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General considerations for implementing an artificial insemination program or other reproductive technologies.

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Introduction

The development of effective methods of synchronizing estrus and ovulation has been based on our understanding of the physiological and hormonal mechanisms controlling the estrous cycle and the initiation of estrous cyclicity and prepuberty in heifers and postpartum cows. Although estrus synchronization products ($PGF_{2\alpha}$, MGA, CIDR and GnRH) and protocols have changed over time, the basic physiological principles underlying how these products work have not. An understanding of how these products impact the bovine estrous cycle and an understanding of how management decisions impact pregnancy success will have an effect on the success of any reproductive program.

Estrus synchronization and artificial insemination (AI) are among the most powerful and applicable technologies for genetic improvement of beef herds (Seidel, 1995). When we consider the food demands to feed the 9 billion people expected to be on the earth in 2050 the efficiency of beef production needs to increase or beef may become a luxury item and not a commodity to feed the world. Beef is currently 22% of the world meat production, and with meat production estimated to need to be over 455 million tons to feed the 2050 population beef production will need to double to maintain its current share of the production system.

When it comes to reproductive management the things you do well will not compensate for the mistakes you make. Instead, the mistakes you make cancel out all the things you do well. This is best illustrated by examining the primary factors that affect pregnancy success, and this can be done using the "Equation of Reproduction." The equation looks at 4 main topic areas: 1) Percentage of animals detected in standing estrus and inseminated, 2) Inseminator efficiency, 3) Fertility level of the herd, and 4) Fertility level of the semen. We will utilize these four main areas in this paper to examine the keys to a successful synchronization and AI program.

Important Management Considerations before Implementing an Estrus Synchronization or Artificial Insemination Program

Are my heifers and cows good candidates for an estrus synchronization protocol?

To determine if you are ready for a synchronization and AI program, the first question to ask is "Over the past few years what has been the pregnancy rate in my heifers or cows after a 60 to 80 day breeding season?" If the pregnancy rate at the end of this length of a breeding season has been less than 85% there may be management issues that should be addressed before initiating a synchronization and AI program. If the pregnancy rate has been ≥ 85% during a 60 to 80 day breeding season then you need to evaluate whether your heifers and cows are good candidates for an estrus synchronization and AI program.

Criteria for heifers. Heifers that will be used for breeding purposes should not have received growth promoting implants. Previous studies report that implanting heifers within 30 days of birth impairs uterine function and decreases subsequent pregnancy rates. Heifers should have attained 65% of their mature body weight by the start of breeding. Some recent studies

have proposed that heifers can be developed to lighter weights prior to the first breeding season. However, fewer heifers that were developed to 53% of mature weight were cycling prior to the start of the breeding season compared to heifers developed to 58% of mature weight, but the percentage pregnant in a 45 d breeding season was not different between treatments (Funston and Deutscher, 2004). While this might indicate that heifers can be developed to a lighter weight without negatively impacting reproductive performance, Creighton et al. (2005) reported that when heifers were developed to 50% of mature weight, 15.7% fewer of them conceived in the first 30 days of the breeding season compared to heifers developed to 55% of mature weight. Therefore, consideration should be given to the possibility of heifers conceiving later in the breeding season when trying to decrease heifer development costs and developing heifers to a lighter weight. Knowing the mature weight of the cows in your herd can be helpful in calculating an appropriate target weight. This can practically be determined by looking at the sell weight of cows you have culled from your herd over the past couple of years.

Pregnancy success during the breeding season has been correlated with the percentage of heifers that reached puberty before or early in the breeding season (Short and Bellows, 1971), and it has been reported that pubertal status is one of the main factors that impacts conception rates (Bridges et al., 2014). A minimum of 50% of your heifers need to have reached puberty and have started normal estrous cycles. This can be determined by reproductive tract scoring your heifers 4 to 6 weeks prior to the breeding season. A reproductive tract score (RTS) is a subjective measurement of the sexual maturity of a heifer that is normally performed by a veterinarian. The score is obtained by palpation per rectum and is based on the degree of uterine development and ovarian status (size of dominant follicle and presence or absence of a CL). Each heifer is assigned a score of 1 to 5 (1 = immature; 5 = presence of a corpus luteum) with a RTS of 1 referring to a prepubertal heifer, 2 or 3 referring to a peripubertal heifer (transitional stage), and 4 or 5 referring to a pubertal (cycling) heifer. The uterine and ovarian dimensions of heifers for each of the RTS are described in **Table 1**.

Table 1. Description of uterine and ovarian measurements for different reproductive tract scores (RTS).

RTS	Uterine horns (diameter, mm)	Ovarian length (mm)	Ovarian height (mm)	Ovarian width (mm)	Ovarian structures
1	Immature, < 20 mm, no tone	15	10	8	No palpable follicles
2	20-25 mm no tone	18	12	10	8 mm follicles
3	20-25 mm slight tone	22	15	10	8-10 mm follicles
4	30 mm good tone	30	16	12	> 10 mm follicles, CL possible
5	> 30 mm	> 32	20	15	CL present

Criteria for postpartum cows. To maintain an annual calving interval (≤ 365 days), conception must occur within 80 days of calving; however, the period of anestrus following calving is frequently greater than 60 days. Based on data from Missouri beef herds only 60% of postpartum beef cows were cycling at the start of the breeding season. In beef cattle, prolonged postpartum intervals decrease the proportion of cows that are cycling at the start of the breeding season and thereby decrease pregnancy rates and pounds of calf weaned per cow exposed during a breeding season. Postpartum interval length is influenced by a variety of factors including suckling, nutrition, age, dystocia, genetic variation, stress, and disease (Short et al., 1990; Crowe et al., 1993; Yavas and Walton, 2000).

Suckling: Postpartum beef cows that are suckled ad libitum have a longer postpartum anestrous period than cows that are suckled once daily, or not suckled at all (see review by Williams, 1990). This extended anestrous period is a direct function of suckling and the bond that develops between a cow and her own calf. The ability of a cow to recognize her calf prolongs postpartum interval length in addition to the neural stimulation of the suckling stimulus. Luteinizing hormone (LH) is an important reproductive hormone that is secreted from the anterior pituitary gland into the blood and is required for the establishment and maintenance of normal estrous cycles in numerous mammals, including cattle. An increase in LH pulse frequency is required for growth and maturation of an ovulatory follicle. As time from calving increases so does the frequency of LH pulses in the circulation and this culminates in a short luteal phase followed by the first normal estrous cycle postpartum. Interestingly, the biological changes from calving to the first ovulatory estrus in a postpartum cow are similar to the physiological changes in a heifer as she approaches puberty. For example, initiation of normal estrous cycles in prepubertal heifers and cows is frequently preceded by an ovulation, without estrus, that results in a short luteal phase (Perry et al., 1991; Werth et al., 1996). This short exposure to progesterone is believed to be necessary for reprogramming the reproductive axis to resume normal estrous cycles. Therefore, in herds that have a large proportion of prepuberal heifers or anestrous cows, progestin pretreatment (Melengestrol acetate or CIDR treatment) before induction of ovulation can initiate estrous cycles by simulating a short luteal phase.

Nutrition: Short et al. (1990) proposed the following biological priorities for nutrient utilization (nutrient partitioning) by cattle: 1) basal metabolism, 2) motor activity, 3) growth, 4) basic energy reserves, 5) maintenance of pregnancy, 6) lactation, 7) additional energy reserves, 8) estrous cycles and initiation of pregnancy, and 9) excess reserves. The preceding priorities for nutrient partitioning demonstrate that reproduction (resumption of estrous cycling and pregnancy) is a low priority, particularly for heifers calving at two years of age. Consequently, underfeeding energy and/or protein precalving and post calving reduced both pregnancy rates and first service conception rates, and increased the postpartum interval (see review by Randel, 1990). Both suckling and nutrition interact to have a powerful effect on return to estrus in beef cows.

A simple method of assessing bovine energy reserves is through a subjective body condition scoring (**BCS**) system, which ranges from 1 (emaciated) to 9 (obese). The scoring system evaluates the amount of fat cover at specific locations on the female. Cow body condition at calving has a critical role in determining postpartum interval length compared to body condition score at the start of the breeding season (see review by Dziuk and Bellows, 1983). Consequently, prepartum nutrition level and maintenance of nutrition level postpartum has an important effect on subsequent reproductive performance (see review by Randel, 1990). Cows

having a body condition score ≥ 5 at calving returned to estrus sooner than cows having a lower body condition score (Spitzer et al., 1995), and cows with a body condition score of six or seven had higher pregnancy rates compared to cows with a body condition score of four or five (DeRouen et al., 1994).

A strategic time to assess cow body condition is at weaning since a cow's nutrient demands are significantly reduced after weaning and this is the most economical time to improve cow body condition. In general, a cow needs to gain approximately 80 lbs (not including the weight of a gestating calf and the associated fluids) to increase one condition score. Consequently, if a cow has a BCS of 3 at weaning and you want her to have a BCS of 5 at calving she will need to gain 160 lbs. By knowing how much weight she needs to gain and the number of days from weaning to calving you can calculate an expected average daily gain to achieve the targeted BCS goal by calving.

Precalving nutrition has an important effect on cow body condition at calving and subsequent postpartum interval length. The effects of poor body condition in cattle can be overcome by feeding cows prepartum to obtain a good body condition score at parturition (Morrison et al., 1999). Cows fed a high energy diet for 135 to 142 days prior to calving had higher pregnancy rates, conceived earlier in the breeding season, had a shorter interval from calving to conception, and exhibited estrus earlier postpartum than cows fed half the energy of the high energy ration (Dunn et al., 1969). Increased energy content of feed as late as two months before calving increased BCS, percent cycling and pregnancy rates during the first half of the breeding season (Espinoza et al., 1995).

Whereas precalving nutrition is an important determinant of postpartum interval length, postcalving nutrition has an important effect on conception rate. Increasing energy content in a ration after calving resulted in higher pregnancy rates and cows conceived earlier in the breeding season, but cows did not exhibit estrus earlier postpartum compared to control animals (Dunn et al., 1969). Waiting until 4 weeks after calving and 11 days before breeding to increase energy supplementation had no effect on concentrations of LH or estradiol, but did increase the size of the largest follicle 7, 9, and 12 days after feeding was initiated, and also increased pregnancy rates and maintenance of the embryo (Khireddine et al., 1998). Therefore, supplementation of cattle following calving resulted in a shorter duration of negative energy balance and increased reproductive performance.

Age of the Cow: As previously discussed, growth is a higher priority for nutrient partitioning than reproduction, and heifers consistently had longer postpartum intervals than multiparous cows (Doornbos et al., 1984; Fajersson et al., 1999). In addition, the first ovulation postpartum in primiparous cows was delayed relative to multiparous cows (Sharpe et al., 1986; Guedon et al., 1999). Consequently, as animals reach mature body size nutrients that were previously partitioned for growth can be utilized for lower priority functions including reproduction. Consequently, feeding first calf heifers separate from older cows and providing supplemental nutrition to first calf heifers can be effective strategies for negating the effect of cow age on rebreeding.

Dystocia: Heifers calving at two years of age have increased incidence of dystocia compared to older cows. Furthermore, heifers that experienced calving difficulty at two years of age weaned fewer calves that were younger and lighter (Brinks et al., 1973). Cows experiencing dystocia resulted in a lower percentage of cows exhibiting standing estrus within 45 days of calving, decreased AI pregnancy rates, and decreased total pregnancy rates (Laster et al., 1973). Therefore, minimizing the incidence of dystocia through proper heifer development

and use of "calving ease" bulls as well as being proactive in providing obstetrical assistance will help reduce postpartum interval length and increase reproductive performance.

Postpartum cows that are good candidates for an estrus synchronization programs normally meet each of the following criteria: 1) body condition score at calving of ≥ 5 (1= emaciated; 9 = obese), 2) mean postpartum interval of the cows to be synchronized should be ≥ 40 days at the beginning of the protocol. This does not mean that each cow should be ≥ 40 days postpartum but that the mean of the entire group to be synchronized should be ≥ 40 days. If the estrus synchronization protocol you plan to use includes CIDR administration, each cow should be a minimum of 21 days postpartum at the time of CIDR insertion, and 3) low incidence of calving difficulty since dystocia will lengthen the postpartum interval.

Impact of Timing of Vaccination on Pregnancy Success

The question is often asked; can the time and labor involved in heifer development be reduced by vaccinating heifers at the start of the synchronization protocol?

The effects of vaccination on estrus synchronization and conception are variable. A study in which the vaccination history was not reported and titer concentrations were not determined indicated that vaccination with a MLV at time of the start of a synchronization protocol (day -9, with AI on day 1 to 5) did not impact estrous response or pregnancy success (Stormshak et al., 1997). In another study, animals were vaccinated with a MLV vaccine at least two times prior to synchronization protocol (the second dose being administered at day -90 prior to peak breeding day). The heifers were then revaccinated either at -40 d or -3 d prior to peak breeding (three doses total) and no differences in conception rates were observed (Bolton et al., 2007). However, several studies have reported negative impacts on pregnancy success by vaccinating naïve heifers with a MLV around time of breeding (Miller et al., 1989; Chiang et al., 1990; Miller, 1991; Perry et al., 2013; Perry et al., 2018).

Naïve Animals

Decreases in fertility by vaccination of naïve heifers around the onset of standing estrus are likely mediated through negative effects on corpus luteum (CL) function (Van der Maaten and Miller, 1985; Smith et al., 1990), with the hypothesis that the virus can get inside large dominant follicles and disrupt the formation and development of the corpus luteum. However, recently developed estrous synchronization or fixed-time AI protocols in heifers and cows try to control follicular development by inducing ovulation at the start of the synchronization protocol; therefore, insemination should occur on the second ovulation after the start of the protocol (Lamb et al., 2010; Grant et al., 2011). Therefore, a recent study investigated the effect that vaccinating naïve heifers with either a Modified Live Vaccine (MLV) or inactivated virus vaccine (IVV) at the time of the first induced ovulation of a fixed-time AI synchronization protocol has on changes in hormone production, estrous cycle length, and pregnancy success (Perry et al., 2013).

In this study, no control heifers (nonvaccinated) experienced an abnormal estrous cycle following AI. An abnormal estrous cycle was defined as an estrous cycle less than 15 d (concentrations of P4 decreased to < 1 ng/mL prior to day 15 after AI) or concentrations of P4 never increased above 1 ng/mL. Heifers vaccinated 36 and 8 days before AI with an IVV (ViraShield® 6VL5HB) experienced 10% (2/21) abnormal cycles and heifers vaccinated 8 days before AI with an IVV (ViraShield® 6VL5HB) experienced 14% (1/7) abnormal cycles. There was no difference between these groups (P = 0.72), and both were similar to the control group (P = 0.31 and 0.22, respectively). A greater percentage of heifers vaccinated with a MLV 8 days before AI (BoviShield Gold® FP 5 VL5) had abnormal estrous cycles [38% (8/21)] compared to control heifers (P = 0.02). In addition, bulls were with the heifers for only14 d following AI, thus heifers only had one chance to conceive unless they experienced an abnormal estrous cycle. Of the heifers that experienced an abnormal estrous cycle, 100% of heifers vaccinated 36 and 8 days before AI with an IVV (2/2) and heifers vaccinated 8 days before AI with an IVV (1/1) conceived during the breeding season. However, only 38% of heifers vaccinated with a MLV 8 days before AI (3/8) conceived during the return cycle.

When heifers that conceived following an abnormal estrous cycle were considered open to allow comparison of conception rates following the synchronization protocol, pregnancy rates were similar (P = 0.52) between control heifers [90% (9/10)] and heifers vaccinated 36 and 8 days before AI with an IVV [81% (17/21)]. Both control and heifers vaccinated 36 and 8 days before AI with an IVV had greater pregnancy rates compared to heifers vaccinated with a MLV 8 days before AI [33% (7/21); P < 0.01 and < 0.01, respectively]. Pregnancy rates for heifers vaccinated only 8 days before AI with an IVV [71% (5/7)] were intermediate. They were similar to control (P = 0.32) and heifers vaccinated 36 and 8 days before AI with an IVV (P = 0.59), but tended (P = 0.08) to be greater than heifers vaccinated with a MLV 8 days before AI.

Table 2. Impact of vaccine on luteal function and pregnancy success in naïve animals.

Vaccine	Abnormal luteal function	AI Pregnancy Success (%)	Pregnancy Success (%) to second service
1 dose Modified Live	8/21 (38%)[b]	7/21 (33%)[b]	3/8 (38%)
1 dose Inactivated	1/7 (14%)[a]	5/7 (71)[ab]	1/1(100%)
2 doses Inactivated	2/21 (10%)[a]	17/21 (81%)[a]	2/2 (100%)
Saline	0/10 (0%)[a]	9/10 (90%)[a]	-----

Means within a column having different superscripts are different [ab]P < 0.05
Adapted from Perry et al., 2013

Thus, it has been well established that vaccination of naïve heifers with a MLV around time of breeding has negative impacts on corpus luteum development and on pregnancy success (Miller et al., 1989; Chiang et al., 1990; Miller, 1991) even when utilizing a synchronization protocol that induces ovulation of the dominant follicle at the start of the

protocol (Perry et al., 2013). This negative impact on pregnancy success has been reported on not only first service conception rates, but also on a low percentage of animals conceiving during the second service following vaccination (Chiang et al., 1990; Perry et al., 2013), and in some heifers infected with BHV-1 at or near estrus, normal estrous cycles were delayed for up two months (Miller and Van der Maaten, 1985). Furthermore, BVDV antigen has been detected in the ovary up to 30 d post-vaccination [(Grooms et al., 1998) although the impact of this finding is not clear.

Previously Vaccinated Animals

The same effect of abnormal luteal function that occurs following vaccination of naïve animals has not been reported when previously vaccinated heifers were vaccinated with a MLV (Spire et al., 1995). Few studies have attempted to measure the effect of vaccinating well vaccinated (non-naïve) beef animals (Stormshak et al., 1997; Bolton et al., 2007), and one deficiency in these studies is the lack of true control (non-vaccinated animals) against which to measure conception rates. In this regard, it is difficult to draw a conclusion regarding vaccination timing and its effect on ovarian function and conception rates in well vaccinated animals. A recent study in dairy cattle reported no difference in conception rates between vaccinating previously vaccinated primiparous dairy cows (3 MLV as calves and 1 prebreeding as a heifer) with either a MLV or inactivated vaccine 45 days prior to FTAI (Walz et al., 2015b). In another recent study (Walz et al., 2015a), heifers were vaccinated with either a MLV or inactivated vaccine 40 and 10 d prior to a 45 d breeding season (n = 30) or 61 and 31 d prior to a 45 d breeding season (n = 30). Among heifers vaccinated 40 and 10 d prior to breeding, heifers vaccinated with the inactivated vaccine had a 20% greater pregnancy success compared to MLV vaccine, and heifers vaccinated at 61 and 31 d prior to breeding with an inactivated vaccine had a 15% greater pregnancy success compared to heifers vaccinated at 61 and 31 d prior to breeding with a MLV vaccine. However, in this study animal numbers were small, limiting their ability to detect small differences in pregnancy success. Another recent study (Walz et al., 2017), reported a 20% decrease in pregnancy success between heifers vaccinated with 2 doses of MLV compared to heifers vaccinated with 2 doses of saline, but again the animal numbers were small (n = 60 and 15; respectively). However, with the large numerical differences noted between those vaccinated with a MLV vaccine and non-vaccinated controls in these two studies, the question arises, does vaccination 30 days prior to the start of an AI breeding season negatively influence breeding season pregnancy success? Therefore, a study was conducted to examine the differences in pregnancy success between beef females vaccinated with either a MLV (BoviShield Gold® FP 5 L5 HB) vaccine or an inactivated (ViraShield® 6 L5 HB) vaccine 30 days before the breeding season, with sufficient power to detect a difference of less than 10 % in pregnancy success between groups (9 herds with 1436 animals) (Perry et al., 2016).

Conception rates to the fixed-time AI tended to differ between MLV treated animals and IVV treated animals ($P = 0.055$), but control animals were intermediate with no difference in conception rates between MLV and Control ($P = 0.21$) or between IVV and Control ($P = 0.49$). When pregnancy was determined on day 56 of the breeding season (AI conceptions plus 1 return estrus) conception rates in the IVV group were greater ($P = 0.01$) compared to the MLV group. Animals treated with MLV also had decreased pregnancy success compared to the Control ($P \leq 0.01$), but there was no difference between IVV and Control. Following the breeding season, pregnancy success was similar between MLV and Control ($P = 0.34$) as well as between the Inactivated and Control ($P = 0.14$), but there was still a difference between MLV and IVV (P = 0.01).

Table 3. Impact of vaccine on pregnancy success among previously vaccinated animals.

Vaccine	AI Conception (%)	Day 56 Pregnancy Success (%)	Breeding Season Pregnancy Success (%)	Early Embryo Loss (%)
Modified Live	40.0 ± 4^{a}	88.9 ± 2^{c}	95.2 ± 2^{c}	2 ± 1
Inactivated	46.5 ± 4^{b}	93.2 ± 2^{d}	98.0 ± 1^{d}	2 ± 1
Saline	43.3 ± 4^{ab}	92.5 ± 2^{d}	96.4 ± 1^{cd}	2 ± 1

Means within a column having different superscripts are different $^{ab}P = 0.055$, $^{cd}P \leq 0.01$
Adapted from Perry et al., 2016

It is commonly thought that IVV provide some protection against these viruses, but the same level of protection as a MLV is not achieved (Zimmerman et al., 2007; Rodning et al., 2010). However, a recent publication reported that heifers vaccinated with a MLV prior to their first breeding season and then vaccinated with a Chemically Altered/Inactivated vaccine CA/IV (CattleMaster Gold FP5) before their second breeding season had similar levels of abortions following both a BVD and IBR challenge as animals vaccinated with a MLV (Bovi-Shield Gold 5 FP) before their second breeding season (Walz et al., 2017).

Table 4. Impact of BVD and IBR challenge following vaccination with either a MLV or IVV.

Vaccine	Abortions following BVD and IBR challenge (%)	Detection of BVDV in fetuses and/or calves	Detection of IBR in fetuses and/or calves	Detection of BVD and/or IBR in fetuses and/or calves
Modified Live	3/23 (13%)[a]	2/23 (9%) [a]	2/23 (9%) [a]	4/24 (17%) [c]
Inactivated	1/22 (5%)[a]	0/22 (0%) [a]	0/22 (0%) [a]	0/22 (0%) [d]
Saline	11/15 (73%)[b]	14/15 (93%) [b]	8/15 (53%) [b]	15/15 (100%) [b]

Means within a column having different superscripts are different $^{a,c,d\ vs\ b}P < 0.01$, $^{cd}P = 0.045$
Adapted from Walz et al., 2017

Therefore, with CattleMaster Gold FP5's ability to protect the fetus from abortion and virus, a field study was conducted to examine the differences in pregnancy success between beef females vaccinated with either a MLV (BoviShield Gold® FP 5 L5 HB) vaccine or a CA/IV (CattleMaster Gold FP5) vaccine between 27 and 89 days before the breeding season, with sufficient power to detect a difference of less than 10 % in pregnancy success between groups (10 herds with 1565 animals)(Perry et al., 2018). Conception rates to AI were greater in the CA/IV vaccine group compared to the MLV vaccine group ($P = 0.05$; 60% vs 52%). Furthermore, interval from vaccination with either vaccine until AI also influenced conception rates ($P = 0.02$). Animals vaccinated 27 to 30 d prebreeding and animals vaccinated 30 to 37 days prebreeding had similar ($P = 0.98$; 52% and 52%) conception rates; however, both were decreased compared to animals vaccinated 38 to 89 d prebreeding ($P < 0.03$; 64%). There was no treatment by interval interaction ($P = 0.79$), indicating at all three intervals conception rates to the CA/IV vaccine were increased compared to the MLV. Furthermore, there was no effect of treatment ($P = 0.18$) or treatment by interval interaction ($P = 0.17$) on breeding season pregnancy rates. In summary, vaccination of well-vaccinated beef cows and heifers with a MLV vaccine pre-breeding (28 to 89 d) decreased AI conception rates compared to a CA/IV vaccine.

Table 5. Impact of vaccine and timing of vaccine on pregnancy success among previously vaccinated animals.

Vaccine	AI Conception (%)	Breeding Season Pregnancy Success (%)	Breeding Season Pregnancy Success (%)
Modified Live	52.0%[a]	95.2 ± 2	95.2 ± 2
Chemically Altered/Inactivated	60.0%[b]	96.4 ±1	96.4 ±1
27 to 30 days	52%[a]		
30 to 37 days	52%[a]		
38 to 89 days	64%[b]		

Means within a column having different superscripts are different [ab]P < 0.05
Adapted from Perry et al., 2018

So where do these studies leave us on the impact of virus vaccines on reproductive success? Vaccines against infectious reproductive diseases are valuable tools in the prevention of these diseases, as outbreaks of these diseases can be potentially devastating to a beef herd. This emphasizes the importance of proper vaccination of females before they enter the breeding herd. However, evidence is growing that MLV versions of these vaccines can have negative effects on reproductive management in well managed herds. Studies utilizing different pre-breeding vaccination protocols and intervals indicate that MLV vaccines, even when given at labeled pre-breeding intervals, may negatively affect reproductive parameters compared to cattle vaccinated with inactivated vaccines. In light of this research, it appears the

choice of pre-breeding vaccine product type and timing is one to carefully consider. Important to this consideration is the level of exposure that a given herd may have, as none of these large prebreeding studies were carried out in the face of disease challenge and do not address the question of protection in the face of an infectious reproductive disease exposure. Future research will help determine how to strike the best balance between appropriate disease protection and minimizing harmful effects from the vaccines themselves. It is reasonable to expect that striking this balance will be different for each individual cattle operation, making it imperative that cattle producers consult their veterinarian and weigh all available information when making decisions about pre-breeding vaccinations in their herds.

Important Management Considerations at time of Estrus Synchronization and/or Artificial Insemination

Which estrus synchronization protocol should I choose?

When choosing an estrus synchronization protocol there are a number of issues to consider including whether you want to detect estrus and inseminate according to the AM/PM rule, inseminate at a predetermined time, or detect estrus for 72 to 84 hours (depending upon the protocol) and inseminate any cows not detected in estrus at a fixed-time. There is an estrus synchronization protocol sheet for heifers and cows that appears in the catalogs of the major AI companies and there are protocols that fit each of the preceding approaches to estrus synchronization. Other items to consider include the proportion of females that are cycling as well as the time, labor, and cost of the protocol.

Implementation of an estrus synchronization protocol.

Estrus synchronization protocols must be followed precisely. Each product must be administered at the correct dose on the correct day (refer to protocol sheet) and in some cases at the right time of day. For example, the interval from prostaglandin $F_{2\alpha}$ (PGF) to gonadotropin releasing hormone (GnRH) and insemination must be in accordance with what is recommended in the protocol sheet (e.g. 66 ± 2 hr for the CO-Synch + CIDR protocol). The recommended time of insemination relative to PGF injection is based on research trials and should be strictly adhered to. In addition, estrus synchronization products must be stored, handled, and administered correctly. For specific tips on estrus synchronization products see Figures 1 and 2. Should a mistake occur in product administration or the treatment timeline seek advice immediately from a veterinarian, an extension agent specializing in reproduction, or a representative from an AI company. To minimize the probability of making a mistake, a good practice is to write each of the days of treatment, the product name, dose to be administered, and the day of insemination on a calendar and ask a trusted veterinarian, extension specialist, or AI company representative to review it before beginning the protocol.

Understanding the basic principles of the bovine estrous cycle and how the products synchronize estrus and ovulation can be helpful in reducing the probability of administering the wrong product on a certain day. For more information on how estrus synchronization protocols synchronize estrus and ovulation refer to the web based course entitled “Fundamentals of Beef Reproduction and Management:Focus on Estrus Synchronization (http://animalsciences.missouri.edu/extension/beef/estrous_synch/).

Figure 1. Proper handling and administration of GnRH and PGF products.

- All injections of GnRH and PGF products should be given intramuscularly (IM)
- Wear latex gloves when administering GnRH and PGF products
- An 18 gauge 1 ½ inch needle is recommended for these injections
- Change needles frequently
    - Make sure that injection sites are free of manure and dirt, which may cause infection
    - Injecting cattle during wet weather increases the potential for infection
- **Always** follow manufacturer's recommended storage, dosage and administration procedures

Figure 2. Proper handling and administration of progestins for estrus synchronization.

Controlled Internal Drug Release (CIDR)

1) Wear protective (e.g. latex) gloves when handling CIDR inserts.

2) The CIDR applicator should be rinsed in a disinfectant solution (Nolvasan or Chlorohexidine). There should be two buckets each containing a disinfectant solution. The applicator should be washed free of debris in the first bucket and then rinsed clean in the second. This sequence of events will improve sanitation from animal to animal and reduce the likelihood of infection.

3) Fold the wings of the CIDR and insert it into a clean applicator. The CIDR will protrude approximately one inch from the applicator.

4) Apply lube to the end of the applicator.

5) Wipe the vulva clean before inserting the applicator.

6) When inserting the CIDR make sure that the nylon tail is curved downward. If the tail is pointed upward it will be easier for other animals to pull out the CIDR thus reducing retention rate. To prevent other animals from removing the CIDR, the nylon tail can be clipped such that only 2 ½ inches protrude from the vulva.

7) Gently insert the applicator containing the CIDR in an upward manner similar to the insertion of an AI catheter.

8) Push the applicator as far forward as possible, deposit the CIDR by pressing the plunger, and slowly remove the applicator.

9) At CIDR removal, gently but firmly pull on the nylon tail until it is removed. Be sure to dispose of the CIDR properly.

Melengestrol Acetate (MGA)

1) MGA is an orally active feed additive that should be fed once a day at the recommended dose - 0.5 mg in a 3 to 5 lb carrier. Do not top dress MGA on other feeds. Provide adequate bunk space - 18-24 inches per animal.

2) Allow heifers to adjust to carrier prior to MGA administration.

3) MGA is approved by the FDA for heifers intended for breeding (suppression of estrus) and for heifers fed in confinement for slaughter for increased rate of weight gain, improved feed efficiency, and suppression of estrus.

4) Use of MGA as part of any estrus synchronization protocol in beef cows constitutes and extra label use of medicated feed that is prohibited by the Animal Medicinal Drug Use and Clarification Act and regulation 21 CFR 530.11(b).

How do I choose an AI sire and where do I obtain the semen?

Sire selection is of critical importance and can have a long term effect within a herd, particularly when heifers are retained as replacements. When choosing a sire the following questions need to be addressed: 1) Will I raise my own replacement heifers or purchase them?, and 2) How will I market my calves? Answers to the preceding questions will determine the traits that need to be emphasized. If a producer raises his or her own replacement heifers then selection pressure should be placed on maternal traits such as milk, maternal calving ease, stayability, etc. However, if replacement heifers are purchased off the farm then emphasis on maternal traits in your herd would not be important. When selecting a sire, you need to think about how you will be paid (e.g. pounds of weaning weight, carcass weight, carcass quality) and let this affect your sire selection decisions. Producers that sell their calves at weaning need to place selection pressure on preweaning growth; whereas, producers that retain ownership and market their calves on a grid should emphasize carcass weight, marbling, and ribeye area.

Expected progeny differences (EPDs) are an effective selection tool and are reported to be 7 to 9 times more effective at generating a response to selection than focusing on measurements of individual performance, which is strongly affected by environment. Use AI sires with high accuracy EPDs and where the semen has been collected from a certified semen services (CSS) facility. Avoid using unproven bulls and do not be hesitant to seek advice from individuals in the AI industry to help make this important management decision.

Another consideration when selecting a sire is whether the bull's semen has worked in FTAI programs. Differences among sires in pregnancy rate to FTAI have been noted; however, the same differences in pregnancy rate may not occur when cows are detected in estrus and inseminated according to the AM/PM rule. Therefore, just because an AI sire has a good conception rate following estrous detection does not ensure he will perform equally well when ovulation is induced and insemination occurs at a predetermined time. It is a good idea to ask an AI representative if there is information available regarding how a bull has worked in a FTAI program.

Estrus Expression and the Establishment of Pregnancy

Detection of estrus: In cattle, the estrous cycle normally varies from 17 to 24 days and the duration of standing estrus is generally 12 to 15 hours; however, considerable variation exists among individual animals (range < 8 to > 30 hours; (O'Connor and Senger, 1997). The primary sign of estrus in cattle is standing to be mounted and secondary signs of estrus include frequent mounting, watery mucus from the vulva, and restlessness. Maximizing the estrous detection rate is dependent upon accurate detection of animals in standing estrus. Estrus was synchronized in a group of animals at Colorado State University and monitored for standing estrus 24 hours a day with a computer assisted estrous detection system (HeatWatch®) or twice a day for 30 minutes by visual observation. By day 5 after estrus synchronization, 95% of animals monitored 24 hours a day were detected in standing estrus; whereas, only 56% of animals observed twice a day for 30 minutes were detected in standing estrus (Downing et al., 1998). With an estrous detection rate of 95% and a conception rate of 70% (95% X 70% = 67%) approximately 67% of the animals would be pregnant; whereas, only 39% would be pregnant (56% X 70% = 39%) with a 56% estrous detection rate.

Therefore, the success of any estrus-based artificial insemination program requires detecting animals in standing estrus and inseminating them at the correct time relative to detection of estrus. Failing to detect estrus or errors in accurately detecting estrus can result in

significant economic losses. Accurate detection of estrus can be a difficult and time-consuming activity. When estrus was detected in 500 Angus cows with the HeatWatch® estrus-detection system, the length of estrus averaged 10 hours (range: 0.5 hours to 24 hours); however, 26% of cows exhibited estrus for less than 7 hours and had fewer than 1.5 mounts per hour (Rorie et al., 2002). To maximize detection of standing estrus, it is important to visually monitor cattle as much as possible. Observations should occur as early and as late as possible as well as during the middle of the day. Continuous observation of over 500 animals exhibiting natural estrus in 3 separate studies indicated that 55.9% of cows initiated standing estrus from 6 p.m. to 6 a.m. (Table 6). Furthermore, when cows were observed for standing estrus every 6 hours (6 a.m., noon, 6 p.m., and midnight), estrous detection increased by 10% with the addition of a mid-day observation and by 19% when observed four times daily (every 6 hours) compared to detecting standing estrus at 6 a.m. and 6 p.m. alone (Hall et al., 1959). Therefore, detection of standing estrus can be one of the most time-consuming chores related to artificial insemination.

Table 6. Time of day when cows exhibit standing estrus.

6 a.m. to 12 noon	26.0 %
12 noon to 6 p.m.	18.1 %
6 p.m. to midnight	26.9 %
Midnight to 6 a.m.	29.0 %

Data adapted from (Hurnik and King, 1987; Xu et al., 1998), G.A. Perry unpublished data).

There are commercially available estrus detection aids that can be used in conjunction with visual observation to increase estrous detection efficiency in beef herds. The HeatWatch Estrus Detection System is probably the only tool that can replace visual observation, since this system provides precise data on the onset, intensity, and duration of estrus. Some of the more common estrus detection aids include tail chalk/paint, pressure mount detectors, gomer (spotter) bulls (teaser bulls; rendered sterile by vasectomy, epididectomy, and (or) penile deviation), and androgenized cows. Table 7 provides a list of common estrus detection aids, a description of how they work, some potential concerns, and relative cost. A comparison between visual estrous detection every 3 hours (8 times daily), a marker animal (a bull with a deviated penis), and Estrotect® patches resulted in a similar ($P > 0.79$) percentage of animals correctly identified in standing estrus (92%, 92%, and 91%, respectively; (Perry, 2005). Increased visual observation, in addition to the use of estrus-detection aids, can improve pregnancy rates by determining the most appropriate time for insemination.

The number of mounts per estrus increases as the number of females in estrus increases (Helmer and Britt, 1985; Landaeta-Hernandez et al., 2002). This is likely due to the formation of sexually active groups of cattle which is known to increase the number of mounts per female (Hurnik et al., 1975; Galina et al., 1994). In nonsynchronized cattle there will be fewer sexually active groups (or fewer animals per group) and less mounting activity. Therefore, improved estrous detection efficiency is an advantage of an estrus synchronization program. However, it is also true that frequent animal handling and restraint are stressors (Dobson and Kamonpatana, 1986) and that increased handling and restraint of heifers during a synchronized estrus decreased the number of mounts per estrus (Lemaster et al., 1999). Depending upon the estrous

synchronization protocol, a fixed-time insemination protocol should reduce the amount of animal handling associated with sorting estrual heifers at the time of insemination.

Table 7. A list of estrus detection aids in beef cattle, a description of how they work, potential concerns, and relative cost.

	How it Works	**Potential Concerns**	**Relative Cost**
Tail Chalk	Chalk is applied to tailhead. When animal is mounted the color will be rubbed off and hair will be ruffled.	Removal by trees, water, fences, or licking by other animals	$
Heat Mount Detectors	Detectors are applied to tailhead and turn a different color when mounted.	Partial activation or loss of detector requires interpretation, false activation (e.g. trees, fences, other animals)	$$
Heat Watch	Transmitters are attached to tailhead region. When transmitter is depressed a signal is sent to receiver.	Expensive to replace lost sensors, data interpretation, appropriate facilities/terrain	$$$
Gomer Bulls	Vasectomized, epididyectomized, and (or) penile-deviated animals are used as teaser animals and will mount females in estrus.	Feeding and maintenance expense, potential loss of desire to mate, and disease transmission by non penile-deviated animals	$$$
Chin Ball Marking Harness	Detector animal is fitted with harness leaving an inkmark on the back and neck of females that have been mounted.	Maintenance of equipment, feeding and maintenance of animal, ill-defined markings	$$
Androgenized cows	Testosterone injections before and during the breeding season or androgen implant causes cow to mount other females in heat.	Cost and labor of administering drug, variable response to hormone	$$

Effect of estrus expression on pregnancy rate: When insemination is performed at a fixed-time there will be heifers or cows that are in estrus and those that have not displayed estrus. Of those that are not in estrus some will show estrus if the GnRH injection (to initiate the ovulatory process) and insemination are delayed; whereas, some may not express estrus at all. It is still possible for some heifers and cows in the latter group to conceive since ovulation can be induced following GnRH injection. There is considerable data indicating that heifers and cows in estrus around the time of fixed-time AI have a higher pregnancy rate than those not in estrus, and a review of 10,116 animals using the top 5 recommended fixed-time AI protocols indicated a 27% ± 5% improvement in conception rates among animals that exhibited estrus prior to the time of fixed-time AI (Figure 3).

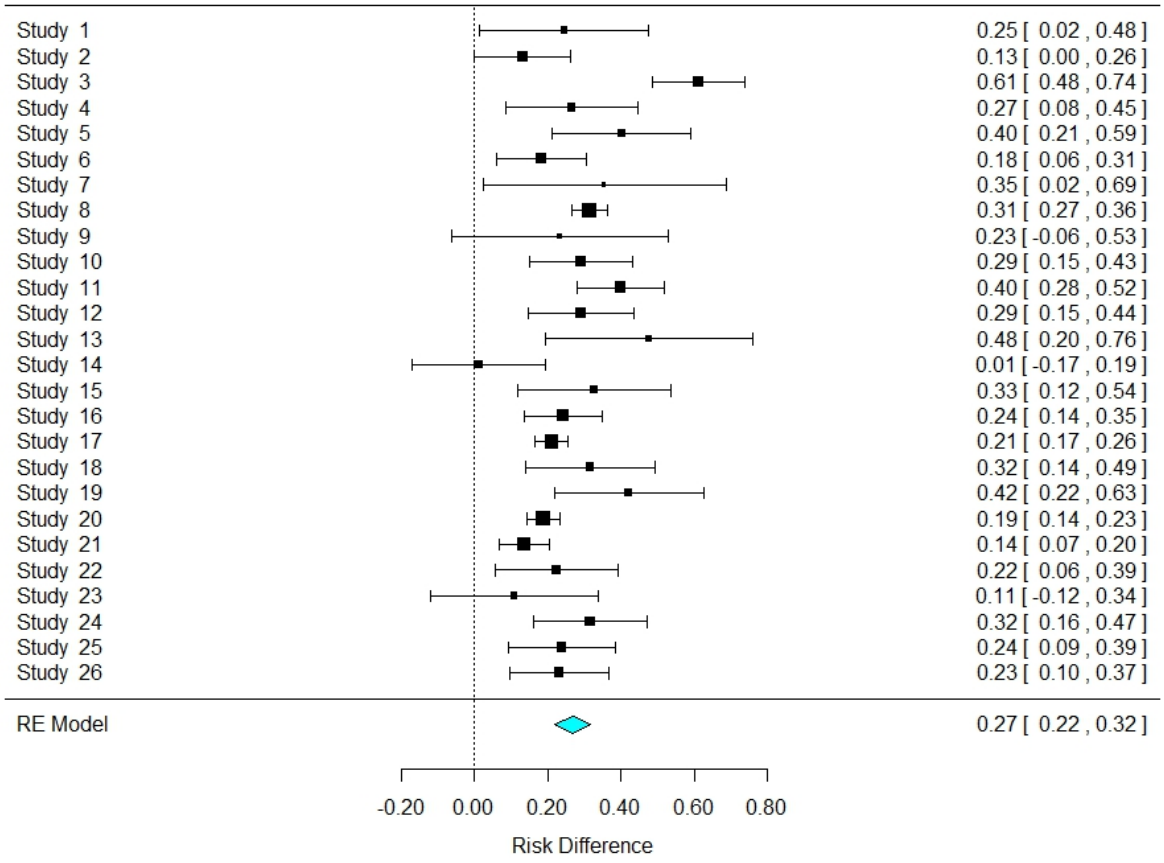

Figure 3. Effect of estrus expression around the time of fixed-time AI on pregnancy rate in beef heifers and postpartum cows. In each case animals that were detected in estrus around the time of fixed time AI. A meta-analysis of these 26 studies (10,116 animals) indicate a 27% improvement in fixed-time AI conception rates when animals exhibit standing estrus compared to when animals do not show estrus ($P < 0.01$; 95% Confidence Interval 22% to 32%).

Why does estrus expression at fixed-time AI increase pregnancy rate?: Expression of estrus is stimulated by increasing concentrations of estradiol (a follicular hormone) at a time when progesterone (secreted by the corpus luteum) is low. Estradiol secretion is higher in heifers and cows that show estrus compared to those that are not detected in estrus. Preovulatory secretion of estradiol by a dominant follicle coordinates a number of physiological processes that are required for the establishment of pregnancy. Some of these effects occur during the preovulatory period (e.g. estrus expression, induction of the gonadotropin surge that induces ovulation, sperm transport, and embryo survival); whereas, other effects are manifested during the luteal phase (e.g. preparation of maternal environment for pregnancy). In general, the secretion of estradiol increases as the physiological maturity of a dominant follicle increases. Consequently, during the development of a fixed-time AI protocol emphasis is placed on

maximizing the proportion of females that have a physiologically mature ovulatory follicle at insemination.

Timing of insemination following estrous detection or fixed-time AI: When utilizing an estrus synchronization protocol that requires estrous detection, insemination occurs approximately 8 to 12 hours following detection of estrus (AM/PM rule). In other words, if a cow is detected in estrus in the AM then AI should occur the following PM; whereas, if a cow is detected in estrus in the PM then AI should occur the following AM). It is essential that the presence of fertile sperm in the oviduct coincide with the time when the oocyte is viable (8 to 10 hour period following ovulation). Insemination (AI) too soon, following detection of estrus, can decrease the probability that viable sperm are present at ovulation. However, insemination too late, relative to detection of estrus, may result in the oocyte dying before the sperm complete capacitation (process, within the female tract, by which sperm gain the capacity to fertilize the egg) and are capable of fertilizing the oocyte. The time of insemination is based on an understanding of the relationship among the following biological parameters: duration of estrus, interval from the gonadotropin (LH) surge to ovulation, lifespan of the oocyte (egg), lifespan of frozen-thawed sperm in the female tract, and duration of capacitation. For pregnancy to occur it is essential that fertile sperm be present in the vicinity of the oocyte when it is still alive. The duration of the preceding factors are shown in table 8 below and relationship among these factors when insemination twelve hours after estrus detection (AM/PM rule) is depicted in Figure 4.

However, with FTAI protocols, time of insemination becomes a compromise between maximizing the proportion of females that show estrus before insemination and not waiting too long such that heifers or cows that were the first to show estrus end up being inseminated too late. There can be variation in the fertility of sires used in a FTAI protocol. Sires that achieve high fertility when insemination occurs approximately 12 hours after detection of estrus (AM/PM rule) do not always achieve high pregnancy success following fixed-time AI. Although the exact reasons for the difference are not known, it is likely that sperm longevity in the female tract is a primary reason.

Table 8. Duration of biological factors that affect the time of artificial insemination with frozen-thawed semen in cattle.

Biological factor	Duration
Duration of standing estrus	Highly variable but normally 12 to 15 hr
Time of the gonadotropin (LH) surge which initiates the ovulatory process	Begins around the onset of standing estrus and lasts a few hours
Time from the LH surge to ovulation	25 to 30 hr
Lifespan of the oocyte (egg)	8 to 10 hr
Lifespan of frozen-thawed semen in the female reproductive tract	Approximately 24 hr but can be variable among bulls
Duration of capacitation within the female tract	4 to 6 hr following insemination but may vary among bulls.
Lifespan of fertile (capacitated) sperm in the female tract	18 to 20 hr

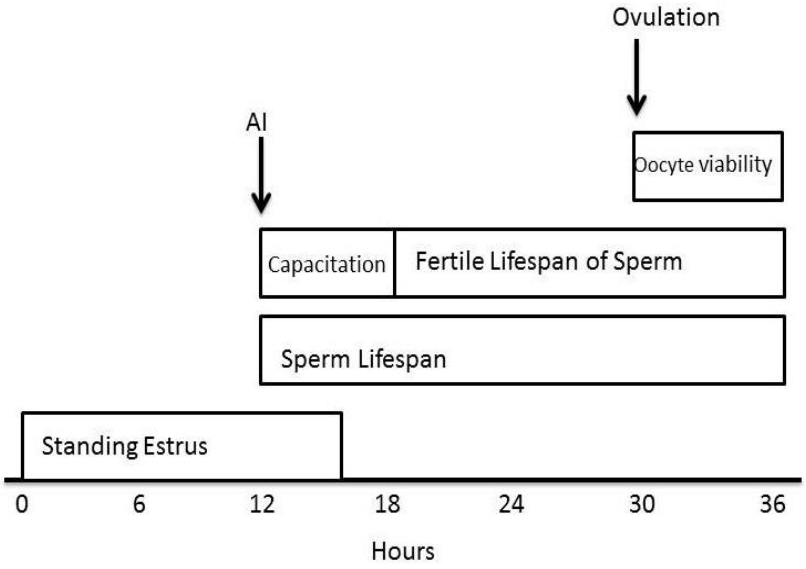

Figure 4. Illustration of the relationship among duration of estrus, duration of the sperm lifespan, length of capacitation, duration of fertile lifespan of sperm, time of ovulation, and duration of oocyte lifespan. Time periods are based on data from table 4.

Inseminator Efficiency

With AI, inseminator efficiency is influenced by semen handling and the ability of the technician to deposit semen in the correct location. A detailed inventory of semen should be easily accessible, so that straws may be located and removed from the tank quickly to avoid exposure of semen to ambient temperature. Sperm injury (as judged by sperm motility) occurs at temperatures as warm as -79 ^{o}C/-110 ^{o}F (Etgen et al., 1957; Bean et al., 1963; deJarnette, 1999), and injury to sperm cannot be corrected by returning semen to the liquid nitrogen (Berndtson et al., 1976; Saacke et al., 1978). Proper semen handling has been discussed in another chapter of this proceedings.

When numerous cows must be inseminated on a given day, multiple straws of semen are routinely thawed simultaneously to facilitate AI. Dalton et al. (2004) conducted a trial to determine: a) the effect of simultaneous thawing of multiple 0.5-mL straws of semen and sequence of insemination (1st, 2nd, 3rd or 4th) on conception rates, b) whether conception rates achieved following AI by professional AI technicians (PAI) and herdsman-inseminators (HI) differed, and c) the effect of elapsed time from initiation of thawing straws of semen to seminal deposition on conception rates. Average conception rate differed between PAI and HI (45% vs. 27%, respectively), but simultaneous thawing and sequence of insemination (1st, 2nd, 3rd or 4th), and elapsed time from initial thaw to completion of fourth AI had no effect on conception rate within inseminator group (Dalton et al., 2004). Conception rates are most likely maximized when personnel: a) accurately identify and administer the appropriate treatments to all cows to synchronize estrus or ovulation, b) accurately identify cows in estrus, c) follow the AI stud's recommendations for thawing semen, d) prevent direct straw-to-straw contact during thawing of multiple straws simultaneously to avoid decreased post-thaw sperm viability as a result of straws sticking together (Brown et al., 1991), e) use appropriate hygienic procedures, f) maintain thermal protection of straws during AI gun assembly and transport to the cow, and g) deposit semen in the uterus of the cow within approximately 15 minutes after thawing.

Using conventional semen, many studies have compared semen deposition near the greater curvature of the uterine horns with traditional deposition into the uterine body. Although Senger et al. (1988), López-Gatius (1980), and Pursley (2004) reported increased conception rates when semen was deposited in the uterine horns rather than the uterine body, Hawk and Tanabe (1986), Williams et al. (1988), and McKenna et al. (1990) found no difference in fertility when comparing uterine body and uterine horn inseminations. Furthermore, Diskin et al. (2004) reported an inseminator and site of semen deposition interaction, with evidence of either an increase, decrease, or no effect of uterine horn deposition on conception rate for individual inseminators.

Unfortunately, it is not clear why some studies have shown an advantage following uterine horn insemination while others have not. A possible explanation for the positive effect of uterine horn inseminations may be related to the minimization or elimination of cervical semen deposition. Cervical insemination errors account for approximately 20% of attempted uterine body depositions (Peter et al., 1984). Macpherson (1968) reported that cervical insemination resulted in a 10% decrease in fertility when compared with deposition of semen in the uterine body. Clearly, all AI technicians must develop sufficient skill to recognize when the tip of the AI gun remains in the cervix. To maximize conception rates, AI technicians must continue to manipulate the reproductive tract until the tip of the AI gun is past the cervix and deposition into the uterus can be accomplished.

Management Factors affecting Pregnancy Rate After Insemination

In cattle, fertilization generally occurs > 90% of the time when animals are inseminated following detection in standing estrus, but pregnancy rate at the earliest possible detection (day 27) is generally < 70%. Cows induced to ovulate smaller follicles with GnRH have reduced pregnancy rates and experience greater embryonic loss, even after pregnancy has been established (Atkins et al., 2013). These inefficiencies are likely due to either ovulation of an immature oocyte that compromises fertilization and embryo survival or ovulation occurs before the follicular cells have fully matured to produce sufficient estradiol during the preovulatory period and subsequently, progesterone to adequately prepare the uterus for pregnancy. The preceding study (Atkins et al., 2013) was designed to differentiate between follicular effects on oocyte quality and uterine environment on pregnancy success in beef cattle and the primary results are summarized below.

In order to understand how stress may increase embryonic mortality, one must first understand the development of the embryo (**Table 9**). Just like the estrous cycle, embryo development begins on day 0, or the day of standing estrus. This is the day the female is receptive to the male and insemination occurs. Ovulation occurs on day 1 or about 30 hours after the first standing mount (day 0 Wiltbank et al., 2000). If viable sperm is present, fertilization occurs inside the oviduct shortly after ovulation. The first cell division occurs on day 2, and by day 3 the embryo has reached the 8-cell stage (Shea, 1981). Between days 5 and 6 the embryo migrates into the uterine horn and by day 7 to 8 it forms into a blastocyst (Flechon and Renard, 1978; Shea, 1981; Peters, 1996). At this stage two distinct parts of the embryo can be seen: 1) the inner cell mass, which will form into the fetus and 2) the trophoblast, which will form into the placenta.

Between days 9 and 11 the embryo hatches from the zona pellucida, a protective shell that has surrounded the embryo to this point (Shea, 1981; Peters, 1996). Then, on days 15 to 17, the embryo produces a chemical signal to prevent corpora lutea destruction and allow the cow to remain pregnant (Peters, 1996). The embryo attaches to the uterus beginning on day 19, and around day 25, placentation, an intricate cellular interface between the cow and the calf, begins. By day 42 the embryo has fully attached to the uterus of the cow (Peters, 1996).

Table 9. Time course of early bovine embryo development

Event	Day
Estrus	0
Ovulation and Fertilization	1
First cell division	2
8-cell stage	3
Migration to uterus	5-6
Blastocyst	7-8
Hatching	9-11
Maternal recognition of pregnancy	15-17
Attachment to the uterus	19
Adhesion to uterus	21-22
Placentation	25
Definitive attachment of the embryo to the uterus	42
Birth	285

Data adapted from: (Flechon and Renard, 1978; Shea, 1981; Telford et al., 1990; Peters, 1996)

Mechanisms Associated With Pregnancy Establishment: After examining the effect of twelve or more factors on pregnancy rate at day 27, Atkins et al., (2013) were only able to account for about 10% of the variation in pregnancy rate. Therefore, much of the biology underlying establishment and maintenance of pregnancy in cattle remains to be determined. The establishment of pregnancy by day 27 was positively affected by serum progesterone at day 7 (day 0 = insemination), and serum estradiol at insemination. The positive effects of estradiol and progesterone were independent and likely aid in the establishment of a maternal environment that is conducive to pregnancy establishment (Inskeep, 2004).

Mechanisms Associated With Pregnancy Maintenance: GnRH-induced ovulation of small dominant follicles resulted in increased late embryonic/early fetal mortality in postpartum beef cows (Perry et al., 2005). The majority of the preceding late embryonic/fetal loss occurred around the time of embryo uterine attachment (day 27 to 41 Inskeep, 2004). This is a time when late embryonic/early fetal mortality has been reported by others and might be due to improper placentation. Pregnancy maintenance was directly affected by embryo quality and cow age. Consequently, late embryonic/fetal mortality was associated with poorer quality embryos and younger cows.

Shipping Stress and Embryonic Mortality

With the knowledge of the critical time points in embryonic development, it is possible to completely understand how stress from shipping can result in increased embryonic mortality

in cows (**Table 10**). When animals are loaded on a trailer and hauled to a new location, they become stressed and release hormones related to stress. These hormones lead to a release of different hormones that change the uterine environment in which the embryo is developing. During blastocyst formation, hatching, maternal recognition of pregnancy, and attachment to the uterus, the embryo is vulnerable to these changes. The most critical time points are between days 5 and 42 after insemination. Before day 5, the embryo is in the oviduct and is not subject to changes in the uterine environment. Therefore, stress does not influence embryo survivability at this time. The greater the length of time after day 42, the less severe the influence of shipping stress on embryonic loss appears to be. At the time of complete attachment of the embryo to the uterus the embryo is supported by the dam and appears to be not as easily affected by changes in its environment. On the other hand, in between these time points (5 – 42 days), the embryo is at greatest risk. Shipping during this time can cause detrimental changes to the uterine environment and may result in embryonic mortality. Administration of the prostaglandin inhibitor flunixin meglumine to cows and heifers 10 to 13 days after AI (when they were transported) reduced pregnancy losses about 9% (Merrill et al., 2007). However, administration of flunixin meglumine 10 to 15 d after breeding did not increase pregnancy establishment in cows. In another study, handling heifers to administer flunixin meglumine (compared to leaving them in the pasture) reduced pregnancy rates by 6% (Geary et al., 2010). Taken together, these studies provide evidence that some heifers are more susceptible to the stress of handling.

When should I not ship cows? Shipping cows between days 5 and 42 can be detrimental to embryo survival and cause around a 10% decrease in pregnancy rates (**Table 10**). Critical time points such as blastocyst formation, hatching, maternal recognition of pregnancy, and adhesion to the uterus take place during this early time of pregnancy. If any of these time points are disturbed, then the result would lead to increased embryonic mortality and decreased pregnancy rates. Research has also demonstrated that shipping cattle 45 to 60 days after insemination can result in 6% of embryos being lost. Therefore, it is important to plan on transporting cattle before the breeding season or immediately after insemination.

When can I ship cows? Shipping between days 1 – 4 is best. The embryo is still in the oviduct during this time; therefore, it is likely not subjected to uterine changes. Also after day 45, the embryo is well established and fully attached with the placenta; therefore it is less susceptible to the changes resulting from stress. Shipping at this point is less risky. However, embryonic loss from shipping has been reported up to 60 days after insemination. Care should always be taken to try to reduce the stress involved when animals are shipped. Do not overcrowd trailers and handle cattle as gently and calmly as possible.

Table 10. Effect of time of transport after insemination on pregnancy rates

	Days after insemination that transportation occurred			
	1 to 4	8 to 12	29 to 33	45 to 60*
Synchronized pregnancy rate	74%	62%	65%	
% pregnancy loss compared to transportation on days 1 to 4		12%	9%	6%*
Breeding season pregnancy rate	95%	94%	94%	

*Loss in heifers compared to percentage pregnant prior to transportation (pregnancy determined by transrectal ultrasonography)

Data adapted from Harrington et al., 1995, and T. W. Geary unpublished data

Heat Stress and Embryonic Mortality

The best time to ship cattle is during early stages of development. However, this is also the time point when the embryo is most susceptible to increased temperatures. Temperature, humidity, radiant heat, and wind all affect heat stress in cows. The rectal temperature of cattle is normally 102.2^{o}F, and an increase in rectal temperature but as little as 2^{o} F can result in decreased embryonic development (Ulberg and Burfening, 1967). When rectal temperatures reach 105.8^{o}F for as little as 9 hours on the day of insemination, embryonic development can be compromised (Rivera and Hansen, 2001). Heat stress has also been reported to change follicular waves, resulting in reduced oocyte quality (Wolfenson et al., 1995). Researchers have reported that heat stress 42 days prior to (Al-Katanani et al., 2002) and up to 40 days after breeding can affect pregnancy rates (Cartmill et al., 2001). This illustrates how important it is to plan ahead for the breeding season.

Several methods have been researched to reduce the effects of heat stress. Shade, fans, and misters can all reduce the effects of heat stress in natural service or AI programs. These methods allow animals to stay cooler during the hottest parts of the day. In humid areas, misters may not actually benefit the animals. If the water cannot evaporate, it will not be effective at cooling the animal.

Producers that utilize AI can also implement timed AI (TAI) protocols to increase pregnancy rates during the hot summer months. Timed AI has increased pregnancy rates over animals inseminated 12 hours after estrous detection in conditions of heat stress (Arechiga et al., 1998; de la Sota et al., 1998). This is most likely due to fewer animals showing signs of estrus when under heat stress. When the weather is too hot, animals tend not to move around as much and do not show signs of standing estrus. Heat detection is a vital part of getting more animals pregnant. Since fewer animals are seen in heat, fewer animals can be inseminated. In this case, TAI protocols that synchronize ovulation would be the best choice because of the lack of necessity for heat detection.

Using embryo transfer during times of heat stress can also increase pregnancy rates. High quality, fresh embryos have been proven to increase pregnancy rates over AI in heat stressed cows (Putney et al., 1989). Embryos at time of embryo transfer can adapt to the elevated temperatures. Therefore, use of embryo transfer during times of heat stress can improves pregnancy success.

Stress from Change in Diet

Changes in nutritional status can also have a tremendous influence on embryonic survival through many mechanisms. Heifers fed 85% maintenance requirements of energy and protein had reduced embryo development on day 3 and day 8 compared to heifers fed 100% maintenance (Hill et al., 1970) indicating decreased embryonic growth. Therefore, changes in nutrition can have a tremendous impact on embryo survival and the ability of heifers to conceive during a defined breeding season.

Previous research has indicated that grazing skills are learned (Flores et al., 1989a, b; Flores et al., 1989c) early in life (Provenza and Balph, 1988). This learning resulted in the development of preferences or aversions to plants and in the development of the skills necessary to harvest and ingest forages efficiently (Provenza and Balph, 1987). Heifers that grazed forage from weaning to breeding rather than being placed in drylots appeared to retain better grazing skills and had increased average daily gains into the subsequent summer (Olson et al., 1992):

Perry et al., 2014). A decrease in feed intake from 120% of maintenance to 40% of maintenance resulted in a loss of 56.3 lbs over 2 weeks (4.03 lbs/day \Mackey, 1999 #1372}; similar to the losses reported by Perry et al., (**Figure 7**) when heifers that were developed in a feedlot from weaning until the next spring were moved from a feedlot to grass. However, heifers that were developed from weaning until the next spring on range with supplementation showed no weight loss the following spring. Furthermore, heifers that were kept in a drylot until AI (n = 214) had decreased (P = 0.04) pregnancy rates compared to heifers that had previous grazing experience (n = 207; 59.4% vs. 49.1%). Therefore, post-insemination nutrition may influence embryonic survival. Nutritionally mediated changes to the uterine environment can occur by changing components of uterine secretions or by influencing the circulating concentrations of progesterone that regulate the uterine environment (see review by Foxcroft, 1997).

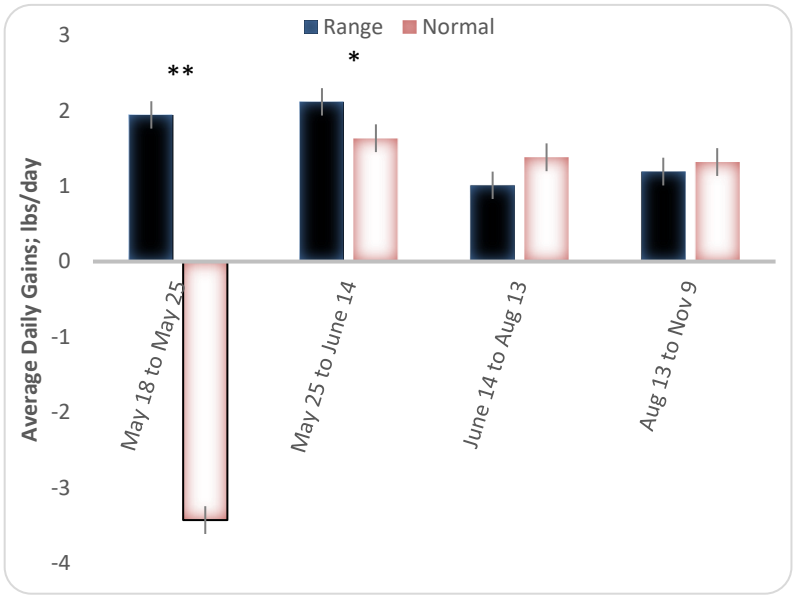

Figure 7. Average daily gain (lbs/day) of heifers weaned and developed on range (Range) compared to heifers weaned and developed in a drylot (Normal). All heifers were moved to the same pasture on May 18th (*P = 0.06; **P < 0.05)

In another recent study (Perry et al., 2015), beef heifers (n = 164) were developed in a feedlot from weaning to breeding. At time of insemination heifers were randomly allotted to one of two treatments: 1) heifers were moved from the feedlot to graze spring forage, or 2) heifers were moved to graze spring forage and supplemented with DDGS (5 lbs/hd/day) for 42 days. Pregnancy success was determined 42 days after AI. Heifers that were grazing spring forage alone lost 37 ± 4 lbs, but heifers that were grazing spring forage and were supplemented gained 45 ± 3 lbs from AI to pregnancy determination (P < 0.01). Pregnancy success was different between treatments (P = 0.02). Heifers that were not supplemented after AI had decreased pregnancy success (61%) compared to heifers that were supplemented (76%). Therefore, when heifers were developed in a feedlot, pregnancy success tended to be influenced by supplementation and subsequent weight gain after moving heifers to grass.

To investigate the idea that the decrease in AI pregnancy success may be due to grazing behavior and not just a change in diet, we conducted an experiment where heifers were moved from a grazing environment to a drylot following AI. Beef heifers at one location (n= 333) were developed on a forage diet from weaning to breeding. All heifers were brought into a feedlot and synchronized with a 7-d CO-Synch + CIDR protocol. At time of insemination heifers were randomly allotted to one of three treatments: 1) heifers were moved to graze spring forage, 2)

heifers were moved to graze spring forage plus supplemented with DDGS (5 lbs/hd/day) for 42 days, or 3) heifers were returned to the feed lot for 42 days. Pregnancy success was determined 42 days after AI. Body condition increased ($P < 0.01$) from the day synchronization began (day -7; 5.4 ± 0.05) to day 42 in both the heifers that were supplemented on pasture and the heifers that were kept in the feed lot (5.9 ± 0.04 and 5.8 ± 0.04, respectively; Table 4). Body condition did not change from day -7 to day 42 among the heifers that were on grass alone (5.4 ± 0.05 and 5.4 ± 0.04 for day -7 and day 45, respectively; Table 4). Pregnancy success did not differ among treatments [59% (65/111), 57% (63/111), and 56% (62/111) for heifers on grass alone, heifers on grass plus supplemented, and heifers in the feed lot, respectively). Therefore, when heifers were developed on grass, there was no effect on pregnancy success whether they were returned to grass with or without supplementation or even kept in the feed lot.

To further investigate if method of heifer development could impact grazing behavior, we conducted an experiment to measure daily activity between drylot developed heifers that had been moved to grass before AI compared to heifers that were moved to grass on the day of AI (Perry et al., 2015). Sixty-nine drylot developed heifers were randomly allotted to one of two treatments 42 days before AI: 1) heifers remained in the drylot until AI, or 2) heifers were moved to graze spring forage for the 42 days prior to AI. Daily activity was measured by a pedometer. Prior to AI, heifers that were grazing spring forage took more ($P < 0.01$) steps per day compared to heifers in the drylot (**Figure 8**). However; following AI, heifers that had remained in the drylot until AI had increased activity compared to heifers that had previous experience grazing spring forage (**Figure 9**). When activity is increased energy requirements are also increased. Cows that were forced to walk 3.2 km per day had a greater than 30% increase in energy requirements compared to cows that were held in a drylot (Bellows et al., 1994). Hence, heifers switched from a drylot to pasture are not accustom to grazing, forced to eat a novel diet, and exert increased energy during the period following AI. These factors combined may be the reason some heifers developed in a drylot and moved to forage after insemination have reduced conception rates. Therefore, keeping consistency in management during the breeding season is important to achieving optimum pregnancy success.

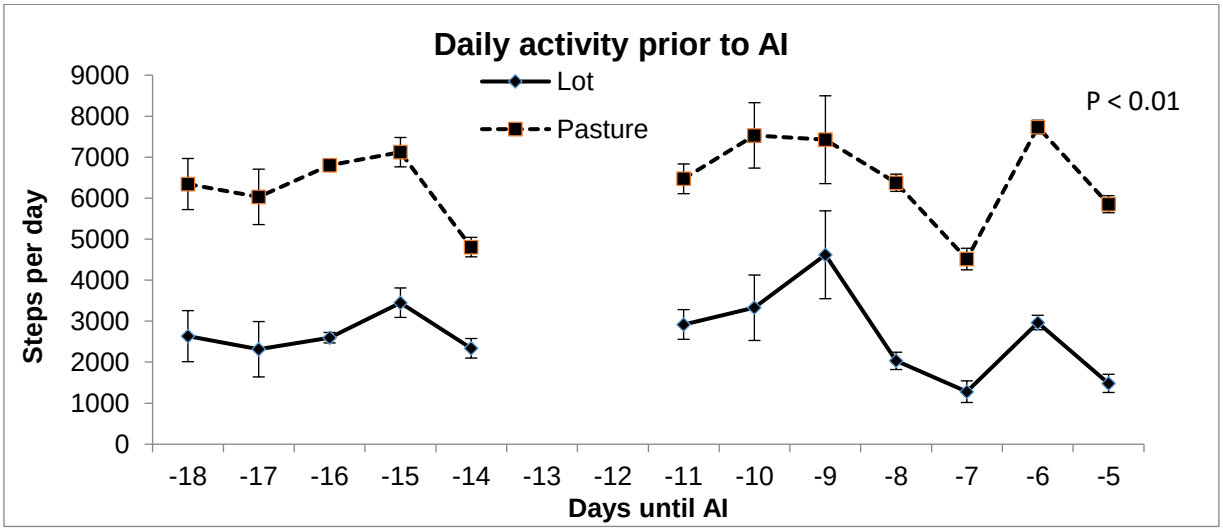

Figure 8. Daily activity for heifers that remained in the drylot until AI (LOT), and heifers that were moved to graze spring forage for the 42 days prior to AI (Pasture).

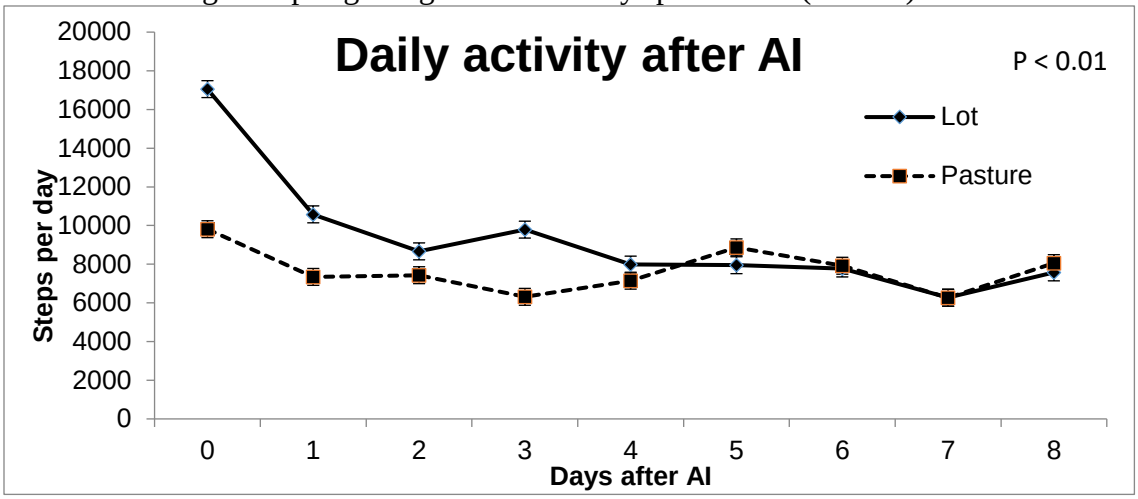

Figure 9. Daily activity for heifers that remained in the drylot until AI (LOT), and heifers that were moved to graze spring forage for the 42 days prior to AI (Pasture).

How do I determine what may have gone wrong during a FTAI program?

Occasionally the pregnancy rate following FTAI is much lower than expected. Trying to identify the root cause of a decreased pregnancy rate can be a daunting task due to the countless factors that can impact pregnancy rate following AI. When trying to trouble shoot what went wrong you should systematically work through the possibilities and not assume anything was done correctly – evaluate all the possibilities! A list of questions that may provide a systematic approach to identifying the problem is provided in Figure 10. Additional points to consider are included below.

Figure 10. Questions to ask when the pregnancy rate to FTAI is lower than expected. • What was the pregnancy rate following estrus synchronization and fixed-time AI (FTAI)? • Was the pregnancy rate low or do you have unrealistic expectations? Consider asking the following questions to an AI company representative, your veterinarian, or a beef reproduction specialist to identify potential causes of the reduced pregnancy rate.
1) What was the pregnancy rate in your heifers or cows after 60 to 80 days over the past few years? If less than 85% there may be other issues that should be addressed before initiating an estrus synchronization and AI program.
2) What was the nutrition (protein, energy, phytoestrogens, sulphates, etc) and mineral program before and after FTAI?
3) Did the animals meet the criteria for being good candidates for an estrus synchronization protocol (see earlier section)?
4) Did you use fixed-time AI or did you breed following detection of estrus? If you inseminated following detection of estrus, how frequently did you detect estrus (when did you begin and when did you end), what criteria did you use for detecting estrus, and when did you inseminate relative to detecting estrus?
5) What bull did you use and is there evidence that semen from this sire has resulted in acceptable pregnancy rates when using fixed-time AI or AI following estrous detection?
6) What protocol did you use and exactly when did you administer each of the products? You will need to confirm that the correct products were administered at the correct dosages and at the correct times. It is helpful to record on a calendar which product was administered on a particular day so you can check back to see if a mistake was made.
7) Was the biological activity of the various products compromised? You will need to verify that the products were not out of date and were stored and administered properly.
8) If using fixed-time AI, when did you inseminate the heifers or cows? Did you record who inseminated each animal? This will be helpful in identifying if there is a technician problem.
9) Where did you obtain the semen, how was it stored, and was the semen thawed correctly?

What are the most common mistakes when implementing an estrus synchronization and AI program?

1. One of the most common problems accounting for reduced pregnancy rates following FTAI is that the heifers or cows do not meet the criteria for being good candidates for an estrus synchronization and AI program (see previous section).
2. The second problem is poor choice of an estrus synchronization protocol and(or) protocol compliance. If you have a mixture of cycling and anestrus animals at the beginning of estrus synchronization treatment, you need to use a protocol that includes a progestin (e.g. CIDR or MGA). Progestin treatment will increase the proportion of prepuberal heifers and anestrus

cows that will respond to the protocol. Furthermore, it is essential that each heifer or cow receives the correct estrus synchronization product, at the correct dose, and on the appropriate day.

3. A third problem is that the facilities don't allow the cattle to be inseminated properly within a 2 to 3 hour time period and(or) cause undue stress on the cattle. With a FTAI protocol you have to carefully consider how many animals you can inseminate properly within the designated time period (e.g. 66 ± 2 hr for CO-Synch + CIDR protocol) with a minimum of stress.

Summary

Artificial insemination in beef cattle is more practical than ever due to advances in estrus synchronization protocols, identification of sires with highly accurate EPDs, and a market structure that can identify and reward producers for the quality of their cattle. Above all, a successful estrus synchronization and AI program is dependent upon being proactive, well organized, and attention to detail. The success of these systems hinges on many factors. A check list of management tips that should be implemented before, during, and after estrus synchronization and AI is provided in Figure 11.

Figure 11. Check list of tips for a successful estrus synchronization and AI program.

Things to do before fixed-time artificial insemination

- Keep accurate calving, breeding, and pregnancy records.
- Animal identification should be clear and easily readable.
- Ensure herd health and disease prevention with a well-designed prebreeding vaccination protocol. Vaccinate females a minimum of 30 days before the breeding season begins.
- Decide which estrus synchronization protocol best fits your breeding program, facilities, and personnel (see protocol sheets in AI catalogs).
- Ensure all products are purchased and on-hand prior to initiation of the protocol.
- Prepare the calendar of actions to ensure protocol compliance.

Sire selection

- Determine if you will purchase or raise replacement heifers.
- Decide how you will market your calves.
- Select proven AI sires with high-accuracy EPDs that match performance goals.
- Purchase semen from a Certified Semen Services (CSS) collection facility.
- Prepare or update your semen inventory.
- Make sure females meet the criteria for being good candidates for estrus synchronization.

Heifer criteria

- Heifers should weigh 65% of their mature body weight by the start of breeding.
- At least 50% of heifers should have a reproductive tract score (RTS) ≥ 4 by two weeks prior to the start of synchronization or 6 to 8 weeks prior to the breeding season.

Cow criteria

- Synchronize and inseminate only cows with BCS at calving of ≥ 5 (1 = emaciated; 9.0 = obese).
- The average days postpartum of the group of cows to be synchronized should be ≥ 40 by the start of estrus synchronization and experience a minimum of dystocia.

Things to do at the time of estrus synchronization and artificial insemination

- Meticulously follow the estrus synchronization protocol!
- If detecting estrus, spend as much time observing the animals as possible.
- Use a minimum of one person to detect estrus per 100 head of cattle.
- Use estrous detection aids to facilitate visual observation of estrus.
- Use a properly trained technician for AI.

Things to do after fixed-time artificial insemination

- AVOID STRESS – keep things consistent and calm.
- To distinguish between AI and bull bred pregnancies at pregnancy diagnosis, you should wait approximately 10 days to turn in clean up bulls after AI.
- Pregnancy check by 75 days after AI via ultrasound or 80 to 90 days after AI via rectal palpation to distinguish AI from bull bred pregnancies.
- If cattle need to be shipped do so between days 1 to 4 after AI and avoid shipping cattle between days 5 to 42 after AI.
- Maintain breeding females on an adequate nutrition and mineral program.

PAY ATTENTION TO DETAILS!

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