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Dairy Reproductive Synchronization Notes

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Achieving excellent reproductive performance is a high priority on dairy operations. In recent years significant progress has been made in clarifying basic reproductive physiology, leading to new approaches for manipulating the estrous cycle and the timing of ovulation. These newer approaches utilize products available for decades as well as newly approved products.

This paper will present short discussions on:

1. A review of follicular waves and the hormonal sequence of the estrous cycle.
2. Label indications and legal implications for exogenous GnRH and progesterone.
3. Potential effects of exogenous GnRH and progesterone.
4. Effect of presence of anovulatory cows on reproductive programs.
5. Use of early pregnancy diagnostics in reproductive programs.
6. Common issues leading to synchronization program failures.

A comprehensive review of the recent literature on these subjects is beyond the scope of this presentation. However, the resources utilized to prepare this paper and some selected references are included at the end of this paper.

Follicular waves, follicular development, and hormonal patterns

There have been many publications, reviews, and textbook discussions in recent years on follicular development and follicular waves in dairy cattle. For more information and more comprehensive bibliographies see reviews by Lucy 1992, Ginther 1997, Wiltbank 2002, and Senger 2003.

It appears that most adult dairy cows (especially higher producing animals) have two follicular waves of about 10-11 days duration during a given 21 day cycle. A minority of cows may have three waves, with the first wave about 10-11 days in duration and the middle wave of shorter duration. This outline will only consider animals with two waves. Follicular development in a two wave cycle can be divided into several phases.

<u>First Wave</u>

Recruitment

Immediately after an ovulation has occurred, estradiol and inhibin levels drop sharply and FSH levels begin to rise rapidly. The FSH rise is due to removal of estradiol inhibition of GnRH release from the hypothalamus and removal of inhibition by estradiol and inhibin of FSH release from the anterior pituitary. The rising FSH levels "recruits" a group of follicles to begin development. These follicles grow and begin producing low levels of estradiol. This phase lasts until the follicles are about 6 mm.

Selection

The growing follicles continue to slightly increase estradiol production and another hormone called inhibin. This combination of hormones inhibits the secretion of FSH, slowing the growth of most of the follicles. The decreasing FSH, rising estradiol, and increasing inhibin stop the recruitment of new follicles and cause all but one of the originally recruited follicles to begin to degenerate (become atretic).

However, one follicle is "selected" to become dominant and continues to grow. At this same time increasing levels of LH begin to be released from the anterior pituitary. It appears that the increasing LH levels encourage continued growth of the selected follicle.

Dominance
The selected follicle continues growing and becomes dominant, while the rest of the follicles in the wave continue to degenerate. Estradiol and inhibin are still being produced and depressing FSH levels. LH continues to encourage growth of the dominant follicle.

Atresia of the dominant follicle
During the first wave presence of progesterone from the CL prevents a final large surge of GnRH and the subsequent ovulatory surge of LH. Lack of an LH surge leads to atresia rather than ovulation of the first-wave dominant follicle. No ovulation occurs in the first wave in a normal cycle if no exogenous hormones are administered.

Second Wave
After atresia of the first wave's dominant follicle begins, estradiol and inhibin levels drop, allowing FSH levels to rise. A new wave of follicular recruitment, selection, and dominance is initiated. However, prostaglandin produced by the non-pregnant uterus lyses the CL about 6-7 days into the second wave (about day 17 of the cycle), causing progesterone levels to drop rapidly. The removal of progesterone allows continued growth of the dominant follicle through ovulation. The dominant follicle begins to produce estradiol in increasing quantity. The rising estradiol eventually causes release of GnRH in a sudden surge. This GnRH "surge" in turn causes a sharp release of LH. Ovulation follows in about 28 hours. FSH from the anterior pituitary is also released in a sharp surge after the GnRH surge, but its function is unclear.

After ovulation, estradiol and inhibin levels drop sharply. A sharp rise in FSH levels follows closely after ovulation and the sequence of the first wave begins again.

Additional Notes on the Hormonal Sequence in Dairy Cattle

Effects of endogenous progesterone
During diestrus, progesterone is secreted by the CL. Progesterone has the following effects:

- Inhibits GnRH release from "surge" center thereby inhibiting an LH surge
- Inhibits expression of estrus activity (even if estradiol is present)
- Dampens release of GnRH from the "tonic" center in hypothalamus
- Prevents developing follicles from completing their maturation through ovulation.
- Primes the brain for increased responsiveness to later exposure to estradiol for demonstration of behavioral estrus. (May help explain the lack of estrous activity at the first ovulation post-partum and after a period of non-cyclicity.)
- Prior presence of progesterone appears to have influence on the responsiveness of the hypothalamus to estradiol for the surge release ~~of GnRH and LH.~~

Pattern of release of GnRH from the hypothalamus

- Release of GnRH from the hypothalamus can be in both a pulsatile (or "tonic") manner and in a "surge" fashion. It appears these releases come from separate areas of the hypothalamus.
- GnRH is released at lower levels in a pulsatile fashion from a "tonic" center throughout the cycle, with the frequency of the pulses influenced by the progesterone level.
- GnRH is also released at a much higher level from a "surge" center just before ovulation.
- Decreased progesterone level allows the "tonic" center of the hypothalamus to increase the frequency of small releases of GnRH from the hypothalamus.
- Rapidly rising estradiol and a low level of progesterone trigger release of GnRH in a large pulse from the surge center. Remember that the previously present progesterone helped "prime" the hypothalamus for the estradiol, thus increasing the effect of estradiol on GnRH/LH release and on behavioral display of estrus.

Prostaglandin release

- If no maternal recognition of pregnancy is present in late disestrus, prostaglandin $F_{2\alpha}$ is released by the uterus and causes regression of the corpus luteum, thereby dramatically dropping progesterone levels over the next 12 hours or so.
- Oxytocin produced by CL plays a role in prostaglandin production by the uterus.

Follicular waves and exogenous GnRH

Exogenous GnRH has been used extensively in the past few years to help control the estrous cycle and the timing of ovulation. The response to exogenous GnRH is dependent on the maturity of the follicle(s) at GnRH administration because a follicle only acquires ovulatory capacity after selection and establishment of dominance during a follicular wave. Typically, a follicle should be capable of ovulating if it is more than 10 mm in size.

<u>Cycling animals with 2 follicular waves</u>

Cycling animals with 2 follicular waves in theory would have about two-thirds of the cycle time where there would be a follicle mature enough to ovulate in response to exogenous GnRH. This has been borne out in most experimental studies. (Pursley 1995, review Fricke 2003b).

- If there is a follicle (>10mm) capable of ovulating, the LH surge associated with GnRH injection will ovulate that follicle and luteinization will follow. The resultant CL may be the primary CL or may be an accessory CL if a CL was already present at the time of ovulation. Removal of a dominant follicle by ovulation allows for recruitment of a new follicular wave.
- If there are smaller follicles present (<10mm) not capable of ovulating (insufficient LH receptors), the FSH release associated with GnRH injection may encourage continued growth of these follicles without ovulation. If there is a primary CL already present, it will not be affected.

A majority of the above animals should have luteal tissue capable of responding to prostaglandin in 7 days. Even more animals should have a follicle large enough to ovulate to a second GnRH 48 hours after the prostaglandin, whether a CL was present or not at the time of prostaglandin. This forms the basis for the classical OvSynch scenario (Pursley et al 1995).

However, there are animals that may not have their ovulations synchronized. Examination of one study's results might help explain some of these failures. All animals were cycling.

Ovulation in response to first and second GnRH by day of cycle. (Vasconcelos et al, 1999).

		1st GnRH	2nd GnRH
Days	**N**	**Ovulation**	**Ovulation**
1-4	31	23%	94%
5-9	47	96%	89%
10-16	52	54%	85%
17-21	26	77%	81%
Overall	156	64%	87%

As proposed by Fricke (2003b), the data in this table suggests:

- About 87% of cycling animals in the classic OvSynch program have synchronized ovulation to the second GnRH.
- Failure to ovulate to the first GnRH does not have a negative impact on ovulation synchronization to the second GnRH, as the highest synchronization for the second GnRH occurred with the lowest ovulation rate for the first GnRH.
- Cows in the second half of the estrous cycle at first GnRH have lower synchronization rates than cows in the first half.

The table below may help clarify these results (Vasconcelos et al, 1999)

	1st GnRH		2nd GnRH Ovulation		
Days	**Ovulation**	**N**	**Before**	**Synchronized**	**None**
1-12	No	42 (39%)	0 (0%)	39 (36%)	3 (3%)
	Yes	65 (61%)	0 (0%)	58 (54%)	7 (7%)
	Total	107	0 (0%)	97 (91%)	10 (9%)
13-22	No	14 (29%)	9 (18%)	5 (10%)	0 (0%)
	Yes	35 (71%)	0 (0%)	34 (69%)	1 (2%)
	Total	49	9 (18%)	39 (80%)	1 (2%)
All		156	9 (6%)	136 (87%)	11 (7%)

Note that the failures to synchronized fell into two large categories (Fricke 2003b):

- *Cows that failed to ovulate to the second GnRH.* Almost all of these cows received the first GnRH in the first half of their cycle. Also, the majority of these animals did ovulate in response to the first GnRH. One explanation would be that animals ovulating in response to the first GnRH initiated a new follicular wave that grew more rapidly and lost dominance prior to the second GnRH nine days later. These animals would not have a follicle capable of ovulation 2 days after the prostaglandin injection.
- *Cows ovulating prior to second GnRH.* All of these cows received the first GnRH in the second half of the cycle. Also, there was no ovulation in response to the first GnRH. An explanation for this situation would be that these animals were in the process of naturally regressing their CL a day or so prior to the prostaglandin injection and had ovulated the dominant follicle prior to the second GnRH.

Commercially FDA Approved GnRH Products

At least four NADAs have been granted by the FDA for products containing GnRH, shown in the table below. Also note there are two forms: a diacetate tetrahydrate and a hydrochloride.

NADA	Brand Name	Company	Active Ingredient
098-379	Cystorelin[1]	Merial	Gonadorelin Diacetate Tetrahydrate
200-069	Fertelin/OvaCyst[1]	Phoenix Scientific	Gonadorelin Diacetate Tetrahydrate
200-134	Fertagyl[1]	Intervet	Gonadorelin Diacetate Tetrahydrate
139-237	Factrel[2]	Fort Dodge	Gonadorelin Hydrochloride

The approved label indications are identical or very similar for all of these products:

[1]*The drug is used for the treatment of ovarian cysts.*
[2]*For the treatment of cystic ovaries (ovarian follicular cysts) in cattle to reduce the time to first estrus.*

Note there is no mention of use in synchronization programs or in any animal not having an ovarian cyst. Remember AMUDA does *not* grant permission for production drugs (e.g., CIDRs, GnRH, rBST) for extra-label purposes, even for veterinarians. Even though these uses may make very logical sense, be in extremely widespread use, and have a low regulatory enforcement priority, the veterinarian must remember that the manufacturer will not support them in any claim of adverse reactions and the FDA need not recognize the above reasons.

Use of Exogenous Progesterone

Based on the outlined hormonal sequence, it would appear that there is opportunity to control the timing of return to estrus using exogenous progesterone. The presence of exogenous progesterone would affect the sequence of events that occur after the release of prostaglandin and the regression of the CL, thereby delaying estrus until removal of the source of progesterone. The exogenous progesterone likely acts by depressing GnRH and FSH release, slowing follicular development and suppressing ovulation.

Ideally, the exogenous progesterone would be in a form that releases progesterone over a period of time, but would also allow abrupt removal of the source. Progesterone delivery devices in the form of intravaginal progesterone inserts have been developed to meet these needs. Controlled internal drug-releasing (CIDR®) devices have been utilized for several years in New Zealand and other countries for use in reproductive programs. In the United States an intravaginal progesterone insert is currently marketed by Pfizer Animal Health under the brand name of EAZI-Breed™ CIDR® Cattle Insert (NADA 141-200). The product contains 1.38 grams of progesterone in a silicon device and is designed to release a controlled amount of progesterone over a period of days. A supplemental approval for lactating dairy cows was granted on July 29, 2003.

The levels of progesterone achieved by these devices are roughly 1.5 nanograms/ml while a mature CL will produce a level of progesterone in the 4-7 nanograms/ml range. The exogenous progesterone is sufficient to block ovulation and estrous behavior, but does not exert quite as much effect the progesterone from a fully mature CL.

EAZI-Breed™ CIDR® Cattle Insert – label and label uses

The specific label indication in lactating dairy cows for this product reads as follows (1):
Synchronization of the return to estrus in lactating dairy cows inseminated at the immediately preceding estrus.

In the directions these additional statements are made for lactating dairy cows:

- *Administer one EAZI-BREED CIDR Cattle Insert per animal 14±1 days after insemination and remove the EAZI-BREED CIDR Cattle Insert 7 days later.*
- *Observe animals for signs of estrus on days 1 to 3 after removal of the EAZI-BREED CIDR Cattle Insert and inseminate animals about 12 hours after onset of estrus.*
- *Reminder: do not administer LUTALYSE Sterile Solution or other prostaglandin products to cows, as this will interrupt pregnancy that may have occurred at the immediately previous insemination.*

The above label use fits into this sequence:

Previous insemination
↓ 14 days (13 to 15 days)
CIDR insertion
↓ 7 days
Observe for estrus next 72 hours and inseminate 12 hours after onset of estrus

Strictly speaking, this is the only permitted sequence. Remember that AMUDA does *not* allow use for "production" purposes in an extra-label manner, even for veterinarians. A summary of trials leading to approval of the CIDR product was published (Chenault et al, 2003a).

	Control		**CIDR**		
	Percent	**Number**	**Percent**	**Number**	**p Value**
Synchronization	19.30%	544	34.10%	589	0.001
Pregnancy Rate (previous cycle)	36.70%	863	32.70%	881	0.044
Conception Rate	30.90%	194	26.70%	266	0.271
Pregnancy Rate	11.10%	540	12.20%	583	0.650

Synchronization was defined as the percent in estrus during the 3 d after CIDR insert removal for the treated group and the highest 3-d cumulative percent in estrus for controls. Mean time to estrus was approximately 2 days earlier (since prior insemination) for treated versus control.

These results would indicate the effectiveness of the product to increase synchronization of estrus (19.3% vs. 34.1%, $p<0.001$) without a statistically significant change in either conception rate (30.9%, 26.7%) or pregnancy rate (11.1%, 12.2%) at the resulting estrus after CIDR removal. The management advantages would include better timing of estrus observation, more predictability of labor needs, and more cattle in estrus at the same time.

One item of note would be the apparent increase in pregnancy loss for cows pregnant when the CIDR was inserted. This conclusion was derived from the decreased percent of pregnant cows in the CIDR group versus the control group at the time of CIDR insertion (control 36.7%, CIDR 32.7%). However, this evidence is indirect in that the true pregnancy status was not known at the time of CIDR insertion. Therefore, it is not certain that this difference was due to the presence of the CIDR or was an artifact due to other factors that lead to differences in conception or pregnancy rates in the control and treated groups at the insemination prior to CIDR insertion.

Management Considerations for CIDRs

Reuse: In the recent dairy economy it is always tempting to cut corners to save costs. One common practice is reuse of CIDRs for more than one time. While there is likely residual progesterone activity present in a previously used CIDR, the manufacturer does not recommend reuse for both efficacy and hygienic reasons. If a veterinarian recommends reuse of CIDRs, they will not be supported by the manufacturer in any case where a claim of lack of efficacy or an increase in vaginal infections occurs.

Labor: Use of CIDRs has potential to either increase or decrease labor. More importantly, like all synchronization programs, it likely requires increased management attention to timing of tasks, careful tracking of cattle, and attention to detail and hygiene when inserting and removing the CIDR.

Costs: While costs are always a consideration, some or all of the increased product costs can be offset by a decrease in labor costs and/or an improvement in reproductive performance. Given the value of a pregnancy, a strong argument can be made for consideration to determine if CIDRs would be a useful addition to a reproductive program.

ELDU: AMUDA does *not* grant permission for production drugs (e.g., CIDRs, GnRH, rBST) for extra-label purposes, even for veterinarians.

Future Potential Uses for Exogenous Progesterone

With the foregoing cautions about label usage in mind, there may be opportunities in the future to incorporate progesterone inserts into reproductive programs.

Use #1 – Initiation of normal length cycles in anovulatory post-partum cows

In the immediate post-partum and early lactation periods there may be a significant population of non-cycling (anovulatory) cows. In a recent review (Rhodes 2003), the percent of anovulatory cows at 50 to 60 days post-partum range from 11% to 38% in published reports. Given the effects of progesterone in the time prior to an estrus on behavioral estrus and perhaps on release of hypothalamic hormones, the reviewers stated: *"the use of progesterone alone may be beneficial for initiating normal length estrous cycles and may have a synchrony effect when used with PGF2α, but the response is variable and does not include synchronization of ovulation."*

While the results from inseminations of the first ovulation in formerly anovulatory animals treated with CIDRs may have been disappointing in many trials, the initiation of cyclicity should have much potential value for future cycles. Efforts will still need to be made to identify and remove causes of non-cyclicity, but this use of progesterone inserts has the potential to lessen the on-going costs of avolulatory cows by initiating cyclicity. For more information see Lamb 2001, Lucy 2002, Pursley 2001, Xu 2000a, Xu 2000b.

Use #2 – In conjunction with GnRH and PgF2alpha in post-partum cows in an OvSynch program

Inclusion of progesterone in the familiar OvSynch scheme at the time of the first GnRH may make physiological sense. Potential benefits would include the initiation of normal cycling in anovulatory cows and perhaps enhanced fertility and/or synchrony in cycling animals. Studies examining this use have been conducted (e.g., Lamb 2001, Xu 2000a, El-Zarkouny 2004) and other trials are currently underway. Most of these trials show little advantage over OvSynch in pregnancies in animals that are already cycling, but some advantage in pregnancies in formerly anovulatory animals.

However, one additional advantage of this approach is the resolution of existing cystic ovarian disease by the GnRH injection and to help initiate normal cycling in these animals. The luteinization of the cystic follicle would also expose the animal to increased progesterone. The resulting luteal tissue would then be responsive to prostaglandin. Even if the animal was not bred in a TAI program such as OvSynch, normal cycling could be re-established.

Identifying anovulatory cows

The percent of cow that are not cycling at the time of the end of the voluntary wait period has been reported to range from 11% to 38% (Rhodes 2003). Since most of these anovulatory cows will eventually resolve the condition with advancing DIM, the majority of the effects on reproduction will occur early in the rebreeding period.

Since it appears that anovulatory animals demonstrate the most economic benefit from use of CIDRs in early lactation, it would be useful to be able to target those animals for CIDR use. However, without repeated measures of blood or milk progesterone levels, the level of anovulatory cows can be difficult to estimate for a given herd. There have been a couple of approaches suggested by dairymen and their advisors:

1. Use of ultrasound to diagnose presence or absence of a CL at time of first GnRH injection in an OvSynch program that was preceded by a two injections of prostaglandin 14 days apart (i.e., PreSynch). The animals without a CL would get a CIDR plus GnRH. Obviously, this is not perfect, but since the majority of animals that have gone through a PreSynch program should have a CL, this approach would at least decrease the number of animals received CIDRs considerably. However, this does involve additional labor and handling of the cattle.

2. Use of tail paint at time of first prostaglandin in a two shot PreSynch program. Animals that had no evidence of tail paint being removed at the end of the 28 days would receive a CIDR and GnRH. Note that the purpose of the tail paint in this case is NOT heat detection. While one should not confuse anestrus and anovulation, this might be another approach to concentrate the truly anovulatory animals into a smaller group. This is less labor than ultrasound, but is not a proven strategy.

Effect of anovulatory cows on conception rates in synchronization programs

The presence of these anovulatory cows in herds using synchronization programs can lead to skewing of results and has sometimes lead to disappointment with synchronization programs. To illustrate the impact of anovulatory cows, examples will be shown below. **These examples are for first service only.**

A common approach on many operations is what is sometimes referred to as "backdoor" OvSynch. These programs usually involve intensive visual heat detection and insemination of detected heats for a short period of time following the voluntary wait period. At the end of the period designated for heat detection, all remaining animals are enrolled in the OvSynch program.

Example #1

- 100 eligible cows enter the breeding population at the VWP.
- Heat detection period is 14 days long.
- Assume percent anovulatory cows are 15% of all eligible cows.
- Good heat detection intensity
  - Assume normally would have detected 60 cows over full 21 days
  - Means 40 eligible cows are submitted for insemination in the 14 days.
- Excellent heat detection accuracy (assume 100%)
- Conception rate on the detected heats is 30%. Results in 40*30%=12 pregnant cows.
- If full heat detection for 21 days, 60*30% = 18 pregnant cows
- Assume anovulatory cows have zero chance of becoming pregnant even after OvSynch.
- Assume OvSynch conception of cycling animals is 85% of detected, due to ovulation exceptions. Means cycling animals at 25% CR.

What would be the expected conception rate on the cows enrolled in backdoor OvSynch?

- 100 cows start – 40 cows detected = 60 cows in OvSynch
- Out of the 60 cows enrolled, 15 are anovulatory.
- If remaining 45 have 25% conception, will be 45*25% = 11 pregnant cows
- 11 pregnant out of 60 in OvSynch is an 18% conception rate.
- Total pregnant cows in combined is 23 versus 18 expected in 100% heat detection.

If one directly compares 30% versus 18%, it makes the OvSynch performance look disappointing. Should the conclusion be that use of OvSynch be discontinued? Not necessarily – in this scenario there is 5 more pregnant cows than would have been present otherwise.

Example #2

- 100 eligible cows enter the breeding population at the VWP.
- Heat detection period is 14 days long.
- Assume percent anovulatory cows are 15% of all eligible cows.
- Medium heat detection intensity
  - Assume normally would have detected 36 cows over full 21 days
  - Means 24 of eligible cows are submitted for insemination in the 14 days.
- Excellent heat detection accuracy (assume 100%)
- Conception rate on the detected heats is 30%. Results in 24*30% = 7.2 pregnant cows.
- If full heat detection for 21 days, 36*30% = 11 pregnant cows
- Assume anovulatory cows have zero chance of becoming pregnant even after OvSynch.
- Assume OvSynch conception of cycling animals is 85% of detected, due to ovulation exceptions. Cycling animals at 25% CR.

What would be the expected conception rate on the cows enrolled in backdoor OvSynch?

- 100 cows start – 24 cows detected = 76 cows in OvSynch
- Out of the 76 cows enrolled, 15 are anovulatory.
- If remaining 61 have 25% CR, will be 61*25% = 15 pregnant cows
- 15 pregnant out of 75 in OvSynch is a 20% conception rate.
- Total pregnant cows in OvSynch 22 versus 11 expected in 100% heat detection.

Again, if one directly compares 30% versus 20%, it makes the OvSynch performance look disappointing. Should the conclusion be that use of OvSynch be discontinued? There are 16 more pregnant cows than would have been present otherwise.

Example #3

- 100 eligible cows enter the breeding population at the VWP.
- Assume no heat detection, but assume conception rate would have been 30%
- Assume percent anovulatory cows is 15% of all eligible cows.
- Assume anovulatory cows have zero chance of becoming pregnant even after OvSynch.
- Assume OvSynch conception of cycling animals is 85% of detected, due to ovulation exceptions. Cycling animals at 25% CR.

What would be the expected conception rate on the cows enrolled in total OvSynch?

- 100 cows start – 0 cows detected = 100 cows in OvSynch
- Out of the 100 cows enrolled, 15 are anovulatory.
- If remaining 85 have 25% CR will be 85*25% = 21 pregnant cows
- 21 pregnant cows out of 100 in OvSynch is a 21% conception rate.

If one could direct compare 30% versus 21%, it makes the OvSynch performance still look somewhat disappointing. Should the conclusion be that use of OvSynch be discontinued? Notice that in this scenario we end up within 2 pregnant cows of the other two scenarios. It still beat the best heat detection by several cows. In many cases the complete OvSynch can lead to much better organization of the labor and simplify delivery. Cost of drugs must be weighed against cost of labor and the ability of the dairy to deliver the tasks is critical for the success.

What if the first service OvSynch conception rate was the same as the conception rate on detected heats?

This could arise under some widely differing circumstances:

1. Low levels of anovulatory cows and excellent heat detection accuracy

In this case the conception rates might be acceptable in both groups, but could still be low if there was a conception issue not related to heat detection accuracy or anovulation. Examples would include semen viability, semen handling, and insemination techniques.

2. Poor heat detection accuracy

Poor heat detection accuracy will lead to breeding of cows at inappropriate times. In most cases this will lead to a decrease in conception. If there are very few anovulatory cows, the conception rates at first service on OvSynch may actually be higher.

Notes on OvSynch

- Conception rates on OvSynch should be expected to be lower if the potentially anovulatory have been concentrated into the synchronized group, as in backdoor OvSynch. The differences are due to multiple factors, but some major ones are heat detection intensity prior to OvSynch, heat detection accuracy, and the level of anovulatory cows.
- If conception rates on OvSynch at first service are higher than detected heats, one might become suspicious of the accuracy of heat detection.
- The conception rates on total OvSynch at first service should be expected to be lower than heat detection due to anovulatory cows, but since 100% of animals are submitted for service, the end result should be more pregnant animals.

- Overall conception rate for OvSynch should be expected to be slightly lower than heat detected inseminations, but not to the degree as at first service. This is because the percent of anovulatory cows decreases with increasing DIM as cows recover from post-partum difficulties. The hormonal programs themselves may also help initiate cyclicity.

Resynchronization

These examples were for first service only. It is assumed that the number of anovulatory cows drops as DIM progresses, with a lesser impact on conception rates. However, one of the common problems with backdoor OvSynch programs is the failure to track cows after first service and get them re-enrolled as promptly as possible. This failure to implement some form of "resynching" can negate the positive benefits of getting more cows pregnant in the first cycle. The resynching of open cows has as much if not more impact on economics than even the first cycle impact.

Resynchronization programs (such as GnRH 7 days prior to the 40 day pregnancy check) are available and have proven to be effective on dairies. These programs allow reinsemination as quickly as 2 days after an open diagnosis. While it may appear to be "wasting" GnRH, remember that the GnRH is only "wasted" on the animal that is pregnant at 33 days. Therefore, in a 100 cow herd there might be 100 pregnant cows receiving this extra GnRH in the course of a year. With pregnancies worth $200 to $400, the extra $4-$6 per pregnancy is fairly small.

Considerations when Adopting Early Pregnancy Diagnosis

A centerpeice of reproductive programs has been the detection of open cows and confirmation of pregnant cows in a timely manner. The recent availability of ultrasound has allowed these tests to be performed at an earlier time than rectal palpation. Other technologies for early pregnancy diagnosis may soon become available, including factors detectable in milk or blood.

At first glance there would no potential downside to early pregnancy diagnosis (EPDx) in order to identify open cows as early as possible for re-enrollment into the breeding program. However, there may be some variables to consider prior to adopting EPDx.

For example, compare these programs (for this discussion, assume 40 day rectals are 100% correct and all diagnoses are done exactly on the day stated):

1. Rectal at 40 days post-breeding and confirmation at 68 days post-breeding
2. EPDx at 26 days and confirmation at 68 days post-breeding

There would be these possible comparisons:

1. EPDx open and 40 day rectal open
2. EPDx pregnant and 40 day rectal pregnant
3. EPDx open and 40 day rectal pregnant
4. EPDx pregnant and 40 day rectal open

What would be the costs and benefits of each of these comparisons?

Comparison #1: EPDx open and 40 day rectal open

In this case the EPDx would detect an open cow 14 days earlier than the 40 day pregnancy. Using $2 for the cost of a days open, this would give about a $28 advantages for EPDx for an animal in this category. If 60% of the animals tested fell into this category, this would be about $17 benefit each for all animals tested, plus or minus any differences in costs. (The calculated benefit assumes that the time to re-enrollment is the same for both situations.)

However, if the comparison is:

- GnRH at day 33, rectal at day 40, open cows - prostaglandin at day 40, rebred at day 42
- EPDx at day 26, GnRH to open cows, prostaglandin at day 33, rebred at day 35

There would be a 7 day advantage or about an $8 advantage.

Another potential downside emerges if there are potential conception differences in animals re-enrolled at the two points with available re-synchronization schemes. These conception differences may be great enough in some cases to essentially offset the gains. For example, a difference of 5% in conception rates could change the days open as much as 15 days. In those cases the gains from the early open diagnoses would be negated by repeatedly exposing the animals for insemination at times with lower conception rates. (Fricke 2003a).

While there may be advantages to the EPDx for this comparison, care must be taken to not lessen the advantages by becoming more lax in getting cows re-enrolled or by choosing times for re-insemination where large conception differences exist in the two programs.

Comparison #2: EPDx pregnant and 40 day rectal pregnant

In this case the EPDx would confirm a pregnant cow 14 days earlier than the 40 day rectal. However, since there is no action taken or needed, there is little benefit to the EPDx, with the possible exceptions of management peace of mind or grouping of animals.

Comparison #3: EPDx open and 40 day rectal pregnant

In this case the cow would have been pregnant at day 40. However, the EPDx at day 26 incorrectly missed the pregnancy. In most cases this cow would have been given prostaglandin and/or other action taken that would have lead to iatrogenic abortion. The cost in days open for this loss of pregnancy is considerable. The costs include not only the original 26 days of gestation, but also the time needed for re-conception to occur. As shown in the table below, the median days open after the voluntary wait period at a 15% pregnancy risk is about 90 days. An animal in this category would have 26+90 or approximately 125 additional days open. At $2 per day this is $250 per pregnancy lost. If present in 1-2% of the original cases (~3-6% of pregnant cows), would be $3-$5 cost for early pregnancy diagnosis on average for all animals. The high cost per lost pregnancy means that extreme caution must be used to minimize this mistake.

Pregnancies per 21 day period at 15% pregnancy risk if no culling.

Days	Open at Start of Period	Pregnancies In Period
0	100	15
21	85	13
42	72	11
63	61	9
84	52	8
105	44	7

Comparison #4: EPDx pregnant and 40 day rectal open

In this comparison the EPDx test pronounced the cow pregnant but the 40 day rectal found her open. Days to re-enrolment for the 40 day rectal could be as low as 2 days with available re-synchronization programs. Days to re-enrolment for the EPDx cows in this category would be 26 (68-42) days plus about 10 days to re-enrolment for a difference of about 36 days. This would be about $70 per animal in this category.

Note that this comparison occurs in these two scenarios, as the end result is the same:

- Incorrect original diagnosis of pregnancy at EPDx.
- Correct diagnosis of pregnancy with subsequent loss prior to 40 days.

The degree of pregnancy losses that occur in early pregnancy (between day 26 and day 40) is currently an area of very active investigation. In many studies 10% to 20% of animals pregnant at day 26 may lose the pregnancy prior to day 40. If one assumes 40% of examined animals would be diagnosed pregnant at day 26, there would be about 32-36% of them pregnant at day 40 This would mean about 4% to 8% of the original animals would fall into this comparison. A 6% estimate at $70 per original animal in this category would be an average of $4.00 cost for EPDx per examination.

However, in some cases the percent can be considerably higher not due to truly increased pregnancy loss, but due to the caution needed to avoid the mistake in comparison #3, the iatrogenic loss of pregnancy. One solution would be to retain the 40 day rectal. While this would lessen the days open lost, it would mean there would be additional costs due to the early pregnancy diagnosis being present in addition to the 40 day rectal.

Early Pregnancy Diagnosis in "Total" Synchronized Programs versus Traditional Programs

The above discussion assumes that every EPDx occurred at day 26 and every rectal occurred at day 40. This situation would normally occur only in total synchronization programs with no heat detection. It also likely involves weekly pregnancy examinations.

More traditional programs have a majority of breedings based on daily heat detection and have pregnancy examinations on a 2 or 4 week interval. In those cases the average pregnancy examination conducted by the EPDx method might still start at 26 days, but the average would be about 6-7 days longer for the biweekly checks and about 12-14 days for the monthly (depending on heat detection intensity, conception rate, etc.). Likewise, the average for the rectals would be higher in these cases.

Care must be taken when comparing biweekly traditional programs to total synchronization programs with EPDx. If all pregnancy examinations occur at 26 days in total synch programs and on average at 45-50 days in traditional programs, it may well appear that there is a much increased degree of pregnancy loss occurring in the total synchronization programs. The temptation is to blame the synchronization program as the cause of the losses. However, the increase in apparent losses may be merely an artifact of the time of diagnosis, not as a result of the programs. The losses were always present, but were undetected.

On the other hand, EPDx is often unduly created with a positive increase in pregnancy risks. The reason for this is that in traditional programs the pregnancies that were lost early went undetected but also uncredited. However, in total synchronization programs with EPDx these formerly unrecognized pregnancies are detected and may be credited in a positive manner. There must be some recognition and adjustments made in the pregnancy risk calculations to make fair comparisons to more traditional programs.

Early Pregnancy Diagnosis Summary

Models can be useful for estimating costs and benefits. However, when comparing two programs, the accuracy of the original estimates can often sway the results. The point was to introduce potential costs and benefits of early pregnancy diagnosis versus a more traditional rectal diagnosis program. The discussion was not intended to be an advocate either for or against early pregnancy diagnosis, but to merely raise some cautions. It is unclear at this point whether early pregnancy diagnosis has an overwhelming advantage to more traditional rectal programs.

One of the largest issues surrounding early pregnancy diagnosis is the true degree of pregnancy losses occurring in the 26 to 40 day range. Early pregnancy diagnosis has greatly increased the awareness of these losses and has stimulated investigation into this area. It is uncertain whether these losses can be reduced, as the causes may be genetic defects, embryonic developmental defects, hormonal abnormalities in the dam, pathologic conditions in the uterine environment, infectious agents that kill the embryo/fetus, or one of many other factors.

Another issue with early pregnancy diagnosis is the differences in conception that may be present at various points since previous insemination, especially in resynchronization programs. If the early pregnancy diagnosis results in re-insemination at a time of substantially poorer conception rate, the average time to conception for these animals may be substantially increased, offsetting some or all of the gains made by an earlier diagnosis of openness.

A final issue is the accuracy of the early pregnancy diagnosis. One needs to avoid iatrogenic loss of pregnancy. This means that the test must essentially be close to 100% sensitive for pregnancy (close to zero percent false negatives). However, tests with sensitivities this high often have specificities that are lower (higher false positives). Since the majority of economic benefit to EPDx arises from earlier detection of open cows and quicker re-enrolment, lower specificity would lead to decreased benefits.

Implementation or Delivery Issues Leading to Decreased Success

In many cases failure of synchronization programs arise due to problems with implementation and not due to the underlying physiology/endocrinology/logic of the program itself. A short, incomplete list of implementation problems would include:

Problems with injections
Not given at all or given to wrong cows
Given at variable times during the injection day
Too busy/forgot so given next day rather than the correct day
Improper injection techniques
Reduction of dosage in pursuit of cost savings
Improper list generation/inadequate data entry
No list
Incomplete or wrong cows on lists
List generation too complicated
Data entry too cumbersome
Problems with insemination of cattle
Semen handling
Thawing too many straws ahead of time
Prolonged environmental exposure (cold/heat)
Insemination techniques
Physical ability to inseminate large number of cows
Problems with sticking to a schedule
Weekly
Daily
Too much rough handling of cattle during injections or inseminations

In other cases there may be problems with the logic of the program or incompatibilities with multiple programs simultaneously in place. In some cases no single program is ever fully implemented as new wrinkles are added regularly. In other herds there is the temptation to micromanage each cow, leading to each cow being an exception with her own unique program. A program that is a little bit of everything is likely the toughest to manage. Another problem facing synchronization acceptance is that herds that do not manage heat detection well may also not manage injections and other tasks very well either.

Finally, as noted above, programs that do not also place emphasis on resynching cows in a timely manner as well as the first service risk losing or negating the economic benefits that synchronization programs may offer.

Summary

Research into the underlying physiology has lead to practical application of available reproductive hormones to influence either estrus or ovulation synchronization. There is still much to be learned, but a solid understanding of the basics will help the practitioner implement programs in a logical manner.

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