A Comparison of the Standard 5-day CO-Synch with CIDR Protocol and a 14-day CO-Synch with CIDR Protocol in Primiparous and Multiparous Beef Cattle

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ABSTRACT:

Utilization of estrus synchronization protocols aims to induce a new follicular wave in order to improve response and enhance pregnancy rate in heifers and cows. The objective of this study was to determine the effectiveness of GnRH administered d 0 and 9 during a controlled internal drug release (CIDR) protocol. Two CIDR protocols were utilized in this experiment: a 14-day CO-Synch + CIDR and a 5-day Co-Synch + CIDR. Although previous work has shown variability in differences between these two protocols in terms of TAI pregnancy success, this experiment aims to test the hypothesis that a 14-day CO-Synch treatment may have an improved pregnancy success rate in both heifers and cows due to the initiation of a second follicular wave, as opposed to the single follicular wave induced in the 5-day CO-Synch protocol. Beef cows (n=97) and beef heifers (n=42) at two separate locations respectively, were randomly assigned to 1 of 2 treatment protocols. Treatment 1 administered a 14-d progesterone (PG) treatment, in which they received a CIDR with 100 μg GnRH analog intramuscularly (i.m.) on d 0, 100 μg GnRH analog i.m. on d 9, and CIDR removal with 50 mg $PGF_2\alpha$ i.m. on d 14. Treatment 2 administered a standard 5-d CO-Synch + CIDR, in which they received a CIDR with 100 μg GnRH analog i.m. on d 9, followed by CIDR removal with 25 mg $PGF_2\alpha$ i.m. on d 14. Cows and heifers in all treatments received 100 μg GnRH analog i.m. and timed AI (TAI) 72 h after CIDR removal. Pregnancy status to TAI was determined by ultrasound examination approximately 60 d after TAI. Averaged over all locations, pregnancy rates to TAI for treatment 1 and treatment 2 were 44.1% and 38.0% respectively.

INTRODUCTION:

Recent estrus synchronization protocols for timed AI (TAI) such as the 5-d CO-Synch + controlled internal drug release (CIDR) have reported success in improving pregnancy rates up to 70% in beef cows when compared to other protocols (Bridges et al., 2008; Gunn et al., 2009). The purpose of this protocol is to induce one new follicle by use of a single GnRH injection at the initial starting period, followed by the specific treatment of CIDR placement at d 0 and removal at d 5. Contrarily, the 14-d CO-Synch + CIDR protocol induces a second follicular wave by the addition of a second GnRH injection at d 9, and subsequent CIDR removal at d 14. These protocols do not address the stage of the estrous cycle when cows are nonresponsive to GnRH.

In the event of a follicular wave, the ovulation of a cow leads to an increase of follicle stimulating hormone (FSH), and the formation of a corpus hemorrhagicum. Follicles begin to grow on this corpus hemorrhagicum, which soon becomes a corpus luteum (CL), or a structure created with the discharge of the ovum that secretes the hormone progesterone. The CL secretes progesterone starting at day 5 of the Estrous cycle, and continues until day 18 unless pregnancy is achieved (West Virginia University, 1914). With the initial secretion of progesterone, one follicle in the follicular wave becomes dominant and leads to the regression of the remaining follicles. The dominant follicle secretes the hormones estrogen and inhibin, but ovulation cannot occur while the CL is present and simultaneously secreting progesterone. When the follicle has sufficiently developed, it produces enough estradiol, an estrogen hormone, to initiate a surge of luteinizing hormone (LH) that causes ovulation. In order for a dominant follicle to proceed to ovulation, it must be at least 10 mm in size, and its growth much coincide with the regression of the CL caused by the release of prostaglandin from the uterus. Additionally, with the degradation of the CL, progesterone levels will decline and estradiol levels will rise until the LH surge occurs (Parish et. al, 2010).

An increase in the number of follicular waves during an Estrous cycle in cattle should increase the probability that the ovulation of the dominant follicle will coincide with the abovementioned events. Chances are that a single follicular wave, as seen with the 5-d CO-Synch, will cause ovulation to occur before the complete degradation of the CL and therefore before the decline of progesterone, whereas a second follicular wave induced by the additional GnRH injection of the 14-d CO-Synch would surpass the day 18 mark of progesterone secretion. This allows the dominant follicle to be more likely to be ovulated, and should increase the chances of attaining a successful pregnancy (Sirois & Fortune, 2013). It is important to note that follicles ovulated during the second wave cycle may be more fertile than those from the previous wave, because the follicles are younger. This leads us to the hypothesis that the 14-d CO-Synch will be more effective than the standard 5-d CO-Synch, as well as the hypothesis that heifers should perform better in pregnancy success rates than cows, as there is a lesser depletion of the primordial follicle pool (Abreu, 2011).

Our primary objective was to assess the efficiency of utilizing an additional GnRH injection during a 14-d CIDR program compared to a single GnRH dose during a 5-d CIDR program. The 14-d CIDR treatment is designed to induce a second follicular wave during the influence of progesterone, which we hypothesize to increase the pregnancy success rate overall by increasing the amount of time the follicle is present during progesterone availability.

In accordance with observing the influence that the two CIDR treatments will have upon the pregnancy rates in heifers and cows after TAI performed by students, data will be collected for the overall pregnancy rates between both TAI and clean up bull utilization. Artificial insemination was performed by a group of students, so data will reflect the amount of assistance provided to them as well as the percentage of successful TAI performed by the selected students.

MATERIALS AND METHODS:

All experimental procedures involving cows and heifers were approved by California Polytechnic State University beef department before initiation of the experiment.

Animals and Treatments

Multiparous and primiparous Angus, Angus cross, and Limousine beef cows (n=139) at 2 ranches in Central California (location 1, n=97; location 2, n=42) were used in this experiment. Cows were assigned to one of two treatments on the basis of a preplanned randomization process. Treatment 1 included a total of 19 heifers and 49 cows (n=68); Treatment 2 included a total of 23 heifers and 48 cows (n=71). During the initial randomization process, animals that were deemed unfit or underage were removed from the experiment. All cattle included in the study and given a GnRH shot had their tail heads painted. Following the day of initial GnRH analog injection and CIDR placement, three separate groups of students observed the primiparous group (n = 42) over a period of two days for estrus detection, and recorded data based upon observed standing heat as well as tail head paint presence. Tail head paint was scored at TAI on d-17 at all locations using a 1 to 3 scale based upon the amount of paint that was removed (1= completely untouched, 2 = some slight paint removed, 3 = almost all or 100% removed).

Pregnancy Diagnosis

Pregnancy status to TAI was diagnosed between 60 and 65 d after TAI using transrectal ultrasonography. Cows and heifers were exposed to intact bulls beginning 5 days after TAI for the remainder of the breeding season.

Statistical Analyses

Data was analyzed via the logistic program JMP. The model included location, treatment, parity (primiparous or multiparous), AI technician, assistance needed in TAI process, pregnancy success rate due to TAI, and overall method of pregnancy (TAI or clean up bull).

RESULTS:

The first objective was to evaluate the efficiency of the second GnRH analog injection used in the 14-d CIDR (treatment 1) compared to the standard 5-d CO-Synch + CIDR protocol (treatment 2). Overall, the pregnancy rate to TAI in treatment 1 involving heifers was 31.6%, while treatment 2 had a pregnancy rate of 34.8% (Fig. 1). Between these two treatments, there was not a significant difference in success of TAI (P>0.80).

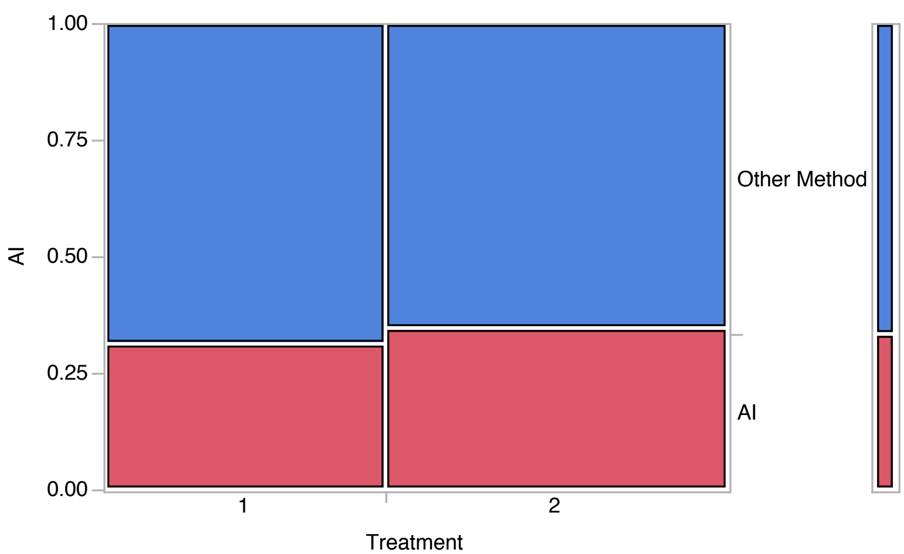


Fig. 1. Percentages of pregnancy success rate through TAI in primiparous heifers compared to other methods. 'Other method' category includes pregnancy by clean up bull exposure, and all open heifers at the end of breeding.

Utilization of a clean up bull after TAI revealed treatments 1 and 2 to both have pregnancy success rates of 16.7% of the remaining heifers in the herd (Fig. 2).

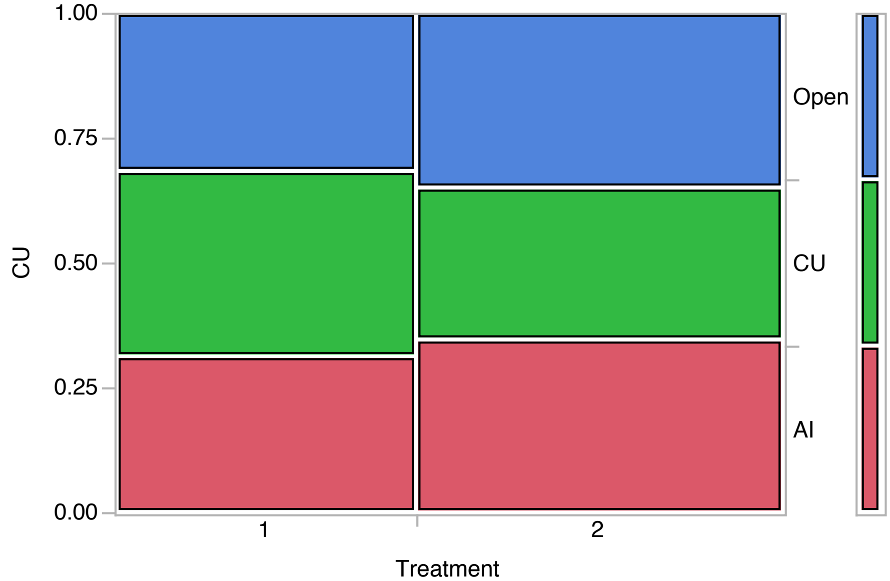


Fig. 2. The percentages of all open heifers at the end of breeding, compared against the percentages of heifers that were pregnant by TAI, and those that were pregnant after exposure to the clean up bull.

Overall, the pregnancy success rates of the heifers in treatments 1 and 2 showed no difference in efficiency of utilizing a 14-d CIDR versus the standard 5-d CO-Synch + CIDR. While 66.7% of the heifers attained a pregnancy status by either TAI or clean up bull (CU), only 33.3% of them were impregnated through TAI.

The pregnancy rate to TAI in treatment 1 involving multiparous cows was 24.7%, while treatment 2 has a pregnancy rate of 19.6% (Fig. 3). Again, there was not a significant difference in success of TAI (P>0.35) between the two treatment types.

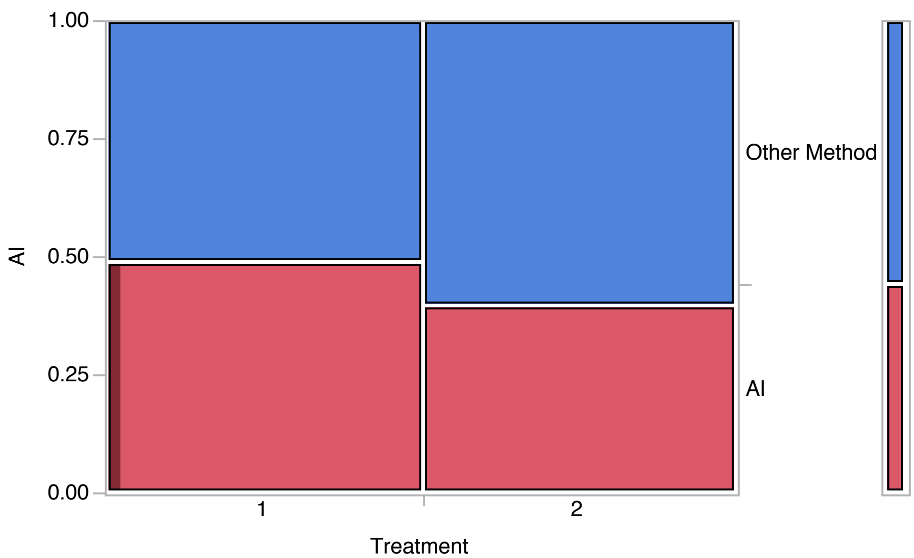


Fig. 3. Percentages of pregnancy success rate through TAI in multiparous cows compared to other methods. 'Other method' category includes pregnancy by clean up bull exposure, and all open cows at the end of breeding.

Utilization of a clean up bull after TAI revealed treatments 1 and 2 in cows to have pregnancy success rates of 15.5% and 14.5% respectively of the remaining herd (Fig. 4).

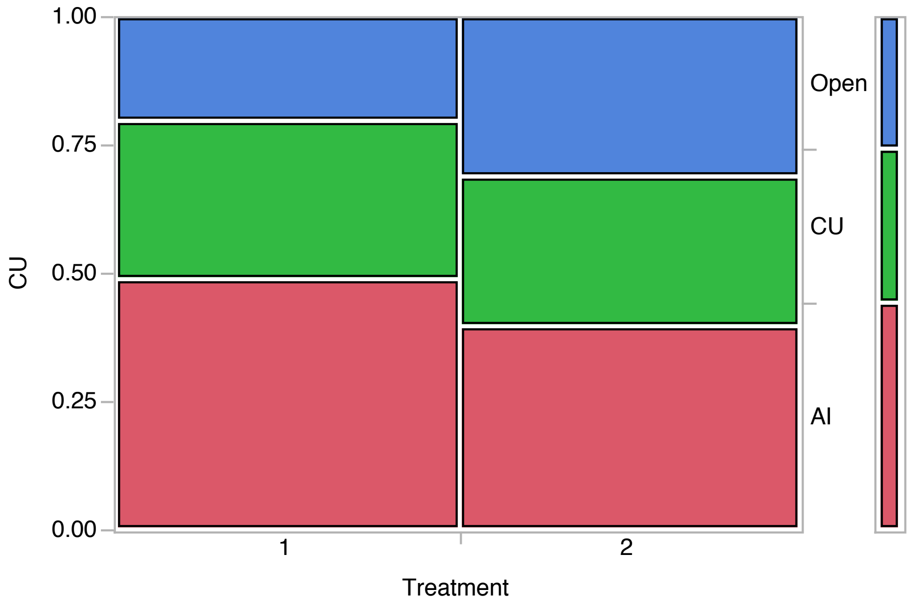
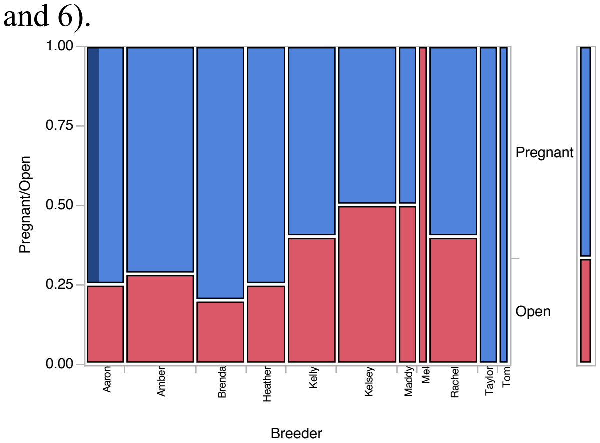


Fig. 4. The percentages of all open cows at the end of breeding, compared against the percentages of cows that were pregnant by TAI, and those that were pregnant after exposure to the clean up bull.

DISCUSSION:

The results from TAI in cows supports the idea that pregnancy rates do not significantly differ based upon utilization of a 14-d CIDR versus the standard 5-d CO-Synch + CIDR. While 74.23% of the entire cow herd used in this study resulted in pregnancy, only 44.3% of the herd was impregnated through TAI.

Certain factors need to be taken into consideration when reviewing the collected data, and the results of this experiment. First, the entire AI breeding program was performed by Cal Poly students learning the appropriate technique, leading to some variation in success based upon individual understanding of how to properly AI (Figs. 5 and 6).



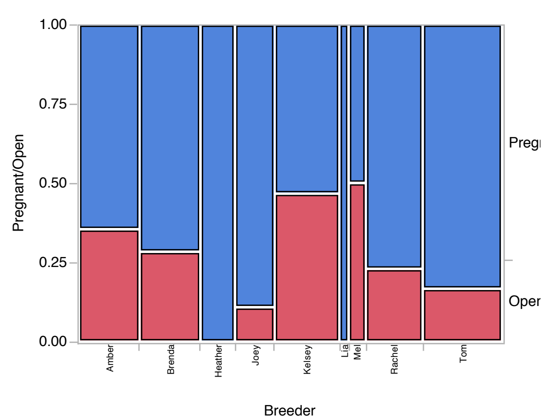


Fig. 5. (left) Percentages of heifers pregnant through TAI based upon who performed the insemination.

Fig. 6. (right) Percentages of cows pregnant through TAI based upon who performed the insemination.

Second, heifers and cows were on different diets, with heifers being fed a grain-based diet while cows were set out to graze and supplemented with hay when necessary. Finally, equipment failure may have been responsible for some failed AI attempts, in which semen was exposed to extreme high temperatures during the thawing processes. All of these factors may have variable effects upon the results obtained in this experiment, and should be adjusted for future reproduction of this experiment.

In conclusion, there was not a significant difference between the two treatments utilized with the heifer and cows groups based upon their pregnancy rates, time of estrus detection, and pregnancy rate based upon TAI. This is perhaps due to the limited heifer and cow numbers for adequate power to test the experiment. With further research based upon data received using the number of cattle we had, it became clear that in order to achieve optimal pregnancy rates with CIDR based estrous synchronization protocols, regardless if they are 14-d or 5-d, certain conditions with the cattle must be met first. Cows should be in good body condition (BCS $\geq$ 5), avoiding using cattle that are too thin or too fat (Iowa Beef Center, 2004). Management and diet can influence the time of postpartum anestrus in cattle, as well as the likelihood of a successful pregnancy and number of days open (Roche et. al, 2009). Nutrition is an extremely important aspect in a cow-calf operation, and all cattle expected to breed should be somewhere near a body condition score of 5 (AgriLife Today, 2013). Branching from the nutrition aspect of maintaining appropriate body condition scores in breeding cattle, other key aspects that must be met with the herd includes pasture management, weaning management and appropriate lactation periods, as well as supplementation methods and health management.

On average, pregnancy rates for timed-insemination heifers are generally between 50 to 68%, averaging at 57% (Watches et. al, 2014). Therefore, the results of TAI pregnancy rates in the heifers used in this study were uncharacteristically low. It is suggested that factors such as management systems, milk yield, parity, body condition score, and ambient temperature could significantly influence the rate of pregnancy loss in cattle (Zobel, et. al., 2011). One or more of these factors may lead to a better comprehension of why heifers in both treatments achieved such a low combined pregnancy rate compared to the average. However, one of the obvious reasons behind the highly reduced pregnancy success rate in the heifers is their body composition due to their daily diets. When visually assessing the heifers used in this study, they were above the standard body condition score (BCS) of a 5, carrying too much fat around their tail-head, udders, and other areas of their body. While a BCS of at least a 5 is ideal at the time of breeding, a heifer that is carrying too much weight may experience related hormonal imbalances that affect the Estrous cycle and prevent ovulation. Better known as polycystic ovarian syndrome, ovaries of cattle that are obese do not produce enough of the hormones to stimulate follicle development and ovulation (Barrett, 2014). Taking into consideration the importance of timing during CIDR protocols, as well as the complex mechanism and timing requirements during the Estrous cycle to even achieve pregnancy, issues with weight causing a severe decrease in hormone production can account for the significant decline in the pregnancy success rate seen in the heifers used in this study.

In the study, the strategy to induce follicular waves by utilizing a 14-d treatment with a CIDR showed no significant difference in TAI pregnancy rate when compared with the 5-d CO-Synch + CIDR protocol. The latter protocol is considered to have the best results for fixed TAI, according to the Beef Reproduction Task Force associated with ABS (2013).

Heat detection was observed only in the heifer herd, in which students facilitated the observation and data collection. Of the forty-two heifers participating in the study, only 22 were noted to be in standing heat during a student's observation period. On average, most heifers that were observed in standing heat were observed around 41 hours after CIDR removal, although it ranged anywhere between 41 and 47 hours after CIDR removal. Over half of the heifers were witnessed in standing heat at 41 hours, most notably heifers that were given treatment 1. Of the 22 heifers observed in standing heat at some period, 13 were observed in heat at 41 hours, 76.92% of which were included in the treatment 1 group.

It should also be noted that of the 22 heifers witnessed in standing heat, only 8 of them achieved a successful TAI pregnancy status. Therefore, only 36.3% of the heifers actually observed in estrus achieved pregnancy through TAI. 68.2% of the heifers observed in estrus achieved pregnancy through TAI or CU. On average, heifers can be witnessed in estrus from anywhere between 36-60 hours after given $PGF_2\alpha$, although the highest percentage of heifers in estrus is seen between 48-60 hours (Lamb and Larson, 2004).

Heat detection was not observed in the cow herd due to the large pasture in which they were grazed. All cows received tail paint at the time of CIDR removal, which was analyzed on a scale of 1 to 3 at breeding. The time and degree of estrus was correlated with this scale, assuming that a 1 (paint untouched) potentially meant no heat, while a 3 (paint completely removed) meant the cow was in standing heat for a longer period of time.

The ability to induce cyclicity was expected to be similar between the two treatment types, as both stimulate a hormonal response to produce a follicle that is to be available during the period of TAI. However, previous studies have shown that the additional GnRH injection and extra 9 days of progesterone presence during the 14-d Synch treatment could lead to added benefits. Pretreatments with both of these could induce cyclicity as well as the resumption of follicular growth, ensuring an adequate response to the second GnRH injection on day 9. This response would not be possible with a 5-d CO-Synch, since only one GnRH injection is available in that treatment (Giles et al., 2013). Therefore, we would assume that pregnancy rates should increase with the utilization of a 14-d CO-Synch treatment. However, there was no difference ($P>0.73$) between the treatment types and TAI pregnancy rates.

The response to the initial GnRH injection in a standard 5-d CIDR-based estrus synchronization protocol is a key factor in the initiation of a new follicular wave, which will ovulate a fertile oocyte once the CIDR is removed. The presynchronization of estrous cycles can lead to a cow having a better response to GnRH, and ultimately lead to an increase in pregnancy rates in both cows and heifers (Stegner et al., 2004, Leitman et al., 2009). Similarly, the utilization of two GnRH injections used in the 14-d Synch protocol leads to the idea that cows with a responsive follicle to GnRH on d 0 should ovulate again and start a brand new follicular wave. This would lead to a new follicle that would also be responsive to GnRH on d 9, which would be ovulated during progestin influence to initiate a second follicular wave before CIDR removal occurs on d 14. This method could ensure that any cows or heifers that did not respond to the initial GnRH injection on d 0 would have a follicle that is responsive to GnRH by d 9, and again on d 14 (Giles et. al., 2013). However, since we saw no increase in TAI pregnancy rates overall with the use of the 14-d Synch protocol, we cannot assume that this is entirely accurate. Further research must be done on the method to fully understand the benefits of inducing a second follicular wave.

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