

Strategic Interventions for Reproductive Management

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■ Take Home Messages

- ▶ The foundation of a high pregnancy rate is laid in the transition with active management to prevent, detect, and treat ketosis, metritis, and purulent vaginal discharge.
- ▶ Approximately 20% of cows are anovular at 60 DIM, so a program to attempt to identify and intervene in these cows should be part of routine reproductive management.
- ▶ A deliberate decision must be made to set an upper limit for the time of first insemination for both cows and heifers, and to implement the interventions needed to achieve this policy. Good programs exist to ensure that practically all cows are inseminated by about 85 DIM and heifers by 14 months.
- ▶ Programs exist to ensure that animals are re-inseminated by a maximum of 42 days after previous service, and these should be used to ensure that essentially all cows and heifers meet this target.

■ Introduction

It is critically important to herd profitability to optimize the proportion of heifers that are on-target for growth and pregnant by 13 to 15 months of age, and the proportion of cows that are pregnant between 85 and 120 days-in-milk (DIM). While probability of pregnancy per insemination (“conception risk”, (CR)) is important, the intensity or efficiency of insemination (insemination rate (IR) per 21 days) comes first and is much more readily changed with practical management programs. This paper briefly reviews elements of setting cows up to achieve reproductive targets of performance and opportunities or requirements for strategic interventions to increase consistent attainment of herd goals.

■ Metabolic Health in the Transition Period

The mechanisms of pathogen detection, immune response, and to a lesser degree, regulation of inflammation in the uterus of dairy cows in the postpartum period have been described (LeBlanc, 2012). It is hypothesized that reproductive tract disease represents a failure of the immune system to switch fast enough or far enough from the down-regulated state necessary for maintenance of pregnancy to a heightened state of function for postpartum clearance of bacteria and tissue debris, and then back to a basal state 3 to 4 weeks later. A desirable response seems to be a prompt and effective immune and inflammatory response in the uterus after calving. Excessive inflammation early during the postpartum period seems to be a key feature of cows with endometritis about 1 month later (LeBlanc, 2012). Generally, worse postpartum negative energy balance is associated with more severe or prolonged uterine inflammation.

It is generally understood that bacterial infection of the uterus initiates inflammation of the uterus. This inflammation is a normal adaptive response, but it may be inadequate for the task (i.e., the balance tips in favor of bacterial growth, inflammation, and tissue damage rather than clearance and healing – insufficient response) or inflammation may be disproportionate in degree or duration (excessive response). It is not clear if excessive or persistent inflammation is provoked by the type or quantity of bacterial infection, by genetic or metabolic influences on immune function and regulation, or both. Presently, more data are available to support the importance of immune response as a critical variable in the development of reproductive tract disease. This underlines the importance of understanding what determines the variation in the effectiveness of the inflammatory response to parturient tissue trauma and postpartum pathogen challenges.

■ Postpartum Reproductive Tract Health

Metritis

By definition, metritis is an obvious systemic illness that reduces production and cow wellbeing in the short-term. Limited data exist on the longer-term (full lactation) effects of metritis on production, reproductive performance, and culling. Diagnosis, impacts and treatment were recently reviewed (LeBlanc 2013). Briefly, metritis may practically be identified based on at least 2 of fetid discharge, fever, and signs of systemic illness (dullness, inappetence, or decreased milk production). Routine, systematic screening of fresh cows is likely useful to increase early detection of health problems, especially in large herds, but it is likely most useful if training and experience of personnel and facilities allow for assessment of the cows' attitude, appetite, ketosis status (once or twice weekly), rumination, and abomasal displacement.

Treatment of metritis is presently based on administration of systemic antibiotics. Data exist to support treatment of cows having metritis with ceftiofur or penicillin but clinical cure is only around 75 to 80% and impacts on subsequent health and reproductive performance are unclear. Drug concentrations are not maintained consistently above the target levels in the uterus of cows with metritis. Preliminary data indicate that delay in the onset of treatment of metritis – a “wait-and-see” approach to allow for spontaneous resolution in perhaps 20% of cases – is worth testing in larger randomized clinical trials.

Purulent Vaginal Discharge (PVD) and Endometritis

Each case of PVD and endometritis is associated with substantial reductions in subsequent reproductive performance and their effects are additive (LeBlanc et al., 2011). There is only moderate agreement between PVD and endometritis defined by uterine cytology. This leads to the question of the source of the pus in the vagina if it is not always from the uterus. Cervicitis exists as a distinct condition that is associated with both separate and additive impaired reproductive performance. Approximately one-half of cows with PVD have cervicitis and vice versa, and 50 to 75% of cows with endometritis have cervicitis and vice versa.

Accurate diagnosis of purulent vaginal discharge (formerly called clinical endometritis) requires examination of discharge in the vagina after a minimum of 3 wk postpartum, which may be done with a vaginoscope, clean gloved hand, or a Metricheck device. Subclinical endometritis is diagnosed by endometrial cytology obtained trans-cervically either by uterine lavage or cytobrush. Neither technique for subclinical endometritis is sufficiently rapid or practical for widespread use in clinical practice, although rapid cow-side tests have been explored.

Consistent evidence exists that cows with PVD have improved reproductive performance when treated with a single intra-uterine (IU) infusion of cephalosporin approximately 1 month before first insemination, relative to receiving no treatment. Several studies reported that 1 or 2 injections of prostaglandin F_{2α} (PGF) improved reproductive performance or produced clinical outcomes similar to IU antibiotics, but PGF consistently did not improve reproductive performance in cows with PVD, although many of these studies lacked valid case definitions, statistical power, or both. In a clinical trial with more than 2,000 cows, including more than 600 cows with PVD, cytological endometritis, or both, cows were assigned randomly to receive PGF at postpartum weeks 5 and 7 (Dubuc et al., 2011). Overall, or among cows with reproductive tract disease, no difference was detected in time to pregnancy between PGF-treated and control cows. Data from Dubuc et al. (2011) were re-analyzed to examine cows with PVD specifically and without regard to endometritis status i.e., to address the clinical question of treatment

of cows examined only for PVD (which is practical) but without diagnosis of endometritis by cytology (which is well validated, but impractical for routine clinical application). Among 323 cows with PVD at 5 weeks postpartum, clinical resolution (absence of PVD) at 8 weeks postpartum was 72% in cows that received PGF at weeks 5 and 7 and 58% in untreated controls ($P = 0.01$). Among these cows with PVD, 43% had a corpus luteum (CL; serum progesterone > 1 ng/ml) at week 5 and 63% had a CL at week 7; 69% had a CL at least once before either of the PGF injections. Accounting for parity, BCS at calving, occurrence of dystocia, retained placenta or twins, and herd, cows with PVD that received 2 injections of PGF tended ($P = 0.07$) to become pregnant sooner than untreated cases (hazard ratio = 1.2, 95% confidence interval 0.95 to 1.6). There was no interaction of the effect of PGF with the presence of a CL. Therefore, these results join others pointing to an equivocal effect of PGF for treatment of PVD. A recent trial showed that among cows with PVD, a program of treating those with a palpable CL with PGF produced equivalent reproductive performance as treating all with IU cephalosporin (McDougall et al., 2013). Different strategies for PGF as therapy for reproductive tract inflammatory disease merit further investigation.

Taken together, it seems that IU cephalosporin is beneficial for reproductive performance in cases of PVD (which may be associated with cervicitis or endometritis), but the benefit of PGF as therapy for PVD is unclear. Although 1 study (Kasimanickam et al., 2005) reported a benefit to reproductive performance of either PGF or IU cephalosporin relative to no treatment, further investigation of rapid cow-side diagnostic tests and treatments for cytological endometritis are needed.

Management practices to prevent metritis, PVD, and endometritis are summarized in Figure 1 and Table 1.

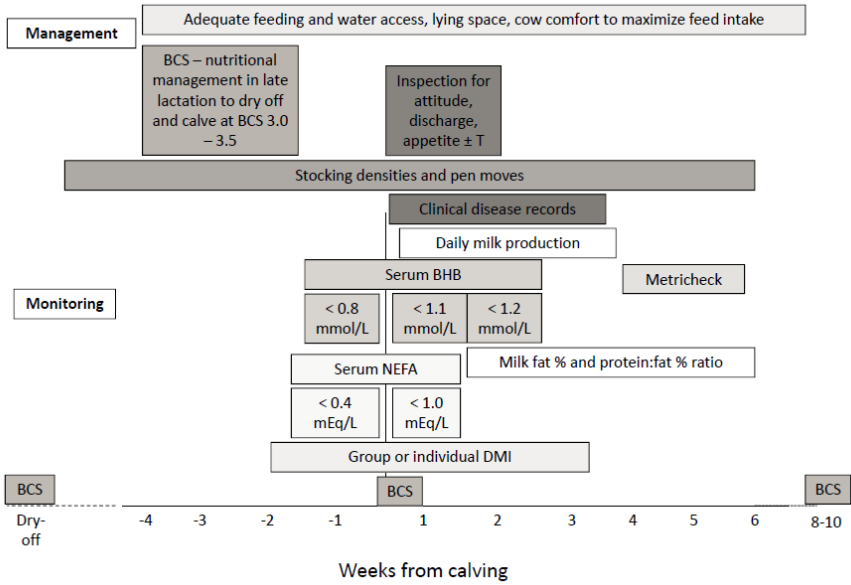


Figure 1. Suggested key management practices and monitoring metrics for transition dairy cow health. Good metabolic and reproductive health lay the foundation for good reproductive performance.

Table 1. Summary of management practices and monitoring targets to reduce the risks of reproductive tract disease in dairy cows

Recommendation
Prevent consumption of dietary energy above requirement in the “far-off” dry period (weeks 8 to 3 before calving)
Provide for unrestricted feed bunk access (i.e., all cows able to eat at the time of fresh feed delivery)
Provide 30 inches (75 cm) of linear bunk space per cow or no more than 8 cows per 10 headlocks
Provide space to allow for lying 11 to 12 h per day ≥ 1 free stall per cow or > 100 ft ² (~10 m ²) of bedded pack per cow
Minimize pen moves and social group changes
Build dry cow and fresh pens for approximately 130 to 140% of the expected average number of calvings per month
Provide heat abatement (fans and sprinklers) when the THI exceeds 68
Manage nutrition so cows calve at BCS of 3.0 or 3.25, and maintain a minimum BCS of 2.5
<u>Monitoring methods and targets (Serum or plasma tests)</u>
NEFA < 0.4 mmol/L during the week before expected calving
BHB < 1.1 mmol/L during week 1 and < 1.4 during week 2 after calving
Haptoglobin < 0.8 g/L during week 1 after calving

■ Anovular Condition

Numerous studies have documented that approximately 20% of cows have not ovulated (not resumed a normal estrus cycle) by the typical start of the breeding period at 9 weeks postpartum and that these cows have impaired reproductive performance at first AI (even if synchronized for timed AI) and longer time to pregnancy (e.g. Walsh et al., 2007; Dubuc et al., 2013). To identify anovular condition with accuracy typically requires 2 examinations, 11 to 14 days apart, with the absence of a functional corpus luteum (CL) on the ovaries at both times. This can be done with milk or blood tests to measure progesterone (P₄) or ultrasound examination of the ovaries. However, presently, there are no widely available, accurate, validated, convenient, rapid, and cost-effective cow-side tests for determination of progesterone status. Ultrasound examination of the ovaries can be about 90% accurate for CL status and if examination were very efficient (minimal extra time to find and scan cows during routine herd health), this might be a viable strategy. However, the economics of this are not clear. With cows on a double PGF

presynchronization program, a single ultrasound examination at the start of Ovsynch when cows should have a CL was 69 to 90% (mean of 6 locations was 84%) accurate to assess ovular status relative to 3 blood measurements of P4 (Stevenson et al., 2008). In a similar study, Silva et al. (2007) found a single ultrasound examination at the start of Ovsynch had 87% accuracy for ovular status. Heat mount detectors had overall accuracy of 71% to classify anovular status but were inaccurate (specificity of 34%) to predict CL at the start of Ovsynch (Stevenson et al., 2008), which may be the practical decision point for intervention.

Even if ovular history in the weeks before first AI or CL status at the start of Ovsynch can be determined accurately and practically, there are inconsistent results of addition of a P4 insert (CIDR or PRID) to Ovsynch protocols. Briefly, some studies indicated improved CR in cows that were anovular at the start of Ovsynch for first AI and received a CIDR (e.g., Stevenson et al., 2006). Others report a benefit of addition of a PRID to Ovsynch in non-presynchronized cows (Colazo et al., 2013). Presynchronized cows without a CL at the start of Ovsynch that received a CIDR had improved pregnancy to the timed AI relative to cows without a CL that did not receive a CIDR (32 vs. 24%; 38% of cows that had a CL at the start of Ovsynch were pregnant at 33 days, which was not statistically different to cows without CL with a CIDR) (Stevenson et al., 2008). However, as summarized by Stevenson et al. (2008), the apparent benefit of addition of CIDR to PreSynch-Ovsynch was in cows that had ovulated before the start of Ovsynch but had low P4 at that time or at the time of the final PGF during Ovsynch. Unfortunately, overall there is not consistent evidence that the effect of anovular status can be mitigated with addition of a CIDR for first AI. Therefore, strategies to detect and intervene in cows that have not expressed estrus or have not ovulated before the time limit for first AI await both more accurate practical means of diagnosis (selection of animals to treat) and more consistently effective treatments.

■ Control of First Insemination

The single most influential variable in time to pregnancy is time to first insemination, or the efficiency of managing first AI. The economic goal is to have cows pregnant by 150 DIM, and under most circumstances, to maximize the proportion becoming pregnant between 85 and 120 DIM. Given that typical insemination rates in North American herds are around 40% per 21 days, an important starting point is to set a target for time to first AI, specifically an upper limit, and to rigorously implement interventions to achieve the goal. This may be as simple as enrolling all cows that have not been inseminated by 65 to 75 DIM on the Ovsynch protocol. Whether used in combination with estrus detection, with or without activity monitors, or systematically for all first services, use of an ovulation synchronization and

timed AI protocol to enforce a thoughtful upper limit on time to first AI would be a valuable starting point for many herds.

Enhancing Pregnancy at First Insemination in Lactating Cows

Once control has been taken of the timing of first insemination, a next step is to consider selection and implementation of amendments to the basic Ovsynch protocol to increase the probability of pregnancy to first insemination. The principles as currently understood are to:

- ▶ Have a CL at the start of Ovsynch
- ▶ Ovulate a follicle in response to the first shot of GnRH
- ▶ Have a CL at the time of PGF and have it fully regressed following PGF
- ▶ Ovulate at the predicted time after the second GnRH

Practically, this means aiming to have cows between days 5 and 10, ideally day 6 or 7, after ovulation at the start of Ovsynch.

The two main tools to help achieve these goals are:

1. Presynchronization

Presynchronization (“Presynch”) with 2 injections of PGF 14 days apart, 11 to 14 days before the start of Ovsynch. The benefit to conception risk (CR; i.e. probability of pregnancy per AI) to Ovsynch, by adding Presynch, ranged in several studies from 0 to 12% points. The mean benefit appears to be approximately 6% points but is subject to the prevalence of anovular cows in which no benefit of the Presynch is expected. Galvao et al. (2007) showed that an interval of 11 days rather than 14 from the second PGF of Presynch to the start of Ovsynch improved CR from 34 to 41%. Stevenson (2011) compared Presynch with intervals of 14, 12, or 10 days to Cosynch alone (i.e. without Presynch). With ~150 cows per treatment, statistical power was limited but the CR in these groups were not different to Cosynch alone: 32, 37, 35, and 34%, respectively. There may be some advantage to Presynch when a hybrid program of estrus detection (after one or both PGF) followed by Ovsynch is employed. Limited data (Chebel and Santos, 2010) from one herd with freestalls and dirt lots (i.e. relatively good opportunity for estrus expression and detection) showed, despite lower CR on detected heats, no difference in overall time to pregnancy with “cherry-picking” heat detection after the second Presynch PGF (59% of cows bred this way) compared to 100% timed AI to Presynch-Ovsynch for first service. This may be a viable option that satisfies the wish to breed cows on heat while still controlling time to first AI. In theory, and sometimes in practice (discussed by Santos, 2007) CR to detected heat may be higher than to Ovsynch. To the extent that cows that are potentially more fertile are detected in heat, and relatively more

anovular cows and cows that failed to express heat are bred by Ovsynch, this may confound the apparent success of the timed AI. Given that herds with fewer than about 1000 cows are unlikely to be able to formally compare their own data on CR with or without Presynch, some caution is warranted in implementing this protocol to increase CR at first AI.

2. *Double Ovsynch (DO)*

This consists of an Ovsynch protocol (GnRH – 7 d – PGF – 72 h – GnRH) without AI, followed 7 days later by Ovsynch (GnRH – 7 d – PGF – 56 h – GnRH – 12-16 h – AI). Addition of GnRH to the presynchronization regime should in principle improve the response among anovular cows by inducing some of them to ovulate early in the program. Souza et al. (2008) produced remarkable CR in primiparous cows (65% with DO vs. 45% with Presynch) but there was no difference in CR (39%) in multiparous cows. In a larger follow-up study, striking CR was achieved in primiparous cows (53% with DO vs. 42% with Presynch) but only a tendency for improvement in multiparous cows (CR of 40 vs. 34%, respectively). The mechanism for this interaction with parity is not fully understood, and this influences the practical application of this tactic if cows in first and greater parities are housed together. Nevertheless, DO may improve fertility in anovular cows and produce CR that are more consistently improved over Ovsynch alone than does Presynch.

5-day CIDR Ovsynch in Cows

By shortening the period of dominance of the follicle to be ovulated for insemination, the quality of the follicle may be improved. Practically, this may be achieved by shortening the Ovsynch program from 7 days between the first GnRH and PGF to 5 days. This, with a CIDR and Cosynch (final GnRH and AI concurrent at 72 hours after CIDR removal) improved CR from 31 to 38% (Santos et al., 2010; Figure 2). However, to fully regress the younger CL, this program requires 2 doses of PGF. Moreover, there is not a lot of field data to assess whether the 5-day CIDR Ovsynch option consistently yields better CR (reports vary from 38 to over 50%), pregnancy rates or economic return than other approaches to control of first AI. Interestingly, there are inconsistent data on whether various presynchronization methods improve CR with this program (Ribeiro et al., 2012, 2013). This protocol is applicable to first and repeat services but more data are required in intensively managed, housed Holstein cows to establish where 5-day programs, with or without presynchronization, fit into the toolkit of reproductive management interventions.

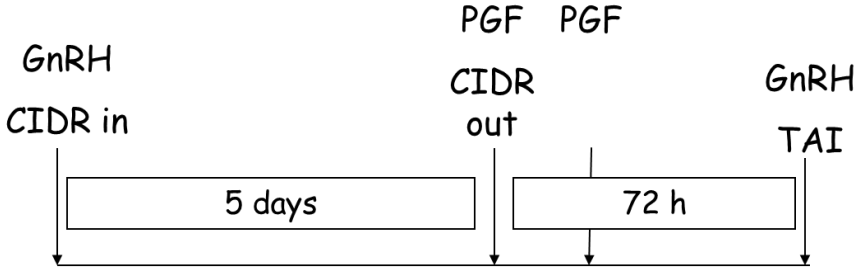


Figure 2. 5 day CIDR synchronization program for lactating dairy cows. In cows, 2 doses of PGF 8 to 24 h apart are required (Ribeiro et al., 2012 *Therio* 78: 273-284). (A double dose of PGF administered all at once on day 6 (1 day after CIDR removal) may also be effective (Stevenson et al 2013 *J. Dairy Sci.* 96: 5769–5772.) After CIDR removal, the program can be completed as shown, or with the final GnRH 56 hours after CIDR removal and the first PGF, with AI 16 hours after the last GnRH (CR was not different: 46% Bisinotto et al 2010 *J. Dairy Sci.* 93: 5798–5808).

Control of Insemination in Heifers

Traditionally, heifers are expected to express estrus more intensely, leading to higher heat detection rates, and to have higher CR than lactating cows. However, to achieve the economic target of having heifers at 55% of their target mature size and pregnant at 13 to 15 months old requires proactive management. Heifer growth should be planned from birth and measured at 2, 6, and 10 months of age to ensure that it is on track to meet this target. Because of limited labour or housing of heifers in more remote locations, insemination rate or timing may be less than optimal. Therefore, assuming heifers are well grown, synchronization and timed AI may be relevant or necessary tools to achieve reproductive targets. In the last few years, a program has become available that offers attractive CR (around 50%) with fixed time AI (Figure 3).

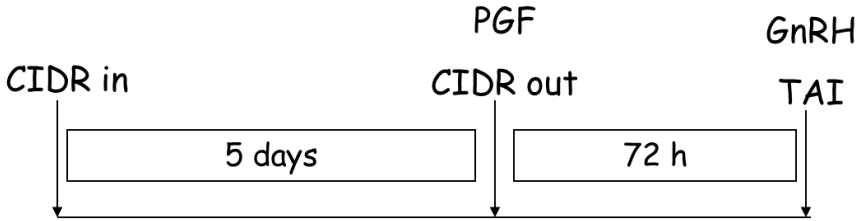


Figure 3. 5 day CIDR synchronization for timed AI in heifers. In heifers, a single administration of PGF is sufficient, and GnRH at the start of the program does not appear to be necessary (Colazo & Ambrose 2011 Theriogenology 76: 578-588).

■ Control of Repeat Inseminations

With a program well in place to control the timing and pursue high CR for first service, the next step is to set an upper limit of no more than 42 days for re-insemination of open cows. A simple and often under-used tactic is to focus attention on detecting returns to estrus 20 to 24 days after the last AI. Next, Ovsynch allows for essentially all cows to be re-bred within 3 to 10 days of being diagnosed open, which can achieve the goal of a maximum interval of 42 days for almost all cows if pregnancy diagnosis is conducted weekly or bi-weekly between 28 and 42 days. A simple policy of placing cows on Ovsynch upon being diagnosed open guarantees timely re-insemination. As for first service, timing the start of Ovsynch can optimize the stage of the estrous cycle and potentially improve CR from < 30% to 30% or so. Starting Resynch before 26 days is likely to reduce CR (Wiltbank et al., 2008) and there was no advantage in CR to starting at 39 vs. 32 days (Bilby et al., 2013). Practically, administration of GnRH between days 28 and 33 allows for nearly optimal timing and flexibility in aligning pregnancy diagnosis with groups of cows that were previously synchronized together. If producers are willing to give GnRH before pregnancy diagnosis, this provides for greater flexibility as well as greater accuracy of pregnancy diagnosis and fewer embryonic losses that occur after pregnancy diagnosis. For example, GnRH could be given at day 32, with pregnancy diagnosis anywhere between days 32 and 39 and PGF to open cows at day 39 and re-insemination at day 42. A recent study showed no pregnancy rate or economic difference between ultrasound pregnancy diagnosis and Resynch at day 31 vs. GnRH for Resynch at day 31 and pregnancy diagnosis by palpation at day 38 (Pereira et al., 2013).

The latest approach has been to pursue greater CR through presynchronized Resynch (Bilby et al., 2012), following the same principles and, more or less, the same protocols as used for first service. Small gains in CR at first service can easily pay for additional hormonal interventions. However, for repeat services the trade-off between increased CR but delay of insemination by 1

week or more to implement extra steps to pursue higher CR must be carefully assessed. For example, Double-Ovsynch resynch started at day 22 (AI at day 49) improved CR at 39 days to 35% from 27% with simple Resynch at day 32 (AI at day 42) (Giordano et al., 2012), yet an economic model of this (assuming Double-Ovsynch for all cows for first AI) showed a very small benefit (1% difference) to the Double-Ovsynch Resynch (Giordano et al., 2011). Application of resynch strategies should consider the extent to which heat detection is desired for re-insemination. Administration of GnRH will decrease expression of estrus in the following week, whereas PGF-based presynchronization will increase the proportion of cows detected in heat before timed AI to up to 60%.

Suggested programs that synthesize and apply the information above are presented in Figure 4.

If 21-day heat detection rate > 50% with CR to detected heats > 35%

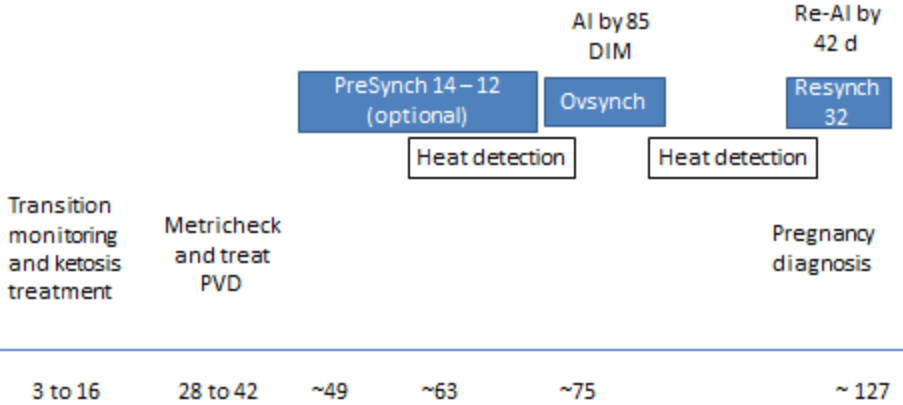


Figure 4a. A strategy to manage reproduction where good heat detection can be achieved. Days in milk (DIM) for each intervention are shown along the time line. See the text for details. PVD = purulent vaginal discharge

If 21-day heat detection rate < 50%

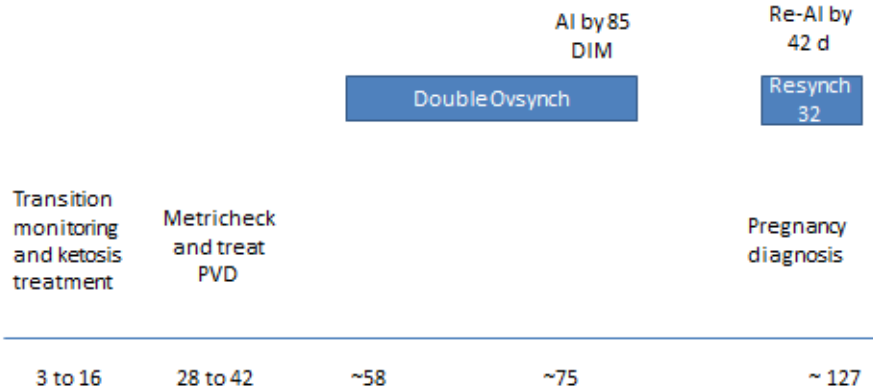


Figure 4b. A strategy to manage reproduction where less than above-average heat detection is present. Days in milk (DIM) for each intervention are shown along the time line. See the text for details. PVD = purulent vaginal discharge.

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