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Evaluation of Estrous Response Patches as a Tool to Determine Optimum Timing for Artificial Insemination and if Gonadotropin-Releasing Hormone is Needed at Timed-AI in Beef Cattle

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EVALUATION OF ESTROUS RESPONSE PATCHES AS A TOOL TO DETERMINE OPTIMUM TIMING FOR ARTIFICIAL INSEMINATION AND IF GONADOTROPIN-RELEASING HORMONE IS NEEDED AT TIMED-AI IN BEEF CATTLE

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ABSTRACT

Two experiments evaluated using an estrous-detection patch to identify animals that are in standing estrus at split-timed AI (STAI) and the necessity of a gonadotropin-releasing hormone (GnRH) injection at STAI on a 7-Day CO-Synch + CIDR protocol. In experiment 1 (n=216) and in experiment 2 (n=101), multiparous lactating crossbred beef cows were stratified by age, BW, BCS, and post-partum interval to 2 treatment groups: CTRL=timed-AI (TAI) at 72h post CIDR removal, or TRT=STAI at 72 or 84h post CIDR removal. All females received GnRH plus a CIDR on d0, prostaglandin-F2α, CIDR removal, and an Estrotect estrous-detector patch on d7. At 72h post-CIDR removal, a patch score was assigned (PS1 <50% removed; PS2 ≥ 50% removed) to all females. Cows in the CTRL group were administrated a second GnRH injection at 72h TAI. Cows in the TRT group with a PS2 were not administrated GnRH at TAI. At 84h, the remaining TRT cows were given a second PS and cows with a PS1 received a GnRH injection and cows with a PS2 did not. Blood samples for Progesterone concentration were collected on d-11 and 0 to determine percent of cows cycling. Data were analyzed using Proc Genmod with treatment and AI technician as fixed effects, sire as a random effect, and BW, BCS, age, and PPI as covariates. In experiment 1, TAI pregnancy rates were similar (*P*=0.81) between the CTRL (40.8%) and TRT (43.4%) groups. Pregnancy rates tended to be greater (P=0.07) for cows with a PS2 (50.3%) compared with a PS1 (29.4%). However, by extending TAI to 84h in unresponsive cows, 82.0% of the TRT cows did not receive a second injection of GnRH at TAI. In experiment 2, TAI pregnancy rates were similar (*P*=0.80) between the CTRL (32.3%) and TRT (38.6%) groups. Pregnancy rates were greater (P=0.04) for cows with a PS2 (70.6%) compared with a

PS1 (19.4%). By extending TAI to 84h in unresponsive cows, 37.1% of the TRT cows did not receive GnRH at TAI. Using a heat-detector can reduce the percentage of cows that require GnRH at TAI without compromising pregnancy rates.

CHAPTER I

INTRODUCTION

Over the past decades, researchers have been able to develop several new assisted reproductive technologies (ART) that directly improved the reproductive performance of several domestic species. The first technology to be used was artificial insemination (AI), pointing the scientific world towards a better understanding of the reproductive tools to select farm animals on a genetic point of view (Foote, 2002). By 2016, technologies such as artificial insemination (AI), fixed-time AI, split-time AI, semen and embryo cryopreservation, embryo transfer and in vitro fertilization are widely available for producers. These technologies contribute by maximizing the production potential of farm animals while reducing environmental impact (Dick et al., 2015; Troy et al., 2016) and shortening the intervals between progenies (Burns et al., 2010). The genetic value of animals is due to the availability of superior genetics from animals all over the world (Corebima and Rengkuan, 2012; Thundathil et al., 2016), which is only possible by the easiness of acquiring semen from the best bulls of the desired breed (Lamb et al., 2016).

For several years, producers were reluctant to use AI in their herds, as fertility rates were lower than expected (Foote, 2002). More recently, newer technologies such as fixed-time AI, split-time AI, semen, and cryopreservation have been developed that the use of AI increased throughout the United States. With improved pregnancy rates, the techniques started to be more desirable by producers, who were looking to improve their herd value and efficiency.

With this technology, researchers began looking into beef cattle as beef cows outnumbered dairy cows in the US. Artificial insemination is still not well spread among beef cattle producers (Lamb et al., 2016) due to several herds being part of extensive ranges where detecting estrus and managing animals in estrus for insemination can be rather challenging (Foote, 1981; Dziuk and Bellows, 1983).

The present study is a result of several attempts to improve pregnancy rates on protocols that rely on estrous synchronization as researchers still face problems such as inaccurate estrous detection (Foote, 1975), adjustment of the timing of insemination (Ulberg et al., 1951; Hansel and Trimberger, 1952) and low fertility rates (Hansel and Convey, 1983; Lamb et al., 2016). Even though fertility might be low in certain scenarios, AI is extremely facilitated by the ability of inseminating the animals at a fixed time without the observation of estrus (Nebel et al., 2000). This study evaluated using an estrous detection patch as a simple, cost-effective reproductive management tool to identify animals that have been or are in standing heat at split-timed AI (STAI) and the necessity of a second gonadotropin-releasing hormone (GnRH) injection at STAI synchronized with a 7-Day CO-Synch + controlled intravaginal drug release (CIDR) protocol for beef cattle.

CHAPTER II

LITERATURE REVIEW

The History of Artificial Insemination

The history of artificial insemination dates back to 1678, when Leeuwenhoek published a paper about identifying sperm for the first time with the help of a 270x magnification lens that he built himself. One century after Leeuwenhoek's discovery, in 1784, an Italian researcher named Spallanzani performed the first successful AI in a bitch, which resulted in the birth of three pups after a 62-day pregnancy. Artificial insemination did not become a world disseminated technique until 1897, when Heape and many other researchers reported that artificial insemination was being seen in research done by Ivanoff (also known as Ivanow or Ivanov) involving rabbits, dogs and horses (1907 and 1922).

Ivanoff created the first substances that we know today as semen extenders and developed techniques to train people to identify and select stallions which carried superior genetics to multiply their progenies via AI. Ivanoff encouraged other scientists such as Dr. Ishikawa, from Japan, to study with him and applied AI to cattle, sheep, swine and poultry. However, most of the research performed by the Japanese researchers was not disseminated to the world due for the fact it was written in Japanese and was only translated to English by Niwa (1958) and Nishikawa (1962, 1964 and 1972) in later years.

Walton (1933) described the extensive use of AI in Russia by Ivanoff which helped to disseminate its use all over the world. In 1936, Sørensen, a researcher at The

Royal Veterinary College in Copenhagen, Denmark organized the first artificial insemination cooperative and enrolled 1,070 cows the first year with a 59% pregnancy rate. Sørensen (1940) and his fellow Danish researchers also contributed to the development of artificial insemination by creating the method of rectovaginal fixation of the cervix, thus reducing the amount of semen utilized per insemination and the straw to store semen. Those straws would end up being modified to production for a commercial use by a French researcher named Cassou in 1964 and they are still the ones used today. During this time, an Italian researcher Spallanzani developed the first artificial vagina for dogs, which was the base for the creation of artificial vaginas for bulls, stallions and rams (Perry, 1968).

Artificial insemination we know today is a result of an exorbitant growth that occurred in the 1940s with several dairy herds throughout the country utilizing this technology. The first AI cooperative in the US was established in 1938 in New Jersey (Perry, 1968). The development of the New York Artificial Breeders Cooperative Inc., which is currently known as Genex, in Ithaca, New York was a result of the collaboration between researchers and farmers. This allowed for the insemination of thousands of dairy cows and the publication of over one hundred scientific manuscripts describing the selection of sires, testicular evaluation, semen collection and preservation and fertility testing (Foote, 1998). This information gave researchers the knowledge to detect and synchronize estrus and timing the insemination for an easier approach on inseminating cattle herds.

The success of an insemination protocol depends on several factors, including proper detection of estrus, skilled AI technicians, animals that have reached puberty or are cycling among other factors. Trimberger (1948) reported that visually detecting an animal in estrus at morning or evening and inseminating that animal 12 hours later resulted in acceptable pregnancy rates, later named as the AM-PM rule. This is still widely used among producers and was developed as a result of observation, ovarian palpation and breeding data from several producers.

Factors Controlling Fertility

The reproductive performance of the herd is impacted by several factors that must be correctly managed. Most times, factors such as age at puberty and first conception, duration of post-partum anestrus and total lifespan of the animal in the herd are not given the necessary attention by the producer due to inconsistent or inexistent data collection.

Puberty is defined as the time when an individual becomes sexually mature. For a heifer, puberty is achieved when the ovaries go from an inactive to an active state which is represented by the first estrus and ovulation followed by a normal luteal phase. This directly impacts the longevity of the herd, as the animals that conceive first will produce more calves during their lifetime when compared to the ones that take a longer time to reach puberty. Fordyce et al. (1994) suggested that early puberty heifers requires a higher level of nutritional management, resulting in costs to maintain the growth of these animals until mature size (Burns et al., 1997). It is known that several factors, such as the percentage of mature body weight of the animal, control the achievement of puberty in beef heifers. The percentage of mature body weight of that animal at the time of AI is critical, with a target of 65% (Morgan et al., 1989).

Poor nutrition results in reduced body condition scores (BCS) in a herd that is not ideally managed, can contribute to a deficiency of nutrients to the herd. This deficiency negatively impacts the cyclicity due to the negative energy balance resulting in an increase of anestrus animals (Vanroose et al., 2000; Bilodeau-Goeseels and Kastelic, 2003). These factors have a significant impact on fertility in postpartum beef cattle (Short et al. 1990) and are composed by general infertility, which is an idiopathic factor and is responsible for 20-30% of herd infertility, lack of uterine involution, short estrous cycles and anestrus.

The post-partum interval (PPI) is very important when regarding primi- and multiparous animals and is represented by the period between parturition and the return to a regular estrous cycle and first ovulation (Arthur et al., 1996). Shorter post-partum intervals are seen as major economic losses due to the insufficient time that animal will have to recover from the parturition and get ready to carry another pregnancy. On the other hand, longer postpartum intervals are seen as a well-managed herd as the animals are able to give birth early on the calving season due to good nutritional and reproductive management, giving them more chances to conceive early on the breeding season. As expected, nutrition plays one of the major roles in fertility of beef cattle and is responsible for most failures with pregnancy.

Postpartum Interval

Managing the PPI, which is the time from calving to the subsequent conception, is critical to ensure a high percentage of females that are cycling at the beginning of the breeding season. The ideal situation for a producer is to have his cows calving early in the calving season allowing those animals to have a longer interval between calving and breeding. The longer the interval is between calving and breeding, the greater the chances of this animal to be cycling (Whitman, 1975). Nutritional recovery from gestation is another factor that prevents animals from conceiving if the PPI is not ideal as this interval will allow for the complete uterine involution after parturition, (Burns et al., 2010).

Uterine Involution

After giving birth, the animal goes through a period called uterine involution, in which the uterus regresses to its standard structure and function so it will be able to carry on another pregnancy. During this process, the uterus will revert to its non-pregnant size, format and location while reconstruct the uterine tissues, taking approximately 20-40 days (Stevenson et al., 2015). The length of this process makes conception during early postpartum difficult. However, researchers have shown that a delayed uterine involution will not interfere with the animal's PPI period (Kiracofe, 1980). As El-Din et al. (1995) points out, the most important step for the resumption of ovarian activity is the increase in daily intake of total digestible nutrients (TDN) as dams with no puerperal issues ovulated earlier (22d) than those with some complications (>30d).

Short Estrous Cycles

After parturition, most females will undergo a period of uncommon ovarian activity, which is characterized by an unusual luteal activity that results in the first ovulation postpartum and won't have the visual expression of estrus. A short estrous cycle is determined by the life span of the corpus luteum (CL), which is approximately 10d (compared to the normal 14-18d). The first postpartum ovulation will end in a completely functional CL, which produces regular progesterone levels to support a pregnancy (Kirakofe 1980; Bischoff et al., 2012). The uterus is still producing abnormal quantities of prostaglandin ($PGF_{2\alpha}$) as a result of its own involution, which causes a premature CL regression. This means that if the oocyte was indeed fertilized, the regression of the CL would occur before recognition of pregnancy by the dam (≈ d16 to 18 of pregnancy) and cause loss of the embryo (Smith et al., 1982).

Nutrition

Nutritional factors are indispensable in all fronts of the beef cattle production system and prepartum nutrition plays a major role during the period of the postpartum anestrus. It is extremely difficult for an animal to come out of anestrus, meaning to start having normal estrous cycles, if that animal is in a negative energy balance, which is characterized by a lower energy intake when compared to 5-8% of mature body weight necessary maintenance energy (NRC, 1996). Selk et al. (1986 and 1988) and Kunkle et al. (1994) concluded that prepartum nutrition, prior to calving, is more critical for postpartum recovery than postpartum nutrition and will affect cyclicity even if the animal is receiving adequate nutrition postpartum. According to Bischoff et al. (2012) females with an inadequate energy intake, lower than maintenance requirements, have lower pulses of luteinizing hormone which will prevent ovulation.

Cyclicity

According to Lamb et al. (2001) approximately 50% of postpartum cows are not cycling by the beginning of the breeding season. To have a successful response to a synchronization protocol, the animals must be exhibiting estrus before the protocol begins (Wheaton and Lamb, 2007). Perea et al. (2008) suggests that non-cyclic animals prior to a synchronization protocol have a delayed estrous response, reduced ovulation and poor conception rates. The use of an controlled internal drug release (CIDR) impregnated with progesterone can induce non-cyclic animals to initiate cycling and increase the percentage of heifers attaining puberty (Lamb et al., 2006).

Body Condition Score (BCS)

Body Condition Score (BCS) is a very useful tool to determine the energy balance of a cow, which impacts fertility. It is critical to achieve a minimum BCS before the breeding season to ensure that a high percentage of animals are cycling (Lamb et al., 2001). An animal that is too thin or too fat for breeding can negatively impact pregnancy rates (Stevenson et al., 2003). Body condition score (BCS) is based on a 1 to 9-point scale with 1 being emaciated and 9 being obese (Richards et al., 1986). Marques et al. (2016) concluded that to optimize cow-calf productivity and wean heavier calves, dams need to gain BCS during pregnancy which will result in greater performance of the offspring. The BCS will also directly impacts the length of the PPI and is directly related to a cow's previous calving date (Larson and White, 2016). Selk et al. (1986) reported a 30% greater incidence of estrous cycles 80 d postpartum for cows that calved at a BCS of 5 to 6 compared with cows that calved at a BCS of 4 or lower. Also, as BCS increases from 3.5 to 6.0, the percentage of cows cycling increases by 18 ± 2 % by each unit of BCS gained (Stevenson et al., 2003).

Fertility Impact of BCS

Nutrition factors have one of the biggest impact on fertility during estrous synchronization protocols. Wettemann (1994) reported that any factor described to cause postpartum anestrus besides BCS and nutrition is simply a result of the effects caused by these last two major factors. Nutrient intake before and after parturition is critical to achieve acceptable pregnancy rates in cows (Montiel and Ahuja, 2005) and heifers. This lack of nutrient intake results in a major loss of BCS and an increase in anestrous in beef cattle (Richards et al., 1989; Bishop and Wettemann, 1993). Similarly, Perry et al. (1991) reported that under nourished cows didn't have ovarian follicles larger than 5 or 8mm that can produce enough estradiol to overcome the anestrus period, resulting in infertility. This also impacts the post-partum and calving-to-conception intervals, which will be longer for cows suffering from any kind of nutritional problems (Laflamme and Connor, 1992). According to Lowman et al. (1976) it is desirable for beef cows to be in a BCS between 5 to 6 at the time of artificial insemination, and heifers to be at a BCS 6 to 7.

Ovarian Follicle Diameter

The size of the dominate follicle is also an important factor affecting fertility (Sá Filho et al., 2010a) as it is directly related to the size of the CL after ovulation, meaning that a smaller dominant follicle will result in a smaller CL and consequently a reduced production of progesterone (Siqueira et al., 2009; Sá Filho et al., 2010b). This directly affects pregnancy rates, as the concentration of progesterone which is about 0.5 ng/ml (Arreguin et al., 1997) is not sufficient to carry on a pregnancy (Vasconcelos et al., 2001) therefore pregnancy is lost. Perry et al. (2014) reported that follicle size has a direct impact on preovulatory estradiol concentrations in cows that exhibit estrus compared with cows that did not meaning that ovulation is more likely to occur in those animals. Madsen et al. (2015) performed a study evaluating to establish the role of estradiol on post-fertilization embryo survival and pregnancy establishment. He reported that animals with lower estradiol concentrations prior to ovulation had greater embryonic losses (Pereira et al., 2014) after maternal recognition of pregnancy.

Regulation of Estrous Cycle

Estrus Detection

To achieve optimum timing between ovulation and AI, it is important to observe for a standing estrus. Estrus is defined as an animal standing when mounted. The average length of standing estrus ranges from 15 to 18 hours but it may vary from 8 to 30 hours among cows. During estrus, the female will be mounted by other animals approximately 20 to 55 times, allowing the technicians, although rather inefficiently (Andersson et al., 2016) to visually identify which animal is in standing estrus.

Animals in standing estrus are typically more active that those who are not in heat being more alert to their surroundings and even more nervous. In addition, an increase in phonation and attempts to mount other animals can be interpreted as a sign that the animal might coming into or out of estrus (Senger, 1994; Torres-Júnior et al., 2014). Other physical signs of estrus may include a clear mucus discharge from the vulva, dirt or mud on the side of hips and roughed up tail head on the animal in standing estrus (De Silva et al., 1981; Bikker et al., 2014; Dolecheck et al., 2015). Animals in standing heat will also be receptive to be mounted by other females and bulls (Roelofs et al., 2005).

Newer methods of estrus detection include the use of an accelerometer attached to the animal to measure its physical activity and it can transfer wirelessly to a computer server on which the physical activity data from the animals can be read on a software like Microsoft Excel (Chanvallon et al., 2014). There is also a patch (Estrotec® Heat Detector, Rockway Inc., Spring Valley, WI) that is applied to the tail head of the animal that when activated following multiple mounts indicates the female is in standing estrus. This patch turns into a very bright color which allows for easy detection. Davis et al. (2015) analyzed the Estrotect patches and concluded that they were highly reliable (80% or more) to identify cyclic or noncyclic heifers, and 99% reliable to detect animals pregnant by AI or animals in estrus. It has been reported (Rorie et al., 2002) that visual observation of estrus is only 50 to 70% efficient. Tsai and Huang (2014) reported that video can also be utilized to detect estrus in cows. This system is a standalone system that captures video from the herd and records animals that have either followed or mounted indicating standing estrus resulting in the identification of females in estrus through a computer.

Silper et al. (2015) combined the use of an accelerometer to measure physical activity (steps per day) and video surveillance to achieve a more automated method compared with the Tsai and Huang video surveillance method only. These authors reported that the behaviors that accounted for the most reliable indicators of estrus were chin rest (can be considered a test of receptivity for mounting), genitalia sniffing, back mount, crossover (unquiet animal walking across the pasture), accept chin rest and follow could be measured and reported by this system. However, they also point out, that the variation in this experimental groups were large and that these methods can have a large amount of error when detecting estrus (Dolecheck et al., 2015). While automated methods of estrus detection can be beneficial to a farm management, the decision to utilize each must align with producer objectives and financial due to the higher costs when compared to estrus detection by visual observation (Chanvallon et al., 2014).

Timing of Insemination

The AM-PM rule of estrous detection was established in 1948 and has become the industry standard as the most reliable method to achieve optimum timing for AI. The AM-PM rule states that the animal visualized standing estrus should be inseminated 12 h post first observed standing estrus. Beef heifers were utilized by Dorsey et al. (2011) to determine if the AM-PM rule was accurate to achieve optimum timing for increased pregnancy rates in beef heifers. Results indicated that heifers should be inseminated at 16 h, ranging from 4 to 20 h, after displaying estrus to improve AI pregnancy rates. Robbins et al. (1978) achieved a 63.4% of pregnancy rates in beef cows when insemination occurred at 12 h after displaying estrus while pregnancy rates were reduced to 57% for the animals inseminated after 26h of standing estrus. Maatje et al. (1997) conducted a similar study with dairy cows. In that study pedometer data complied with standing estrus was used to determine optimum timing for AI. These authors report the chance of pregnancy was highest between 6 and 17h after increased pedometer activity and estimated best timing for AI at 11.8h after standing estrus.

Bishop et al. (2016) used the method of a split-time AI to verify if it was possible to achieve optimum timing at AI and consequently higher pregnancy rates. The study split a regular TAI protocol according to animals that exhibit estrus and utilized the AM-PM rule until 90 hours after PG focusing on giving gonadotropin-releasing hormone (GnRH) only to animals that had displayed estrus and did not find any differences in pregnancy rates among groups: however estrous expression in animals that had the AI delayed was greater. It is worth noting that beef cows that display estrus have 3.3 times more chances of becoming pregnant when compared to the beef cows that don’t (Kasimanickam et al., 2016).

Calf Separation Versus Luteinizing Hormone Pulses

Calf separation after the estrous synchronization protocol is one of the factors that can impact the frequency of pulses of luteinizing hormone (LH) by increasing the ovarian luteal activity (Wettermann et al. 1986) and affect the timing of ovulation. Dunlap et al. 1981 reported that LH pulses decrease with the increase of calf suckling, meaning that the suckling stimulus actually decreases ovarian luteal activity. Several researches done in beef cattle found an increase on the frequency of LH pulses after a 48-hour calf removal but no difference in pregnancy rates when compared to cows that were not separated from their progenies (Waltens et al. 1982, Whisnant et al. 1985, Fanning et al., 1995) increasing even more 72 hours after removal (Dunn et al. 1985).

Martins et al. 2012 reports greater pregnancy rates on beef cows that had their calves removed for 48 hours every 20 days when compared to cows that only had their calves removed during one 48-hour period. Other studies show that a short period of calf separation does not impact these pulses nor the pregnancy rates of synchronizing females. Peel et al., 2010 published data reporting that fixed-time AI pregnancy rates did not differ between cows who had their calves removed for 12 hours from cows who were never separated from their calves. It was then concluded that calf separation for 12 hours had no effect on TAI pregnancy rates but did simplify the splt-time AI synchronization protocol by not having to sort cows and calves two times during this period.

Estrous Synchronization

Most estrous synchronization protocols use a combination of three hormonal classes, progestins (Progesterone), prostaglandins ($PGF_{2\alpha}$) and gonadotropins (GnRH), that mimic certain episodes that occur during the estrous cycle of a cow. Progesterone is used during the synchronization protocol to prevent cycling females from coming into heat, as well as initiate cyclicity in anestrous females. The use of $PGF_{2\alpha}$ is intended induce luteolysis it when given to the animal and therefore, causing estrus to occur. However, timing of $PGF_{2\alpha}$ is critical as it is possible to cause termination of pregnancy if given to a pregnant animal (Zerobin et al., 1973) or as pointed by Lauderdale (1972) and Rowson (1972) failure to initiate luteolyis may occur due to the CL still producing low amounts of progesterone following ovulation (days 1 to 6 post ovulation) or during days 17-21 post ovulation due not having a functional CL yet therefore won't be affected by $PGF_{2\alpha}$. The injection of $PGF_{2\alpha}$ at the day 7 will normally cause luteolysis of the CL and allow the animal to come into standing estrus.

Heat-Detection Protocols

The primordial step in synchronizing females is knowing how much labor is available for heat detection is possible to be done or even warranted by the producer. Heat Detection is labor intensive and depends not only on farm personnel but also on facilities and the production system the farm utilizes. Heat-Detection protocols require that animals be observed for standing estrus and subsequently inseminated according to the AM-PM rule. These protocols require heat-checking for up to 5 or 7 days after $PGF_{2\alpha}$ injection 3 times per day with a duration of about 30 minutes to 1 hour each time. Heat detecting protocols for use in cows include Select Synch, Select Synch + CIDR and PG 6-day CIDR, while heifers are recommended to be synchronized with the 7-day CIDR + PG or MGA-PG protocols. Johnson et al. (2016) recommended that the Select Synch + CIDR protocol be used on young, thin, and late-calving cows or when heat detection before PG administration is not possible.

In nulliparous heifers, the 7-day CIDR + PG or MGA-PG protocols are recommended as CIDR may induce some prepubertal heifers to start cycling (Nielson et al., 2016). Peel et al. (2013) suggests that the initial GnRH injection is not needed at the beginning of the treatment by not having any significant effects (P = 0.18) on pregnancy rates (59 vs 71%) at the beginning of the Select Synch + CIDR protocol. The MGA-PG protocol may also have inconsistent response because it relies on the ideal setup of the facilities and adaptation of the heifers by the producer to uniformly consume MGA for 14 days and another 19 days after the last day of MGA consumption until PG injection making it a total of 33 days. After the feeding period is over and the animals already received a single injection of PG, it is necessary to heat check for twice as long when compared to other protocols, due to the synchronization being less accurate when compared to other protocols.

Heat-Detection + TAI Protocols

These are protocols that utilize heat-detection in addition to TAI of females that are nonresponsive 72 to 84 hours following PG. Success of these protocols depend on accurate heat detection specially for early heats in the Select Synch protocol and by having an injection of GnRH at TAI these protocols will allow females not detected in heat to have a chance on getting pregnant. Protocols used for beef cows include the Select Synch (Select Synch and TAI), Select Synch + CIDR (Select Synch + CIDR and TAI) and the PG 6-day CIDR and TAI protocols, all those with TAI at 72 to 84 hours.

Heifers again have different protocols when compared to the ones used in beef cows. These protocols include the MGA-PG and TAI at 72 to 84 hours, 14-day CIDR-PG and TAI at 70 to 74 hours or the Select Synch + CIDR and Tai at 72 to 82 hours. The first two protocols are similar; however, the 14-day CIDR-PG and TAI has a shorter interval between CIDR removal and PG due to a quicker estrous response. The Select Synch + CIDR and TAI protocol has a higher cost when compared to the other two because it relies on a single injection of GnRH at CIDR insertion, which may increase pregnancy rates in heifers that respond to the GnRH injection.

Fixed-Time AI (FTAI) Protocols

The biggest difference between FTAI protocols and the ones mentioned above is that no heat detection is required. Instead, all animals are inseminated at a previously defined time. Pregnancy rates between FTAI and heat detection protocols are similar for cows but when taking heifers into consideration it tends to be 5 to 10 percent lower (Johnson et al., 2016). One factor that is important is that timing of TAI for the 5-day CO-Synch + CIDR, 7-day CO-Synch + CIDR, 14-day CIDR – PG and MGA-PG are approximate and dependent on the size of the group being inseminated as well labor and facilities available. This interval should be no more than 3 or 4 hours.

In multiparous females, there are two protocols being currently used (Nash et al. 2012), the 7-day CO-Synch + CIDR and the slightly shorter 5-day CO-Synch + CIDR. Both protocols rely on an injection of GnRH on day 0, and the insertion of an intravaginal progesterone implant for 5 or 7 days, according to the protocol. In the 7-day CO-Synch + CIDR protocol synchronized females will receive a single injection of $PGF_{2\alpha}$ on day 7 upon CIDR removal while the 5-day CO-Synch + CIDR protocol requires CIDR removal on day 5 and two injections of $PGF_{2\alpha}$ 8 hours apart beginning at CIDR removal. Animals are TAI at 60 to 66 h post $PGF_{2\alpha}$ administration on the 7-day protocol and 72 ± 2 hours post $PGF_{2\alpha}$ administration on the 5-day protocol.

Utilization of Estrus Detection Aids

Heat detection aids become a very useful tool in helping producers and veterinarians to easily identify animals in heat, providing less economical losses due to missing the correct insemination period (Senger, 1994). Senger listed what the ideal estrous detection aid method should provide, which includes 24 h/day surveillance, accurate and automatic identification of cows in estrus, minimized labor, and high accuracy in identifying the appropriate physiologic or behavioral events that correlate with ovulation. It is important to know that no detection method will replace the required knowledge to correctly identify all signs of estrus or upcoming estrus and that the detection accuracy will help determine the optimum time for AI (Tsai and Huang, 2014).

Types and Accuracy of Estrus Detection Aids

In 1975, Foote describes two methods available that are capable of providing 24-h surveillance: a pressure sensitive device mounted on the back of the cow that activates when the cow is in standing estrus and a marking device that is worn by an infertile bull which paints the back of the cow in standing estrus. In 1993, Redden et al. tested the use of body temperature and pedometer-monitored activity to improve the accuracy of detecting estrus and detected 66% of the females in heat. By 2005, the HeatWatch® (HW; DDx Inc., Denver, CO) system was already well established and was tested by Peralta et al. (2005) and compared to two other methods such as the ALPRO® (ALPRO; DeLaval Inc., Kansas City, MO) and the traditional visual observation of estrus. The HeatWatch® system consisted of small pressure sensitive sensors that transmit mounting data to a base station and from there to a computer on which the technician can analyze individual cow activity. The ALPRO® system is pretty similar, consisting of an activity meter instead of a mounting detection sensor, but not as accurate on detecting estruses as data from Peralta et al. 2005 shows (37.2% vs 48% on the HeatWatch®). Follow-up studies evaluating accuracy reported detection of 91 to

100% of the females in standing estrus (Dolecheck et al., 2015; Madureira et al., 2015 and Andersson et al., 2016).

Newer technologies such as the Ultra-Wideband (UWB; Thales Research & Technology Ltd., Reading, UK), creates a 3-dimentional position of the animals and monitors continuously the activity of the animals and Homer et al. (2013) was able to detect 9 out of 10 animals in estrus. Other systems were also reported to have good accuracy (96%) on detecting increases on physical activity of the females, such as a 3-dimensional accelerometer (SensOor; Agis Automatisering BV, Harmelen, the Netherlands) which is attached to the ear identification tag and showed promising results pointing that it could be used to help detect animals in standing estrus (Bikker et al. 2014). Chanvallon et al. (2014) concluded that most heat detection aids are not as accurate on the first postpartum ovulation but have a higher accuracy on the subsequent heats and Sliper et al. (2015) reported a lot of variation between individuals when regarding the increase or not of physical activity of that animal during estrus.

O'Neill et al. (2014) evaluated a new technique using an ultra-high frequency (UHF) proximity logger telemetry device to evaluate the interactions between males and females and identify the reproductive cycle phenotype (duration and estrous expression). He reports that both number and duration of interactions were greater in estrus cows when compared to anestrus cows. Whether methods of automatic estrous detection are preferred, Tsai and Huang (2014) evaluated the use of a video recording system that didn't require any devices to be attached to the animals but required personnel to closely watch the footage for a few hours every day to accurately identify animals in estrus.

Davis et al. (2015) utilized estrous-detection patches (Estrotect Heat Detector; Rockway Inc., Spring Valley, WI) that are glued to the tail head of the cow and the surface is scratched by other cows when the animal is standing estrus. This scratching removes the exterior film of the patch exposing the bright colored layer that is easily seen from great. Perry, 2005 reported a 97% estrous detection accuracy with the use of heat-detection patches and Walker et al. (2014) reported that on a 7-day CO-Synch + CIDR the patches can be used to determine GnRH use at TAI.

Estrous Synchronization and Estrus Detection Aids Costs

Estrous synchronization protocol cost will vary according to timing of AI and protocol. Most TAI protocols rely on the use of an intravaginal progesterone implant (CIDR), a single injection of $PGF_{2\alpha}$ and two injections of GnRH. Average cost for the CIDR implant is $11.80, $3 for a $PGF_{2\alpha}$ injection and $3 for a GnRH injection. If we compare cost between protocols, cost will range from $20-25/head, not including the semen which can vary between $10-15 for an average or around $25-40 for above average bulls (Beef Sires Catalog. Genex Cooperative, Inc., Shawano, WI, USA).

Additional costs include labor, materials and AI technician which is around $15/head. Narrowing down all costs, the complete synchronization protocol will cost an average of $50 per head. Protocol costs are based on research published by Johnson and Jones (2008) and Edwards et. al. (2015).

The most common heat-detection aid systems utilized currently are the HeatWatch® and the Estrotect® Heat Detector. The first costs an average $25.70 per cow, not counting the equipment costs which will add about $8000 for the antennas, transmitters, patches and computers utilized for a herd size of 100 cows while the Estrotect Heat Detector costs an average of $1.16 per patch. All costs were provided by the heat-detection aid system manufacturers at the present date of the study.

CHAPTER III

EVALUATION OF ESTROUS RESPONSE PATCHES AS A TOOL TO DETERMINE OPTIMUM TIMING FOR ARTIFICIAL INSEMINATION AND IF GONADOTROPIN-RELEASING HORMONE IS NEEDED AT TIMED-AI IN BEEF CATTLE

Introduction

Artificial insemination is currently not well adopted among beef cattle producers (Lamb et al., 2016) due to several herds being part of extensive ranges where detecting estrus and managing animals in estrus for insemination can be rather challenging (Foote, 1981; Dziuk and Bellows, 1983). Artificial insemination is facilitated by inseminating the animals at a pre-determined time without the observation of estrus (Nebel et al., 2000).

The present study is a result of several attempts to improve pregnancy rates from protocols that rely on estrous synchronization and have problems such as inaccurate estrous detection (Foote, 1975), adjustment of the timing of insemination (Ulberg et al., 1951; Hansel and Trimberger, 1952) and low fertility rates (Hansel and Convey, 1983; Lamb et al., 2016) impacting the pregnancy rates.

This study evaluated using an estrous detection patch as a simple, cost-effective reproductive management tool to identify animals that have been or are in standing estrus at split-timed AI (STAI). We hypothesized that animals in standing estrus with an activated patch do not need a second gonadotropin-releasing hormone (GnRH) injection at STAI synchronized with a 7-Day CO-Synch + controlled intravaginal drug release (CIDR) protocol for beef cattle.

Materials and Methods

The Louisiana State University Agricultural Center Institutional Animal Care and Use Committee approved the research protocols for all animal procedures, protocol # A2016-07. This study was conducted at the LSU Hill Farm Research Station (Homer, Louisiana) and at the Double P Ranch (Sibley, Louisiana). The use of animals in this experiment was in accordance with the proper humane animal handling procedures approved by the National Cattlemen's Beef Association and the Louisiana Cattlemen's Beef Association.

Experimental Design

Experiment 1

Estrus was synchronized in multi-parous Angus-crossbred beef cows (MEAN ± STD DEV) at the Hill Farm Research Station (n = 216, BW = 581 ± 67 kg, BCS = 5.3 ± 0.8, PPI = 78.5 ± 15.5 days, age = 5.9 ± 2.5 years. Animals were stratified to two treatments by BCS (1-9 scale) and BW collected on days -11 and 0, age and PPI. Technicians that performed AI (2 technicians) and AI sires (2 sires) were preassigned to treatments based on BCS and BW to ensure that treatments were not biased. Treatments for cows included: 7-day CO-Synch + CIDR (Figure 1) estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR (Figure 2) estrous synchronization protocol with 72- or 84-h split-timed AI (TRT).

All animals were managed on cool-season pastures through May and warm-season pastures through October and had ad libitum access to water, salt and loose trace minerals available throughout the experiment. All cows received the 7-day CO-

Synch + CIDR (Eazi-Breed CIDR insert, 1.38 g progesterone; Zoetis, Madison, NJ, USA) protocol and included a CIDR insert + 100 µg (i.m.) GnRH (Cystorelin, Merial, Athens, GA, USA) injection given on d 0, followed by CIDR removal and 25 mg (i.m.) $PGF_{2\alpha}$ (Lutalyse, Zoetis) administered on d 7; An estrous detection aid (Estrotect, Rockway Inc., Spring Valley, WI, USA) were applied at CIDR removal/PG injection on d 7 for all cows in both treatment groups. All animals were assigned a patch score (PS) of 1 (< 50% of the coating rubbed off of the patch) or 2 (≥ 50% of the coating rubbed off of the patch) at 72-h post CIDR removal by the AI technician. Animals in the CTRL were inseminated and received GnRH at that time. The remaining animals in the TRT group were sorted out and penned separately (without calves) for 12 h. At 84 h post CIDR removal, the remaining TRT animals were assigned a new PS and inseminated: those with a PS of 1 received a GnRH injection and those with a PS of 2 did not receive a second injection of GnRH. All cows were exposed to fertile bulls beginning 14 days after TAI. Diagnosis of TAI and final pregnancy was performed on d43 and d120 after TAI via ultrasonography (Aloka SSD-500v Ultrasound ®, 5-Mhz, Corometrics, Wallingford, CT).

Blood was collected via coccygeal venipuncture using an 18 gauge 2.54-cm collection needle (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria) into a 10-mL BD Vacutainer® Glass Serum Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for analysis of plasma progesterone. As blood samples were collected, they were placed on ice until centrifuged for 15 min at 4,235 x *g* at 0° C. Plasma was pipetted into plastic vials before being frozen until samples were analyzed for plasma progesterone levels via radioimmunoassay (Abraham et al., 1971).

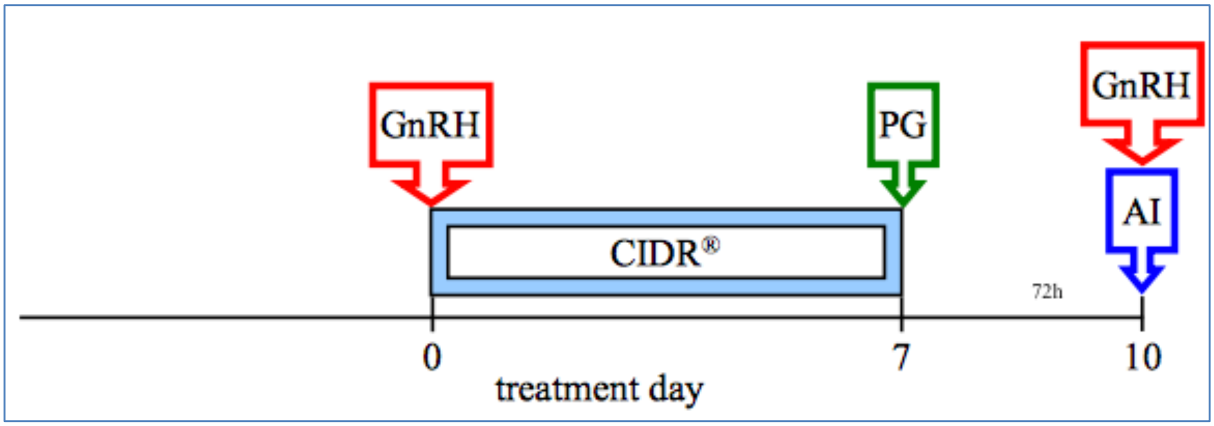

Figure 1. 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL).

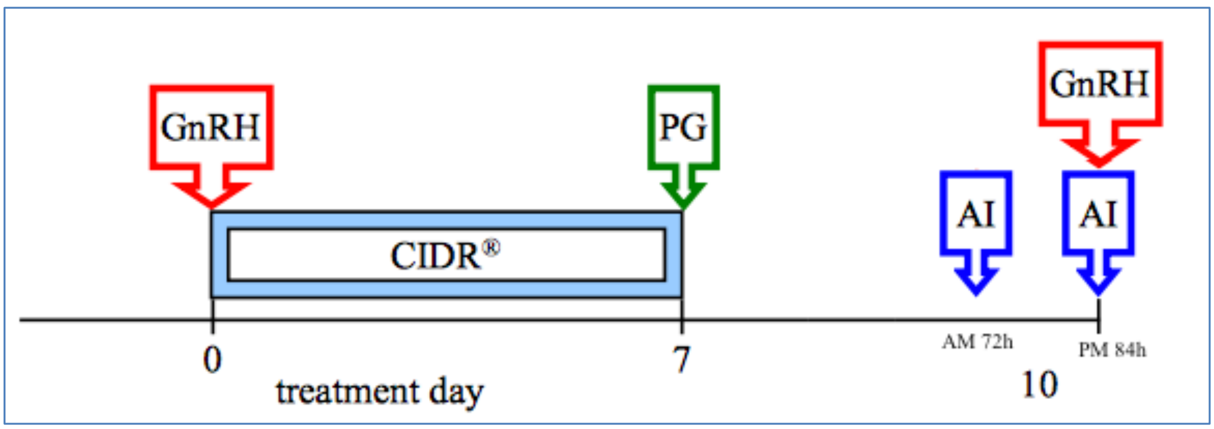

Figure 2. 7-day CO-Synch + CIDR estrous detection protocol with estrous detection patches and split-time AI (TRT).

Statistical Analysis

Treatment effects on the proportion of heifers and cows pregnant to TAI or final pregnancy rates were tested using Proc GENMOD procedure (SAS Institute, Cary, NC) for binomial data. Fixed effects included treatment, AI technicians (2 technicians), patch score (1 = < 50% patch film removed or 2 = ≥ 50% patch film removed), PPI group (1 =

≤ 70 d postpartum or 2 = > 70 d postpartum) and cyclicity (cycling if plasma progesterone concentrations were ≥ 1 ng/ml), and their 2-way interactions with treatment. Body weight, BCS, age and PPI were included as covariates in all models and backward elimination was performed to identify if variable remained in the final model using a significance level set at $P < 0.05$. Cyclicity status and percentage of cows with a PS 2 were included in the model as response variable with fixed effects of treatment. Timed AI pregnancy rate was calculated as the proportion of females that were pregnant to AI at 72 or 84 hours following $PGF_{2\alpha}$ administration. Overall pregnancy was calculated as the proportion of females that were pregnant to TAI or natural service at the end of the breeding season. Significance of main effects was determined using Chi-squared at $P < 0.05$ and tendencies were discussed at $0.10 > P > 0.05$.

Experiment 2

Estrus was synchronized in multi-parous Angus-crossbred beef cows (MEAN ± STD DEV) at the Double P Ranch (n = 101, BW = 513 ± 55 kg, BCS = 3.8 ± 0.9, PPI = 67.9 ± 15.3 days, age = 7.5 ± 2.2 years). Animals were stratified to two treatments by BCS (1-9 scale) and BW collected on days -11 and 0, age and PPI. AI sires were preassigned to treatments based on BCS and BW to ensure that treatments were not biased. Treatments for cows included: 7-day CO-Synch + CIDR (Figure 1) estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR (Figure 2) estrous synchronization protocol with 72- or 84-h split-timed AI (TRT).

All animals were managed on cool-season pastures through May and warm-season pastures through October and had ad libitum access to water, salt and loose trace minerals available throughout the experiment. All cows received the 7-day CO-Synch + CIDR (Eazi-Breed CIDR insert, 1.38 g progesterone; Zoetis, Madison, NJ, USA) protocol and included a CIDR insert + 100 µg (i.m.) GnRH (Cystorelin, Merial, Athens, GA, USA) injection given on d 0, followed by CIDR removal and 25 mg (i.m.) $PGF_{2\alpha}$ (Lutalyse, Zoetis) administered on d 7; An estrous detection aid (Estrotect, Rockway Inc., Spring Valley, WI, USA) were applied at CIDR removal/PG injection on d 7 for all cows in both treatment groups. All animals were assigned a patch score (PS) of 1 (< 50% of the coating rubbed off of the patch) or 2 (≥ 50% of the coating rubbed off of the patch) at 72-h post CIDR removal by the AI technician. Animals in the CTRL were inseminated and received GnRH at that time. The remaining animals in the TRT group were sorted out and penned separately (without calves) for 12 h. At 84 h post CIDR removal, the remaining TRT animals were assigned a new PS and inseminated: those with a PS of 1 received a GnRH injection and those with a PS of 2 did not receive a second injection of GnRH. All cows were exposed to fertile bulls beginning 14 days after TAI. Diagnosis of TAI and final pregnancy was performed on d43 and d120 after TAI via ultrasonography (Aloka SSD-500v Ultrasound ®, 5-Mhz, Corometrics, Wallingford, CT).

Blood was collected via coccygeal venipuncture using an 18 gauge 2.54-cm collection needle (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria) into a 10-mL BD Vacutainer® Glass Serum Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for analysis of plasma progesterone. As blood samples were collected, they were placed on ice until centrifuged for 15 min at 4,235 x *g* at 0° C. Plasma was pipetted into plastic vials before being frozen until samples were analyzed for plasma progesterone levels via radioimmunoassay (Abraham et al., 1971).

Statistical Analysis

Treatment effects on the proportion of heifers and cows pregnant to TAI or overall pregnancy rates were tested using Proc GENMOD procedure (SAS Institute, Cary, NC) for binomial data. Fixed effects included treatment, patch score (1 = < 50% removed or 2 = ≥ 50% removed), PPI group (1 = ≤ 70 d postpartum or 2 = > 70 d postpartum) and cyclicity (cycling if plasma progesterone concentrations were ≥ 1 ng/ml), and their 2-way interactions with treatment. Body weight, BCS, age and PPI were included as covariates in all models and backward elimination was performed to identify if variable remained in the final model using a significance level set at $P < 0.05$. Cyclicity status and percentage of cows with a PS 2 were included in the model as response variable with fixed effects of treatment. Timed AI pregnancy rate was calculated as the proportion of females that were pregnant to AI at 72 or 84 hours following $PGF_{2\alpha}$ administration. Overall pregnancy was calculated as the proportion of females that were pregnant to TAI or natural service at the end of the breeding season. Significance of main effects was determined using Chi-squared at $P < 0.05$ and tendencies were discussed at $0.10 > P > 0.05$.

RESULTS

Experiment 1 Results

The current study evaluated the use of an estrous detection aid to determine if administration of GnRH at TAI for beef cows is required in a 7-Day CO-Synch + CIDR protocol with split-time AI. There was no treatment by technician ($P = 0.78$), PPI Group ($P = 0.15$), PS ($P = 0.28$) or cyclicity interaction ($P = 0.26$); thus, interactions were removed from all models. Breeding season (final) pregnancy rates tended to be different ($P = 0.05$) among the CTRL and TRT groups for cows (86.6 and 76.4%; Figure 3). AI technicians tended to be different ($P = 0.07$) between each other at TAI (28.2 and 50.3%; Figure 4). There was a 22.1% difference in pregnancy rates between the two AI Technicians; however, sire was analyzed as a random variable and likely accounted for some of the differences seen between the two AI Technicians.

Pregnancy rates to TAI (Figure 3) were similar ($P = 0.81$) for cows in the TRT group (45.6%), where 82.0% of the cows did not receive GnRH at TAI due to an activated estrous detection patch, compared with cows in the CTRL group (44.8%), where all cows received GnRH at TAI. There was a difference ($P < 0.01$) on the percentage of cows with a PS of 2 between CTRL (64.2%) and TRT (82.0%) groups (Figure 5); however, pregnancy rates were not impacted based on this response from both groups. Cyclicity did not differ ($P = 0.14$) among CTRL and TRT groups (83.1 and 74.0% - Figure 6).

Timed artificial insemination pregnancy rates based on patch score tended to be different ($P = 0.07$) for cows assigned a PS of 2 when compared with a PS of 1 (50.3

and 29.4%) however final pregnancy rates were similar (*P* = 0.30; 81.8 and 72.5% - Figure 7), respectively. Timed artificial insemination pregnancy rates were similar (*P* = 0.68) among anestrous cows (42.9%) compared with cycling cows (46.9%); however, final pregnancy rates tended to be greater (*P* = 0.06) in cows cycling when compared to anestrous cows (84.5 and 67.3%; Figure 8).

Bishop et al. (2016) reported an increase on estrus response in beef cows when delaying TAI from 66 to 90 h post $PGF_{2\alpha}$ administration in a 7-day CO-Synch + CIDR protocol. In that study, Estrotect patches were applied at PG administration (day 7) with delayed TAI from 66 to 90 h post PG administration according to estrus expression and estrus was defined as having at least 50% of the coating rubbed off of the Estrotect patch. GnRH was administered according to estrous response (animals in estrus did not receive GnRH at TAI). However, there was no difference in pregnancy rates between the two groups when inseminated at 66 h regardless of GnRH administration (57 and 58%). There was also no difference between TAI at 66h with GnRH versus TAI at 90h with GnRH (44 and 49%). Pereira et al. (2016) reported that animals that displayed estrus had a higher probability of getting pregnant from TAI compared with those that did not display estrus (38.9 and 25.5% respectively). It was also reported that that the percentage of pregnancy loss in animals that had conceived from TAI was lower on animals that displayed estrus from those that did not display estrus (14.4 and 20.1% respectively). Stegner et al. (2004) reported greater pregnancy rates for cows inseminated at 72 (64%) vs 80h (50%) synchronized with a MGA-Select protocol.

In the current study, there was no difference in TAI pregnancy rates between CTRL and TRT groups that were assigned a PS of 2 at TAI (Figure 9), suggesting that

GnRH may not have any beneficial effects on ovulation at TAI in beef cows that are exhibiting a standing estrus at TAI. It is worth mentioning that TRT cows inseminated at 84 h were penned up separate from their calves for 12 h; however this did not impact pregnancy rates, as it is consistent with literature (Waltens et al., 1982; Dunn et al., 1985; Whisnant et al., 1985; Fanning et al., 1995; Peel et al., 2010; Martins et al., 2012) that pregnancy rates are not impacted if calf separation less than 48h.

Prior to CIDR insertion on d 0, the cyclicity status of the herd was similar among the CTRL and TRT groups (83.1 and 74.0%) which is even better than the usual 50% of cows cycling at the beginning of every synchronization protocol (Lamb et al., 2006).

Pregnancy rates to TAI tended to stay the same in cows that had a PS 2 not receiving GnRH at TAI in the TRT group when compared to the CTRL group on which all animals received GnRH at TAI (50.0 and 51.2%; Figure 9). This result is complacent to the literature available (Walker et al. 2014; Bishop 2015) pointing out that GnRH is not needed at TAI if the animal is in standing estrus. Estrus detector patches loss was very low in this study (0.46%; 1 animals out of 216 lost their patch), indicating that the adhesive held the patches on very successfully. The data generated by this study suggests that cyclicity status has a major influence on pregnancy rates compared with GnRH administration at TAI. The CO-Synch protocol (Geary and Whittier, 1998) is a result of a modification of the Ovsynch protocol (Pursley et al., 1995) to reduce the number of times animals are handled during the synchronization protocol. The addition of a CIDR insert in the CO-Synch protocol has been shown to increase the percentage of females displaying estrus within an 84-hour period (Larson et al., 2006) supporting the objective of our study to inseminate at 72 and 84 hours.

Several studies in the past couple decades have evaluated different estrous synchronization protocols aiming to increase pregnancy rates to TAI or FTAI in beef cows but not focusing on reducing costs. According to the National Animal Health Monitoring System (NAHMS, 2008), cost is still considered as one of the main reasons why producers do not use these reproductive technologies. Researches have recently dedicated more time on evaluating management tools that can reduce costs without compromising pregnancy rates (Walker et al. 2005; Lamb et al. 2006; Howard et al. 2009; Walker et al., 2014; Bishop, 2015).

The current study focused on evaluating a heat detection aid, the Estrotect Heat Detector patch as a reproductive management tool to determine if GnRH is needed at TAI according to estrous response. Considering that a CIDR implant cost on average of \$11.80 each, $PGF_{2\alpha}$ cost \$3.00/dose, a patch cost \$1.16 each, and GnRH cost \$3.00/dose, total cost for the estrous synchronization protocol used in the CTRL group was \$20.80/animal and the TRT group was \$19.50. The cost of protocol for the CTRL group was calculated as the total cost of injections and CIDR implant only, considering that each animal received one CIDR, two doses of GnRH and one dose of $PGF_{2\alpha}$. To calculate the cost for the TRT group, it was considered that each animal received one CIDR, one dose of GnRH, one dose of $PGF_{2\alpha}$ and one Estrotect patch. In this case, the cost of the extra GnRH dose in the TRT animals that didn't display estrus was divided by the total number of animals within that group to estimate a total protocol cost if using the Estrotect patches to determine the use of GnRH at TAI. Using the heat detector patch to determine the necessity of GnRH at TAI is an effective method to significantly reduce the number of animals that receive a second injection of GnRH at TAI, thus reducing costs without compromising pregnancy rates.

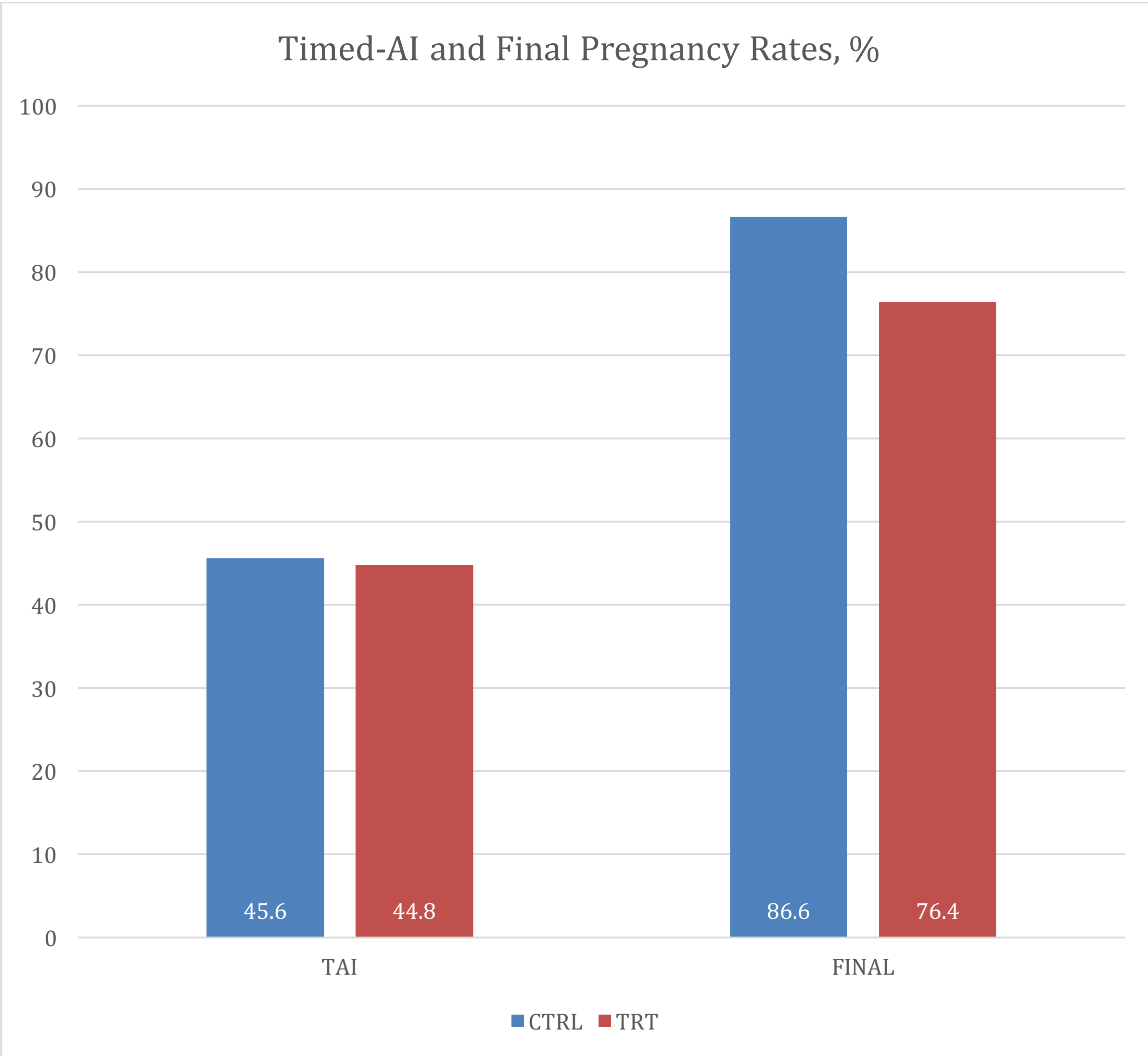

Figure 3. Timed AI and final pregnancy rates. Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT). Pregnancy rates for TAI are measured by the percentage of animals pregnant at d43 after TAI (P = 0.81) and final pregnancy rates are measured by the percentage of animals pregnant at d120 after TAI (P = 0.05).

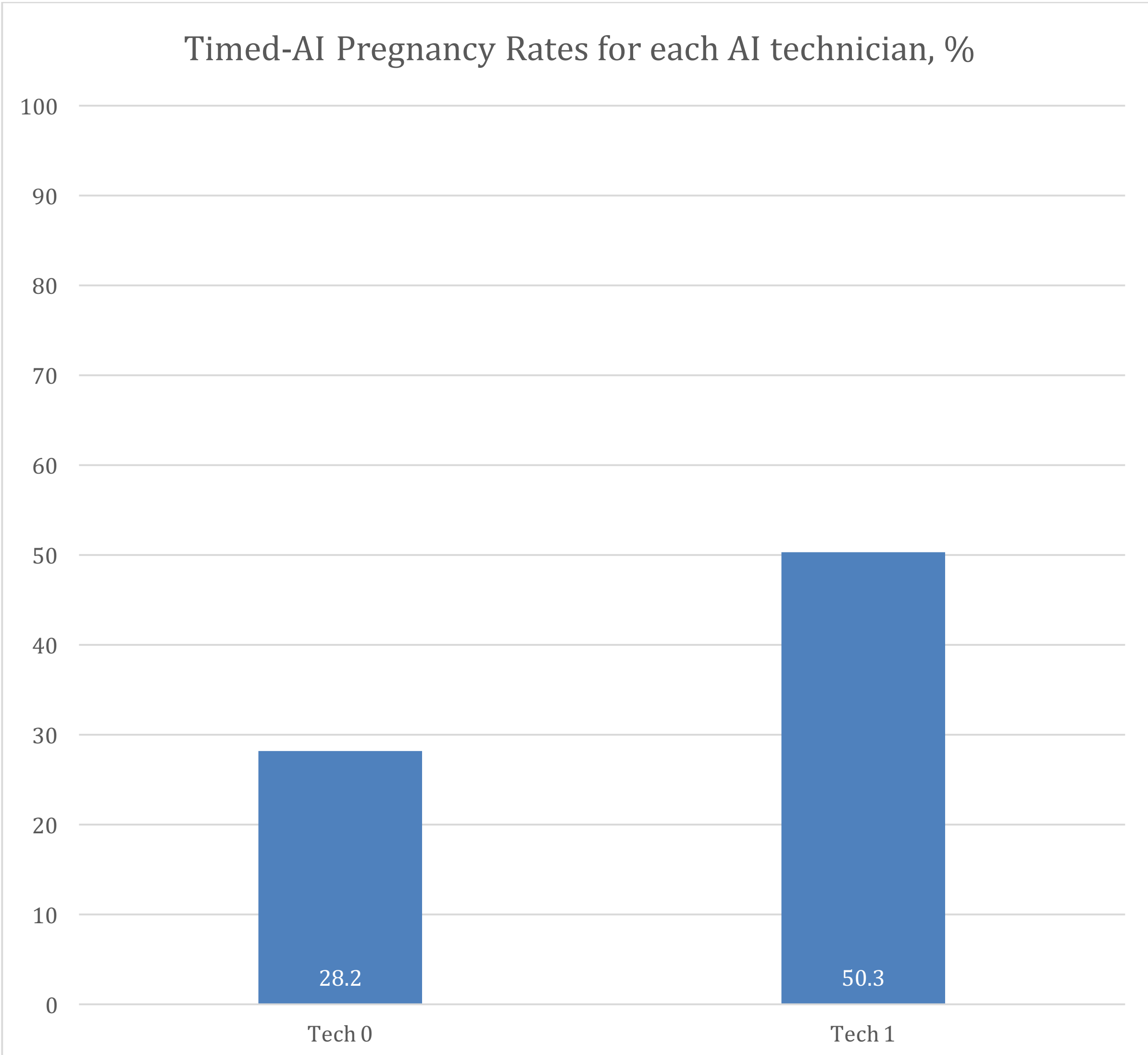

Figure 4. Timed-AI pregnancy rates for each AI technician. Pregnancy rates for TAI are measured by the percentage of animals pregnant at d43 after TAI (P = 0.07).

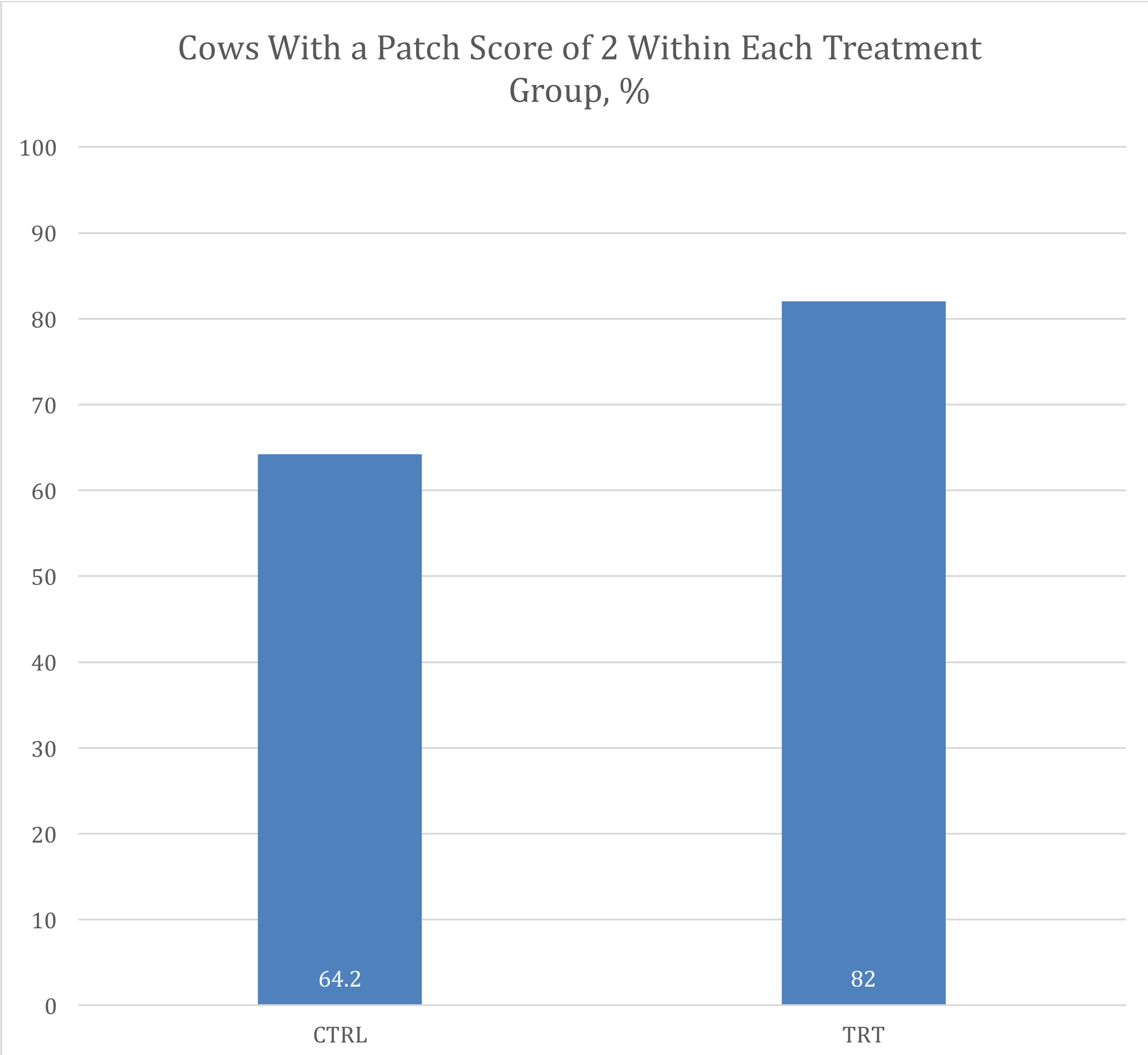

Figure 5. Percentage of cows with a patch score (PS) of 2 within each treatment group. ($P < 0.01$). Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT).

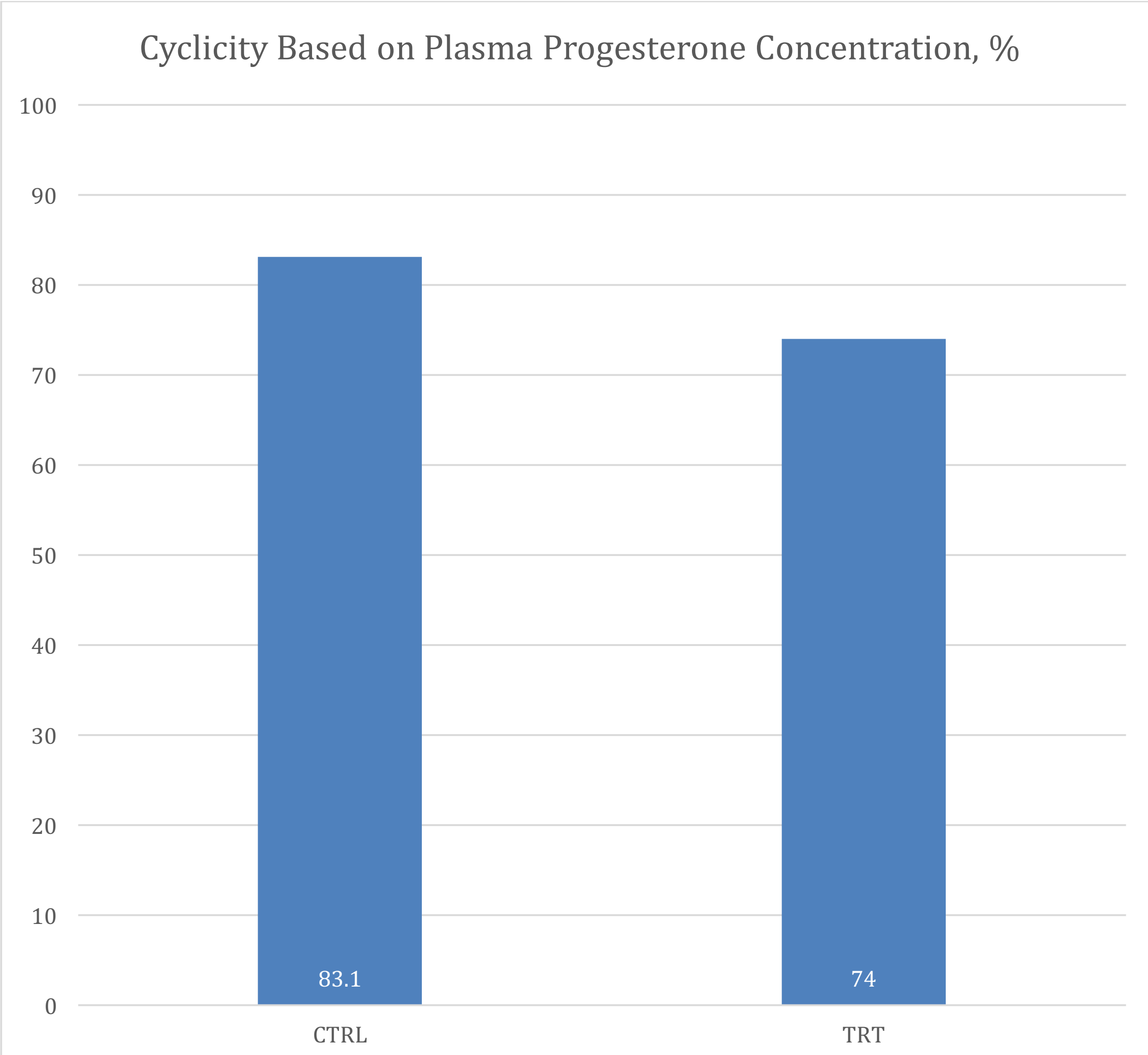

Figure 6. The percent of cows cycling based on plasma progesterone concentration within each treatment group. Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT). Cows were determined to be cycling if plasma progesterone concentrations were ≥ 1 ng/ml (P = 0.14).

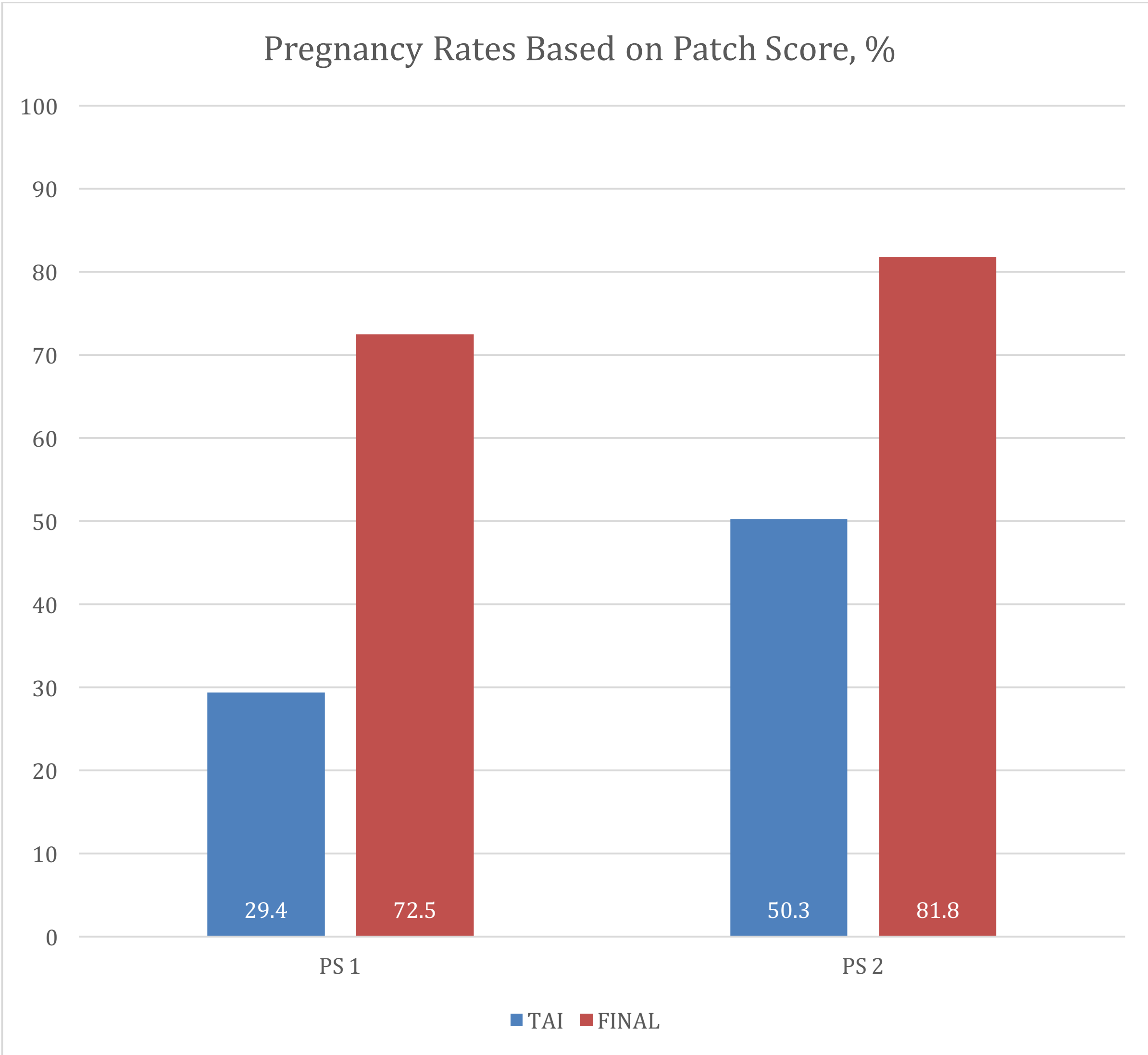

Figure 7. Timed-AI (P = 0.07) and final (P= 0.30) pregnancy rates based on patch score. Patch score were assigned 1 (< 50% patch film removed) or 2 (≥ 50% patch film removed).

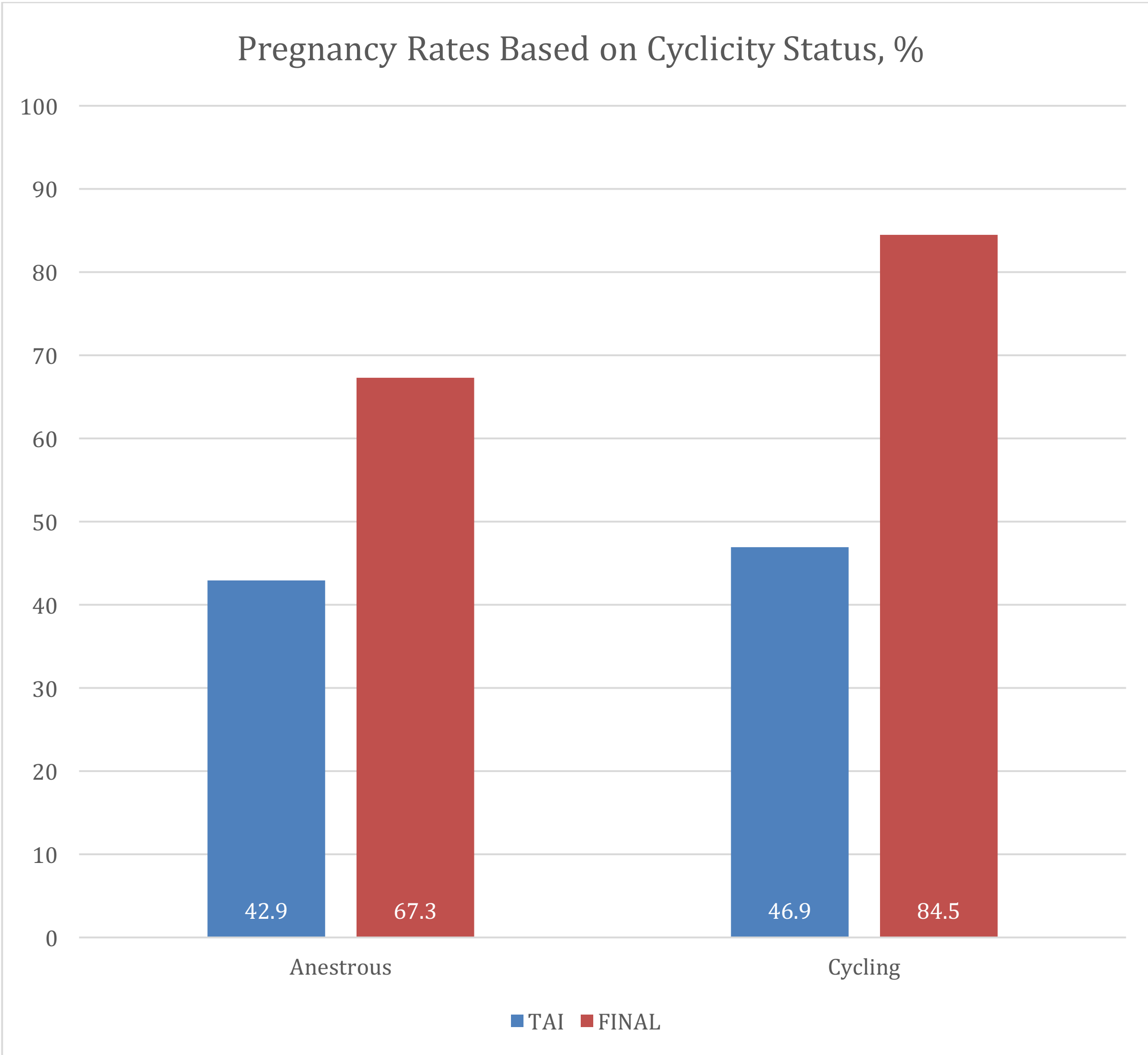

Figure 8. Timed-AI (P = 0.68) and final (P = 0.06) pregnancy rates in cows based on cyclicity status. Cows were determined to be cycling if plasma progesterone concentrations were ≥ 1 ng/ml.

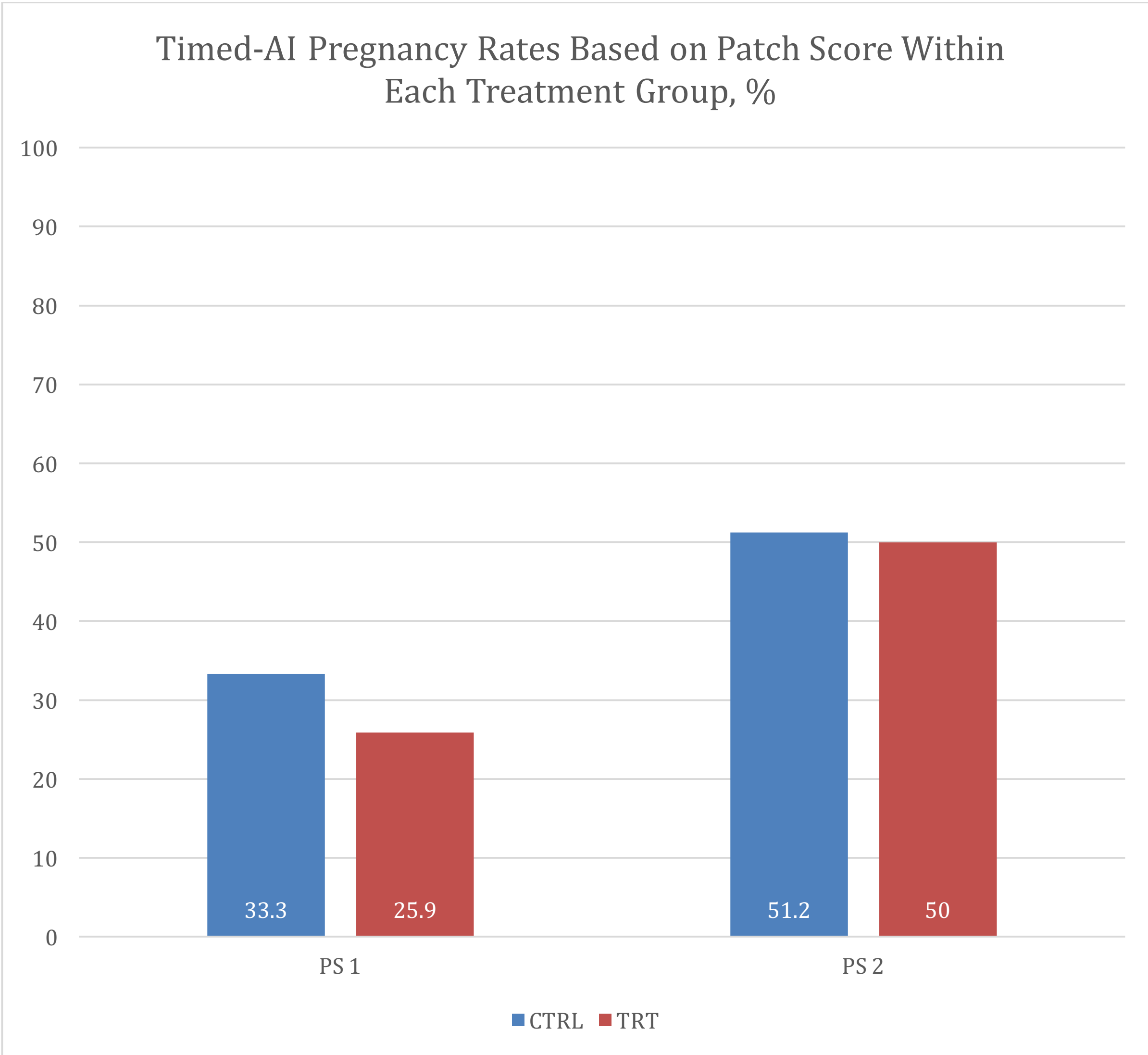

Figure 9. Pregnancy rates to TAI for cows based on patch score within each treatment group. Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT). Patch scores were assigned 1 (< 50% patch film removed) or 2 (≥ 50% patch film removed).

Experiment 2 Results

The current study evaluated the use of an estrous detection aid to determine if administration of GnRH at TAI for beef cows is required in a 7-Day CO-Synch + CIDR protocol with split-time AI. There was no treatment by PPI Group (*P* =0.82), PS (*P* = 0.85) or cyclicity interaction (*P* = 0.93); thus, interactions were removed from all models. Final pregnancy rates were similar (*P* = 0.82) among the CTRL and TRT groups for cows (48.4 and 51.4%; Figure 10). The author believes that calf separation between TAI at 72 h and TAI at 84 h for the TRT group did not impact pregnancy rates, as it is consistent with literature (Waltens et al., 1982; Dunn et al., 1985; Whisnant et al., 1985; Fanning et al., 1995; Peel et al., 2010; Martins et al., 2012) indicating that pregnancy rates are not impacted if calf separation is less than 48 h.

Pregnancy rates to TAI (Figure 10) were similar (*P* = 0.80) for cows in the TRT group (38.6%), where 37.1% of the cows did not receive GnRH at TAI due to an activated estrous detection patch (Figure 11), compared with cows in the CTRL group (32.3%), where all cows received GnRH at TAI. Estrous response based on a PS 2, was similar (P = 0.85) between the CTRL (28.8%) and TRT (37.1%) groups (Figure 11). The percent of cows cycling did not differ (*P* = 0.79) among CTRL and TRT groups (12.9 and 22.1%; Figure 12) and likely accounted for the low pregnancy rates seen in this experiment.

Timed-AI pregnancy rates were similar (*P* = 0.87) on cows cycling (57.9%) when compared to cows that were in anestrous (35.6%). Final pregnancy rates were similar (*P* = 0.35) on cows cycling when compared to cows that were in anestrous (81.8 and 92.3%; Figure 13). Timed-AI pregnancy rates were greater (*P* = 0.04) for cows with a

PS of 2 (70.6%) compared with PS 1 (19.4%), regardless of treatment. There were no differences (*P* = 0.11) in final pregnancy rates based on a PS 2 when compared to a PS 1 (83.3 and 100% respectively) between both treatment groups. There were no differences (*P* = 0.85) on TAI pregnancy rates based on patch score between CTRL and TRT groups with a PS 1 (17.4 and 20.5%; Figure 15) and no differences (*P* = 0.58) between CTRL and TRT cows with a PS 2 (75 and 78.3%; Figure 15).

Bishop et al. (2016) reported an increase on estrous response in beef cows when delaying TAI from 66 to 90 h post $PGF_{2\alpha}$ administration in a 7-day CO-Synch + CIDR protocol. In that study, Estrotect patches were applied at PG administration (day 7) with delayed TAI from 66 to 90 h post PG administration according to estrous expression. Estrus was defined as having at least 50% of the coating rubbed off of the Estrotect patch. GnRH was administered according to estrous response (animals in estrus did not receive GnRH at TAI). However, there was no difference in pregnancy rates between the two groups when inseminated at 66h regardless of GnRH administration (57 and 58%). There was also no difference between TAI at 66h with GnRH versus TAI at 90h with GnRH (44 and 49%). Pereira et al. (2016) reported that animals that displayed estrus had a higher probability of getting pregnant from TAI compared with those that did not display estrus (38.9 and 25.5%, respectively). It was also reported that that the percentage of pregnancy loss in animals that had conceived from TAI and displayed estrus was lower than those animals that displayed estrus from those that did not display estrus (14.4 and 20.1%, respectively).

In the current study, there was no difference (*P* = 0.80) in TAI pregnancy rates between CTRL and TRT groups, suggesting that GnRH may not have any beneficial effects on cows exhibiting a standing estrus at TAI. Prior to CIDR insertion on d 0, the cyclicity status of the herd was similar among the CTRL and TRT groups (12.9 and 22.1%) which disagrees with Lamb et al. (2006) who reported approximately 50% of the animals were cycling at the beginning of every synchronization protocol. This can be explained by the poor BCS (3.8) of the animals at this location when compared to the animals in experiment 1, which averaged a BCS of 5.3 and had higher pregnancy rates at TAI for the CTRL (44.8%) when compared to the CTRL animals in experiment 2 (32.3%) and for the TRT (45.6%) when compared to the TRT animals in experiment 2 (38.6%). This agrees with literature, suggesting that cows with a lower PPI and a lower BCS will have lower cyclicity rates (Larson et al., 2006).

Pregnancy rates to TAI were not improved ($P = 0.35$) in cows that had a PS 2 not receiving GnRH at TAI in the CTRL or TRT group (75.0 and 78.3%). This result is complacent to the literature available (Walker et al. 2014; Bishop 2015) pointing out that GnRH is not needed at TAI if the animal is in standing estrus. Heat detector patches loss was very low in this study (2.97%; 3 animals out of 101 lost their patch), indicating that the adhesive held the patches on very successfully. The data generated by this study suggest that cyclicity status has a major influence on pregnancy rates compared with GnRH administration at TAI. The CO-Synch protocol (Geary and Whittier, 1998) is a result of a modification of the Ovsynch protocol (Pursley et al., 1995) to reduce the number of times animals are handled during the synchronization protocol. The addition of a CIDR insert in the CO-Synch protocol has been shown to increase the percentage of females displaying estrus within an 84-hour period (Larson et al., 2006).

Several studies in the past couple decades have evaluated different estrous synchronization protocols aiming to increase pregnancy rates to TAI or FTAI in beef cows but not focusing on reducing costs. According to the National Animal Health Monitoring System (NAHMS, 2008), cost is still considered as one of the main reasons why producers do not use these reproductive technologies. Researches have recently dedicated more time on evaluating management tools that can reduce costs without compromising pregnancy rates (Walker et al. 2005; Lamb et al. 2006; Howard et al. 2009; Walker et al. 2014 and Bishop, 2015).

The current study focused on evaluating a heat detection aid, the Estrotect Heat Detector patch as a reproductive management tool to determine if GnRH is needed at TAI according to estrous response. Considering that a CIDR implant cost on average $11.80 each, $PGF_{2\alpha}$ cost $3.00/dose, a patch cost $1.16 each, and GnRH cost $3.00/dose, total cost for the estrous synchronization protocol used in the CTRL group was $20.80/animal and the TRT group was $21.23. The cost of protocol for the CTRL group was calculated as the total cost of injections and CIDR implant only, considering that each animal received one CIDR, two doses of GnRH and one dose of $PGF_{2\alpha}$. To calculate the cost for the TRT group, it was considered that each animal received one CIDR, one dose of GnRH, one dose of $PGF_{2\alpha}$ and one Estrotect patch. In this case, the cost of the extra GnRH dose in the TRT animals that didn’t display estrus was divided by the total number of animals within that group to estimate a total protocol cost if using the Estrotect patches to determine the use of GnRH at TAI. Using the heat detector patch to determine the necessity of GnRH at TAI is an effective method to reduce significantly the number of animals that receive a second injection of GnRH at TAI;

however, in this experiment it was not possible to reduce the protocol cost as only 37.1% of the animals in the TRT group did not require a second administration of GnRH and this percentage was not sufficient to pay off the extra cost of the Estrotect Heat Detector patch.

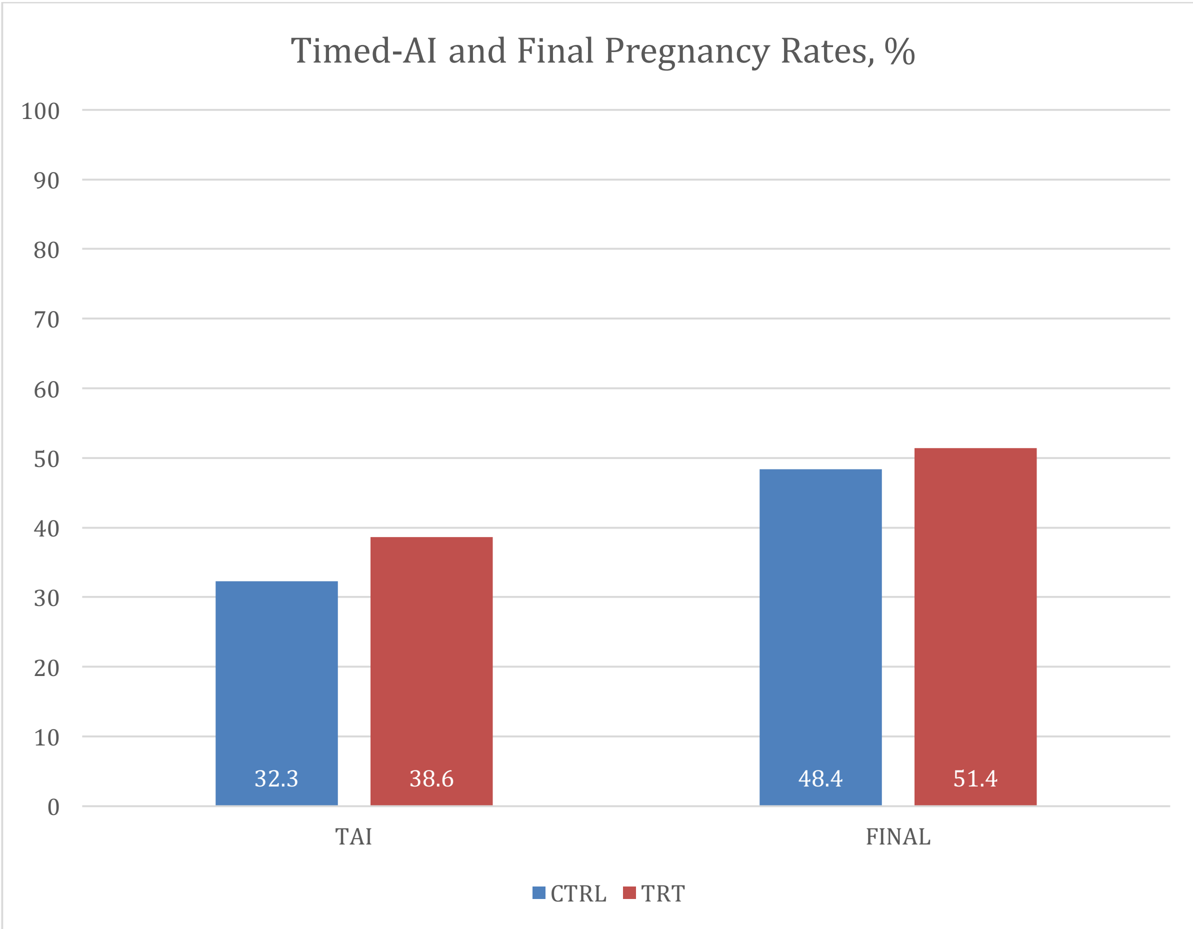

Figure 10. Timed AI and final pregnancy rates. Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT). Pregnancy rates for TAI are measured by the percentage of animals pregnant at d43 after TAI ($P = 0.80$) and final pregnancy rates are measured by the percentage of animals pregnant at d120 after TAI ($P = 0.82$).

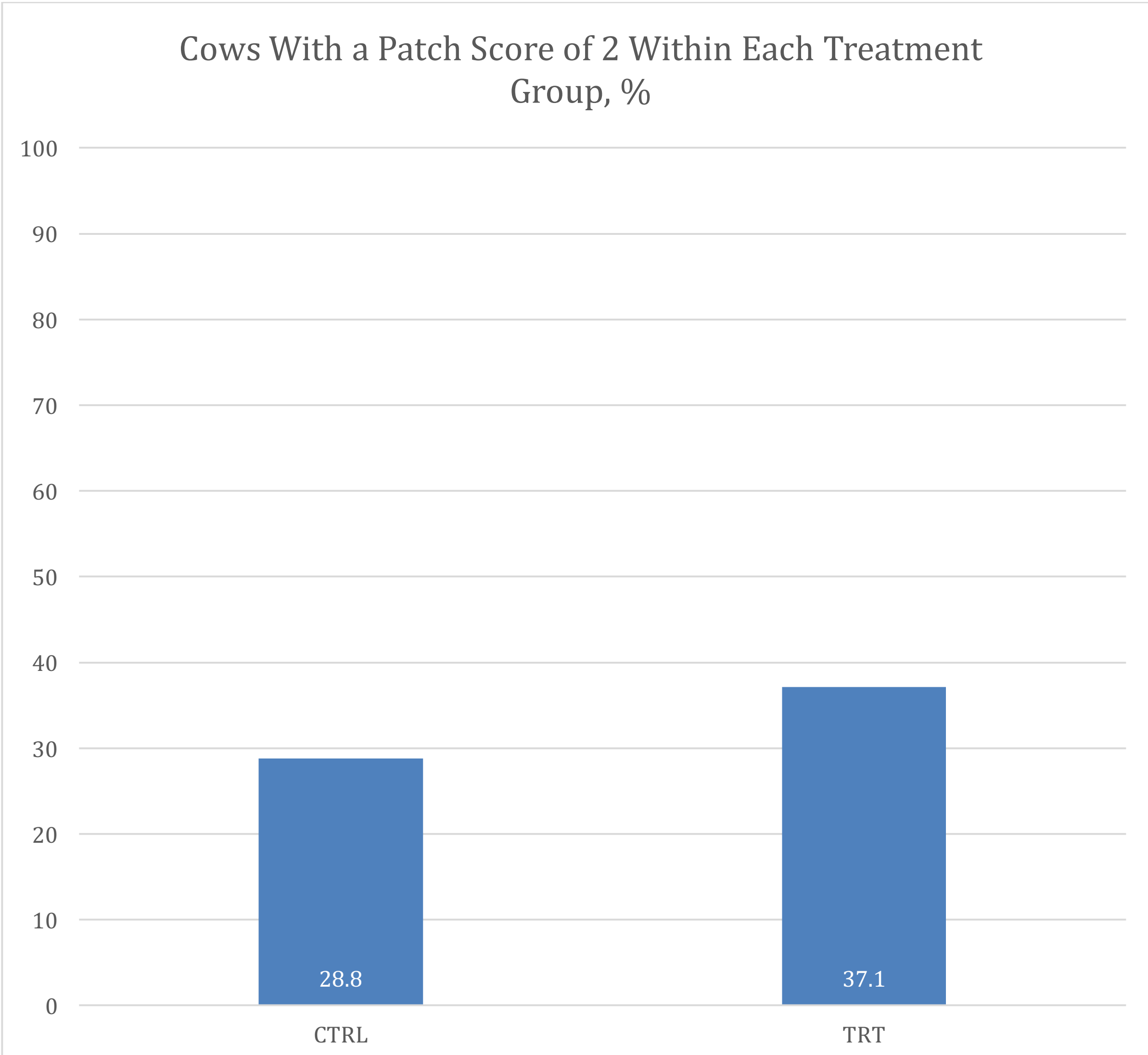

Figure 11. Percentage of cows with a patch score (PS) of 2 within each treatment group. (P = 0.85). Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT).

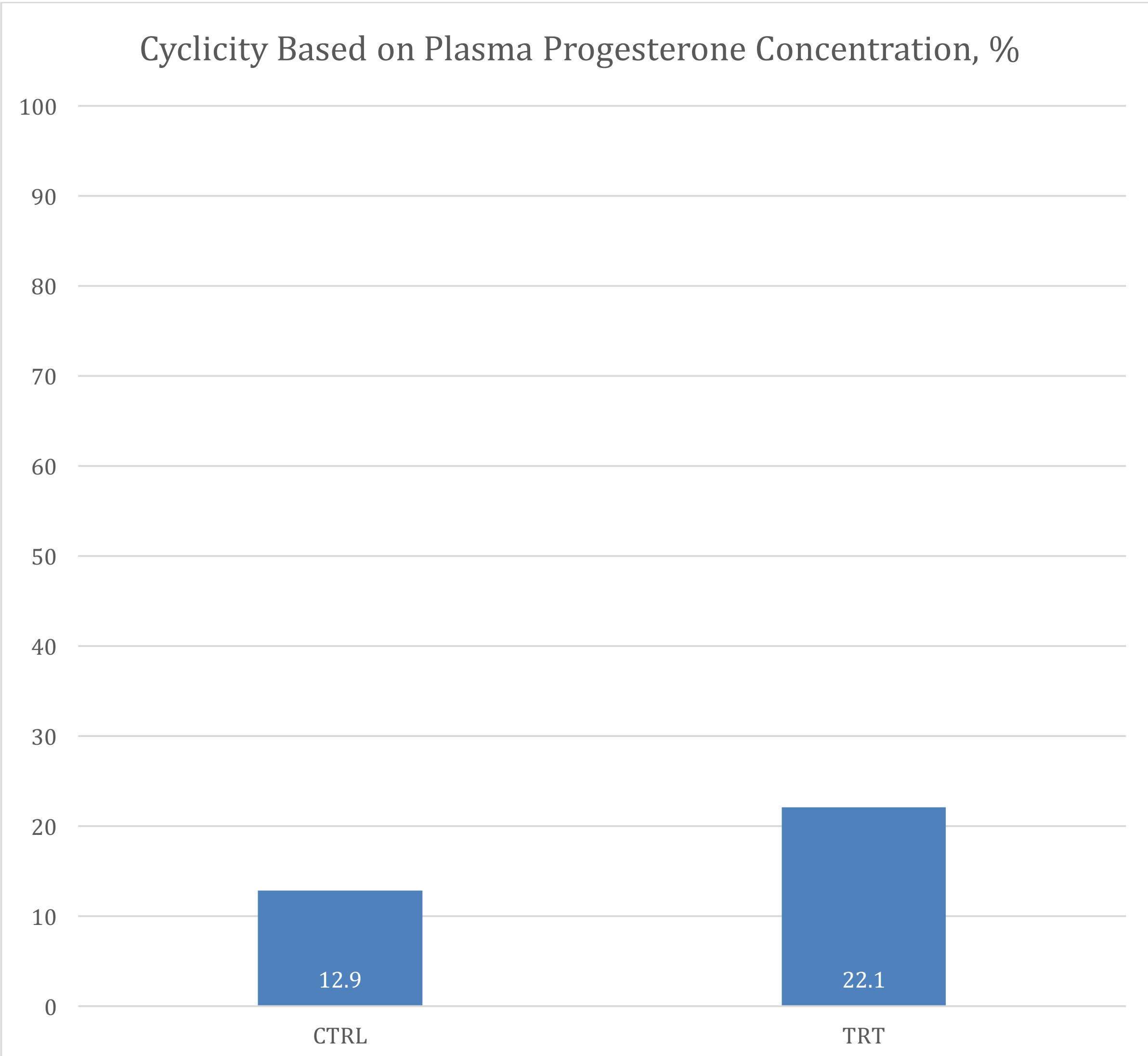

Figure 12. The percent of cows cycling based on plasma progesterone concentration within each treatment group. Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT). Cows were determined to be cycling if plasma progesterone concentrations were ≥ 1 ng/ml (P = 0.79).

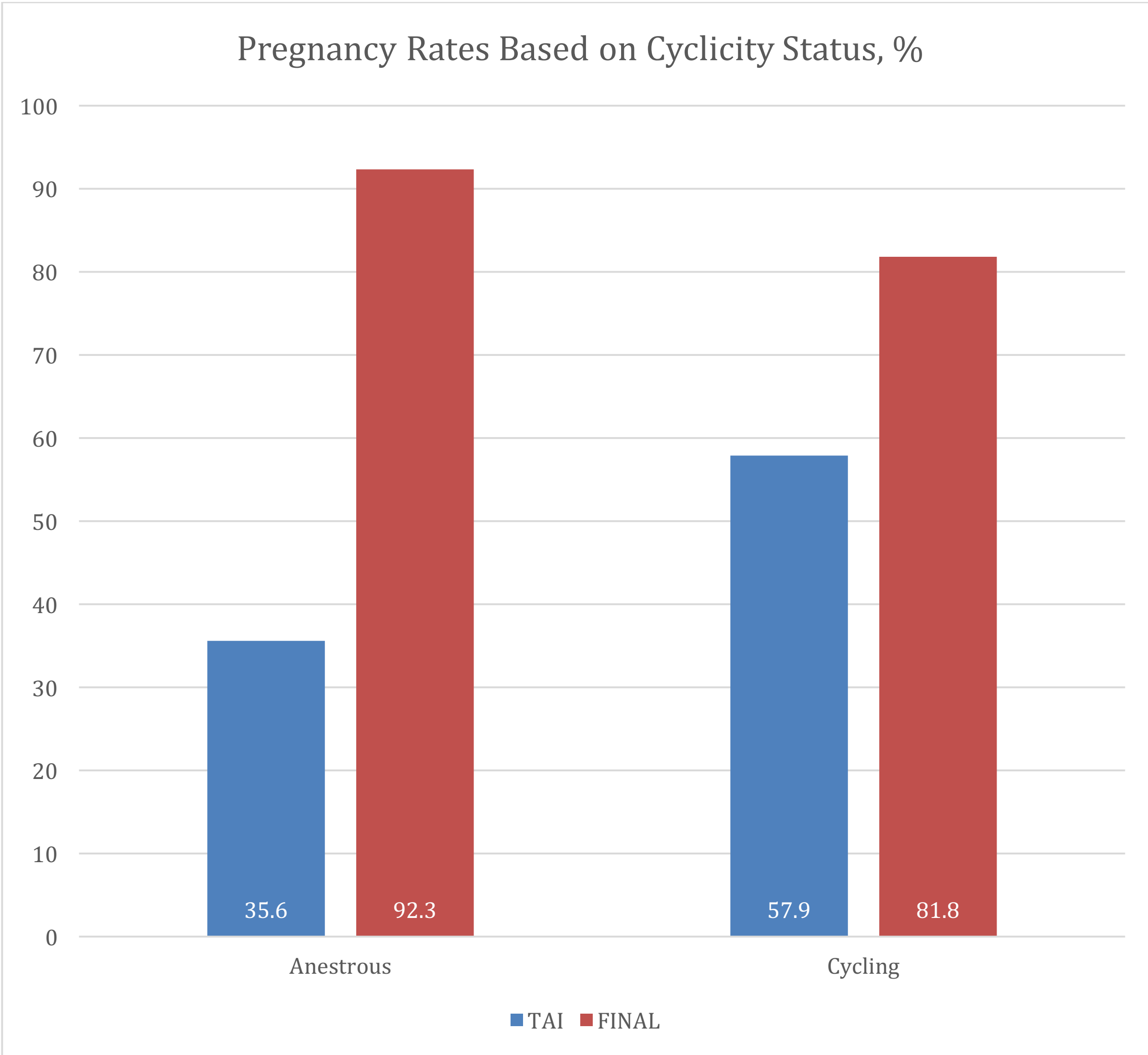

Figure 13. Timed-AI (P = 0.87) and final (P = 0.35) pregnancy rates in cows based on cyclicity status. Cows were determined to be cycling if plasma progesterone concentrations were ≥ 1 ng/ml.

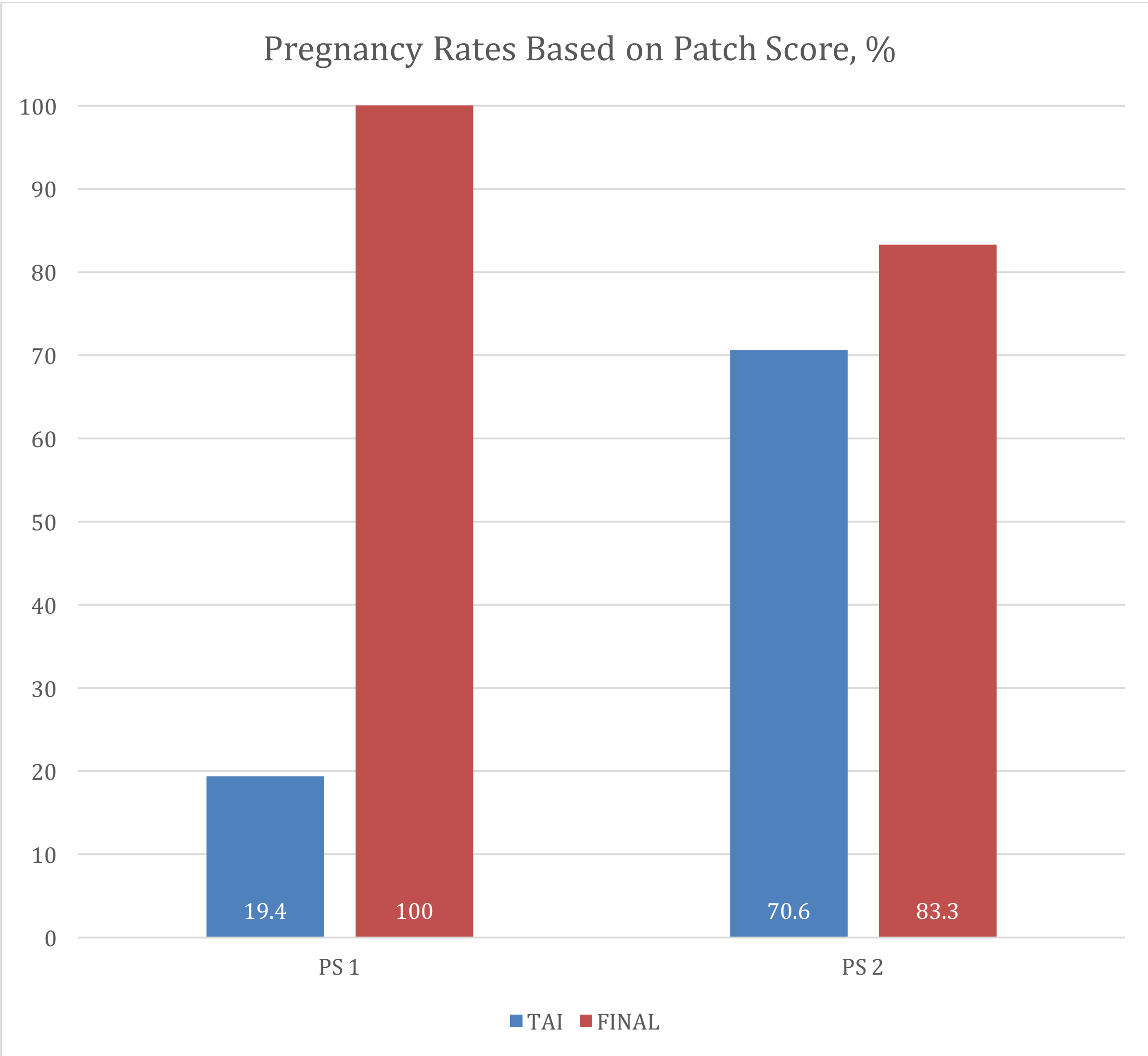

Figure 14. Timed-AI (P = 0.04) and final (P= 0.11) pregnancy rates based on patch score. Patch score were assigned 1 (< 50% patch film removed) or 2 (≥ 50% patch film removed).

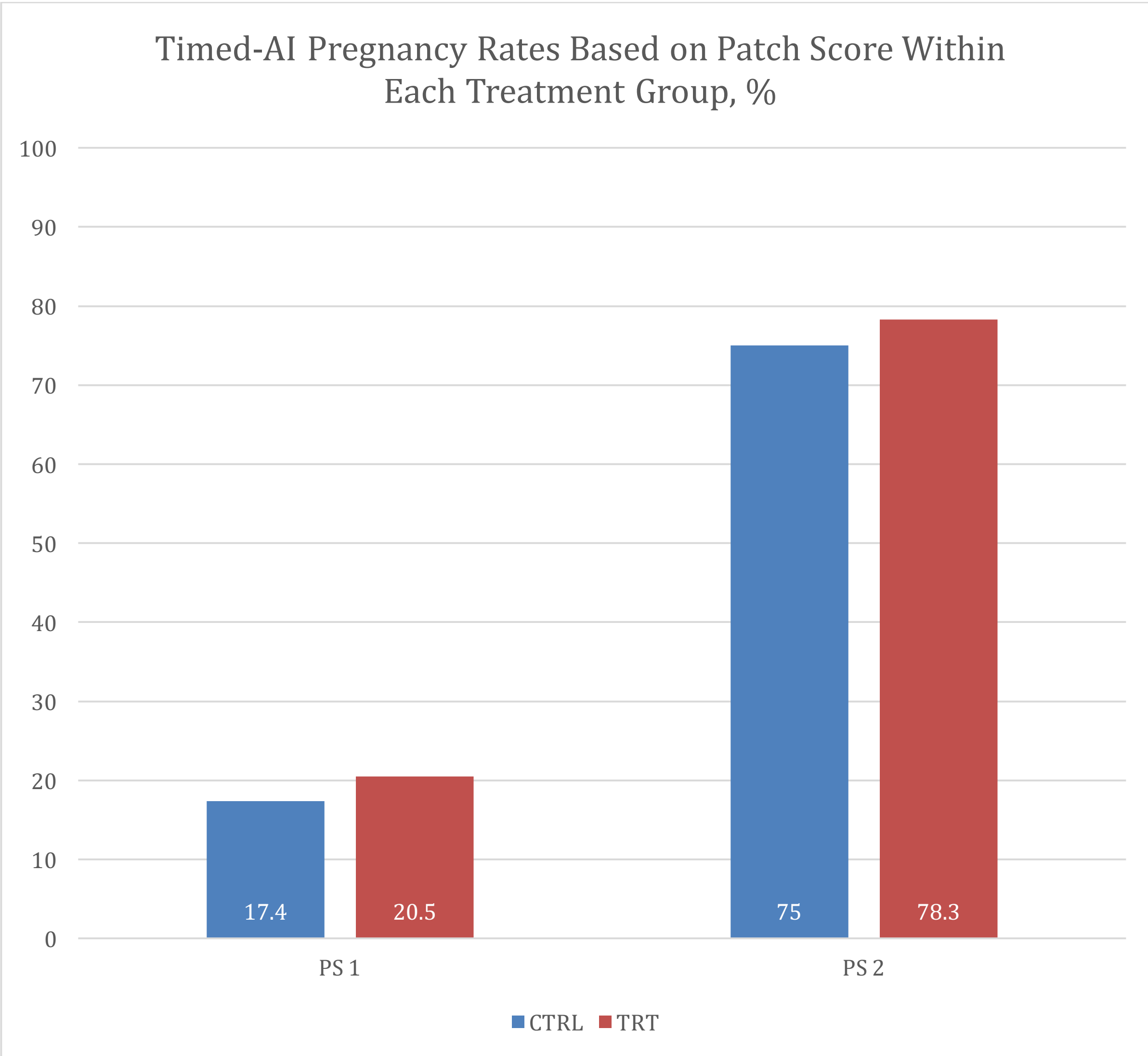

Figure 15. Pregnancy rates to TAI for cows based on patch score within each treatment group. PS 1 (P = 0.85) and PS 2 (P = 0.58). Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72-h TAI or a 7-day CO-Synch + CIDR estrous synchronization protocol with 72- or 84-h split-timed AI (TRT). Patch scores were assigned 1 (< 50% patch film removed) or 2 (≥ 50% patch film removed).

SUMMARY AND CONCLUSIONS

Estrous synchronization is a reproductive technique that synchronizes estrus and facilitates the handling of the animals during breeding season, in addition to shortening the breeding and calving season. In Experiment 1, BCS of the animals were greater compared to animals used in Experiment 2 (5.3 vs 3.8) which resulted in greater cyclicity rates, greater estrous response, and consequently higher pregnancy rates. This study evaluated the necessity of a second GnRH administration at TAI according to estrus response, which was measured by the the Estrotect Heat Detector patches response. In Experiment 1, 82% of the cows in the TRT group did not receive GnRH at TAI indicating that with the use of the Estrotect patch and delaying TAI to 84 h in non-responsive cows, only 18% of these cows required GnRH at TAI. However, in Experiment 2 only 37.1% of the cows in the TRT group did not receive GnRH at TAI, indicating that BCS also had a significant impact on the percentage of cows responding to the estrous synchronization protocol. Using the heat detector patch to determine the necessity of GnRH at TAI is an effective method to significantly reduce the number of animals in a BCS of 5 or greater that receive a second injection of GnRH at TAI.

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VITA

Danilo Demeterco was born in Curitiba, Parana - Brazil, to Sueli and Carlos. After graduating from Colegio Bom Jesus in December 2006, he began his undergraduate studies in 2007 in Law School at UniCuritiba and Business Administration at UNIFAE before finding out his true passion on Veterinary Medicine at Pontificia Universidade Catolica do Parana – PUCPR in 2008. In February 14th, 2013, he received the degree of Medico Veterinario (D.V.M. equivalent). In 2014, he travelled to the United States of America to begin an internship with Dr. Cliff Lamb at University of Florida which resulted on another internship with his major professor and mentor, Dr. Ryon Walker at Louisiana State University. In 2015, he started his graduate studies at the School of Animal Sciences at Louisiana State University in beef cattle reproduction. After graduating, he will go back to his home country to start working with estrus synchronization and continue his career as a veterinarian.