RESYNCHRONIZATION PROTOCOLS FOR TIMED-INSEMINATION IN LACTATING DAIRY COWS

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Introduction

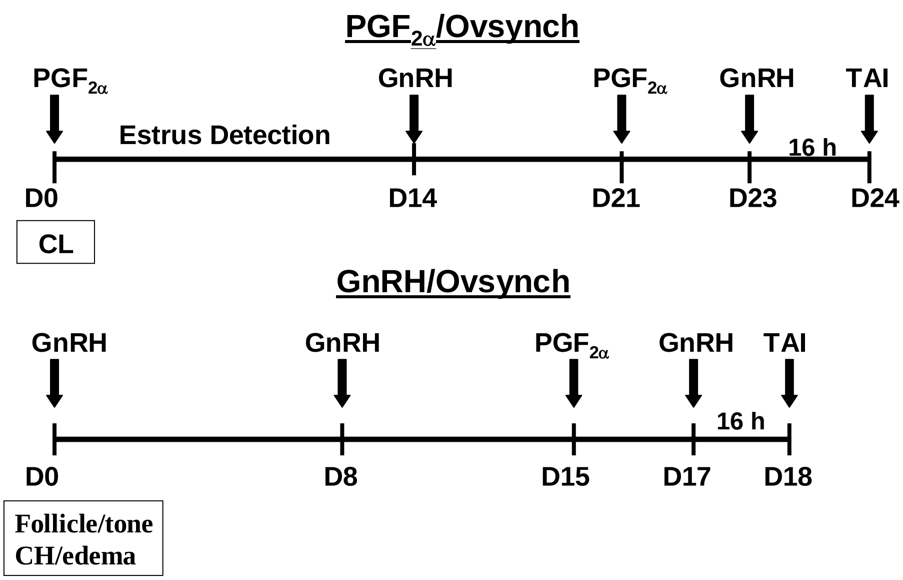
Traditionally, reproductive efficiency in dairy herds has been based on high estrus detection (EDR) and conception rates (CR) after a voluntary waiting period of 40-45 days postpartum. However, it is not uncommon for dairy herds to establish a voluntary waiting period based on both recovery of the reproductive tract and production characteristics. Consequently, lactating dairy cows are usually inseminated around 75 days postpartum such that rapid resumption of ovarian cyclicity becomes less of a problem. Since estrous detection is very low and inaccurate in intensely managed dairy herds[20], protocols for synchronization of ovulation and timed-insemination (TAI) have been developed to ensure that all cows will be inseminated at the designated voluntary waiting period. The Ovsynch protocol is most effective when initiated between days 5 and 12 of the estrous cycle[18,32]. Therefore, TAI programs involving pre-synchronization such as Presynch-Ovsynch[19] and Presynch-Heatsynch[21] can obtain a higher pregnancy rate (PR) to first service. After first service, enhanced reproductive efficiency depends on enhancing embryo survival and prompt re-insemination of cows that either did not conceive, or failed to maintain a pregnancy. The various methods used to detect nonpregnant cows include estrus detection, determination of progesterone (P_4) concentrations, biochemical/immunological assays, ultrasonography (US), and per rectum examination of the genital tract. The objective of this presentation is to provide examples of combining per rectum examination and US of the genital tract to detect nonpregnant cows, and the application of re-synchronization protocols for TAI in lactating dairy cows.

Re-synchronization of cows detected nonpregnant following per rectum examination of the genital tract

Per rectum examination of the genital tract at 35 to 45 days after breeding[33] allows for detection of cows that did not conceive and were not detected in estrus, and cows that conceived but lost their pregnancy. Since the rate of estrus detection is usually very low in modern dairy herds, nonpregnant cows detected by rectal palpation need to be effectively re-synchronized for insemination. Synchronization of ovulation and timed-insemination using the Ovsynch protocol[25] generates acceptable PR in normal lactating dairy cows[5,9,17,26,27,28,29] and lactating dairy cows with ovarian cysts[1]. By modeling reproductive efficiency using different conception and estrous detection rates, it was concluded that timed-insemination programs are economically beneficial in dairy herds when estrus detection is compromised[14].

Per rectum examination of the genital tract allows not only for detection of pregnancy but also for detection of ovarian structures and uterine characteristics that reflect stages of the estrous cycle[33]. Such information could be used to initiate various re-synchronization protocols that are tailored to stage of the estrous cycle. In a study involving 340 reproductively normal lactating dairy cows, during the period of September 2000 to May 2001, two different protocols for re-synchronization were applied based upon stage of the estrous cycle as determined by per rectal examination[2]. Cows with a corpus luteum (diestrus; n=216) were treated with $PGF_{2\alpha}$ and inseminated at estrus within 2 to 7 days. Those cows not seen in estrus by day 14 (days 7 and 12 of the estrous cycle if they responded to the $PGF_{2\alpha}$) were subjected to Ovsynch. Cows with a palpable ovarian follicle and uterine tone or a corpus hemorrhagicum (proestrus and metestrus) were treated with GnRH and 8 days later (days 7 and 12 of the estrous cycle) subjected to the Ovsynch protocol (n=119; Figure 1).

Figure 1. Protocols for re-synchronization of lactating dairy cows diagnosed nonpregnant at rectal palpation on Day 45 after insemination



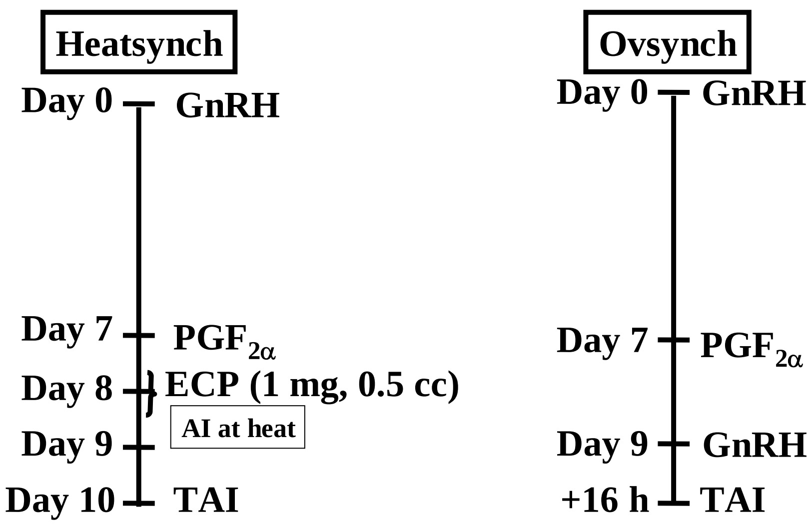
For cows in diestrus, 39.3 % (85/216) were detected in estrus after $PGF_{2\alpha}$ and 60.7 % (131/216) were timed inseminated. Pregnancy rate at Day 45 was similar for cows in diestrus subjected to the protocol $PGF_{2\alpha}$+Ovsynch and time-inseminated (35.9% [47/131]), and cows in proestrus and metestrus subjected GnRH+Ovsynch and time-inseminated (32.8% [39/119]). Among cows in diestrus there was no statistical difference in CR between cows inseminated at detected estrus (37/85; 43.5%) or cows time-inseminated (35.9%). This numerical difference (7.6%) may have been significant if there were a greater number of cows in each classification. However, even though CR for cows inseminated at estrus was high, the hypothetical PR (EDR x CR) would be 17% (39.3% x 43.5%). Since the cows not showing estrus were subjected to Ovysnch on Day 14, overall PR was increased (38.9%; 84/216). A valid criticism of this study was the lack of a group of nonpregnant cows subjected to the Ovsynch protocol at different stages of the estrous cycle. However, this was a conscious decision since CR is reduced when Ovsynch is started at stages of the estrous cycle other than early diestrus. The advantage of the protocols used resides with the fact that cows were subjected to the Ovsynch protocol at potentially optimal stages of estrous cycle. In addition, cows that were treated with $PGF_{2\alpha}$ on

Day 0 ($PGF_{2\alpha}$+Ovsynch) had the opportunity to be inseminated at detected estrus within 14 d. Otherwise they were time-inseminated following the Ovsynch protocol. Even though these protocols allow for selective re-synchronization based upon rectal palpation with acceptable PR, there are several disadvantages: there is extensive time between insemination and pregnancy diagnosis (35-45 days) and the protocols are rather lengthy. Consequently, we evaluated the use of US to diagnose pregnancy earlier and in combination with rectal palpation to determine stage of the estrous cycle with subsequent assignment to different protocols for TAI.

Re-synchronization of cows detected nonpregnant at Ultrasonography of the genital tract

Ultrasonography allows for early detection of nonpregnant cows after breeding[13,23]. Ultrasonographic evaluation of the ovaries[22] and uterus[24] in combination with per rectum palpation of the genital tract[33] allows for a staging of the estrous cycle based upon a collective assessment of ovarian structures and uterine characteristics. A study[3] conducted from August to December, 2001 including 332 lactating dairy cows, evaluated PR for Ovsynch (n=166) and Heatsynch (n=166; Figure 2) in cows diagnosed nonpregnant at US on d 27 after AI (Day 0).

Figure 2. Protocols for re-synchronization of lactating dairy cows diagnosed nonpregnant at ultrasonography on day 27 after insemination



Note: cows AI at estrus on Day 8 and 9 were included.

On Day 0, cows were classified according to different stages of the estrous cycle combining rectal palpation and US to determine ovarian estructures and uterine characteristics (Table 1). Detection of a functional CL included morphology at palpation (line of demarcation and distortion in the shape of the ovary) and visualization at US, detection of follicles was based on US, and uterine tonicity and edema was detected by rectal palpation. Ultrasonography further contributed to the differentiation of follicular and luteal cysts[12].

Table 1. Criteria for Determination of the Stage of the Estrous Cycle by US and Rectal Palpation of the Genital Tract

Stage	Clinical Findings	
	Ovaries	Uterus
Diestrus	Functional CL Follicle > 10 mm	Moderate tonus
Metestrus	Corpus hemorrhagicum Follicle < 10 mm	Edema and tonus
Proestrus/Estrus	Follicle > 18 mm Regressing CL	High tonus
Ovarian Cysts	Multiple follicles >18 mm No CL	Flaccid
Anestrus	Follicle < 18 mm	Flaccid

Adapted from Zemjanis 1962, Pierson and Ginther 1987

The number of cows in different stages of the estrous cycle was as follow: diestrus (46.4% [154/332]), metestrus (14.8% [49/332]), proestrus (22% [73/332]), cystic (14.1%, 47/332), and anestrus (2.7%, [9/332]). There was no difference in PR for re-synchronized services at Day 27 and Day 50 for Heatsynch and Ovsynch (Tables 2).

Table 2. Pregnancy rate at Days 27 and 50, and pregnancy losses for Ovsynch and Heatsynch for re-synchronization in nonpregnant cows at US on Day 27 post-AI

	PR at Day 27		PR at Day 45		Pregnancy losses	
Protocol	N	%	N	%	N	%
Ovsynch	33/127	26.0	30/166	18.1	8/33	24.2
Heatsynch	34/132	25.8	33/166	19.9	6/34	17.6

Furthermore, pregnancy losses between days 27 and 50 were not different between protocols (Table 2). These results are in agreement with a similar study[30] in which PR at Day 45 was 30% (n=466) for Ovsynch and 30% (n=188) for Heatsynch.

Pregnancy rates according to stage of the estrous cycle are presented in Tables 3. For cows in metestrus, PR at Days 27 and 50 were higher for Heatsynch (52.2% and 44.4%) compared to Ovsynch (15.4% and 9.1%). When cows are in metestrus, a follicular wave already has been initiated, and the GnRH injection will not initiate a new follicular wave. Consequently, these cows will ovulate earlier after injection of $PGF_{2\alpha}$ such that those in Heatsynch are likely to show estrus and be inseminated to a detected estrus. Indeed a high percentage of cows in metestrus were inseminated on Day 9 of Heatsynch (11/27) compared to Ovsynch (0/22). Cows in the Ovsynch group were not detected in estrus and were inseminated on day 10 which was likely to late. For cows with ovarian cysts, PR at Days 27 and 50 were higher for the Ovsynch (31.6% and 27.3%) compared to Heatsynch (8.0% and 5.3%). Cows with ovarian cyst are anovulatory cows that fail to release hypothalamic GnRH. Therefore Ovsynch, which uses GnRH to induce ovulation, acts in a manner that is independent of the hypothalamus and appears to be more efficient than Heatsynch which is dependent upon estradiol to induce hypothalamic release of GnRH.

Table 3. Pregnancy rate at Days 27 and 50 for cows re-synchronized at different stages of the estrous cycle

	PR at Day 27				PR at Day 50			
	Ovsynch		Heatsynch		Ovsynch		Heatsynch	
Stage	N	%	N	%	N	%	N	%
Diestrus	20/64	31.2	14/58	24.1	17/82	20.7	12/72	16.7
Metestrus	2/13	15.4^{b}	12/23	52.2^{a}	2/22	9.1^{b}	12/27	44.4^{a}
Proestrus	5/26	19.2	7/29	24.1	5/35	14.3	6/38	15.8
Cystic	6/19	31.6^{d}	1/19	5.3^{c}	6/22	27.3^{d}	2/25	8.0^{c}

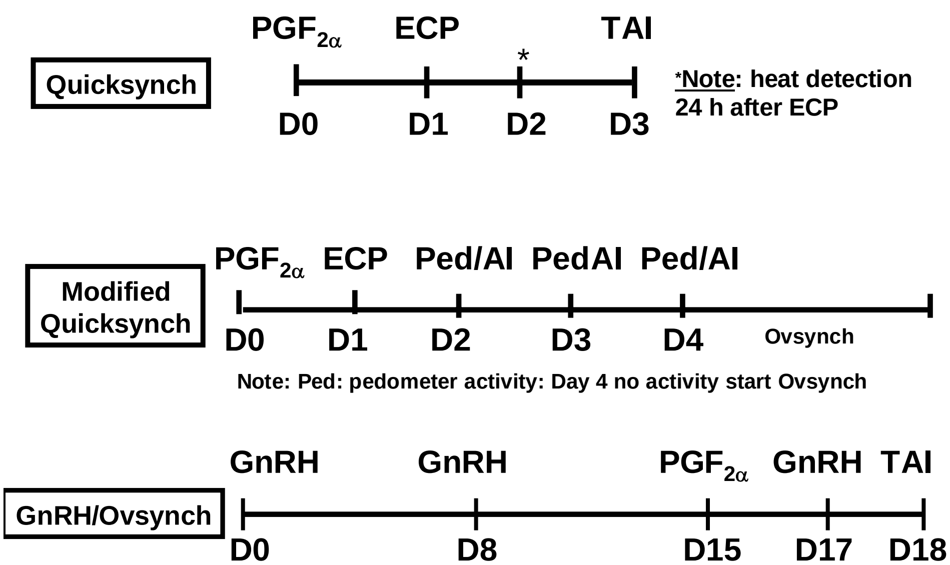
Note: At Day 27 a > b ($P<0.04$); d > c ($P<0.06$); At Day 50: a > b ($P<0.01$), d > c ($P<0.11$).

In conclusion, cows responded differently to the two protocols (Heatsynch and Ovsynch) depending upon their ovarian status or stage of cycle. It is important to recognize that at day 27 after insemination cows will not have a random distribution across all stages of the cycle but will tend to be in early diestrus or metestrus. With the capability to categorize cows as to their estrous cycle stage at pregnancy diagnosis, it may be feasible to select re-synchronization programs that are most suitable for the stage of the estrous cycle and more timely relative to reducing the interval to repeated insemination[4].

Re-synchronization of cows ACCORDING TO stage of the estrous cycle as determined by ultrasonography and per rectum exam of the genital tract

A study was conducted from January to December 2002 in a 3,000 milking cow herd in which 877 cows were detected as nonpregnant based upon an US diagnosis at 30 d post-AI (Day 0). The diagnosis of pregnancy was shifted from Day 27 to Day 30 to increase the number of cows with a CL, and to accelerate the pregnancy diagnosis process with conceptuses that are more advanced in development. On Day 0, cows were classified according to stages of the estrous cycle as previously described in Table 2, and a blood sample was collected for subsequent analysis of plasma P_4 concentration. Based upon stage of the estrous cycle or ovarian status, cows were assigned to various protocols described in Figures 2 and 3. Cows in diestrus were assigned to either Ovsynch (n=216), Quicksynch (n=92, January to May), or Modified Quicksynch (n=110, June to December) protocols (i.e., Figure 3). Cows in metestrus were assigned to Ovsynch (n=54), Heatsynch (n=50) or GnRH+Ovsynch (n=50). Cows in proestrus were assigned to Ovsynch (n=71) or GnRH+Ovsynch (n=73). Cows with ovarian cysts were assigned to Ovsynch (n=75) or GnRH+Ovsynch (n=78) protocols (i.e., Figures 2 and 3).

Figure 3. Protocols used for re-synchronization depending upon stage of the estrous cycle at the time of a nonpregnant diagnosis at day 30 after insemination



Pregnancy diagnosis to the re-synchronized insemination was determined at Day 30 by US and evaluated by multiple logistic regression using cows in diestrus that were submitted to the Ovsynch protocol as an overall referent (i.e., control) and adjusting for time of the year, parity, number of previous services and inseminator. The distribution of cows classified according to stage of the estrous cycle was as follows: diestrus 47.7%, metestrus 16.9%, proestrus 16.4%, ovarian cysts 17.4% and anestrus 1.6%. Mean plasma concentrations of P_4 for the different stages are depicted in Table 4. For cows in diestrus, 95.5 % of the cows had P_4 concentrations greater that 1 ng/ml indicating an accurate staging of diestrus cows. Cows in proestrus and metestrus had a wider variation in P_4 concentrations, and cows with ovarian cysts had a greater frequency of follicular than luteal cysts (85% versus 15%). These results are in agreement with our previous experiment in which 46 % of the nonpregnant cows were in diestrus. It was reported[31] that 61 % of nonpregnant cows had P4 > 1 ng/ml when evaluated ultrasonographically at 27-29 days after first service and injected with $PGF_{2\alpha}$. However, in that study, no distinction was made between stages of the cycle and that 61 % may have included cows in metestrus, proestrus and with a luteal cyst.

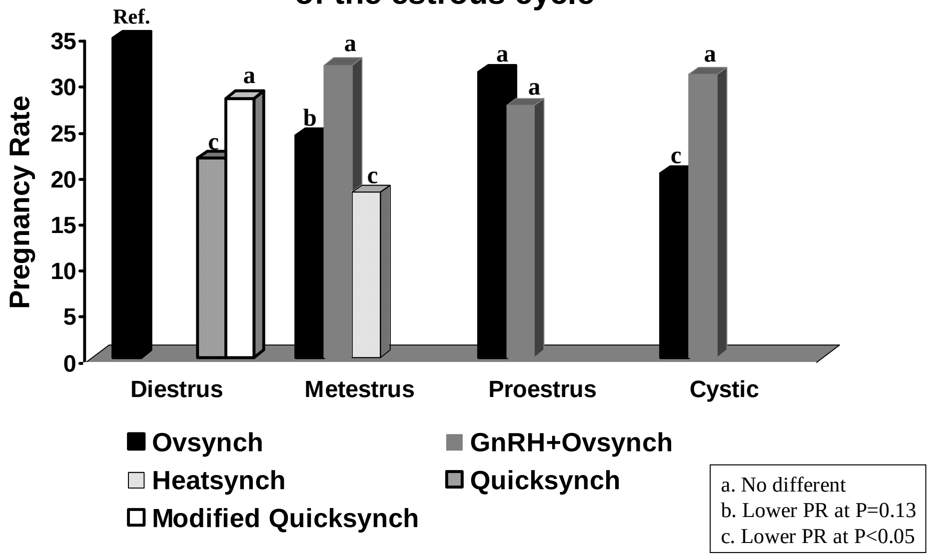
Table 4. Plasma Progesterone Concentrations Within Different Stages of the Estrous Cycle

Stage	P4 ng/ml	N	%
Diestrus	**<1**	**5/112**	**4.5**
	>1(1.05-16.7)	**107/112**	**95.5**
Metestrus	**<1**	**18/48**	**37.5**
	1 to 6	**21/48**	**43.8**
	>6 (6.37-11.28)	**9/48**	**18.7**
Proestrus	**<1**	**36/47**	**76.7**
	>1(1.03-8.67)	**11/47**	**23.3**
Cystic	**<1**	**22/26**	**85.0**
	>1 (2.38-8.81)	**4/26**	**15.0**

There were no differences in PR of cows in diestrus subjected to Ovsynch (34.7%), Modified Quicksynch (28.2%), or cows in metestrus subjected to GnRH+Ovsynch (31.8%), or proestrus cows receiving Ovsynch (31.0%) or GnRH+Ovsynch (27.4%) protocols, and cows with ovarian cysts subjected to GnRH+Ovsynch (30.8%; Figure 4). However, PR was reduced for cows in either diestrus subjected to Quicksynch (21.7%), cows in metestrus subjected to Ovsynch (24.1%) or Heatsynch (18.0%) and cows with ovarian cysts receiving Ovsynch (20.0%). Stevenson et al (2003) reported similar CR between cows that received a shortened Ovsynch protocol ($PGF_{2\alpha}$ at US on Days 27-29, GnRH 48 h later, and TAI 16-20 h later), cows that received $PGF_{2\alpha}$ and were AI at detected estrus or TAI 72-80 h, and control cows (23 %, 22 % and 23 % respectively). In our study, the reduction in PR for the Quicksynch protocol indicated that follicular wave synchronization is necessary before $PGF_{2\alpha}$ and ECP are administered for luteolysis and synchronization of ovulation, respectively.

For cows in diestrus that were subjected to the Modified Quicksynch protocol, 72% were inseminated based upon pedometer activity and PR was 32.9% (26/79). Thirty one (28%) cows did not show pedometer activity and were subjected to Ovsynch on Day 4 and PR was 16% (5/31). The Modified Quicksynch protocol for cows in diestrus reduces re-synchronization time when estrus detection is acceptable and reduces the cost of the treatment without compromising PR (i.e., an overall 28% PR).

Figure 4. Pregnancy rate for inseminations following various assigned re-synchronization protocols after determining stages of the estrous cycle



For cows in metestrus, GnRH+Ovsynch improved PR and this was expected since Ovsynch was initiated at the right stage of the cycle regardless of whether the cow would have received GnRH. It was anticipated, based upon the previous experiment (i.e., high PR in Heat synch cows inseminated on Day 9), that the Heatsynch protocol would improve PR in metestrus cows. However, in the present experiment, only 10 of the 50 cows were inseminated on Day 9. These results indicate that a high PR would be achieved when the Ovsynch protocol is initiated at the right stage of the estrous cycle.

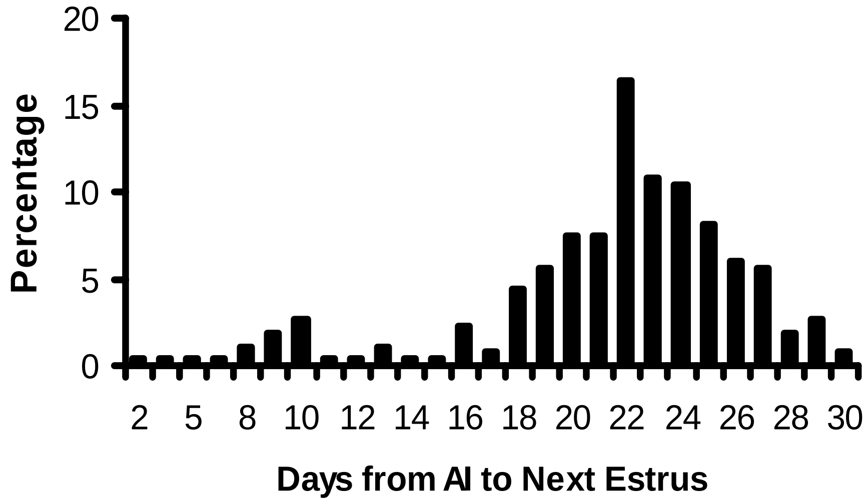
Future strategies for re-synchronization and timed insemination

The importance of re-synchronization to reduce the interval between first and second insemination has stimulated the development of different strategies to re-synchronize returns to estrus to increase the number of cows re-inseminated in a timely manner[16,31]. Administration of 1 mg of estradiol benzoate on Day 13 after estrus synchronizes the ovarian follicular wave and subsequent estrus[6]. Such a treatment in combination with a medroxyprogesterone acetate impregnated sponge increased the number of cows re-inseminated between Days 18 and 25 after insemination[7]. Application of a used CIDR (controlled internal drug releasing insert) device that releases P_4 when inserted into the vagina has been a strategy for re-synchronization. Insertion of the CIDR by itself or in combination with an estrogen injection at CIDR insertion or at is removal (or 24 h after removal) can resynchronize the return estrus following a prior insemination[10,11,16]. However, such programs require detection of estrus. Conception rates to the re-synchronized service have been reported to be normal[11,19],or decreased[7,10] when compared to cows re-inseminated at a spontaneous estrus.

The administration of GnRH before pregnancy diagnosis by US will synchronize the follicular wave, increase the number of cows with a CL that is responsive to $PGF_{2\alpha}$ when inducing luteolysis, and permit synchronization of ovulation for TAI[8,19]. Administration of GnRH on Day 20 post-AI for re-synchronization in cows diagnosed for pregnancy at Day 27 decreased the PR to the initial insemination in cows treated with bST, but not in control cows not treated with bST[19]. It was demonstrated[8] that PR to first service at US on Day 28 was similar when Ovsynch was initiated on Day 21 (33.1%) or Day 28 (33.6%) and similar at rectal palpation on Day 42 (27.0 vs 26.8%). Furthermore, PR to the second re-synchronized service was the same between the two treatments. Therefore, it is safe to initiate Ovsynch on Day 21 before pregnancy diagnosis on Day 28 by US.

In our previous studies we evaluated the interval from AI to return to estrus in cows that were nonpregnant (Figure 5).

Figure 5. Distribution in intervals from insemination to estrus in cows diagnosed nonpregnant at day 30 after insemination



Based on this distribution of intervals to estrus, it is feasible to inject GnRH at day 23 (i.e., 22-24 days) after insemination to synchronize the follicular wave and ensure that corpora lutea are present at day 30 that will be responsive to $PGF_{2\alpha}$. Currently, a study is underway (J. Bartolome and W.W. Thatcher, personal communication) in which GnRH is administered between days 22 and 24, and pregnancy diagnosis using US is conducted between days 29 to 32 (i.e., mean 30 days). Cows diagnosed nonpregnant receive $PGF_{2\alpha}$, and ovulation is synchronized with either ECP or GnRH (Figure 6 and 7).

Figure 6. Future Strategy for Re-synchronization

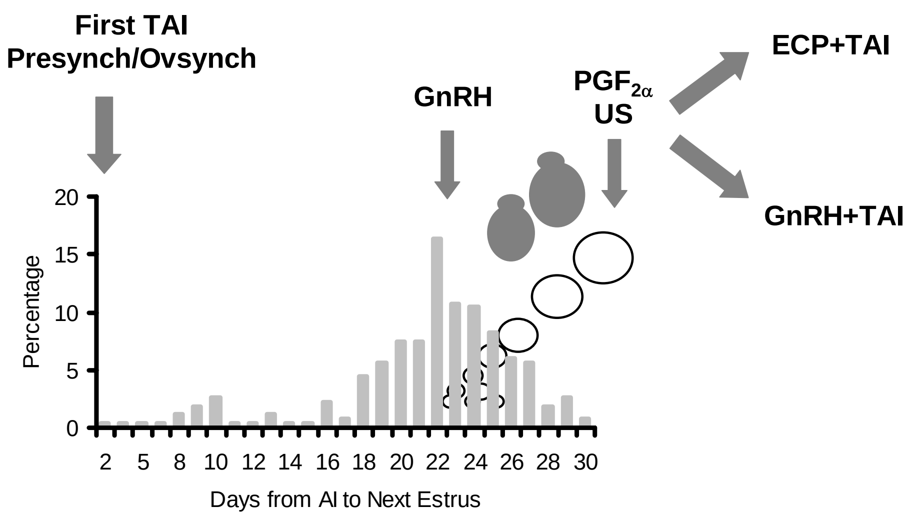
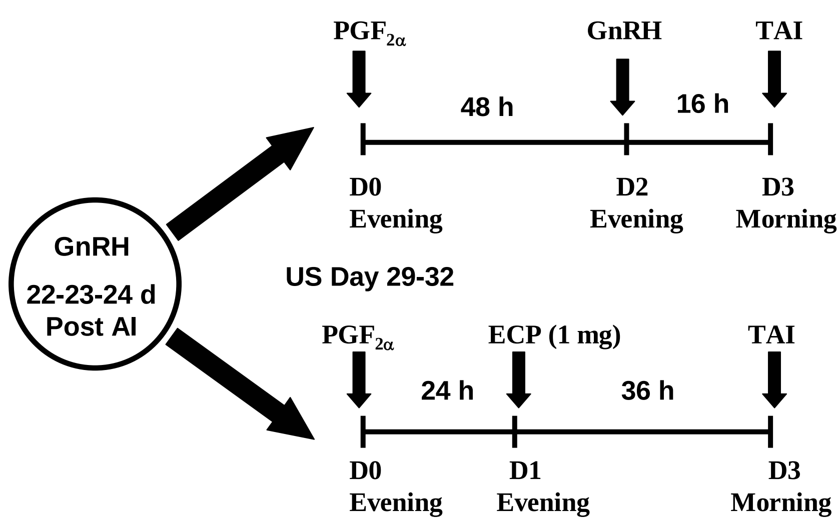


Figure 7. Re-synchronization protocols for cows receiving GnRH at 22-24 days after insemination and diagnosed for pregnancy between days 29-32



Note: The protocols do not included estrus detection, therefore 100 % of the cows are TAI

The timing of the Ovsynch protocol is standard with the ovulatory dose of GnRH given 48 h after injection of $PGF_{2\alpha}$ and a TAI at approximately 16 h after GnRH. Our experiences with ECP for resynchronization is such that we have chosen to time-inseminate all of the cows at approximately 36 h after injection of ECP. Preliminary results evaluating 638 cows indicate the following distribution according to stages of the estrous cycle at the time of pregnancy diagnosis: diestrus 73 %, metestrus 6.4 %, proestrus 9.8 %, ovarian cysts 8.6 % and anestrus 1.7 %. In addition, 50 % of the ovarian cysts showed luteinization at US. Clearly, there is an increase the number of cows in diestrus (i.e., mid-luteal phase) at the time of US. Preliminary PR for the re-synchronization treatments are 28.4 % (33/116) for cows subjected to PGF-ECP-TAI and 28.3 % (32/113) for cows subjected to PGF-GnRH-TAI. Since we still have cows without a corpus luteum despite the fact they received a GnRH injection 7 d prior to pregnancy diagnosis, alternative protocols are being evaluated for cows in metestrus, proestrus and with ovarian cyst.

Perspective

Early detection of nonpregnant cows is crucial for enhancing reproductive performance. An understanding of uterine and ovarian function allows one to choose a resynchronization program that is suitable for a particular farm. Ultrasonography and rectal palpation are tools that can be used to determine stages of the estrous cycle in nonpregnant cows and selection of the most appropriate resynchronization protocol. In summary, we suggest that cows with a CL at the time of diagnosis of nonpregnancy which were treated with GnRH 7 days earlier can be re-synchronized with $PGF_{2\alpha}$ and either ECP or GnRH and TAI within 3 days. However, cows with CL at diagnosis of nonpregnancy which were not pre-treated with GnRH can be re-synchronized with protocols for TAI (Heatsynch and Ovsynch), or protocols that combine estrus detection and TAI (e.g. Modified Quicksynch, $PGF_{2\alpha}$/Ovsynch, $PGF_{2\alpha}$/Heatsynch) based on estrus efficiency of the particular farm. Cows coming in estrus (proestrus/estrus) can be successfully re-synchronized with Heatsynch or Ovsynch. Cows without CL (metestrus, ovarian cyst, anestrus) are less likely to respond to GnRH and they need to be pre-treated with GnRH in order to set up the stage to be responsive to TAI protocols (GnRH/Ovsynch; GnRH/Heatsynch). In addition, US and rectal palpation allow for detection of reproductive tract abnormalities that will contribute to treatment and culling decisions. Future cow-side pregnancy tests may allow the detection of nonpregnant cows at an early stage (e.g., day 23) so that resynchronization protocols can be initiated only in cows known to be nonpregnant. Therefore, choosing the proper stage to initiate the program (e.g., day 23) takes advantage of the re-occurring follicular wave and CL to reduce the time for re-insemination of all cows.

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