FERTILITY OF BEEF RECIPIENTS FOLLOWING A FIXED-TIME EMBRYO TRANSFER PROTOCOL THAT INCLUDES FOLLICLE STIMULATING HORMONE DILUTED IN HYALURONAN

A Thesis

by

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ABSTRACT

This study was performed to test the viability of administering a single 40 mg dose of Folltropin-V® (FSH, Bioniche Animal Health) diluted in SRF (MAP-5 50, Sodium Hyaluronate, Bioniche Animal Health) on day 5 of a recipient synchronization protocol to beef cows to evaluate its effect on recipient fertility. All recipients were administered an estradiol 17β (2.5 mg, IM) and progesterone (50 mg, IM) combination injection on day 0 and a CIDR® (progesterone 1.34 g, Pfizer Animal Health) was inserted. Lutalyse® (dinoprost tromethamine, Pfizer Animal Health, 25 mg, IM) was administered at the time of CIDR removal on day 7, and estradiol 17β (1 mg, IM) was administered on day 8. On day 16, the presence of at least one corpus luteum (CL), detected via ultrasound, resulted in the recipient receiving an embryo (both fresh and frozen-thawed embryos were used). Embryos were not transferred into cows that did not show the presence of a CL. Dependent variables for which data were collected included circulating progesterone levels at the time of transfer, number of CLs and CL diameter, circumference, and area; measured in millimeters. The study (n=572) consisted of a treatment group (n=268) and a control group (n=304), and included both *Bos indicus* (Brahman influenced) crossbred (n=115) and *Bos taurus* (Angus based) cows (n=457). Pregnancy rates for Treated recipients ($40.67\%^{A}$) and Control recipients ($52.96\%^{B}$) differed ($P<.05$). There was no difference in the mean number of CLs per recipient for Treated ($1.14 \pm .03$) and Control ($1.10 \pm .02$) cows, nor was there a difference in

progesterone (P4) at the time of transfer for Treated (3.14 ± .40 ng/mL) and Control (3.23 ± .18 ng/mL) recipients. Overall, the inclusion of Folltropin-V® diluted in hyaluronan in a FTET synchronization protocol did not improve the fertility of beef recipients.

DEDICATION

This thesis is dedicated to my parents. To my mom who has always provided unwavering support of my goals and my dad for providing guidance through life and my educational journey. Thanks for the unconditional love from you both.

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TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Historically, the beef industry has overcame many obstacles to establish itself as one of the leading protein providers, yet as we emerge into the 21st century, the task of maintaining production with fewer resources than ever presents a formidable challenge. Models predict the world population to reach 9.5 billion by 2050 and in turn a 70% increase in world food production will be required to feed the populace (Keyzer et al. 2005). Progress has certainly been made in the last few decades, as the industry is able to produce 1 billion kg of beef with less than 69.9% of the total animal numbers, 81.4% of the available feedstuffs, 87.9% of the water resources, and 67% of the land that was available in 1977 (Capper, 2011). Still, a likely increase in meat consumption is forecasted as incomes begin to rise (Keyzer et al., 2005), yet the beef industry will have to also improve inefficiencies at all levels in order to still be able to compete with the vertically integrated pork and poultry industries. Management of beef cattle will need to be heavily researched to refine methods of production with reduced resources available. One potential answer may be more rapidly advancing the genetic merit of beef herds and employing stringent selection and culling practices. To date, the most efficient way to maximize production from genetically superior beef cows is through the use of advanced reproductive techniques, specifically embryo transfer (ET).

Embryo transfer has been steadily increasing in popularity since its initial commercial use in the 1970s, especially in the last decade, as the American Embryo

Transfer Association reports that in 2002 3.6% (5,105) of registered angus calves were developed through ET, yet by 2007 that figure had risen to 11.5% (40,000) (Lamb and Black, 2011). It's estimated that annually over 500,000 embryos are transferred globally, with around 200,000 of those transfers taking place in the United States (AETA, 2009). Though over the last four decades ET breakthroughs have included the adoption of non-surgical techniques, reliable cryopreservation and thawing methods, embryo splitting, embryo sexing, in-vitro methods, and cloning, actual efficiency of these methods in terms of offspring produced has not progressed nearly as much (Hasler, 2010). In America, as of 2006, the average number of recipients pregnant out of those that received an embryo is 62.4% for fresh embryos and 56.9% for frozen (AETA, 2009).) In an attempt to quantify what percentage of the time the inability of a live calf to develop is due to recipient factors or when pregnancy loss is a result of embryo factors, McMillan et al., 1998 developed a statistical model that utilized a binomial distribution to measure such responsibilities. The authors determined that beyond day 60 of the pregnancy, in almost all cases, the embryo is the determinant for pregnancy loss, however, prior to the day 60 benchmark, the recipient is mostly at fault. Though the exact amount is hard to measure, it is clear that whether or not an embryo develops full term is highly dependent upon the uterine environment and many physiological aspects of the recipient, including fertility, nutritional plane, body condition, age, health, parity, and post-partum period (Lamb and Black, 2011). To test their model, McMillan et al., 1999 identified a group of recipients who had a high propensity to carry their embryo to the 60 day mark and a group of recipients that had a low propensity to carry their

embryo to the same day. By examining when the greatest variation in the percentage of females in each group were pregnant the authors were able to determine that the majority of ET pregnancies that do not result in a live calf are occurring early on, within 25 days of the transfer, indicating "the critical period", or days 15-17 when maternal recognition of pregnancy occurs are appropriately named. Prior to this work, much of the early research primarily focused on superovulation techniques and the donor, yet it has been shown that a large percentage of the inefficiencies is due to the recipient, which the maintenance of happens to be the most expensive part of an ET protocol (Looney, 2006; Hinshaw, 1999). Though more studies are needed, success has been seen utilizing several hormonal treatments to not only synchronize the recipient with the donor, which alone greatly improves pregnancy rate (Spell et al., 2001), but improve fertility. It is necessary to both improve recipient cow management as well as further develop protocols to improve pregnancy rate in order for continued expansion of this technology beyond the purebred sector and ultimately to help support the future of the beef industry.

Recipient Selection and Management

For every recipient that fails to become pregnant, there is an estimated loss of $100 in drugs and time (Looney, 2010), thus selecting the right females to become recipients, and properly managing them, is critical. Though there is much debate on what traits are significant when it comes to selecting recipients, most practitioners and producers agree that recipients must be highly fertile, possess calving ease, and have the ability to raise a quality calf (Lamb et al., 2001) and that females who have a history of

either cystic ovaries, retained placenta, and/or poor lactation records should be avoided (Stroud and Hasler, 2006). Perhaps the most effective purchasing method of recipients is to buy pregnant cows, calve them out, and palpate for reproductive soundness, and then no sooner than 60 days utilize them as recipients (Stroud and Hasler, 2006).

One of the most beneficial aspects of managing recipients is the employment of a synchronization protocol of which some practitioners would call the most significant advancement in the ET field of the last 15 years (Looney et al., 2005). Recipients' estrus should be synchronized with that of the donor within ($\pm$) 24 hours in order to avoid a reduction in pregnancy rate (Hasler, 2001, Spell et al., 2001). It is also imperative that recipients receive their embryo between days 6 and 8 of the synchronization protocol (Hasler, 2001).

It was discovered in the 1940s that exogenous progesterone, primarily produced by a functional CL, would delay estrus in sheep and cattle (Looney et al., 2005). Subsequent studies primarily focused on shortening the luteal phase through the use of prostaglandin and progesterone (Looney et al., 2005). Therefore, initial treatments for synchronization primarily included the administration of PGF_{2a} with the understanding that the prostaglandin would regress the CL (Odde, 1990). Estrus detection was required for at least 5 days post PGF_{2a} administration. Nonetheless, as technology and research advanced it became clear that there were inefficiencies with this protocol that needed to be corrected as variability in estrus detection and other factors led to only approximately 50% of treated recipients being determined suitable for transfer (Bo et al., 2012).

Suitability of the recipients for transfer, which has remained the standard even in most current practice methods, is synchronization with the donor and the presence of a functional CL at the time of transfer (Looney et al., 2005). Currently, estrus detection has been eliminated from most protocols utilized. Fixed-timed embryo transfer (FTET) protocols vary dependent upon practitioner preference and government laws and regulations, as many drugs including equine chorionic gonadotropin and estradiol are not legal in some countries (Lamb and Black, 2011), however, the most common and effective protocol developed to date utilizes a combination of estradiol and progesterone as these two steroid hormones allow for the most controlled suppression of follicular growth of the dominant follicle and consistently induce a new follicular wave 2 to 3 days post ovulation (Looney et al., 2005). FTET protocols have been shown to have similar conception rates as estrus detection protocols for both heifers (Lamb, 2000) and cows (Bo et al., 2012), plus the FTET protocols actually increase overall pregnancy rates because a larger percentage of potential recipients actually receive an embryo (Looney et al., 2005).

Fertility of beef cattle has been shown to be correlated to age, especially in females who are greater than 10 years old, where a major drop off in fertility occurs (Renquist et al., 2005). However, opinions vary on whether heifers or cows of less than 10 years of age are more effective recipients. Heifers have been shown to have similar pregnancy rates as parous cows (Hasler, 2001; Benyei et al., 2006), yet the conception rates for pre-pubertal heifers and pubertal heifers, 36% vs. 76% respectively, are significantly different (Stroud and Hasler, 2006). Besides the obvious benefits of

utilizing heifers who have undergone puberty, it is also recommended that they have a pelvic area of 140 cm^2 or greater at 12 months of age in order to more effectively avoid dystocia (Lamb et al., 2011).

Nutritional plane and body condition can have a major impact on beef cow fertility, mainly due to its direct connection with cyclicity, both with lactating and non-lactating cows. It is well known that cows must establish maintenance and lactation prior to becoming reproductively functional (Short and Adams, 1988). Not surprisingly, condition and nutrition are major factors in recipient management as well. It has been determined that a moderate body condition score (BCS) is the most desirable for recipients at the time of transfer (Mapletoft, 1986). It is also highly important that recipients do not go through a negative energy deficit during the first 45 days after ET (Stroud and Hasler, 2006). Sufficient forage containing both adequate protein and energy levels are essential to the maintenance of recipient pregnancies, a fact often overlooked in poorly managed operations (Stroud and Hasler, 2006). This intermediate BCS (~6 on a scale of 1-9, ~3-3.5 on a 1-5 scale) is also important prepartum to ensure a timely return to estrous, as postpartum infertility is almost exclusively due to anestrous (Short et al, 1990). An increase in late gestation nutrition, in cows that have an intermediate BCS, is also associated with a shortened postpartum period (Jones and Lamb, 2008), as well as an increase in conception and pregnancy rate (Wiltbank et al., 1962). Anestrous multiparous cows, even utilizing a FTET protocol, have been shown to have much lower conception rates even though they may exhibit signs of estrous following treatment (Stroud and Hasler, 2006).

In regards to lactation status, as with age, there is no industry-wide standard as to whether lactating or non-lactating recipients are more desireable. Perhaps the greatest benefit of utilizing lactating recipients is that they were recently reproductively competent to raise a calf. However, in most cases cows do not return to cyclicity, with all other limiting factors status quo, earlier than 45 days postpartum (Short et al., 1990). In the dairy industry, significant issues arise with utilizing lactating females as recipients due to the rate of metabolism of progesterone by the liver (Wiltbank et al., 2011) but limited literature is available about the rate of P4 matabolism in lactating beef cattle. One study, Meyer, 2002 via review by Looney et al., 2005, did show no significant difference in first service pregnancy rates of recipients whether they were lactating or not, 43.4% vs. 43.3%, respectively.

Though some have been discussed, there are a number of factors that can cause a recipient to unsuccessfully become pregnant. Beyond lack of proper synchronization, nutrition, recipient age, and improper embryo development, it is also important to note that calm handling practices of the recipients can improve conception rates as well. Studies have shown that stress and nervousness can cause infertility in beef cattle (Adams and Lamb, 2008). This can be induced by comingling cattle just prior to transfer, which has adverse effects on the established hierarchal status of the group (Lamb, 2011). When transferring, it is imperative that the females are secure in the squeeze chute in a comfortable fashion and not free to move to avoid nervousness (Stroud and Hasler, 2006).

Embryo Factors

From an experimental design standpoint, it is also important to limit the number of factors that are outside the scope of the study that could affect results. The embryo itself can also play a significant role in the success of an ET program. Two major reviews were published in 2001, Hasler, 2001 and Spell et al., 2001, that evaluated the factors that affected pregnancy rates in ET. From their results, fresh embryos resulted in a pregnancy a greater percentage of the time versus frozen embryos with Hasler, 2001 finding the average number of recipients pregnant out of the number transferred to be 68.3% in a sample size of over 9,000. From the same study, frozen embryos were reported to result in a sustained pregnancy 58.4% of the time. The same advantage for fresh vs. frozen was reflected in Spell et al, 2001, where results were 82.9% and 69%, respectively.

Industry wide, it is generally accepted that stage 4-6 embryos are the most successful in ET. Both reviews also showed positive benefits to utilizing stages 4 (morulla) and 5 (early blastocyst) embryos versus stage 6 (blastocyst), though only the results from Hasler, 2001 were significant. In terms of embryo quality, on a scale of 1-3 with 1 being excellent, grades 1 and 2 embryos had no significant difference in pregnancy rate in either study, however grade 3 embryos reported a significantly lower pregnancy rate in Hasler, 2001.

Follicular and Luteal Function as Related to Fertility

A major cause for embryonic loss is the inability of the corpus luteum (CL) to produce enough progesterone to prepare the endometrium for embryo implantation and maintenance of pregnancy (Looney, 2010). It's been reported that a larger preovulatory follicle may generate a larger CL that will secrete more progesterone, and thereby have a positive effect on pregnancy (Binelli et al., 2001). The notion that the size of the preovulatory follicle dictates the size of the CL is further reinforced by Echternkamp et al., 2009 and Vasconcelos et al., 2001. Echternkamp et al., 2009 also concluded that progesterone levels correlated positively with ovulation rate and number of subsequent CLs. Along with this, the authors determined conception rates were higher for cows with multiple ovulations.

Traditionally, the estrous cycle of bovine consists of two to three follicular waves (Bo et al., 2003), with three to six follicles undergoing growth throughout each wave (Fortune et al., 2001). It's been reported that preovulatory follicles are smaller in GnRH induced ovulations, and subsequently, there is a negative impact on fertility (Perry et al., 2005). Even though GnRH is replaced with estradiol/progesterone in some fixed-timed embryo transfer protocols, Looney et al., 2010 states there is still concern amongst practitioners that the resulting CL(s) are smaller, especially in *Bos indicus* cattle who traditionally have smaller CLs as it is. One possible answer to this issue is to not induce ovulation until the preovulatory follicle grows into a persistant follicle by extending the implantation time period of a CIDR to 14 days (Mantovani et al., 2005). In AI, persistant

follicles have compromised conception rates, due to the extended age of the oocyte, however in ET protocols the recipients oocyte does not matter (Wherman et al., 1997). Even with elevated levels of progesterone however, conception rates to ET were not improved in recipients who had persistent follicles. Mantovani et al. 2005 actually found the conception rates to be lower (59.1% in control vs. 38.9% and 37.1% in treated groups) though there was a greater transfer rate in recipients with persistent follicles (51.4% in control vs. 77.4% and 74.6% in treated groups) as well as increased plasma progesterone rates (2.3 $\pm$ .2 in control vs. 3.8 $\pm$ .2 and 3.8 $\pm$ .3 in treated groups).

Importance of Progesterone in Establishing and Maintaining Pregnancy

Progesterone (P4), the hormone associated with pregnancy, is produced primarily by the corpus luteum and metabolized by the liver (Wiltbank et al., 2011). The amount of progesterone circulating in the body is correlated to the amount of luteal tissue present (Echternkamp et al., 2009, Wiltbank et al., 2011), however, inadequate levels of P4 are generally a result of a high rate of metabolism resulting from elevated blood flow. Amount of circulating progesterone present is directly related to the conceptus' ability to produce bovine interferon-tau (bIFN-τ), which is the primary regulator of pregnancy recognition (Mann et al., 1999).

Throughout a large portion of the estrous cycle, P4 levels are peaked and inhibiting the increase of other hormones, and throughout this time, the uterine environment is very similar in both pregnant and non-pregnant females (Binelli et al., 2001). A default prostaglandin (PGF2α) secretion system is in place; meaning that

unless PGF2α production is blocked, luteolysis will occur at approximately day 16, which is deemed the "critical period" as this is the turning point for the body to either "reset" and go through estrus or maintain a pregnancy. If conception does indeed occur, it is important for the conceptus to occupy the majority of the uterine horn ipslateral to the CL and that bIFN-τ production from the trophectoderm of the conceptus is adequate to block luteolysis. bIFN-τ, a 172 amino acid protein, acts on the uterine epithelium to prevent the pulsatile secretions of PGF2α that induce the regression of the CL (Bazer et al., 1994). Exactly how bIFN-τ prevents prostaglandin secretions isn't entirely known. Bazer et al., 1994 provides four possible answers; 1) the up-regulation of endometrial P4 receptors and prevention of E_2 or Oxytocin receptors from forming on the endometrium; 2) direct inhibition of E_2 from generating PGF pulses; 3) direct inhibition of endometrial Oxytocin production; 4) inhibition of post-receptor mechanisms which prevent Oxytocin from releasing pulses of PGF.

It is important to note that mortality of the conceptus up to and through the critical period is the most prevalent cause of fertility loss in cattle (Maurer et al., 1985). Due to its importance in the maintenance of pregnancy, P4 supplementation has been researched since the 1950s (Wiltbank et al., 2011), including its use in recipient protocols. It's been reported that P4 treatments on days 3-6 prior to embryo transfer on day 7 resulted in longer embryos on day 14, just prior to the critical period (Clemente et al., 2009). Previous research in sheep found that recipient ewes that were treated with supplemental progesterone were capable of taking older embryos that were more elongated at the time of transfer (Garret et al., 1988).

Supplemental Gonadotropins and Other Treatments to Increase Fertility

With the commonly used FTET synchronization protocol that utilizes either a progesterone-estradiol combo or GnRH administration on day 0, a progesterone releasing intravaginal device for seven days and concurrent prostaglandin administration, there has been significant amounts of research worldwide involving the inclusion of an additional gonadotropin treatment to this protocol. The idea behind this is the added gonadotropin will help induce the growth of secondary follicles that will eventually ovulate and provide additional corpora lutea. Another theory is that the dominant follicle will benefit from supplemental gonadotropin and eventually ovulate and result in a larger CL and ultimately increase circulating progesterone. The elevated progesterone has multiple benefits on fertility but primarily has been shown to reduce early embryonic loss (Lopez-Gaitus et al., 2004).

Equine Chorionic Gonadotropin (eCG) has been used in this role frequently in South America and in various countries internationally. eCG is a glycoprotein gonadotropin that consists of two noncovalently linked peptide chains; a 96 amino acid alpha subunit and a 149 amino acid beta subunit (Murphy and Martinuk, 1991). eCG has a half-life that is rather long for most hormones, 40 hours, and to many researchers this extended half-life in conjunction with its similar characteristics to FSH and LH is the reasoning behind its beneficial effects on maintaining pregnancy (Baruselli et al., 2011). According to Bo et al. 2011, 400 IU of eCG on either day 5 or 8 is the most common way to increase pregnancy rates in South America. Utilizing this protocol, it is common

to see a transfer rate of 75-85% and pregnant/transferred rates typically exceeding 50% (Bo et al 2002). South American eCG trials (Nasser et al, 2004, Bo et al, 2011, Mayor, 2008) are not the only ones to find the treatment beneficial to fertility, as similar results have been observed in China (Remillard et al., 2006) and Mexico (Looney et al., 2010). On the contrary, Small et al. 2007 and Pinheiro et al, 2008 found no significant difference in pregnancy rates. Despite the conflicting research, it is apparent that in most cases eCG appears to benefit the fertility of recipients, however, eCG is not FDA approved and therefore is not legal for use outside of research in the United States. Because eCG binds to FSH receptors in most mammals other than the horse (Murphy and Martinuk, 1991), and its strong FSH-like action, it has been hypothesized that FSH may have similar effects as eCG in FTET protocols. FSH, which also is a glycoprotein consisting of two polypeptide chains, an alpha and a beta, is traditionally administered exogenously to stimulate follicular growth and super-ovulate donors (Demoustier et al., 1988). Mixed results have been reported in regards to the utilization of FSH in this role. Martins et al., 2010 reported anestrous *Bos Indicus* cows that were treated with 10 mg of Folltropin-V® on day 8 of a FTAI protocol had a similar ovulation rate to that of cows treated with 300 IU eCG (85.5% vs. 82.5%) at the same time. Not surprisingly, pregnancy rates were also similar in the two groups; 51.4% for the FSH group and 55.9% for the eCG group. For both ovulation rate and pregnancy rate a control group that received no additional treatment was significantly lower; 71.8% and 38.9% respectively. However, in two other separate Fixed-Timed Artificial Insemination (FTAI) studies, no significant increase in fertility was observed using supplemental FSH

(Sa Filho et al., 2009, Sales et al., 2011). Follicular growth also was shown to not be advanced by the administration of supplemental FSH (Sales et al., 2011). A potential reason behind the lack of benefit in fertility in some cases may be due to the short half-life of FSH (Sa Filho, 2009), which can range from 5 to 12 hours (Demoustier et al, 1988). Interestingly, FSH has been reported to have similar benefits on fertility as eCG in FTET (Zanenga et al., 2010). The authors found that a day 8 injection of 10 mg FSH (Folltropin-V®) resulted in a transfer rate of 72.6%, pregnant/transferred rate of 49.2%, and a pregnancy rate of 35.7%. 400 IU of eCG administered on the same day for a second group of heifers resulted in a transfer rate of 87.3%, a pregnant/transferred rate of 43.5%, and a pregnancy rate of 35.7%, with only the difference in transfer rate being significant. In a second trial, both 10 mg and 20 mg doses were tested against 400 IU of eCG with results being similar; with respective transfer rates being 76.8%^{a}, 83%^{ab}, and 89.5%^{b}, conceptions rates of 39.7%^{a}, 39.7%^{a}, 38.8%^{a}, and pregnancy rates of 30.5%^{a}, 32.9%^{a}, and 34.7%^{a}. Again, only transfer rates were significantly different.

One potential downfall to the repeated use of eCG treatments is the buildup of antibodies to eCG and subsequent decline in added benefit to fertility (Drion et al., 2001). *Bos taurus* cattle appear more likely to buildup antibodies than *Bos indicus* (Mantovani et al., 2010).

Slow Release Formula Folltropin®-V

Traditionally, superovulation in bovine includes either a once daily administration of eCG or twice daily administration of Porcine FSH (pFSH) for four

days. The extended half-life of eCG allows for fewer animal handlings than the pFSH protocol and obtaining optimal superovulation of the donors while further reducing stress on the animal and possibility of injury to both humans and the donor is also desirable.

Folltropin®-V (Bioniche Animal Health, Bogart, GA) reconstituted with a slow releasing formula (SRF), consisting of reduced concentrations of Hyaluron (HA) and administered in a two shot protocol has been shown to have similar effects on super-stimulatory response as a traditional 8 shot protocol (Hasler and Hockley, 2012). Tibulo et al., 2011 also showed that a similar number of transferrable embryos and corresponding CL size were generated with a one time intramuscular injecton of FSH in HA vs. a 8 shot protocol. HA is a biodegradeable polymer of disaccharides (i.e. glycosaminoglycan) and is found in the mammalian uterus, connective, epithelial and neural tissues, and has an extended half-life in relation to FSH diluted with saline (Looney and Pryor, 2012). Along with its potential benefits in superovulation, HA can also be useful in culture media as it has the ability to elevate the viscosity of the media and regulate water distribution and binding to the cell (Looney and Pryor, 2012). Finally, a third potential use for FSH may be as a carrier for pFSH but utilized in the recipient synchronization protocols as discussed in the previous chapter.

Reproductive Differences in Bos taurus and Bos indicus Cattle

Though primarily only found in significant numbers in the gulf coast region of the United States, *Bos indicus* cattle are dominant in South America and tropical regions,

where most of the world's cattle population resides (Baruselli et al., 2004). Hot temperatures, humidity, and extensive populations of ectoparasites can hinder the performance of most Continental and British cattle, yet *Bos taurus* derived breeds such as the Brahman and Nellore appear to thrive in these conditions. There also appears to be slight reproductive differences between the two types of cattle as *Bos indicus* tend to have a shorter duration of estrus, a higher incidence of silent estrus, and commonly show signs of estrus only at night (Looney et al., 2010). *Bos indicus* also have longer gestations than *Bos taurus* with the average time period being 292 days (Looney et al., 2005). Following calving, *Bos indicus* cattle have longer postpartum periods and durations of anestrous (Sales et al., 2011), which is the main factor negatively affecting reproductive performance (Baruselli et al., 2004). Ruiz-Cortes, 1998 reported an average time period for cyclicity to return to normal in *Bos indicus* cows of 217 -278 days, extending the calving interval to 17-19 months. Not to mention, Brahmans and Brahman crosses have been reported to have smaller CLs than do *Bos taurus*, and subsequently have lower circulating progesterone (Randel et al., 2005).

In regards to FTET synchronization protocols and treatments, it is also important to note that *Bos Indicus* appear to be more sensitive to FSH (Barros and Noguera, 2001). Most cattle, throughout the estrous period have two to three follicular waves emerge before a dominant follicle is selected, matured, and ovulated (Sartori et al., 2010). However, there is a much higher incidence of four waves and even five waves observed in *Bos indicus* (Bo et al, 2003) (Sartori et al., 2010). Each wave in *Bos taurus* cattle

consists of approximately 24 small (2 to 5mm) antral follicles (Ginther et al., 1996), yet in *Bos indicus*, up to 50 antral follicles have been reported (Buratini Jr et al., 2000).

Interestingly, Saldarriaga et al., 2006 and Zuluaga et al., 2006 both report that the Co-synch with a CIDR protocol is much less effective in *Bos indicus* cross females as opposed to straight *Bos taurus*. In an attempt to solve this problem, Pack et al., 2005 replaced the GnRH with Estradiol Benzoate (EB) and reported results of 91% of *Bos indicus* females having new follicular wave emergence compared to 53% in *Bos taurus*. Looney et al., 2010 utilized a protocol involving a progesterone and estradiol benzoate combination administered on day 0, CIDR for 7 days, PGF concurrent with removal and EB on day 8, and reported a transfer rate of 89% and pregnancy rate of 53%.

CHAPTER II

DEVELOPING A FIXED-TIME EMBRYO TRANSFER PROTOCOL WHICH INCLUDES FOLLICLE STIMULATING HORMONE SLOW RELEASE FORMULA

Introduction

Follicle stimulating hormone (FSH) is produced by the anterior pituitary and in conjunction with luteinizing hormone (LH) is responsible for the selection and growth of oocytes during diestrus. In embryo transfer, supplemental FSH is typically given to a donor female in successively decreasing doses over a 2-4 day period, primarily to induce multiple oocyte production/ovulations and ultimately increase the production of a genetically superior female.

It has been hypothesized that due to the increase in luteal tissue formed from cows that have been superovulated (SOV), there are potential benefits to supplementing FSH into recipient synchronization protocols to increase progesterone production, and ultimately increase fertility. There has been reported success of international studies that included equine chorionic gonadotropin (eCG) in recipient protocols (Bo et al., 2011), yet eCG is not approved for use in the United States. eCG has FSH-like action when administered to recipients, thus supplemental FSH could potentially increase the fertility of recipients as well.

Materials and Methods

The data of this study was collected in collaboration with OvaGenix, LP, a private embryo transfer company located in Bryan, TX. All cattle were provided by customers of OvaGenix, LP and the cost of materials and labor were funded by Bioniche Animal Health (Bogart, GA). Recipients included in the study (n=572) were located on ranches in Texas and Oklahoma and consequently were managed under slightly differing climate conditions. Nutrition, age, and health can all play a substantial role in the fertility of the recipients (Lamb, 2011), however, the author, ranch owner and ET practitioner all agreed that recipients utilized in the study were of good health, acceptable age (parous, <8 years old), and nutritional plane was suitable to maintain a body condition score of 4-7 (on a 1-9 scale). Nonetheless, in order to minimize the skewedness of data due to environmental and forage conditions, this study was carried out over two separate seasons; the fall of 2011 and spring of 2012. Ranches in the fall study included Goudeau Livestock (Wharton,TX), Suzi Q Angus (Center, TX), 714 Ranch Angus (Nacogdoches, TX), Pollard Angus (Enid, OK), Howdy U Cattle (Bagwell, TX), and Collier Farms (Chappell Hill, TX). Ranches included in the spring study included Howdy U Cattle (Bagwell, TX), Kiamichi Link Ranch (Antlers, OK), Heritage Cattle Co. (Wharton, TX), Forgason Cattle (Wharton, TX), Pollard Angus (Enid, OK), and Goudeau Livestock (Wharton, TX). Due to drought-like conditions in the summer of 2011 forage levels during the fall study on the ranches involved in the study were poorer than during the spring study. Late winter rainfalls improved the forage conditions prior to the spring study.

The objective of this study was to determine the effectiveness of including a 40 mg injection of Folltropin-V® (IM, Bioniche Animal Health, Bogart, GA) diluted in hyaluronan on day 5 of a FTET synchronization protocol to increase fertility of the recipients. Recipients included in the study were randomly placed in either the Treated or Control groups, with the Treated group having the day 5 injection of FSH included in their synchronization protocol. The estrous cycles of all the recipients, both Treated and Control, were synchronized using a progesterone-estradiol based protocol (outlined in Figure 1.) that included a day 0 injection of estradiol 17β (2.5 mg, IM) and progesterone (50 m, IM) combination, a CIDR® (1.34 mg, Pfizer Animal Health) was also inserted at this time and removed 7 days later. Concurrent with CIDR removal recipients were administered Lutalyse® (dinoprost tromethamine, Pfizer Animal Health, 25 mg, IM), and then on day 8 were administered estradiol 17β (1 mg, IM). On day 16, the presence of at least one functional corpus luteum, detected via rectal ultrasound, resulted in the recipient receiving an embryo. Both fresh and frozen embryos were utilized. Embryos were not transferred into cows that did not show ultrasonic evidence of a CL.

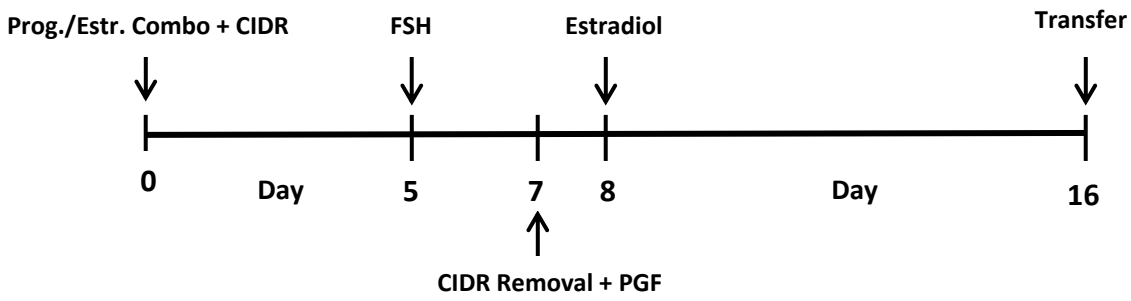

Figure 1. **Timeline of the recipient synchronization protocol with day 5 FSH SRF injection included (Treated group protocol)**

A 40 mg dose was chosen due to the results of an unpublished pilot study performed by the author and Ovagenix, LP, of which the results are displayed in Table 1. Most notably, the number of recipients with multiple CLs appeared to favor treated recipients, though the results of the study were never formally analyzed with SAS. The number of cows that received an embryo did not vary much between the groups, however of those that did receive an embryo, the cows treated with a 40 mg dose of FSH fared the best. From the results it also appeared that the 20 mg treatement was not nearly as effective as the 40 mg dose, and thus the 20 mg dose was not included in the present study.

Table 1. **Fertility data in beef recipients following synchronization for fixed-time embryo transfer with a protocol that included (Treated) 40 mg, 20 mg or did not include (Control) FSH in hyaluronan**

Group	N	No. Transferred	No. Pregnant	Preg./Prog.	Pregnancy Rate	% Recips with Mult. CL
40 mg	23	17	12	12/17 (71%)	12/23 (52%)	7/17 (41.18%)
20 mg	23	18	8	8/18 (44%)	8/23 (35%)	2/18 (11.11%)
Control	29	24	14	14/24 (58%)	14/29 (48%)	1/24 (4.17%)
Total	75	59	34	34/59 (57.6%)	34/75 (45.3%)	10/59 (16.95%)

The present study consisted of grade cows of *Bos taurus* (n=457) and *Bos indicus* crossbred (n=115) breed composition. Breed type was proportionally distributed in each group, with the Treated group (n=268) consisting of 53 *Bos indicus* crossbred and 215 *Bos taurus* cows. The Control group (n=304) consisted of 62 *Bos indicus* crossbred cows and 242 *Bos taurus* cows. The author both collaborated with the owners and visually appraised the recipients to determine their breed type. Females that appeared to be 50% or greater *Bos indicus* blood were classified as *Bos indicus* crossbreds. All other recipients were classified as *Bos taurus*. The primary breed type of the *Bos indicus* crossbred recipients was F1 Hereford X Brahman crosses, and all recipients in the *Bos taurus* classification were Angus based.

Other independent variables for which data was collected consisted of; body condition score (BCS, 1-9 scale), postpartum interval, and lactation status. A total of 206 lactating cows (103 Treated; 103 Control) and 366 dry cows (165 Treated; 201 Control) were included in the study. A breakdown of the lactation status and number of *Bos indicus* and *Bos taurus* in each group for the Treated and Control groups is presented in Table 2.

Table 2. **Breed type and lactation status of recipients in the 2011 fall study and 2012 spring study by group; Treated (40 mg FSH SRF) or Control**

	N	*Bos Taurus* (Lactating)	*Bos Taurus* (Dry)	*Bos Indicus* (Lactating)	*Bos Indicus* (Dry)
Fall Treated	122	34	50	11	27
Fall Control	152	31	66	7	48
Spring Treated	152	61	84	4	3
Spring Control	146	48	83	10	5
Total		174	283	32	83

Due to the fact that clientele of OvaGenix, LP, were the providers of recipients for the study, as opposed to university owned livestock, scheduling conflicts did arise in regards to the date of the additional FSH SRF injection. At the advice of the ET

practitioner a small number of recipients received the injection of the additional FSH SRF on day 3 (n=35), day 4 (n=72) and day 6 (n=23), in addition to those who received the injection on day 5 (n=165).

Ranch personnel were responsible for administering the recipients with the day 0 combo shot, CIDR insertion and removal, and the lutalyse and estradiol injections. The additional injection of FSH SRF to the Treated group was administered by the author. The FSH SRF was injected using 3 mL syringes with 18 gauge 1.5 inch needles and administered intramuscularly in the lower quarter of the recipient. The transfer of embryos into recipients was performed by ET practitioners employed by OvaGenix, LP. In the Treated group, 118 fresh embryos and 78 frozen embryos were transferred and in the Control group 164 fresh embryos and 100 frozen embryos were transferred. Fresh embryos were grades 1 and 2, and frozen embryos were all grade 1. Only embryos that were stage 4, 5, and 6 were utilized. Thawing of frozen embryos was performed following the guidelines described in the IETS manual, as well as Hasler, 2011.

Ultrasounding was performed by OvaGenix, LP, personnel, using a SonoSite MicroMaxx® (Sonosite Inc., Bothell, WA) with a 7mm rectal imaging probe. Split-screen images of each ovary of the recipients who received an embryo were stored and the author used the on-screen caliper calculation measuring tool to measure the CL diameter, area, and circumference. Other dependent variables for which data was collected included number of CLs, plasma progesterone levels at the time of transfer, pregnancy status, transfer rate (no. cows transferred/no. of cows synchronized),

conception rate (no. cows pregnant/ no. cows transferred), and pregnancy rate (no. cows pregnant/ no. cows synchronized). To determine plasma progesterone (P4), whole blood (approximately 5-8 mL) was drawn from all recipients that which an embryo was transferred to on day 16, at the time of transfer. Samples were extracted via tail bleeding, and immediately placed on ice. Within 24 hours, all blood samples were inverted for mixing and then centrifuged for 20 minutes at 3,300 rpm at a temperature of 5°C. Blood plasma was extracted and stored in 1.5 mL microcentrifuge tubes at -20°C. All plasma samples were then assayed for P4 using radioimmunoassay (RIA) procedures (Abraham et al., 1971). For the spring study, the low pool and high pool coefficients of variation (CV) for the assays were .73 and 12.05%, respectively. For the fall study, the low pool and high pool CV were 3.47 and 9.79%.

CHAPTER III

RESULTS

Results of Overall Study

Transfer rate, pregnant/transferred rate, and pregnancy rate results are presented in Table 3. Transfer rates and pregnancy rates of the Treated and Control groups were significantly different ($P < .0001$, and $P < .0006$, respectively), while conception rates were not ($P > .1590$).

Table 3. **Transfer rate, pregnant/transferred rate, and pregnancy rate of recipients in the Treated and Control groups**

	No. Programmed	No. Transferred	No. Pregnant	Transfer Rate	Preg./Trans. Rate	Pregnancy Rate
Treated	268	196	109	73.13%[A]	55.61%	40.67%[A]
Control	304	264	161	86.84%[B]	60.98%	52.96%[B]
Total	572	460	270	80.42%	58.70%	47.20%

[AB]Differing superscripts denote a difference (P<.05)

In total, 48 cows had multiple ovulations during the experiment, resulting in multiple CLs. The mean number of CLs per cow and breakdown of the number of cows within each group that had multiple CLs is presented in Table 4. There was no

significant difference in the mean number of CLs per cow nor the percentage of cows within the group who had multiple CLs between the Treated and Control groups.

Table 4. Number of recipients with multiple corpora lutea and mean number of CLs per cow in the Treated and Control groups

	No. Cows	Mean No. CL
Treated	22/196 (11.22%)	1.14 + .03
Control	25/264 (9.47%)	1.10 + .02
Total	47/460 (10.44%)	1.12 + .02

CL diameter, circumference, area per CL, and total luteal area means are presented in Table 5**.** Differences in circumference, area per CL, and total luteal area did not differ between the two groups, however mean diameter was significantly different between the two groups ($P < .004$).

Table 5. **Mean (±SEM) CL diameter, circumference, area per CL, and total luteal area per cow**

	No.	Diameter (mm)	Circumference (mm)	Area per CL (mm^2)	Total Luteal Area per cow (mm^2)
Total Treated	196	17.07 ± .32[A]	53.09 ± .93	23.76 ± .68	26.23 ± .84
Total Control	263	18.32 ± .29[B]	56.21 ± .67	24.94 ± .52	27.36 ± .74
Total	459	17.79 ± .22	54.88 ± .56	24.44 ± .42	26.87 ± .56

[AB]Differing superscripts denote a difference (P<.05)

At the time of transfer blood samples were taken and radioimmunoassayed for serum progesterone, with results being shown in Table 6. The mean circulating progesterone level, presented in ng/mL, of recipients within the Treated and Control groups were not significantly different.

Table 6. **Mean (± SEM) circulating progesterone (P4) for recipients at the time of transfer for the Treated and Control groups**

	No.	Mean P4 (ng/mL)
Treated	189	3.14 ± .40
Control	251	3.23 ± .18
Total	440	3.18 ± .20

Correlation coefficients between continuous variables for which information was collected are provided in Table 7. Of note, there is only a slight correlation between total luteal area and P4. Expectedly, number of CLs was correlated with mean total luteal area and negatively correlated with mean CL diameter, circumference, and area. Number of CLs nor mean total CL area were significantly correlated with mean circulating P4 at the time of transfer.

Table 7. **Correlation values of continuous variables collected on recipients**

Pearson Correlation Coefficients

Prob > |r| under H0: Rho=0

	Mean P4	No. of CLs	Mean CL Diameter	Mean CL Circumference	Mean CL Area	Mean Total Luteal Area
Mean P4		.01201 .8007	-.03628 .4462	-.01388 .7708	.08357 .0789	.09521 .0452
No. of CLs	.01201 .8007		-.14748 .0015	-.13197 .0046	-.15975 .0006	.46687 <.0001

Lactation Status

Treated cows that were lactating had a significantly lower pregnancy rate (36.97%^{A}) than both lactating (52.24%^{B}) and non-lactating (54.37%^{B}) control recipients. The pregnancy rate of treated cows that were non-lactating was intermediate (46.60%). However, both treated lactating and non-lactating recipients had a significantly lower transfer rate than control lactating and non-lactating recipients. None of the groups differed in pregnant/transferred rate. Detailed results are presented in Table 8. No significant difference was determined in CL measurements between lactating and non-lactating recipients (Table A1.). Though lactating recipients tended to have higher amounts of circulating progesterone than non-lactating recipients, the difference was not significant (Table A2).

Table 8. **Transfer rate, pregnant/transferred rate, and pregnancy rate of recipients by lactation status in the Treated and Control groups**

	No. Programmed	No. Transferred	No. Pregnant	Transfer Rate	Preg./Trans.	Pregnancy Rate
Treated Lact.	165	118	61	71.52%^{A}	51.69%	36.97%^{A}
Treated Non-Lact.	103	78	48	75.73%^{A}	61.54%	46.60%
Control Lact.	201	174	105	86.57%^{B}	60.69%	52.24%^{B}
Control Non-Lact.	103	90	56	87.38%^{B}	65.12%	54.37%^{B}
Total	572	460	270	80.42%	58.70%	47.20%

^{AB}Differing superscripts denote a difference (P<.05)

Breed

Pregnancy/transferred rate and pregnancy rate for *Bos taurus* control recipients differed from *Bos taurus* treated, *Bos indicus* crossbred treated and control groups. Both treated *Bos taurus* and *Bos indicus* crossbred cows differed from *Bos taurus* and *Bos indicus* crossbred control cows. Full transfer, pregnancy/transferred, and pregnancy rates are shown in Table 9.

Table 9. **Transfer rate, pregnant/transferred rate, and pregnancy rate of *Bos taurus* (BT) and *Bos indicus* crossbred (BIX) cows in the Treated and Control groups**

	No. Programmed	No. Transferred	No. Pregnant	Transfer Rate	Preg./Trans.	Pregnancy Rate
BT Treated	215	159	92	73.95%^{A}	57.86%^{A}	42.79%^{A}
BIX Treated	53	37	17	69.81%^{A}	45.95%^{AB}	32.08%^{A}
BT Control	242	207	138	85.54%^{B}	66.67%^{C}	57.02%^{B}
BIX Control	62	57	23	91.94%^{B}	40.35%^{B}	37.10%^{A}
Total	572	460	270	80.42%	58.70%	47.20%

^{ABC}Differing superscripts denote a difference (P<.05)

Number of cows with multiple CLs and the mean number of CLs per cow did not differ by breed and treatment (Table A3). *Bos taurus* treated cows did differ in CL diameter and circumference than *Bos taurus* control and *Bos indicus* control recpients, however mean area per CL and total luteal area did not differ amongst the groups (Table A4).

Table 10 shows the mean circulating progesterone levels at the time of transfer for *Bos indicus* crossbred and *Bos taurus* cows. *Bos taurus* Treated cows and *Bos taurus*

Control cows differed from both Treated and Control *Bos indicus* crossbred but did not differ with one another.

Table 10. **Mean (± SEM) circulating progesterone (P4) at the time of transfer of *Bos taurus* (BT) and *Bos indicus* crossbred (BIX) cows in the Treated and Control groups**

	No.	Mean P4 (ng/mL)
BT Treated	152	2.82 ± .35[A]
BIX Treated	37	4.48 ± .56[B]
BT Control	194	2.64 + .31[A]
BIX Control	57	5.26 ± .45[B]
Total	440	3.19 ± .20

[AB]Differing superscripts denote a difference (P<.05)

Injection Day

Results in regards to transfer rate, conception rate, and pregnancy rate are further explained in Table 11. Recipients injected on day 6 had a significantly higher pregnancy rate than Day 3, Day 4, and Day 5 treated recipients, however Day 6 did not differ from the other days in pregnancy/transferred rate and pregnancy rate. Day 4 treated recipients differed from day 5 treated recipients in pregnancy rate. Day 6 treated recipients did not differ from the other days, but did record the highest pregnancy rate.

Table 11. **Transfer rate, pregnant/transferred rate, and pregnancy rate of recipients in the Treated group by day of the synchronization protocol that they received FSH-SRF**

	No. Programmed	No. Transferred	No. Pregnant	Transfer Rate	Preg./Tran.	Pregnancy Rate
Day 3	35	20	14	57.14%^{A}	70.00%^{B}	40.00%
Day 4	72	51	22	70.83%^{AB}	43.14%^{A}	30.56%^{A}
Day 5	138	103	62	74.64%^{B}	60.19%^{B}	44.93%^{B}
Day 6	23	22	11	95.65%^{C}	50.00%	47.83%
Total	268	196	109	73.13%	55.61%	40.67%

^{ABC}Differing superscripts denote a difference (P<.05)

The number of cows with multiple CLs and the mean number of CLs per cow for each injection day are shown in Table 12. Day 5 treated recipients differed from the other days in mean number of CLs.

Table 12. Number of recipients with multiple corpora lutea and mean number of CLs per cow by injection day in the Treated group

Injection Day	N	Mean No. CLs
Day 3	0/20 (0.0%)	$1.00 \pm .09^{A}$
Day 4	5/46 (10.87)	$1.10 \pm .05^{A}$
Day 5	16/103 (15.53%)	$1.21 \pm .04^{B}$
Day 6	1/21 (4.76%)	$1.05 \pm .08^{A}$
Total	48/460 (10.44%)	$1.12 \pm .02$

^{AB}Differing superscripts denote a difference (P<.10)

Mean circulating progesterone appeared to be higher in days 5 and 6 treated recipients versus days 3 and 4 treated recipients, though only days 5 and 4 differed. Full results of circulating progesterone by injection day are shown in Table 13.

Table 13. **Mean (+ SEM) circulating progesterone (P4) for recipients at the time of transfer for cows in the Treated group by day of the synchronization protocol that they received FSH-SRF**

	No.	Mean P4 (ng/mL)
Day 3	20	1.82 ± 1.26
Day 4	51	1.91 ± .81[A]
Day 5	103	3.88 ± .55[B]
Day 6	22	3.52 ± 1.18
Total	189	3.14 ± .40

[AB]Differing superscripts denote a difference (P<.05)

Season

Transfer rates, conception rates, and pregnancy rates, broken down by season and treatment group are shown in Table 14. Treated recipients from the spring were significantly lower for pregnancy rate but actually had the highest pregnant/transferred rate, though none of the groups differed. Both fall and spring treated recipients were significantly lower for transfer rate.

Table 14. **Transfer rate, pregnant/transferred rate, and pregnancy rate of recipients in the Treated and Control groups by season**

	No. Programmed	No. Transferred	No. Pregnant	Transfer Rate	Preg./Tran.	Pregnancy Rate
Fall Treated	122	95	57	77.87%[A]	60.00%	46.72%[B]
Spring Treated	146	128	81	69.18%[A]	63.28%	35.62%[A]
Fall Control	152	131	80	86.18%[B]	61.07%	52.63%[B]
Spring Control	152	101	52	87.50%[B]	51.49%	53.29%[B]
Total	572	455	270	79.55%	59.34%	47.20%

[AB]Differing superscripts denote a difference (P<.05)

Table 15 shows the mean circulating progesterone levels at the time of transfer. Fall Control and spring Control cows differed, with fall control recipients being significantly greater.

Table 15. **Mean (+ SEM) circulating progesterone (P4) at the time of transfer of recipients in the Treated and Control groups by season**

	No.	Mean P4 (ng/mL)
Fall Treated	94	3.43 ± .43
Spring Treated	95	2.87 ± .43
Fall Control	127	3.98 + .37[A]
Spring Control	124	2.47 ± .38[B]
Total	440	3.19 ± .20

[AB]Differing superscripts denote a difference (P<.05)

Mean CL measurements by season and treatment group are depicted in Appendix Table 5. Mean CL diameter measurements of Control cows from the spring portion of the study differed from spring Treated, fall Control, and fall Treated cows. Spring Treated and fall Treated cows also differed for mean CL diameter. Spring Treated cows differed from the other three classifications for mean CL circumference. Spring Treated and Spring Control cows differed in mean area per CL. Spring Treated cows also differed from fall treated and fall Control cows in total luteal area per cow.

The number of cows with multiple CLs by season and treatment group and the mean number of CLs per cow are shown in Table 16**.** Both fall treated and fall control

tended to be higher than spring treated and spring control classifications though only fall treated differed.

Table 16. **Number of recipients within the Treated and Control groups with multiple corpora lutea and the mean number of CLs per cow**

	No. of cows with multiple CLs	Mean No. of CLs per cow
Fall Treated	15/94 (15.96%)	1.22 +.04[B]
Spring Treated	7/95 (7.37%)	1.07 + .04[A]
Fall Control	18/127 (14.17%)	1.15 + .03
Spring Control	8/124 (6.45%)	1.06 + .03[A]
Total	48/460 (10.44%)	1.12 ± .02

[AB]Differing superscripts denote a difference (P<.05)

Other Independent Variables

Included in Table 17 are the P-values for the effect of other independent variables, for which data was collected, on pregnancy rate of the recipients. Only body condition score tended to have an effect (P < .10).

Table 17. **The effect of post-partum interval of the recipients prior to the initiation of the synchronization protocol, body condition score of the recipients, and whether a fresh or frozen embryo was transferred on the overall pregnancy rate of the recipients.**

Variable	P-Value
Post-Partum Interval	.7601
Body Condition Score	.0660
Fresh vs. Frozen Embryo	.5547

CHAPTER IV

DISCUSSION

Discussion of Overall Study

The intention behind administering supplemental FSH to the recipients was to increase circulating progesterone in the ET recipients by inducing multiple ovulations and increasing subsequent luteal tissue. Day 5 was chosen as the timing of administration for the FSH in order to maximize the effect, as this time period is synonymous with the first follicular wave emergence. During follicular wave emergence, prior to the selection of a dominant follicle, FSH is especially important to the development of the emerging follicles (Mihm and Bleach, 2003). Thus, we hypothesized that the FSH would stimulate more follicular growth prior to the follicles switching from FSH-dependency to LH-dependency, and in turn, generate multiple ovulations. A similar approach with eCG being utilized instead of FSH has shown positive results in many international studies (Bo et al., 2011).

The hypothesized advantage in fertility of recipients who received FSH diluted in hyaluronan versus control cows proved to be false in the present study as pregnancy rates for Treated and Control cows were 40.67% and 52.96%, respectively. However, previous studies that included supplemental FSH given to recipients on day 8 of a synchronization protocol had pregnancy rates of 35.7%, 30.9%, and 32.9% (Zanenga et al., 2010). The higher pregnancy rate of treated cows in the current study compared to those of the aforementioned studies may be attributed to the fact that the FSH was

diluted in hyaluronan thus lengthening the half-life of the hormone within the body. Transfer rates and pregnant/transferred rates of the current study followed these same trends of being in line or slightly above those of related studies, yet control cows appeared more fertile, with the difference being significant for transfer rate and pregnancy rate.

The authors also hypothesized that the inclusion of FSH diluted in Hyaluronan would increase both the size of each corpus luteum and number of CLs, due to the enlarged preovulatory follicle, and subsequently we would see a higher mean progesterone value at the time of transfer on day 16 of the protocol. Nonetheless, no significant difference was observed in mean CL circumference, area, and total luteal are per cow. CL diameter did differ between the two groups but the control cows had the larger CL diameters. The means for both groups were in line with those of previous studies of recipients who did not receive any additional gonadotropins in their synchronization protocol. (Echternkamp et al., 2009). However, they CL measurements appeared to be slightly lower than those in eCG trials (Bo et al., 2011). For instance, Peres et al., 2011 via Bo et al., 2011 review, found 400/736 recipients treated with eCG had CLs with diameters greater than 18 mm. In regards to CL numbers, the findings of the present study (Table 2) were intermediate with eCG trials. In two studies, Nasser et al., 2004 reported the mean number of CLs possessed by eCG treated recipients to be 1.36 and 1.35.However, in a study with a much larger sample size, Peres et al., 2011 via Bo et al., 2011 review, found that eCG treated recipients had a mean number of CLs of 1.10.

Though the means didn't differ significantly, Control cows did have a slightly higher amount of circulating progesterone at the time of transfer. Our means, 3.14 $\pm$ .40 for Treated cows and 3.23 $\pm$ .18 for Control cows, were similar to P4 means found in Mantovani et al., 2005, Pfeiffer et al., 2008, Carter et al., 2008, Pursley and Martins, 2012.

Due to numerous findings in published literature that stated a correlation between luteal tissue and progesterone output, the author created Table 7, to show the strength of the correlation between P4 and the various CL measurements that were recorded. There did appear to be a correlation between mean total luteal area per cow and progesterone, though the correlation was not strong (.09521). Also of note, from our study it appeared that as the number of CLs increased, there was a negative correlation to size of the CLs.

Lactation Status

Lactation status of the recipients has not been heavily researched in regards to FTET, but one known study, Meyer, 2002 via Looney et al., 2005 review, reported that lactation status did not affect pregnancy rate of recipients. In the present study, perhaps the variable is confounded with other variables, such as owner and management, because CL number, size, and P4 did not differ between lactating and non-lactating recipients, regardless if they were in the Treated or Control group.

Breed

Table 9 shows the differences in transfer rate, pregnant/transferred rate, and pregnancy rate for the recipients by treatment and breed. *Bos taurus* recipients appeared to be much more fertile than the *Bos indicus* Due to the findings reviewed in Bo et al., 2003, it was hypothesized that the mean diameter of CLs in the *Bos indicus* recipients would be smaller than those of the *Bos taurus* cows. Subsequently, due to the correlation between CL diameter and P4 in previous research, we expected lower average circulating progesterone in the *Bos indicus* females due to less luteal tissue being present. It was also believed that the *Bos indicus* recipients would be more sensitive to the FSH, and perhaps show a greater response to the treatment by generating either larger CLs or a greater number of CLs.

From Appendix Table 4, the mean diameter of *Bos indicus* cows was not lower than the *Bos taurus* females. Interestingly, the mean diameter reported for the treated and control *Bos indicus* cows was in line with the mean diameters reported in Bo et al., 2003. However, the *Bos taurus* cows in the present study tended to have CL diameters lower than those reported in Bo et al., 2003. However, the lower mean diameter of the *Bos taurus* appeared to not affect pregnancy rate as the control *Bos taurus* group was significantly higher than the *Bos taurus* treated and *Bos indicus* control and treated groups.

Contrasting with the statements in Bo et al., 2003, the present study found there a significant difference in the circulating progesterone levels of *Bos indicus* and *Bos*

taurus females, with the *Bos indicus* cows reporting much higher means, as described in Table 10. Our results are more in line with the findings of Bastos et al., 2010 via Sartori et al.,2010 review, which reported higher progesterone concentrations in *Bos indicus* cows versus *Bos taurus*. Though mixed results have been reported and more research is required, it may be plausible that *Bos indicus* cows metabolize estradiol and progesterone more slowly than do *Bos taurus*.

The findings in the present study also contrast much literature that states higher progesterone levels at the time of transfer are positively correlated with pregnancy rate, as *Bos indicus* recipients, both Treated and Control, had higher P4 levels than *Bos Taurus* but had lower pregnancy rates than *Bos taurus* recipients.

Injection Day

Day 5 of the synchronization protocol was chosen as the time period when the FSH would be administered to the recipients due to the concurrency with the first follicular wave of the estrous cycle. However, the author was forced to alter the protocol in certain instances due to scheduling conflicts with the ranches that owned the recipients, therefore data was collected on recipients who received the FSH injection on days 3, 4, 5, and 6. From Table 11, though only day 5 and day 4 treated recipients differed in pregnancy rate, it appears those recipients who received the injection on days 5 or 6 fared better in regards to pregnancy rate, though sample sizes varied significantly. Interestingly, day 5 recipients differed from the recipients treated on the other days in number of CLs, which certainly implies that the timing of our intended treatment may

have positive benefits on generating accessory CLs. This is also validated by the fact that day 5 treated recipients differed for circulating progesterone. Day 5 treated recipients also differed for number of CLs per cow, 1.21 $\pm$ .04, which is in line with most published eCG research, as referenced earlier in the literature review chapter.

However, it should also be of note that the data compared in regards to injection date may be confounded with other unforeseen independent variables, such as management/ownership and location. Recipients were not randomized as to the date when they received the FSH and as such, all of the day 3 recipients were owned by Kiamichi Link Ranch while all of the recipients that were treated on day 6 were also all owned by Howdy U Cattle Co. Both ranches also had recipients who were treated on different days within the study as well.

In order to provide an accurate comparison of the day 3 and day 6 treated recipients while under common management, Table 18 shows the comparison in pregnancy rate, total luteal area, and circulating progesterone at the time of transfer of these two ranches recipients.

Table 18. **Comparison of transfer rate, pregnant/transferred rate, and pregnancy rate, as well as mean total luteal area and mean P4 at the time of transfer for Kiamichi Link Ranch recipients and Howdy U Cattle Co. recipients**

	Howdy U		KL Ranch	
Season	Spring	Fall	Spring	Spring
Injection Day	4	6	3	4
No. Programmed	30	23	35	32
No. Transferred	22	22	19	19
No. Pregnant	14	11	14	5
Transfer Rate	83.33%	95.65%	57.14%	65.63%
Preg./Trans. Rate	56.00%	50.00%	70.00%^{B}	23.81%^{A}
Pregnancy Rate	46.67%	47.83%	40.00%^{B}	15.63%^{A}
Mean Total Luteal Area	24.80 ± 2.33	30.43 ± 2.48	25.43 ± 2.61	21.92 ± 2.54
Mean P4	1.51 ± .83	3.52 ± .83	1.82 ± .89	1.35 ± .89

^{AB}Differing superscripts denote a difference (P<.05)

From Table 18, there appears to be no conclusive connection between injection day and pregnancy rate. Not surprisingly, the group with the largest mean total luteal area also had the highest circulating progesterone mean, reaffirming the findings of Binelli et al., 2001.

Season

The present study was carried out over two separate breeding seasons, in part to offset management and nutritional variables that could have impacted the study. Data

was essentially analyzed as a 2 x 2 factorial with season (spring vs. fall) and treatment group (Treated vs. Control). The author was unable to find any published literature that looked at the effectiveness of FTET based on the season, and from the finding of the present study it is unclear whether seasonal factors had an effect on fertility of the recipients. It appeared Treated recipients from the spring fared the worst in pregnancy rate (Table 14) and also generated the least amount of total luteal area (Appendix Table 5). The only consistent pattern observed was that fall Treated recipients appeared to generate a greater number of CLs per cow.

Seasonal data was analyzed more thoroughly in an attempt to explain the results. As previously mentioned, the pregnancy rates of the treated cows were significantly lower than those of the control cows, but from Table 14 it is clear that those cows that were treated in the spring were especially lower than those in the fall. Table 19 below shows data for recipients from each ranch that had cows included in the spring portion of the study.

Table 19. **Transfer rate, pregnant/transferred rate, pregnancy rate, mean total luteal area, and mean P4 for each of the ranches in the spring study**

	Pollard		Howdy U		KL Ranch		Heritage		Forgason	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
No. Prog.	30	15	30	44	67	83	9	6	10	4
No. Tran.	22	15	25	42	41	67	8	5	5	4
No. Preg.	11	12	14	26	19	39	5	3	3	3
Transfer Rate	73.33%	100.00%	83.33%	95.45%	61.19%	80.72%	88.89%	83.33%	50.00%	100.00
Preg./ Tran. Rate	50.00%	80.00%	56.00%	61.90%	46.34%	58.21%	62.50%	60.00%	60.00%	75.00%
Preg. Rate	36.67%	80.00%	46.67%	59.09%	28.36%	46.99%	55.56%	50.00%	30.00%	75.00%
Mean Total Luteal Area	24.44 ± 3.06	26.89 ± 2.07	24.80 ± 1.57	30.18 ± 1.49	23.63 ± 1.17	26.31 ± 1.14	25.51 ± 3.96	20.68 ± 5.00	28.54 ± 7.74	17.23 ± 4.58
Mean P4	2.34 ± .34	3.01 ± .43	1.51 ± .23	1.76 ± .30	1.58 ± .21	2.28 ± .22	12.33 ± 8.19	6.50 ± 1.78	5.81 ± 2.90	4.33 ± 2.14

From Table 19 it is clear that all ranches excluding Heritage Cattle Co. had a considerable decrease in pregnancy rate from control to treated groups. Pollard Farms, Howdy U Cattle Co., and Kiamichi Link also reported having larger mean total luteal area and a greater mean P4 value in control cows versus treated cows.

Geographically, the ranches with cows in the spring portion of the study represented different regions, with Pollard Farms being located in northwest Oklahoma, Howdy U Cattle Co. in northeast Texas, Kiamichi Link in east Oklahoma, and Heritage Cattle Co. and Forgason Cattle Co. in south Texas. Cattle at all ranches were managed in large pastures, winter precipitation alleviated most drought conditions in these areas suggesting nutritional decline should have not been much of a negative influence on the fertility of the recipients. A decrease in fertility would have been observed proportionately between the control and treated groups if management or nutritional intake was at fault for the poor results, but it was primarily just the treated group that was negatively impacted. To fully understand the reasoning behind the results that were observed in this study, more research is required.

It is well documented that FSH levels spike at the time of follicular wave emergence and exogenous FSH can cause co dominant (or more) follicle formation. However, as Fortune et al., 2001 explains, extended FSH may inhibit the dominant follicles ability to secrete estradiol and complete its maturation process. A more accurate understanding of metabolization of FSH diluted in hyaluronan is needed to understand if the hyaluronan caused the metabolization to be delayed *too much.*

Other Independent Variables

Table 17 offers the p-values for the effect of other independent variables for which data was collected and its effect on overall pregnancy rate. Only body condition score tended to have an effect, yet there is potential for this to be confounded with other variables due to the limited variability between BCS of 4 (n=122), 5 (n=310), and 6 (n=108).

CHAPTER V

CONCLUSIONS

Results from the present study indicate that administering FSH diluted in Hyaluronan on day 5 of a synchronization protocol is not a viable way to increase fertility of recipient cows. In fact, administering the slow-release FSH may have a *negative* impact on fertility, as pregnancy rates were significantly lower in the treated cows versus the control cows. The numerous variables, both dependent and independent make indentifying the cause of the lowered response in treated cows rather inconclusive. Due to the success of similar protocols that utilized eCG, as opposed to FSH, further work is needed to determine the correct conversion rate for the two supplemental gonadotropins, as well as timing of the dosage. To the best of our knowledge no other domestic studies have utilized FSH diluted in hyaluronan and thus we have no true comparison of our findings, but the author suggests repeating the study utilizing cows under common management so as nutrition and other unforeseen factors may not skew results.

Another factor to consider is that most work with eCG has been conducted with Nellore cattle in South America. The present study consisted of Brahman crossbred and Angus based cows. Breed differences in regards to sensitivity to gonadatropins, even within the *Bos taurus* and *Bos indicus* classifications, may exist but potentially are not as well understood.

Another adjustment to the experimental design of the current design that should be considered for future work is to take multiple blood samples to analyze the rate of metabolization of FSH diluted in HA. With multiple blood samples it would also be possible to determine if any increase or decreases in P4 due to treatment are sustained over a period of time.

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APPENDIX

Table A1. **Number of recipients with multiple corpora lutea and mean number of CLs per cow by lactation status in the Treated and Control groups**

	No.	Mean No. CL
Treated Lact.	13/119 (10.92%)	1.15 ± .04
Treated Non-Lact.	9/78 (11.54%)	1.13 ± .04
Control Lact.	13/174 (7.47%)	1.09 ± .03
Control Non-Lact.	13/89 (14.61%)	1.15 ± .04
Total	47/460 (10.22%)	1.12 ± .02

Table A2. **Mean (± SEM) circulating progesterone (P4) at the time of transfer of recipients by lactation status in the Treated and Control groups**

	No.	Mean P4 ng/mL
Treated Lact.	115	3.46 ± .39
Treated Non-Lact.	74	2.65 ± .49
Control Lact.	167	3.49 ± .33
Control Non-Lact.	88	2.64 ± .49
Total	444	3.18 +.20

Table A3. **Number of recipients with multiple corpus lutea and mean number of CLs per cow by breed type in the Treated and Control groups**

	No.	Mean No. CL
BT Treated	20/159 (12.58%)	1.15 + .03
BIX Treated	1/37 (2.70%)	1.08 + .06
BT Control	21/208 ((10.10%)	1.11 + .03
BIX Control	5/56 (8.93%)	1.11 + .05
Total	47/460 (10.22%)	1.12 + .02

Table A4. **Mean (± SEM) CL diameter, circumference, area per CL, and total luteal area per cow of *Bos taurus* (BT) and *Bos indicus* crossbred (BIX) cows in the Treated and Control groups**

	No.	Diameter (mm)	Circumference (mm)	Area per CL (mm^2)	Total Luteal Area per cow (mm^2)
BT Treated	**159**	16.88 ± .37^{A}	52.63 ± .96^{A}	23.52 ± .70	26.23 ± .88
BIX Treated	**37**	17.88 ± .72	55.03 ± 1.76	24.77 ± 1.48	26.17 ± 2.43
BT Control	**207**	18.17 ± .33^{B}	56.21 ± .85^{B}	24.99 ± .62	27.22 ± .78
BIX Control	**57**	18.71 ± .58^{B}	56.04 ± 1.43^{B}	24.23 ± 1.20	27.54 ± 1.97
Total	**460**	17.79 + .22	54.88 + .56	24.44 + .42	26.87 + .56

^{AB}Differing superscripts denote a difference (P<.05)

Table A5. **Mean (±SEM) CL diameter, circumference, area per CL, and total luteal area per cow of recipients in the Treated and Control groups by season**

	No.	Diameter (mm)	Circumference (mm)	Area per CL (mm^2)	Total Luteal Area per cow (mm^2)
Fall Treated	95	16.35 ± .46^{B}	55.90 ± 1.20^{A}	24.43 ± .91	28.07 ± 1.22^{A}
Spring Treated	101	17.75 ± .44^{C}	50.44 ± 1.16^{B}	23.13 ± .88^{B}	24.49 ± 1.19^{B}
Fall Control	130	16.88 ± .39^{BC}	56.34 ± 1.03^{A}	23.98 ± .78	27.61 ± 1.04^{A}
Spring Control	128	19.72 ± .39^{A}	56.01 ± 1.04^{A}	25.68 ± .79^{A}	26.96 ± 1.05
Total	454	17.79 + .22	54.88 + .56	24.44 + .42	26.87 + .56

^{ABC}Differing superscripts denote a difference (P<.05)