INFLUENCE OF MANAGEMENT FACTORS ON REPRODUCTION IN BEEF CATTLE: 1. EFFECTS OF MELENGESTROL ACETATE AND GROWTH PROMOTING IMPLANTS ON OOCYTE QUALITY AND SUBSEQUENT IN VITRO EMBRYO DEVELOPMENT 2. EXPOSURE OF PREPUBERTAL BEEF BULLS TO CYCLING FEMALES TO ENHANCE SEXUAL DEVELOPMENT

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Abstract

This thesis involves two separate studies that evaluate the effects of different beef cattle management practices on reproduction. The objective of the first study was to determine if feedlot heifers administered melengestrol acetate (MGA) and growth promoting implants could serve as viable oocyte donors for in vitro embryo production. Ovaries from heifers administered MGA and growth promotants (MGA-Implant) and ovaries from heifers not administered either substance (Control) were collected from heifers post-slaughter. Oocytes were harvested and in vitro maturation, in vitro fertilization (IVF), and in vitro culture were completed. Treatment and time interacted to affect the number of oocytes aspirated per ovary ($P = 0.07$) and the number of zygotes per ovary ($P = 0.07$). Fertilization ($P = 0.90$) and cleavage rates ($P = 0.80$) did not differ between treatments. Blastocyst rates ($P = 0.30$) and the number of embryos per ovary ($P = 0.50$) did not differ between treatments. We concluded that beef feedlot heifers fed MGA and implanted with growth promotants seem to be a viable source of oocytes for in vitro embryo production.

In the second study, we hypothesized that continuous fenceline exposure of prepubertal beef bulls to cycling beef females would hasten the onset of puberty as well as increase the percentage of bulls passing their initial breeding soundness examination (BSE). Bulls were either exposed to estrous females (exposed) or were not exposed (control). Monthly scrotal circumference (SC) measurements, blood samples, semen evaluations, and bull behavior assessments were conducted. Age at puberty ($P = 0.40$), SC at puberty ($P = 0.50$), and weight at puberty ($P = 0.30$) did not differ between treatments. A similar ($P = 0.50$) percentage of bulls passed their initial BSE at 363 ± 21.5 d of age (exposed: 87.8%; control: 74.2%). Treatment,

month, and stage of the estrous cycle of cows interacted to affect the number of mount attempts ($P = 0.05$) and the number of flehmen responses ($P < 0.001$). In conclusion, bulls given continuous fenceline exposure to cycling beef females were neither younger at puberty nor did a greater percentage pass their initial BSE.

Table of Contents

List of Figures

List of Tables

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Dedication

I dedicate this thesis to my parents and grandparents who taught me the value of a good work ethic and what it means to work with faith and diligence towards a goal. Through their example, I have always been able to achieve my dreams.

Chapter 1 - General Review of Literature: Factors Influencing In Vitro Embryo Production

Introduction

During recent years, in vitro embryo production has become an increasingly popular technology used by the cattle industry to improve genetic merit of beef and dairy cattle. Production of large numbers of cattle with superior genetics has made technologies such as multiple ovulation and embryo transfer, ovum pick-up, and in vitro fertilization (IVF) important procedures utilized by commercial embryo production companies (Merton et al., 2003). Superior genetics from subfertile bovine females can be salvaged and used for in vitro embryo production (Hasler, 1998). Much of the commercial embryo production that is performed in industry today is the result of ovum pick-up procedures in live donors; however, slaughterhouse females also serve as oocyte donors for bovine embryo production.

In vitro embryo production involves the individual processes of in vitro maturation (IVM), IVF, and in vitro culture (IVC), respectively. Research involving the in vitro embryo production process in cattle is relatively recent. The first mammalian specie to be produced by IVF was the rabbit in 1959 (Chang, 1968), with mice following nearly 10 years later (Whittingham, 1968). In vitro fertilization in cattle did not occur until 1977 with semen that had been capacitated in the oviduct or uterus of cows displaying estrus or in the uterus of a rabbit. The first successful live birth of a calf resulting from IVF occurred in 1981 when a 4-cell stage embryo was transferred to a recipient female (Brackett et al., 1982). The complete in vitro embryo production system (IVM, IVF, and IVC) did not yield a live calf until 1987 (Fukuda et al., 1990).

As in vitro embryo production has become a more prevalent means of expanding superior beef genetics, sources of oocyte donors are becoming more crucial to ensure the continued use of this technology. Increasing global beef and dairy production in foreign countries without a strong livestock industry with desire to expand cattle production and (or) improve genetic merit, has become a driver in producing large quantities of embryos, thus making oocyte sources critical to large-scale embryo production.

The ovum pick-up method of oocyte collection is utilized on live, donor females that may have poor fertility and perhaps cannot maintain a pregnancy, or in females that are genetically superior, making them more desirable to produce larger numbers of offspring. Ovum pick-up is performed by trained technicians using transrectal ultrasound-guided follicle aspiration. Number and quality of oocytes collected via this method can be influenced by the stage of the developing follicle, age and reproductive status of the donor, and abilities of the ovum pick-up technician (Hasler, 1998). In 2 ovum pick-up sessions per week, it is possible to yield 150 embryos through IVF, which could lead to approximately 70 calves per year (Merton et al., 2003).

Ovaries of slaughtered beef feedlot heifers or slaughtered commercial cull cows can serve as a low-cost, abundant source of oocytes for use in commercial embryo production post-slaughter (Leibfried-Rutledge et al., 1989). Typically, ovaries are obtained immediately post-harvest, and stored in warm saline solution until manual follicle aspiration can be completed. Following aspiration, oocytes are placed in maturation media for a period of approximately 24 h; following fertilization, in vitro culture allows the presumptive zygotes to develop through the early embryo stages before being deemed usable for frozen or fresh transfer to a recipient female. Gordon and Lu (1990) reported recovering 4,511 oocytes from 646 ovaries (7 oocytes/ovary), which may have been collected during a few days.

Generally, assisted reproductive technologies such as embryo transfer and ovum pick-up with IVF are utilized in the seedstock sector of the beef cattle business. From the perspective of seedstock producers, these technologies allow them to sell large quantities of high-quality genetics from females that are in high demand. These superior females that may be reproductively challenged still have the potential to earn income for producers through the sale of embryos generated using these procedures. In addition to the seedstock sector, oocytes obtained via the ovum pick-up method of collection also are used by commercial IVF companies. Commercial embryo production companies, such as Sexing Technologies, Inc. (Navasota, TX), however, have an added interest in utilizing oocytes from slaughterhouse ovaries for in vitro embryo production. Much of the income generated through the use of these oocytes for embryo production comes from exporting embryos to foreign countries with developing beef and dairy production systems.

Use of slaughterhouse oocytes for in vitro embryo production is appealing to commercial IVF companies from the standpoint that they provide a large, cost-efficient source of ova. Ability to collect more oocytes in a shorter time frame relative to ovum pick-up, without maintaining a population of donor females, makes slaughterhouse ovaries an attractive source of oocytes. Management and genetic background of the donor female is often unknown. Age of the donor, nutritional status before slaughter, and the stage of her estrous cycle, all have the potential to alter the outcome of IVF. In addition, the process of in vitro embryo production itself has many critical control points that could influence the number of transferable embryos produced. For instance, time from slaughter to follicle aspiration, the oocyte maturation process, and overall handling techniques influence the ability to produce embryos of sufficient quality to

be used for transfer to recipient females. All of these variables can impact the quality and number of oocytes collected and the degree of IVF success.

Harvested beef feedlot heifers may have the most potential as an abundant source of oocytes for use with in vitro embryo production; however heifer management techniques at the feedlot may influence oocyte quality, quantity and subsequent in vitro embryo production. Beef heifers traditionally maintained in the feedlot for the purpose of entering the food chain are administered melengestrol acetate (MGA), a feed additive that suppresses estrus, in addition to implantable steroid-based growth promotants. The effect of these 2 substances on oocyte quality and in vitro embryo development has not been thoroughly researched. The objective of this review is to discuss important factors that potentially affect the success of in vitro embryo production, as well as evaluate the physiological effects of conventional feedlot heifer management practices on in vitro embryo production.

Factors Affecting the Success Of In Vitro Embryo Production

Source of oocytes used for embryo production can differ with regard to fertility status of the donor, breed, age, method of oocyte collection, and handling techniques involved (Merton et al., 2003). Often, the genetic makeup, nutritional background, and age of the donors are unknown when oocytes from slaughterhouse ovaries are used for in vitro embryo production. In ovum pick-up procedures, however, the donor female is most often of elite genetics and the management practices employed before follicle aspiration can be more carefully controlled to ensure optimal results. These factors and management practices likely cause variation in blastocyst rates (the percentage of zygotes achieving blastocyst stage of development) between embryo production using slaughterhouse ova and ovum pick-up. Differences in blastocyst results from laboratories that perform in vitro embryo production using slaughterhouse oocytes

range from 15 to 60% blastocyst rate at d 7 post-IVF. Blastocyst rates for ovum pick-up in in vitro embryo production are less variable than in vitro embryo production using slaughterhouse oocytes, ranging from 10 to 30% at d 7 post-IVF (Merton et al., 2003). Factors influencing the outcome of IVF, including follicular and donor management aspects, will be discussed in this section.

Maturation of the Oocyte

Perhaps the most critical factor affecting the success of in vitro embryo production is the maturation of the oocyte. Maturation of the oocyte occurs in 2 stages; the first stage occurs prenatally, with the primordial germ cell entering into a series of mitotic and meiotic divisions. After the primary oocyte is formed, it enters into nuclear arrest, where it temporarily stops developing. In the second stage, the nuclear arrest is removed once the follicle becomes dominant and the LH surge has occurred. The oocyte then continues to mature in vivo and has the ability to be fertilized (Senger, 2003). Ovaries contain antral follicles including a dominant follicle(s). Antral follicles (from slaughterhouse ovaries) contain oocytes that are not fully mature. If the LH surge occurred before slaughter, then it is likely that the oocyte from the preovulatory follicle is over-matured. With so many potential degrees of oocyte maturation upon oocyte harvest, the in vitro maturation process becomes a critical step in the embryo production system.

In the bovine estrous cycle, approximately 80% of all ovulated oocytes that are fertilized will develop to the embryo stage in vivo (Merton et al., 2003). The outcome for oocytes matured in vitro is not as high. Within in vitro embryo production systems, approximately 30 to 40% of harvested oocytes are fertilized and achieve the blastocyst stage of development (Rizos et al.,

2002). In a study evaluating the development potential of oocytes obtained from slaughterhouse ovaries matured in vitro versus oocytes from superovulated heifers that were later slaughtered and matured in vivo, it was reported that fertilization rates were greater for those matured in vivo (Leibfried-Rutledge et al., 1987). In addition, oocytes matured in vitro that were later fertilized, resulted in a lesser percentage of normal 2 to 4 cell embryos compared with oocytes matured in vivo; in vitro matured oocytes also failed to develop to the morulae or blastocyst stage of embryo development, unlike the oocytes matured in a normal follicular environment (Leibfried-Rutledge et al., 1987). In another study by Rizos et al. (2002), similar results were reported. A greater percentage of oocytes matured in vivo achieved the blastocyst stage 7 d post-IVF compared with oocytes harvested from slaughterhouse ovaries and matured in vitro (48.5% vs. 31.7%, respectively). These studies indicate that the natural follicular environment provides ideal conditions for oocyte maturation to ultimately allow successful blastocyst development post-fertilization; however, it is possible for oocytes to mature in vitro with reduced development to the early embryo stages.

Effect of Follicle Size on Oocyte Quality

Follicle size is associated with the maturation stage of the oocyte contained therein. In spontaneous bovine estrous cycles when large dominant follicles are approaching ovulation, the preovulatory LH surge removes the nuclear inhibitors that prevent oocyte maturation from occurring before ovulation, and oogenesis resumes. Smaller subordinate follicles contain oocytes that are further from maturity than oocytes within dominant, preovulatory follicles. Therefore, follicle size can play a pivotal role in the capability of the contained oocyte to mature in vitro and sustain embryogenesis.

The effect of follicle size on IVM and IVC was evaluated in a study by Lonergan et al. (1994). Ovaries were collected from postpubertal, nonpregnant heifers shortly following slaughter. Two categories of follicles were collected: 2 to 6 mm and greater than 6 mm in diameter. Oocytes were then classified into the following categories based on the presence or absence and (or) appearance of the cumulus cell mass:1) denuded, 2) expanded cumulus cells, 3) 2 or 3 layers of cumulus cells, 4) 4 or 5 layers of cumulus cells, and 5) many layers of compact cumulus cells. Authors found that the 2 to 6 mm follicle group yielded a greater number of oocytes compared with the group of follicles greater than 6 mm in diameter. In contrast, larger follicles (> 6 mm) yielded higher-quality oocytes that had more than 4 layers of cumulus cells (Lonergan et al., 1994). Similar cleavage and hatching rates were observed during embryogenesis when comparing oocytes originating from small (2 to 6 mm) and large (> 6 mm) diameter follicles, but oocytes obtained from large follicles (> 6 mm) produced more blastocysts compared with oocytes originating from smaller follicles (2 to 6 mm; Lonergan et al., 1994). These results indicate that oocyte quality is related to follicle size and affects overall embryo yield.

Hagemann et al. (1999) found that blastocyst rates were similar for oocytes harvested from follicles that were 3 to 5 mm in diameter compared with follicles that were 6 to 8 mm in diameter. They also found that blastocyst rates were greater when oocytes were collected from dominant follicles that were greater than 13 mm in diameter compared with oocytes collected from growing follicles (3 to 8 mm diameter; Hagemann et al., 1999). To an extent, these results agree with those reported by Lonergan et al. (1994), in that the larger follicles (> 6 mm diameter) yielded greater blastocyst rates; however Lonergan et al. (1994) did not specify the extent to which follicles were larger than 6 mm in diameter, so it is difficult to make a direct comparison.

Hagemann et al. (1999) used dominant follicles that contained oocytes that were likely more mature than those contained in smaller follicles. Also in agreement with Lonergan et al. (1994) were the results from Tan and Lu (1990). Authors reported that oocytes from large follicles (> 6 mm) that were subsequently fertilized had more blastocysts compared with fertilized oocytes from follicles < 6 mm in diameter. McCaffrey et al., (1992) also reported that oocytes collected from follicles 6 to 8 mm in diameter had a greater potential for development than follicles < 6 mm. It seems that the ideal follicle size for in vitro embryo production based on the development of the subsequent embryo is greater than 6 mm in diameter. Groups of follicles smaller than this may yield more oocytes, but their potential for development in vitro post-fertilization is compromised.

Oocyte Quality

Oocyte quality is typically determined by the structure of the cumulus cell mass surrounding the oocyte. Cumulus cells are granulosa cells that surround the oocyte in an antral follicle. These cumulus cells serve as a communication medium to the oocyte and are critical in preventing premature oocyte maturation, as well as aid in initiating resumption of meiosis, and support maturation of the ooplasm (Tanghe et al., 2002). Quality of the oocyte is generally assessed based on the morphology of these cumulus cells. Several classification systems exist for assessing cumulus-oocyte quality, but in general, a compact and bright cumulus cell mass is most desirable. Cumulus cells that are slightly expanded tend to have the next greatest quality, followed by cumulus-oocyte complexes that show great expansion and signs of degeneration (Wurth and Kruip, 1992; Blondin and Sirard, 1995).

Oocytes from slaughterhouse ovaries tend to have better quality than oocytes collected via ovum pick-up, according to classification systems, with slaughterhouse oocytes having a

tight cumulus-oocyte complex (Merton et al., 2003). It has been reported that cumulus cells displaying slight expansion actually have greater blastocyst rates (Blondin and Sirard, 1995), indicating they may be more competent for early embryo development compared with oocytes having a tight cumulus cell mass. The reason for oocytes collected from slaughterhouse ovaries having a tighter cumulus-oocyte complex may result from post-mortem effects. Mullaart et al. (1999) suggested that following slaughter, the cumulus-oocyte complex becomes less tightly connected to the follicle wall, allowing the cumulus cells to be collected by technicians in a more complete fashion without sacrificing morphology. Therefore, while the cumulus cells and oocyte are of better quality initially, these post-mortem effects need to be maintained throughout the entire in vitro embryo production process in order to have successful early embryo development.

Effect of Time and Temperature on Oocyte Quality

Other post-mortem effects associated with cumulus-oocyte quality include temperature and the length of time the oocytes remain in the ovary post-slaughter. Blondin et al. (1997) found that oocyte aspiration 4 h post-slaughter was optimal for embryo production. Any shorter (2 h) or longer period (6 or 7 h) of time between animal harvest and oocyte retrieval decreased the number of 64-cell embryos, suggesting that oocytes are subject to the changing follicular environment of the post-mortem ovary (Blondin et al., 1997). If oocyte aspiration occurs too early or too late relative to slaughter, the responsiveness of the oocyte to culture conditions is lessened. In a similar study, it was determined that cleavage and blastocyst rates were significantly decreased if ovaries are stored for 8 h at 37°C (Yang et al., 1990) compared with ovaries that are stored at 25°C for 8 h.

Effect of Donor Age on In Vitro Embryo Production

In vitro embryo production utilizing ovaries obtained from a slaughterhouse includes a significant variation in the age of the donors being used. Relatively little research has been conducted regarding the effect of donor age on in vitro embryo production using slaughterhouse oocytes, and what has been reported, has been contradictory. In a study by Mermillod et al. (1992), oocytes from female cattle ranging in age from 1 to 3 yr yielded greater blastocyst rates compared with oocytes obtained from cows that were older than 4 yr. Similarly, heifers ultimately yielded a greater percentage of viable embryos through the processes of IVM, IVF, and IVC than cows (Lonergan et al., 1990;, Moreno et al., 1992), whereas oocytes obtained from cull cows and then fertilized in vitro, yielded more freezable and thus transferable embryos than oocytes obtained from heifer ovaries (Galli et al., 2003). In that same study, the authors reported that through the process of ovum pick-up during a 4-yr period, cow oocytes produced twice as many freezable and transferable embryos per cow per ovum pick-up session compared with heifers (Galli et al., 2003).

In a study by Rizos et al. (2005), 1 experiment was devoted to evaluating differences between crossbred beef heifers and beef cows in the number of ovarian follicles collected, oocyte morphology, and the developmental competency of those oocytes. Oocytes were obtained from the ovaries of slaughtered crossbred beef heifers (< 30 mo of age) and beef cows (> 4 yr of age), and oocyte numbers, cleavage and blastocyst rates on d 6, 7, and 8 post-IVF were recorded. The number of oocytes collected and cleavage rate did not differ between age groups. In contrast, blastocyst rates on d 7 and d 8 post-IVF were greater for oocytes from cows than from heifers. Based on the results from this experiment, a second experiment was conducted to determine if heifer age affected oocyte quality. Heifers were categorized into 1 of 3 age groups:1) 12 to 18 mo, 2) 19 to 24 mo, and 3) 25 to 30 mo. Ovaries were collected at slaughter from heifers as well

as from slaughtered cull cows (> 4 yr of age). Heifer age did not affect number of oocytes harvested, cleavage rate, or blastocyst yield. In addition, oocytes from heifers, regardless of their age, resulted in lesser blastocyst yields on d 8 post-IVF than oocytes from cows. Earlier published results indicated that heifers have greater success with overall oocyte yield and subsequent early embryo development compared with cows (Lonergan 1990; Moreno et al., 1992; Mermillod et al., 1992); however, more recent studies (Galli et al., 2003; Rizos et al., 2005) reported that cows older than 4 yr produce more oocytes and greater blastocyst yields. Contradicting evidence makes it difficult to draw conclusions; however, older females that have been mature for a longer period of time may be more reproductively efficient and therefore yield greater quantities of higher quality oocytes.

Effect of Donor Nutrition on In Vitro Embryo Production

Nutritional status of a breeding female is a major factor influencing her ability to reproduce. It is well established that nutrition affects a variety of reproductive functions including hormone production, fertilization, and early embryonic development (Boland and Lonergan, 2005). Although nutritional impacts on oocyte quality are not generally considered at the feedlot level, it might be of interest when feedlot heifers are to be used as donors for commercial in vitro embryo production. Generally, feedlot cattle are fed a high-concentrate, high-energy finishing diet that allows them to have optimal performance at the feedlot as well as optimal carcass quality and yield. Yaakub et al. (1999) reported that beef heifers that had been stimulated with FSH before slaughter and were fed silage ad libitum had greater cleavage rates than beef heifers fed silage ad libitum with 6 kg of added concentrate per day. Nolan et al. (1998) found that under restricted dietary intake, beef heifers yielded greater cleavage and blastocyst rates than control heifers fed a high-energy diet. Postpartum lactating dairy cattle that

do not consume adequate energy to sustain production and maintenance requirements will suffer reproductively as well. Postpartum dairy cows that have greater negative energy balances have longer periods of anovulation and more prolonged days to conception (Staples and Thatcher, 1990). Similarly, beef cows in poor body condition can have decreased fertility (prolonged anestrous) because they are deficient in energy and protein to support maintenance and reproduction. In addition, the number of large follicles in postpartum dairy cows, as well as oocyte quality, has been found to be proportional to improved energy balance and greater body condition scores (Kendrick et al., 1999).

In a study investigating nutritional effects on oocyte and embryo development of sheep, it was found that overfed (200% above control diet; ad libitum) and underfed (60% of control diet) ewes produced fewer zygotes, morula, and blastocyst stage embryos, and had poorer cleavage rates than ewes consuming a control diet (Grazul-Bilska, et al., 2006). Underfed ewes tended to yield fewer oocytes that were fertilized and then achieved the blastocyst stage than overfed ewes (Grazul-Bilska, et al., 2006). From these studies, females fed low-energy diets or females that cannot consume adequate amounts of energy may be more likely to produce embryos that achieve blastocyst stage compared with those animals on a high-energy diet. Therefore, feedlot heifers on high-energy finishing diets may be less likely to produce as many blastocyst stage embryos in vitro than other females fed less energy-dense diets.

Feedlot Management Practices Potentially Influencing In Vitro Embryo Production

Feedlots across the country commonly incorporate melengestrol acetate (MGA) and steroid-based growth promoting implants into their feeding and management regimens when finishing beef heifers. These substances are steroid-based, and therefore alter the function of the

hypothalamic-pituitary-ovarian axis. In a normal bovine estrous cycle, GnRH is secreted in a pulsatile manner from the hypothalamus and causes subsequent release of the gonadotropins (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) from the anterior pituitary. These 2 gonadotropins are involved in stimulating growing ovarian follicles to synthesize estradiol. Once estradiol reaches threshold concentrations, it causes a surge release of GnRH from the hypothalamus. This surge of GnRH causes a subsequent surge of LH that causes ovulation. A negative feedback control is in place when progesterone concentrations from the corpus luteum are elevated and follicle-derived estradiol is limited, preventing surge releases of GnRH. When steroid-based substances (i.e., MGA and growth promotants) are administered, they increase circulating blood concentrations of progestin or progesterone, estrogen, or testosterone. Increases in concentrations of these steroids can alter the normal function of the hypothalamic-pituitary-ovarian axis.

Melengestrol Acetate

Melengestrol acetate (MGA) is an oral progestin that is commonly used in estrus-synchronization protocols for both dairy and beef heifers. This orally-administered progestin is also used in feedlots across the U.S. to suppress estrus, which, if not inhibited, can be a disturbance to other animals in the feedlot and can decrease growth performance of nonestrous cattle (O'Brien et al., 1968). When offered to feedlot heifers, MGA is administered in the form of a feed additive at a dosage of 0.5 mg/hd/d (Zimbelman and Smith, 1966). In addition to being an effective estrus suppressant, MGA also has been shown to be effective at increasing weight gains and feed efficiency by 7 to 10% (Glimp and Cundiff, 1971). These production responses are associated with elevated blood estrogen concentrations that occur in postpubertal ovarian function (Bloss et al., 1966; Zimbelman and Smith, 1966).

It is well established that administration of MGA at appropriate doses will prevent the display of estrus and inhibit ovulation. In a study by Zimbelman and Smith (1966), heifers that were determined to be pubertal by ovarian palpation per rectum, were fed MGA (0.5 mg/hd/d) during a period of 106, 110, 113, or 117 d. Incidence of a progesterone-secreting corpus luteum (CL) at the start of MGA treatment was 73%, and by the end of the study, none of the heifers had a CL present in their ovaries. In addition, while the presence of corpora lutea decreased, the incidence of detectable follicles increased from 20 to 100% of treated heifers having ovaries that contained a large follicle (Zimbelman and Smith, 1966). This shift in follicular structures during the treatment period indicated complete inhibition of ovulation in response to treatment with MGA.

Melengestrol acetate prevents ovulation from occurring. Naturally, progesterone is secreted from the CL that develops after ovulation occurs. Progesterone asserts negative feedback on the hypothalamus, inhibits production and release of GnRH, and thus, also inhibits release of large or surge quantities of LH and FSH from the anterior pituitary (Senger, 2003). During the diestrous stage of the estrous cycle progesterone dominates hormonally. Although progesterone concentrations remain elevated during diestrus, follicular development still occurs via FSH and LH stimulation from the anterior pituitary. In a natural estrous cycle, when the female is not pregnant, $PGF_{2\alpha}$ is secreted by the uterus to start luteolysis (death of the CL), which occurs before the onset of estrus (Senger, 2003).

Persistent Follicles

In a typical MGA protocol used for synchronization of estrus, females are administered MGA for an extended period of time (usually 14 d). During this time, MGA prevents ovulation of a developing follicle by partial suppression of LH pulses from the anterior pituitary (Kinder et

al., 1996). In contrast, synthetic progestins, including MGA, when given to cows at the recommended dosage and in combination with commercial estrogens for the purpose of synchronizing estrus, fail to suppress the LH pulse frequency to the same extent as naturally occurring progesterone from the CL (Kinder et al., 1996). As a result, persistent ovarian follicles develop. Compared with normal developing ovarian follicles, persistent follicles will grow to a larger size and remain in the ovary for a longer period of time (Lucy et al., 1990). Cupp et al. (1993) found a greater number of LH receptors on granulosa and theca cells of the developed persistent follicle. It is thought that more LH receptors on the persistent follicle results from a greater frequency of LH pulses from females with persistent follicles on their ovaries (Cupp et al., 1993). Therefore, MGA treatment and a continued LH pulse pattern could be the main driver behind development of persistent ovarian follicles (Kinder et al., 1996).

Early research involving MGA and ovarian development suggested that minimal doses of an exogenous progestin (i.e., MGA) cause an increase in follicular weight (Zimbelman, 1963), larger diameter follicles (Ulberg et al., 1951; Trimberger and Hansel, 1955), or atretic follicles (Guthrie et al., 1970). In addition to causing similar or more frequent LH pulses (in comparison with a similar stage of an untreated estrous cycle) minimal progesterone dosages can also work to maintain follicles (Yelich et al., 1997), which naturally leads to prolonged secretion of estradiol-17-β from the preovulatory follicle. Greater concentrations of estradiol that persist for longer periods of time have been shown to be related to decreased fertility (Wehrman et al., 1993) and abnormal embryo development (Breuel et al. 1993). Ahmad et al. (1995) found that 43% of embryos resulting from oocytes ovulated from persistent follicles, contained 2 to 8 cells on d 6 post-mating, and 43% had 9 to 15 cells on d 6 post-mating, compared with 86% of embryos produced from growing follicles (non-persistent) having greater than 16 cells on the

same day. These data indicated that death of the embryo in very early stages may be the reason for decreased pregnancy rates in cattle that ovulated a persistent follicle or whose estrous cycle is synchronized using low doses of progestins (Ahmad et al., 1995). Likewise, oviductal or uterine environment exposed to extended periods of estradiol could be harmful to early embryonic development (Ahmad et al., 1995). In a similar study, Wishart and Young (1974) found that applying norgestomet implants (another type of synthetic progestin) in beef cows for more than 21 d resulted in delayed cleavage of the early embryo providing support to the idea that persistent follicles producing estradiol for extended periods of time can lead to early embryonic death. Although administration of MGA to beef feedlot heifers has a different purpose than synchronizing estrus in breeding females, its long-term use could have potential detrimental effects on subsequent embryo development.

Growth Promotants

Use of growth-promoting implants in feedlot cattle is a common practice employed by most feedlots in the U.S. Very little research exists on the effects of growth-promoting implants on oocyte quality for use in in vitro embryo production systems because these substances are not typically administered to breeding females. Oocytes from breeding females collected via ovum pick-up are not a concern because they are not exposed to growth implants. In contrast, with the increasing need for large sources of oocytes and slaughterhouse ovaries serving that purpose, evaluation of potential effects of growth-promoting implants on oocyte quality and embryo production is not known.

A majority of growth implants contain estrogenic or androgenic activity, or a combination of the two (Kreikemeier and Mader, 2004). Benefits of implanting finishing cattle include increased average daily weight gains and protein deposition (Popp et al., 1997). An 8 to

20% increase in average daily gain can be achieved depending on the sex of the animal, implant method, and duration of the feeding period (Anderson, 1990). Several types of promotants and feed additives are available for use in feedlot management programs specific to either steers or heifers. Trenbelone acetate (TBA) is a potent analog of the sex steroid testosterone and has been shown to be 8 to 10 times more anabolically active than testosterone (Bouffault and Willemart, 1983). Administered either independently or in combination with MGA, growth promotants elevate circulating blood steroid concentrations, including testosterone and estrogen, in both steers and heifers. Increased concentrations of circulating sex steroids could negatively impact the development of a young embryo. It is thought that elevated concentrations of estradiol are a potential reason for reduced conception rates in cows (Wehrman et al., 1993). Greater steroid concentrations, such as estradiol, are associated with alterations in oocyte maturation and related structural changes of the oocyte that could be detrimental to successful fertilization (Wehrman et al., 1993).

It has been reported that administration of TBA and estradiol implants (a common feedlot management practice) is detrimental to pubertal development in heifers. Trenbolone acetate and estradiol implants have been reported to limit or reduce gonadotropin secretion from the anterior pituitary (Moran, 1988). Early work involving the use of anabolic steroids in heifers showed that puberty was delayed in dairy heifers compared with those heifers not implanted (Heitzman, et al., 1979). Moran et al. (1990) also reported that beef heifers implanted with TBA and estradiol had delayed puberty and fewer ovulations than those heifers not treated with TBA and estradiol. In addition, the first ovulation that did occur in those heifers coincided with the time the blood concentrations of TBA and estradiol implant would have dissipated (Moran et al., 1990). Similarly, TBA also has been shown to inhibit LH secretion from the anterior pituitary in

castrated bulls (Gettys et al., 1984). Inhibition of the gonadotropins, in combination with elevated concentrations of androgen (TBA) and estradiol (implants and MGA) may have deleterious effects on oocyte quality and subsequent in vitro embryo production. It is likely that, because the hypothalamic-pituitary axis is altered by use of growth-promoting implants, ovarian follicular dynamics and follicular environments are changed, and thus, quality and quantity of retrievable oocytes could also be compromised.

Summary

In vitro embryo production is becoming an increasingly popular technology as the demand for production of progeny from cattle with high genetic merit continues to grow. The practical application of utilizing beef feedlot heifers as a source of oocytes for large-scale in vitro embryo production is an attractive and economically feasible option for commercial IVF companies. Management practices that are commonly employed at the feedlot, however, may be detrimental to oocyte quality and subsequent embryo development in vitro. The effectiveness of using MGA as part of an estrous synchronization program has been demonstrated, but its effects, in combination with steroid-based growth promotants, on in vitro processes have yet to be determined. Furthermore, several important factors exist that play a crucial role in the success of in vitro embryo production including age and nutritional status of the donor female, oocyte maturation, including the time and temperature of this process, and the size of the follicle from which the oocyte originates. All of these important determinants may influence the outcome of in vitro embryo production.

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Chapter 2 - Effect of Melengestrol Acetate and Growth Promotants on Oocyte Yield, Quality, Fertilization Rate, and Developmental Competence of In Vitro Embryo Production in Beef Heifers

Abstract

Oocyte quality affects success of in vitro embryo production and is influenced by factors such as age, follicle size, and reproductive and nutritional status of donor females**.** Beef feedlot heifers have potential to serve as viable donors of oocytes post-slaughter for in vitro embryo production; however, it is unknown if traditional feedlot heifer management practices of feeding melengestrol acetate (MGA) and using steroid-based growth promotants impact oocyte numbers and quality**.** The objective was to determine if feedlot heifers fed MGA and implanted with growth promotants could serve as viable donors for in vitro embryo production. Oocytes recovered, oocyte loss, fertilization rate, total embryos and stages of early embryo development were measured through d 7 post in vitro fertilization (IVF)**.** Three hundred and fifty beef feedlot heifers were fed either MGA (0.05 mg/hd/d) and implanted with growth promotants (Revalor IH, 80 mg of trenbolone acetate, 8 mg of estradiol; MGA-Implant) during a 120 d period before slaughter, or served as untreated controls (not fed MGA nor implanted with growth promotants during 120 d before slaughter; Control). Heifers were slaughtered over 2 days and ovaries were obtained post-harvest. Because treatment identification was lost, ovaries from the first day of heifer harvest were grouped based on the presence of ovarian structures. Ovaries with a corpus hemorraghicum (CH), corpus luteum (CL) or corpus albican (CA) indicating that the heifer had ovulated at least once were maintained retrospectively as controls, and heifers lacking a CH, CL, or CA were categorized as the MGA-Implant group. Ovaries from heifers harvested the second

day were grouped (14 to 18 ovaries per group) by time of slaughter within treatment for oocyte collection (MGA-Implant: n=88; control: n=84). Aspirated oocytes were put into maturation media within 6.5 h post-slaughter and fertilized 24 h later. Data was not statistically analyzed from d 1 because of treatment misidentification and technical problems with the shipping containers that were used. On the second day, treatment and time interacted ($P = 0.06$) to affect number of oocytes yielded and zygotes produced per ovary**.** A similar ($P > 0.10$) percentage of zygotes cleaved by d 2 post-IVF (MGA-Implant: 46.8, Control: 47.9). A similar ($P > 0.10$) percentage of embryos per ovary resulted for both treatments (MGA-Implant: 0.34%; Control: 0.39%). In conclusion, beef feedlot heifers fed MGA and implanted with growth promotants seem to be a viable source of oocytes for in vitro embryo production**.**

Introduction

In today's commercial in vitro fertilization (IVF) industry, bovine ovaries obtained from a commercial slaughterhouse are used for in vitro embryo production. Gordon and Lu (1990) indicated that oocytes attained from slaughterhouse ovaries for the use of in vitro embryo production are highly heterogeneous in their quality and developmental competence. Origin, age, or genetic background of the donor females is often unknown and makes it difficult to determine their effects on oocyte quality. Beef feedlot heifers provide an opportunity for a source of oocytes to be utilized for IVF post-slaughter. Potential effects of standard feedlot practices on the growth, recovery, and developmental competence of harvested oocytes harvested from feedlot heifers, however, are questions of concern for IVF utilizing slaughterhouse oocytes (Merton et al., 2003; Rizos et al., 2005).

Melengestrol acetate is an oral progestin that has proven to be effective in reproductive management, including estrous synchronization (Patterson et al., 1989) and is also capable of

improving feedlot heifer performance and feed efficiency (Bloss et al., 1966). In a conventional feedlot system, heifers are administered melengestrol acetate (MGA) to suppress estrous behaviors, which can be a disturbance to other animals in the feedlot, causing them to have reduced growth and poorer feed efficiencies (O'Brien et al., 1968). Administration of MGA to beef heifers at a dose of 0.5 mg/hd/d suppresses estrus (Bloss et al., 1966; Zimbelman and Smith, 1966; O'Brien et al., 1968) by inhibiting the preovulatory surge of luteinizing hormone (LH). Feeding of MGA may lead to changes in ovarian characteristics, including increased ovarian weights, a greater number of large follicles (> 12 mm diameter), and subsequently, increased volume of follicular fluid (Zimbelman and Smith, 1966).

Another effect of feeding MGA is the development of persistent follicles. Persistent follicles develop when synthetic progestins are administered allowing an increase in LH pulse frequency when there is no negative feedback of endogenous progesterone from an endogenous corpus luteum (CL; Yelich, et al., 1997). Melengestrol acetate does not suppress LH pulse frequency from the anterior pituitary to the same degree as endogenous concentrations of progesterone or another progestin. Because of increased LH pulse frequency, follicles develop and persist on the ovary, and they continue to produce estradiol in response to LH (Custer et al., 1994). In breeding programs that utilize MGA as a method to synchronize estrus, estradiol concentrations may be elevated for a prolonged period before ovulation. Prolonged increases in estradiol during the estrous cycle have been linked to decreased fertility and early embryonic death (Ahmad et al., 1995). Retarded growth of the embryo and a reduction in implantation rates have been reported in conjunction with elevated estradiol concentrations in rats (Butcher and Pope, 1979). Similarly, Ahmad et al. (1995) attributed early embryonic death to high intrafollicular concentrations of estradiol that may have altered oocyte maturation. Oocyte

maturation resumes within the follicle once the preovulatory surge of LH has taken place. Large concentrations of estradiol may not only alter the oocyte maturation process within a persistent follicle, but could potentially affect other growing subordinate follicles that are utilized for IVF either directly via altered estradiol profiles, or by indirect effects on the pattern of gonadotropin secretion.

Generally, a majority of growth implants that are utilized in feedlots have a combination of estrogenic and androgenic activity (Kreikemeier and Mader, 2004). Trenbolone acetate (TBA) and estradiol are commonly administered together in one implant. The inclusion of TBA with estradiol allows for a slower release rate of estradiol, thus extending its effectiveness in steers (Brandt, 1997). Implants generally have a lifespan of approximately 100 d (Gill, 1997). Trenbolone acetate has been characterized as anti-estrogenic and anti-gonadotrophic (Neumann, 1976). Reynolds et al. (1981) concluded that TBA could potentially alter the estrous cycle by acting directly in its androgen nature, inhibiting LH secretion through indirect anti-estrogenic activity, or directly through anti-gonadotrophic activity. Similarly, Moran (1988) reported reduced LH and FSH secretion from the anterior pituitary when anabolic steroids are given to beef heifers. Administration of the combination growth promotants (TBA + estradiol) has been shown to delay puberty in beef and dairy heifers (Heitzman et al., 1979; Moran et al., 1990). It is likely then, that if administration of TBA + estradiol implants alters secretion of the gonadotropins, follicle growth is compromised and ovulation is prevented in beef heifers, potentially altering oocyte quality.

The effects of these steroid-based growth promotants in combination with the effects of administering MGA on oocyte quality, ability to be fertilized and early embryo development are unknown. The objectives of the study were to compare yield and quality of oocytes of beef

feedlot heifers administered MGA and steroid-based growth promotants with oocytes from heifers not receiving either substance and to determine potential effects on oocyte fertility and subsequent early embryonic development.

Materials and Methods

Heifer Management

Three hundred and fifty crossbred yearling beef heifers of various backgrounds were maintained at the Kansas State University Beef Cattle Research Unit according to the Institutional Animal Care and Use Committee (IACUC) standards. Heifers (initial BW = 374 ±0.6 kg) were housed in 48 concrete-surfaced pens (36 m^2) containing 6 to 8 heifers per pen. Heifers were blocked by previous growing treatment and assigned randomly to either of 2 treatments: 1) heifers administered MGA (0.5 mg/hd/d) and implanted with growth promotants (Revalor-IH; 80 mg trenbolone acetate and 8 mg estradiol; Intervet Inc., Millsboro, DE; MGA-Implant), or 2) heifers did not receive either MGA or growth promotants (Control). Heifers were fed a finishing diet for 120 d. MGA-Implant heifers were fed MGA for the entire 120 d finishing period and implanted once with Revalor-IH at the start of the finishing period.

Ovary Collection

Heifers were harvested at a commercial abattoir in Holcomb, Kansas on 2 consecutive days. Because of the nature of a commercial harvest facility and the speed with which they operate, not all ovaries were recovered from each heifer. Pairs of ovaries were collected from most heifers; however, in some cases, only one or no ovary was obtained. The following paragraphs describe each day of heifer harvest and ovary collection.

Day One

One hundred and seventy-eight heifers were slaughtered on the first day of ovary collection. Heifer identification was lost during processing at the abattoir, and in turn, correct identification of treatment was lost. Because it is well established that heifers fed 0.5 mg of MGA/hd/d do not ovulate (Bloss et al., 1966; Zimbelman and Smith, 1966; O'Brien et al., 1968), ovaries were assigned retrospectively to either of 2 categories based upon the presence or absence of specific ovarian structures. Control heifers not fed MGA were expected to have ovaries with either a corpus hemorrhagicum (CH), corpus luteum (CL), or a corpus albican (CA), indicating that they had ovulated at least once and were maintained as the control (n = 53). Ovaries lacking a CH, CL, or CA (n = 100) were assumed to be in the MGA-Implant treatment. Ovaries were placed in individual plastic bags containing a small amount of phosphate-buffered saline (PBS) solution maintained at 35 to 37°C until all ovaries had been collected. During ovary collection, we obtained either pairs of ovaries, single ovaries, or did not collect any ovaries from heifers, due to loss of the animal identification and speed of the production line.

Day Two

Heifers (n= 172) were harvested and pairs of ovaries were obtained in random order. Ovaries were labeled by treatment and were assigned a group number based on the time of heifer harvest (8 ± 1 pairs of ovaries per time period). The time that oocytes spend in the follicle post-slaugther has shown to significantly affect the quality of the oocyte and subsequent in vitro embryo development (Blondin et al., 1997, Yang et al., 1990). Therefore, the time from heifer harvest at the abattoir to the time that all oocytes were put into maturation media was accounted for in data analyses. Heifers harvested first were the first to have oocytes collected from their ovaries and were first to have oocytes put into maturation media (oocytes were maintained for less time in ovary but longer in the holding media). Conversely, heifers harvested last were last

to have oocytes aspirated and put into maturation media (oocytes were maintained for a longer time in the ovary but less time in holding media). The MGA-Implant treatment contained ovaries from heifers (n = 88) given MGA and implanted with growth promotants (n=11 time groups), whereas ovaries from heifers not receiving either MGA or growth promotants (n = 84) were controls (n = 11 time groups). Ovaries were evaluated for the presence of ovarian structures, such that ovaries with a CH, CL, or CA were identified. All pairs of ovaries from heifers in the MGA-Implant treatment lacked a CH, CL, or CA, whereas all pairs of ovaries from the control treatment displayed a CH, CL, or CA. These observations are consistent with early research illustrating that heifers fed MGA at a level of 0.5 mg/hd/d do not ovulate, and therefore would not have a CH, CL or CA present on their ovaries (Zimbelman and Smith, 1966). Our classification on d 2 also verifies our categorization from the first day of collection. Some error in this classification is still possible for heifers assigned to the MGA-Implant treatment that may have ovulated before feedlot treatments were imposed.

Oocyte Recovery and Culture

On both days, and within 45 min of heifer harvest, ovaries were rinsed twice in warm saline solution (Sexing Technologies, Inc., Navasota, TX) and kept in a water bath (35°C) until oocytes were aspirated. Oocytes were aspirated into 50 mL conical tubes from follicles 3 to 8 mm in diameter (estimated visually) by using a vacuum pump system and 21-g butterfly needles (3.81 cm). Oocytes from d 1 were maintained in the categories they were assigned post-slaughter based on ovarian structures, whereas oocytes from d 2 were maintained by time group identification within treatment. Oocytes were rinsed twice in TL-HEPES media (Sexing Technologies, Inc., Navasota, TX), placed in 60 mm petri dishes, and evaluated under a microscope at 400 x magnification. Any oocytes that were denuded, had discolored cytoplasm,

or appeared to be degenerating, were discarded. Remaining oocytes were placed in M199 holding media (Sexing Technologies, Inc., Navasota, TX) in a 35-mm petri dish until all remaining oocytes had been aspirated and evaluated.

Between 5 and 6 h post-slaughter, all collected oocytes were put into 1 mL maturation media (70-100 oocytes/mL; Sexing Technologies, Inc., Navasota, TX) for in vitro maturation (IVM). Oocytes were incubated overnight in cryovials at 39°C under a 5% CO_2 atmosphere and shipped to the Sexing Technologies laboratory (Laceyville, PA). Each cryovial contained between 70 and 100 oocytes. Oocytes were allowed to mature for 23.5 h.

One of the shippers from the first day of oocyte collection malfunctioned during the shipping process, and therefore all oocytes categorized as controls were lost. Thus, a treatment comparison could not be made on the number of matured oocytes, fertilized oocytes, or total embryos. All oocytes that were not damaged during shipping were subjected to IVF; however, because a large portion of the control categoryt oocytes were lost, statistical analysis could not be conducted for d 1 oocytes.

In Vitro Fertilization and Early Embryo Development

Upon arrival the Sexing Technologies, Inc. laboratory, all oocytes were put into fertilization media (50 oocytes/150 µL of media; Sexing Technologies, Inc., Navasota, TX) and fertilized with semen from a Holstein bull at 1.0 x 10^6sperm/mL. After 18 h in fertilization media, the presumptive zygotes were washed twice and cumulus cells were removed from zygotes. Any zygotes that had shrinking cytoplasm, cracked zona pellucidas, or were lacking clear polar bodies were not used. Remaining zygotes were placed in culture media for 7 d. On d 2 post-IVF, developmental stages were assessed, including the number that achieved 1-cell, 2 to

4-cell, 8-cell stages of development or were lysed. Any cells that had not divided by d 2 post-IVF were discarded.

On d 7 post-IVF, embryo grades were assigned using Sexing Technologies, Inc. method of grading. Morula, early blastocyst, and blastocyst stages of development were classified as C2 embryos (insufficient quality for freezing); C1- embryos (blastocyst or expanded blastocyst stages that were freezable); and C1 embryos had a very compact inner cell mass and were beginning to hatch, and thus, freezable.

Statistical Analyses

Data from the first day of collection were not statistically analyzed because of difficulties throughout the process and loss of an entire shipper containing the control oocytes. Data from d 2 of the study (oocytes per ovary; zygotes per ovary; embryos per ovary; 8-cell, 2 to 4 cell, 1-cell, and lysed cells per zygotes; cleavage rate; embryo grades per 8-cell stage; embryos per 8-cell stage) were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Main effects of treatment, time, as well as treatment by time interactions, were assessed. Each conical tube containing harvested oocytes (groups) served as experimental unit due to the nature of oocyte collection. A *P*-value of ≤ 0.05 was considered statistically significant and a *P* value $\leq$ 0.10 was considered to be a tendency.

Results

Day One

The MGA-Implant categorized group yielded a total of 741 oocytes from 100 ovaries. They also averaged 7.4 ± 0.8 oocytes per ovary, 6.5 ± 0.6 zygotes per ovary, and 0.60 ± 0.10 embryos per ovary. Ovaries assigned to the control category based on the presence of a CH, CL, or CA, yielded 432 oocytes from 53 ovaries. This category averaged 8.2 ± 0.8 oocytes per

ovary, 7.1 ± 0.4 zygotes per ovary, and 0.04 ± 0.02 embryos per ovary. Results from the first day of heifer harvest are reported in Table 2.1.

Day Two

A total of 1,820 oocytes were aspirated from 152 ovaries in the MGA-Implant treatment (Table 2.2). The control yielded 1,272 oocytes from 145 ovaries. Treatment and time interacted to affect the total number of oocytes harvested per ovary (P= 0.07; Figure 2.1), the number of oocytes placed in maturation media per ovary (P= 0.07), and the number of zygotes (successfully fertilized oocytes) per ovary (P= 0.06; Figure 2.2). Fertilization rates (the percentage of oocytes fertilized) did not differ (P = 0.90) between treatments (MGA-Implant: 79.9 ± 4.6; Control: 82.3 ± 5.5; Table 2.3). Cleavage rates (percentage of zygotes that divided by d 2 post-IVF) did not differ (P = 0.50) between treatments (MGA-Implant: 46.8 ± 5.9; Control: 47.9 ± 7.1; Table 2.3).

A similar (P = 0.90) percentage of zygotes did not cleave by d 2 post-IVF (MGA-Implant: 50.8 ± 3.9; Control: 49.1 ± 3.9; Table 2.4). A similar (P = 0.90) percentage of zygotes achieved the 2 to 4-cell stage of development for both treatments by d 2 post-IVF (MGA-Implant: 24.7 ± 2.7; Control: 26.1 ± 2.7; Table 2.4). The percentage of zygotes achieving the 8-cell stage of development by d 2 post-IVF was also similar (P = 0.60; MGA-Implant: 19.5 ± 2.7; Control: 22.7 ± 2.7; Table 2.4). A similar (P = 0.5) percentage of zygotes lysed by d 2 post-IVF (MGA-Implant: 5.0 ± 1.8; Control: 2.1 ± 1.8; Table 2.4).

A similar (P = 0.50) number of embryos per ovary was produced for both the MGA-Implant and Controls (MGA-Implant: 0.34 ± .07; Control: 0.39 ± 0.07; Table 2.5). A similar (P = 0.30) percentage of oocytes with the opportunity for fertilization yielded embryos by d 7 post-IVF (MGA-Implant: 3.2 ± 1.0; Control: 5.1 ± 1.0). Blastocyst rates (the percentage of zygotes

achieving blastocyst stage by d 7 post-IVF) did not differ (P = 0.30) between treatments (MGA-Implant: 3.9 ± 1.1; Control: 6.1 ± 1.1). Across treatments, the percentage of 8-cell stage embryos at d 2 post-IVF that developed to the morula or blastocyst stage by d 7 post-IVF, was affected (P= 0.05) by time from heifer harvest to the time that all oocytes were put into maturation media. The number of embryos generally decreased as the time from heifer harvest to the time the oocytes were put into maturation media increased (Figure 2.3).

Embryo grades were assigned on d 7 post-IVF according to grading procedures utilized by Sexing Technologies, Inc. Embryos receiving grades of either C1 or C1- were deemed to be of sufficient quality to freeze. Time and treatment interacted (P = 0.04) to affect the percentage of C1 embryos that developed from the 8-cell stage at d 2 post-IVF (Table 2.6); however it should be noted that only 2 control C1 grade embryos resulted from this study. A similar (P = 0.9) percentage of embryos that developed from the 8-cell stage of development at d 2 post-IVF received a grade of C1- on d 7 post-IVF (MGA-Implant: 8.5 ± 2.4; Control: 7.0 ± 2.4; Table 2.6). A majority of the remaining embryos received a grade of C2. The percentage of C2 embryos that developed from the 8-cell stage at d 2 post-IVF decreased (P = 0.05) as the time from heifer harvest to the time oocytes were put into maturation media increased (Figure 2.4).

Discussion

Success of in vitro embryo production is dependent on a vast array of factors. In the present study, we focused primarily on the effects of management of the heifers at the feedlot before slaughter. This is the first study to evaluate the effects of administration of MGA and growth promoting implants on the success of in vitro embryo production from oocytes harvested from slaughterhouse ovaries. The results of this study indicate that oocytes collected from feedlot heifers post-harvest that were fed MGA and implanted with growth promotants have the

ability to mature in vitro, be successfully fertilized in vitro, and produce viable embryos to be used for transfer to recipient females.

Unfortunately, results from d 1 could not be analyzed. The correctness of our decision, however, to categorize the ovaries based on structures present on the ovary was reaffirmed on the second day of heifer harvest. All ovary pairs from the MGA-Implant heifers harvested on d 2 had large ovarian follicles and lacked a CH, CL, or CA, whereas all control ovaries had one or more CH, CL, or CA.

The interaction between time and treatment that was observed for the number of oocytes per ovary and the number of zygotes yielded per ovary on d 2 of collection is difficult to explain. In general, the number of oocytes and zygotes yielded per ovary in the control did not change as the time from heifer slaughter to oocyte placement into maturation media increased. The MGA-Implant treatment displayed a general trend of decreasing in numbers while time advanced. The treatment differences occurred at approximately 6 h post-slaughter, at a time when some ovaries were waiting to be aspirated and others had already been aspirated, in which case those oocytes were being held in a holding media. Perhaps a reason for this interaction is that MGA and growth promotants alter oocyte quantity and/or quality only after a certain time threshold is reached post-slaughter. The amount of time that oocytes remain in ovaries post-slaughter affects the oocyte's ability to develop to early embryo stages. Blondin et al. (1997) reported that oocyte aspiration 4 h post-slaughter was optimal for successful embryo production, with an increased rate of 64-cell embryos, compared with 2 or 6 h of storage. In a similar study, Yang et al. (1990) found that temperature also influenced ovary storage time; ovaries that were stored for 8 h post-slaughter at 37°C were more likely to have detrimental effects on cleavage and blastocyst rates than ovaries stored for 8 h at 25°C. In the current study, a 6.5 h window of time existed between

heifer slaughter and the time that all oocytes were placed in maturation media. Heifers that were slaughtered first were the first to undergo follicular aspiration (approximately 2 h post-slaughter), whereas those heifers slaughtered last were the last ones to undergo follicular aspiration (approximately 5 h post-slaughter). Disruptions in the oocyte maturation process may have occurred while oocytes were being held in holding media. Perhaps if oocytes had been put into maturation media immediately following follicular aspiration, the observed interactions may not have occurred.

The interaction between time and treatment observed for the number of zygotes yielded per ovary could possibly be an illustration of the oocyte's ability to be fertilized, but it could also simply be a reflection of the number of oocytes that were harvested and thus available to be fertilized. When plotted, both variables display similar patterns (Figures 2.1 and 2.2), indicating that a more likely reason for the time and treatment interaction observed for the number of zygotes yielded per ovary was the number of oocytes that were available to be fertilized. The general decrease in the number of oocytes per ovary harvested from the MGA-Implant treatment over time may be the result of decreased efficiency in oocyte recovery, rather than fewer follicles available for oocyte aspiration.

The process of oocyte maturation is a key event influencing successful fertilization, either in vivo or in vitro (Rizos et al., 2002). Most of the oocyte maturation process in nature occurs prenatally. Oocyte maturation has been described in three phases. Meiotic maturation occurs once the LH surge has been initiated. It is comprised of a series of nuclear events, in which nuclear inhibitors are removed, and the oocyte is able to resume meiosis. The cytoplasmic maturation stage occurs a few days before the LH surge. Changes in transcriptional and translational activity, as well as adaptations in the cell organelles in the cytoplasm, define

cytoplasmic maturation. Finally, the molecular maturation stage is made up of molecular changes that help prepare the oocyte for fertilization and development post-fertilization. This stage is thought to be the largest contributor to an oocyte's developmental competence and can determine whether or not it can reach the blastocyst stage post-fertilization (Sirard et al., 2006). Molecular maturation of the oocyte is likely linked to the stage of follicular development and the environment within that follicle.

The follicular environment can positively or negatively impact the quality of the oocyte, depending on when the oocyte is harvested relative to the follicular status. For the present study, we collected all visible antral follicles. Follicles that are similar in size, however, can be quite different in terms of their physiological development (Vassena et al., 2003). During the growing phase of follicular development, small antral follicles are dependent on stimulation of FSH from the anterior pituitary for growth. As follicles continue to grow, they become less dependent on FSH for growth and develop the ability to respond to LH stimulation. Oocytes collected from FSH dependent or independent growing follicles generally do not have sufficient competence to reach the blastocyst stage post-fertilization. In contrast, oocytes from follicles in the early atretic phase are competent enough to achieve the blastocyst stage (Sirard et al., 2006). A study by Vassena et al. (2003) found that oocytes collected from follicles 5 d after the emergence of a follicular wave are the most competent as indicated by having the greatest blastocyst rate (22.7%) compared with oocytes from follicles 2, 3, or 7 d after the emergence of a new follicular wave. Follicles at d 5 post-wave emergence are in the early stages of atresia (Vassena et al., 2003). Late stage atretic follicles have extremely poor developmentally competent oocytes, limiting the number of oocytes that achieve blastocyst post-IVF (Sirard et al., 2006). Growing and atretic follicles can be similar in size, making it difficult to determine the physiological

phase of follicle development, and whether the oocytes within those follicles have better or worse developmental competence. While we may have been collecting oocytes from follicles comparable in diameter, the follicular environments were likely different, and thus affected the oocyte's ability to develop post-IVF. Hence, the population of oocytes recovered from follicles in the MGA-Implant treatment and control was likely heterogeneous, making it difficult for the in vitro maturation process to be effective for all oocytes. This may have contributed to our overall lowered blastocyst rates (5%) for this study. In fact, it has been demonstrated that even through cautious selection of a homogenous group of oocytes, only 35% of those oocytes complete maturation of the cytoplasm and are able to yield a viable embryo (Blondin and Sirard, 1995).

Another way to evaluate the success of maturation is to look at fertilization and cleavage rates. In this study, fertilization and cleavage rates were similar for both treatments, indicating that MGA and growth promotants that were administered before slaughter played no significant role in the oocyte's ability to mature in vitro and to allow fertilization and normal development to occur. Overall cleavage rates for this study were 46.4%, which is much less than what has been reported in other studies. Ward et al. (2000) collected cumulus-oocyte complexes from ovaries of slaughterhouse cows, and had overall cleavage rates ranging from 61 to 85.7%, depending on the duration of the in vitro maturation process (16 to 32 h). Similar results were reported by Merton et al. (2003), with cleavage rates again ranging from 61.1 to 67.7%, depending on the duration of oocyte maturation (19 and 24 h, respectively). In both studies, 24 h was found to be the optimum time for in vitro maturation.

Our original hypothesis was that a high estradiol environment caused by administration of MGA, as well as altered gonadotropin secretion resulting from administration of steroid-based

growth promotants, would affect subordinate follicles present on the ovary, either directly or indirectly, thus compromising oocyte quality. In contrast, we found this hypothesis to be incorrect. We did not detect any treatment differences in overall fertilization, cleavage, or blastocyst rates that would indicate that oocyte quality was altered. In our study the overall blastocyst rate was 5%. Relative to other studies, this rate is extremely low. Rizos et al. (2002) found blastocyst rates to be 31.7% for oocytes from follicles 2 to 6 mm in diameter collected from slaughterhouse ovaries. In that same study, oocytes that had the opportunity to mature in vivo had blastocyst rates of 48.5%. It is difficult to say how these exogenous substances (MGA and implants) physiologically interacted with each other and how they influenced follicular growth without having information about whole animal blood steroid concentrations during the feeding period. Perhaps a reason for the lack of treatment differences throughout the study was because the effects of MGA and growth implants negate each other, causing no observed differences. Because no treatment differences were observed in any of our measured variables, oocyte quality was sufficient to produce large quantities that resulted in viable in vitro produced embryos.

Besides management practices at the feedlot (administration of MGA and growth promotants), age of the female also plays a role in oocyte and embryo quality in vitro. It has been previously reported that mature bovine females tend to have oocytes of greater quality when collected via ovum pick-up (OPU) or from slaughterhouse ovaries (Zhang et al., 1991; Galli et al., 2003). However, there have been conflicting reports suggesting that oocytes recovered from heifers yield higher or similar blastocyst rates compared with those from cows. Mermillod et al. (1992) found that animals between 1 and 3 yr of age and animals > 4 yr of age had similar blastocyst rates following the complete in vitro process. It was observed that oocytes

obtained from slaughtered crossbred beef heifers yielded similar numbers of oocytes and cleavage rates compared with cows, whereas cow oocytes achieved a greater blastocyst percentage than heifers (Rizos et al., 2005). Although we did not compare oocytes from heifers with oocytes from cows, we found the number of oocytes harvested per ovary to be greater than those from heifers reported by Rizos et al. (2005; 4.7 oocytes per animal). In contrast, our overall blastocyst rates were considerably less than the heifers used in that study (5% vs. 20 to 29%, respectively). Mermillod et al. (1992) reported blastocyst rates of roughly 9% from fertilized oocytes originating from slaughtered heifers (1 to 3 yr of age), which is much closer to our results (5%).

Overall nutrition of the feedlot heifers used in this study also may have influenced the overall reduced cleavage and blastocyst rates. All heifers in this study were fed a finishing diet, containing high percentage of concentrate. It has been previously reported that beef females fed diets high in concentrate have reduced oocyte quality as demonstrated by reduced cleavage and blastocyst rates. Nolan et al. (1998) reported that beef heifers fed a diet with 7 kg of concentrate per day had significantly reduced cleavage and blastocyst rates compared with heifers fed a diet with 1 kg of concentrate per day. Similarly, Yaakub et al. (1999) reported that beef heifers fed silage ad libitum as well as 6 kg of concentrate, had a lower fertilization rate compared with heifers fed only forage, as well as a lower cleavage rate compared with heifers fed just silage. Beef heifers that are fed limited or no concentrate in their diets, seem to have increased cleavage and blastocyst rates compared with heifers fed high levels of concentrate. The nature of feeding beef heifers for the purpose of slaughter may limit the success of in vitro embryo production. The overall low cleavage and blastocyst rates observed in our study could simply be attributed to the dietary regimen to which the heifers were subjected.

Conclusion

In conclusion, our results showed no significant differences between the MGA-Implant treatment and the control for the total number of oocytes per ovary, zygotes per ovary, or total embryos per ovary. Similarly, fertilization, cleavage, and blastocyst rates were unaffected by treatment. The lack of differences indicate that commercial IVF companies, such as Sexing Technologies, Inc., have the opportunity to use beef feedlot heifers as a source of oocytes for in vitro embryo production. Perhaps, because our overall cleavage and blastocyst rates were poor in general, any treatment differences that may have existed were actually suppressed. More research involving greater numbers of cattle and oocytes may be needed to test this idea. Furthermore, the in vitro maturation process itself has been documented to be inefficient (30% of all oocytes develop to the blastocyst stage; Rizos et al., 2002), and could be the reason for overall reduced cleavage and blastocyst rates. The intricate maturation process may have been the limiting step in this study that affected our overall results. Traditional feedlot heifer management practices including administration of MGA and steroid-based growth promotants seem to play no detrimental role in potential development or quality of oocytes and embryos.

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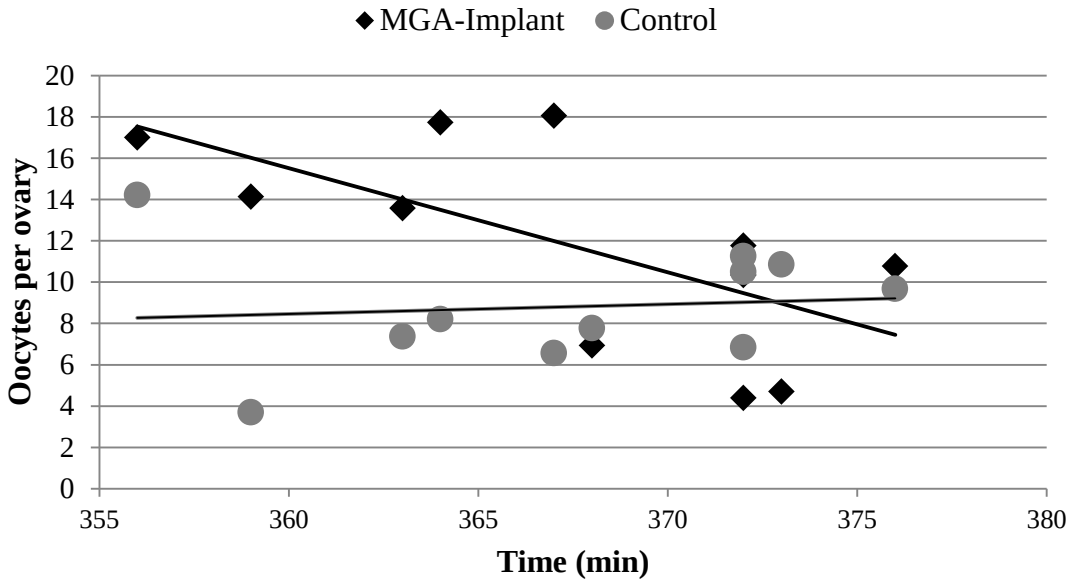

Figure 2.1 Number of harvested oocytes per ovary from heifers administered melengestrol acetate (MGA) and growth promotants (MGA-Implant), or from heifers not receiving either substance (Control). Treatment and time interacted ($P = 0.07$) to affect the number of oocytes harvested per ovary. Oocytes were counted immediately following follicular aspiration and put into holding media until all ovaries had been aspirated. The time above reflects the time from heifer harvest to the time that all oocytes were put into maturation media.

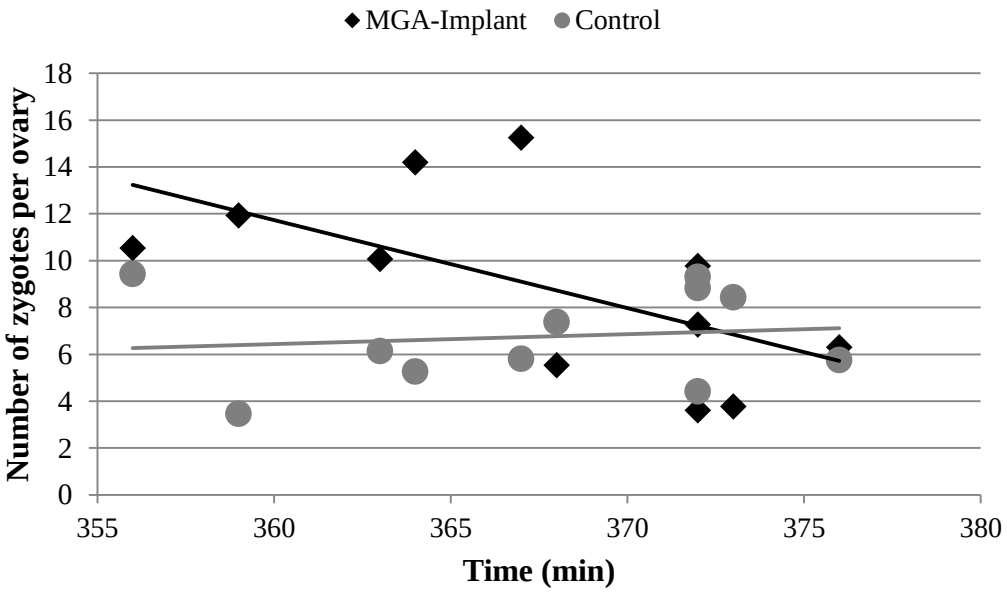

Figure 2.2 Number of zygotes per ovary from heifers administered melengestrol acetate (MGA) and growth promotants (MGA-Implant), or from heifers not receiving either substance (Control). Time and treatment interacted ($P = 0.06$) to affect the number of zygotes per ovary. Zygotes (fertilized oocytes) were counted 18 h post-in vitro fertilization.

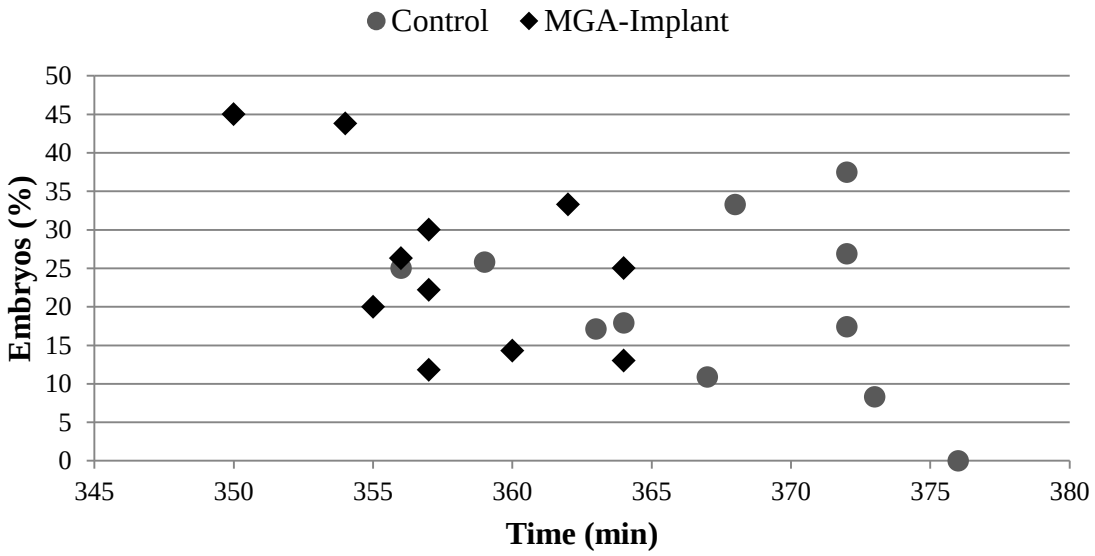

Figure 2.3 Percentage of 8-cell stage embryos from d 2 post-IVF that developed to either morula or blastocyst by d 7 post-IVF. A treatment by time interaction ($P > 0.10$) was not detected. The percentage of embryos decreased ($P = 0.05$) as the time from heifer harvest to the time all oocytes were put into maturation media increased.

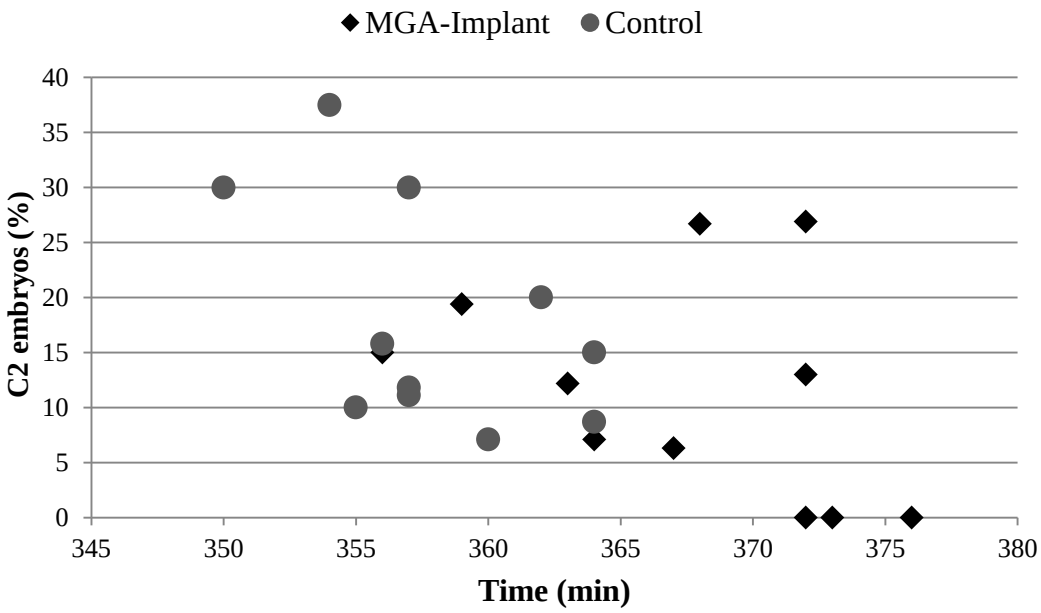

Figure 2.4 Percentage of 8-cell stage embryos from d 2 post-IVF receiving a grade of C2 on d 7 post-IVF. The percentage of grade C2 embryos decreased ($P = 0.05$) as the time from heifer harvest to the time that all oocytes were put into maturation media increased. Treatment and treatment by time interactions ($P > 0.10$) were not detected.

Table 2.1 Oocytes, oocytes and zygotes per ovary, and embryo yield from d 1 of ovary collection from heifers with ovaries containing either large follicles, or ovaries with a corpus hemorraghicum (CH), corpus luteum (CL), or corpus albican (CA).

Ovary pair classification	Ovaries, n	Oocytes, n	Oocytes /ovary	Zygotes/ ovary	Total Embryos	Embryos /ovary
No visible CH, CL or CA	100	741	7.4 ± 0.8	6.5 ± 0.6	60	.60 ± .10
Visible CH, CL, or CA	53	432	8.2 ± 0.8	7.1 ± 0.4	2	.04 ± .02

Statistical analysis was not performed on data from d 1 because of loss of treatment identification during heifer harvest. Ovaries were classified according to their structures; ovaries without a CH, CL, or CA were assumed to be from the MGA-Implant treatment, while the presence of a CH, CL or CA indicated the control treatment. The shipper containing oocytes harvested from ovaries with a visible CH, CL, or CA malfunctioned during shipment to the IVF lab, resulting in immature oocytes that yielded only 2 embryos for that group.

Table 2.2 Heifers, ovaries, and oocyte yield from d 2 of ovary collection for heifers administered melengestrol acetate (MGA) and growth promotants (MGA-Implant) or from heifers not receiving either substance (Control).

Treatment[1]	Heifers, n	Ovaries, n	Oocytes, n	Oocytes/ovary[1]	Maturing oocytes/ovary[1]
MGA-Implant	88	152	1,820	14.2 ± 1.4	13.6 ± 1.3
Control	84	145	1,272	9.2 ± 1.6	8.7 ± 1.6
P-value	-	-	-	0.07	0.07

[1]Treatment and time interacted to affect the number of oocytes per ovary and number of maturing oocytes per ovary. Maturing oocytes per ovary were the number of oocytes given the opportunity to mature in vitro.

Table 2.3 Zygotes per ovary, fertilization and cleavage rates produced from oocytes from heifers administered melengestrol acetate (MGA) and growth promotants (MGA-Implant), or from heifers not receiving either substance (Control).

Treatment[1]	Zygotes/ovary	Fertilization rate[1] (%)	Cleavage rate[2] (%)
MGA-Implant	10.7 ± 1.1	79.9 ± 4.6	46.8 ± 5.9
Control	7.3 ± 1.3	82.3 ± 5.5	47.9 ± 7.1
P-value	0.06	0.9	0.5

Treatment by time interaction ($P = 0.06$) affected the number of zygotes per ovary.

[1] Percentage of oocytes that were successfully fertilized.

[2] Percentage of zygotes that had divided by d 2 post-IVF.

Table 2.4 Early stages of embryo development at d 2 post-IVF from heifers administered melengestrol acetate (MGA) and growth promotants (MGA-Implant), or from heifers not receiving either substance (Control).

Treatment[1]	% 1-cell	% 2 to 4 cell	% 8-cell	% lysed
MGA-Implant	50.8 ± 3.9	24.7 ± 2.7	19.5 ± 2.7	5.0 ± 1.8
Control	49.1 ± 3.9	26.1 ± 2.7	22.7 ± 2.7	2.1 ± 1.8
P-value	0.90	0.90	0.60	0.50

No treatment differences or treatment by time interactions were detected for any of the variables measured on d 2 post-IVF.

Table 2.5 Embryo yields, percentage 8-cell stage embryos achieving morula or blastocyst by d 7 post-IVF, and percentage blastocyst from d 2 of ovary collection from heifers administered melengestrol acetate (MGA) and growth promotants (MGA-Implant), or from heifers not receiving either substance (Control).

Item	Total Embryos	Embryos/Ovary	% 8-cell stage embryos[1]	% Blastocyst[2]
MGA-Implant	52	0.336 ± 0.069	20.0 ± 2.0	3.9 ± 1.1
Control	54	0.390 ± 0.069	25.9 ± 2.0	6.1 ± 1.1
P-value	NA	0.50	0.60	0.30

No treatment or treatment by time interactions were detected for any of the variables measured.

[1]The percentage of 8-cell stage embryos at d 2 post-IVF that achieved morula or blastocyst by d 7 post-IVF.

[2]The percentage of zygotes achieving blastocyst by d 7 post-IVF.

Table 2.6 Percentage of 8-cell stage embryos receiving a grade of C1, C1-, or C2 at d 7 post-IVF from d 2 of ovary collection from heifers administered melengestrol acetate (MGA) and growth promotants (MGA-Implant), or from heifers not receiving either substance (Control).

	Freezable Embryos[1]		Fresh Use Embryos[2]
	% C1[3]	% C1$^-$	% C2
MGA-Implant	0 ± 0.3	8.5 ± 2.4	11.5 ± 3.0
Control	0.9 ± 0.3	7.0 ± 2.4	17.9 ± 3.0
P-value	0.04	0.90	0.40

[1]Freezable embryos are those that have achieved blastocyst, expanded blastocyst, have a tight inner cell mass, or have begun to hatch. C1 embryos are blastocysts that have a very tight inner cell mass and have begun to hatch. C1$^-$ embryos are either blastocyst or expanded blastocyst.

[2]Fresh use embryos are those that are morula, early blastocyst and blastocyst stages of development (C2 embryos).

[3]Time and treatment interacted ($P = 0.04$) to affect the percentage of C1 embryos that developed from the 8-cell stage of development at d 2 post-IVF.

Chapter 3 - General Review of Literature: Sexual Development of Young Beef Bulls

Introduction

In today's beef industry, it has become important to utilize genetically superior breeding animals to maximize the genetic opportunity that exists to produce a high-quality beef product with fewer cattle. The seedstock sector of the beef industry serves as the source of the latest genetics available to commercial cattlemen who choose not to AI their females, through the production and sale of bulls and females that are of superior genetics. The importance of the seedstock industry remains high, as natural service breeding programs continue to be the primary way of mating cattle in the U.S, with less than 10% of U.S. cattle producers utilizing artificial insemination (AI; Anderson et al., 2008).

In general, seedstock producers who focus their program on bull development will manage bulls from weaning until approximately 1 year of age, at which time they are sold to commercial cattlemen. Selling yearling bulls allows for reduced production costs, shorter generation intervals, and potential genetic gains (Barth et al., 2008). The intense nature of this bull development, however, calls for bulls to be fed high-energy diets during the postweaning period in order to maximize growth performance (Barth et al., 2008), an important criterion commercial cattlemen. Because bulls are being challenged nutritionally for maximum gains, their scrotal circumference (SC) increases more quickly and is subsequently larger as yearlings than for bulls not managed for maximal performance. It is thought that this increased SC results from excess fat in the scrotum, which could have detrimental effects on sperm morphology because of altered thermoregulation of the testes (Barth et al., 2008).

Before being sold, bulls are required to pass a breeding soundness examination (BSE) that is performed by a veterinarian. Breeding soundness examinations evaluate the physical soundness of the bull, semen quality, including a minimum sperm motility of 30% and minimum normal morphology of sperm of 70%, and a SC that is considered to be acceptable for the bull's age (Society of Theriogenology, 1993). Because semen quality can be greatly affected by age at puberty and management of the bull (i.e., nutritional status), it can be difficult for yearling bulls to successfully pass their first BSE. For this reason, some seedstock producers delay their bull sales until bulls are approximately 18 mo of age and have a greater chance of passing their BSE. By waiting to sell bulls when they are older, however, producers incur a greater rearing cost and potentially lose an opportunity to sell bulls that are of superior genetic value at a younger age.

It is important to recognize that besides the strict management of yearling bulls that may influence their ability to pass the BSE, bulls are likely undergoing the pubertal process during the time at which BSEs are conducted. Sexual development of bulls occurs on a continuum, such that when they achieve puberty, undergo a BSE, and are actually sexually mature, are all different. Evaluations that are performed before a yearling bull sale capture a moment of time in spermatogenesis, in which some bulls have the ability to pass; however, some bulls at this time are not yet pubertal. Sexual maturation of bulls (maximum sperm output) occurs well beyond a year of age. Age can greatly influence the passing rates of bulls, and ultimately cost the seedstock producer more money for repeated examinations.

Age at puberty is the main factor influencing semen quality and thus influences the bull's ability to pass their semen evaluation. Puberty has been defined as a semen sample containing at least 50×10^6 sperm/mL with at least 10% progressive sperm motility (Wolf et al., 1965). The onset of puberty in beef bulls has been shown to range from 8 to 13 mo of age, but is variable

among and within breed by as much as 88 d (Arteaga et al., 2001). Previous work in female swine and cattle has shown that age at puberty can occur earlier in life with different management practices including plane of nutrition, treatment with exogenous hormones, and social interactions with the opposite sex (Brooks and Cole, 1970; Roberson et al., 1991). A potential opportunity may exist to reduce the age at puberty in developing beef bulls that may allow for a greater percentage of them to pass their BSE at a younger age.

The objectives of this review are to 1) define the pubertal process in beef bulls, including spermatogenesis and steroidogenesis; 2) discuss bull breeding soundness examinations; and 3) discuss management factors that influence the age at puberty and success of BSEs, including the importance of biostimulation in breeding livestock.

Pubertal Process in Bulls

Puberty in beef bulls is a culmination of hormonal and neuroendocrine signals from the hypothalamus, anterior pituitary, and testes acting in concert to result in sperm production and output. Several definitions exist to define or measure puberty in bulls, including the age at which sexual behaviors are displayed, age at the first ejaculation, and age at which the ejaculate contains spermatozoa (Lunstra et al., 1978). The most widely accepted definition of puberty in bulls is when the ejaculate first contains a threshold amount of sperm (50×10^6 sperm/mL) with at least 10% progressively motile sperm (Wolf et al., 1965). Using this definition, *Bos taurus* bulls will generally achieve puberty between 8 and 13 mo of age (Lunstra et al., 1978; Lunstra and Echternkamp, 1982; Pruitt et al., 1986). Furthermore, the ability of a bull to produce this threshold concentration of sperm results from a complex maturation of the hypothalamic-pituitary-testes axis, initiation of spermatogenesis, and production of sex hormones (i.e., androstenedione and testosterone).

Hypothalamic-Pituitary-Testes Axis

Sexual development of beef bulls has been described to occurr in 3 stages, including the infantile period (birth to 2.5 mo of age), prepubertal period (2.5 to 6 mo of age; also described as the 'early gonadotropin rise'), and finally, the pubertal period (6 mo of age to puberty attainment), which is the period of accelerated sexual development (Brito, 2006). During the infantile period, serum concentrations of luteinizing hormone (LH) are very low. This gonadotropin, as well as follicle stimulating hormone (FSH), are released from the anterior pituitary in response to gonadotropin-releasing hormone (GnRH) secreted by neurons in the hypothalamus. Reduced concentrations of gonadotropins early in a bull calf's life are attributed to decreased GnRH secretion from the hypothalamus (Miller and Amann, 1986; Chandolia et al., 1997; Madgwick et al., 2008).

The prepubertal period begins around 2.5 to 3 mo of age. During this time, the pulse frequency of LH begins to increase (McCarthy et al., 1979; Amann and Walker, 1983; Amann et al., 1986). At approximately 4 to 5 mo of age, LH secretion considerably increases, as the frequency of LH pulses increase (Rawlings and Evans, 1995). These more frequent episodes of LH peaks are caused by increased GnRH pulsatile secretion at the level of the hypothalamus (Rodriguez and Wise, 1989). Amann et al. (1986) conducted 1 of the first studies to suggest that early in a bull calf's life the hypothalamus was sensitive to negative feedback of a steroid that seemed to prevent the secretion of GnRH. Over time, the sensitivity to this negative feedback decreased, concomitantly with increasing GnRH secretion, thus driving increased pulse frequency of LH secretion from the anterior pituitary.

During both the infantile and prepubertal periods, testosterone is secreted in small concentrations from the testes and continues to increase during the prepubertal period. It is the concentrations of testosterone causing negative feedback at the level of the hypothalamus that

limit the secretion of GnRH, and in turn, limits LH secretion from the anterior pituitary (Schanbacher, 1980). The hypothalamus is extremely sensitive to negative feedback of both testosterone and estradiol. As testosterone concentrations continue to increase, LH secretion begins to decline (McCarthy et al., 1979) resulting from the negative feedback of testosterone. Beginning approximately 4 to 5 mo of age, the hypothalamus becomes less and less sensitive to the negative feedback of testosterone (Rawlings and Evans, 1995), allowing for increased GnRH secretion ultimately increasing LH secretion. This increase in LH acts on the testes, and drives increased testosterone production, testicular growth, and spermatogenesis.

Leydig cells and Sertoli cells are the primary testicular cells supporting the production of testosterone and process of spermatogenesis, respectively. Leydig cell mass and proliferation increases from about 4 wk of age to 30 to 40 wk of age, and remains unchanged until about 52 wk of age. At this point, Leydig cells increase drastically in size (diameter and volume) and will maintain these characteristics through adulthood (Wrobel, 1990). Undifferentiated Sertoli cells are present in the seminiferous tubules at birth and will develop into adult cells very early in life. A bull will have developed all of the Sertoli cells for his lifetime by 25 wk of age (Bagu et al., 2004).

Luteinizing hormone acts on the Leydig cells that are located in the interstitial space of the seminiferous tubules within the testes. Leydig cells contain membrane-bound LH receptors that respond to pulsatile secretions of LH from the anterior pituitary (Schanbacher, 1979). When LH binds to its receptors, Leydig cells initiate steroidogenesis, by which they produce progesterone from cholesterol. Progesterone is then converted enzymatically to testosterone. In turn, testosterone diffuses out of the Leydig cell into the seminiferous tubules and binds to

Sertoli cells as well into testicular capillaries that govern circulating concentrations of testosterone.

Sertoli cells are located within the seminiferous tubules and respond to FSH secreted by the anterior pituitary. Under this stimulation, Sertoli cells produce androgen binding protein (ABP), which is responsible for binding testosterone once it has been produced by Leydig cells. It is important for the seminiferous tubules to maintain high local concentrations of testosterone so that spermatogenesis can occur. Once testosterone binds to Sertoli cells, it is converted to estradiol. Testosterone and estradiol are both transported through the bloodstream to the hypothalamus where negative feedback effects are exerted. This process continues, ultimately driving spermatogenesis.

Spermatogenesis

Spermatogenesis occurs within the seminiferous tubules. In bulls, the complete process of spermatogenesis is 61 d long and is initiated when bulls are 16 to 24 wk of age (Curtis and Amann, 1981). The beginning of spermatogenesis at this age marks the end of the prepubertal period and onset of the pubertal stage (Curtis and Amann, 1981). Spermatogenesis can be divided into 3 phases. The first phase is composed of a series of mitotic divisions of spermatogonia. A continuous pool of stem cells exists so that spermatogenesis can continue without interruption. These stem cells undergo mitosis to provide a consistent source of type A-spermatagonia (Senger, 2003). Through mitotic divisions, type A-spermatogonia proliferate to form type B-spermatogonia. The second phase (meiotic phase) of spermatogenesis results in primary and secondary spermatocytes. Meiosis is initiated when bulls are approximately 20 to 24 wk of age. The number of primary spermatocytes continues to increase until 32 wk of age. Secondary spermatocytes undergo meiosis II to form haploid spermatids. Elongated spermatids

appear around 30 to 32 wk of age and continue to increase at an accelerated pace. At 40 wk of age, spermatids outnumber any other germ cells undergoing spermatogenesis (Abdel-Raouf, 1960; Curtis and Amann, 1981). Sperm cell differentiation (or spermiogenesis) is the third and final phase of spermatogenesis. It is during this phase that a spermatid differentiates into spermatozoon, containing a head and a tail (Senger, 2003). Spermatozoa are released from the Sertoli cells to which they are associated into the lumen of the seminiferous tubules during a process known as spermiation. From there, spermatozoa travel to the head of the epididymis where they continue to undergo maturation. Sperm in this section of the epididymis contain a proximal cytoplasmic droplet. As sperm travel through the body of the epididymis to the tail, the cytoplasmic droplet moves down the tail of the sperm and is lost during ejaculation. Ejaculated sperm containing a proximal cytoplasmic droplet indicates incomplete maturation of the sperm (Senger, 2003), whereas those have distal droplets are considered to be normal.

Breeding Soundness Examinations

Breeding soundness examinations (BSEs) are a reproductive management tool for cattle producers utilizing bulls for natural service mating in their herd. Because more than 90% of all cattle are naturally mated (Thompson et al., 2004; Anderson et al., 2008), this important tool is crucial to ensure that bulls possess the capability to breed large numbers of females. According to a 2009 report from USDA-APHIS, approximately 18% of beef cattle operations with 1 to 49 cows have semen tests conducted on their bulls before the breeding season; in operations with over 200 cows, approximately 61% semen tested bulls before breeding. Even fewer operations include scrotal measurements as part of their routine examination before breeding season. Passing rates for bulls that are subjected to a BSE range from 65 to 80% (Barth and Waldner, 2001; Kennedy et al., 2002; Waldner et al., 2010). This range largely depends on the age of the

bull, with older bulls generally having a greater chance of successfully passing the BSE than younger bulls (yearling or younger; Barth and Waldner, 2002; Kennedy et al., 2002). Kennedy et al. (2002) classified reasons for failure into 4 categories, including inadequate SC, inadequate sperm motility, inadequate morphology, or a physical abnormality. The greatest reasons for failing a BSE are when bulls have less than minimum SC and substandard sperm morphology (Kennedy et al., 2002). As bulls age, the likelihood of passing a BSE increases, because they have larger SC and improved morphology (Barth and Waldner 2002; Kennedy et al., 2002). Generally, BSEs should be performed well ahead of a breeding season to allow producers to replace any bulls that may be deemed unsatisfactory. For seedstock producers developing bulls for sale to commercial cattle producers, BSEs are typically conducted 30 to 45 d before the sale. A BSE consists of 3 main components, including a physical examination, determining SC, and semen evaluation. The BSE can provide a snapshot of a bull's breeding potential and aid them in bull selection. A physical examination evaluates the bull's body condition, structural soundness, and overall health status. The reproductive tract evaluation uses trans-rectal palpation as a way to ensure that the internal reproductive organs are free from problems that could prevent breeding. External genitalia are also evaluated (Society for Theriogenology, 1993). One major limitation of the BSE, however, is its lack of observation of a bull actually being able to complete a successful mating also known as serving capacity (Chenoweth 1997).

Scrotal circumference measurements are assessed to predict the bull's sperm output. Scrotal circumference has a positive association with total sperm output, percentage of live sperm, and sperm motility (Gipson et al., 1985). As SC increases, the number of sperm, percent motile, and percent normal sperm morphology also increase. It has also been reported that bulls with small testes at 12 to 16 mo of age will actually have small testes as 2-yr old bulls (Cates et

al., 1981). Larger SC during a BSE also has been associated with a greater frequency of cows the bull mated being diagnosed pregnant via transrectal palpation, likely resulting from increased percentage of normal sperm (Waldner et al., 2010). Scrotal circumference measurements, as well as the other components of a BSE, can provide a glimpse into the bull's semen quality and sperm-producing ability (Rusk, et al., 2002), thus aiding in determining the potential fertility of that bull. Minimum acceptable SC measurements for a BSE are dependent on the bull's age. Bos taurus bulls 15 mo of age or younger should have SC > 30 cm; 15 to 18 mo of age should have SC > 31 cm; and 19 to 21 mo of age should have SC > 32 cm. A SC less than the minimum requirement at the time of a BSE can be indicative of poor fertility at an older age.

Evaluation of a semen sample is perhaps the most critical component of a BSE. Semen from bulls is generally collected via electroejaculation, and the semen sample that is obtained is examined under a microscope. Sperm motility and normal morphology are the 2 primary characteristics evaluated. Motility is the percentage of sperm moving in a forward direction, and morphology is the percentage of normal composition and structure of the sperm (Thompson et al., 2004). The required minimum motility is 30%, and the minimum requirement for normal morphology is 70% (Chenoweth et al., 1993).

Once all of these factors have been thoroughly evaluated by a veterinarian, the bull is deemed either 'satisfactory,' 'deferred,' or 'unsatisfactory' as to his expected breeding potential. Satisfactory bulls have achieved at least the minimum required SC, semen characteristics, and passed their physical examination. Deferred bulls are those that are neither satisfactory nor unsatisfactory, but are expected to likely pass a BSE in the near future and thus require further testing before being deemed acceptable or unacceptable to breed females. Unsatisfactory bulls

are those that failed to meet the minimum requirements for at least 1 part of the examination and may not easily recover before onset of the breeding season (Thompson et al., 2004).

In today's modern beef production system, most bulls are developed to be sold as yearlings to commercial cow-calf producers. Yearling bulls, however, are less likely to pass a BSE compared with older mature bulls. Multiple studies have indicated that of the bulls that are tested at 12 mo of age, only 25 to 35% are deemed satisfactory, with the remaining 65 to 75% having poor semen quality traits according to the standards set forth by the Society for Theriogenology (Cates et al., 1981; Perry and Patterson, 2011). A similar study (Arteaga et al., 2001) evaluated the proportion of bulls that obtained mature semen samples from 11 to 15 mo of age. In this study, bulls that were considered sexually mature had semen samples with a sperm concentration of at least 400 x 10^6 sperm/mL, 60% progressively motile sperm, and a minimum of 70% normal sperm morphology. The motility standards for mature bulls are greater than those recommended by the Society for Theriogenology in order to pass a BSE; nonetheless, it was reported that 20% of bulls at 11 mo of age were considered sexually mature based on semen characteristics. As age of the bulls advanced, a greater percentage of bulls were classified as mature according to their semen requirements. By 15 mo of age, more than 60% of bulls tested were considered mature (Arteaga et al., 2001). Cates et al. (1981) demonstrated that 100% of all bulls tested had mature semen evaluations at 16 mo of age. These data support the dogma that bulls develop on a continuum, and with age, a greater percentage of bulls are deemed to have acceptable semen characteristics needed to breed females. For some beef producers, this fact alone drives them to sell bulls closer to 18 mo of age when bulls are more likely to pass their BSEs rather than as yearlings.

It is important to recognize that SC and semen standards for passing a BSE are different from that of a mature bull. Hahn et al. (1969) found that Holstein bulls did not achieve their maximal SC until about 5 yr of age, at which time, the SC reached a plateau and remained relatively constant thereafter. It also has been reported that dairy bulls can achieve mature rates of spermatogenesis by 1 yr of age, but SC continues to increase beyond that age and is the primary reason for increased sperm output after this time (Hahn et al., 1969).

The greatest reason for yearling bulls struggling to pass a BSE because of inadequate semen quality is age at which puberty is attained. Bulls achieve puberty when semen has obtained a sperm concentration of 50 x 10^6 sperm/mL with at least 10% progressive motility (Wolf et al., 1965). Age when bulls reach puberty can range from 8 to 13 mo of age (Wolf et al., 1965; Lunstra et al., 1978); however, significant variation exists among breeds and individual bulls within breed, making it difficult to determine when bulls are capable of successfully passing the semen evaluation portion of their BSE. Lunstra et al. (1982) reported that the percentage normal morphology, progressively motile sperm, and overall semen concentration increases in the 3 to 4 mo post-puberty attainment. This indicates that bulls are more likely of having a mature semen sample as they age.

Scrotal circumference, while it is a factor all on its own for the BSE, can also influence a bull's ability to pass the semen evaluation. Bulls that have a larger SC reach puberty earlier (Lunstra et al., 1982). It is well established that SC is highly correlated with sperm output (Hahn et al., 1969; Almquist et al., 1976). Logic suggests that bulls with a larger SC at the time BSEs are conducted are more likely to have greater sperm output and thus a greater opportunity to pass their BSE. Scrotal circumference, however, also is influenced by nutrition. Bulls that are on test to be sold as yearlings are generally fed a high-energy diet in order to obtain maximum gains and

performance. A study by Seidel et al. (1980) found that Angus bulls fed 133% of their requirements for TDN had larger SC than Angus bulls fed 95% of their TDN requirements (over 154 d). They also found testes weight to be similar for both treatments, with the high-energy group having heavier scrotal weights at slaughter. Barth et al. (2008) suggested that high-energy diets affect SC by depositing more fat in the scrotum rather than strictly increasing testicular size. Increased scrotal fat could potentially alter thermoregulation of the testes and thus affect sperm production and output. , regardless of SC measurement, however, bulls on a high energy diet had the same scrotal hide thickness compared with bulls on a 100% forage diet, indicating no difference in scrotal fat with high energy diets (Coulter et al., 1987).

Overall, BSEs are meant to be a useful tool in reproductive management and have great value in predicting the ability of bulls to successfully breed females. With the greatest percentage of producers using natural service to breed females, and a large portion of bulls being sold as yearlings, it is crucial to ensure that bulls have the best opportunity to pass their BSE as yearlings.

Potential Management Factors Influencing Age at Puberty and Subsequent Breeding Soundness Examinations

Nutritional Effects

It has been well established that nutrition plays a role in the onset of puberty in bovine females. When females are fed the correct plane of nutrition, such that they have obtained sufficient energy stores to supply more than beyond maintenance requirements, metabolic indicators, such as leptin, signal to reproductive neurons that the female has achieved adequate nutritional status. Thus, correct nutritional management can influence the age at which females begin cycling and are considered pubertal. Much of the research evaluating nutritional effects on

the age at puberty in bulls has involved scrotal circumference and sperm output. Bulls that are sold as yearlings are typically fed a high-concentrate diet post-weaning through sale time. This allows for bulls to achieve maximum gains and can add more value to those bulls at sale time. A scrotal circumference of 28 cm has been considered pubertal (Lunstra et al., 1978). Scrotal circumference has a large negative correlation with age at puberty (-0.65), inferring that a larger SC is associated with a younger age at puberty attainment (Lunstra et al., 1978).

With SC being indicative of age at puberty, it was thought that SC could be influenced by energy intake of the bull, and thus affects age of puberty. In a study by Pruitt et al. (1986), Hereford and Simmental bulls were fed different amounts of a similar diet, such that bulls were receiving low, moderate, or high levels of energy during the postweaning period. Scrotal circumference, body weight, and backfat were measured every 25 d; serving capacity tests were completed 1 wk before SC measurements and semen was evaluated for pubertal characteristics as described by Wolf et al. (1965). Authors reported larger SC in Simmentals on a high-energy diet versus Herefords, but diets high in energy did not seem to reduce age at puberty in either breed.

In a study by Mwansa and Makarechian (1991), bulls were fed either a high-concentrate diet or a low-energy diet comprised of ad libitum access to hay and half the concentrate of the high-energy diet post-weaning for 168 d. After 77 d on feed, half of the bulls switched diets for another 77 d, such that 4 different energy combinations resulted (high-high; high-low; low-high; and low-low). At the completion of the study, bulls fed the high-high energy content diet had larger SC, but a larger percentage of abnormal sperm was detected in the high-high bulls than in bulls in other dietary treatment combinations. This result could be explained by the variability in the age of bulls (11.5 to 13 mo) during semen evaluations. Therefore, poor semen characteristics

may have occurred because some bulls were still developing. Increased scrotal fat deposition also may have influenced the thermoregulation ability of the testes and negatively altered semen quality. Coulter et al. (1997) found that bulls on a high energy diet (80% grain, 20% forage) had larger SC, decreased testicular tone, and decreased percent motile and percent normal sperm morphology compared with bulls fed a moderate energy diet (100% forage). In a more recent study by Brito et al. (2012), the effect of rate of gain on age at puberty and maturity in beef bulls was evaluated. Beginning at 6 mo of age, bulls were fed 1 of 3 diets in an effort to obtain different rates of gain. Bulls received no concentrate, 14% concentrate, or 37% concentrate in addition to ad libitum access to forage. Semen samples were obtained every 2 wk for evaluation of pubertal characteristics (Wolf et al., 1965) once bulls achieved a SC of 26 cm. Bulls were collected until a measure of maturity was achieved (30% sperm motility and 70% normal morphology). Authors reported that average daily gain of bulls was unrelated to their sexual development. In addition, negative correlations were detected between body weight at various ages and age at puberty and age at maturity. The authors interpreted these negative correlations to mean that body weight at initiation of the study was a more profound factor in sexual development than growth rate after 6 mo of age.

It is thought that nutrition during calfhood when the early gonadotropin rise is occurring may have the largest impact on puberty attainment in bulls. Bulls were weaned at 2 mo of age and fed low, medium, or high amounts of a base diet, with all 3 diets providing forage ad libitum, from 2.5 to 17.5 mo of age. All bulls were challenged with GnRH every 4 wk from 10 to 26 wk of age and again at 44 to 48 wk of age. Bulls in the low nutrition group were lighter and had a smaller SC than bulls in the high nutrition group. Low nutrition bulls, however, were also older at puberty than both the medium and high nutrition groups. Calves having reduced LH secretion

achieved puberty at an older age (low nutrition group). The authors concluded that nutrition mediated the LH pulse frequency during the early gonadotropin rise. Low nutrition bulls were found to have lower concentrations of IGF-1 which may play a role in Leydig cell function affecting the release of LH from the anterior pituitary (Brito et al., 2007b). Similarly, bull calves that were supplemented feed from 10 to 30 wk of age had increased LH pulse frequency as well as advanced body and testicular development that resulted in greater sperm production at 16 mo of age (Barth et al., 2008). It is thought that the early gonadotropin rise may drastically affect the number of Sertoli cells available and thus drive increased sperm production (Brito et al., 2007a). If nutrition during calfhood plays such a crucial role in regulating the early gonadotropin rise, there may be opportunity to influence the age at which bulls achieve puberty by potentially increasing the number of Sertoli cells available to produce spermatozoa.

Social Interactions

Extensive research has led to the accepted dogma that female livestock species that are given an allotted time of exposure to their male counterparts can show a variety of responses, including synchronized estrus, accelerated puberty, and decreased postpartum anestrus. Many livestock production systems have adapted management practices that include exposing females to mature males as a method to achieve those results. These physiologic responses are mediated through pheromones that signal the olfactory system. A pheromone is an airborne substance that is excreted in urine, feces, or other mucus secretions. Males of the same species react to these pheromones with a specific behavior or physiological change (Izard, 1983). The term biostimulation is used to explain the stimulatory effect males have on estrus and ovulation in females, via pheromones, genital stimulation, or other external prompts (Chenoweth, 1983).

Effects of Male Exposure on Female Sexual Development

In swine, it has been clearly demonstrated that prepubertal gilts will achieve puberty at a younger age if given boar exposure. Brooks and Cole (1970) were the first to report that exposing gilts starting at 165 d of age to boars for 30 min per day accelerated their pubertal development, allowing them to achieve puberty 30 d earlier. Kirkwood et al. (1981) further supported this, as they observed that gilts exposed to boars starting at 160 d of age were younger at puberty than those gilts that had their olfactory bulbs removed before boar exposure. These experiments clearly indicate that boar stimulation has no effect when the olfactory system is not intact. Similarly, it has been shown that boar exposure can induce estrus in sows during the postpartum anestrus (Hughes et al., 1990).

In beef cattle, the importance of biostimulation has proven to be substantial. The influence of male exposure on female reproduction ranges from decreased age at puberty in heifers to a shorter postpartum anestrus in beef cows. It has been demonstrated that beef heifers treated with oronasally-applied bull urine achieved puberty at a younger age (Izard and Vandenbergh, 1982). Roberson et al. (1987) was first to investigate the duration and type of bull exposure on the response of prepubertal beef heifers. In that study, heifers were placed in a pen with 2 mature teaser bulls until they achieved puberty. Heifers exposed to bulls had a similar age at puberty as those not exposed. The authors concluded that this could have occurred because of the low ratio of bulls to heifers and that the pheromone excreted in bull urine may have been diluted. In another study the effects of growth rate and mature bull exposure were evaluated (Roberson et al., 1991). Heifers were assigned to receive 1 of 2 diets that would allow them to have a high or moderate growth rate, as well as exposure or no exposure to mature vasectomized bulls. A combination of high or moderate growth rate and bull exposure led to a decreased age at puberty compared with high or moderate growth rate heifers not exposed to bulls.

Bull exposure also can influence the duration of postpartum anestrus in primiparous beef cows by hastening resumption of cyclic ovarian activity (Custer et al., 1990, Fernandez et al., 1993). Fike et al. (1996) illustrated that primiparous cows given fenceline contact with intact yearling bulls had a shorter postpartum anestrus than those cows not given fenceline contact to bulls. Similarly, Berardinelli and Tauck (2007) investigated the influence of close physical contact or fenceline contact exposure of primiparous suckled beef cows to mature bulls. Cows were either penned with bulls, had fenceline contact, or had no contact with bulls for a period of 35 d. The authors reported a greater proportion of cows with either close physical contact or cows with fenceline contact resumed luteal function by the end of the exposure period compared with cows not exposed. In addition, the interval in which cows resumed estrous activity from the start of the study was 13 d shorter for cows given close physical contact with bulls compared with cows without bull exposure.

Effect of Female Exposure on Male Sexual Development

It is obvious that biostimulation could be an important reproductive management tool utilized by swine and cattle producers to aid in accelerating the cyclicity of their breeding females whether it is influencing age at puberty or shortening the duration of postpartum anestrus. Relatively little research has been conducted investigating this phenomenon in males when exposed to their mature cycling female counterparts. Considering the evidence in the success of exposing females to mature males, it is possible that exposing young males to adult females of their species also may prove beneficial to altering various reproductive capabilities of males.

Research involving exposure of immature boars to cycling females failed to improve reproductive performance. Nelssen et al. (1982) penned 30 boars adjacent to 50 cycling females

beginning when boars were 5 wk of age until 22 wk of age. Sexual development was assessed via evaluation of the testes collected from randomly chosen boars sent to slaughter at 22 wk and 30 wk of age. Boars exposed to cycling sows tended to have smaller testes at 22 and 30 wk of age, but heavier accessory sex glands. The authors concluded that accelerated puberty was not achieved. In behavioral tests that were conducted, however, it was observed that boar sexual behaviors such as sheath sniffs, mount attempts, and total sexual behaviors from 16 to 22 wk of age were positively correlated with mating test scores conducted at 26 and 30 wk of age, although there was no treatment differences detected. This is a critical age for boars to be developing sexual behaviors; however, advanced puberty and sexual behaviors of boars were not influenced by the presence of cycling females.

The effects of female exposure on the semen collection of buck rabbits have been investigated. Sexually experienced, mature buck rabbits were either exposed to mature does continuously or were not exposed (7 d interval from exposure to first semen collection). Semen was collected twice per week and several variables were assessed, including semen volume, sperm motility, normal alive, normal dead, and total normal alive motile sperm. Libido also was assessed based on the time it took for bucks to ejaculate once introduced to a teaser doe during collection. Buck rabbits that were exposed to does had increased libido (as determined by time to first collection) as well as increased semen volume, increased percent motile sperm, total sperm per ejaculate, and increased normal alive motile sperm. It was determined that controlled doe exposure can be a useful tool in managing mature buck rabbits for reproductive efficiency (Rodriguez-De Lara et al., 2010).

When males are given the opportunity to view other mounting activity (either homosexual or heterosexual mounting) before semen collection, they generally have greater

success in generating a semen sample. Male goats allowed to view either estrus-induced goats mounting one another or estrus goats housed with 1 male, had a greater occurrence of sexual behaviors as well as a greater ejaculation frequency compared with male goats not allowed to observe mounting activity before collection (Shearer and Katz, 2006). Rams from 4.5 to 9.5 mo of age reared with ovariectomized ewe lambs that were induced to show estrus every 2 wk had increased mounting and ejaculation frequencies during their sexual performance tests at 10 mo of age. In addition, rams that were not raised with estrus-cycling ewes during the postweaning period had a greater incidence of homosexual mounting and showed less sexual interest in ewes during the performance tests, indicating that those rams reared as a group during the prepubertal period without contact with ewes may develop preference for rams versus ewes (Katz et al., 1988). Similarly, rams that were exposed to cycling ewes at 7 to 8 mo of age for 17 d had increased mounts, ejaculations, and sexual behaviors during serving capacity tests that were conducted at 16 to 19 mo of age compared with rams not exposed to estrus-cycling ewes (Stellflug and Lewis, 2007). Furthermore, a large proportion of rams that were not exposed to ewes as ram lambs failed to display sexual activity during the serving capacity test at 16 to 19 mo of age. Overall, exposing ram lambs to estrus-cycling ewes at a young age was proven to be a beneficial reproductive management tool for sheep breeders.

Little research has been reported on the effects of exposing young beef bulls to cycling females to enhance their sexual development. Early research in which young beef bulls were exposed at an average age of 376 d to cycling females twice weekly for a period of 30 min indicated no effect of female exposure on bull mating behavior or libido in subsequent mating tests (Lane et al., 1983). In the study, bulls were housed either individually (with or without female exposure) or in large groups (with or without female exposure) beginning 6 mo after

weaning (d 0 of study). Single-bull or multi-bull mating tests were conducted in alternating weeks beginning at 10 wk. Attempted mounts, disoriented mounts, service, and sniffing and licking behaviors were assessed during these tests. Bulls that were penned individually had more mount attempts than bulls housed in large groups and it was found that exposure to cycling females twice weekly for 30 min was unsuccessful at boosting libido or mating behavior (Lane et al., 1983). In a similar study, Price and Wallach (1990a) found that rearing bulls beginning at 7 mo of age with ovariectomized females had no effect on the bull's sexual performance test at 18.5 mo of age; however, when those bulls were housed individually for a short period of time at 21 mo of age, they had decreased libido scores. This would suggest that short-term individual housing may potentially alter the sexual behaviors of bulls before collection (Price and Wallach 1990a). Another study by Price and Wallach (1990b) placed bulls in pens with heifers from 6 to 9 mo of age, and from 12 to 18 mo of age bulls were exposed to cycling heifers. These heifers were brought into estrus using injections of estradiol benzoate, and bulls were allowed access to cycling heifers for an entire day. Sexual performance tests were conducted when bulls were 18.5 mo of age. Bulls reared with heifers and given exposure to cycling heifers from 12 to 18 mo of age did not have advanced sexual development as was evidenced by having similar sexual behaviors as bulls not exposed during sexual performance tests (Price and Wallach, 1990b).

It seems that short-term exposure to cycling females has no advantageous effect on the sexual development of young beef bulls. Ample evidence suggests, however, that visual stimulation and sexual preparation proves to be beneficial in subsequent semen collections (Geary and Reeves, 1992; Hale and Almquist, 1960). Dairy bulls that are given sexual preparation such as false mounting or restraint have increased sperm output for use in AI (Hale and Almquist, 1960). Beef bulls also have increased sperm output when given the opportunity to

have 3 false mounts before collection (Almquist, 1973). Furthermore, significant improvements in both sperm concentration and semen volume were observed when 3 false mounts were employed in that study. Mader and Price (1984) evaluated the effects of visual stimulation on the sexual performance of 12 Hereford bulls that were 22.5 mo of age. Bulls that were allowed to observe their conspecifics mating before their own mating test had greater ejaculation frequency and less time from between their introduction to an estrous female and first ejaculation. In addition, bulls that are restrained in close proximity to an estrous female also may have enhanced sexual performance (Mader and Price, 1984). Bailey et al. (2005) reported that bulls had advanced sexual behaviors when they were introduced to novel estrous females during serving tests; however, as time advanced bulls had a lessened interest in stimulus females (Bailey et al., 2005). In conclusion, bulls given the opportunity to observe mating or mounting activity by their conspecifics have increased sperm output and display greater sexual behaviors. This practice is employed in all major AI stud companies, where bulls are allowed to observe other false mounting activity and subsequent semen collection, as well as being allowed a set number of false mounts themselves before being collected.

Summary

Proper management of young beef bulls is essential to the success of the seedstock sector of the beef cattle industry. Bulls that are managed for maximum gains and performance in order to add value at the time of sale (typically as yearlings), often struggle to pass a BSE because of poor semen quality. In contrast, when yearling bulls are being challenged to pass their semen evaluation, they are likely still undergoing the pubertal process, causing substandard semen characteristics that prevent them from passing their BSE. Age at puberty is the primary factor determining whether or not a bull will pass a BSE. Puberty attainment has been shown to be

positively influenced in both prepubertal gilts and heifers through proper nutrition and exposure to the opposite sex. Previous research suggests that males may not elicit as great of a response to exposure to their female counterparts; however, it has been documented that sexual performance and sperm output is enhanced when bulls are given the opportunity to visualize mating activity of other bulls. In those studies, however, bulls were mature, and when exposure to females was allowed, it was for brief periods of time. There is yet to be a study evaluating the effects of continuous, long-term exposure of prepubertal beef bulls to estrus-cycling females during sexual development. Perhaps exposing bulls continuously to females exhibiting estrous cycles will decrease the age of puberty in bulls, and thus increase the percentage of bulls having a successful BSE as yearlings. Economic value may be found if such a study resulted in more bulls reaching puberty as yearlings. Thus, commercial cattlemen could subsequently capitalize on those superior genetics available at an earlier age.

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Chapter 4 - Exposure of prepubertal beef bulls to cycling females to enhance their sexual development

Abstract

We hypothesized that continuous, fenceline exposure of prepubertal beef bulls to cycling beef females would decrease the age at which bulls attain puberty as well as increase the percentage of bulls passing their initial breeding soundness examination (BSE) at approximately 1 yr of age. Seventy-seven bulls (Angus, n = 37; Simmental, n = 22; Hereford, n = 10; Simmental x Angus, n = 8) averaging 196 ± 21.5 d of age were either given fenceline and visual exposure to cycling females (exposed; n= 41), or were not exposed (control; n=36). Estrus was induced in cycling beef females so that 2 to 5 females were in standing estrus each week during the 179-d study. Body weights, scrotal circumference (SC), and blood samples were collected every 28 d. When bulls had a scrotal circumference ≥ 26 cm, semen samples were obtained monthly via electroejaculation until puberty was achieved. Bulls were considered to be pubertal when sperm concentration was ≥ 50 x 10^6 sperm/mL and motility was at least 10%. Behavioral observations were conducted twice monthly, once during diestrous and once during estrus stages of the beef females exposed to bulls. Homosexual mounting, flehmen responses, and penis extension was recorded for each observation period. Breeding soundness examinations were conducted when bulls averaged 363 ± 21.5 d of age. Normal sperm morphology of at least 70% and sperm motility of at least 30% were required to pass the BSE. Age, body weight, and SC at puberty were analyzed using the mixed model procedure in SAS, with fixed effects of treatment and random effect of pen. Breeding Soundness Examination data were analyzed using the Glimmix procedure in SAS with fixed effect of treatment. Sexual behaviors were analyzed using

the mixed model procedure in SAS with fixed effects of treatment, month of assessment, and stage of estrous, and random effect of pen. Age at puberty, SC at puberty and weight at puberty did not differ (P =0.38; P = 0.49, and P = 0.13, respectively) between exposed and control bulls (320 ± 28 d and 311 ± 29 d; 34.4 ± 2.5 and 34.9 ± 2.5; and 466.2 ± 12.2 and 437.7 ± 13.5 kg, respectively). Percentage of bulls passing their initial BSE did not differ (P = 0.52) between treatments (exposed: 87.8%, control: 74.2%). Treatment, month, and female estrous stage interacted (P = 0.05) to affect the number of mount attempts. Across treatments, penis extension occurred more often (P = 0.01) during estrus than diestrus during month 3. Treatment, month, and stage of estrous interacted (P < 0.001) to affect the number of flehmen responses. Exposed bulls entered the cow area more times (P < 0.0001) during estrus than diestrus in months 1, 2 and 3. We concluded that bulls given continuous, long-term, fenceline exposure to cycling beef females did not have earlier puberty, nor did a greater percentage pass their initial BSE, indicating that housing bulls next to cycling females would not be beneficial to reduce age at puberty.

Introduction

Use of beef bulls for natural service is still extremely prevalent in the U.S. cattle industry today, with less than 10% of breeding occurring via the use of artificial insemination (Anderson et al., 2008). Through the use of yearling beef bulls, commercial cattle producers are able to make use of the latest superior beef genetics. In general, seedstock producers work to develop bulls to be sold at approximately 1 yr of age to commercial cattle producers. The potential breeding success of these yearling beef bulls is generally evaluated via a breeding soundness examination (BSE). This is a critical step in a bull's reproductive life because they are carefully assessed to ensure they have achieved the ability to breed females (Arteaga et al., 2001).

Age at puberty is a crucial factor influencing a young bull's ability to pass the BSE. Sexual maturity of a beef bull occurs on a continuum, making it difficult for young bulls to pass their initial BSE at such an early age (approximately 1 yr old) compared with mature bulls (Arteaga et al., 2001). Wolf et al. (1965) defined puberty as the first ejaculate that contains 50 x 10^6 sperm with at least 10% being progressively motile. Age at which puberty occurs varies in bulls and can range from 231 d to 371 d of age (Almquist et al., 1976). Even if this threshold of sperm production has been achieved, great variability in the sperm morphology still exists (Barth and Brito, 2004). A successful BSE requires that bulls produce semen that has a minimum of 30% sperm motility and at least 70% normal morphology (Society for Theriogenology, 1993). The time from pubertal onset to the time that ejaculates meet the BSE standard is 3 to 4 mo (Lunstra et al., 1982), indicating that many bulls, at the time of their first BSE, may not be capable of achieving the standard.

Sperm motility and morphology have been shown to improve with bull age (Cates et al., 1981; Chenoweth et al., 1996; Arteaga et al., 2001). Arteaga et al. (2001) reported a dramatic increase in sperm concentration from 11 to 15 mo of age, as well as a concurrent decrease in the percentage of abnormal sperm. A large percentage of abnormalities were associated with proximal cytoplasmic droplets, which are common in young bulls during the pubertal period (Lunstra et al., 1982). Although proximal cytoplasmic droplets generally decrease with age, they are a cause of unsatisfactory semen evaluations for young bulls, making it difficult for yearling beef bulls to pass their BSE.

Reducing the age that bulls achieve puberty may benefit producers by increasing the percentage of bulls that can pass their initial BSE before being sold. Much research has been conducted in swine and cattle evaluating methods to reduce age at puberty in females, including

nutritional management and interactions with the opposite sex. It has been established that beef females with high to moderate growth rates can achieve puberty at earlier ages when their olfactory systems are stimulated by exposure to mature bulls (Roberson et al., 1991). Similarly, exposure of mature beef females to bulls or bull excretory products reduces duration of postpartum anestrus (Tauck et al., 2010; Berardinelli and Joshi, 2005). Puberty is also hastened in prepubertal gilts when exposed to boars, reducing age at puberty by 30 d (Brooks and Cole, 1970).

Relatively little research has evaluated the effects of female exposure on bovine male sexual development. Most research conducted has been primarily explored in other species. For example, exposure of young boars for 14 wk to mature, nonpregnant sows and gilts displaying estrus did not seem to influence the boar's sexual development (Nelssen et al., 1982). Rams reared with ewes after weaning or rams that had exposure to ewes at an early age had enhanced sexual performance compared with rams reared in unisexual groups or not exposed to ewes (Katz et al., 1988; Price et al., 1994).

In cattle, most of what has been reported involves mature bulls. It is well documented that stimulation (visual or false mounting) prior to semen collection will enhance sperm output by bulls (Hale and Almquist, 1960; Almquist 1973; Mader and Price 1984) and is widely used in the commercial AI industry. Price and Wallach (1990) reported that bulls, having exposure to females (weaning to 18 mo of age) exhibiting estrus at various times throughout the study, did not seem to have any advantage over nonexposed bulls in their sexual performance at an older age.

Considering previous research, the hypothesis for this study was that exposure of young beef bulls to cycling females would accelerate the pubertal process, thus positively influencing

the percentage of bulls passing their first BSE at approximately 1 yr of age. Therefore, the objectives of this study were to: 1) determine if continuous, long-term, fenceline exposure of prepubertal beef bulls to mature beef females exhibiting estrous cycles influence age at puberty, and 2) quantify the percentage of bulls passing their initial BSE compared with bulls not exposed to cycling females. In addition, we determined how estrual females affected bull sexual behavior.

Materials and Methods

This study was conducted at the Kansas State University Purebred Beef Teaching Unit in accordance with the Kansas State University Institutional Animal Care and Use Committee (IACUC). Seventy-nine bulls (Angus, n=39; Simmental, n=22; Hereford, n=10; Simmental x Angus, n=8) born between January 1 and April 30, 2011 were used after weaning beginning in September 2011 (average age of 196 d ± 21.5 d). Bulls were stratified by age and breed and assigned randomly to either of 2 treatments: 1) continuous fenceline and visual contact with beef females exhibiting estrous cycles (Exposed; n = 41); or 2) no visual or fenceline contact with beef females (control; n = 38). Treatments were administered for 182 d when bulls were 196 ± 21.5 to 363 ± 21.5 d of age. Two control bulls were removed from the study because 1 of the bulls had only 1 testicle, and the other bull was a carrier of a genetic defect. Hence, 41 bulls were in the exposed treatment and 36 served as controls. Exposure to cycling females began on d 0 of the study and continued through the second BSE at d 182.

Bull Management

Bulls were housed in 4 pens, each measuring 97.6 m x 42.7 m, with 2 pens per treatment (Figure 4.1). A 3.4 m high plywood wall served as a visual barrier between the exposed and control bulls, preventing the control bulls from observing the cycling beef females (minimum of

42.7 m from control bulls to penned females). Dietary rations were similar for all bulls, consisting of 4 ration phases: starter (46.3% wet corn gluten, 44.7% prairie hay, 2.4% flaked corn); grower (47.5% wet corn gluten, 34.7% prairie hay, 15.3% flaked corn); finisher (44.6% wet corn gluten, 24.5% prairie hay, 28.5% flaked corn); and concluded with the grower diet. Diets were formulated for bulls to achieve an approximate average daily gain of 1.6 kg.

Female Management

Beef females (n = 9) that continued to be exhibiting estrous cycles were divided into 2 groups for use in this study. Transrectal ultrasound was performed and presence of a corpus luteum (CL) identified to ensure that females were exhibiting estrous cycles before beginning the study. Females with a CL at the time of the ultrasound were administered a 2 mL injection of a $PGF_{2\alpha}$ analog (EstroPlan, Agrilabs, St. Joseph, MO) to cause luteolysis and bring females into estrus. All other females without a CL were subjected to an initial estrous synchronization protocol before the study in which a 2-mL injection of GnRH (Factrel, Fort Dodge Animal Health, Overland Park, KS) was given in conjunction with an intravaginal controlled internal drug release (CIDR) insert for 5 d. Upon removal of the CIDR, a 2 mL injection of prostaglandin (EstroPlan, Agrilabs) was given to cause luteolysis and bring females into estrus. Females were divided into 2 groups after this initial synchronization. Each week beginning on d 0, a group (n = 4 or 5) of females were estrus-synchronized using a 2mL injection of EstroPlan (Agrilabs, St. Joseph, MO) and the second group was synchronized the following week. On d 46 of the study, 7 of the original 9 females were replaced with females that had been confirmed to be exhibiting estrous cycles. The females that were replaced were then managed to be bred and sold. The pattern of weekly estrus-synchronization continued throughout the study. Estrotect patches (Rockway, Inc., Spring Valley, WI) were utilized to aid in detection of estrus each week

to confirm females were exhibiting estrus and standing behavior. Females were housed in a 10.6 x 54.9 m pen located within the exposed bull pen (Figure 4.1). Synchronization of estrus continued through the completion of the study in February 2012 (d 182). Bulls had visual and nose-to-nose contact at any given time with cycling females. Cycling females shared an automatic waterer with bulls from pen 3 (Figure 4.1).

Body Weights, Scrotal Circumference and Blood Sampling

When bulls averaged 196 ± 21.5 d of age (d -3 of study), an initial BW, scrotal circumference (SC), and blood sample was collected before each bull was enrolled in the study. On d 0, exposure to cycling females began, and on d 9, a second BW, SC, and blood sample was collected and repeated every 28 d until d 149 of the study. Scrotal circumference was measured using a standard scrotal measuring tape by pulling the testicles to the bottom of the scrotum and measuring the largest circumference. Blood samples were collected via jugular puncture to assess testosterone concentrations. One 5 mL blood sample was collected from each bull and allowed to coagulate at 5°C for 12 h overnight. Samples were centrifuged approximately 24 h post-collection at 1,000 x g for 15 min in a refrigerated centrifuge at 5°C to harvest serum. Serum was stored at −20°C until assayed.

Radioimmunoassay

Concentrations of testosterone in blood serum were measured by direct quantitative (nonextracted) radioimmunoassay using Coat-A-Count testosterone kits (Catalog #TKTT; Siemens Medical Solutions Diagnostics, Los Angeles, CA) previously validated for bovine serum. The antibody (supplied by the manufacturer) did not cross-react with aldosterone, 11-deoxycortisol, prednisone, progesterone, and spironolactone. Crossreactivity of the antibody was < 3.4% for 5-beta-androstan-3α, 17-beta-diol, androstenedione, 5α-dihydrotestosterone, 4-estren-

7α-methyl-17-beta-ol-3-1, ethisterone, methyltestosterone, and 11-beta-hydroxytestosterone. Kit standards (0.16, 0.96, 4.14, 7.92, and 16.85 ng/mL), unknowns, and assay pools were added (100 µL each) in duplicate to antibody-coated tubes. One mL of ^{125}I labeled testosterone was added to each tube, vortexed for 5 s, and incubated overnight at 4°C. The next day, tubes were decanted, blotted on paper towels, and then radioactivity of each tube was quantified for 1 min in a gamma counter. Recovery of added mass (0.08, 0.48, 2.07 and 3.96 ng) added in quadruplicate (50 µL) to 4 different bovine serum samples (0.07, 0.55, 3.28, and 4.70 ng/mL) averaged 102%. Parallelism was demonstrated by assaying 75 and 100 µL aliquots of bovine serum in quadruplicate and recovering 91% added mass. Average assay sensitivity was 30 pg/mL. Inter and intra-assay coefficients of variation for 5 assays were 6.49% and 3.90%, respectively, for a pooled serum sample that averaged 6.98 ± 0.28 ng/mL (n = 5).

Semen Collection and Evaluation

Beginning when bulls had obtained a SC ≥ 26 cm, a semen sample was obtained via electroejaculation using a SireMaster Professional electronic ejaculator (SireMaster, Manhattan, KS) with a 6.35 cm diameter probe (SireMaster, Manhattan, KS). Semen collection continued monthly until the bull achieved puberty (50 x 10^6 sperm/mL with ≥ 10% motility). All semen collections and evaluations were conducted at the Kansas Artificial Breeding Service (KABSU) unit. Sperm concentration was assessed using a NucleoCounter SP-100 (Chemometec, Denmark). Sperm motility was analyzed under a microscope (Olympus CX41, Center Valley, PA) at 20x magnification. Morphology was analyzed at 40x magnification. Bulls were considered pubertal if they achieved: 1) ≥ 26 cm SC; 2) 5.0 x 10^6 sperm/mL: and 3) ≥ 10% progressive motility (Wolf, et al. 1965). Once all criteria for puberty were met, semen

collections ceased until BSEs were conducted in early February 2012 (d 163) when bulls averaged 363 ± 21.5 d of age.

Breeding Soundness Examinations

Breeding soundness examinations were conducted by a veterinarian when bulls averaged 363 ± 21.5 d of age on d 163 of the study. Semen samples were obtained from all bulls via electroejaculation (as described previously) regardless of whether they had previously achieved puberty. Sperm motility and morphology were assessed under 20x and 40x magnification, respectively. Bulls passed their BSE if their semen sample had at least 30% progressive motility and a minimum 70% normal morphology in addition to all other BSE criteria including an acceptable SC for their age (> 30 cm; Society for Theriogenology, 1993). Bulls that did not meet these minimum criteria at the initial examination were retested 20 d later.

Bull Behavior Assessments

Assessments of bull behavior were conducted twice monthly when cycling females were in estrus and in diestrus. Each day of assessment consisted of 3, 1-h observation periods, with a minimum of 1 observer per pen. The number of mounting attempts, penis extensions, and flehmen responses were recorded for all bulls in each pen. Mounting attempts were defined as any movement by a bull towards another bull in which both front feet were raised from the ground and contact was made between bulls. In addition to these behaviors, exposed bulls were also assessed for the number of times they entered a specified cycling female bovine area, which was defined as 3.05 m from each side of the cow pen, during each hour of observation. Behaviors were summed over the 3, 1-h assessments for each observation period. Observers used in this study were subjected to an observer reliability test before beginning the study, in which all observers were placed in the same pen with a partner and recorded behaviors during 1

h to minimize variation between observers that could alter assessment outcomes. The same observers conducted each assessment throughout the study, and alternated which pen they observed for a given assessment.

Statistical Analysis

All statistical analyses were conducted using procedures in SAS (version 9.2, SAS Inst. Inc., Cary, NC), with nonsignificant variables being removed from the model (i.e., breed). Age, BW, SC, and semen characteristics at puberty, as well as all repeated measures (body weight, SC, and testosterone concentrations) were analyzed using the mixed model procedure in SAS, with fixed effect of treatment and random effect of pen. Breeding soundness data were analyzed using the general linearized mixed model (Glimmix) procedure in SAS, with fixed effects of age, treatment, and their interactions, and random effect of pen. Behavior data were analyzed using the mixed model procedure in SAS, including treatment, month of assessment, and stage of estrous cycle, as well as their interactions, as fixed effects. Random effects were pen, and interactions between pen and bull, and 3-way interactions with pen, animal, and month of assessment. A P-value of ≤ 0.05 was considered significant and $0.05 < P\text{-values} \leq 0.10$ were considered to be a tendency.

Results

Treatment and day interacted ($P = 0.0003$) to affect bull BW (Figure 4.2). Exposed bulls were heavier ($P = 0.02$) at d 149 of the study compared with control bulls (510.2 ± 7.4 kg and 485.5 ± 7.9 kg, respectively; Table A.1). Scrotal circumference (Figure 4.3; Table A.2) increased ($P < 0.001$) from d -3 to 149 of the study but was unaffected by treatment ($P = 0.7$). Day of the study and treatment interacted ($P = 0.03$) to affect testosterone concentrations (Figure

4.4). Control bulls had greater (P = 0.002) testosterone concentrations at d 93 than exposed bulls (11.87 ± 0.78 ng/mL and 8.20 ± 0.77 ng/mL, respectively; Table A.3).

Age at Puberty

A total of 51 bulls (65.4%) achieved puberty during the study (between d 0 and d 149; Table 4.1). Of the bulls achieving puberty by d 149, 43.1% were controls (n = 22) and 56.9% were exposed (n = 29; Table 4.1). Overall, 70.7 ± 9.7% of the exposed bulls achieved puberty, whereas 60.8 ± 10.1% of the controls achieved puberty during the study (Figure 4.5), but this difference was not significant (P = 0.6). Age at puberty did not differ (P = 0.39) between treatments (Table 4.1; Exposed: 320.3 ± 5.1 d; Control: 311.2 ± 5.9 d). Scrotal circumference was also similar (P = 0.49) at puberty (Table 4.1; Exposed: 34.4 ± 0.5 cm; Control: 34.9 ± 0.5 cm). Body weight at puberty did not differ (P = 0.25; Exposed: 466.2 ± 12.2 kg; Control: 437.7 ± 13.5 kg). Sperm concentration at puberty was similar (P = 0.35) for both treatments (Exposed: 85.5 ± 9.6 million sperm/mL; Control: 106.1 ± 10.8 million sperm/mL). The percentage motile sperm in an ejaculate at puberty was similar (P = 0.23) for exposed and control groups (45.2 ± 4.3, 34.2 ± 4.5, respectively). The percentage of sperm with normal morphology was similar (P = 0.18) for both treatments at puberty (Exposed: 33.8 ± 4.3, Control: 24.8 ± 4.9).

Breeding Soundness Examinations

Breeding soundness examinations were completed when bulls averaged 363 ± 21.5 d of age (Table 4.2). The percentage of bulls that passed their BSE was similar (P = 0.54) for both treatments (Exposed: 87.8%; Control: 74.2%). A total of 14 bulls failed their BSE because of inadequate normal sperm morphology or percentage motile sperm. Eight bulls were retested 20 d after the first BSE. Of those 8, 4 bulls failed a second time, with the primary reason for failure for 3 bulls being that sperm motility and normal morphology were below the minimum passing

standards (30% motility, 70% normal morphology). The fourth bull that was retested had white blood cells in the ejaculate and no sperm. Age affected ($P = 0.03$) whether a bull passed or failed the BSE. Of the bulls that had achieved puberty during the study (n = 51), 47 passed their BSE (96.1%) and only 2 failed (3.9%). Pubertal exposed bulls had a BSE passing rate of 93.1%, whereas 7.4% of the exposed bulls that achieved puberty failed their first BSE. Of the control bulls that achieved puberty during the study, 100% of them passed their first BSE.

Bull Behavior Assessments

Bull reproductive behaviors were assessed twice monthly (12 assessments) during the study at both estrus and diestrus stages of the female estrous cycle (Table 4.3). A 3-way interaction ($P = 0.05$) between treatment, month of assessment, and stage of the estrous cycle in which the assessment was conducted was observed for the number of mounting attempts. Exposed bulls exhibited more mounting activity (Figure 4.6) when females were in estrus compared with diestrus in months 1 ($P = 0.02$), 3 ($P < 0.001$), 4 ($P = 0.01$), and 5 ($P = 0.07$). Mounting activity was similar ($P = 0.28$) in month 2 for exposed bulls during stages of female estrus and diestrus, and was also similar ($P = 0.57$) in month 6 during stages of female estrus and diestrus. Similarly, control bulls exhibited more mounting activity ($P = 0.03$) in month 4 when females were in estrus versus diestrus (Figure 4.7). The remaining months for the control bulls were similar in terms of the number of mount attempts observed during each assessment for estrus and diestrus. Exposed bulls tended ($P = 0.07$) to have more mounting attempts than control bulls during diestrus assessments (Figure 4.8). Mounting attempts were similar ($P = 0.20$) for both treatments during estrus assessments (Figure 4.9). A month by stage of estrous cycle interaction ($P = 0.02$) was observed for the number of times penis extension was observed, indicating that during month 3 of assessments, penis extension occurred more often during estrus

assessments than during diestrus assessments, regardless of treatment (Figure 4.10). A 3-way interaction ($P < 0.001$) between treatment, month of assessment, and stage of the estrous cycle in which the assessment was conducted was observed for the number of flehmen responses. During months 1 and 2, exposed bulls had more ($P < 0.05$) flehmen responses when females were in estrus than during diestrus. In contrast, in month 4, more ($P = 0.004$) flehmen responses were observed when females were in diestrus than estrus (Figure 4.11). Control bulls during month 1 had greater ($P = 0.09$) flehmen responses during the diestrus assessment than during the estrus assessment, but during month 5, had greater ($P = 0.03$) flehmen responses during the estrus assessment than during diestrus assessments (Figure 4.12). Exposed and control bulls had similar ($P = 0.4$) numbers of flehmen responses when females were in estrus (Figure 4.13) and when they were in diestrus (Figure 4.14). The distribution of these behaviors was not normally distributed. There were multiple bulls over multiple assessments that did not display reproductive behavior, whereas a few bulls displayed a majority of the sexual behaviors.

A month by stage of estrous cycle interaction ($P < 0.001$) was detected for the number of times that exposed bulls entered the designated cycling beef female area (Figure 4.15). During months 1 and 3, exposed bulls entered the cow area more times ($P < 0.001$ and $P = 0.007$, respectively) when females were in estrus than when females were in diestrus. During month 2, exposed bulls tended ($P = 0.09$) to enter the cow area more times during estrus than during diestrus. In contrast, during month 5, bulls entered the cow area more ($P = 0.04$) during diestrus than during estrus. There was no difference in the number of times exposed bulls entered the cow area when females were in estrus as compared with diestrus during months 4 ($P = 0.4$) and 6 ($P = 0.3$) of the study.

Discussion

Puberty in beef bulls has been defined as 50 x 10^6 sperm/mL with at least 10% progressive motility (Wolf et al., 1965). This definition served as our indicator of puberty attainment in this study. Semen was collected beginning when a SC of ≥ 26 cm was achieved. Eighteen bulls were electroejaculated on the first collection day, but none had attained puberty at that time. Overall, only 65% of all of the bulls achieved puberty before the BSE conducted at the conclusion of the study. Age at puberty in beef bulls is variable, ranging from 231 to 371 d of age (Barth and Brito, 2004). Age at puberty in this study actually ranged from 260 to 376 d, in agreement with previous literature (Wolf et al., 1965; Lunstra et al., 1978; Barth and Brito, 2004) and did not differ between treatments.

It is well documented that prepubertal beef heifers exposed to mature bulls or to bull excretory products attain puberty at a younger age than heifers not exposed to bulls or their excretory products (Roberson et al., 1991; Izard and Vandenbergh, 1982). We hypothesized that bulls exposed to cycling females also would have a decreased age at puberty. The females used in the present study were managed such that a group of at least 3 females were displaying estrus each week. Exposed bulls were able to observe the mounting activity of these females, in conjunction with having fenceline contact, allowing for tactile stimulation. It was thought that this contact and visualization would be beneficial to attainment of puberty because these stimuli have been shown to be important in the process of detection of estrus by bulls (Rekwot et al., 2001). The importance of stimulation is also prevalent in today's commercial AI industry, with the sexual preparation of bulls before semen collection serving as a widely practiced reproductive tool. Almquist (1973) proved that carefully managed false mounting and a prolonged period of sexual preparation by bulls observing other bulls mounting before semen collection can increase sperm output of beef bulls collected once or twice weekly.

Our hypothesis was that bulls exposed to females exhibiting estrous cycles would attain puberty at an earlier age because visual and olfactory cues would stimulate the release of neuronal signals that would then stimulate the release of GnRH and drive increased LH and testosterone secretion. Age at puberty, however, was not affected by exposure to cycling females in the present study. The method by which we isolated the control bulls from the females was by erecting a plywood barrier that was high enough to completely prevent bulls from having contact with the females. Geary and Reeves (1992) reported that bulls use visualization as the preferred and primary way to detect females in estrus. Because of this, it was thought that the barrier and the space between control bulls and cycling females (42 m) was sufficient for this study; however, pheromonal communication may still have occurred between bulls and females as the 2 pens of control bulls were located at least 42 m from the pen of cycling females. Perhaps the distance between the control bulls and the cycling females was not sufficient to eliminate the possibility of pheromones influencing both control and exposed bulls. Another study of this nature with control bulls at a separate location would need to be conducted to test this theory.

Another reason for similar ages at puberty may simply be because cycling beef females do not influence the sexual development and pubertal process of bulls to the extent that is observed when prepubertal heifers are exposed to bulls during pubertal development. It has been established that prepubertal beef heifers exposed to mature bulls can achieve puberty at a younger age than heifers not exposed to mature bulls (Roberson et al., 1991). In contrast, a similar effect on bulls was not seen in our study. The unaffected age at puberty in the present study is in agreement with a study that investigated boar pubertal development, in which boars that were penned next to mature, cycling females did not have accelerated pubertal development

compared with boars that were not penned near cycling females (Nelssen et al., 1982). Authors of that study found that boars penned next to cycling sows and gilts tended to have smaller testes and heavier accessory sex glands at 22 and 30 wk of age compared with boars not penned next to cycling females. Even though boars did not have a reduced age at puberty, their sexual behaviors were positively correlated with their mating test scores that were conducted when boars were 26 and 30 wk of age.

In our study, behavior assessments were conducted as potential indicators of any influence that females had on developing bulls. Even the results of the behavior assessments showed that the exposed bulls did not exhibit more or earlier sexual behaviors in response to cycling females, indicating that exposure to females may not play a significant role in the pubertal process of beef bulls. Although data were not recorded, it was observed that on the first day that exposed bulls had contact with cycling females, almost all of the bulls stood near the female pen and were extremely active in their homosexual mounting and flehmen behaviors. As the study progressed, the incidence of bulls crowding the female pen decreased, as did the occurrence of reproductive behaviors. The number of times that bulls entered the designated cow area during estrus assessments was the greatest during the first month of the study, and generally decreased with each subsequent estrus assessment (Figure 4.15). A reason for this observation might be that bulls during the first month found the females to be a novel stimulus. At this point in the study, their interest in the cycling females was greater as indicated by the number of times they stood near the penned females (1.36 ± 0.12 times per bull in month 1 during female estrus). In addition, flehmen responses also were greatest during month 1 when females were in estrus (2.27 ± 0.34 per bull).

Before the month 3 assessments, a new group of estrous females were introduced to replace the females that had been used during the first 2 mo of the study. These females were introduced roughly 30 d before estrus assessments occurred in month 3. Mounting activity of exposed bulls was greatest when females were in estrus during month 3 but exposed bulls did not have greater flehmen responses when females were in estrus. In fact, exposed bulls had more flehmen responses when females were in diestrus during month 3 assessments. Similarly, exposed bulls entered the designated cow area more when females were in estrus during month 3 than during diestrus. Increased mounts and time spent in the cow area during month 3 may be a reflection of the change in females used for synchronization. Assessments were conducted, however, almost a month after the new group of females were introduced, which would have given time for the bulls to adjust to their presence before our observations.

Bailey et al. (2005) reported that mature bulls were exposed to novel estrual females for 60 min at a time elicited a greater sexual behavioral response than those bulls exposed to either the same female in estrus or to a female in diestrus. After the introduction of the new females in our study during month 3, reproductive behaviors either remained relatively constant, or decreased. As the study progressed, perhaps the bulls became less interested or more accustomed to the presence of the cycling beef females. It is also worth noting that at the beginning of the study, the cow pen was slightly smaller, with the cow's hay feeder being in closer proximity to the bulls. Bulls may have been crowding the cow pen because of overflowing hay or because they were closer to females. This problem of reduced pen space was resolved during month 2 of the study. Perhaps the introduction of novel estrual females every month would have altered our reproductive behavior outcomes to be more similar to those found in Bailey et al. (2005).

Bulls exposed to cycling females displayed more mounting attempts and flehmen responses during estrus than during diestrus for all but 2 and 3 mo of the study, respectively, even though no differences detected between exposed and control bulls for mounting and flehmen activity when females were in estrus. This was surprising, because we had expected bulls in the exposed treatment to display more sexual behaviors in response to estrous females than the control bulls simply because of the opportunity they had to observe females as well as the fenceline interaction. Perhaps a reason the expected results were not seen was again due to the decrease in overall behaviors observed for the exposed bulls. As they became less responsive to the cows, their display of reproductive behaviors was similar to that of the control bulls.

Control bulls not exposed to cycling females had relatively the same amount of sexual behaviors during estrus as during diestrus, with the exception of mounting activity being greater during estrus in month 4, and greater flehmen response during estrus in months 1 and 5. It seemed that the flehmen responses displayed by control bulls were often times in response to other bulls urinating. These responses were still recorded, but it was noted that more frequently, flehmen responses were not always associated with female interactions. If flehmen activity in response to other bulls had been eliminated from our observations, it is possible that the exposed bulls would have displayed greater flehmen responses during estrus than diestrus compared with control bulls; however we did not differentiate the motivation for flehmen responses.

Puberty in the bull occurs in a continuum of time rather than occurring with 1 specific event. From birth until approximately 2.5 mo of age, the hypothalamus is extremely sensitive to the negative feedback of testosterone, thus causing small amounts of GnRH and subsequently LH, to be released. As the bull develops, this sensitivity to negative feedback of testosterone decreases, allowing larger concentrations of the gonadotropins to be secreted, ultimately driving

increased testosterone production and spermatogenesis. This increase in testosterone and sperm production occurs during several months. In our study, treatment and time interacted to affect testosterone concentrations (Figure 4.4). A treatment difference in testosterone concentrations was seen at d 93 of the study when bulls averaged 294 ± 21.5 d of age, with control bulls having greater concentrations than exposed bulls. The observed concentration for control bulls on this day was actually the greatest concentration detected in either treatment during the entire study. The exact reason for the difference at 93 d and not during any other day is unknown. After testosterone of control bulls peaked at 11.87 ng/mL on d 93, the concentrations returned closer to that of d 65 and the exposed bulls. Exposed bulls had peak testosterone concentrations on d 65 (8.68 ng/mL; bulls averaged 266 ± 21.5 d of age). Pruitt et al. (1986) reported lesser testosterone concentrations (3.8 to 7.2 ng/mL depending on breed and energy level) at puberty (one successful mating and 50 x 10^6 sperm/mL with at least 10% motility) than what was found in the present study. Testosterone concentrations begin to rise during the early gonadotropin rise, or the prepubertal period, concurrent with increases in LH and FSH (Amann, 1983) and continue to increase until puberty. McCarthy et al. (1979) reported that testosterone is released in a pulsatile manner following pulsatile release of LH beginning when bulls are approximately 5 mo of age. The frequency and peaks of testosterone pulses increased with age and by 10 mo of age, bulls had episodic releases of testosterone every 3 to 4 h (McCarthy et al., 1979). The pulsatile release of testosterone during this time frame may make it difficult to truly explain any differences in testosterone concentration in our study, when a single sample was collected on a single day. Our study shows that testosterone concentrations continued to increase with age, and eventually plateaued once peak concentrations were achieved by both treatments. This is supported by previous studies (Lunstra et al., 1978; Evans et al., 1996) in which mean serum testosterone is

basal when bulls are 1 to 4 mo of age, but then increases until puberty is achieved when mean serum concentrations remain relatively constant.

Increasing testosterone concentrations drive testicular development. Scrotal circumference measurements (used to estimate testes size) are indicative of a bull's ability to produce semen and can be used to predict the age at which bulls will attain puberty (Rusk, et al. 2002). Scrotal circumference at the onset of puberty has been shown to average 27.9 ± 0.2 cm (Lunstra et al., 1978; Barth and Brito, 2004). In our study, the average SC at puberty was 34 cm, but it ranged from 29 to 40 cm. This average SC is greater than that reported previously (Lunstra et al., 1978; Barth and Brito, 2004). Our results were similar to those reported by Pruitt et al. (1986). Bulls in that study were fed varying energy levels to determine its effect on attainment of puberty and mating ability in post-weaning sexual development. In contrast, their definition of puberty included the successful completion of at least 1 mating test in addition to producing a semen sample with at least 50 x 10^6 sperm/mL and a minimum of 10% progressive sperm motility. In their study, bulls were able to complete a mating test before they were deemed pubertal, indicating that although they were able to satisfy the requirement for the mating test, their semen quality was not yet acceptable to be deemed pubertal. Therefore, their SC at puberty was larger. Similar to Pruitt et al. (1986), bulls in our study had large SC at puberty, but were not younger at puberty.

We hypothesized that exposed bulls would achieve puberty at a younger age and would therefore yield a greater percentage of bulls successfully passing their first BSE conducted when bulls averaged 1 yr of age. A similar percentage of exposed bulls passed their first BSE compared with control bulls (87 vs. 74%). This passing rate and lack of difference between treatments is likely a reflection of exposed bulls not attaining puberty at a younger age than

control bulls. The semen evaluation component of a BSE is more demanding than the semen characteristics required for a bull to be considered pubertal. Bulls needed to have at least 30% progressive sperm motility and at least 70% normal sperm morphology (Society for Theriogenology, 1993) to pass the BSE. Of the 14 bulls (18%) not passing their BSE, the primary reason for failure was having less than 70% normal sperm morphology. In fact, exactly half of the bulls that failed their BSE had no normal appearing sperm in their ejaculates. Of those 7 bulls, 3 had 100% proximal cytoplasmic droplets. Cytoplasmic droplets are usually lost before ejaculation as a final step in sperm maturation, and their presence in a semen sample indicates immature sperm and is common in young bulls undergoing puberty (Arteaga et al., 2001). Poor semen evaluations are the primary reason for failure of a BSE by yearling beef bulls (Coe, 1999; Arteaga et al., 2001; Kennedy et al., 2002). In all of these studies, less than 70% normal sperm morphology was the main cause of inferior semen evaluations. Occurrence of morphologically abnormal spermatozoa in semen is related to SC. Barth and Waldner (2002) reported bulls with below average SC or a SC that was below their minimum requirement for a specified breed (Barth, 1994), had a greater percentage of abnormal sperm and decreased sperm motility. Sperm abnormalities included head and midpiece defects, detached heads, and proximal cytoplasmic droplets. As SC increased, however, the percentage of sperm abnormalities decreased. Another study found that of 338 bulls ranging from 8 to 15 mo of age with unacceptable SC to pass the BSE (< 30 cm), 96.7% of them produced a semen sample with less than 70% morphologically normal sperm (Coe, 1999). Our study echoed those findings because as our bulls developed and SC increased, the percentage of abnormal sperm present in an ejaculate declined.

It is difficult for yearling bulls to pass a semen evaluation at such a young age because some are likely still undergoing the pubertal process. Arteaga et al. (2001) illustrated that bulls at 11 mo of age had the greatest incidence of abnormal sperm compared with 15 mo old bulls, with proximal droplets and midpiece reflexes being primary causes of abnormality. In that study, mature semen samples needed to have a sperm concentration of at least 400 x 10^6 sperm/mL, a minimum of 60% progressively motile sperm, and at least 70% normal sperm morphology. Authors reported that the passing rates for these mature semen evaluations increased from 20% at 11 mo of age to 61.5% when bulls were 15 mo of age. A breeding soundness examination is an important reproductive tool that can help predict a bull's fertility and ability to breed females. It only illustrates, however, one specific time point of a bull's sexual development. As bulls age, BSE results could be vastly different such that a greater passing rate occurs simply because bulls have had the opportunity to develop. The likelihood of bulls passing their semen evaluation increases with age (Arteaga et al., 2001). In our study, bulls ranged in age from 10.5 to 13 mo at the time of first BSE, and age was a significant source of variation in the percentage of bulls that passed. Given time, however, it is likely that a larger portion of bulls would pass their semen evaluation portion of the BSE and be deemed acceptable to settle females.

The goal of this study was to reduce the age at which bulls attain puberty by giving them constant, long-term exposure to cycling beef females, and ultimately increase the percentage of those bulls passing their BSE as yearlings. Although age at puberty and BSE passing rates were not influenced by continuous fenceline exposure of bulls to females exhibiting regular estrous cycles, opportunities for further research studies exist. For example, it may be worth investigating the duration or timing of bull exposure to cycling females. Perhaps allowing only

several hours of exposure at a time rather than continuous exposure would alter bull responsiveness. In addition, the early gonadotropin rise is a critical time period in the sexual development of bulls, in which Sertoli cells, crucial for spermatogenesis, differentiate. It may be worth investigating whether the presence of estrous females during this significant time point influences this process by exposing bulls at a younger age than what was done in the present study. Another idea would be to alternate the females used in the study. Novel, estrual stimuli have been shown to increase sexual behaviors of bulls (Bailey et al., 2005). Perhaps introducing the females multiple times per week rather than keeping the same 2 groups of estrous females in the pen continuously would increase bull interest and stimulate accelerated pubertal development and spermatogenesis.

Conclusions

In conclusion, constant, long-term exposure of prepubertal beef bulls to cycling females neither reduced age at puberty nor influenced the ability of a bull to pass a BSE. Occurrence of sexual behaviors elicited by bulls in both treatments indicated that the presence of cycling females had no role in advancing the sexual development of young bulls. Females displaying estrus did influence the sexual activity of exposed bulls but no related effects of sexual behavior were observed in testosterone, age at puberty, or BSE success. There may be opportunities for further research investigating the duration and frequency of exposure to cycling females, as well as housing bulls at completely separate locations. From this study, it was determined that seedstock producers developing bulls for the purpose of sale to commercial cattlemen would see no benefit in age at attainment of puberty or greater BSE passing rates by housing bulls next to cycling females.

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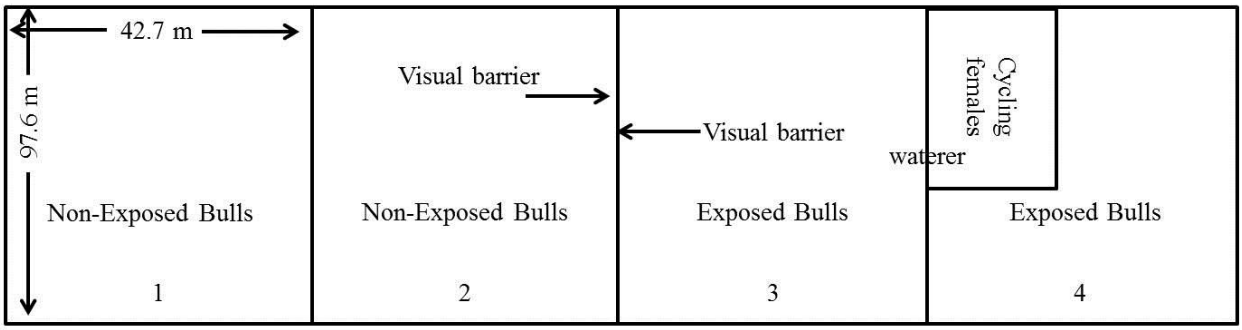

Figure 4.1 Diagram of bull pens and cycling beef female area. Bulls with exposure to cycling beef females (exposed bulls) were housed in pens 3 and 4 and were allowed fenceline and visual contact with cycling females. Nonexposed control bulls were housed in pens 1 and 2 and were separated from cycling beef females by at least 42.7 m with a solid visual barrier standing 3.4 m high.

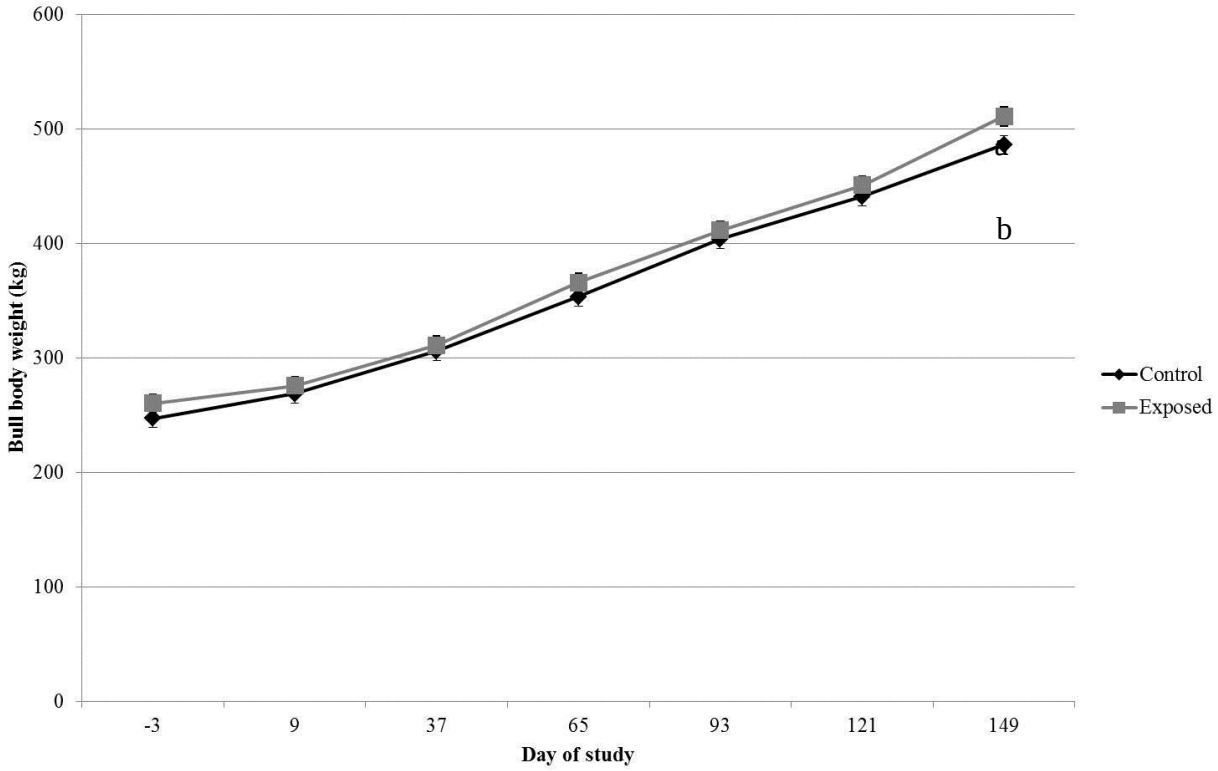

Figure 4.2 Mean bull BW from d -3 to d 149 for bulls with continuous fence line exposure to cycling beef females (Exposed) and bulls not exposed to females (Control). A treatment by day interaction ($P = 0.0003$) existed. Exposed bulls were heavier at d 149 than control bulls ($P = 0.02$). Day 0 was onset of exposure to cycling beef females. Day 149 was the last day of monthly semen collections before breeding soundness examinations.

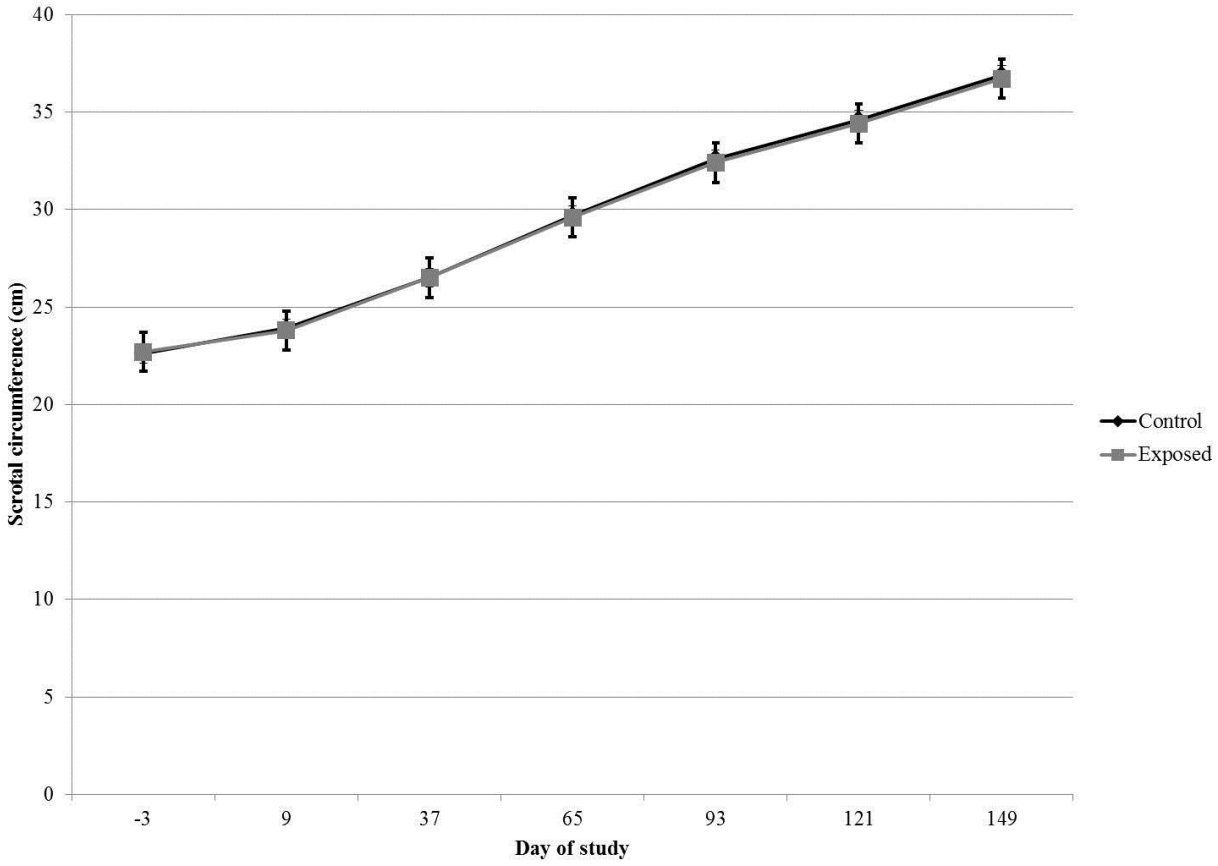

Figure 4.3 Mean scrotal circumference from d -3 to d 149 for bulls with continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Control). Scrotal circumference increased ($P < 0.0001$) as day of the study increased. A treatment by day interaction ($P = 0.40$) was nonexistent. Day 0 was onset of exposure to cycling beef females. Day 149 was the last monthly semen collection before breeding soundness examinations.

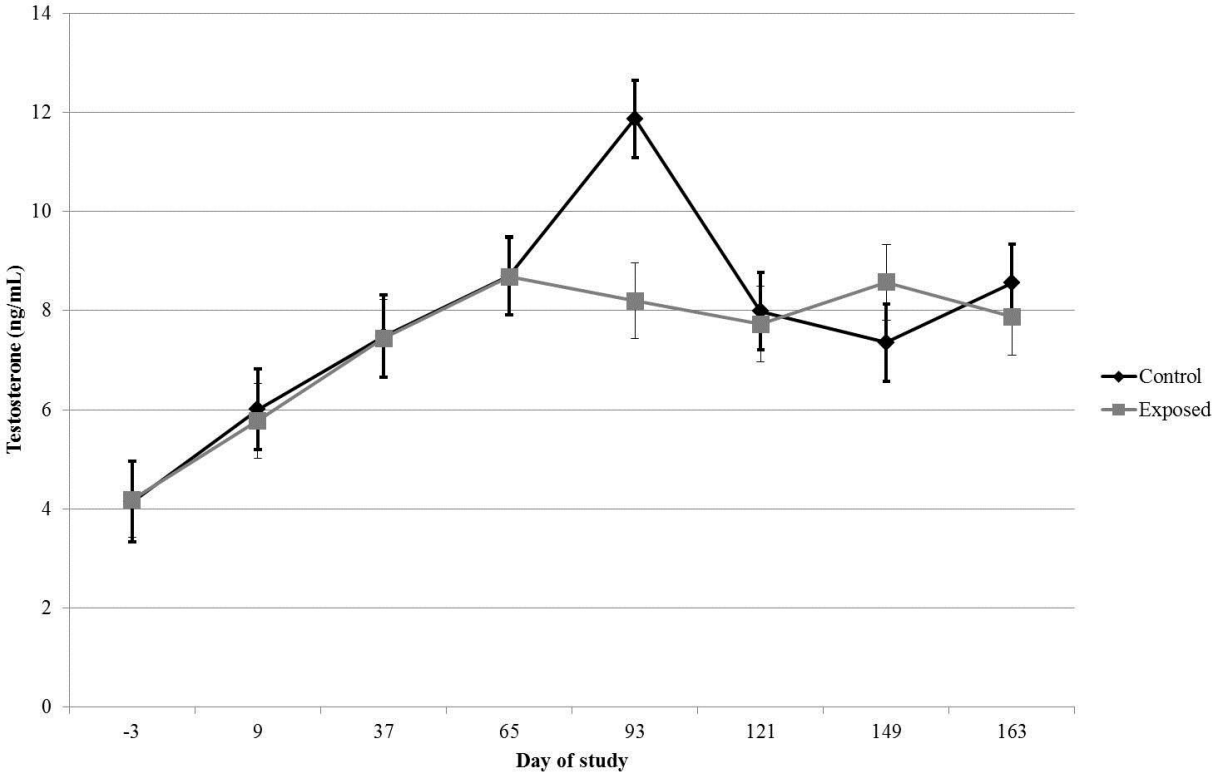

Figure 4.4 Mean serum testosterone concentrations from d -3 to d 163 for bulls with continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Control). A treatment by day interaction ($P = 0.03$) existed. Serum testosterone concentrations were greater for exposed bulls compared with control bulls at d 93 of the study ($P = 0.002$). Day 0 was onset of exposure to cycling beef females. Day 163 was the day of breeding soundness examinations.

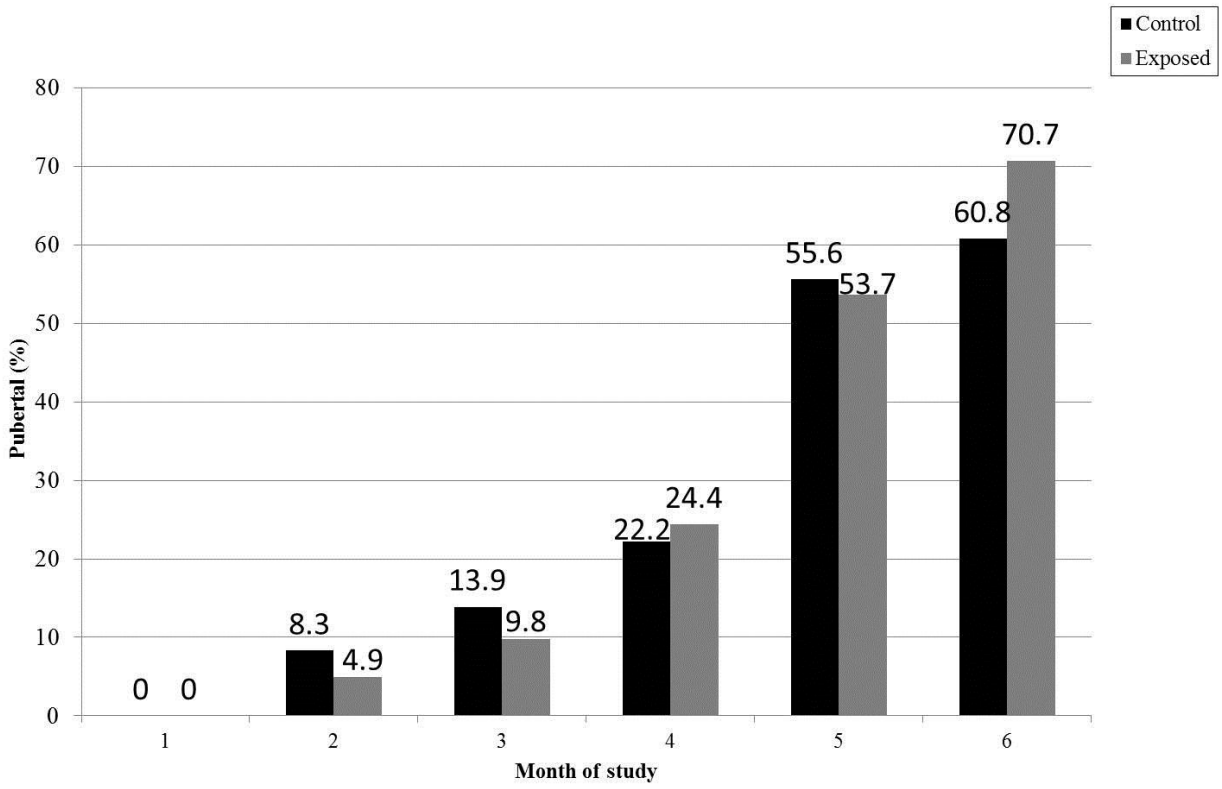

Figure 4.5 Cumulative percentage of bulls achieving puberty during each month of the study for bulls with continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Control). The percentage of bulls achieving puberty by month 6 did not differ ($P > 0.10$) between treatments.

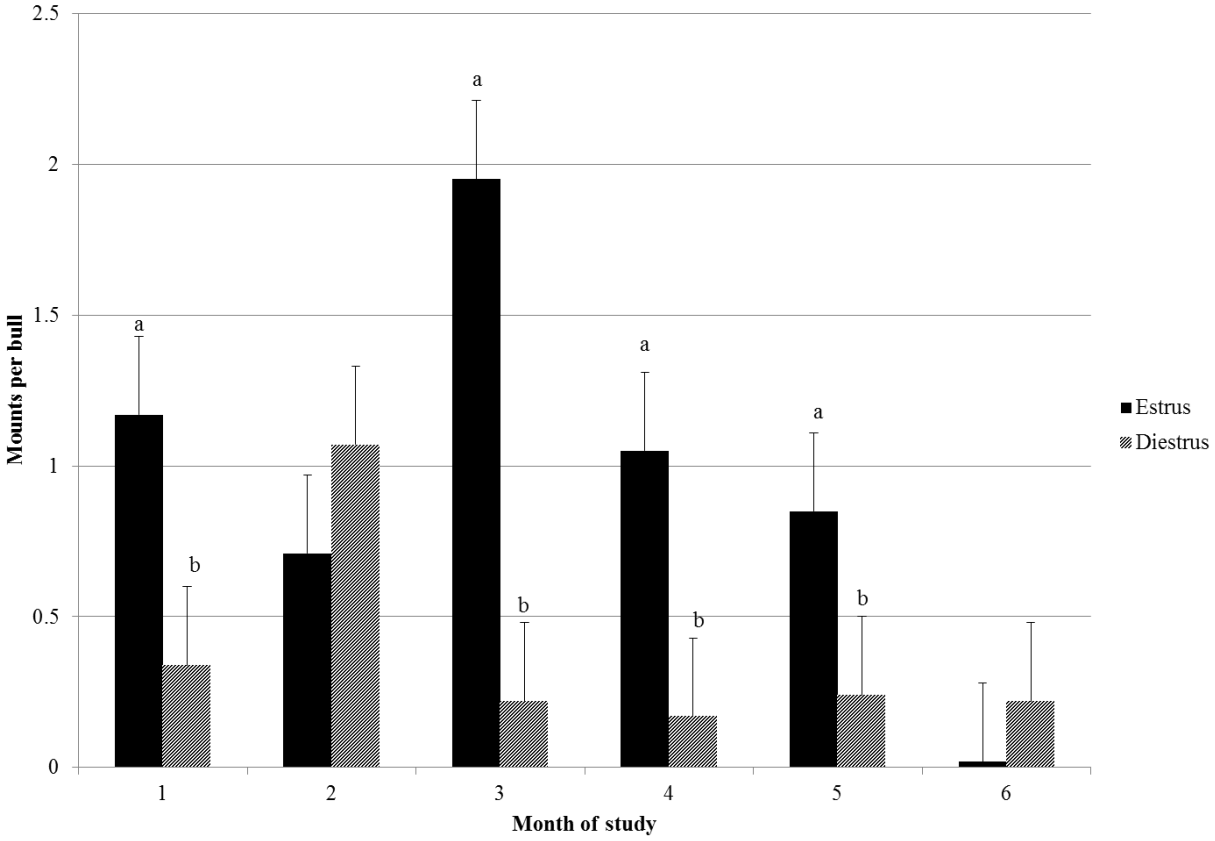

Figure 4.6 Mean mounts exhibited by bulls with continuous fenceline exposure (Exposed) to cycling beef females when females were either in estrus or diestrus. Treatment, month of study, and stage of female estrous interacted ($P = 0.05$) to affect the number of mounts exhibited. Bars with different superscripts within month are different ($P < 0.05$).

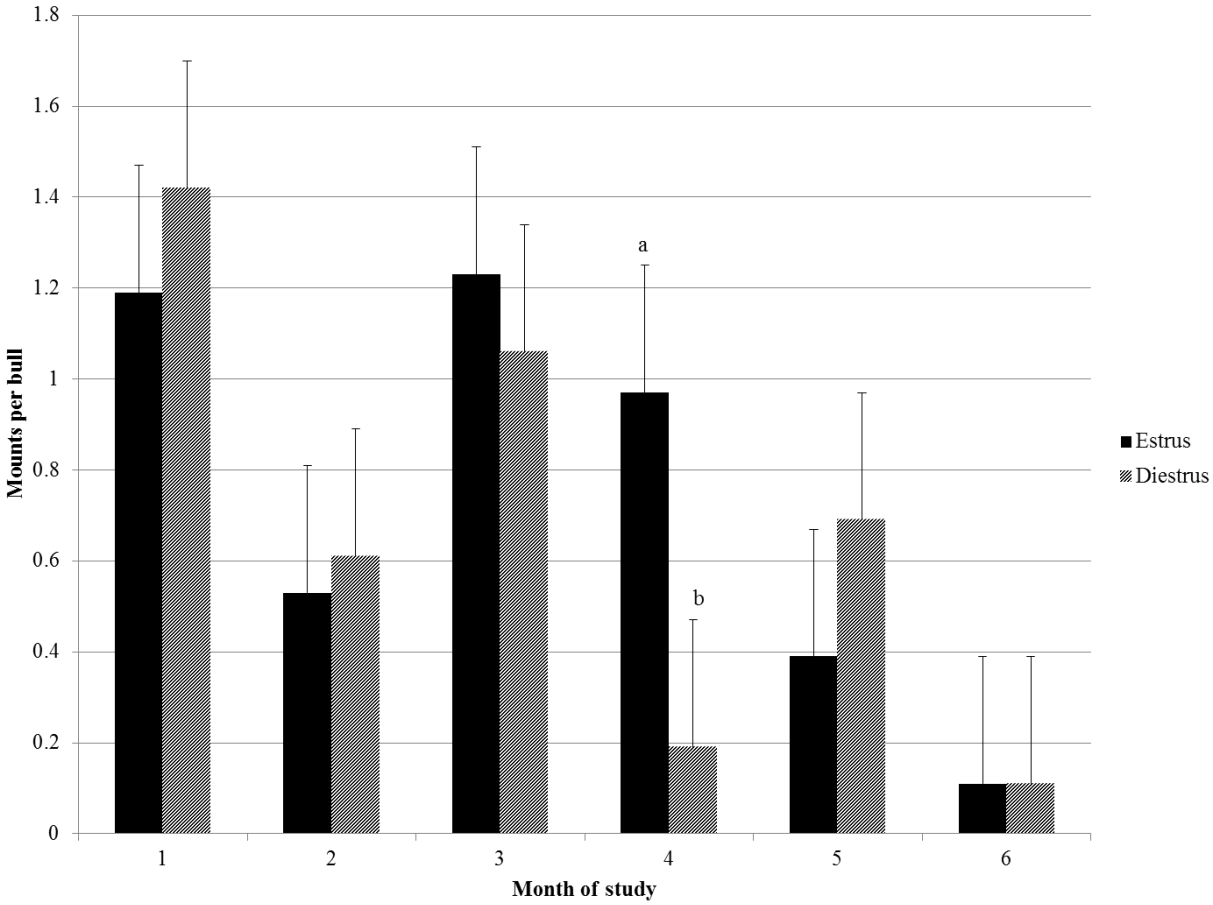

Figure 4.7 Mean mounts exhibited by bulls not exposed to cycling beef females (Controls) when females were either in estrus or diestrus. Treatment, month of study, and stage of female estrous interacted ($P = 0.05$) to affect the number of mounts exhibited. Bars with different letters within month differ ($P = 0.03$).

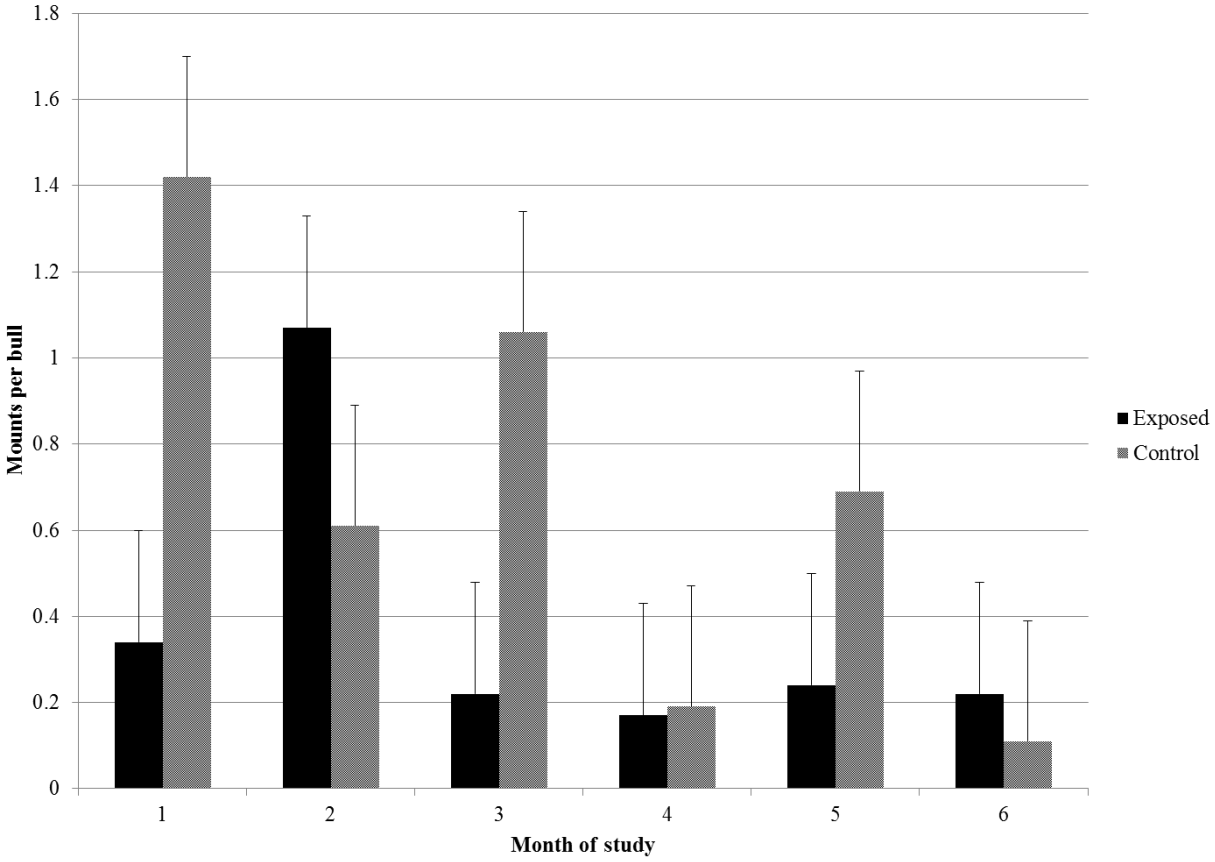

Figure 4.8 Mean mounts exhibited for bulls with continuous fenceline exposure to cycling beef females (Exposed) and bulls with no exposure to females (Control) when females were in diestrus. Treatment, month of study, and stage of female estrous interacted ($P = 0.05$) to affect the number of mounts exhibited. Exposed bulls tended ($P = 0.07$) to have more mount attempts than control bulls when females were in diestrus.

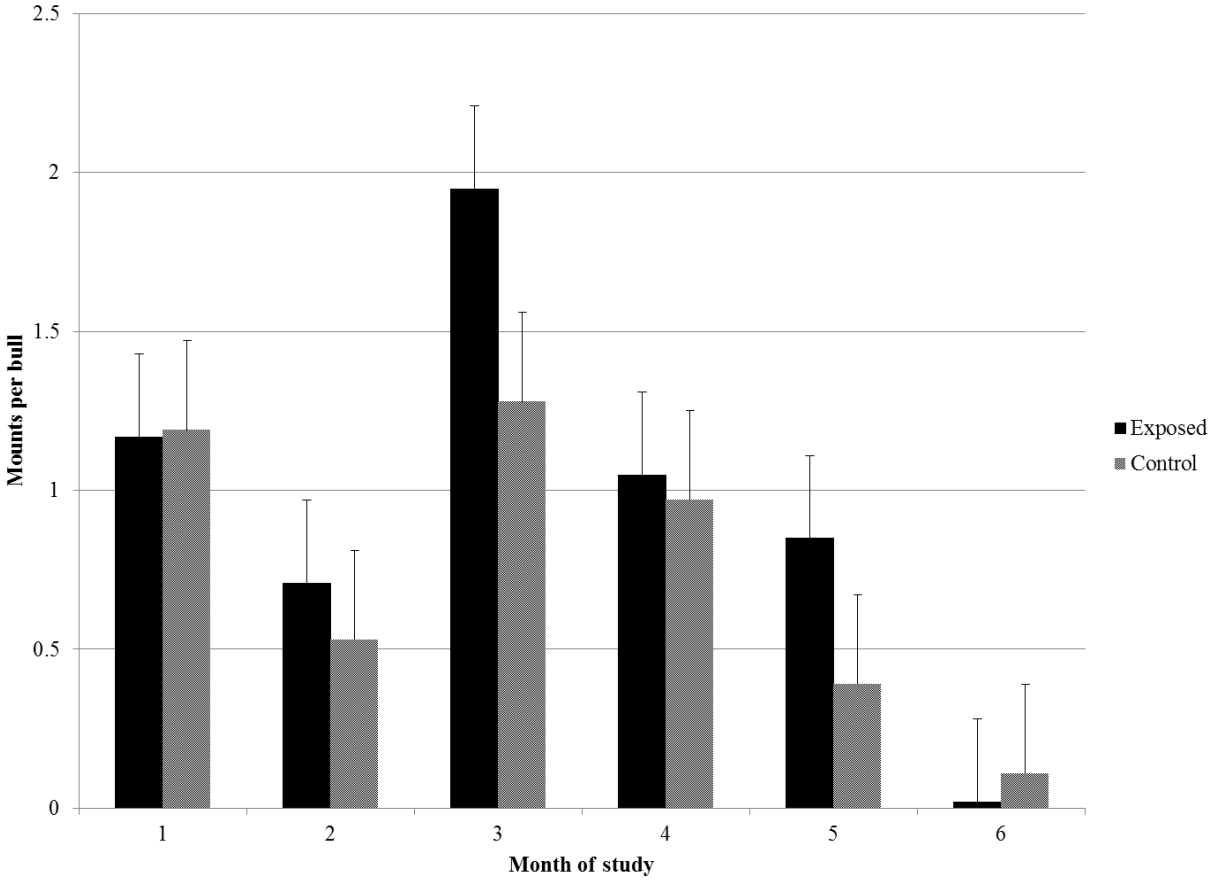

Figure 4.9 Mean mounts exhibited by bulls with continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Control) when females were in estrus. Treatment, month of study, and stage of female estrous interacted ($P = 0.05$) to affect the number of mounts exhibited. No difference ($P = 0.70$) between exposed and control bulls for the number of mounts per bull when females were in estrus.

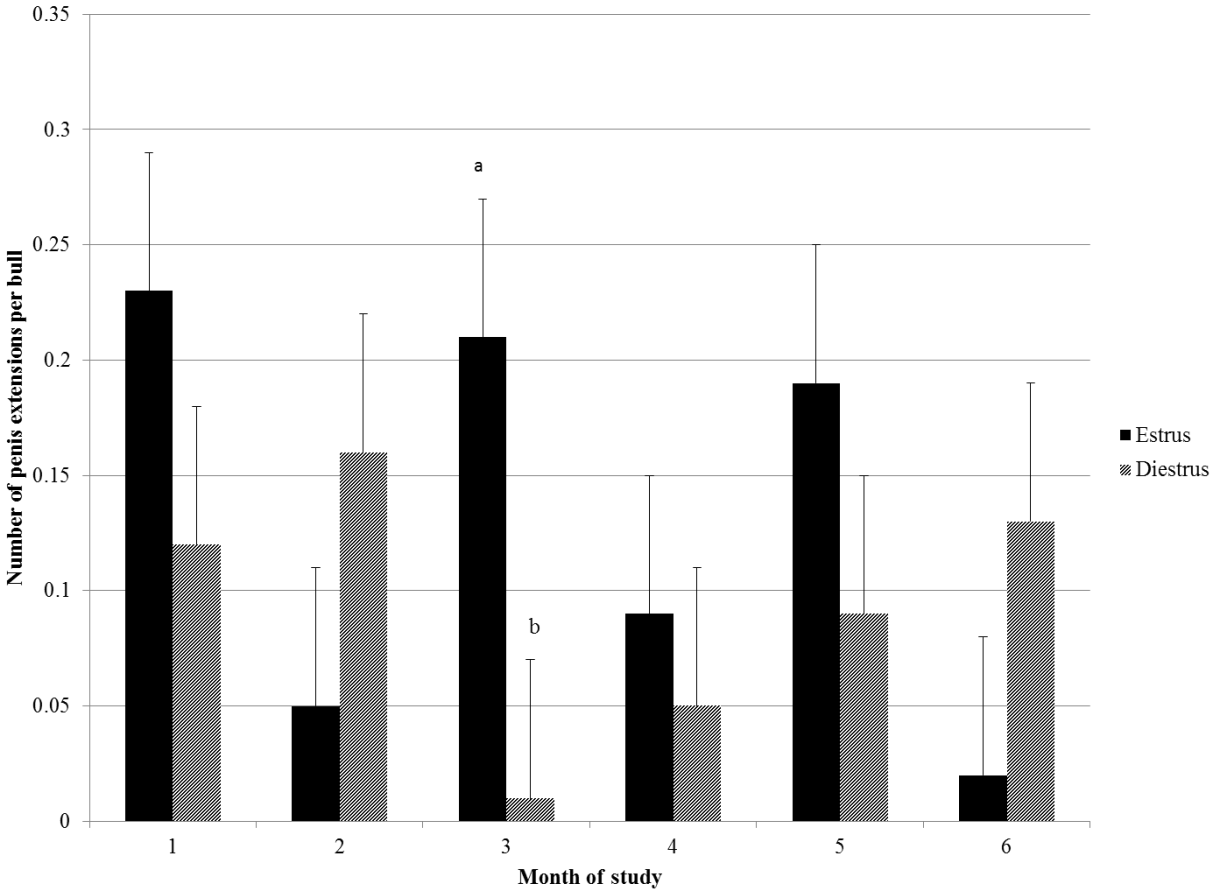

Figure 4.10 Mean number of penis extensions per bull when females were either in estrus or diestrus. Month of study and stage of female estrous interacted (P = 0.02) to affect the number of penis extensions. Across treatments, the number of penis extensions was greater (P = 0.02) when females were in estrus compared with diestrus, during month 3 of the study.

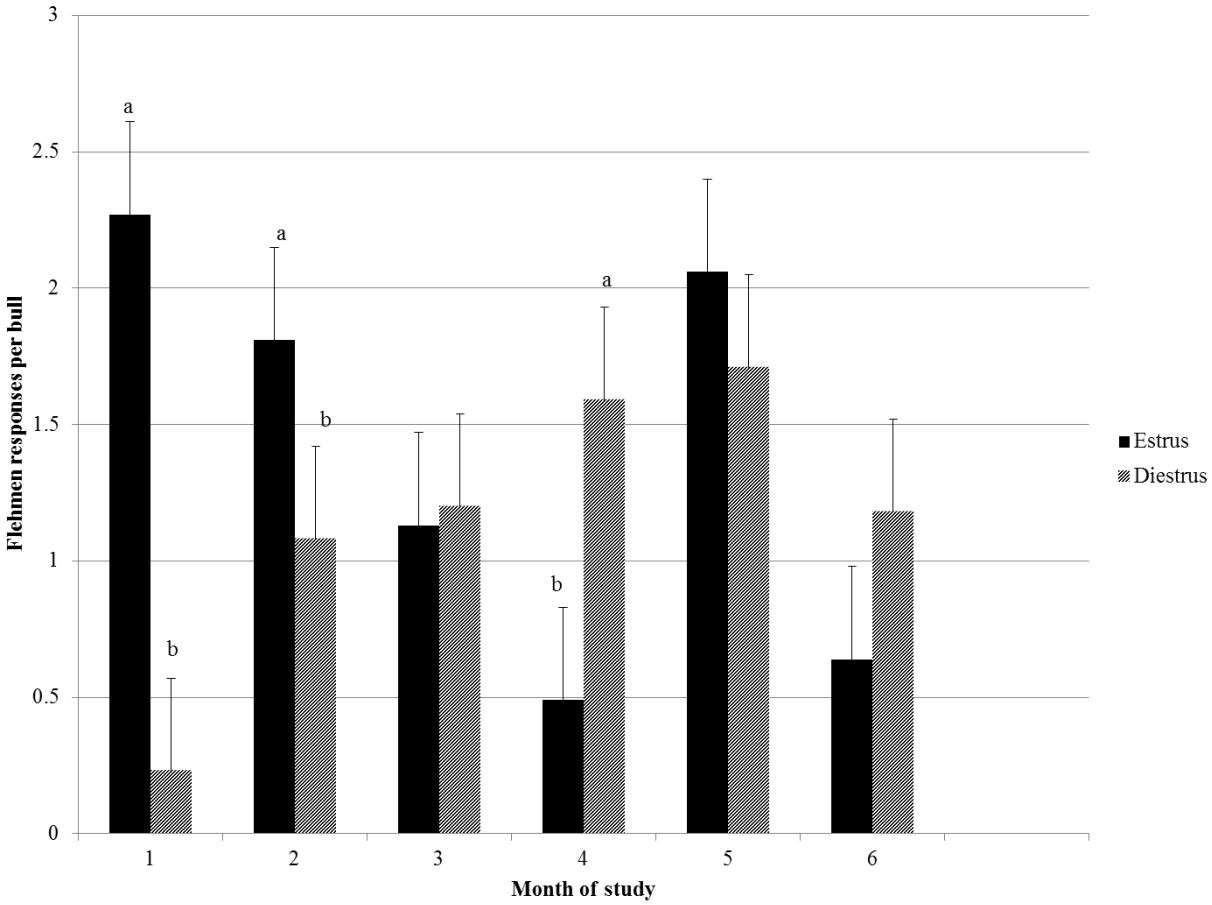

Figure 4.11 Mean number of flehmen responses for bulls with continuous fenceline exposure to cycling beef females (Exposed) when females were either in estrus or diestrus. Treatment, month of study, and stage of female estrous interacted ($P < 0.001$) to affect flehmen response. Bars with different letters within month were different ($P < 0.05$).

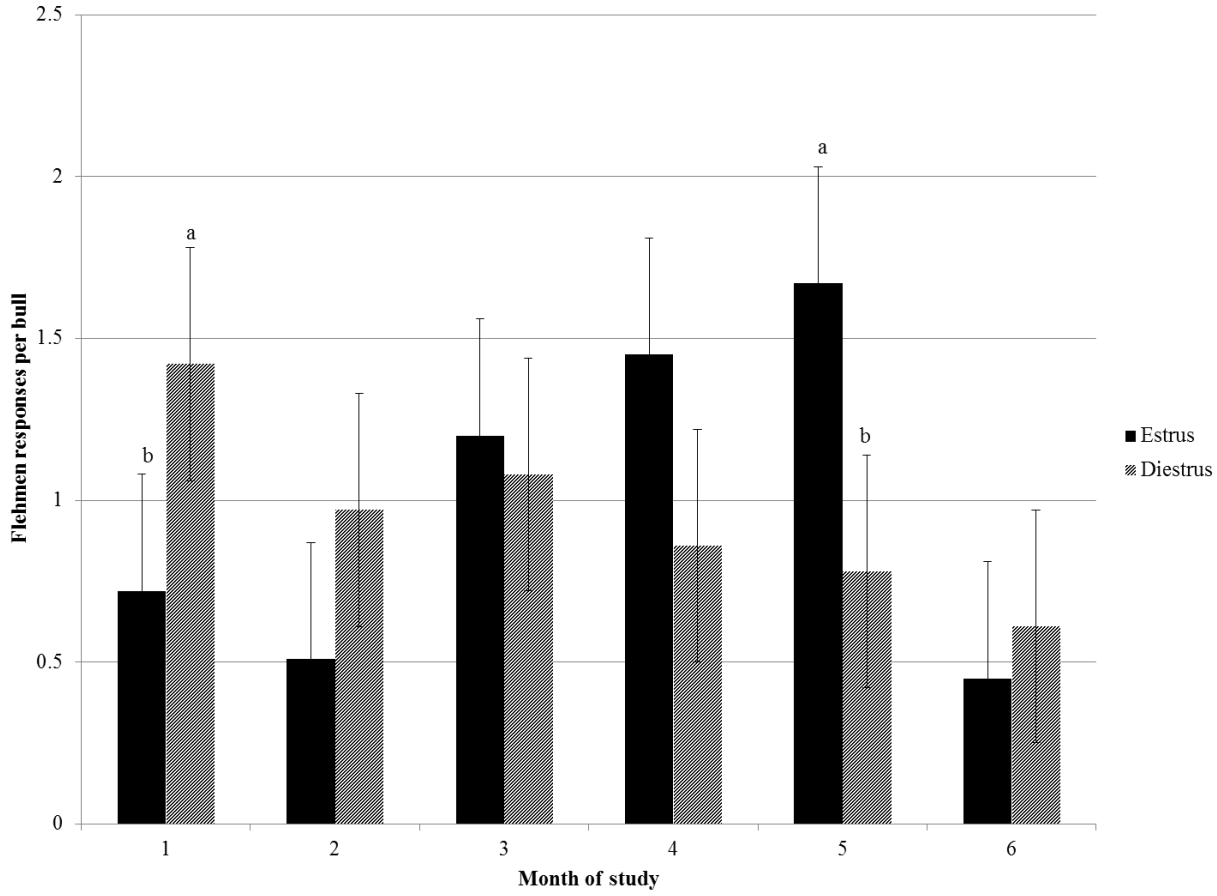

Figure 4.12 Mean number of flehmen responses for bulls without exposure to cycling beef females (Control) when females were either in estrus or diestrus. Treatment, month of study, and stage of female estrous interacted ($P < 0.001$) to affect flehmen response. Bars with different letters within month are different ($P < 0.05$).

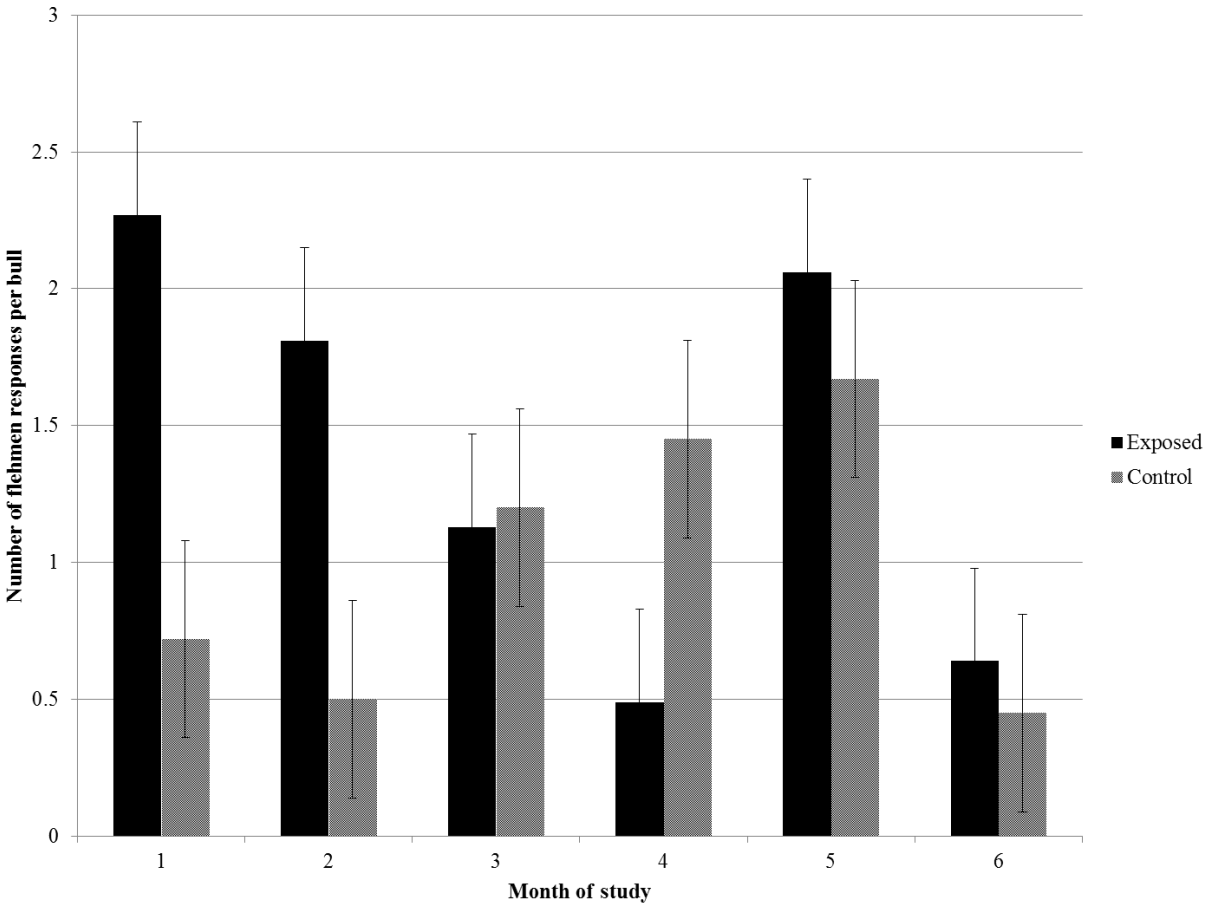

Figure 4.13 Mean number of flehmen responses exhibited by bulls with continuous fenceline exposure to cycling beef females (Exposed) or bulls without exposure to females (Control) when females were in estrus. Treatment, month of study, and female stage of estrous interacted ($P < 0.0001$) to affect flehmen response. No difference was detected in the number of flehmen responses between exposed and control bulls when females were in estrus ($P = 0.40$).

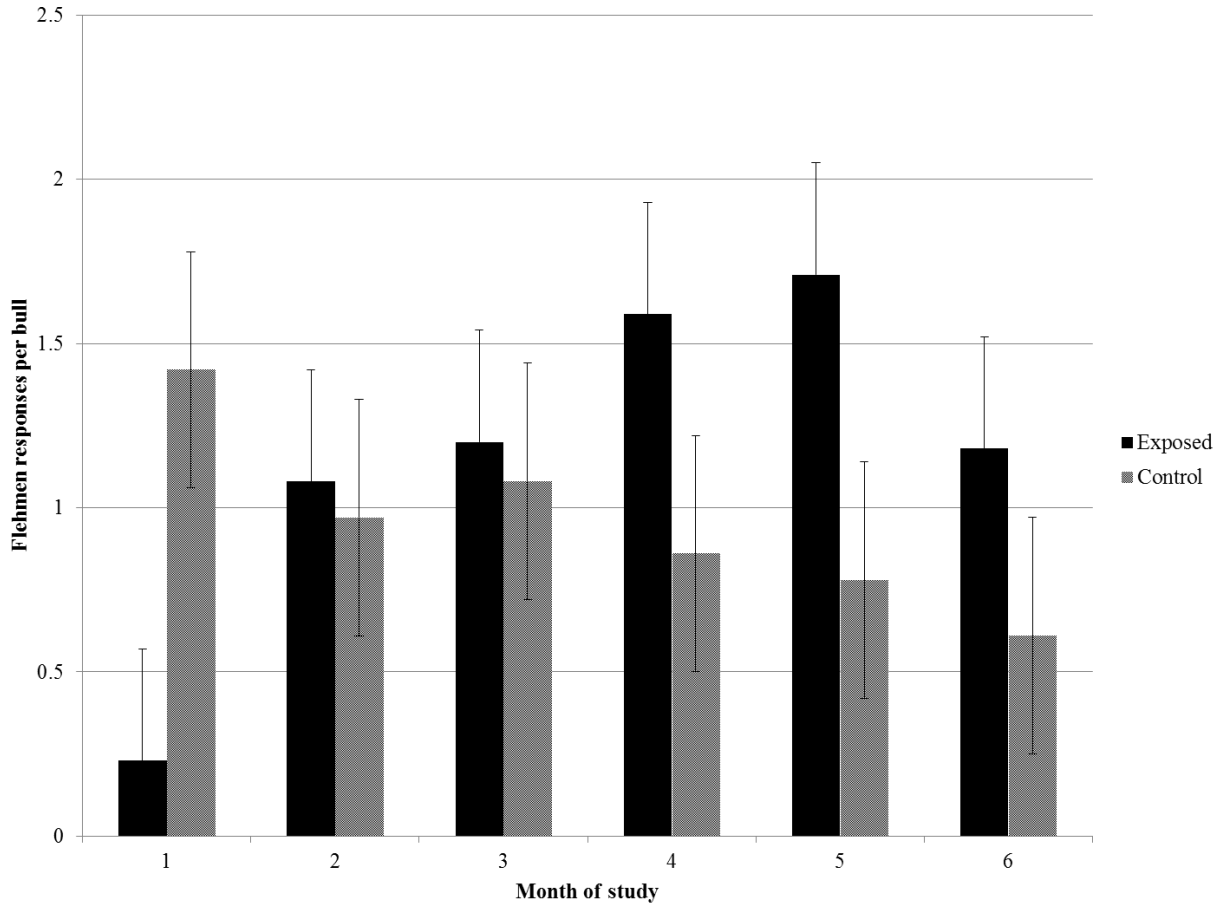

Figure 4.14 Mean number of flehmen responses exhibited by bulls with continuous fenceline exposure to cycling beef females (Exposed) or bulls without exposure to females (Control) when females were in diestrus. Treatment, month of study, and stage of female estrous interacted ($P < 0.001$) to affect flehmen response. No difference ($P = 0.40$) was detected between exposed and control bulls when females were in diestrus.

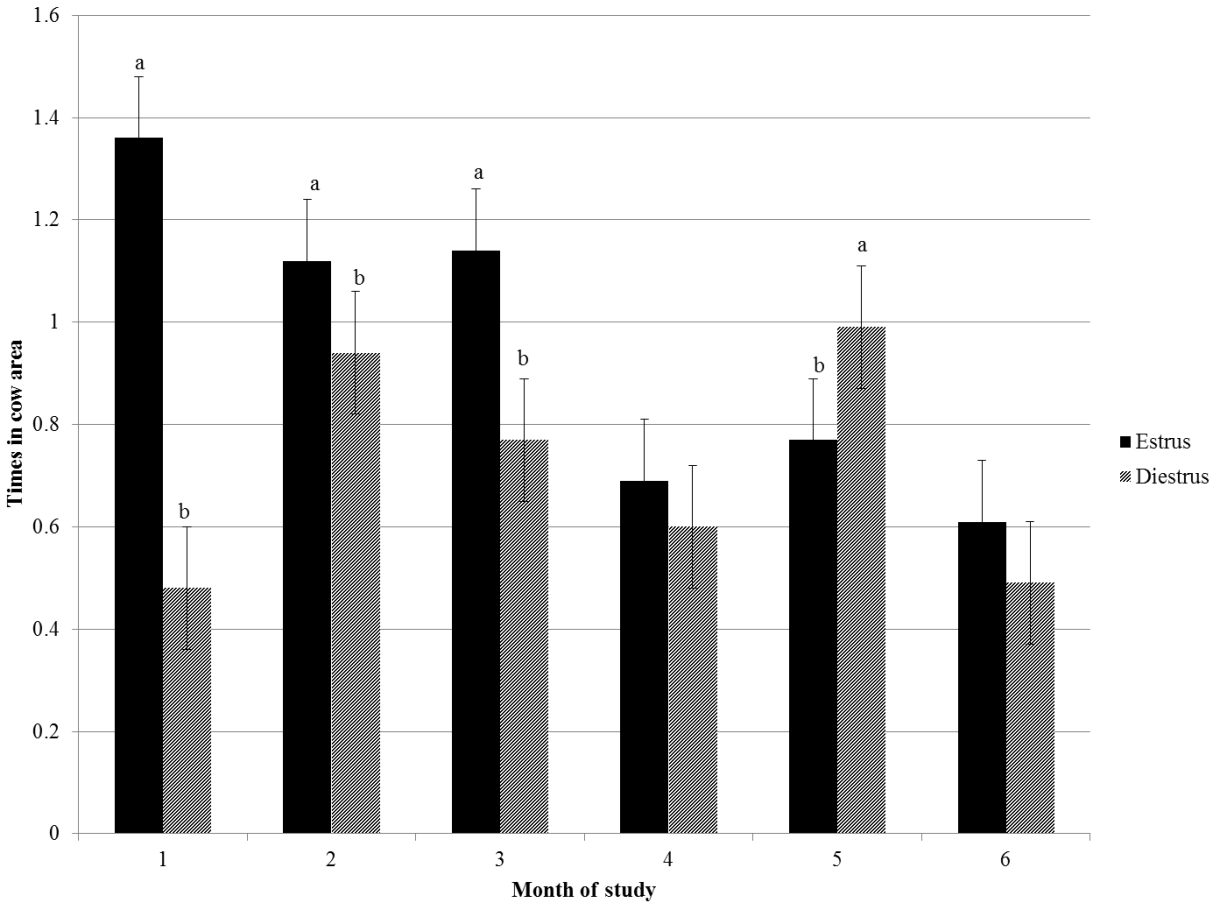

Figure 4.15 Mean number of times bulls with continuous fenceline exposure to cycling beef females (Exposed) entered the designated cow area when females were either in estrus or diestrus. Month of study and stage of female estrous interacted ($P < 0.001$). Bars with different letters within month were different ($P < 0.10$).

Table 4.1 No. of bulls reaching puberty by d 149, mean age, scrotal circumference, body weight and semen characteristics at puberty for bulls with continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Control).

Item	Exposed	Control	*P*-value
No. of bulls pubertal (%)	29 (56.9)	22 (43.1)	…
Age (days)	320.3 ± 5.3	311.3 ± 5.9	0.28
Scrotal circumference (cm)	34.6 ± 0.5	34.9 ± 0.5	0.65
Weight (kg)	466.2 ± 12.2	437.7 ± 13.5	0.25
Sperm concentration (10^6/mL)	85.53 ± 9.6	106.12 ± 10.8	0.35
Motility (%)	45.2 ± 4.3	34.2 ± 4.5	0.23
Normal morphology (%)	33.8 ± 4.3	24.8 ± 4.9	0.17

Table 4.2 Pass/fail rates of bulls during breeding soundness examinations (BSE) previously exposed to cycling beef females (Exposed) or not exposed (Control) including bulls that were deemed pubertal by d 149.

	% Pass (n)	% Fail (n)
Exposed	87.8 (36/41)	12.2 (5/41)
Pubertal	93.1 (27/29)	7.4 (2/29)
Control	74.4 (28/36)	25.0 (9/36)
Pubertal	100.0 (22/22)	0 (0/22)

There were no treatment differences ($P = 0.52$) detected in passing rates.

Age influenced ($P < 0.01$) a bull's ability to pass the BSE.

Table 4.3 Sexual behaviors exhibited by bulls with continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Control) when females were either in estrus or diestrus.

	Stage of the estrous cycle for beef females during assessment					
	Estrus			Diestrus		
	Mounts	Flehmen	Extension	Mounts	Flehmen	Extension
Exposed	.96 ± .11[a]	1.40 ± .24	.14 ± .03	.38 ± .11[†b]	1.16 ± .24	.09 ± .03
Control	.75 ± .12	.99 ± .24	.13 ± .03	.68 ± .12[†]	.96 ± .24	.10 ± .03
Overall mean	.86 ± .09	1.21 ± .09	.13 ± .02	.52 ± .07	1.06 ± .08	.10 ± .02

[ab] Means within trait and stage of the estrous cycle having different superscript letters differ ($P < 0.05$).

[†]Mounting activity tended ($P = 0.07$) to be greater for control than exposed bulls when females were in diestrus.

Table 4.4 Mean mount activity, flehmen response, and penis extension when females were in estrus and diestrus for bulls not exposed to cycling beef females (Control).

	Estrus			Diestrus		
Month	Mount	Flehmen	Extension	Mount	Flehmen	Extension
1	1.19	0.72[a]	0.17	1.42	1.42[b]	0.22
2	0.53	0.50	0.03	0.61	0.97	0.08
3	1.28	1.19	0.17	1.06	1.08	0
4	0.97[a]	1.45	0.14	0.19[b]	0.86	0.06
5	0.39	1.67[c]	0.25	0.69	0.78[d]	0.14
6	0.11	0.45	0	0.11	0.61	0.11
SEM	0.27	0.36	0.08	0.27	0.36	0.08

[a,b] Means within trait and stage of the estrous cycle having different superscript letters differ (P = 0.03).

[c,d] Means within trait and stage of the estrous cycle having different superscript letters differ (P = 0.09).

Table 4.5 Mean mount activity, flehmen response, penis extension, and the number of times bulls entered the cycling beef female area when females were either in estrus or diestrus for bulls with continuous fenceline exposure to cycling beef females (Exposed).

	Estrus				Diestrus			
Month	Mount	Flehmen	Extension	Cycling Female Area	Mount	Flehmen	Extension	Cycling Female Area
1	1.17[a]	2.27[a]	0.29	1.36[a]	0.34[b]	0.23[b]	0.02	0.48[b]
2	0.71	1.81[a]	0.07	1.12[c]	1.07	1.08[b]	0.24	0.94[d]
3	1.95[a]	1.13	0.24	1.14[a]	0.22[b]	1.20	0.02	0.77[b]
4	1.05[a]	0.49[b]	0.05	0.69	0.17[b]	1.59[a]	0.05	0.60
5	0.85[c]	2.06	0.12	0.77[a]	0.24[d]	1.71	0.05	0.99[b]
6	0.02	0.64	0.08	0.61	0.22	1.18	0.15	0.49
SEM	0.26	0.34	0.08	0.12	0.26	0.34	0.08	0.12

[a,b] Means within trait and stage of the estrous cycle having different superscript letters differ ($P < 0.05$).

[c,d] Means within trait and stage of the estrous cycle having different superscript lettersdiffer ($P < 0.10$).

Appendix A - Additional data not included in Chapter 4

Table A.1 Body weight (kg) of bulls given continuous fenceline exposure to cycling beef females (Exposed) and bulls not given exposure to females (Control) from d -3 to d 149.

	Sampling Days							
	-3	**9**	**37**	**65**	**93**	**121**	**149**	**SEM**
Exposed	260.2	275.7	311.0	365.8	411.0	450.7	510.9[a]	7.4
Control	247.3	268.7	305.0	353.3	403.6	440.8	486.1[b]	7.9

Columns with different superscripts differ ($P < 0.05$).

Day 0 was onset of exposure to cycling beef females. Day 149 was the last monthly semen collection before breeding soundness examinations.

Table A.2 Scrotal circumference (cm) for bulls given continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Controls) from d -3 to d 149.

	Sampling Days							
	-3	**9**	**37**	**65**	**93**	**121**	**149**	**SEM**
Exposed	22.8	23.8	26.5	29.6	32.2	34.1	36.4	0.4
Control	22.6	23.9	26.5	29.7	32.6	34.6	36.9	0.5

Scrotal circumference significantly increased ($P < 0.01$) from d -3 to d 149.

No treatment differences were detected at any of the days throughout the study.

Day 0 was onset of exposure to cycling beef females. Day 149 was the last monthly semen collection before breeding soundness examinations.

Table A.3 Serum testosterone concentrations (ng/mL) of bulls given continuous fenceline exposure to cycling beef females (Exposed) and bulls not exposed to females (Control) from d -3 to d 163.

	Sampling Days								
	-3	**9**	**37**	**65**	**93**	**121**	**149**	**163**	**SEM**
Exposed	4.19	5.78	7.44	8.68	8.20[a]	7.73	8.57	7.89	0.8
Control	4.15	6.01	7.48	8.70	11.87[b]	7.99	7.36	8.56	0.8

Columns with different superscripts differ ($P < 0.05$).

Day 0 was the onset of exposure to cycling beef females. Day 163 was the day breeding soundness examinations were conducted.