Improving reproductive management in cow herds

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Introduction

Heifers should calve by 24 months of age to achieve maximum life-time productivity,[1] and heifers that lose a pregnancy or conceive late in the breeding season are unlikely to have adequate time to rebreed during a defined breeding season. However, heifers that conceive and calve early have more time to resume normal estrous cycles by the start of the subsequent breeding season. Therefore, early calving heifers are more likely to breed back as 2-years and continue to calve early in the calving season. This is economically important since heifers that calved during the first 21 days of the calving season had increased longevity in the herd compared to heifers that calved in the second 21-day period or later.[2] Furthermore, analysis of 3,700 calves at the USDA-Meat Animal Research Center indicated that for each day after the beginning of the breeding season that a calf is born, 2.4 pounds of weaning weight is lost (personal communication, R. Cushman).

As production costs increase, so does the importance of maintaining a cow. Research has indicated it takes the net revenue from ~ 6 calves to cover development and production costs of each replacement heifer (E. M. Mousel, unpublished data). Any cow that misses a single calving is not likely to recover the lost revenue of that missed calf. Therefore, longevity of a beef female is important to the sustainability and profitability of any beef operation. Considering the importance of longevity, an important question arises: 'Why are females culled from a beef herd?' According to the 2007-08 NAHMS survey, the greatest percentage of cows were culled due to pregnancy status (33.0%). Additionally, other reasons for culling included age or bad teeth (32.1%), economic reasons (14.6%), other reproductive problems (3.9%), producing poor calves (3.6%), temperament (3.6%), injury (2.9%), udder problems (2.7%), bad eyes (1.8%), and other issues (1.8%). Therefore, understanding how management decisions impact pregnancy success and longevity will affect the profitability and sustainability of an operation.

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 this rate is < 85%, there may be management issues that should be addressed before initiating a synchronization and AI program.

Criteria for heifers

Goal of all heifer development methods is to have heifers that have reached puberty and are of good fertility at the start of the breeding season. However, one should also consider how the method of development can influence management after insemination (via AI or natural service), and how this management can impact pregnancy success.

Puberty is influenced by genetics,[3] but developing heifers to lighter weights results in heifers being older when they reach puberty.[4,5] However, the timing of puberty is dependent on both age and weight.[4,6,7] Therefore, the idea of developing heifers to a specific target weight (i.e., 65% of mature weight) has become a typical management practice. Specific target weights vary across breeds because the age of puberty and mature weight will differ among breeds.[8] Across several breeds, heifers were 55 - 60% of mature weight when puberty was attained.[9]

Some recent studies have proposed that heifers can be developed to only 50 - 55% of mature weight prior to the breeding season. In one study, fewer heifers that 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 percent pregnant in a 45-day breeding season was similar between treatments.[10] In another study, heifers developed to 55% compared to 65% of mature weight and there was no difference among groups in percentage of pubertal heifers at 12 months of age, or yearling pregnancy rates after an 80-day breeding season.[11] However, more heifers developed to 65% of mature weight were pregnant during the first 45 days of the breeding season compared to heifers developed to 55% of mature weight.[11] Heifers developed to 55% of mature weight also tended to have increased postpartum intervals, taking longer to reinitiate estrous cyclicity after calving.[11] When crossbred heifers were developed to 50% of mature weight, 15.7% fewer heifers conceived during the first 30 days of the breeding season compared to heifers developed to 55% of mature weight.[12] These studies indicate that heifers should reach 65% mature body weight in order to conceive early in the breeding season.

Pregnancy success during the breeding season has been correlated with the percentage of heifers that reached puberty before or early in the breeding season,[13] and it has been reported that pubertal status is 1 of the main factors impacting conception rates.[14] This can be determined by reproductive tract scoring heifers 6 to 4 weeks prior to the start of the breeding season. A reproductive tract score (RTS), a subjective

measurement of the sexual maturity of a heifer, is obtained by transrectal palpation, and is based on the degree of uterine development and ovarian status (size of dominant follicle and presence or absence of a corpus luteum (CL). Each heifer is assigned a score of 1 - 5 with an 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. Approximately 50% of the heifers should have an RTS ≥ 4 by the start of breeding season. The uterine and ovarian dimensions of heifers for each RTS are described (Table 1).

Table 1. Uterine and ovarian characteristics for reproductive tract scores (RTS) in cattle

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

Early heifer management

There are many methods used to develop replacement heifers, but in many locations, heifer development usually involves placing heifers into a confined feeding situation from weaning to breeding. This allows for intensive management of nutrient intake and growth to ensure proper development for successful breeding. However, this heifer development method usually results in a diet transition from the development diet to grazing forage at the start of the breeding season which has the potential to influence reproductive efficiency and lifetime performance of the heifer.

Grazing skills and dietary habits are learned early in life,[15] resulting in the development of motor skills necessary to harvest and ingest forages,[16] and allowing animals to increase their consumption of forages.[17] Skills learned between weaning and breeding have been reported to carry through to the next grazing season.[18] Furthermore, the willingness to try novel feedstuffs declines with age.[15] Young livestock ingest small amounts of novel feedstuffs and gradually increase the amount ingested, if no adverse effects occur.[19,20] When introduced to novel feeds and/or environments, livestock may spend more time and energy foraging,[21] but ingest less feed.[22-24] Additionally, when heifers grazed forage from weaning to breeding rather than being placed in a confined feeding situation, they appeared to retain better grazing skills and had increased average daily gains into the subsequent summer.[18,25]

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 is influenced by a variety of factors including suckling, nutrition, age, dystocia, genetic variation, stress, and disease.[26-28]

Suckling

Postpartum beef cows that are suckled ad libitum have a longer postpartum anestrous period than cows suckled once daily or not suckled at all (reviewed[29]). 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 postpartum cows is frequently preceded by an ovulation without estrus, resulting in a short luteal phase.[30,31] 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 [MGA] or CIDR treatment) can initiate estrous cycles by simulating a short luteal phase and increase the number of females that respond to a synchronization protocol.

Nutrition

Proposed biological priorities for nutrient utilization (nutrient partitioning) in cattle include:[26] i. basal metabolism; ii. motor activity; iii. growth; iv. basic energy reserves; v. maintenance of pregnancy; vi. lactation; vii. additional energy reserves; viii. estrous cycles and initiation of pregnancy; and ix. excess reserves. These priorities demonstrate that reproduction, both resumption of estrous cycling and pregnancy, is a low priority, particularly for heifers calving at 2 years of age. Consequently, underfeeding energy and/or protein pre- and post-calving reduced both first-service conception rates and overall pregnancy rates, and increased the postpartum interval (reviewed[32]). Both suckling and nutrition interact to have a powerful effect on return to estrus in beef cows.

Assigning a subjective body condition score (BCS; 1 = emaciated to 9 = obese) is a simple method of assessing bovine

energy reserves. The scoring system evaluates the amount of fat cover at specific locations on the female. Cow BCS at calving has a critical role in determining postpartum interval length compared to BCS at the start of the breeding season (reviewed[33]). Consequently, prepartum nutrition level and maintenance of nutrition level postpartum has important effects on reproductive performance.[32]

Which estrus synchronization protocol should I choose?

When choosing an estrus synchronization protocol, there are several considerations. These include whether one wants to detect estrus and inseminate according to the AM/PM rule (heat detect), inseminate at a predetermined time (fixed time artificial insemination [fixed-time AI]), or detect estrus for 72 - 84 hours (depending upon the protocol) and inseminate any cows not detected in estrus at a fixed time. There are protocols that fit each of the preceding approaches. Other items to consider include the proportion of females that are cycling as well as the time, labor, and cost of the protocol. A tool that takes these items into consideration and helps in the decision-making process is available at https://www.BeefRepro.org.

Follicular development

In cattle, follicular formation and development begins during fetal development, but more is known about regulation of antral follicle growth. Follicular waves consist of the following 3 stages: recruitment, selection, and dominance. The bovine estrous cycle usually consists of 2 or 3 follicular waves, and each wave begins with the recruitment of a cohort of small antral follicles from the pool of growing small antral follicles. Recruitment of a cohort of follicles, ~ 3 mm in diameter, is stimulated on each ovary by a transient rise in FSH.[34] One follicle is subsequently selected from this cohort for continued growth and becomes dominant, whereas the remaining follicles in the cohort become atretic (die off). Dominance occurs when a single follicle has been selected and continues to grow at a faster rate than the largest subordinate follicle, and inhibits emergence of a new follicular wave.[35] Following selection and establishment of a dominant follicle, follicular recruitment is inhibited until dominance is lost, generally via atresia or ovulation. During a nonovulatory follicular wave, the dominant follicle eventually becomes atretic, and a new follicular wave is initiated.

Oocyte growth and development

When considering follicular maturity, one must first consider the oocyte, when it is capable of being fertilized, and its subsequent development into a viable embryo. Bidirectional communication between the oocyte and surrounding follicular cells likely has a role in the growth, regulation, and nutrition of the oocyte, and stimulating the production of regulatory factors by the oocyte and cumulus/granulosa cells (reviewed[37]). In cattle, oocyte growth begins in primary follicles and continues until the preovulatory stage of follicular growth.[38,39] Bovine oocytes grow from ~ 27 µm in diameter in primordial follicles, to 110 - 120 µm in diameter in tertiary follicles; at that point, the oocyte is capable of undergoing nuclear maturation (reviewed[38]). Transcriptional activity in the oocyte was detected as early as the secondary follicle stage (1-2 layers of cuboidal granulosa cells),[40] and was reduced once oocytes attained a diameter of approximately 110 µm.[39] During oocyte growth and development, mRNA and proteins are produced and stored,[41] developmental competence is gained with increased follicular diameter,[42] and low levels of RNA synthesis occurred in large oocytes.[39] Thus, developmental competence continued to increase with increased follicular diameter,[42] and oocytes > 110 µm in diameter (follicles > 1 mm) had a greater capacity to undergo nuclear maturation[39] compared to smaller diameter oocytes. When oocytes were collected from early atretic follicles (≥ 15% pycnotic nuclei) they were developmentally competent, but when oocytes were collected from small healthy follicles (≤ 3 mm in diameter) they were not fully competent.[43]

Ovulatory capacity

Knowing that an oocyte reaches a state that allows maturation (nuclear and cytoplasmic) and fertilization before follicular selection or dominance, we now have to ask the question: 'In a breeding program, when is a follicle capable of ovulating or being induced to ovulate?' Bovine follicles appear to acquire ovulatory capacity at a diameter of ~ 9 - 10 mm, coincident with several physiological changes including increased circulating estradiol concentrations.[44,45] Ovulation was inducible in healthy dominant follicles larger than 10 mm, but fewer follicles ≤ 11 mm ovulated (1 out of 25) with a 4 mg dose of LH compared to follicles ≥ 12 mm (16 out of 16).[44] However, follicles ≤ 11 mm ovulated in response to a larger dose of LH. A single injection of gonadotropin releasing hormone (GnRH) to cows on various days throughout the estrous cycle only induced ovulation and initiated a new follicular wave in 66% of animals.[46] Furthermore, an injection of GnRH does not rescue an atretic follicle from atresia.[47]

Mechanism mediating the acquisition of ovulatory capability may be expression of LH receptors on granulosa cells. Expression of LH receptor mRNA on granulosa cells increased from nondetectable levels (~ 6.7 mm) to detectable levels ~ 10.8 mm.[48] There was no detectible increase in LH receptor expression in granulosa cells of follicles from ~ 9 - 11 mm in diameter.[49] However, expression of LH receptor mRNA increased in the granulosa cell layer nearly 2-fold as follicles grew from 10.8 to 13.2 mm, and increased another 2-fold as they grew to 15.0 mm.[50] Therefore, the quantity of LH required to ovulate healthy dominant follicles is likely related to the quantity of LH receptors present on granulosa cells. Thus, ovulatory capacity is acquired around the time when the dominance phase of follicular development begins.

Oocyte quality

The endocrine environment of a preovulatory follicle has been correlated with oocyte quality and ability to undergo germinal vesicle breakdown.[51] Follicles containing oocytes that were more capable of being fertilized and developing to the blastocyst stage contained decreased follicular fluid concentrations of progesterone,[52] 3 - 8 fold greater aromatase activity, and increased amounts of the α subunit of inhibin.[53] Additionally, the ability of human oocytes to develop into embryos increased when they were collected from follicles having increased follicular fluid concentrations of estradiol, compared to oocytes collected from follicles that had lower concentrations of estra-

diol.[54] Thus, the timely utilization of RNA transcripts and proteins stored during oocyte development affects the ability of an oocyte to develop into a viable embryo.[41]

Subsequent progesterone production

Luteal progesterone is required for the establishment and maintenance of pregnancy,[55] and lower serum progesterone concentrations were coincident with embryonic loss between days 24 and 28.[56] Progesterone treatment from days 1 - 5 of the bovine estrous cycle enhanced embryo development and synthesis of interferon tau (IFN-τ) that corresponded to altered synthesis and release of polypeptides from the endometrium.[57] Progesterone stimulated endometrial secretions[58,59] and embryonic growth and development.[57,60] Furthermore, cows that had an earlier rise in progesterone had embryos that were further developed and produced more of the antiluteolytic protein, IFN-τ, by day 16 than cows that had a delayed rise in concentrations of progesterone.[61]

Estrus expression and detection of estrus

In cattle, the estrous cycle normally varies from 17 to 24 days, and the duration of standing estrus is generally 12 - 15 hours, with considerable variation among individual animals (range: < 8 - > 30 hours).[62] Primary sign of estrus in cattle is standing to be mounted. Secondary signs of estrus include frequent mounting, watery mucus from the vulva, and restlessness. Maximizing the estrus 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.[63] With an estrus detection rate of 95% and a conception rate of 70% (95 x 70 = 67%) ~ 67% of the animals would be pregnant, whereas only 39% would be pregnant (56 x 70 = 39%) with a 56% estrus detection rate.

Success of any estrus-based AI program requires detecting animals in standing estrus and inseminating at the correct time relative to the 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 - 24 hours); however, 26% of cows exhibited estrus for < 7 hours and had fewer than 1.5 mounts per hour.[64] 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 pm to 6 am. Furthermore, when cows were observed for standing estrus every 6 hours (6 am, noon, 6 pm, and midnight), estrus 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.[65] Therefore, detection of standing estrus can be one of the most time-consuming chores related to AI.

There are commercially available estrus detection aids that can be used in conjunction with visual observation to increase estrous detection efficiency in beef herds. 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. The number of mounts per estrus increases as the number of females in estrus increases.[66,67] This is likely due to formation of sexually active groups of cattle, which are known to increase the number of mounts per female.[68,69] In unsynchronized cattle, there will be fewer sexually active groups (or fewer animals per group) and less mounting activity. Therefore, improved estrus detection efficiency is an advantage of an estrous synchronization program. However, it is also true that frequent animal handling and restraint are stressors,[70] and increased handling and restraint of heifers during a synchronized estrus decreased the number of mounts per estrus.[71] Depending upon the estrus synchronization protocol used, FTAI should reduce the amount of animal handling associated with sorting estrual heifers at the time of insemination.

Effect of estrus expression on pregnancy success

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 exhibit 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 not displaying estrus to conceive since ovulation can be induced with GnRH treatment. There are 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 FTAI (Figure 1).

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 CL) 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 several physiological processes 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 subsequent 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.

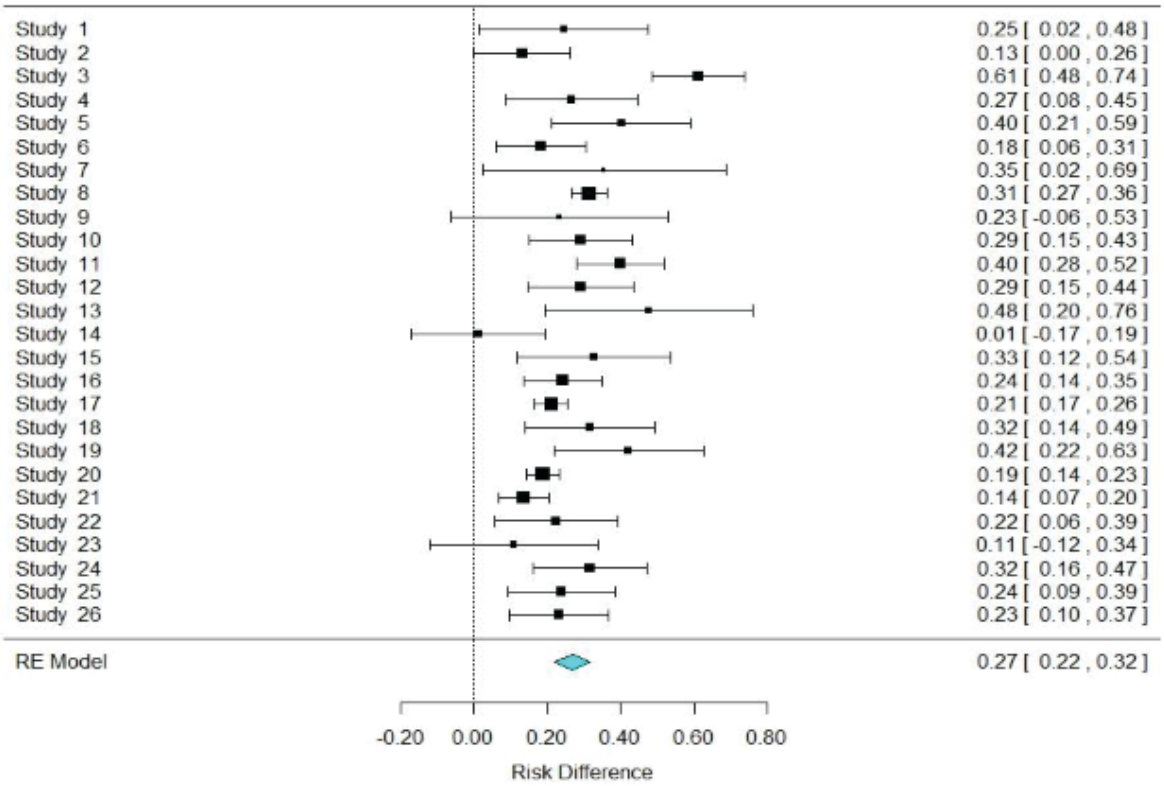

Figure 1. Effects of estrus expression around the time of fixed-time AI on pregnancy rate in beef heifers and postpartum cows. In each case animals that were detected in estrus around the time of fixed-time AI. A meta-analysis of these 26 studies (10,116 animals) indicates a 27% improvement in fixed-time 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%)

A study involving reciprocal embryo transfer of embryos to and from cows induced to ovulate either a large or small follicle with GnRH revealed some interesting results about the factors affecting fertility.[72] Although ovulatory follicle size and serum estradiol concentrations were highly correlated ($r = 0.49$), follicle size and estradiol concentrations had independent positive effects on fertilization success. Furthermore, donors with greater estradiol concentrations at the GnRH-induced ovulation were more likely to yield a fertilized embryo than an unfertilized oocyte.[73]

Preovulatory estradiol has a pivotal role in initiating standing estrus and the ovulatory cascade (reviewed[74]), programming uterine hormone receptors during the subsequent estrous cycle,[75-77] and uterine sperm transport.[78,79] When an injection of estrogen, preceding long-term progesterone treatment was omitted in ovariectomized ewes, embryo survival following embryo transfer,[80] uterine weight, uterine protein, RNA to DNA ratio, and the rate of protein synthesis were decreased.[81] Concentrations of estradiol during the preovulatory period has also been associated with the number of endometrial progesterone receptors during the luteal phase,[76] and may permit progesterone to coordinate the timing of prostaglandin $F_{2\alpha}$ (PGF) secretion.[76] Thus, the ability of a dominant follicle to produce and secrete sufficient estradiol to initiate estrus behavior is likely a good indicator of follicular maturity in an in vivo breeding program and may have a critical role in fertility.

Timing of insemination following estrus detection or fixed-time artificial insemination

When utilizing an estrus synchronization protocol requiring estrus detection, insemination occurs ~ 8 - 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 that same 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 coincides with the time when the oocyte is viable (8 - 10 hours 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 completes capacitation (process within the female tract where sperm gain the capacity to fertilize the egg) and are capable of fertilizing the oocyte. Insemination time 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 while it is still alive.

With fixed-time AI protocols, time of insemination becomes a compromise between maximizing the proportion of females showing estrus before insemination, and not waiting too long such that heifers or cows that were first to show estrus are in-

seminated too late. There can be variation in the fertility of sires used in a fixed-time AI 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.

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, and in some cases at the right time of day. For example, the interval from PGF to GnRH and insemination must be in accordance with what is recommended in the protocol sheet. The recommended time of insemination relative to PGF treatment is based on research trials and should be strictly followed. In addition, estrus synchronization products must be stored, handled, and used correctly. Should a mistake occur in product use or the treatment timeline, seek advice immediately. 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 given, 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. Additionally, producers can visit https://www.iowabeefcenter.org/estrussynch.html, to have a calendar auto-calculated for them.

Impact of vaccination on pregnancy success

The question is often asked, 'Can I save time and labor by vaccinating cattle at the start of the synchronization protocol?'

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,[82,83] with the hypothesis that the virus can enter large dominant follicles and disrupt the formation and development of the CL. However, recently developed estrus synchronization or fixed-time AI protocols control follicular development by inducing ovulation at the start of the synchronization protocol. Thus, insemination should occur on the second ovulation after the start of the protocol.[84,85] Consequently, a study was conducted to determine the effect of vaccinating naïve heifers with either a MLV or inactivated virus vaccine (IVV) at the start a fixed-time AI protocol.[86]

No control heifers (nonvaccinated) experienced an abnormal estrous cycle following AI (an estrous cycle where concentrations of progesterone decreased to < 1 ng/ml prior to day 15 after AI or concentrations of progesterone never increased above 1 ng/ml). However, heifers vaccinated 36 and 8 days before AI with an IVV (ViraShield® 6VL5HB) experienced 10% abnormal cycles. Heifers vaccinated only 8 days before AI with the same IVV experienced 14% abnormal cycles. There was no difference between these groups, and both were similar to control. Heifers vaccinated with a MLV 8 days before AI (BoviShield Gold® FP 5 VL5) had 38% abnormal estrous cycles compared to control heifers. In addition, bulls were with the heifers for only 14 days following AI, thus heifers had only 1 chance to conceive unless they experienced an abnormal estrous cycle. Of the heifers that experienced an abnormal estrous cycle, 100% of heifers vaccinated with an IVV conceived during the breeding season, whereas only 38% of heifers vaccinated with a MLV conceived during the return cycle (Table 2).

When heifers that conceived following an abnormal estrous cycle were considered open to allow comparison of conception rates following artificial insemination, pregnancy rates were similar among control heifers (90%) and heifers vaccinated 36 and 8 days before AI with an IVV (81%). 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%). Pregnancy rates for heifers vaccinated only 8 days before AI with an IVV (71%) were intermediate.

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]	-----

Within a column, means without a common superscript are different ([ab] $p < 0.05$)
Adapted from Perry et al., 2013[86]

Thus, it has been well established that vaccination of naïve heifers with a MLV around the time of breeding has negative impacts on CL development and pregnancy success,[87-89] even when utilizing a synchronization protocol that induces ovulation of the dominant follicle at the start of the protocol[86] This negative impact on pregnancy success has been reported on not only first service conception rates, but also on animals conceiving during the second service following vaccination.[86,87] In some heifers infected with BHV-1 at or near estrus, normal estrous cycles were delayed for up 2 months.[90] Furthermore, BVDV antigen has been detected in the ovary up to 30 days post-vaccination,[91] illustrating the duration of estrous cycle dysfunction that may occur following viral infection.

Previously vaccinated animals

The same effect of abnormal luteal function that occurs post-vaccination of naïve animals was not reported when previously vaccinated heifers were vaccinated with a MLV.[92] Few studies have attempted to measure the effect of vaccinating

on previously vaccinated (nonnaïve) beef animals,[93, 94] and 1 deficiency in these studies is the lack of true control (unvaccinated animals) to compare 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 reported no differences in conception rates between vaccinating nonnaïve primiparous dairy cows (3 MLV as calves, and 1 prebreeding as a heifer) with either a MLV or IVV 45 days prior to FTAI.[95] In a study,[96] heifers were vaccinated with either a MLV or IVV 40 and 10 days, or 61 and 31 days prior to a 45-day breeding season (n = 30). Among heifers vaccinated 40 and 10 days prior to breeding, those vaccinated with the IVV had a 20% greater pregnancy rate compared to those which received the MLV. Heifers vaccinated at 61 and 31 days prior to breeding with an IVV had a 15% greater pregnancy rate compared to those vaccinated with an MLV. Another recent study[97] reported a 20% decrease in pregnancy rate between heifers vaccinated with 2 doses of MLV compared to heifers given 2 doses of saline, but in these studies, animal numbers were small, limiting their ability to detect small differences in pregnancy success. However, the large numerical differences between those vaccinated with a MLV and non-vaccinated controls poses the question, "Does vaccination 30 days prior to the start of an AI breeding season negatively influence breeding season pregnancy success?" A study was conducted to examine differences in pregnancy success between beef females vaccinated with either a MLV (BoviShield Gold® FP 5 L5 HB) or an IVV (ViraShield® 6 L5 HB) 30 days before the breeding season, with sufficient power to detect a difference of less than 10% in pregnancy success (9 herds with 1436 animals) between groups.[98] Conception rates to fixed-time AI tended to differ between MLV treated animals and IVV treated animals, but control animals were intermediate. 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 compared to the MLV group. Animals treated with a MLV also had decreased pregnancy success compared to controls, but there was no difference between IVV and controls. Following the breeding season, pregnancy success was similar between MLV and controls as well as between the IVV and controls, but there was a difference between MLV and IVV (Table 3).

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

Within a column, means without a common superscript are different ($^{ab}p = 0.055$; $^{cd}p \leq 0.01$)
Adapted from Perry et al., 2016[86]

It is commonly thought that IVVs provide some protection against viruses that cause infectious reproductive diseases, but not the same level of protection that a MLV provides.[99,100] However, a recent publication reported 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 BVDV and IBR challenge as animals vaccinated with a MLV (Bovi-Shield Gold 5 FP) before their second breeding season.[97] Therefore, with the ability of CA/IV's (CattleMaster Gold FP5's) 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) or a CA/IV (CattleMaster Gold FP5) between 27 and 89 days before the breeding season, with sufficient power to detect a difference of less than 10% in pregnancy success (10 herds with 1565 animals) between groups.[101] Conception rates to AI were greater in the CA/IV group compared to the MLV group (60 versus 52%). Furthermore, interval from vaccination to AI also influenced conception rates. Animals vaccinated 27 - 30 days prebreeding and animals vaccinated 30 - 37 days prebreeding had similar conception rates; however, both were decreased compared to animals vaccinated 38 - 89 days prebreeding (64%; Table 4).

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 such diseases, as outbreaks can be potentially devastating to a beef herd. This emphasizes the importance of proper vaccination of females prior to entering a 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 MLVs, even when given at labeled prebreeding intervals, may negatively affect reproductive parameters compared to cattle vaccinated with IVVs.

Table 4. 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 - 30 days	52[a]		
30 - 37 days	52[a]		
38 - 89 days	64[b]		

Within a column, means without a common superscript are different ([ab] $p < 0.05$)
Adapted from Perry et al., 2018

How do I choose a sire for the breeding season?

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: i. Will I raise my own replacement heifers or purchase them?; and ii. How will I market my calves? Answers to the preceding questions will determine the traits that need to be emphasized. If a producer raises their own replacement heifers, then selection pressure should be placed on maternal traits such as milk, maternal calving ease, stability, etc. However, if replacement heifers are purchased, then emphasis on maternal traits in the herd may not be as important. When selecting a sire, a producer should also consider how they will be paid (e.g., pounds of weaning weight, carcass weight, carcass quality) and let this affect sire selection decisions. Producers who sell their calves at weaning should 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.

A single bull's genetic contribution to a herd can be much greater than that of any female due to an individual sire's ability to service, both naturally and artificially, numerous females. Therefore, it is not only important for a herd bull to be capable of breeding, but also provide genetic improvement to the herd. For the previously mentioned reasons, and because > 90% of beef cows in the US are bred by natural service, it is important that bulls be managed to optimize breeding performance.

In 2017, the percentage of operations that acquired bulls for the breeding season and performed a semen test, took scrotal measurements, or tested for *Tritrichomonas foetus* (trich) was 66.8, 57.0 and 53.6%, respectively. These percentages were greatly decreased when looking at operations that were using bulls residing on farm for at least 2 breeding seasons, such that the percentage of semen tests performed, scrotal measurements taken, and trich tested for was 31.4, 22.1, and 20.8%, respectively.[102] The low percentage of operations implementing reproductive examination procedures on bulls is concerning and demonstrates the importance of educating producers on the large effect sires can have on the economic return of an operation, but also the lack of confidence producers have in the examination procedures available to them.

Attainment of puberty

A bull is considered to have reached puberty once an ejaculate collected via electroejaculation contains a minimum of 50 x 10^6 total sperm with at least 10% progressive motility. When age was used as predictive measurement of the achievement of puberty in several breeds of bulls, age varied by 62 days. However, the scrotal circumference measured at the onset of puberty averaged 27.9 cm and ranged from 25.9 to 30.1 cm, indicating a higher accuracy in predicting the attainment of puberty.[103] Thus, once scrotal circumference reaches 27 - 29 cm, a bull is considered pubertal.[104] However, the ability of a bull to produce semen does not indicate good fertility. The percentage of 12-, 14-, and 16-month-old bulls that were reproductively mature and produced good quality semen was 35, 60, and 95%, respectively.[105] This demonstrates that while a bull may have met the minimum requirements to be considered pubertal, sperm quality and quantity will likely continue to increase for several months following initial sperm production.

Breeding soundness examination

One of the most commonly used methods to assess male fertility is a breeding soundness exam (BSE) that when completed correctly, will evaluate semen quality, scrotal circumference, and physical fitness. A BSE is only effective when the bull is pubertal, and only provides a snapshot of that bull's reproductive potential on that given day, meaning it cannot be reliably used to predict how the bull will continue to perform. This is largely due to the fact that sperm production is a continuous process, and thus when a BSE is performed, the sperm production measured is only capturing that of a specific time; therefore, the classification a bull receives at the completion of one BSE may differ from the classification that same bull receives at the completion of a BSE performed at a later date.

Minimum requirements

The Society of Theriogenology indicates that in order for a bull to pass a BSE, they must have obtained a minimum scrotal circumference based on their age, exhibit > 30% motility, and have at least 70% morphologically normal sperm.[106] Bulls meeting the preceding minimum requirements are classified as **satisfactory potential breeders**. However, when the minimum requirements are not met, the bull will be classified as either **deferred** (indicating that the bull should be tested again at a later date) or as an **unsatisfactory potential breeder** (suggesting the bull should be culled). These examinations should

occur ~ 6 - 4 weeks prior to the start of breeding season, as this will allow time for bulls that are deferred to be retested, or for producers to find a replacement bull.

Factors not evaluated in a breeding soundness examination that affect fertility

Libido, or the desire/willingness to mate, is heritable and has a positive effect on pregnancy rates to natural service but is not commonly measured. Scrotal circumference, semen quality, and mating ability are not correlated to libido and thus while a bull may be classified as a satisfactory potential breeder, his libido could be low. Observing bulls when they are exposed to estrual females could be advantageous to producers, especially in single-sire pastures.

Social dominance should be taken into consideration when deciding which bulls to pair together in multiple sire breeding pastures, as social rankings exist among bulls and may influence the number of females a particular sire is willing and able to breed. Producers should pay special attention to sires that have poorer semen quality (smaller scrotal circumference, borderline motility and morphology) but are socially dominant, as this dominance could prevent a more fertile, but less dominant bull from being able to breed as many females as possible.

Variation among satisfactory potential breeders

A BSE examination and its minimum parameters help producers cull bulls that have suboptimal reproductive qualities; however, research has shown there is significant variation in fertility among bulls who met the minimum requirements of a BSE. An example is the significant differences in pregnancy per timed artificial insemination (P/TAI) that existed among 3 bulls that all passed a BSE, where Bull A (48.1%) and B (47.7%) had greater P/TAI than Bull C (40.7%). In another study, 2 bulls who had similar semen characteristics, such as sperm plasma membrane viability, DNA stability, and percent total and progressively motile sperm, resulted in differing pregnancy rates after AI, with Bull A obtaining 71.2% P/TAI and Bull B obtaining only 27.8%. The previously mentioned results indicate that although a BSE is a tool that should continue to be used in selection against infertile bulls, other tools need to be developed to identify sub-fertile sires.

When considering new tools that may identify subfertile bulls, it is important to keep in mind that male fertility is often correlated with sperm motility, abnormalities, DNA status, mitochondrial function, and membrane integrity.[107] Preferably, the tools developed to evaluate fertility would be noninvasive, while identifying bulls capable of producing sperm that can: i. reach the fertilization site; ii. fertilize the ovum; and iii. contribute to early embryonic development.[108]

Future directions

It was believed that sperm only delivered the paternal genome to the oocyte. Research has since provided evidence that in addition to the sperm's centrosome for reactivation of meiosis II[109] and sperm specific phospholipase C that has a role in activation of embryonic development,[110] ~ 5 - 10 fg of paternal RNA can be delivered to the oocyte.[111] Among the paternal RNA is coding and noncoding RNA,[112] which includes microRNAs (miRNAs).[113]

MicroRNAs

MicroRNAs are small noncoding RNA molecules, highly conserved among species, and approximately 22 nucleotides in length that alter protein translation post-transcriptionally.[114] Upon the discovery that miRNAs from sperm enter the oocyte, the question arose: "Are microRNAs important to early embryonic development?" With the use of intracytoplasmic sperm injection (ICSI), oocytes were fertilized by sperm with partially depleted microRNA profiles. Resulting embryos had reduced developmental potential compared to embryos fertilized by sperm with complete microRNA profiles.[113] This experiment established that paternal microRNAs transferred to the oocyte at fertilization are indeed crucial to proper embryo development.

Proteomics and sperm longevity

As estrus synchronization and FTAI implementation increased, it became apparent that some bulls consistently performed well in a FTAI setting, indicating improved sperm longevity whereas other bulls had poorer conception rates in a FTAI setting compared to when they were used in either a natural service or estrus detection scenario. This observation stimulated inquiry into what factors contribute to sperm longevity and sperm transport.

Plasma membrane of sperm is coated with a plethora of glycoproteins,[115-117] and epididymis contains several enzymatic proteins involved in sperm protection[118] or motility.[119-121] To better understand how these proteins may contribute to longevity and/or transport, proteins from epididymal fluid, and sperm and protein from ejaculated fluid were analyzed. Proteins in the epididymal fluid likely affect motility and mitochondrial activity whereas proteins on sperm located in the epididymis are enzyme inhibitors or catalytic subunits of proteasomes. Meanwhile, tproteins in ejaculated fluid had little interaction with one another but could be involved in functions of the extracellular region. Conversely, proteins on ejaculated sperm are likely involved in enzymatic activities.

A basic BSE is the first step in identifying infertile bulls and will likely always be the starting point in identifying bull fertility as it can be conducted chute-side in a pasture. However, to separate subfertile from fertile bulls, work is completed to advance technologies investigating the role of surface proteins in sperm transport and longevity and the role of microRNAs in early embryo development and survival.

Inseminator efficiency

With AI, inseminator efficiency is influenced by both 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°C – 110°F[122-124], and inju-

ry to sperm cannot be corrected by returning semen to liquid nitrogen.[125,126] Conception rates are maximized when personnel: i. accurately identify and apply appropriate treatments to cows to synchronize estrus or ovulation; ii. accurately identify cows in estrus; iii. follow the AI stud's recommendations for thawing semen; iv. prevent direct straw-to-straw contact when thawing multiple straws simultaneously to avoid decreased postthaw sperm viability as a result of straws sticking together;[127] v. use appropriate hygienic procedures; vi. maintain thermal protection of straws during AI gun assembly and transport to the cow; and vii. deposit semen in the uterine body of the cow within ~ 15 minutes after thawing.

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.[128] Cervical insemination errors account for ~ 20% of attempted uterine body depositions,[129] and resulted in a 10% decrease in fertility when compared to deposition of semen in the uterine body.[130] AI technicians must develop sufficient skill to recognize when the tip of the AI gun remains in the cervix.

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, yet pregnancy rate at 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.[72] These inefficiencies are likely due to either ovulation of an immature oocyte that compromises fertilization and embryo survival, or ovulation occurring before the follicular cells have fully matured to produce sufficient estradiol during the preovulatory period and subsequent progesterone to adequately prepare the uterus for pregnancy. The preceding study[72] was designed to differentiate between follicular effects on oocyte quality and uterine environment on pregnancy success in beef cattle.

To understand how stress may increase embryonic mortality, one must first understand embryonic development (Table 5). Just like the estrous cycle, embryonic development begins on day 0, the day of standing estrus. This is the day the female is receptive to the male and insemination occurs. Ovulation occurs on day 1, ~ 30 hours after the first standing mount day 0.[131] If viable sperm is present, fertilization occurs within 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.[132] Between days 5 and 6, the embryo migrates into the uterine horn, and by day 7 to 8 the embryo forms a blastocyst.[132-134] At this stage, 2 distinct parts of the embryo can be observed: i. the inner cell mass that will form the fetus; and ii. the trophoblast, which will form the placenta. Between days 9 and 11, the embryo hatches from the zona pellucida.[132,134]

Table 5. 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:[132-135]

Then on days 15 - 17, embryo produces a chemical signal to prevent CL destruction, allowing the cow to remain pregnant.[134] The embryo begins attaching to the uterus on day 19, and by day 42 the embryo is fully attached.[134]

Mechanisms associated with pregnancy establishment

After examining the effect of 12 or more factors on pregnancy rate at day 27, ~ only 10% accounted for the variation in pregnancy rate.[72] 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 concentration on day 7, and serum estradiol concentrations at insemination. Positive effects of estradiol and progesterone were independent and likely aid in establishment of a maternal environment that is conducive to pregnancy establishment.[136]

Mechanisms associated with pregnancy maintenance

GnRH-induced ovulation of small dominant follicles resulted in increased late embryonic/early fetal mortality in postpartum beef cows.[137] The majority of the preceding late embryonic/fetal loss occurred around the time of embryonic attachment to the uterus day 27 - 41.[136] Late embryonic/early fetal mortality is commonly reported during this timeframe 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

Knowing the critical time points in embryonic development, it is possible to understand how stress from shipping can result in increased embryonic mortality in cows (Table 6). When animals are loaded on a trailer and hauled to a new location, they become stressed which causes the release of stress hormones. These hormones lead to secretion of factors that alter 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 changes in the uterine environment. The most critical time points are between days 5 and 42 after insemination. Prior to day 5, the embryo is in the oviduct and is therefore not subject to changes in the uterine environment. After day 42, shipping stress is less influential on embryonic loss. Upon complete attachment to the uterus, the embryo is supported by the dam and appears less susceptible to environmental changes. Shipping between days 5 and 42 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 - 13 days after AI (when they were transported) reduced pregnancy losses by about 9%.[138] However, treatment of flunixin meglumine 10 - 15 days 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%.[139] Taken together, these studies provide evidence that some heifers are more susceptible to the stress of handling than others (Table 6).

Table 6. Effect of time of transport after insemination on pregnancy rates

	Days after insemination that transportation occurred			
	1 - 4	8 - 12	29 - 33	45 - 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,[140] and T. W. Geary unpublished data

Heat stress and embryonic mortality

The best time to ship cattle is during early stages of embryonic development. However, this is also when the embryo is most susceptible to heat stress. Temperature, humidity, radiant heat, and wind affect heat stress in cows. The rectal temperature of cattle is normally 102.2°F, and an increase in rectal temperature by as little as 2°F can result in decreased embryonic development.[141] If rectal temperatures reach 105.8°F for as little as 9 hours on the day of insemination, embryonic development can be compromised.[142] Heat stress has also been reported to change follicular waves, resulting in reduced oocyte quality.[143] Researchers have reported that heat stress 42 days prior to[144] and up to 40 days after breeding can affect pregnancy rates,[145] illustrating how important it is to plan ahead for the breeding season.

Several methods have been researched to reduce the effects of heat stress in natural service or AI programs, such as shade, fans, and misters. These methods allow animals to stay cooler during the hottest parts of the day. In humid areas, misters may not be as beneficial as evaporative cooling is not possible.

Producers that utilize AI can also implement fixed-time AI protocols to increase pregnancy rates during the hot summer months. Fixed-time AI has increased pregnancy rates compared to animals inseminated 12 hours after estrous detection in conditions of heat stress.[146,147] This is most likely due to fewer animals showing signs of estrus when under heat stress. When the weather is too hot, animals tend to move around less and do not show signs of standing estrus. Estrus detection is a vital part of improving pregnancy rates. Since fewer animals are observed in estrus, fewer animals can be inseminated. In this case, fixed-time AI protocols that synchronize ovulation would be the best choice because estrus detection is not necessary.

Using embryo transfer during times of heat stress can also increase pregnancy rates. Fresh, high-quality embryos have been proven to increase pregnancy rates compared to AI in heat stressed cows.[148] Embryos at time of embryo transfer can adapt to the elevated temperatures.

Stress from change in diet

Changes in nutritional status can also have a tremendous influence on embryonic survival through many mechanisms. Heifers fed 85% of maintenance requirements of energy and protein had reduced embryo development on day 3 and day 8 compared to heifers fed 100% of maintenance[149] 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[150-152] early in life,[15] resulting in the development of preferences or aversions to plants, and the skills necessary to harvest and ingest forages efficiently.[16] Heifers that grazed forage from weaning to breeding rather than being placed in drylots appeared to retain better grazing skills and had increased ADG into the subsequent summer.[18,25] A decrease in feed intake from 120 to 40% of maintenance resulted in a loss of 56.3 lbs over 2 weeks (4.03 lbs/day),[153] similar to the losses reported[25] (Figure 2) when heifers that were developed in a feedlot from weaning to the following spring were moved to grass. However, heifers that were developed from weaning to the following spring on range with supplementation had no weight loss. Furthermore, heifers kept in a drylot until AI (n = 214) had decreased pregnancy rates compared to heif-

ers that had previous grazing experience (59 versus 49.1%). Therefore, post-insemination nutrition may influence embryonic survival. Nutritionally mediated changes to the uterine environment can occur by changing uterine secretion composition, or by influencing blood progesterone concentrations that regulate the uterine environment (reviewed[154]).

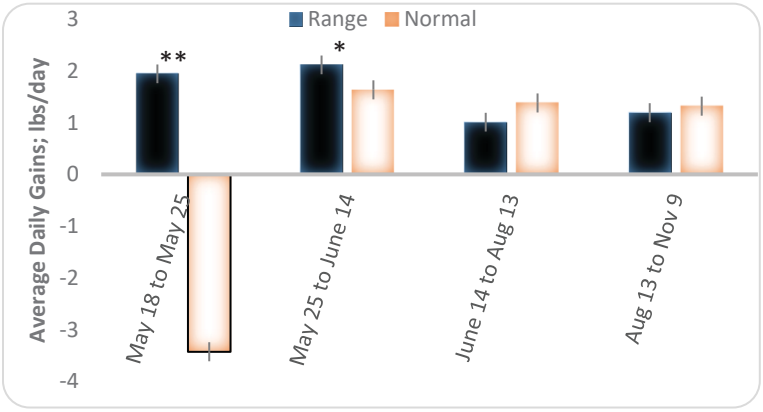

Figure 2. Average daily gain (lbs/day) of heifers weaned and developed on range (Range) compared to heifers weaned and developed in a drylot (Normal). All heifers were moved to the same pasture on May 18th (*p = 0.06; **p < 0.05)

In another study,[155] beef heifers (n = 164) were developed in a feedlot from weaning to breeding. At time of insemination, heifers were randomly allotted to 1 of 2 treatments: i. heifers were moved from the feedlot to graze spring forage; or ii. heifers were moved to graze spring forage and supplemented with Dried Distillers Grains (DDGS; 5 lbs/hd/day) for 42 days, when pregnancy success was determined. Heifers that were grazing spring forage without supplementation lost 37 ± 4 lbs, whereas heifers grazing spring forage with supplementation gained 45 ± 3 lbs from AI to pregnancy determination. 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 decreased pregnancy success to AI 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 1 location 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 1 of 3 treatments: i. heifers were moved to graze spring forage; ii. heifers were moved to graze spring forage plus supplemented with DDGS (5 lbs/hd/day); or iii. heifers were returned to the drylot for 42 days, upon which pregnancy success was determined. Body condition increased 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 drylot (5.9 ± 0.04 and 5.8 ± 0.04, respectively). Body condition did not change from day -7 to day 42 in heifers that were on grass alone (5.4 ± 0.05 and 5.4 ± 0.04 for day -7 and day 45, respectively). 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).

To further investigate if method of heifer development could impact grazing behavior, an experiment was conducted 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.[155] Drylot-developed heifers (n=69) were randomly allotted to 1 of 2 treatments 42 days before AI: i. heifers remained in the drylot until AI; or ii. 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 grazing spring forage took more steps per day compared to heifers in the drylot (Figure 3). Following AI, heifers that remained in the drylot until AI had increased activity compared to heifers that had previous experience grazing spring forage (Figure 4). 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.[156] Hence, heifers switched from a drylot to pasture were not accustomed to grazing, consuming a novel diet, and exert increased energy during the period following AI. These factors combined may be the reason that some heifers developed in a drylot and moved to forage after insemination have reduced conception rates. Therefore, having consistent management during the breeding season is important to achieving optimum pregnancy success.

How do I determine what may have gone wrong during a fixed-time AI program?

Occasionally, the pregnancy rate following fixed-time AI is much lower than expected. Trying to identify the root cause of a decreased pregnancy rate can be a daunting task due to the many factors that can impact pregnancy rate before and after AI. When trying to trouble shoot, you should systematically work through the possibilities and not assume anything was done correctly, but evaluate all possibilities! A list of questions that may provide a systematic approach to identifying the problem is provided in Figure 5. Additional points to consider are included below.

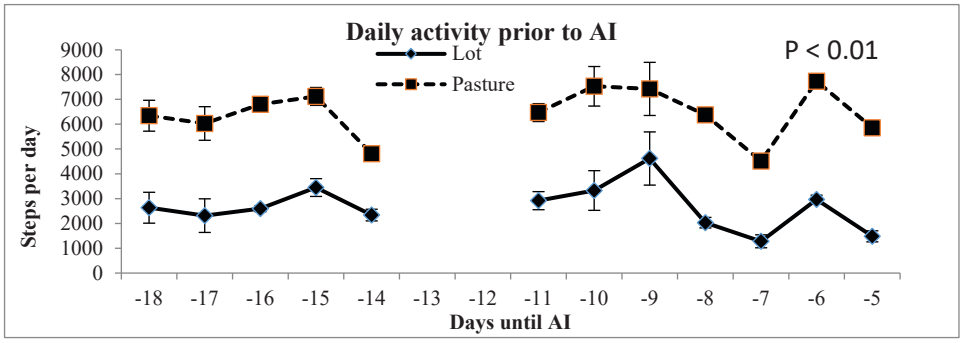

Figure 3. Daily activity for heifers that remained in the drylot until AI (LOT), and heifers that were moved to graze spring forage for the 42 days prior to AI (Pasture).

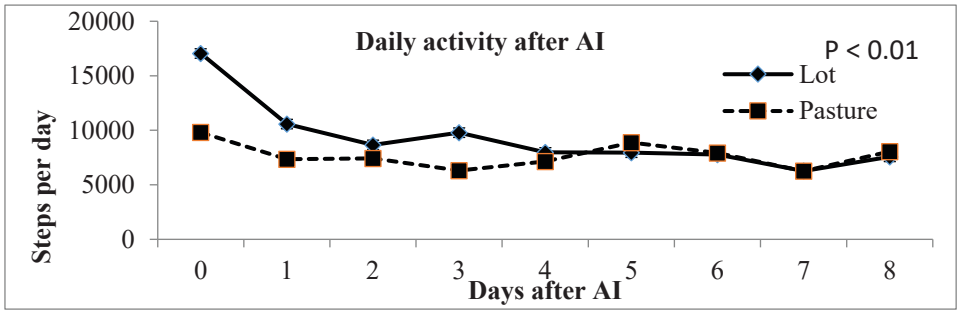

Figure 4. Daily activity for heifers that remained in the drylot until AI (LOT), and heifers that were moved to graze spring forage for the 42 days prior to AI (Pasture).

• What was the pregnancy rate following estrus synchronization and fixed-time AI? • 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 < 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 fixed-time AI?
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?

Figure 5. Questions to ask when the pregnancy rate to fixed-time AI is lower than expected

Things to do before fixed-time AI
• 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 pre-breeding vaccination protocol. • Vaccinate females a minimum of 45 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 at 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!

Figure 6. Check list of tips for a successful estrus synchronization and AI program

Conclusion

Profitability and sustainability are dependent on the longevity of each animal and the production of a live calf every year. Proper heifer development is essential to increase longevity and to maximize productivity. Heifers that are properly developed and conceive early in the breeding season had increased longevity compared to heifers conceiving late in the breeding season. In addition to getting heifers to conceive early in the breeding season, managing heifers to minimize embryonic losses is essential to maximizing productivity. Before implementing an estrus synchronization program, one needs to evaluate the current reproductive status of the herd, and select a protocol based on the goals and available resources of the operation. No matter if animals are inseminated by AI or by natural service, expression of estrus is the visual sign that confirms the body is ready for a pregnancy to begin. Thus, watching for estrus expression during the breeding season will help decrease surprises at pregnancy diagnosis time. Care also needs to be taken in how animals are managed before the breeding season. Animals need to be vaccinated at least 45 days prior to the start of the breeding season. Sires should be selected based on goals of the herd and how calves will be marketed, and sires should have, at minimum, a BSE before the breeding season. During and after the breeding season, abrupt changes in diet (i.e., moving heifers naive to grazing to grass or other forms of nutrient restriction) should be avoided as they can have negative impacts on pregnancy success. Thus, consistency and attention to details are critical for improved AI conception (Figures 5 and 6).

Conflict of interest

None to declare.

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