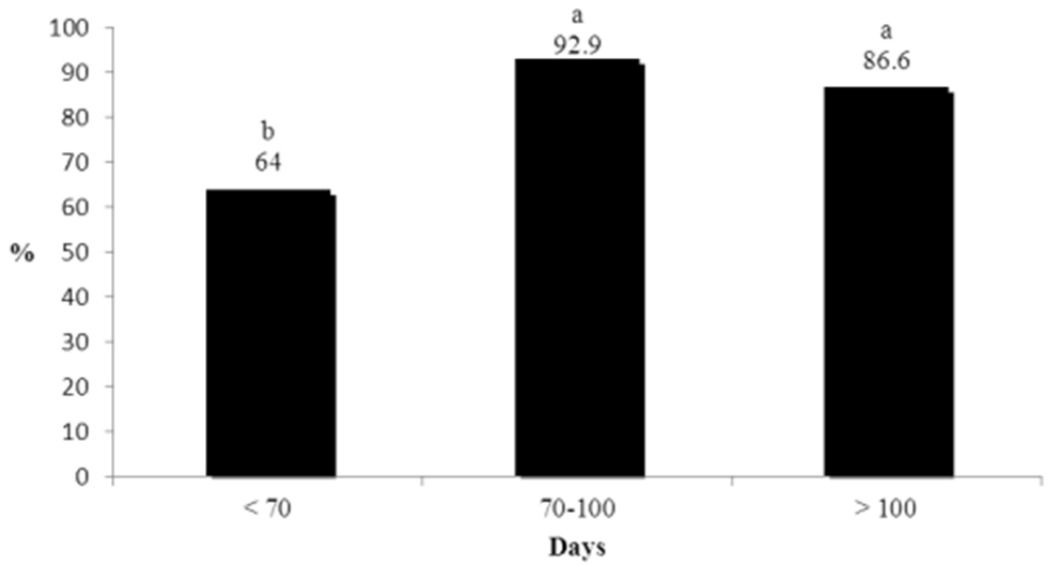

Figure 3.3 Percentage of cows cycling, classified by days postpartum. [a,b] Means without a common superscript differ ($P < 0.05$)

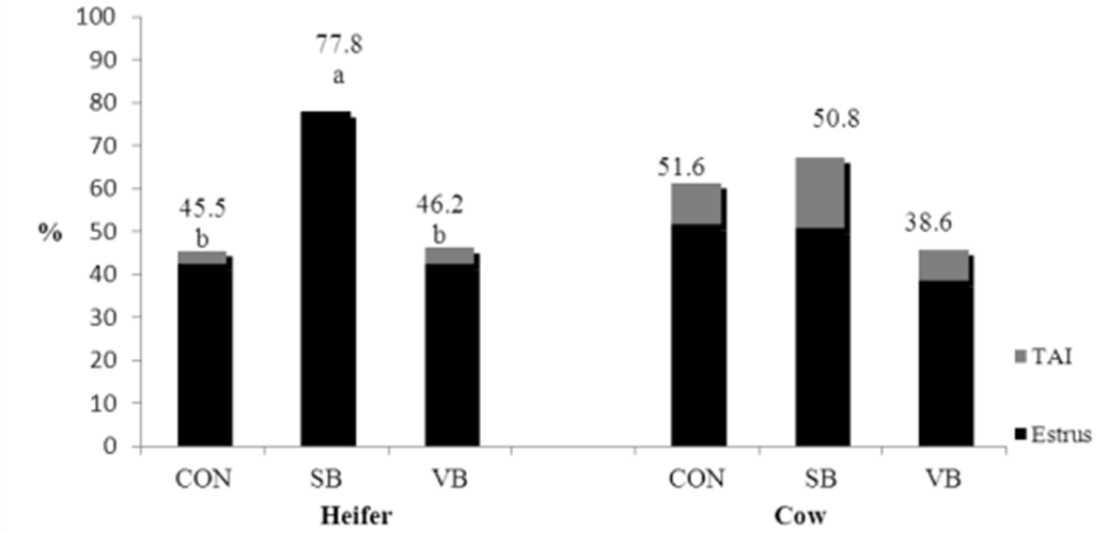

Figure 3.4 Percentage of heifers and cows inseminated at estrus (black bars) or at TAI (gray bars) among treatments. [a,b] Means without a common superscript differ ($P < 0.05$)

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CHAPTER IV

CONCLUSION

The interactions of many factors affect reproductive function. Advancing the understanding of these factors allows for increased management of female infertility. Selection of females based on classification of the ovarian reserve allows for the use of females with increased fertility in cow-calf production. Establishing the use of AMH to assess this reserve requires an understanding of the mechanisms involved in the regulation of this hormone. The concentrations of AMH were similar between a natural and a synchronized estrous cycle indicating the use of estrus synchronization does not have an effect on the concentration of AMH. This allows for further applicability of AMH in future fertility trials in which estrus synchronization can be utilized allowing for the assessment of females.

In addition, the failure of reproduction associated with anestrus negatively impacts productivity of the beef industry. The potential for improvements in reproductive parameters demonstrated by biostimulatory effects on females has supported the need for a greater understanding of the mechanisms involved. Response to biostimulation in this experiment was more evident in heifers compared to cows, as heifers in the DB group had increased rates of expression of estrus and pregnancy compared to other groups. Further studies in heifers may produce results supporting the use of biostimulation as a management practice and elucidate the most efficient method for incorporating bull

exposure into a breeding system. The use of these management factors can increase the efficiency of cattle production increasing profitability within the cattle industry