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Influence of Steroids and Gonadotropins on Reproduction in Beef Cattle

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ABSTRACT

The objective of this thesis was to evaluate the influence of steroids and gonadotropins on reproduction in beef cattle. In experiment 1, beef heifers were used to determine the influence of growth-promoting implants on growth, reproductive development, estrous behavior, and pregnancy rate. Heifers were assigned to 1 of 4 implant treatment groups: control (CON); trenbolone acetate (TBA); trenbolone acetate plus estradiol (TBA+E2) or zeranol (ZER). Heifers were implanted once, A.I. and exposed to bull during this experiment. Body weight, BCS, HH, RTS, estrous behavior and pregnancy data were collected throughout this experiment. Average daily gain of heifers was greater for TBA+E2 heifers. Fewer heifers treated with ZER were classified with a cyclic RTS on d 106 than CON and TBA treated heifers while heifers treated with TBA+E2 were similar to all treatments. Heifers treated with TBA had increased mounts during estrus compared with all other treatments. Overall and A.I pregnancy rates did not differ among treatments.

In experiment 2, superovulated beef donors were utilized to determine the feasibility of performing a cow-side LH assay (Predi′Bov®) on superovulated donors, with emphasis on determining how to use the results in a commercial program. Donors were subjected to superstimulation; blood samples were collected starting at CIDR removal, continuing every 6 h until a positive test was acquired or 36 h after CIDR removal. Whole blood (0.5 mL) was submitted to the assay and donors were inseminated approximately 12 and 24 h after a positive test or onset of estrus. The majority of positive LH tests occurred within 12 to 24 h after CIDR removal. Forty-four percent of the positive tests occurred 0 to 6 h after the onset of estrus. Donors that were inseminated 6 to 10 h after a positive LH test produced more viable and grade

1 embryos than donors inseminated either < 6 or 10 to 14 h after a positive test. There were no differences in embryo production between insemination times from the onset of estrus or between donors inseminated approximately 12 and 24 h after a positive test or the onset of estrus.

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TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The United States cattle inventory as of January 1, 2014 (87.7 million head) was the smallest since 1951 (USDA, NASS, 2014). There were 38.3 million cows and heifers that had calved in the U.S. as of January 1, 2014, and is the lowest January 1 inventory of all cows and heifers calved since 1941 (USDA, NASS, 2014). Of the 38.3 million cows and heifers that have calved, beef cows contributed 29.0 million head to the total; this number is down nearly 12% since 2000 alone. The 2013 calf crop was estimated at 33.9 million head which is the smallest calf crop since 1949 (USDA, NASS, 2014). There were 5.5 million heifers produced for beef cow replacements in the U.S. as of January 1, with 3.3 million expected to calve within the year (USDA, NASS, 2014). The global human population is expected to reach over 9 billion by 2050 and current animal protein production will need to be doubled at that time to meet the increased demand (Neumeier and Mitloehner, 2013).

Beef cows harvested in 2013 totaled 3.1 million; this is the first time since 2007 that the number of replacement beef heifers expected to calve within the year has exceeded the number of harvested beef cows (USDA, NASS). However, since 2001, the number of replacement beef heifers expected to calve (data collection began in 2001) has only surpassed the number of beef cow deaths and harvest twice (USDA, NASS). The number of cattle that exited the feedlot in 2013 was slightly lower than the previous year but down 13% from 2000 and total kg of beef produced has decreased for three years (USDA, NASS). Consequently retail beef prices are at record highs.

In the U.S., 83% of all replacement heifers are raised at the operation where they were born (USDA, APHIS, 2008). In 2010 that cost to take a beef heifer from weaning to pregnancy was approximately $1040 (Hersom et al., 2010). The most influential factor to the cost of raising replacement beef heifers is the opportunity cost of not selling the heifer at weaning and this cost is determined by current market price. Record cattle prices have drastically increased the cost of raising replacement beef heifers and ranged from $1450 (Hughes, 2013) to $1600 (Huedepohl, 2014) in 2013. Replacement heifer cost is the second largest expenditure of a cow calf operation, second only to annual feed cost. Replacement heifer cost represents 30% of the total expenses for a cow calf operation (Hughes, 2013).

Management strategies that increase that increase production efficiency and reproductive performance of cattle will be requirement to meet increasing demands of the U.S. cow herd and the animal protein needs of a growing global human population.

CHAPTER II

REVIEW OF LITERATURE

Introduction

In vertebrates, reproduction is primarily controlled by the hypothalamus-pituitary-gonad axis. The gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus are key regulators of the hypothalamic neuroendocrine system mainly though the synthesis of GnRH (Moenter et al., 2003). The decapeptide GnRH is released from the hypothalamus in a pulsatile manner, and the amplitude and frequency of these pulses change throughout the estrous cycle (McCartney et al., 2002; Moenter et al., 2003). High amplitude and low frequency pulses of GnRH secretion occur during the luteal phase of the estrous cycle with pulse frequency increasing and amplitude decreasing during the follicular phase (Savio et al., 1988; Clarke and Pompolo, 2005). The binding of GnRH to gonadotrope cells in the anterior pituitary stimulates the synthesis and release of the glycoprotein gonadotropins, follicle-stimulating-hormone (FSH) and luteinizing hormone (LH; Schally et al., 1971). The gonadotropins LH and FSH act on the gonads to promote gametogenesis and steroidogenesis and in turn, stimulate gonadal development and function. Generally, gametogenesis is regulated by FSH and steroidogenesis is stimulated by LH. Steroidogenesis in the ovary involves the binding of LH to theca cells which stimulates the synthesis and secretion of androgens, while FSH binds to the granulosa cells and increases aromatase activity that converts androgens to estradiol (Leung and Armstrong, 1980).

Gonadotropin-releasing hormone neurons and subsequent secretion of LH and FSH is regulated by steroids through positive or negative feedback acting either directly on the

gonadotrope cells or indirectly by alteration of GnRH pulses from the hypothalamus (Shupnik, 1996; Sullivan and Moenter, 2005). Decreased estradiol (E2) negative feedback on secretion of LH has been associated with the occurrence of puberty in heifers (Day and Nogueira, 2013). Estradiol suppresses FSH secretion by acting directly on the anterior pituitary to decrease expression of the gene encoding the FSH subunit (Roche, 1996). During the luteal phase of the estrous cycle, negative feedback of progesterone (P4) regulates LH pulses and ultimately ovulation (Rahe et al., 1980). Increased E2 concentrations and decreased circulating concentration of P4 induce a surge in GnRH (Frandson et al., 2003). Furthermore, the hypothalamus forms an interaction between the central nervous system and the endocrine system, which allows for integration of internal (nutrition, metabolism) and external (temperature, photoperiod, pheromones) effects on LH and FSH release (Wray, 2002). Nutritional status plays an important role in reproduction. Heifers, with higher body condition scores (body fat), have been shown to reach puberty earlier than heifers with lower body condition scores (Hall et al., 1995). Leptin, produced by adipose tissue, has been shown to play an important role in regulating GnRH secretion (Zieba et al., 2005).

Puberty in Heifers

Puberty in the female occurs when ovulation is accompanied by estrus and followed by normal luteal function (Perry, 2012). Day and Nogueira (2013) reported occurrence of puberty is the result of decreased estradiol negative feedback on secretion of LH. It has been suggested that the decline in the negative feedback of estradiol on secretion of LH results from the decline of estradiol receptors in the hypothalamus and pituitary as puberty approaches (Day et al., 1987). The hypothalamo-pituitary-ovarian axis and its individual functions have the ability to operate as

a system before puberty occurs (Perry, 2012). In prepubertal heifers, the hypothalamus stimulates the synthesis and secretion of LH and FSH by the pituitary gland through the release of GnRH then, with the appropriate stimuli the ovaries secrete sufficient levels of estradiol to induce a preovulatory LH surge and as a result ovulation occurs (Day and Anderson, 1998). Prendiville et at. (1995) showed that puberty was delayed in heifers that were immunized against gonadotropin-releasing hormone. Thus, alteration in concentration of LH and estradiol should have adverse effects on heifer reproductive performance.

Ovarian Maturation

The time from birth to puberty in heifers can be divided into four periods, beginning with an infantile period (birth to 2 mo of age), developmental period (2 to 6 mo of age), a static phase (6 to 10 mo of age), and the perpubertal period (Day and Nogueira, 2013). Evans et al. (1992) showed that mean circulating concentrations of LH and LH pulse amplitude were increased at 12 and 18 w of age than at 4, 24 and 32 w of age. Luteinizing hormone concentration is decreased during the infantile stage, increased as heifers enter the developmental period and stabilize at low concentrations in the static phase, until the preovulatory surge of LH in the perpubertal period (Day and Anderson, 1998). The changing pattern of LH secretion from birth to puberty is reflected in changes in the population of vesicular follicles on the ovary during this time (Day and Anderson, 1998). Research conducted to study follicle populations at slaughter in Holstein heifers (Desjardins and Hafs, 1969) found that numbers of small (< 5 mm) and large (> 5 mm) ovarian follicles increased from birth to a maximum at 16 w of age, then decreased to 24 w of age and remained fairly constant until puberty. A more resent ultrasonographic examination of beef heifer ovaries (Honaramooz et al., 2004) revealed maximum follicle diameter increased

from 8 to 14 weeks, again from 38 to 42 weeks and finally from 52 to 60 weeks of age. In the heifer, the ovaries produce dominant follicles of increasing diameter during the static period and during the perpubertal increase in LH, which culminates in the pubertal ovulation (Day and Anderson, 1998).

Characteristic of the Estrous Cycle

Hormones from the hypothalamus (GnRH), anterior pituitary (FSH and LH), ovaries (P4, E2 and inhibins) and uterus (Prostaglandin; $PGF_{2\alpha}$) regulate normal estrous cycle function. The initiation of estrous cycles in heifers occurs at the time of puberty. A preovulatory dominate follicle (DF) and corpus luteum (CL) are the two primary ovarian structures that play a role in regulating the estrous cycle. The normal estrous cycle averages 21 d in cows and 20 d in heifers with a normal range of 18 to 24 d (Werner et al., 1938; Olds and Seath, 1951; Beal et al., 1980). Each cycle consist of two phases: the luteal phase (14 to 18 d) and the follicular phase (4 to 6 d). The luteal phase is often further separated as metestrus and diestrus while the follicular phase is further separated as proestrus and estrus. During metestrus of the luteal phase P4 concentrations begin to rise due to CL formation from the collapsed ovulated follicle (corpus haemorragicum) in which the granulosal and thecal cells of the ovulated follicle luteinize and produce P4 (Ireland et al., 1980). Progesterone concentrations remain elevated during diestrus of the luteal phase due to the presence of the CL (Ireland et al., 1980). Follicular development continues and is initiated throughout diestrus by the release of FSH from the anterior pituitary. Dominant follicles that grow during the luteal phase do not ovulate. Through negative feedback, P4 only allows the secretion of greater amplitude but lesser frequency LH pulses that are inadequate for ovulation of the DF (Ireland et al., 1980; Rahe et al., 1980). During proestrus of the follicular phase, PGF2α

secretion from the uterus causes the regression of the CL and progesterone concentrations rapidly decrease (Hansel and Convey, 1983). Luteinizing hormone binds to theca cells of the DF resulting in the synthesis of androgens that diffuse into the granulosa cells of the DF. Follicle stimulating hormone binds to the granulosa cells and increases aromatase activity that converts androgens from the theca cells to estrogens (Fortune and Quirk, 1988). Reports have shown the increased frequency of LH pulses, during the follicular phase, enhances dominate follicle growth, which leads to the production of enough E2 to induce behavioral estrus and a preovulatory surge of gonadotropins (Savio et al., 1988; Bergfeld et al., 1994). This pre-ovulatory GnRH surge induces a LH and FSH surge followed by ovulation of the DF (Sunderland et al., 1994). Follicle growth in cattle occurs in a wave-like fashion with 2 to 3 waves per estrous cycle (Savio et al., 1988; Ginther et al., 1989).

Follicular Wave Dynamics

A wave of follicular growth involves the synchronous development of a group of follicles 4 to 5 mm in diameter followed by selection and growth of a dominant follicle, suppression of the subordinates and either atresia or ovulation of the dominant follicle (Ginther et al., 1989). The length of interovulatory intervals has been shown to be 20.4 d for 2-wave cycles and 22.8 d for 3-wave cycles (Ginther et al., 1989). Ginther et al. (1989) determined the waves begin 0.2 d before and 9.6 d after ovulation for 2-wave cycles while waves begin 0.5 d before ovulation, 0.3 d and 9.0 d after ovulation for 3-wave cycles. Waves of follicular growth are gonadotropin dependent and a surge in FSH necessarily preceded the emergence of a wave (Adams et al., 1992). The FSH surge begins 2 to 4 d before the emergence of a follicular wave, peaked 1 or 2 d before emergence and began to decrease when a dominant follicle is selected (Adams et al.,

1992). When the largest follicle reaches 8 to 9 mm, the follicles of the wave deviate in growth rates with usually the largest one becoming dominant. After a dominant follicle is selected, FSH-dependent subordinate follicles cease to grow because of reduced FSH concentrations, whereas the selected dominant follicle continues to grow by a shift in primary gonadotropin dependency from FSH to LH (Ginther et al., 1996). Inhibin and E2, produced by granulosal cells of the DF, suppress FSH secretion by acting directly at the anterior pituitary to decrease expression of the gene encoding the FSH subunit (Roche, 1996). In the presence of elevated P4, LH pulse frequency is maintained at 1 pulse/4 h and the dominant follicle undergoes atresia. In the absence of P4, LH pulse frequency increases to 1 pulse/h and this stimulates final maturation, increased E2 concentrations and positive feedback on GnRH, LH and FSH, in a surge that induces ovulation (Sunderland et al., 1994).

Ovulation

Ovulation is a complex process that is initiated by the pre-ovulatory LH surge and results in the rupture of follicle at the surface of the ovary and release of the ovum (Richard et al., 2002). Ovulation occurs approximately 28 h after the pre-ovulatory LH surge in beef cattle (Arije et al., 1974; Christenson et al., 1975) and 24 h after in dairy cows (Henricks and Dickey, 1970; Chenault et al., 1974; Ginther et al., 2012). The pre-ovulatory LH surge induces genes involved in ovulation that impacts both theca cells and granulosa cells to activate selective signaling pathways. These signaling pathways rapidly induce transcription of specific genes, which initiate or alter additional cell signaling cascades, resulting in the activation of proteolytic enzymes that digest the follicular wall (follicular rupture) and promote follicular remodeling to form a CL (Richard et al. 2002).

Estrous Behavior

Estrogen, specifically estradiol-17β, is the primary signal to the hypothalamus that induces estrus, but only in the absence of P4 (Vailes et al., 1992). Estrus is characterized as the period when a cow or heifer exhibits sexual desire and acceptance of a bull or other females by standing to be mounted. Accurately detecting estrus is a critical facet of both artificial insemination (A.I.) and embryo transfer programs. For successful estrous detection there are several distinct estrous behaviors and visual signs that need to be understood. Standing to be mounted by herd mate or bull is the most definite and accurate sign that a cow is in estrus (Diskin and Sreenan, 2000). Because standing estrus may not always be observed, other secondary signs that indicate a cow or heifer is coming in to estrus can be utilized in determining the occurrence of estrus. These secondary signs include swelling of the vulva, restlessness, vocalization, walking fence lines and discharge of a clear mucus from the vulva (Diskin and Sreenan, 2000). Behavioral estrus is used by cow herd managers to determine the optimal time to AI by estimating when a heifer of cow will ovulate. Ovulation typically occurs 31 to 32 h after the onset of estrus in beef cattle (Wiltbank et al., 1967; Arije et al., 1974; Christenson et al., 1975; White et .al, 2002) and 27 to 29 h in dairy cattle (Henricks and Dickey, 1970; Swanson and Hafs, 1971; Walker et al., 1996). Onset of estrus can be determined either by visual observation or electronic technologies. Detecting estrus visually is an accurate method but can be inefficient at times. In operations that utilize A.I., estrous detection is an important factor affecting overall reproductive performance. Failure to detect cows in estrus or misdiagnosis of estrus can result in significant economic losses.

Optimal Time of AI

The optimal time to AI depends mainly on the fertile lifespan of spermatozoa and the viable lifespan of the ovum in the female reproductive tract. A minimum of 6 h is required to transport a population of viable spermatozoa large enough, to the oviduct, capable of fertilization (Dransfield et al., 1998). The viable life of bovine spermatozoa in the reproductive tract has been estimated at 24 to 30 h while the optimal period that the ovum retains its capacity for fertilization is estimated to be 6 to 10 h (Dransfield et al., 1998). Seasonal effects on time of ovulation relative to the end of estrus have been reported; whereas, time of ovulation relative to onset of estrus is not affected by season (White et al, 2002). Thus, time of AI should be determined from the onset of estrus. Dransfield et al. (1998) determined optimal time of AI in dairy cows occurred 4 to 12 h after the onset of estrus. Optimal time of AI may differ between dairy and beef cows because dairy cows have been shown to ovulate sooner after the onset of estrus when compared to beef cows. A broad optimum insemination window for beef heifers of 4 to 24 h after onset of estrus (mean pregnancy rate 63.7%) has been reported with greater pregnancy rates when AI occurred 8 to 12 h after the onset of estrus (69.2%; Hall and Dorsey, 2005). Time of ovulation relative to onset of estrus is a major contributor to pregnancy failure and embryo quality and thus a key component in determining the optimal time of AI. Saacke et al. (2000) reported that inseminating early relative to ovulation results in low fertilization rates, but good embryo quality; inseminating late relative to ovulation results in high fertilization rates, but poor embryo quality. This suggests that sperm life could have a greater impact on pregnancy rate and embryo quality when insemination occurs too early before ovulation and age of the ovum after ovulation could have a greater impact when insemination occurs late.

Radiotelemetry

HeatWatch (CowChips LLC, Manalapan, NJ) is a 24 h estrous detection system that utilizes radiotelemetry. The system consists of an individual cow, pressure-sending radio transmitters, a buffer that receives and stores mounting activity information from the transmitters and a personal computer. The transmitter is placed inside a patch that is glued in front of the tail head. The transmitter and animal identification number, together, are logged into the system. When the female stands to be mounted, a signal unique to her transmitter is sent to buffer where it is stored until it is accessed with a computer. The system's default setting defines onset of estrus as three mounts received of ≥ 1 sec each, within a 4 h period. HeatWatch and other mount detectors offer a more efficient method of estrous detection for cow herd managers. A review of electronic estrous detection technology reported that HeatWatch improved the efficiency of estrus detection by 37% over twice daily visual observation in beef heifers (Rorie et al, 2002).

Studies utilizing HeatWatch have determined duration of estrus and mounts received during estrus is variable among cattle. Duration of estrus in dairy and beef cows, and heifers averages between 7.1 to 17.6 h while mounts received during estrus average from 10.1 to 59.0 mounts / estrus (Walker et al., 1996; Dransfield et al., 1998; Stevenson et al., 1998; Xu et al., 1998; White et al., 2002). Landaeta-Hernandez et al. (2002) reported duration of estrus and mounts received during estrus were less for beef cows with unsynchronized estrus (9 ± 1 h and 8 ± 4 mounts) compared to beef cows with synchronized estrus (16 ± 1 h and 34 ± 4 mounts). Season also has been shown to effect duration of estrus and mounts received during estrus in beef cows (White et al., 2002). Also, mounting activity has been reported to increase (10.1 to 63.5) with the number of heifers in estrus simultaneously (1 to 5 heifers; Helmer and Britt, 1985). Breaks or quiescent periods in standing activity also have been observed in beef cows

where multiparous cows (1.9 h) had a greater quiescence period compared with primiparous cows (0.5 h) with no difference between unsynchronized and synchronized cows (Flores et al., 2006).

Predi′Bov®

Predi′Bov® (ReproPharm, Nouzilly, France) is an on-the-farm ovulation test specific to cattle. The Predi'Bov® assay is designed to help predict ovulation by detecting the pre-ovulatory LH surge. The assay can be performed in 40 min with approximately 0.5 mL^{-1} of whole blood. The assay kit is composed of four small tubes and a stick applicator. The first tube is designated for whole blood, while the other tubes contain either a reagent, reactor or rinse solution. Whole blood used in this assay can be collected in various ways and by either collecting blood directly into the tube designated for whole blood or in heparinized blood collection tubes. To perform the assay, the stick applicator is submerged in approximately 0.5 mL^{-1} of whole blood for 15 min. After incubation for 15 min, the stick applicator is washed in the rinse solution for 20 sec. After washing, the stick applicator is subjected to a reagent solution for 15 min followed by another wash for 20 sec. The stick applicator is incubated a final time with the reactor solution for 10 min. After the final incubation, the stick applicator will either turn blue or stay white in color. If the applicator remains white in color, concentration of LH of the whole blood is below the proprietary threshold the assay is designed to detect and indicates the pre-ovulatory LH surge period has not been reached. If the applicator turns blue in color, the concentration of LH is above the threshold, and indicates the pre-ovulatory LH surge period.

The onset of estrus is not always a reliable predictor of ovulation, and at times estrous behavior is difficult to detect. Twenty-five percent of cows may have periods of estrus with few

mounts and short duration and can go undetected (Rorie et al, 2002). Furthermore, interval between the onset of estrus and ovulation has been shown to be quite variable in cattle with less variation between the pre-ovulatory LH surge and ovulation interval (White et al., 2002; Bloch et al. 2006). Bloch et al. (2006) illustrated these variations in dairy cows, with intervals from onset of estrus to ovulation ranging from 23.9 to 42.6 h and intervals from the pre-ovulatory LH surge and ovulation ranging 24.0 to 28.4 h. Evaluation of AI relative to the pre-ovulatory LH peak also has shown that beef heifers inseminated at least 10 h after the pre-ovulatory LH peak had an increased number transferable embryos (7.2 ± 0.9) when compared to heifers inseminated less than 10 h after the pre-ovulatory LH peak (4.2 ± 1.1; Lafri et al., 2002). Detection of the pre-ovulatory LH surge could offer a more consistent and precise method of predicting ovulation in cattle and determining optimal time of AI.

Replacement Heifer Development

Beef cattle production is dependent on the rejuvenation of the cow herd with replacement heifers. Replacement heifers should be able to replace aged and low producing cows while introducing new and advanced genetics into the herd. Replacement heifers are expected to conceive early in the breeding season, calve as 2 yr olds and continue calving within 12 mo intervals for numerous years. Heifers that conceive early in their first breeding season and calve as 2 yr old cows have greater lifetime productivity in operations with a defined breeding season, and produce heavier calves at weaning when compared to heifers that conceive late in the same season (Lesmeister et al., 1973). Failure to conceive is the largest economic loss in the beef cow herd and age at puberty one of the most important factor when heifers are targeted to calve at 2

yr of age (Ferrell, 1982). Thus, heifers must reach puberty at an early age to assure high conception rates in their first breeding season (Lesmeister et al., 1973).

Target Weight

For many years the commonly used target weight concept has been at the forefront in almost all replacement heifer development systems. This concept's premise is that replacement heifers should achieve 60 to 65% of their expected mature body weight by breeding (Patterson et al., 1992). Weight is a major factor affecting age at puberty and heifers fail to reach puberty until significant weight gains are made (Patterson et al., 1992). Short and Bellows (1971) reported that age at puberty is highly correlated with body weight, and heifers reach puberty only when adequate growth and weight gains are made. Furthermore, lighter weight heifers will be older at the time they reach puberty than heifers at heavier weights (Wiltbank et al., 1985). Age and weight at time of puberty varies among breeds (Wiltbank et al., 1966; Short and Bellows, 1971).

With significant advancements in beef cattle genetics, considerable differences in the average size of cows in production today and all-time high expense cost, new development systems are being targeted to reduce production cost without reducing reproductive performance. Endecott et al. (2013) illustrates the increase of the average mature size of cows from 500 kg to 590 kg used in studies in the past 5 decades and suggested a large number of heifers should reach puberty at or below 60% of their mature BW. Freetly et al. (2011) showed that on average, heifers were 55 to 60% of their mature BW at the time of puberty for numerous breeds. Recent research conducted at the University of Nebraska demonstrated the effects of developing replacement beef heifers to 55 and 60% of their mature BW (Funston and Deustscher, 2004). Funston and Deustscher (2004) found that fewer heifers developed to a lower percentage of their

mature BW had luteal activity at the initiation of breeding season, but pregnancy rates were similar to heifers developed to the traditional target weight, at the end of a 45-d breeding season. When heifers were managed to only 50% of their mature BW, a considerably fewer number of heifers conceived in a 30-d breeding season when compared to heifers managed to 55% of the mature BW (Creighton et al., 2005). Development costs are less in heifers managed to a lower percentage of their mature size at time of breeding (Funston and Deustscher, 2004). It is recognized that fertility traits are moderate to low in heritability (Martin et al., 1992; Dearborn et al., 1973) thus, environmental and management strategies have a major impact on replacement heifer development and subsequent reproductive performance.

Hip Height

Hip height (HH) is used to measure skeletal development of growing animals and is a useful tool for determining mature size in cattle. Arango et al. (2002) reported that skeletal growth, specifically mature height, reached earlier than mature weight and most breeds of cattle achieved 96% to 98% of their final height by 3 yr of age. Skeletal size and frame development is often emphasized in replacement heifer development and traits that reflect long-bone growth (i.e. HH) may reflect true size of replacement heifers better than BW (Brown et al., 2001). Body weight fluctuates within the cow herd and is affected by many environmental factors, nutritional status and stage in production while skeletal measurements including HH remain constant (Brown and Franks, 1964). Body weight is influenced by body condition and pregnancy with body condition being the most important source for variation (Marlowe and Morrow, 1985; Brown et al., 2001; Nephawe et al. 2004).

Body Condition

Body condition refers to the stored energy reserve (i.e. fat) of an animal. Body condition scoring (BCS) is a subjective measurement system used to estimate relative fatness in cattle. The mostly widely used BCS scale for beef cattle in the U.S. utilizes scores ranging from 1 to 9, where 1 = emaciated and 9 = obese (Wagner et al., 1988; Encinias and Lardy, 2002). Hall et al. (1995) showed that heifers with more back fat and a greater BCS were 42.7 d younger at puberty than lean heifers with low BCS. Leptin is a protein that is mainly produced in and secreted by adipose tissue (i.e. body fat) and is involved in numerous physiological processes including reproduction (Chillard et al. 2005). Leptin plays an important role in regulating GnRH secretion, and ultimately in reproductive performance (Zieba et al., 2005). Mice injected with leptin had an earlier onset of classic pubertal parameters compared with saline-injected controls (Ahima et al., 1997). Garcia et al. (2002) reported that leptin plays a functional role in maturation of the central reproductive axis. This suggests that adequate body condition may contribute to the initiation of puberty in heifers.

Reproductive Tract Scoring

A non-invasive, 5-point scoring system was developed (Anderson et al. 1991) to estimate pubertal status in heifers. Reproductive tract scoring (RTS) is performed via rectal palpation of the uterine horns, ovaries and ovarian structures. Scores are subjective estimates of sexual maturity, based on ovarian follicular development and palatable size of the uterus (Patterson et al., 2000). Heifers with a RTS of 1 are described as having immature tracts, with a uterine horn diameter < 20 mm, no uterine tone and no palatable ovarian structures. Heifers with a RTS of 2 have a uterine horn diameter of 20 to 25 mm, no uterine tone and follicles 8 mm in

size. Heifers with a RTS of 3 have a uterine horn diameter of 20 to 25 mm, slight uterine tone, and follicles of 8 to 10 mm in size. Heifers with a RTS of 4 have uterine horn diameter of 30 mm, good tone to the uterus, follicles of 10 mm in size or possible CL. Heifers with a RTS of 5 have a uterine horn diameter > 30 mm and a palatable CL. Heifers with a tract score 1, 2 or 3 are considered prepubertal and heifers with a tract score 4 or 5 are considered to be pubertal (Rosenkrans and Hardin, 2003).

Patterson et al. (2000) combined endocrine and ovarian changes that occur as puberty approaches in heifers with RTS. It was concluded that a RTS of 1 corresponds to the point in time which the pattern of LH release is characterized by low frequency pulses. Reproductive tract score of 2 and 3 are associated with the peripubertal phase, at which there is increases in LH pulse frequency, follicle growth and estradiol secretion. Reproductive tract score of 4 and 5 are assigned to heifers that have reached puberty but differ in stage of the estrous cycle (i.e. follicular phase = 4; luteal phase = 5; Patterson et al., 2000).

Growth Promoting Implants

Growth promoting implants containing anabolic steroids have been used extensively in beef production for many years to increase gain in animals destined for harvest. However, few are recommended for the use in heifers intended for replacement because of the potential negative effects on reproduction (Heitzman et al., 1979; Deutscher et al., 1986; Moran et al., 1990). Improved feed efficiency of 5 to 10% and average daily gain response to these products can be expected to increase, 0.05 kg/d for steer calves while responses in heifers are slightly greater (0.06 to 0.07 kg/d; Selk, 1997). The majority of growth implants have either estrogenic or androgenic activity, or a combination of the two (Kreikemeier and Mader, 2004). Growth

promoting implants used in beef cattle are made up of hormones either naturally occurring (estradiol, progesterone and testosterone) or synthetics (zeranol and trenbolone acetate). Anabolic steroids trigger increased growth rates largely by increasing protein accretion and mobilization of fat stores (Meyer, 2000). Growth promoting implants have been shown to induce postnatal skeletal muscle hypertrophy through increased circulating and local concentrations of important growth factors for skeletal muscle and insulin-like growth factor-I (Johnson et al., 2013). Estradiol and zeranol have estrogenic activity, whereas testosterone and trenbolone acetate have androgenic activity (Meyer, 2000). Trenbolone acetate also displays antiestrogenic activity (Moran et al., 1990). Heitzman et al. (1979) reported trenbolone acetate concentrations remain elevated over control values for 11 to 13 weeks while estradiol concentrations remain elevated for 13 to 15 weeks after implantation. It is estimated that two-thirds of all implants marketed in the U.S. contain various concentrations of trenbolone acetate and estradiol (Johnson et al., 2013). Average daily gain has been shown to increase in heifers and steers that received growth promoting implants with a combination of trenbolone acetate and estradiol compared to heifers and steers that received either compound alone (Hunt et al., 1991; Kreikemeier and Mader, 2004). Similar body condition and HH results have been reported in non-implanted heifers and heifers that received growth promoting implants (Staigmiller et al., 1983; Deutcher et al., 1986; Kreikemeier and Mader, 2004). Previous work involving the use of anabolic steroids in heifers showed that puberty was delayed compared with those heifers not implanted (Heitzman, et al., 1979; Deutscher et al., 1986; Moran et al., 1990). Using growth promoting implants in heifers intended for replacements is controversial.

Estradiol

Estrogens seem to improve protein anabolism and mineral retention while stimulating growth hormone secretion, growth hormone receptors and insulin like growth factor 1 (Meyer, 2000). Cattle respond well to exogenous estradiol and enhance growth performance 5 to 15%. The physiochemical and biochemical characteristics of estrogen receptors in the bovine skeletal muscle match those of the uterine estrogen receptors (Meyer, 2000). Moran et al. (1990) reported that heifers implanted with estradiol were older when puberty occurred and pregnancy rate was lower than non-implanted heifers.

Trenbolone acetate

Trenbolone belongs to the group of most efficient anabolic steroids and shows strong binding to androgen receptors, progestin receptors and the glucocorticoid receptors (Meyer, 2000). Trenbolone acetate (TBA) is a potent analog of the sex steroid testosterone and has been shown to be 10 to 50 times more anabolically active than testosterone (Johnson et al., 2013). Trenbolone acetate has been reported to limit gonadotropin secretion from the anterior pituitary in bulls (Getty et al., 1984). Heitzman et al. (1976) reported pregnancy was not influenced by TBA or TBA+E2 implants when heifers were implanted at both 16 and 31 weeks of age. Moran et al. (1990) reported that beef heifers repeatedly implanted with TBA had delayed puberty when compared to non-implanted heifers. Also, TBA implanted heifers had an increase in non-ovulatory estrus compared to non-implanted heifers but fewer occurrences than heifers implanted with TBA+E2; first ovulation coincided approximately with the time the implant expired (Moran et al., 1990). This is perhaps due to the antigonadotropic activity of TBA, which suggests a

single implant of TBA and strategic timing of administration could be beneficial in adding growth to heifers without having detrimental effects on reproduction.

Zeranol

Zeranol is classified as a nonsteroidal macrolide and is in a class of products referred to as β-resorcyclic acid lactones (Johnson et al., 2013). The mode of action of zeranol is similar to that of estradiol but with several differences in receptor binding and elimination, but a more detailed understanding of zeranol biochemistry is needed (Meyer, 2000). When low weight heifers were implanted with zeranol at 8 and 11 mo of age reproductive development and puberty were not influenced by treatment, and pregnancy rates were similar (Staigmiller et al., 1983). Deutscher et al. (1986) reported that reproductive development and puberty was delayed when heifers were implanted with zeranol at 6 mo of age, not effected when heifers were implanted at 9 mo of age and pregnancy rate did not differ between the two dates or control heifers. However, repeated implanting with zeranol decreased conception rates and heifer reproduction performance (Deutscher et al., 1986). Moran et al. (1990) reported heifers implanted 4 times with zeranol had an increase in non-ovulatory estrus and first ovulation did not occur until the implant had expired. Also, reproductive tract development and pregnancy rates were decreased in zeranol implanted heifers (Moran et al., 1990).

Conclusion

Recently, heifers that were developed to a lower percent of their mature body weight at the time of breeding required less input cost and did not differ in subsequent reproductive performance than heifers developed to the traditional 60 to 65%. The ability to accomplish this

may be due to the advancements in both cattle genetics and management practices over the past 40 yr. Furthermore, growth prompting implants have been used extensively in calves intended for market and in the finishing phase of production because of the increase weight gain and feed efficacy associated with these compounds. Very few growth promoting implants are designated for uses in heifers and use of these compounds in heifers is controversial. However, changes in not only management practices but also growth promoting implants have lead studies that utilize these compounds in the production of replacement beef heifers. Strategic timing of administration of growth promoting implants could be beneficial in adding growth to heifer without having detrimental effects on reproduction. In addition, RTS, HH measurements, BCS, defined breeding seasons and reproductive technologies could greatly assist in replacement heifer development.

Major advancement in cattle genetics and management practices has made way for new methods in the development of replacement heifers from birth to puberty and thereafter. These methods could be endless and research should be conducted to determine the best approach in developing replacements for today's economic and production setting. Regardless of the operation, careful consideration of environmental and nutritional factors that affect reproduction is a must for the management of replacement heifers. A rigorous and strategic plan is essential for successful replacement heifer production and ultimately the future profitability of the cow herd. Replacement heifer development and subsequent reproduction is one of the most important factors that influence the profitability of any cow-calf operation. Alterations to the traditional target weight concept of developing heifers to breeding and utilization of growth promoting implants and management tool such as RTS, BCS and reproductive technologies could be advantageous to the bottom line of producers.

The application of new development strategies and biotechnologies will be necessary to meet increasing demands of the U.S. cow herd and the animal protein needs of a growing global human population. The manipulation and relationships between steroids and gonadotropins could offer additional aid in the dilemma facing beef cattle production in the U.S. Future research is needed to determine the most efficient method of replacement heifer development.

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CHAPTER III

GROWTH, REPRODUCTIVE DEVELOPMENT, AND ESTROUS BEHAVIOR OF REPLACEMENT BEEF HEIFERS TREATED WITH GROWTH-PROMOTANTS

Abstract

Charolais x Balancer heifers (n = 65; 179 ± 30 kg; 255 ± 12 d of age) were used to determine the influence of growth-promoting implants on growth, reproductive development, estrous behavior, and pregnancy rate. Heifers were assigned to 1 of 4 implant treatment groups: 1) control, no implant (CON; n = 16); 2) trenbolone acetate (TBA; 200 mg of TBA; n = 15); 3) trenbolone acetate plus estradiol (TBA+E2; 40 mg TBA and 8 mg E2; n = 17); or 4) zeranol (ZER; 36 mg ZER; n = 17). Reproductive tract scores (RTS; scale of 1 to 5) were determined via ultrasonography on d 106 and 195. Estrous behavior was monitored by radiotelemetry. Average daily gain of heifers was greater ($P < 0.03$) for TBA+E2 heifers compared with other treatment groups. A lower percentage ($P < 0.03$; 18%) of heifers treated with ZER were classified with a cyclic RTS on d 106 than CON heifers (53%) and heifers treated with TBA (67%); heifers treated with TBA+E2 (35%) were similar ($P > 0.10$) to all treatments. Heifers treated with TBA had increased mounts ($P < 0.05$; 60.1 ± 10.4 mounts) during estrus compared with all other treatments (mean = 27.0 ± 8.2 mounts). Overall pregnancy rate did not differ ($P > 0.10$) among treatments (mean = 72%). Implanting with TBA+E2 post-weaning resulted in heavier heifers at breeding. Reproductive development was delayed in ZER heifers; however, implant strategy did not decrease pregnancy rates.

Introduction

Efficient beef cattle production requires replacement heifers that conceive early in the breeding season, calve as 2 yr old and continue calving within 12 mo intervals. Heifers that calve as 2 yr old cows maximize their lifetime productivity potential (Lesmeister et al., 1973), and age at puberty is one of the most important factors when heifers are targeted to calve at 2 yr of age (Ferrell, 1982). Heifers must reach puberty at an early age to assure high conception rates in their first breeding season (Lesmeister et al., 1973). Weight is a major factor affecting age at puberty and heifers fail to reach puberty until significant weight gains are made (Patterson et al., 1992). Adequate growth and weight is necessary for the initiation of puberty in beef heifers (Short and Bellows, 1971) and lighter weight heifers will be older at the time they reach puberty than heifers at heavier weights (Wiltbank et al., 1985).

Growth promoting implants have been used extensively in beef production for many years to increase gain in animals destined for harvest. However, few are recommended for the use in heifers that may be retained for replacements. Previous research involving the use of growth promoting implants in heifers have shown that reproductive performance of implanted heifers is determined by dosage of the growth promotant and timing of implantation (Heitzman et al., 1976; Staigmiller et al., 1983; Deutscher et al., 1986; Moran et al., 1990). Minimal data exist on the influence of growth promoting implants on estrous behavior determined by radiotelemetry in beef heifers.

Beef producers need options to meet the increasing demands of beef production and the U.S. cow herd situation and may want to consider adding value to low weight heifers. Therefore, our objective was to determine the influence of growth-promoting implants on

growth, reproductive development, estrous behavior, and pregnancy rate of low weight beef heifers.

Materials and Methods

Management

This study was conducted at the University of Arkansas North Farm, Fayetteville, AR, with sixty-five spring-born Charolais x Balancer heifers (179 ± 30 kg; 255 ± 12 d of age) from the University of Arkansas System, Division of Agriculture, Livestock and Forestry Research Station, Batesville, AR. The University of Arkansas' Institutional Animal Care and Use Committee (#13021) approved the animal procedures used in this study. Ear notches were collected from each heifer before the initiation of the study and submitted to a commercial laboratory (Cattle Stats, LLC; Oklahoma City, OK) for determination of bovine viral diarrhea virus persistent infection with each heifer yielding a negative result. Heifers rotationally grazed orchard grass, novel endophyte-infected tall fescue, and mixed grass pastures as a single group for 307 d. Heifers were supplemented with alfalfa haylage (0.50 kg/heifer on an as-fed basis) for 50 d in the winter months when available forage was limited.

Treatments

Heifers were blocked by BW and assigned to 1 of 4 implant treatment groups: 1) control, no implant (CON; n = 16); 2) trenbolone acetate (TBA; 200 mg of TBA; n = 15); 3) trenbolone acetate plus estradiol (TBA+E2; 40 mg TBA and 8 mg E2; n = 17); or 4) zeranol (ZER; 36 mg ZER; n = 17). Heifers were implanted once according to treatment group on d 0. Growth measurement data including BW, hip height (HH; determined by Altitude Stick, NASCO, Fort

Atkinson, WI) and body condition score (BCS; scale from 1 = very thin to 9 = obese; Wagner et. al., 1988) were determined at d 0, 106 and 195 of the study, with final BW and BCS measurements taken at time of breeding (d 220). Reproductive tract scores (RTS; scale of 1 to 5; Anderson et al., 1991) of heifers were determined via ultrasonography (Aloka 500 V; Corometrics, equipped with a 5.0-MHz transducer) on d 106 and 195. Heifers with BW < 227 kg were not subjected to ultrasound to avoid possible injury to the heifers and categorized as a RTS 2 (Patterson et al., 1995). Reproductive tract scores of 1, 2, and 3 where categorized as noncyclic while scores of 4 and 5 were categorized as cyclic (Rosenkrans and Hardin, 2003). Estrous synchronization was initiated on d 195 when heifers received an intravaginal, controlled internal drug-releasing device (EAZI-BREED CIDR, Zoetis, Kalamazoo, MI) for 16 d, followed by GnRH (Factrel, Zoetis, Kalamazoo, MI) 2 d later (d 213 of experiment); PGF2α (Lutalyse, Zoetis, Kalamazoo, MI) was administered 1 wk after GnRH (d 220 of experiment). Estrous behavior was monitored by radiotelemetry (HeatWatch, CowChips, LLC, Manalapan, NJ) for 96 h post-PGF2α. Duration of estrus, number of standing events and quiescence periods between standing events were calculated. Onset of estrus was defined as the first of three mounts of ≥ 1 sec in duration, received within a 4 h period and the end of estrus was considered to be the last mount, with a mount 4 h before, and no mounts during the next 12 h. Quiescence period was defined as the interval between each successive mount and calculated as mean quiescence period = duration of estrous, h/(number of mounts received – 1) (Flores et al., 2006). Heifers were insiminated with conventional semen, 10 to 19 h after onset of estrus (d 222 to 223 of experiment). All heifers were exposed to Angus bulls (1 bull / 22 heifers) for 28 d starting 12 d after AI (d 235 of experiment) and pregnancy was diagnosed via ultrasonography (Aloka 500 V; Corometrics, equipped with a 5.0-MHz transducer) on d 280 and 301.

Statistical Analyses

Growth performance parameters and estrous behavior data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with heifer as the experimental unit. Variables analyzed were BW, BCS, HH and their change, ADG, duration of estrus, number of standing events and quiescence periods between standing events. Influence of implant treatment group on RTS (proportion of heifers categorized as cyclic and noncyclic at d 106 and 195) and pregnancy rates (proportion of all heifers that became pregnant during the experimental period) were analyzed by Chi-square using FREQ procedure of SAS. Treatment means were reported as least squares means. Least squares means were compared using the PDIFF statement of SAS when protected by a significant ($P < 0.05$) treatment effect.

Results

There were no differences ($P > 0.10$) in BW, BCS or HH between treatment groups at d 106 (mean = 280.3 ± 8.74 kg, 5.0 ± 0.09 and 115.0 ± 1.20 cm; for BW, BCS, and HH, respectively; Table 3.1) and 195 (mean = 320.7 ± 8.11 kg, 5.3 ± 0.08 and 121.8 ± 1.09 cm; for BW, BCS, and HH, respectively; Table 3.1). However, heifers treated with TBA+E2 did have a greater ($P < 0.02$) BW change from d 0 to d 220 than all other treatment groups (Table 3.2). Change in HH from d 0 to d 195 (mean = 12.5 ± 0.59) and change in BCS from d 0 to 220 (mean = 0.6 ± 0.15) was similar ($P > 0.10$) across all treatment groups (Table 3.2). Average daily gain at d 106 was greater ($P < 0.03$) for TBA+E2 treated heifers compared to all the other treatment groups (Table 3.2) and also from d 0 to 220 (Table 3.2).

Fewer ($P < 0.03$) heifers treated with ZER were classified with a cyclic RTS (score 4 or 5) at d 106 than CON heifers and heifers treated with TBA; heifers treated with TBA+E2 were similar ($P > 0.10$) to all treatments (Table 3.3). Percentage of heifers classified with a cyclic RTS did not differ ($P > 0.10$) between treatment groups at d 195.

Forty-one heifers (63%) responded to estrous synchronization with no treatment effect ($P > 0.10$) on synchronization response (Table 3.4). Estrous behavior was only recorded on heifers that responded to estrous synchronization. Duration of estrus (mean = 10.9 ± 1.7 h; range 2.3 to 23.4 h) was similar ($P > 0.10$) among treatment groups (Table 3.4). Heifers treated with TBA had an increased number of mounts ($P < 0.05$) during estrus compared with all other treatments (Table 3.4). Quiescence period between mounts (mean = 0.5 ± 0.1 h; range 0.1 to 1.3 h) did not differ ($P > 0.10$) between treatment groups (Table 3.4). Overall pregnancy rates (mean = 72%) did not differ ($P > 0.10$) among treatments (88, 67, 65 and 71% for CON, TBA, TBA+E2 and respectively; Figure 3.1). Also, A.I. pregnancy rates did not differ ($P > 0.10$) among treatments (54, 14, 50 and 45% for CON, TBA, TBA+E2 and respectively; Figure 3.1).

Discussion

Reproduction is primarily controlled by the regulation of gonadotropins by steroid hormones. Prendiville et al. (1995) showed that reproduction was altered when heifers were immunized against gonadotropin-releasing hormone. Thus, altering the concentration of gonadotropins or gonadotropin-inhibiting compounds, such as the implants used in the current study, should have adverse effects on reproductive performance. Studies conducted to determine the influence of estrogenic and androgenic growth promotants on reproductive development and subsequent reproductive performance in heifers are conflicting. Thus, the use of growth

promoting implants in replacement heifers is controversial. Anderson et al. (1991) developed a RTS system, and Rosenkrans and Harin (2003) suggested categorizing RTS of 1, 2 and 3 as prepubertal and reproductive tract scores of 4 and 5 as pubertal. Reproductive tract development was influenced by treatment at d 106 but not at d 195. Fewer heifers implanted with ZER had reproductive tract scores of 4 and 5 than control and TBA treated heifers but did not differ from heifers treated with TBA+E2 at d 106. Percentage of heifers with a reproductive tract score of 4 and 5 did not differ at d 195. Heifers were likely not under the influence of their respective growth promotant for the approximately 90 d between RTS measurements. This may have allowed for additional development by the time of breeding. In previous research, when low weight heifers (mean BW = 178 kg) were implanted with ZER at 8 and 11 mo of age, reproductive development and puberty was not influenced by treatment (Staigmiller et al., 1983). Deutscher et al. (1986) reported reproductive development and puberty was delayed when heifers were implanted with ZER at 6 mo of age but not effected when heifers were implanted at 9 mo of age. It also has been concluded that TBA, TBA+E2 and ZER implants delay reproductive tract development and puberty in heifers (Heitzman et al. 1979; Moran et al., 1990). However, in these studies heifers received multiple implants at various stages of development.

Few studies include or have been conducted to determine the influence of growth promoting implants on estrous behavior in heifers. This is the first study to utilize radiotelemetry to observe estrous behavior in heifers implanted with growth promotants. In this study, 41 heifers responded to the synchronization protocol and estrous behavior data were collected from these heifers. Also, the ability to respond to estrous synchronization was not influenced by implant treatment. Duration of estrus averaged 10.9 h, ranged from 2.3 to 23.9 h and did not differ between treatment groups in the current study. Similarly, previous research has reported duration

of estrus ranged from 2.6 to 26.2 h and averaged 14 h in synchronized yearling dairy heifers when determined by HeatWatch (Stevenson et al., 1996). Also, average duration of estrus determined by HeatWatch in synchronized *Bos indicus* heifers has been reported to be 14 h with a range from 2.0 to 24.2 h (Lemaster et al., 1999). Duration of estrus in dairy and beef cows and heifers, averaged between 7.1 to 17.6 h (Walker et al., 1996; Dransfield et al., 1998; Stevenson et al., 1998; Xu et al., 1998; White et al., 2002). Landaeta-Hernandez et al. (2002) reported duration of estrus is less for beef cows with unsynchronized estrus (9 h) compared to beef cows with synchronized estrus (16 h).

Number of mounts received during estrus was influenced by treatment. Heifers treated with TBA had increased number of mounts received during estrus compared to all other treatment groups. Average number of mounts received during estrus for the current study was 35.3 and ranged from 3 to 135 mounts received. Stevenson et al. (1996) reported the number of standing events monitored by HeatWatch was 50.1 mounts/estrus, in synchronized yearling dairy heifers. Lemaster et al. (1999) reported an average number of mounts received during estrus of 28.0 and a range from 1 to 121 mounts received in synchronized *Bos indicus* heifers when determined by HeatWatch. Mounts received during estrus in dairy and beef cows and heifers have been shown to average from 10.1 to 59.0 mounts/estrus (Walker et al., 1996; Dransfield et al., 1998; Stevenson et al., 1998; Xu et al., 1998; White et al., 2002). Mounting activity also has been reported to increase (10.7 to 48.9) with the number of heifers in estrus simultaneously (1 to 7; Floyd et al., 2001). Also mounting activity was increased in beef cows when estrus was synchronized compared to unsynchronized cows (Landaeta-Hernandez et al., 2002).

Quiescence between mounts was not influenced by treatment in our study. Mean quiescence between mounts was 0.5 h and ranged from 0.1 to 1.3 h. These results are in

agreement with results observed in primiparous, *Bos indicus* cows where mean quiescence between mounts was 0.5 h and ranged from 0.1 to 1.4 h (Flores et al., 2006).

Pregnancy was not influenced by treatment in this study. The percentage of heifers that were confirmed pregnant (mean pregnancy rate = 73%) at the conclusion of the study did not differ between treatment groups. Similarly, Deutscher et al. (1986) reported that pregnancy was not affected when heifers were implanted once with ZER at 6 mo or 9 mo of age and Heitzman et al. (1976) concluded pregnancy was not influenced by TBA or TBA+E2 implants when heifers were implanted at both 16 and 31 wk of age. However, when low weight heifers (mean BW = 178 kg) were implanted with ZER at 8 and 11 mo pregnancy rates were affected (mean pregnancy rate = 73% and 56%; CON and ZER respectively; Staigmiller et al., 1983). Pregnancy rates have also been shown to decrease when heifers were repeatedly implanted with TBA or ZER and once with TBA+E2 (Moran et al., 1990)

Growth promoting implants have been used extensively in beef production for many years to increase rate of gain and feed efficiency. In the current study ADG was increased in heifers implanted with TBA+E2. Heifers treated with TBA+E2 had increased ADG compared to all other treatment groups. Similarly, feedlot heifers that received an implant with TBA+E2 achieved a higher ADG (1.43 kg/d) than heifers not receiving growth promotants (1.25 kg/d), TBA (1.31 kg/d) or estrogenic (1.33 kg/d) implants (Kreikemeier and Mader, 2004). This same trend has been reported in steers, where ADG in steers implanted with TBA+E2 was greater (1.58 kg/d) than steers not receiving an implant (1.28 kg/d), and steers receiving a TBA implant (1.29 kg/d; Hunt et al., 1991). Average daily gain was not increased when heifers were implanted once at 9 mo of age with ZER (Deutscher et al., 1986). Moran et al. (1991) reported ADG with TBA, TBA+E2, and ZER implants to be 0.82, 0.81, 0.81 kg/d respectively when heifer calves

were implanted once with TBA+E2 or implanted four times with either TBA or ZER in a 368 d experimental period. Heifers in the current study experienced higher ADG (mean = 0.95 kg/d) from d 0 to 106 and (mean = 0.75 kg/d) from d 0 to 220. This is likely due to the length of time growth promotants are biologically active. Heitzman et al. (1979) reported TBA concentrations remain increased over control concentrations for 11 to 13 wk while estradiol concentrations remain increased for 13 to 15 wk after implantation.

Body condition scores averaged 5.3 at the time of breeding and BCS as well as HH were not affected by treatment in the current study. These results are in line with previous research where BCS (Deutcher et al., 1986) and HH (Staigmiller et al., 1983; Deutcher et al., 1986) in heifers were not influenced by ZER implants. Also, fat thickness in (feedlot) heifers did not differ between control heifers and heifers implanted with TBA or TBA+E2 (Kreikemeier and Mader, 2004).

Conclusion

Treatment of low weight beef heifers with TBA, TBA+E2 or ZER did not influence BW, BCS or HH. Heifer implanted with TBA+E2 had a greater change in body weight and consequently an enhanced ADG. Reproductive development was delayed in heifers that were implanted with ZER. The number of mounts received during estrus was increase in heifers implanted with TBA. Duration of estrus and quiescence between mounts was not altered by treatment. Trenbolone acetate TBA+E2 and ZER implants did not affect pregnancy rates. Proper care and management should always be taken during replacement heifer development. Replacement heifers that are identified early in life should not be implanted. Strategic timing of administering growth promoting implants could be beneficial in adding growth to low weight

beef heifers without having detrimental effects on reproduction. Utilizing growth promoting implants to add value or salvage, low weight and mismanaged beef heifers could offer additional marketing options for beef produces. Further research is needed to determine the long term effects of growth promotants on reproduction and subsequent calf performance.

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Table 3.1. Growth measurements of beef heifers not treated (CON) or implanted with growth-promoting implants (TBA, 200 mg of trenbolone acetate; TBA+E2, 40 mg trenbolone acetate and 8 mg estradiol; ZER, 36 mg zeranol).

	Treatment					
Item	CON	TBA	TBA+E2	ZER	SEM[a]	*P*-value
d 106						
BW, kg	277.6	282.9	283.8	277.6	8.74	0.92
BCS[b]	5.0	4.9	5.1	5.1	0.09	0.76
HH, cm	114.4	115.4	114.7	115.1	1.20	0.92
d 195						
BW, kg	316.1	319.4	325.1	322.1	8.11	0.88
BCS[b]	5.3	5.3	5.3	5.2	0.08	0.90
HH, cm	121.3	121.7	122.4	121.6	1.09	0.91

[a] SEM = Pooled standard error of the mean.

[b] Scale from 1 = very thin to 9 = obese (Wagner et al., 1988).

Table 3.2. Average daily gain from d 0 to 106 and d 0 to 220, changes in BW and BCS from d 0 to 220 and change in HH from d 0 to 195, of beef heifers not treated (CON) or implanted with growth-promoting implants (TBA, 200 mg of trenbolone acetate; TBA+E2, 40 mg trenbolone acetate and 8 mg estradiol; ZER, 36 mg zeranol).

	Treatment					
Item	CON	TBA	TBA+E2	ZER	SEM[a]	*P*-value
ADG						
to d 106, kg	0.91[c]	0.93[c]	1.04[d]	0.92[c]	0.03	0.03
to d 220, kg	0.72[c]	0.72[c]	0.80[d]	0.74[c]	0.02	0.03
Δ BW, kg	158.1[c]	158.0[c]	174.4[d]	163.1[c]	4.34	0.02
Δ BCS[b]	0.6	0.6	0.8	0.5	0.15	0.33
Δ HH, cm	12.1	12.1	13.2	12.4	0.59	0.47

[a] SEM = Pooled standard error of the mean.

[b] Scale from 1 = very thin to 9 = obese.

[c,d] Means in a row without common superscripts letter differ ($P < 0.05$)

Table 3.3. Reproductive tract scores (RTS) classifications (4 and 5 = cyclic; 1, 2, and 3 = non-cyclic) at d 106 and d 195 of beef heifers not treated (CON) or implanted with growth-promoting implants (TBA, 200 mg of trenbolone acetate; TBA+E2, 40 mg trenbolone acetate and 8 mg estradiol; ZER, 36 mg zeranol).

	Treatment			
Item	CON	TBA	TBA+E2	ZER
RTS d 106				
cyclic, n (%)	8 (53)[a]	10 (67)[a]	6 (35)[ab]	3 (18)[b]
non-cyclic, n (%)	7 (47)	5 (33)	11 (64)	14 (82)
RTS d 195				
cyclic, n (%)	11 (69)	11 (73)	10 (59)	7 (41)
non-cyclic, n (%)	5 (31)	4 (27)	7 (41)	10 (59)

[a,b] Means in a row without common superscripts letter differ ($P < 0.05$).

Table 3.4. Estrous behavior of beef heifers not treated (CON) or implanted with growth-promoting implants (TBA, 200 mg of trenbolone acetate; TBA+E2, 40 mg trenbolone acetate and 8 mg estradiol; ZER, 36 mg zeranol).

	Treatment			
Item	CON	TBA	TBA+E2	ZER
Synchronization response, %	81	47	59	65
Duration of estrus, h				
Mean	10.6 ± 1.5	10.8 ± 2.1	12.1 ± 1.7	10.1 ± 1.7
Range	2.3 to 17.9	4.0 to 23.4	2.9 to 23.9	6.3 to 14.1
Mounts received/estrus				
Mean	30.5 ± 7.6[a]	60.1 ± 10.4[b]	25.0 ± 8.7[a]	25.5 ± 8.3[a]
Range	3 to 110	5 to 135	7 to 74	12 to 64
Quiescence between mounts, h				
Mean	0.5 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
Range	0.1 to 1.2	0.1 to 1.0	0.2 to 1.3	0.2 to 0.8

[a,b] Means in a row without common superscripts letter differ ($P < 0.05$).

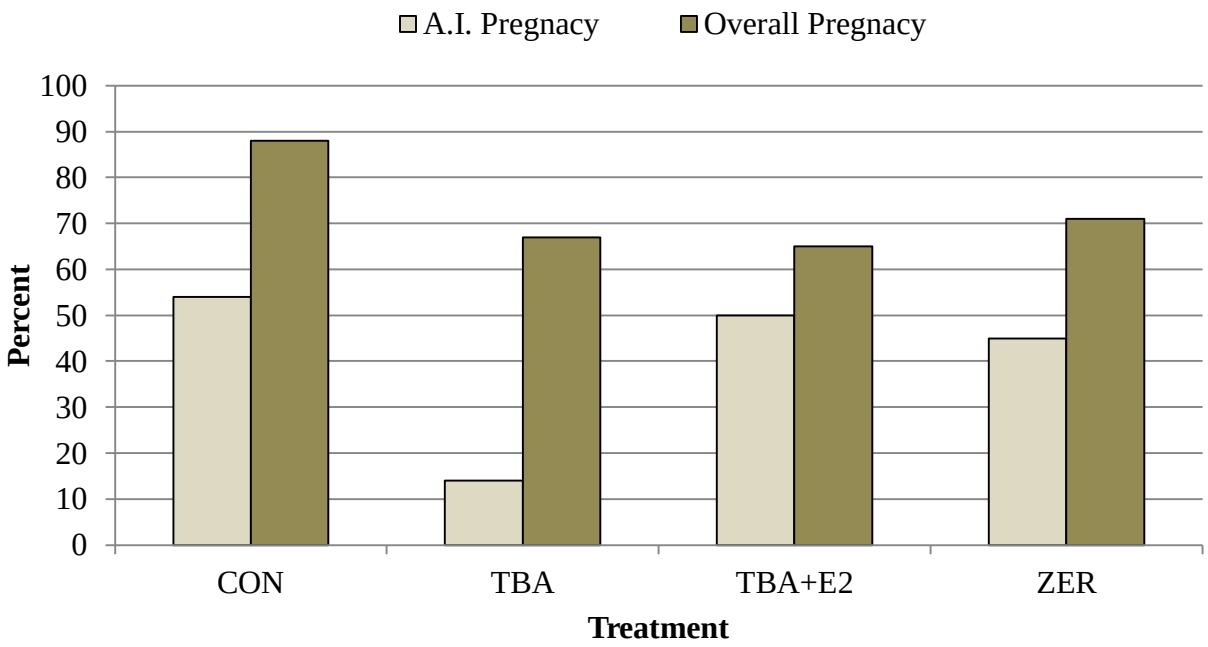

Figure 3.1. Pregnancy rates as determined by ultrasonography at d 280 and 301 of beef heifers not treated (CON) or implanted with growth-promoting implants (TBA, 200 mg of trenbolone acetate; TBA+E2, 40 mg trenbolone acetate and 8 mg estradiol; ZER, 36 mg zeranol).

UNIVERSITY OF
ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Dirk Philipp

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: December 10, 2012

SUBJECT: IACUC Protocol **APPROVAL**
Expiration date : **November 30, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol #**13021** - **"Management strategies for adding value to growing beef heifers ".** You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **11-30-2015,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at atime.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

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CHAPTER IV

COMMERCIAL APPLICATION OF A NEW COW-SIDE LH ASSAY FOR DETERMINING OPTIMUM AI INTERVALS IN SUPEROVULATED BEEF DONORS IN THE USA: A PRELIMINARY STUDY

Abstract

Successful superovulation and embryo collection require donors to be managed for precise insemination intervals. At times, determination of these intervals can become difficult because of estrous detection and ovulation viabilities. The objective of this study was to determine the feasibility of performing a rapid cow-side LH assay (Predi′Bov®, ReproPharm, Nouzilly, France) on superovulated donors, with emphasis on determining how to use the results in a commercial program to time inseminations. This study was conducted at Ovagenix (Bryan, TX) on 52 superovulated beef donors of varying breed and age. Blood samples were collected in heparinized tubes via tail vein puncture starting at CIDR removal, continuing every 6 h until a positive Predi′Bov® test was acquired or 36 h after CIDR removal. Whole blood (0.5 mL) was submitted to a proprietary 3-step procedure (40 min) using a tube-stick applicator to determine whether increased concentrations of LH were present. Donors were artificially inseminated 11.6 ± 0.3 h after a positive test or 12.5 ± 0.3 h after the onset of estrus and again 12.4 ± 0.2 h and 12.5 ± 0.2 h later respectively. The majority (81%) of positive LH tests occurred within 12 to 24 h after CIDR removal. Forty-four percent of the positive tests occurred 0 to 6 h after the onset of estrus, with a range of 13.4 h before to 16.2 h after the onset of estrus. Donors that were inseminated 6 to 10 h after a positive LH test produced a greater (P <0.05) amount of viable

(13.2 ± 1.7) and grade 1 (8.0 ± 1.4) embryos than donors inseminated either < 6 or 10 to 14 h after a positive test. There were no differences ($P > 0.05$) in embryo production between the times of insemination from the onset of estrus or when comparing donors inseminated approximately 12 and 24 h after a positive test or 12 and 24 h after the onset of estrus. In conclusion, Predi′Bov® cow-side usage could offer commercial utility when identifying estrus is difficult or nonexistent. Increased detection of ovulation could help to identify optimal AI intervals, thereby increasing embryo production and limiting the use of expensive semen.

Introduction

Successful superovulation and embryo collection require donors to be managed for precise insemination intervals. The optimal time to AI depends mainly on the fertile lifespan of spermatozoa and the viable lifespan of the ovum in the female reproductive tract. A minimum of 6 h is required to transport a population of viable spermatozoa large enough, capable of fertilization, to the oviduct (Dransfield et al., 1998). The viable life of bovine spermatozoa in the reproductive tract has been estimated at 24 to 30 h while the optimal period that the ovum retains its capacity for fertilization is estimated to be 6 to 10 h (Dransfield et al., 1998). For many years, time of AI has been determined by using the onset of estrus to predict time of ovulation. Ovulation occurs approximately 28 h after the pre-ovulatory LH surge in beef cattle (Arije et al. 1974; Christenson et al., 1975) and 24 h after in dairy cows (Henricks and Dickey, 1970; Chenault et al., 1974; Ginther et al., 2013). Saacke et al. (2000) reported that inseminating early after the onset of estrus results in low fertilization rates, but good embryo quality, whereas, inseminating late after the onset of estrus results in high fertilization rates, but poor embryo quality. The release of many ova from multiple follicles in superovulated females creates a

greater need for the presence of viable spermatozoa in the oviduct at the time of ovulation. The onset of estrus is not always a reliable predictor of ovulation and at times estrous behavior is difficult to detect. Twenty-five percent of cows are characterized as having periods of estrus with few mounts and short duration and can go undetected (Rorie et al., 2002). Furthermore, interval between the onset of estrus and ovulation has been shown to be quite variable between individuals with less variation between the pre-ovulatory LH surge and ovulation. Bloch et al. (2006) illustrated these variations in dairy cows, with intervals from onset of estrus to ovulation ranging from 23.9 to 42.6 h and intervals from the pre-ovulatory LH surge and ovulation ranging 24.0 to 28.4 h. Evaluation of the time of AI relative to the pre-ovulatory LH peak also has shown an increased number transferable embryos when beef heifers are inseminated at least 10 h after the pre-ovulatory LH peak when compared to heifers inseminated less than 10 h after the pre-ovulatory LH peak (Lafri et al., 2002). Detection of the pre-ovulatory LH surge could offer a more consistent and precise method of predicting ovulation in cattle and determining optimal time of AI. The objective of this study was to determine the feasibility of performing a rapid cow-side LH assay (Predi′Bov®, ReproPharm, Nouzilly, France) on superovulated donors with emphasis on determining how to use the results in a commercial program to time inseminations.

Materials and Methods

Animals and Treatment

This study was conducted at Ovagenix (Bryan, TX, USA) on 52 superovulated beef donors of varying breed and age. Donors were examined via ultrasonography (Aloka 500 V; Corometrics, Wallingford, CT, equipped with a 5.0-MHz transducer), 7 d after a preceding reference estrus, for the presence of a CL and to determine eligibility for superstimulation (d 0).

On d 0 donors received an intravaginal progesterone-releasing device (EAZI-BREED CIDR, Zoetis, Kalamazoo, MI) and were administered 25 mg of progesterone and 2.5 mg of estradiol 17β. On d 4 to 7 superstimulation was induced using 150 to 300 mg of FSH (Folltropin-V®, Bioniche Animal Health Inc., ON, Canada) administered with 7 injections at 12-h intervals in decreasing dosages. On d 6, two doses (a.m. and p.m.) of $PGF2_{\alpha}$ (Estrumate®, totaling 1 mg, Zoetis, Kalamazoo, MI) were given, concurrently with FSH injections 5 and 6 with CIDR removal on d 7 (a.m.). HeatWatch® transmitters (CowChips, LLC, Manalapan, NJ) were applied to donors on d 7 to determine onset of estrus.

LH Analysis

The assay used in this study (Predi′Bov®; ReproPharm, Nouzilly, France) is an on-the farm ovulation test specific to cattle. The Predi'Bov® assay is designed to help predict ovulation by detecting the pre-ovulatory LH surge. The assay is performed in 40 minutes with approximately 0.5 mL of whole blood. Whole blood (0.5 mL) was submitted to the Predi′Bov® assay, following manufacture guidelines, to determine whether increased concentrations of LH were present. Blood samples were collected in heparinized tubes via tail vein puncture starting at CIDR removal (0 h) continuing every 6 h until a positive Predi′Bov® test was acquired or 36 h after CIDR removal. Donors were artificially inseminated 11.6 ± 0.3 h after a positive test or 12.5 ± 0.3 h after the onset of estrus and again 12.4 ± 0.2 h or 12.5 ± 0.2 h later respectively. Embryos were nonsurgically collected 7 d after initial insemination.

Statistical analysis

Embryo production data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with donors as the experimental unit. Variables analyzed were number of total, viable, unfertilized, degenerated and quality grade 1 embryos. Time of insemination group means were reported as least squares means. Least squares means were compared using the PDIFF statement of SAS when protected by a significant ($P < 0.05$) time of insemination effect.

Results

Eighty-one percent (42/52) of positive LH tests occurred within 12 to 24 h after CIDR removal; 7.5% (4/52) occurred at 12 h and 11.5% (6/52) at or after 30 h post CIDR removal (Figure 4.1). Forty-four percent of the positive tests occurred 0 to 6 h after the onset of estrus and ranged 13.4 h before to 16.2 h after the onset of estrus (Figure 4.2).

Mean embryo production by donors that either produced a positive LH test, exhibited estrus or a combination of the two is displayed in Table 4.1. Due to age one donor that produced a positive test began displaying estrous behavior was separated from the other donors to prevent injury and was grouped with donors that exhibited estrus and produced a positive test. A total of 428 viable embryos were collected from the 52 donors. Forty-one (79%) donors exhibited estrus, produced a positive test and a total of 364 viable embryos (Table 4.1). Seven (14%) donors did not exhibit estrus but produced a positive test and 47 viable embryos (Table 4.1). Three donors exhibited estrous behavior and never produced a positive test but contributed 17 viable embryos to the total (Table 4.1). One donor did not exhibit estrus; produce a positive test or viable embryos (Table 4.1).

Donors that were inseminated 6 to 10 h after a positive LH test produced more ($P < 0.05$) viable and grade 1 embryos when compared to donors that were inseminated < 6 h or 10 to 14 h after a positive test (Table 4.2). Total, unfertilized and degenerated embryos produced did not differ ($P > 0.05$) between intervals from a positive LH test to insemination (Table 4.2). The interval from the onset of estrus to insemination did not influence ($P > 0.05$) embryo production or quality (Table 4.3). Embryo production and quality from donors inseminated 13 to 14 h and 23 to 25 h after a positive LH test was similar ($P > 0.05$) to donors that were inseminated 13 to 14 h and 23 to 25 h after the onset of estrus (Table 4.4).

Discussion

The onset of estrus is not always a reliable predictor of ovulation and at times estrous behavior is difficult to detect. It has been reported that twenty-five percent of cows are characterized as having periods of estrus with few mounts and short duration and can go undetected (Rorie et al., 2002). Fourteen percent of the donors in our study failed to exhibit behavioral estrous but still produced a positive LH test and viable embryos. The number of viable embryos produced by these donors may have been greatly reduced if the time of insemination was determined using the traditional onset of estrus.

In this study the preovulatory LH surge was detected in 48 donors, by the LH assay, from 12 to 36 h after CIDR removal with 40% being detected at 18 h post CIDR removal. Preovulatory LH peak concentrations have been reported to occur 27 to 42 h post CIDR removal superovulated *Bos taurus* beef heifers (Lafari et al., 2002) and 33 to 45 h in superovulated *Bos* indicus heifers (Occhio et al., 1997). The variation between the present findings and the findings previously reported may be due to the differences in the duration of the preovulatory LH surge.

Stevenson et al. (1998) reported an average preovulatory LH surge duration of 12.2 h while Ginther et al. (2013) reported an average duration of 21.3 h. In the current study LH concentrations and peaks were not determined; the Predi′Bov® assay solely detects increased concentrations of LH at various stages during the preovulatory LH surge. Thus, the preovulatory LH peak could have been well within the ranges previously reported. Furthermore, the LH assay determined the interval between the onset of estrus and the preovulatory LH surge varied greatly between donors. These results are agreement with Swanson and Hafs (1971) observations of the preovulatory LH surge occurring 8 h before to 8 h after the onset of estrus and a more recent observation of 10 h before to 15 h after the onset of estrus (Lafari et al., 2002). This variability further supports the 23.9 to 42.6 h variation from the onset of estrus to ovulation reported by Bloch et al. (2006).

Many studies have determined that ovulation occurs approximately 28 h after the pre-ovulatory LH surge in beef cattle (Arije et al. 1974; Christenson et al., 1975) and 24 h after in dairy cows (Henricks and Dickey, 1970; Chenault et al., 1974; Ginther et al., 2013) and is less variable that the onset of estrus to ovulation (Bloch et al., 2006). Donors in this study that were inseminated 6 to 10 h after the pre-ovulatory LH surge detected by the LH assay, produced more viable and grade 1 embryos than donors inseminated less than 6 h or 10 to 14h after the pre-ovulatory LH surge was detected. Donors that were inseminated less than 6 h, 6 to 10 h or 10 to 14 h after a positive LH test produced 5.1, 1.7 and 3.5 unfertilized embryos respectively. Although not significant, these values are in line with Saacke et al. (2000) who reported early insemination relative to ovulation results in low fertilization rates, but good embryo quality, whereas, late insemination relative to ovulation results in high fertilization rates, but poor embryo quality. A short interval between the pre-ovulatory LH surge and insemination would

create for a long interval between insemination and ovulation and vice versa. This suggests that sperm life is a major factor during early inseminations while ovum age relative to fertilization is major factor during late inseminations (Saacke et al., 2000). Contrary to this, Lafri et al. (2002) reported an increased number of transferable embryos when superovulated beef heifers were inseminated at least 10 h after the pre-ovulatory LH peak when compared to heifers inseminated less than 10 h after the pre-ovulatory LH peak.

Interval between the onset of estrus and first insemination did not influence embryo production in our study. Also there were no differences in embryo production between donors inseminated 11 to 13 h and 23 to 25 h after a positive LH test and donors inseminated 11 to 13 h and 23 to 25 h after the onset of estrus. The lack of differences is likely contributed to the large variation in occurrence of the preovulatory LH surge relative to the onset of estrus. A portion of the donors that were inseminated from the onset of estrus may have been inseminated at approximately the same interval from the preovulatory LH surge as donors inseminated from a positive LH test. Although not significant there was a numerical increase in the number of degenerated embryos produced (3.3 to 6.5) as the interval from the onset of estrus to first insemination increased. Saacke et al. (2000) also reported that as the interval from the onset of estrus to first insemination increased the percent of degenerated embryos also increased. The increase in degenerated embryos could be contributed to an increased interval between ovulation and fertilization in which the aging ovum is continually decreasing in its ability to be fertilized (Saacke et al., 2000).

Conclusion

Donors that failed to exhibit estrous behavior were able to produce viable embryos due to a positive LH test. Viable embryo production and grade 1 embryos increased when donors were inseminated 6 to 10 h after the preovulatory LH surge was detected by the LH assay. Interval from onset of estrus to first insemination did not influence embryo production or quality. Embryo production and quality was similar between donors inseminated 11 to 13 and 23 to 25 h after the preovulatory LH surge was detected by the LH assay or the onset of estrus. The interval from onset of estrus to the detected preovulatory LH surge was quite variable. A commercial LH assay such as Predi′Bov® could offer utility when identifying estrus is difficult or nonexistent. Preovulatory LH surge detection may offer a more consistent and precise method of predicting ovulation in cattle and determining optimal time of AI in superovulated beef donors, thereby increasing embryo production and limiting the use of expensive semen.

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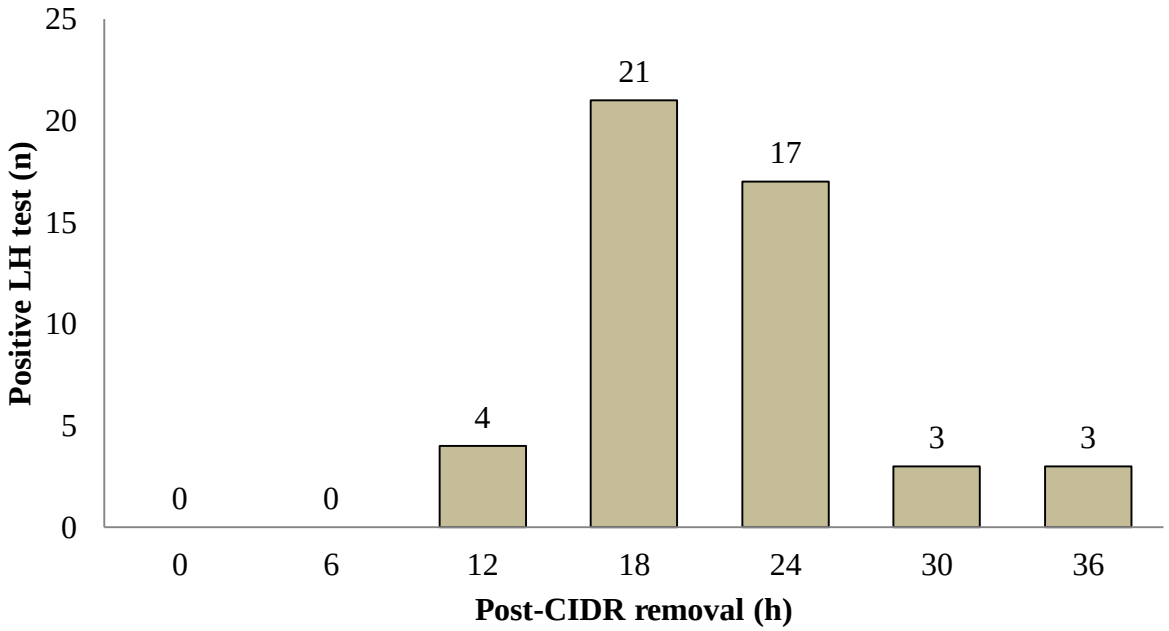

Figure 4.1. Distribution of positive LH test (n) from superovulated beef donors in h post-CIDR removal.

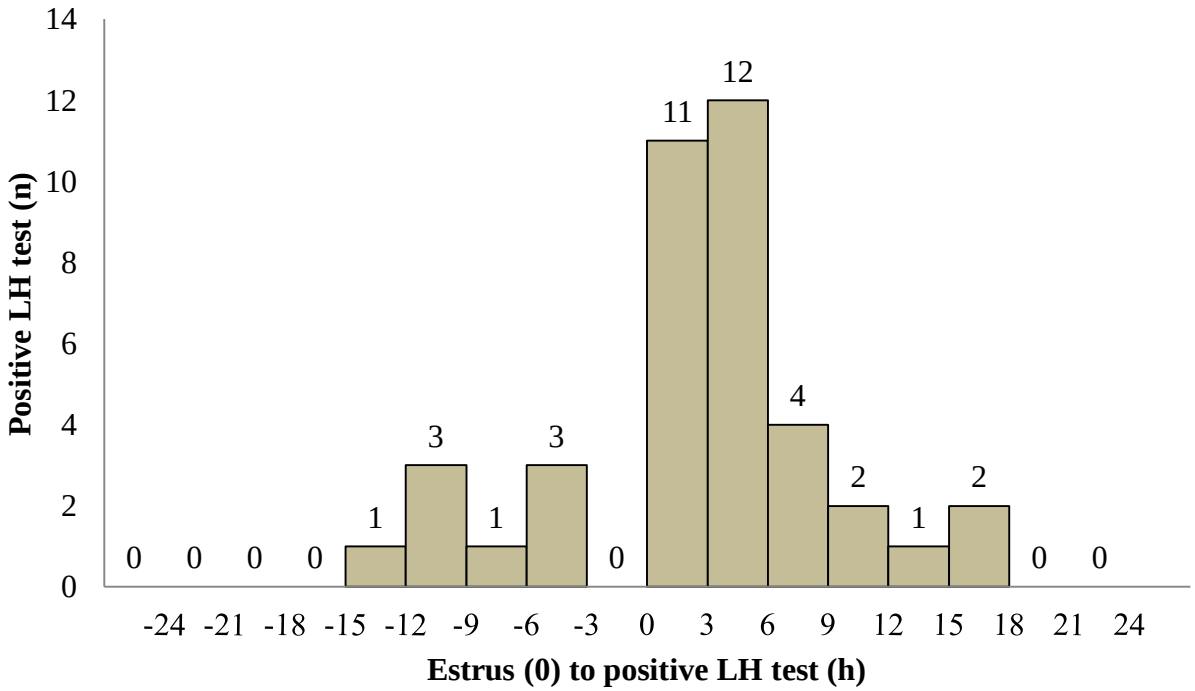

Figure 4.2. Distribution of positive LH test (n) from superovulated beef donors relative to the onset of estrus (0) in h.

Table 4.1. Embryo production by superovulated beef donors exhibiting estrous behavior, producing a positive LH test or a combination.

		Embryo Production				
Donors	n	Total	Viable	Unfertilized	Degenerated	Quality grade 1
Positive test and detected estrus	41	17.5 ± 1.7	8.8 ± 0.9	3.4 ± 0.7	5.2 ± 0.8	5.2 ± 0.7
Positive test and no detected estrus	7	15.1 ± 2.8	6.7 ± 2.2	3.4 ± 1.4	5.0 ± 1.9	3.1 ± 1.3
Negative test and detected estrus	3	6.3 ± 2.2	5.6 ± 2.8	0.7 ± 0.6	0	3.7 ± 1.9
Negative test and no detected estrus	1	0	0	0	0	0
Total	52	16.3 ± 1.4	8.2 ± 0.84	3.2 ± 0.6	4.8 ± 0.7	4.7 ± 0.6

Table 4.2. Embryo production by donors inseminated less than 6, 6 to 10, or 10 to 14 h after a positive LH test.

Positive LH test to first A.I., h	n	Embryo Production				
		Total	Viable	Unfertilized	Degenerated	Quality grade 1
< 6	7	15.6 ± 3.8	6.3 ± 2.1[a]	5.1 ± 1.7	4.1 ± 1.9	3.9 ± 1.7[a]
6 to 10	10	20.8 ± 3.1	13.2 ± 1.7[b]	1.7 ± 1.4	5.9 ± 1.6	8.0 ± 1.4[b]
10 to 14	28	17.5 ± 1.9	8.3 ± 1.0[a]	3.5 ± 0.9	5.6 ± 0.9	4.6 ± 0.8[a]
P - Value		0.47	0.01	0.49	0.48	0.03

Table 4.3. Embryo production by donors inseminated less than 10, 10 to 14, or 14 to 18 h after the onset of estrus.

Onset of estrus to first A.I., h	n	Embryo Production				
		Total	Viable	Unfertilized	Degenerated	Quality grade 1
< 10	8	13.4 ± 3.9	7.9 ± 2.2	1.8 ± 1.7	3.3 ± 1.8	4.0 ± 1.7
10 to 14	14	17.9 ± 2.7	9.8 ± 1.5	3.8 ± 1.1	4.3 ± 1.2	6.1 ± 1.6
14 to 18	17	17.5 ± 2.7	8.2 ± 1.5	2.9 ± 1.1	6.5 ± 1.2	4.9 ± 1.6
P - Value		0.79	0.65	0.37	0.39	0.51

Table 4.4. Embryo production by donors inseminated 13 to 14 and 23 to 25 h after a positive LH test or after the onset of estrus.

		Embryo Production				
Item	n	Total	Viable	Unfertilized	Degenerated	Quality grade 1
Positive LH test to first and second A.I., h						
13 to 14 and 23 to 25	10	16.0 ± 3.5	9.2 ± 2.1	1.8 ± 1.4	5.0 ± 1.7	4.7 ± 1.5
Onset of estrus to first and second A.I., h						
13 to 14 and 23 to 25	9	22.6 ± 4.2	11.4 ± 2.5	4.8 ± 1.7	6.3 ± 2.0	6.0 ± 1.8
P - Value		0.38	0.47	0.37	0.82	0.52

CHAPTER V

SUMMARY AND CONCLUSION

Cattle inventories have drastically declined over the past several years. Environmental conditions in recent history and record beef cattle prices have created for an intensive culling of beef cows. However, the number of replacement heifers entering the herd has not met this severe liquidation. As a result the number of fed cattle and total beef production has suffered while the need for replacement heifers is at an all-time high.

There are many factors that affect the efficiency and profitability of beef production; one of utmost importance is the development of replacement heifers. Replacement beef heifers are extremely important because efficient production is dependent on the rejuvenation of the cow herd. Replacement heifers should be able to replace aged and low producing cows while introducing new and advanced genetics into the herd. Replacement heifer cost represents 30% of the total expenses for a cow calf operation. Major advancement in cattle genetics and management practices over the past few decades have led to new methods in replacement heifer development. Research has also shown that replacement beef heifers can be developed with diverse methods without altering subsequent reproductive performance.

Growth prompting implants such as estradiol, progesterone, testosterone, zeranol and trenbolone acetate, have been used extensively in calves intended for market and in the finishing phase of production because of the increase weight gain and feed efficiency associated with these hormones. Few growth promoting implants are designated for use in replacement heifers and use of these implants in replacement heifers is controversial. However, the use of growth promoting implants to add growth to low weight beef heifers without negatively impacting reproduction

could be advantageous to the bottom line of beef producer. Furthermore, reproductive technologies have allowed producers to utilize superior genetics and increase herd quality in less time than traditional breeding methods. This combine with manipulation and accurate detection of reproductive functions has increased overall reproductive performance and efficiency. The application of reproductive technologies and more efficient methods of replacement heifer production will be necessary to meet not only the increasing demands of the U.S. cow herd but also the animal protein needs of a growing global human population.

In experiment I, treatment of low weight beef heifers with TBA, TBA+E2 or ZER did not influence BW, BCS or HH. Heifer implanted with a combination of TBA+E2 had a greater change in body and consequently an enhanced average daily gain. Reproductive tract development was delayed in heifers that were implanted with ZER. The number of mounts received during estrus was increased in heifers implanted with TBA. Duration of estrus and quiescence between mounts was not altered by treatment. Trenbolone acetate, TBA+E2 and ZER implants did not affected pregnancy rates.

Proper care and management should always be taken during replacement heifer development. Replacement heifers that are identified early in life should not be implanted. Strategic timing of administering growth promoting implants could be beneficial in adding growth to low weight beef heifers without having detrimental effects on reproduction.

In experiment II, donors that failed to exhibit estrous behavior were able to produce viable embryos due to a positive LH test. Viable embryo production and grade 1 embryos increased when donors were inseminated 6 to 10 h after the preovulatory LH surge was detected by the LH assay. Interval from onset of estrus to first insemination did not influence embryo production or quality. Embryo production and quality was similar between donors inseminated

11 to 13 and 23 to 25 h after the preovulatory LH surge was detected by the LH assay or the onset of estrus. The interval from onset of estrus to the detected preovulatory LH surge was quite variable.

A commercial LH assay such as Predi′Bov® could offer utility when identifying estrus is difficult or nonexistent. Preovulatory LH surge detection may offer a more consistent and precise method of predicting ovulation in cattle and determining optimal time of AI in superovulated beef donors, thereby increasing embryo production and limiting the use of expensive semen for desired donors.