Review Report

When the plan goes awry: how to negotiate estrus synchronization errors in beef cattle

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Abstract

Estrus synchronization protocols vary widely, and errors during implementation are not uncommon. When an error is made, recommendations for resolution must consider the initial purpose of implementing the synchronization protocol in the herd. The ideal solution is to convert the protocol to a different published protocol. When this is not possible, the safest solution for maintaining an acceptable pregnancy rate is often either restarting a synchronization program or potentially foregoing artificial insemination for the season. Knowledge of estrous cycle physiology and how hormone treatments manipulate the cycle are foundational when attempting to modify a protocol based on the specific error that is made. Errors made early in a synchronization protocol tend to be more manageable than those made toward the end of the protocol. The use of less valuable semen and immediate introduction of a clean-up bull should be considered to maximize pregnancy outcomes when attempting to correct an error during estrus synchronization.

Keywords: Artificial insemination, bovine, estrus synchronization

Introduction

The goal of estrus synchronization is to create a herd of fertile females that will either express estrus and/or ovulate within an ideal timeframe. If breeding is based on estrus detection, a larger variability of timeframe may be acceptable. However, when fixed-timed artificial insemination (AI) is planned, a tighter synchrony of the group is critical.[1] Ovulation occurs ~ 30 hours after the onset of estrus in *Bos taurus*, providing a very narrow window in which successful pregnancy can occur as a result of insemination.[2] Success of synchronization is practically determined by pregnancy outcome, rather than estrus expression or ovulation rates. The determination of an ovulation rate is not practical, and neither the ovulation rate nor estrus expression is directly predictive of pregnancy outcome.

Numerous factors affect success of estrus synchronization, including the selection of a protocol that fits the herd type.[3] Protocols often differ for *B. taurus* versus *B. indicus*, dairy versus beef, and conventional versus sex-sorted semen. In the presynchronization period, animal health, animal nutrition, liquid-nitrogen tank maintenance, and pharmaceutical storage and date of expiration should be evaluated. Critical factors include proper dosing, route of treatment, and timing of pharmaceuticals during synchronization, and semen handling, AI technique, and AI timing. The ability of the producer to comply with drug treatment and AI timings should be considered when selecting a protocol. After synchronization, animal handling, stress, transport, and nutrition can also impact success.[3]

Errors during synchronization related to pharmaceutical treatment are not uncommon.[4] There are numerous published protocols that can be quite complex, particularly if presynchronization or resynchronization protocols are included. Most protocols use several pharmaceuticals, with varying frequency and/or dosage. Unfortunately, this results in seemingly infinite opportunities for error during implementation of a synchronization protocol. Ultimately, this also means that a ‘one-size fits most’ solution is not realistic or reliable.

Recommendations regarding solutions must be made carefully since a previous bad experience may negatively impact perception of estrus synchronization or AI.[5,6] The purpose of this review is to provide useful information for recommending a feasible solution after an error has occurred during estrus synchronization in a herd. This includes the following:

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1. a brief review of the purposes of implementing estrus synchronization; 2. four general categories of solutions to consider; and 3. core synchronization theory and the underlying estrous cycle physiology. A strong understanding of synchronization mechanisms and estrous physiology is vital to successfully modify an existing protocol. Superstimulation, superovulation, and estrogen-based protocols are outside the scope of the presented information.

Purposes of implementing estrus synchronization

The intended purpose of estrus synchronization must be considered when deciding on a solution for errors in protocol execution. Cost-prohibitive or time-consuming corrections may jeopardize the intended benefits of synchronization. Estrus synchronization protocols are used to increase economic impact, genetic selection, and overall efficiency of production in cattle herds.[7] Synchronization can increase pregnancy rates to fixed-time AI, embryo transfer, or live cover insemination,[8,9] which, in turn, increases profitability and decreases cost of rebreeding. More uniform calf crops of superior genetics can be produced when synchronization is combined with AI. This enhances the marketability of calves and increases their market value.[10–12] Some synchronization protocols can also induce cyclicity in noncycling individuals. However, pregnancy rates were significantly lower in females that were not cycling prior to synchronization/AI compared to those that were cycling.[13]

Critical evaluation of errors and determining solutions

With the purpose of estrus synchronization in mind, consideration can be given to 4 general solutions: restart the protocol, forgo AI for the season, convert to a different published protocol, or modify the protocol based on knowledge of synchronization theory and estrous cycle physiology.

Restarting a synchronization protocol is a straightforward option for the producer. If time in the breeding season allows, and the operation can cover the cost of restarting the synchronization protocol, this may be a feasible solution to circumvent errors made in the initial synchronization attempt. It may reduce the risk of another error resulting from lack of familiarity with a new or modified protocol.[14] However, the cost of restarting may render this option less desirable. Costs may include additional pharmaceuticals, extra labor, and loss of production associated with delayed breeding (i.e. lighter calf crop at weaning and shorter or shifted breeding season). These costs are quite considerable in a commercial beef operation but may be overcome in certain seed stock beef operations.[15] Restarting the protocol allows for a fresh start with an already known and prepared-for protocol.

A second general solution is to forgo AI and solely use clean up bull(s) for that breeding season. Ideally, the bull(s) must be able to breed multiple times while exposed to the herd over a window of time, minimizing negative effects if the error resulted in less synchronous ovulation. Bulls with high libido may achieve multiple services in a routine estrus synchronization program.[16,17] This option may reduce labor and pharmaceutical costs. One concern with this option is ensuring sufficient bull power for the synchrony of expected estrus. In a non-synchronized herd, the serving capacity of a fertile bull is expected to be 1 bull to 25 to 60 cows.[18] In a study of synchronized females, pregnancy rates were not affected by either the serving capacity ratio (ranging from 1:7 to 1:51) or the number of females exhibiting estrus when exposed to bulls classified as high or medium libido.[19] However, serving capacity may still be a concern as libido is not routinely tested,[20] and newer synchronization protocols may have tighter estrus expression compared to more simple protocols (i.e. Syncro-mate B or 'two-shot' prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$]).[19] Therefore, when considering this solution, the type of protocol in use and the stage of the protocol in which the error occurred must be considered.

In some cases, errors during estrus synchronization can be corrected. In an ideal situation, the correction changes the planned protocol to another published protocol. A good starting place to find additional protocols is a local extension website. Insemination, semen sexing, and embryo transfer companies have synchronization protocols listed and diagramed for producer use; these companies may also provide consulting, services, and products useful for executing protocols. The multistate Beef Reproduction Task Force is aimed at bringing research and extension together, and their website (https://beefrepro.org/) contains links for downloadable resources and protocol timelines.[21] Because protocols should be chosen based on the producer's needs and capabilities, the decision to transform an existing protocol once an error has occurred to a different published protocol requires evaluation of both the producer's abilities and the timing of the error in the initial protocol.[22]

Less ideal situations (i.e. unable to switch to another published protocol) are much more common, and modifications of the protocol in these instances are likely to result in poorer synchrony.[23] If breeding is based on estrus detection, lesser synchrony resulting from normal physiologic variability of the estrous cycle may not be of concern. Estrous behavior may be expressed earlier and for a shorter duration in *Bos indicus* cattle compared to *Bos taurus*.[24,25] Ideally, AI should be performed 6 - 24 hours prior to ovulation, which is ~ 2 - 14 hours after the onset of standing heat.[26,27] In a fixed-time AI protocol in which the window of insemination is narrow (± 2 - 4 hours), variability in the synchronization of ovulation is more detrimental.

Regardless of the program type, poorer synchrony that results from either a poorly timed luteinizing hormone (LH) surge or prolonged periods of follicular dominance is of particular concern. A narrow window of oocyte viability occurs after oocyte maturation is triggered by the LH surge.[28] Prolonged periods of follicular dominance reduce embryonic development and quality due to prolonged exposure of the oocyte to follicular estradiol, which has been correlated with delayed meiotic progression and blastocyst rate.[29–31] Because the LH surge and follicular dominance occur toward the end of the protocol, it is easier to correct errors occurring at the beginning of the protocol before these events are underway. Errors toward the end of synchronization tend to be more challenging to overcome. Fertility of the oocyte within a late atretic dominant follicle declines rapidly as crucial estradiol production declines and progesterone production slowly increases with granulosa cell aging.[32] The ovulated oocyte, arrested at metaphase II, is highly susceptible to postovulatory aging, resulting in decreased viability.[33,34] Decreased developmental competence, or the oocyte's ability to be fertilized and give rise to a healthy embryo, is significantly decreased due to postovulatory oocyte aging.[35] Decreasing estradiol concentrations due to the decline of the dominant follicle, and potential postovulatory oocyte aging can both compromise fertility near the end of the estrous cycle. Even females displaying estrus may have aged oocytes and be subfertile.[36] In these cases, modification of synchronization is highly situational, depending on the protocol implemented and the error that was made. Critical evaluation of what was given, not given, and the timing of these events in light of their physiologic purpose is crucial and complex. Therefore, the authors highly recommend use of less valuable semen and immediate introduction of a clean-up bull to attempt to maximize pregnancy outcomes if restarting synchronization and foregoing AI are not options. The only relatively simple error to overcome is when gonadotropin releasing hormone (GnRH) treatment at AI is forgotten. GnRH should be given as soon as possible to help ensure ovulation as close to insemination as possible, but it is likely that the majority of well-synchronized females will ovulate in an acceptable time frame, even if ovulation is less synchronous than initially desired.[37]

Integration of estrous cycle physiology and synchronization protocol

A strong knowledge base of synchronization theory and underlying estrous cycle physiology is critical in cases where modification of the protocol is the reasonable solution. Estrous cycle reviews are available that detail information beyond core concepts related to synchronization.[24,38] As the bovid has a continuous, monoovulatory, and nonseasonal estrous cycle,[24,38] synchronization protocols are designed to manipulate a group of females at variable stages of their cycle into 1 particular part of the cycle (estrus) at the same time. In a synchronization protocol, all individuals receive the same pharmaceutical at the same time, regardless of their status in the estrous cycle. Producers do not keep track of each animal's stage in the estrous cycle for practical reasons, and therefore, the entire herd receives uniform treatment protocol to minimize labor for producers and increase herd synchrony. Because the bovid is continually cycling, certain synchronization protocols may alter hormonal events midcycle and initiate the next cycle in which breeding will occur in an optimal timeframe.[39,40]

At synchronization, the 'ideal' cow (Figure. Cow A: Panels C and D) would already be cycling and nearing the end of her 21-day cycle, such that she would ovulate 6 to 24 hours[26,27] after planned insemination without manipulation. This cow would be 'ideal' because her cycle already aligns with the scheduled breeding at the end of synchronization. She would arrive at the planned breeding date and ovulate, without need for interference. It is impossible, however, to determine which cows, if any, are the 'ideal' member of the herd before synchronization. This means that the 'ideal' cow still received synchronization drugs along with her herdmates. However, most individuals in the herd will not be in this 'ideal' category, and their cycle will need to be manipulated to ensure that they ovulate at the appropriate time. When progesterone is low, ovulation results from a LH surge acting on the dominant follicle.[41] Synchronization of herd-mates (Figure. Cows B through E) is aimed at manipulating cycles to align 3 key requirements: a low progesterone environment, LH surge, and the existence of a dominant follicle containing a fertile cumulus-oocyte-complex.[42,43]

Low progesterone

A low progesterone environment is required for the dominant follicle to ovulate.[41] This environment is necessary for ovulation because high progesterone environments prevent an LH surge from occurring. Progesterone inhibits episodic GnRH secretion from the hypothalamus. Conversely, GnRH exerts a positive effect on the pulsatile release of LH from the anterior pituitary, eventually culminating in a preovulatory LH surge and subsequent ovulation. Under a high progesterone environment, the dominant follicle can become atretic, regress, and a new follicular wave begins.[41]

Progesterone is produced by the corpus luteum.[38] Circulating progesterone concentrations in cattle with a functional corpus luteum must reach serum concentrations > 1 ng/ml to be considered physiologically relevant.[44] In the natural estrous cycle, progesterone concentrations decrease (< 1 ng/ml) due to lysis of the corpus luteum that results from the luteolytic activity of $PGF_{2\alpha}$ as it binds to its receptors. The number of $PGF_{2\alpha}$ receptors on the corpus luteum increases from the early to late luteal phase of the cycle, peaking around day 16 when luteolysis typically occurs.[45] $PGF_{2\alpha}$ is produced by the uterus.[38]

To synchronize a low progesterone environment, $PGF_{2\alpha}$ treatment may be given to cattle. If the number of $PGF_{2\alpha}$ receptors is sufficient, lysis will occur and progesterone concentrations will decline.[46,47] Concentrations of serum progesterone decline to < 2 ng/ml within 12 hours after 30 mg of intramuscular $PGF_{2\alpha}$.[48] Typically, a corpus luteum will be capable of responding to a single dose of exogenous $PGF_{2\alpha}$ by days 5 - 7 of the cycle (if day 0 is the day when ovulation last occurred).[49,50] Although the majority of females in an unsynchronized herd will have a corpus luteum capable of responding (Figure. Panel E and F), a significant remainder will not (Figure Panel G).[51] Therefore, $PGF_{2\alpha}$ may also be used to presynchronize the herd in some protocols. The simplest example of this is a 'two-shot' $PGF_{2\alpha}$ protocol. In this protocol, the first $PGF_{2\alpha}$ treatment synchronizes the majority of females. After enough time has passed to allow their new corpus luteum to mature and be responsive to $PGF_{2\alpha}$, a second $PGF_{2\alpha}$ treatment is given. Females with a less mature corpus luteum at the first treatment likely did not respond. It is expected that these individuals' corpus luteum will be mature enough by the second $PGF_{2\alpha}$ treatment to respond and lyse or be close to natural lysis.[51,52]

Another way to create a low progesterone environment is the timely removal of progesterone treatment. Progesterone treatment for a short period prevents the female bovid from ovulating and subsequently developing a young corpus luteum. This period in which luteolysis is expected to occur in the protocol is depicted (Figure, Panel H). The source of progesterone is via a controlled internal drug release device (CIDR) and removal at $PGF_{2\alpha}$ treatment with the intent of creating a low progesterone environment in all animals at the same time.[53] Notably, *Bos indicus*-influenced cattle may be more sensitive to progesterone's effects during earlier aspects of the protocols. Improved pregnancy rates to fixed-time AI result when a functional corpus luteum is eliminated at the beginning of the protocol.[25]

Luteinizing hormone surge

The hypothalamic-pituitary-gonadal axis is differentially regulated by progesterone and estrogen. The hypothalamic surge center is responsible for inducing a LH surge in low progesterone environments when progesterone's negative inhibition on GnRH release is absent. Progesterone also negatively inhibits follicle stimulating hormone production, yet cohorts of small follicles are recruited due to transient rises in circulating

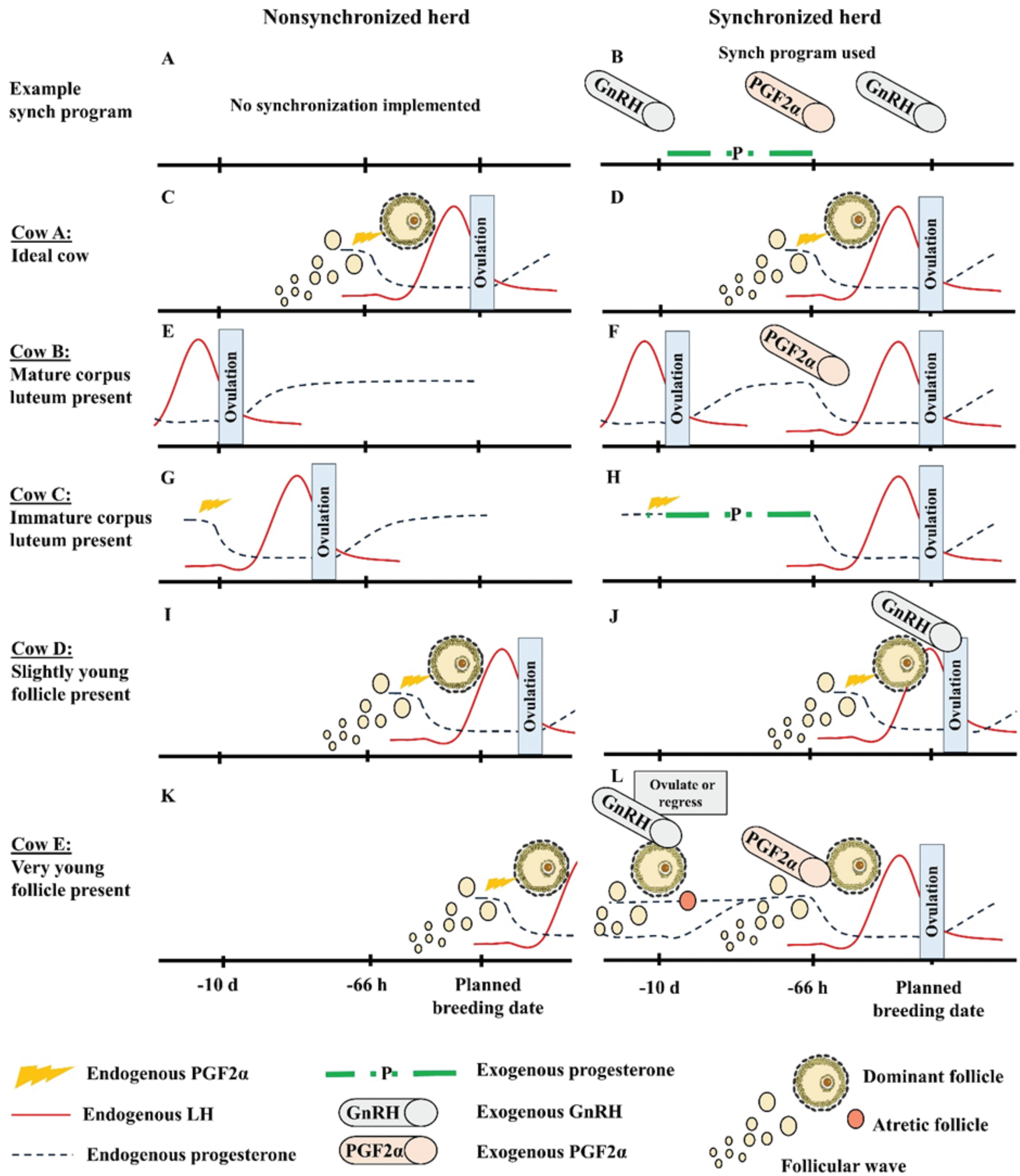

Figure. Use of estrus synchronization to modify cows at various stages of the estrous cycle in an example herd. A herd of unsynchronized females (Panels A, C, E, G, I, and K) may be at different stages of their estrous cycle at the start of a given protocol. Cow A is considered the ideal cow because no modification of her cycle is needed for her to ovulate near the planned breeding date (Panels C and D). In a herd of cycling females, the stage of the cycle in all cows is unknown, and therefore, all cows will be enrolled in an estrus synchronization program. Implementing an estrus synchronization program (e.g. 7-day CO-Synch + CIDR[68,79] [Panel B]), will modify the cycles of herd mates (Panels F, H, J, and L) to eventually coincide with the ideal cow by the time of the planned breeding date (Panel D). Note timeline presented is approximate, and cycles are not exactly to scale.

follicle stimulating hormone, even in a high progesterone environment. Eventually, 1 follicle is selected, and the growing dominant follicle produces increasing concentrations of estradiol during proestrus. After corpus luteum regression, and without the negative feedback of progesterone on GnRH, LH then increases in a pulsatile fashion.[54] These changes culminate in the LH surge and subsequently ovulation.[55] After ovulation, the estradiol producing follicle becomes a progesterone producing corpus luteum.[56] Detailed information on the hypothalamic-pituitary-gonadal axis and effects of progesterone are available.[25,53,57–59]

Although an LH surge may occur within estrus synchronization programs, the timing may be too variable for fixed-time AI programs (Figure. Panel I).[23,28] Theoretically, ovulation of a dominant follicle could be induced by stimulation of various aspects of the hypothalamic-pituitary-gonadal axis. This may include treatment with estrogens, GnRH, LH, or their analogues.[60] As estrogens are illegal in food producing species in the United States, a GnRH analogue is typically given. *Bos indicus*-influenced cattle have a highly variable and relatively low rate of ovulation to exogenous GnRH compared to *Bos taurus*.[61,62] An LH surge is expected to occur ~ 2 hours after exogenous GnRH treatment.[63,64] Ovulation occurs ~ 22 - 34 hours (in *Bos taurus*[63,64]) or 26 - 28 hours (in *Bos indicus*[65]) after the LH surge. Sperm must travel from the site of deposition (i.e. uterus during AI) to the site of fertilization (i.e. ampullary-isthmic junction of the uterine tube). To coordinate travel efforts of both gametes, GnRH is commonly given 16 - 24 hours prior to AI.[64,66,67] However, to reduce labor costs, GnRH is given at AI in some protocols (Figure. Panel J).[21,68,69] The majority of inseminations in North America utilize conventionally frozen semen. Protocols designed for insemination using other semen types (i.e. fresh, chilled, or sex-sorted), number of sperm (i.e. sex-sorted), or in other anatomic locations (i.e. uterine horn breeding) impact the timing of insemination and GnRH treatment.

Dominant follicle

In the natural estrous cycle, *Bos taurus* cattle can have either 2 or 3 follicular waves. The 2 wave cycle produces a follicular wave ~ every 10 days, whereas the 3 wave cycle produces a wave ~ every 7 - 9 days.[70] The average length of the estrous cycle is 21 days ± 2 days.[71] However, cattle with 3 wave estrous cycles tend to have longer cycles, landing in the 22+ day range overall.[70] *Bos indicus* cattle estrous cycle average length is 21 days for 2 wave and 22 days for 3 wave cycles. Four and 5 wave cycles have also been reported.[24] Each wave contains 3 stages: emergence/recruitment, selection, and dominance.[38] A dominant follicle is lined with mural granulosa cells possessing sufficient LH receptors to respond to LH surge.[72] Dominant follicles also produce inhibin, a hormone that prevents progression of other follicles.[32] As previously reviewed, a dominant follicle will become atretic in a high progesterone environment, as it is unable to ovulate.[53] In a low progesterone environment, the growing dominant follicle produces increasing concentrations of estradiol during proestrus, triggering the LH surge, and eventual ovulation.[55]

The natural variability in wave length[24,70] results in considerable challenges when attempting to synchronize a group of females. If wave timing is left unmanaged, some cattle may have significantly delayed ovulation.[73] This could result in subfertility due to aging of the sperm.[27] Currently, no therapies exist to change the rate of follicle development (i.e. speed it up or slow it down). Ovulation of the first wave dominant follicle results in reduced pregnancy per AI compared to the second wave. However, when progesterone was given, similar pregnancies per AI resulted.[74] For practical purposes, wave number is not a consideration related to estrus synchronization, particularly when the protocol includes progesterone treatment.

Altogether, current synchronization protocols rely on inducing a wave at the same time in all herd members, so that dominant follicles, capable of responding to LH surge, will be present at the same time.[75] Variability in ovulation timing due to wavelength can be managed by utilizing an induced LH surge. Induction of LH surge was described in the previous section (Figure. Panel J). It is worth reiterating that only a dominant follicle, with sufficient LH receptors, will ovulate in response to induced LH surge.[72] If the present follicle is much younger and, therefore, has not acquired dominance or the appropriate number of LH receptors, it is not capable of ovulation (Figure. Panel K).

Eliminating a large, inhibin-producing follicle helps to synchronize the next follicular wave in cattle.[32] Initially, this was performed by follicle ablation,[76] but most protocols now use a GnRH analogue for this purpose. GnRH exposure results in the induction of an LH surge and the regression (if high progesterone) or ovulation (if low progesterone) of any existing follicles.[64,77,78] After a new wave begins, sufficient time is allowed for the follicle to progress toward dominance and any young corpus luteum to mature. The corpus luteum is then lysed, followed by a second dose of a GnRH analogue treatment near the time of AI to cause ovulation of the dominant follicle (Figure. Panel L). Because exogenous GnRH induction of a new wave may result in a new corpus luteum,[64,77] subsequent use of $PGF_{2\alpha}$ is mandatory if the cow is to be bred before natural luteolysis would occur.[80]

A female bovid will not respond to the initial dose of GnRH analogue if a dominant follicle is not present at treatment. Therefore, some protocols include initial measures to create a persistent dominant follicle prior to this initial GnRH dose. Long-term (i.e. ~ 14 day) progesterone treatment is used.[81] During this period, the female will lyse her own corpus luteum but may not ovulate due to high concentrations of progesterone. Lysis of the mature corpus luteum may be induced by uterine release of $PGF_{2\alpha}$.[31,49] Under natural circumstances, the lysis of the corpus luteum will cease progesterone production and remove the feedback of progesterone on hypothalamic GnRH release, allowing for eventual ovulation and subsequent LH surge.[82] In cases where progesterone is given and a corpus luteum is not present, circulating progesterone concentrations are at physiologically relevant concentrations that are high enough to prevent LH surge but lower than the concentrations produced by a mature corpus luteum. These lower progesterone concentrations result in persistence of a dominant follicle, rather than follicle turnover. In the absence of LH surge (natural or induced), the dominant follicle persists.[83] When progesterone treatment is terminated, ovulation will occur, a corpus luteum will form, and a new wave will start. Synchrony of later waves is then used for breeding purposes. With these protocols, the initial oocyte in the persistent dominant follicle ages, and the herd will express unfertile estrus after the termination of progesterone treatment. Producers must wait to breed according to protocol for acceptable pregnancy outcomes.[14] A similar concept is used in the relatively new 7 & 7 protocol. Initially, exogenous progesterone and prostaglandin are given to mimic natural corpus luteum lysis and stalling of a dominant follicle for up to 7 days. The GnRH analogue in the middle of the 14 days of progesterone treatment ultimately results in a new wave that is then utilized for breeding.[84]

Conclusion

Errors during synchronization related to pharmaceuticals' treatment are not uncommon. The opportunities for error during implementation of a synchronization protocol can appear quite infinite and ultimately suggest that a 'one-size fits most' solution is not realistic. Recommendations for solutions must be made carefully, considering both the producer and the affected herd. With the purpose of estrus synchronization in mind, 4 general solutions can be considered: restart the protocol, forgo AI for the season, convert to a different published protocol, or modify the protocol. A strong understanding of synchronization theory and estrous cycle physiology is vital if the chosen solution is to modify an existing protocol. Synchronization protocols manipulate cycles to align 3 key requirements: low progesterone environment, LH surge, and the existence of a dominant follicle containing a fertile cumulus-oocyte-complex.

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Conflict of interest

None to declare.

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