Spring 2010

# DELAWARE DAIRY NEWSLETTER

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**Proceedings from the 2010 Delmarva Dairy Days can be found at:**

http://ag.udel.edu/anfs/faculty/kung/Publications.htm

## Silo Safety

Its getting close to making silage again and it's a good time to remind everyone about safety during silo filling. Dr. Keith Bolsen, formerly of Kansas State University just published a great silo safety manual. I will try to summarize some of his recommendations below and feel free to contact us if you would like a copy.

Tractor roll-over safety items:

- Roll over cages create a zone of protection for the tractor operator and seat belts should also be worn.
- Never fill higher than a bunker wall to prevent drop off.
- Sight rails should be installed to indicate where the wall is to the pack tractor operator.
- Use the progressive wedge design when filling bunkers and piles to provide a safe slope.
- To reduce the risk of tractor roll-over, keep the slope a minimum of 3 to 1 on the sides and ends of a drive over pile.
- Tractors should be backed up steep slopes to prevent roll backs.
- Wide front-end tractors equipped with well lugged tires prevent slipping.
- Never use any type of hay or straw bales as temporary bunker walls.
- Add weights to front/back of tractors to improve stability and packing density.
- Keep the center of gravity low when moving silage in a bucket
- Establish a driving pattern when 2 or more tractors are used to prevent collisions

Other items:

- Never stand near the top of a silage overhang as your weight might cause a collapse.
- Never allow a person to ride in the bucket of a front end loader!
- Do not fill a bunker or pile higher than unloading equipment can reach.
- Shave down the face, not up from the bottom.
- Be especially cautious around faces of bunk or pile silos. Cave ins can occur even in small silos that look well packed. Keep children away from silo faces.
- Beware of silo gasses. ..even small amounts can be fatal. (more on silo gasses can be found at http://nasdonline.org/document/64/d001621/silo-gas-dangers.html

# Chromium in Dairy Cattle Diets

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As many of you are likely aware, chromium (Cr) propionate recently received no objection from the FDA for use in cattle diets. The two supplemental forms of Cr propionate currently on the market provide either 0.4 or 0.04% Cr and are mixed into the diet to provide up to 500 ppb (parts per billion) Cr in the total mixed ration.

**What is chromium?**

Chromium is a trace mineral element that can be found in molecules at two different valences, Cr+3 or Cr+6. The primary function of Cr+3 in the body appears to be as a component of the “glucose tolerance factor” that helps to regulate glucose metabolism. On the other hand, Cr+6 can be toxic. Discussion of “Cr” in this article refers specifically to Cr+3. Inorganic dietary forms of Cr including CrCl3 and Cr2O3 are not absorbable, and Cr must be complexed with an organic molecule to be available in the digestive tract (NRC, 2001). Supplementation of human diets with Cr is recommended, but there is currently no requirement for Cr in dairy cattle diets (NRC, 2001).

**Glucose tolerance and insulin sensitivity**

Glucose tolerance relates to the ability of an animal’s body to respond to changes in blood glucose. In monogastrics such as humans, blood glucose rises following a meal and our bodies respond by directing blood glucose to tissues for utilization. Problems with this response, known as glucose intolerance, cause blood glucose concentration to be elevated for longer periods of time following a meal. In fact, much of the tissue damage that occurs in individuals with uncontrolled diabetes is due to negative effects of high concentrations of blood glucose.

The importance of Cr to glucose tolerance was originally discovered when rats fed purified diets were found to have poor glucose tolerance. Inclusion of Cr in their diets restored glucose tolerance and hence was believed to be a part of a “glucose tolerance factor” which was later found to be a complex compound (Schwarz and Mertz, 1959; NRC, 2001). Supplemental Cr appears to have therapeutic effects when glucose tolerance is poor. For example, Cr enhances glucose tolerance and reduces blood glucose concentration in humans with diabetes, but Cr has no effects on blood glucose in healthy humans (Balk et al., 2007).

The ability of Cr to enhance glucose tolerance is believed to be due to its effects on insulin sensitivity. Insulin sensitivity is basically the ability of tissues to mount an appropriate response to the hormone insulin. In humans, production of insulin following a meal results in changes at the cellular level which ultimately result in glucose being absorbed by insulin-responsive cells. A lack of proper response to insulin is called insulin resistance and is associated with metabolic disease states.

**Glucose tolerance and insulin sensitivity in cows**

In cows, ruminal fermentation of feed carbohydrates to volatile fatty acids results in relatively little glucose absorption from the gut compared to monogastrics. However, glucose tolerance and insulin sensitivity are important to cattle metabolism as well. For example, insulin production in response to glucose is reduced in postpartum compared to prepartum cows (Bossaert et al., 2008). They also found that those cows that responded poorly to glucose prepartum carried that poor response over to the postpartum period. Additionally, insulin resistance has been suggested to be a risk factor for development of postpartum metabolic and reproductive diseases (Oikawa and Oetzel, 2006). As a bottom line, if we can find ways to increase tissue responsiveness to both glucose and insulin during the transition period, this should decrease incidence and severity of metabolic diseases such as ketosis.

Supplementation of transition dairy cows with organic Cr sources has the potential to improve insulin sensitivity, glucose increased intake both pre- and post-partum, though there were no effects on milk yield. Smith et al. (2005) found that prepartum Cr supplementation increased postpartum intake and milk production, with no effect on prepartum intake. McNamara and Valdez (2005) found similar results when Cr was supplemented from 21 d before calving to 35 d postpartum, though the increase in milk production was not significant.

Chromium and cattle health

As outlined above, most of the Cr research in dairy cattle has evaluated its effects on glucose tolerance, insulin sensitivity, and production, and most of this work has focused on transition and early lactation cows. However, some work also suggests that Cr may enhance animal health, particularly during periods of stress. Kegley et al. (1996) found that calves given milk replacer supplemented with Cr had an increased rate of recovery from an infectious bovine rhinotracheitis challenge. Moonsie-Shageer and Mowat (1993) found that Cr supplementation following transport stress in beef steers enhanced vaccine response and reduced morbidity. Though few such studies have been conducted in lactating cattle, Burton et al. (1993) found that Cr supplementation from 6 weeks prepartum to 6 weeks postpartum enhanced vaccine response.

**Summary**

- Chromium supplementation increases glucose tolerance and insulin sensitivity in transition cows
- Chromium supplementation during the transition period and into early lactation increases intake and milk production in some studies, but not in others
- Chromium supplementation may be most beneficial in herds with low prepartum and/or postpartum intakes or in herds with a high incidence of ketosis (>5% of cows)

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# POTENTIAL OF ALTERNATIVE DAIRY REPLACEMENT HEIFER NUTRITION PROGRAMS TO REDUCE ECONOMIC COST AND ENVIRONMENTAL IMPACT

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## TAKEHOME MESSAGE

In general, simple manipulation of dietary protein and energy in replacement heifer diets has not yielded profound changes in feed cost or future animal performance. Modestly increasing dietary energy and protein combined with limit-feeding the resulting diet has the potential to reduce feed cost and nutrient excretion without compromising future performance. Recent data also suggest supplementation of P to dairy heifers does not result in improve frame or bone growth and maybe minimally required.

## I N T R O D U C T I O N

The goals of a dairy replacement management program are to rear heifers at a low economic and environmental cost without compromising future lactation performance. To meet these objectives, heifers are commonly fed diets containing high fiber forages (MPS, 2003), which meet the low energy requirement (NRC, 2001) of replacement heifers. Feeding heifers low energy, high fiber forages also helps minimize over-conditioning at calving which can be detrimental to lactation performance (Hoffman et al., 1996). Total feed cost and feed efficiency are however often over-looked with feeding heifers diets containing predominately high fiber forages. Historically, research (Van Amburgh et al., 1998, Hoffman et al., 1996, Radcliff et al., 2000) focused on feeding heifers higher energy diets to reduce calving age below recommended (22-24 mo) as methodology to shorten length of the rearing period and correspondingly reduce feed cost. Although this strategy has the potential to lead to an earlier return on feed investment decreasing the calving age frequently results in a decrease in lactation performance (Van Amburgh et al., 1998, Hoffman et al., 1996, Radcliff et al., 2000). Another strategy to reduce heifer feed cost is to feed higher energy diets but to limit the amount of the diet fed controlling average daily gain (**ADG**) which could effectually yield a calving age and body condition score similar to feeding high forage diets. This management strategy will be referred to as limit feeding for the remainder of this paper. Limit feeding has the potential to reduce feed cost, increase feed efficiency and decrease fecal excretion while preserving the rearing period time course which to date has been difficult to alter without negative health and production effects. In addition, dietary P supplementation to dairy heifers increases diet and environmental cost. If basal feeds contain P levels similar to the P requirement supplemental P may not be required for dairy heifers. This paper will review issues associated with limit feeding and P supplementation to dairy replacement heifers.

## PSEUDO LIMIT-FEEDING RESEARCH

Limit feeding ruminants is not new or novel. Limit-feeding strategies have been successfully employed with ruminants such as beef cows, (Loerch, 1996), ewes (Susin et al. 1995) and beef heifers (Wertz et al. 2001). Likewise limit feeding dairy replacement heifers is not new or novel and has been a research methodology in a number of investigations. What is different about these investigations is limit feeding was not the central hypothesis rather limit feeding was merely a methodology to investigate a related hypothesis. The author has arbitrarily classified these research projects as pseudo limit feeding research.

For example, Lammers et al., (1999) used a limit-feeding as method to control growth rates of prepubertal Holstein heifers to investigate effects of prepubertal growth rates on lactation performance. Differing prepubertal growth rates were achieved by offering different amounts of dry matter (**DM**) of a single diet [(16.0 % CP and 1.21 Mcals/lb of metabolizable energy, (**ME**)]. Prepubertal ADGs were 1.54 and 2.20 lbs/d thus the 1.54 lbs/d treatment was commissural with limit feeding. Heifers limit fed to grow 1.54 lbs/d produced 7.1 percent more milk than heifers fed near ad libitum (1000 g/d) which was attributed to differences in prepuberty mammary development which was the central hypothesis of the experiment. Lammers et al., (1999) observed no negative effects of limit feeding on body weight (**BW**), calf birth weight or dystocia index.

North Dakota researchers (Ford and Park, 2001, Park et al., 1998) have hypothesized that dietary energy restriction followed by realimentation stimulates rapid and greater expression of mammary tissue resulting in improved milk production. The work has demonstrated alteration of hormonal signaling, increased genetic expression of mammary tissue and up to 15.0 % improvements in milk production. Similar to Lammers et al., (1999), the experimental methodology (Ford and Park, 2001) used to implement energy restriction realimentation protocols was limit feeding. Control heifers were allowed ad libitum access to a diet containing 12.0 % CP and 1.07 Mcals/lb of ME while energy restricted realimentation heifers were limited to 70 percent of the same diet during energy restriction phases. Limiting feed intake to 70 percent of the control diet resulted in improving feed efficiency approximately 30 percent. The hypothesis and design of these experiments was to investigate energy restriction which yielded positive lactation responses. The energy restriction however was facilitated by limit feeding not by energy dilution of the diet. Data suggest there were no negative confounding aspects associated with limit feeding to facilitate limiting dietary energy intake.

There are additional examples in the literature (Carson et al., 2000, Hof and Lenaers, 1984, Sejrsen and Foldager, 1992 and Van Amburgh et al., 1998) that employed some form of limit feeding in an experiment to investigate an alternative hypothesis in heifer production and management. While no direct linkage can be made from experimental results to limit feeding per se the limit feeding methodology employed in these experiments did not result in any negative effects on milk production. In all experiments outlined above milk production was numerically greater for any treatment, regardless of hypothesis studied, for heifers that were limit fed as a part of the methodology.

**LIMIT-FEEDING RESEARCH – CENTRAL HYPOTHESIS**

As previously stated limit feeding is not new and has been employed by researchers as a method to execute experimental designs for other hypothesis. Likewise it can be assumed that some forms of limit feeding heifers have been employed by dairy producers over time. Recently, it has been consciously recognized that limit feeding methods applied in experiments appear to have a more robust applied utility. Limit feeding has been utilized in experiments as a method is to control growth rates, decrease energy intake, decrease feed usage, improve feed efficiency or improve lactation performance. These are exactly the same goals as the goals of commercial heifer production. As result two recent experiments have been conducted evaluating limit feeding as a central hypothesis to explore applied applications.

At the University of Wisconsin we explored a simple limit-feeding feeding system for bred replacement heifers (Hoffman et al., 2007). A summary of trial results is presented in Table 1. Bred Holstein heifers were fed diets (C-100, L-90 and L-80) containing 67.5, 70.0 and 73.9 percent TDN respectively but heifers fed the 70.0 and 73.9 percent TDN diets were limit-fed at 90 and 80 percent of their intake potential. The study was designed to provide iso-caloric and iso-nitrogenous intakes. Limit feeding resulted in heifers being fed less DM per day but the total amount of calories consumed per day was equal. We did not observe any differences in the size or body condition scores of the heifers after a 111 day feeding period. The limit-feeding regimen however resulted in a 25 percent improvement in feed efficiency and heifers excreted significantly less manure. We observed no effects of limit feeding heifers on calf BW or dystocia index. As with pseudo limit feeding experiments we observed a numerical trend in improved milk yield but true lactation performance was similar between control and limit-fed heifers.

A second study with limit feeding as a central hypothesis was conducted at the Pennsylvania State University (Zanton and Heinrichs, 2007). This study was uniquely different that our study at the University of Wisconsin. Our study was conducted on bred heifers (1000 lbs) with a short experimental period (111 d). The Penn State study was conducted on heifers weighing 275 lbs and heifers were limit fed for the entire prepubertal period (245 d) and then feed a common diet post puberty. The level of concentrate in the limit fed diet (75 %) was more intensive than the level of concentrate we fed to bred heifers (37 %). A summary of key results of the Penn State study are presented in Table 2. Limit feeding 300 lb Holstein heifers diets containing 25 percent forage as compared to feeding diets containing 75 percent forage ad libitum resulted in no differences in ADG or skeletal growth of heifers. Heifer reached puberty at the same age and had similar reproductive performance. Heifers calved at the same age but limit fed heifers had numerically higher BW at calving and lost more BW after calving. As with previous studies limit fed heifers produced numerically higher amounts of milk with similar milk composition.

It is important to recognize the uniqueness of each of these studies. In the Wisconsin study heifers were limit fed post puberty while the heifers in the Penn State study were limit fed pre puberty. Both limit feeding strategies resulted in similar animal performance. To date there are no data which has evaluated limit-feeding heifers throughout the majority of the rearing period.

**LIMIT FEEDING – CHANGES IN HEIFER BEHAVIOR**

There are some changes in heifer behavior as a result of limit feeding. In our study at the University of Wisconsin (Hoffman et al., 2007) we monitored several aspects of heifer behavior and data are presented in Table 3. First, heifers vocalize to minor extent for approximately one week with vocalization diminishing thereafter. Vocalization is primarily limited to bellowing immediately prior to feeding. In addition, eating time is logically reduced when heifers are limit fed but heifers appear to compensate for reduced eating times by standing more which ultimately reduces lying times. Despite observation of changes in behavior, the behavioral changes we observed when heifers are limit fed appear to be subtle and manageable.

We have observed some undocumented quirks in heifer behavior as a result of limit feeding. In preface to explaining these observations in should be noted that most experiments defined above the heifers were individually fed. For example in the experiment recently published by Zanton and Heinrichs, (2007) the heifers were individually fed via calan gates. Limit feeding heifers individually does not allow observation of group feeding behavior dynamics which could be altered by limit feeding. In our study, heifers were fed in pens (6 heifers/pen) because pen was used as the experimental unit. At the time of the experiment we failed to anticipate changes in bunk (eating) behavior and did not quantify these issues. As a result, changes in bunk behavior noted in this paper forward are empirical but we feel worthy of mention.

Changes in eating behavior of heifers limited to 80-90 percent of ad libitum intake are subtle and overly aggressive eating behavior was not observed. However, heifers while eating, efficiently push feed forward perpendicular to the feed bunk with their muzzle. When fed on a flat feeding surface a large portion of diet will be pushed out of reach by the heifers. If heifers have not reached fill or satiety, heifers will aggressively reach in an attempt to acquire feed which they have displaced too far forward. This reaching behavior requires heifers to splay their fore and hind legs to create torque to lean forward. The long term effect of this behavior on foot and leg health is not known. We corrected this behavior by frequently pushing remaining feed up proximal to the fence line. As a result we would caution that increased feed push ups may be required when limit feeding heifers in a flat manger.

Another undocumented behavioral change we observed, is heifers, appear to become acclimated to limit feeding regimens and eating behaviors carry over for a short time after limit feeding is discontinued. After our experimental period we transitioned the heifers to a common high bulk, high NDF diet. For a short period of time (5-7 days) heifers ate this diet as if limit fed. Visual evidence of additional ruminal distention was obvious. These observations suggest heifers have the ability to rapidly increase rumen volume. Quick and rapid extension of rumen volume has been well documented in lactating dairy cows (Dado and Allen, 1995).

Adequate bunk space is required to assure all heifers have full access to feed because heifers fed to 80 percent of intake potential will consume all feed available within 2-3 hours. Lack of adequate bunk space could result in displacements at the bunk and ultimately result in un-even ADG. We observed small numerical increases in ADG variance when heifers were limit fed but variance in ADG was not significant when 1000 lbs heifers were allowed 24 inches of bunk space/heifer. The critical lower limit of bunk space per heifer under various limit feeding scenarios is not known. Finally limit feeding can not be implemented were edible bedding such as straw, grass, corn stalks etc is used as heifers will consume bedding to reach satiety.

**PHOSPHORUS REDUCTION**

Phosphorus requirements, as percent of dietary dry matter (DM) for heifers (0.20-0.35 %) and endogenous levels of P in feeds (0.20-35 % of DM) are similar suggesting supplementation of P in heifer diets may be infrequently required. Recently, Esser et al., (2009), fed heifers diets with (0.39 %) and without (0.29 %) supplemental P from 4-21 mo of age. Two subpopulations of heifers were selected mid-trial for intensive measurement of bone development and metabolism. Thirty-two heifers were evaluated for bone development and measurements included hip height, length, heart girth, hip width, cannon bone circumference, pelvic length, pelvic height, and pelvic width. Tails of heifers were surgically amputated with the 13 and

14th coccygeal vertebrae retained. After tissue removal, the 13th coccygeal vertebrae were scanned using peripheral quantitative computed tomography with cortical, trabecular and total bone densities determined. A second sub-population (n=64) of heifers were evaluated for serum pyridinoline and osteocalcin to assess systemic bone metabolism. Supplementing P had no effect on external frame measurements, bone density, or bone metabolism markers. Bone P content was lower (18.1 vs 18.6 %) in heifers fed no supplemental P. Phosphorus supplementation to heifers modestly increased bone P content but increased bone P was not reflected in frame growth, bone density or bone metabolism. As a result, if dietary feedstuffs contain P proximal to the P requirement supplemental P may not be required for dairy heifers.

## CONCLUSIONS

To date the following can be concluded about limit feeding and P supplementation to dairy heifers.

Limit feeding decreases feed usage, manure excretion and improves feed efficiency of dairy replacement heifers.

There are no research trials indicating that limit feeding has a detrimental effect on heifer/cow health or future lactation performance.

A hypothesis could be constructed that limit feeding may improve milk production but mechanisms are not known.

Limit feeding does result in some minor changes in heifer behavior and management may need to be modified to account for such behavior.

Limit feeding cannot be implemented when bunk space is limited or in housing systems using edible bedding

Supplemental P can be reduced or eliminated if basal feeds contain P proximal to the P requirements of dairy heifers.

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**Table 2. Penn State limit feeding trial: Summary of results (Zanton and Heinrichs, 2007)**

| | Treatment | | | |
|---|---|---|---|---|
| Item | Control | Limit-fed | SEM | P < |
| Diet | | | | |
| Forage | 75 | 25 | | |
| Concentrate | 25 | 75 | | |
| Gain | | | | |
| Body weight, lbs/d | 1.82 | 1.82 | 0.02 | NS |
| Withers height, in/d | 0.04 | 0.04 | 0.0007 | NS |
| Reproduction | | | | |
| Age @ puberty,d | 333.0 | 320.0 | 6.0 | NS |
| Conception rate, % | 83.0 | 75.0 | 7.0 | NS |
| Parturition | | | | |
| Age @ calving, mo | 23.3 | 23.5 | 0.2 | NS |
| Postpartum BW, kg | 1179.0 | 1232.0 | 24.2 | NS |
| Lactation performance (0-150 DIM) | | | | |
| Milk yield, lbs/d | 69.7 | 78.3 | 3.2 | NS |
| Milk fat, % | 3.71 | 3.95 | 0.11 | NS |
| Milk protein, % | 3.12 | 3.02 | 0.04 | NS |

**Table 1. University of Wisconsin limit feeding trial: Summary of results (Hoffman et al., 2007)**

| Item | Treatment[1] C-100 | R-90 | R-80 | SEM | Effect(P>)[2] Treatment | Linear | C vs R |
|---|---|---|---|---|---|---|---|
| Diet | | | | | | | |
| Forage | 94.3 | 80.3 | 62.7 | | | | |
| Concentrate | 5.7 | 19.7 | 37.3 | | | | |
| NDF | 47.3 | 41.8 | 35.6 | | | | |
| Nutrient intake, lbs/d | | | | | | | |
| DM | 21.3 | 19.9 | 18.3 | 0.4 | 0.01 | 0.003 | 0.006 |
| CP | 2.42 | 2.54 | 2.57 | 0.03 | 0.07 | 0.03 | 0.03 |
| NDF | 10.06 | 8.29 | 6.50 | 0.16 | 0.0003 | 0.0001 | 0.0002 |
| $NE_g$, Mcals/d | 9.4 | 9.4 | 9.5 | 0.2 | ... | ... | ... |
| Weight | | | | | | | |
| Initial, lbs | 1036 | 1021 | 1011 | 21 | ... | ... | ... |
| Final, lbs | 1220 | 1234 | 1217 | 19 | ... | ... | ... |
| Feed efficiency | | | | | | | |
| lbs DM/lb gain | 13.2 | 10.7 | 11.1 | 0.9 | ... | ... | 0.09 |
| Excretion | | | | | | | |
| DM, lbs/d | 7.7 | 6.9 | 5.8 | 0.6 | ... | 0.10 | 0.10 |
| Parturition | | | | | | | |
| Dystocia index[3] | 2.2 | 2.1 | 1.9 | 0.3 | ... | ... | ... |
| Calf BW, lbs | 91.4 | 93.3 | 95.1 | 3.1 | ... | ... | ... |
| Postpartum BW, kg | 1238.0 | 1245.0 | 1275.0 | 20.9 | ... | ... | ... |
| Lactation performance (0-150 DIM) | | | | | | | |
| Milk yield, lbs/d | 68.8 | 68.9 | 72.4 | 1.7 | ... | ... | ... |
| Milk fat, % | 3.89 | 3.74 | 3.68 | 0.09 | ... | ... | ... |
| Milk protein, % | 2.87 | 2.85 | 2.89 | 0.03 | ... | ... | ... |

[1] C100, control heifers fed ad libitum, L90, limited to 90.0 percent of intake, L80, limited to 80.0 of intake. Treatment means expressed as least square means on a per heifer basis.

[2] C=Control (C-100) vs L=Limited (L90,L80). Entries without values are not significant (P>0.10). Trt = treatment.

[3] Dystocia index, 1= no problem, 2 = slight problem, 3 = needed assistance, 4 = considerable force, and 5 = extremely difficult.

**Table 3. Behavior of limit fed heifers when group fed (Hoffman et al., 2007)[1].**

| Item | Treatment[2] C-100 | L-90 | L-80 | SEM | Effect(P<)[3] Trt | TrtxWeek |
|---|---|---|---|---|---|---|
| Eating, % of time | 19.3 | 15.7 | 10.3 | 0.6 | 0.0001 | ... |
| Standing, % of time | 19.6 | 24.4 | 32.9 | 0.7 | 0.0001 | ... |
| Lying, % of time | 60.9 | 59.8 | 56.7 | 0.5 | 0.0001 | ... |
| Vocalization, % of time | 0.02 | 0.04 | 1.10 | 0.2 | 0.0001 | 0.03 |
| Eating, hrs/day | 2.3 | 1.9 | 1.2 | 0.1 | 0.0001 | ... |
| Standing, hrs/day | 4.7 | 5.8 | 7.9 | 0.2 | 0.0001 | ... |
| Lying, hrs/day | 14.6 | 14.4 | 13.6 | 0.1 | 0.0001 | ... |

[1] Time associated with involuntary behavior such as barn cleaning, blood sampling etc. was not recorded therefore percent of time and hours of time will not equal 100 and 24 respectively.

[2] C-100, control heifers fed ad libitum, L-90, limited to 90.0 percent of intake, L-80, limited to 80.0 of intake. Treatment means expressed on a per heifer basis.

[3] Trt = treatment. Entries without values are not significant (P>0.10).

## Anestrous, Anovular Cattle, Synchronization Programs and Reproductive Efficiency

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Pregnancy rate is a function of conception rate and estrous detection rate. In the average herd practicing observational estrus detection, rates of estrus detection generally run between 45-55%. In herds with acyclic, anovulation and anestrous cattle, estrus detection rates can fall precipitously below 45%. Moreover, increased errors in estrus detection result in insemination of cattle that are not in heat. Accordingly, timed artificial insemination programs (TAI) such as Ovsynch and Presynch-Ovsynch have been developed and implemented to increase estrous detection rates to 100%. Hence the popularity of TAI programs in reproductive management on the modern dairy production units. With voluntary wait periods set at 45-60 days post partum, TAI programs are often initiated by 45 days post partum in order to achieve first service dates at 60-75 DIM in the complete absence of estrus detection. The principle drawback to this management approach is the absence of specific knowledge about the cyclicity, ovulatory and fertility status of cattle undergoing service in TAI programs. This can present challenges to reproductive management programs because anovulatory cattle entering TAI programs typically have lower conception rates and higher rates of early embryonic death (EED) during the first 50-60 days post insemination.

Anestrous and anovulatory cows are transition cows that lack estrus behavior, do not cycle and cannot ovulate. Anestrous or anovulatory cattle are typically encountered by 45 days in milk (DIM) when clinical reproductive performance tends to terminate. Anovulatory cattle continue to develop and grow follicle waves but fail to show estrus and successfully ovulate. Anestrous, anovulatory cattle have fewer pregnancies/breeding, fail to cycle and ovulate at 60-100 DIM, show poor follicle development with variable and many times smaller follicle size, generate oocytes with poor development and quality, show higher incidence of anovulatory follicles and cystic follicles and typically generate follicles and corpus luteal bodies with lower capacity for steroidogenisis. Once transition cattle move from the anestrous condition and start to cycle, the first ovulatory estrus is often associated with lower conception rates, multiple ovulations and production of poor quality oocytes and embryos. These cattle often demonstrate shortened luteal phases of the cycle (11 days) and therefore increased percentage of irregular length cycles after resuming cyclicity. Lastly, they benefit from Ovsynch but not Presynch components of a synchronization program.

Herds with difficulties managing cows prepartum and in the transition period typically experience increased prevalence of anovulatory and anestrous cows by the end of the voluntary wait period. These anestrous, anovulatory problems are associated with deep and prolonged nadirs in negative energy balance during the transition period. Prevalence of anovulatory cattle entering into the TAI programs may run as low as 8% in well managed herds or as high as 50-60% in inadequately managed herds. The median level of anovulatory cattle in commercial dairies may lie closer to 18-29% as determined by 2 consecutively low serum levels of progesterone determined 2 weeks apart in commercial herds (Moreira et al., 2001, Pursley et al., 2001). Most evidence indicates anovulatory cattle present a substantial problem and challenge to reproductive efficiency in modern dairies. Prevalence within each herd will depend upon the successful management of nutrition, ration formulation and transition cow programs across herds. The incidence of anovulation is generally greater in primiparous cattle rather than multiparous animals (Chebel et al., 2006) but reports exist with data showing just the opposite incidence as well. In one report, 37% of primiparous cattle compared to only 16% of multiparous animals were anovulatory (Gumen et al, 2003).There is a strong, negative and linear relationship between the incidence of anovulation and BCS. Most cows greater than 3.5 BCS tend to be ovulatory whereas as many as 50-60% of cows with BCS ≤ 2.25 tend to be anovulatory (Gumen et al., 2003, Chebel et al., 2006).

Anovulatory cows are very capable of growing waves of follicles similar to normal animals but the waves do not culminate in production of an ovulation. Failure to ovulate can be attributed to less than normal follicle function and estrogen synthesis by slowly growing follicles in anovulatory cows. Estrogen is a key hormone involved in the induction of pre-ovulatory surges of GnRH and LH that trigger follicular ovulation. Anovular cattle show less than normal production of estrogen as well as an insensitivity of the GnRH and LH releasing centers of the brain to estrogen. For years it has been known that fertility is very low on the first insemination after re-establishing ovulation and cyclicity in anovular cattle. At least part of the low fertility has been associated with a lack of progesterone stimulation from the corpus luteum of the preceding cycle. (By definition, anestrous cattle lack a preceding cycle with the accompanied ovulation and therefore do not produce a corpus luteum to synthesize and secrete progesterone.) The secretion of progesterone during preceding cycles maintains the sensitivity of GnRH and LH producing centers in the brain to estrogen stimulation. When there is no progesterone stimulation during anestrus and anovulation in cattle, low or even normal levels of estrogen produced by anovular cows cannot trigger the ovulatory release of GnRH and LH. As a result these cattle remain anestrus, anovulatory and infertile for prolonged periods post partum.

Smaller than normal follicle growth may also lead to undesirably small corpus luteal body formation after ovulation. Small than normal corpus luteal body formation can lead to low or inadequate synthesis of progesterone and a lack of follicular selection or ovulation. Follicle selection and growth in preparation for ovulation is partially controlled by a progesterone mediated limitation on hormones that drive follicle growth. In concert with progesterone effects on follicular growth hormones, only one follicle in a wave of many follicles develops to ovulatory size. A lack of follicular selection occurs in the absence of sufficient progesterone synthesis. As a result 2 or more follicles in a follicular wave achieve dominance and ovulate. Low progesterone levels from a lack of cyclic activity increase the frequency of twinning in groups of anovular cattle that begin to cycle. Ovulation of smaller, poorly developed follicles also impacts post conception fertility by enhancing EED. Inadequate estrogen synthesis by the selected, dominant follicle that is smaller than normal fails to prepare secretory function of the uterine glandular structures for the synthesis and secretion of nutrients and growth factors necessary to sustain the fetus early in the post insemination period. Smaller sized corpus luteums that accompany ovulation of small, poorly grown follicles results in inadequate progesterone secretion. Low progesterone levels in these cattle fail to properly stimulate uterine gland to secrete nutrients and growth factors supporting fetal growth after insemination. Thus abnormal follicular and corpus luteal body development in poorly cycling, anovulatory cattle can lead to inadequate growth of the conceptus during the first weeks of pregnancy. Inadequate fetal growth results in weak fetal signals of pregnancy to the dam thereby increasing the risk the dam will not recognize fetal presence in the uterus and destroy the corpus luteal body of pregnancy. This results in the high prevalence of fetal death and return to estrus (EED) typically associated with anovulatory cattle that begin to cycle.

There are a number of functionally important differences between ovulatory and anovulatory populations of cattle that impact reproductive efficiency and ultimately dairy profitability. Insemination rates of acyclic cattle can be as low as 30% of those in normally cycling animals over a 21 day period of visual estrous detection. More importantly, errors in estrus detection in groups of anovulatory cattle can be as high as 75% compared to only 5% error in cycling animals. This increases the likelihood acyclic cattle are inseminated at times that are not close to ovulation. Clearly this error with asynchronous insemination indicates tremendous problems with estrous detection in anovulatory cattle.

An issue of economic and practical significance is the ability of TAI programs to produce pregnancies in groups of anovulatory cattle. Herds with higher than normal amounts of anovulatory cows benefit from TAI programs because the first and/or second GnRH injection of Ovsynch for example will drive follicle growth and dominance in cows that otherwise would not achieve follicle growth due to greater than desirable nadirs in negative energy balance and loss in body condition (more than 0.5 BCS loss during the first 30-45 DIM). Follicle growth and dominance driven by exogenous sources of GnRH in Ovsynch or Presynch, Ovsynch programs triggers many anovulatory cows to ovulate and achieve pregnancy when in the absence of these hormones pregnancy is not an option. Studies clearly indicate that anovulatory cows respond to the first dose of GnRH of Ovsynch by forming luteal tissue from preexisting follicles. Thus, even though anovulatory cattle tend to possess follicles with variable and often smaller than normal size, these follicles have the capacity to ovulate, form luteal bodies and secrete progesterone just as the larger sized follicles in normally cycling ovulatory cattle. In addition most (94% anovulatory and 97% ovulatory) cattle can respond to the 2nd dose of GnRH in the Ovsynch program with ovulation (Gumen et al., 2003). The practical and important outcome for producers however is the success of synchronization when TAI programs like Ovsynch and Presynch, Ovsynch are implemented at the end of a 45 voluntary wait period where the two populations of cattle, ovulatory and anovulatory enter the synchronization protocols. The data shows 80-90% of the lactating, ovulatory groups are successfully synchronized (Fricke and Wiltbank,1999). Synchronization of anovulatory groups may be somewhat less consistent as rates as high as 90% and as low as 50-70% have been reported for anovulatory cattle (Cartmill et al., 2001). Inspite of the relatively high rates of synchronization in ovulatory and anovulatory cattle, conception rates and pregnancy rates tend to lie around 30-35% in ovulatory animals and 10% in anovulatory animals. Synchronization programs appear to drive ovulation

Never the less, many anovulatory cows fail to achieve pregnancy following TIA. The reasons are incompletely understood but are associated with some important observations of practical significance. Acyclic, anovular cows generally (but not inevitably) have lower BCS and lose > .5 BCS during the transition period. Low BCS has also been shown to be associated with though not causally related to lower pregnancy rates 33 and 61 days after TAI. Never the less, many anovular cattle successfully synchronized during Presynch, Ovsynch that did not become pregnant possessed BCS comparable to anovular cattle with good BCS that became pregnant after TAI. Thus, even though lower BCS is linearly associated with anestrous and anovulation, low BCS is clearly neither directly causal nor even necessary for development of anovular problems in most transition cattle (Moreira et al., 2000, Lopez et al., 2005, Sterry et al., 2006). Besides BCS, the cyclic condition of cattle entering TAI programs is closely associated with fertility and pregnancy rates in synchronized cattle. Acyclic, anovulatory cattle show fewer pregnancies per service at 30 and 60 days post TAI compared to cycling herd mates in the TAI program. Pregnancy loss due to early and late embryonic death is also considerably greater at 30 and 60 days post TAI in acyclic, anovulatory cows compared with cyclic, ovulatory cattle (Sterry et al., 2006).

Diagnosis of anovulatory cows can be achieved by strategic determination of progesterone levels in blood or milk. Normally cycling cows form a minority of follicles selected from a large number of follicles in a wave of developing follicles for rapid growth and maturation into dominant follicles. A single dominant follicle is ultimately formed that achieves the ability to ovulate and form a functional corpus luteal body. Anovulatory cows do not develop a dominant follicle that is induced to ovulate and form a large, functioning corpus luteal body. Therefore, failure of corpus luteal body formation is a hallmark sequella of anovulation and lends itself to 2 diagnostic approaches. One can utilize ultrasound to determine the presence or absence of a corpus luteal body on the ovary when a CL is predicted to be present on the ovarian surface. Alternatively, one can determine progesterone levels during the time when a functioning corpus luteal body should be active on the ovarian surface.

TAI programs lend themselves to these two diagnostic approaches because ovulatory, cycling cows should always possess a normally functioning corpus luteal body on the ovarian surface at distinct periods during the TAI program(s). Cows placed on a Presynch, Ovsynch program to generate the first post partum ovulation are expected to possess a large corpus luteal body on the ovarian surface at the time of the first Ovsynch dose of GnRH that follows the second PGF2α dose of the Presynch program. Alternatively, cows in the Presynch, Ovsynch or the Ovsynch program should possess a large corpus luteal body on the ovarian surface the time of the administration of the second dose of PGF2α in the Ovsynch protocol. Thus transrectal ultrasound of TAI cows should show a corpus luteal body during either of these times and would render a diagnosis of ovulatory and cyclic cows. Cows lacking a corpus luteal body by transrectal ultrasound would be diagnosed as acyclic and anovulatory.

Synchronization protocols like Ovsynch or Presynch, Ovsynch induce ovulation in cows diagnosed as anovulatory. Data from one study (Sterry et al , 2009) showed 81% (127/156) of anovular cows synchronized with a Presynch, Ovsynch program were induced to ovulate after the second dose of GnRH. Four other cows in the same trail showed evidence of cyclicity 4-11 days post AI but apparently failed to ovulate a follicle after the 2nd dose of GnRH. High progesterone levels in these four animals indicated they may have failed to destroy a functional corpus luteum in respond to the 2nd PGF2α dose of the Ovsynch program or ovulated a follicle and developed a corpus luteal body earlier than anticipated either 5-5 days prior to the 2nd PGF2α dose or 7 days before the 2nd dose of GnRH in the Ovsynch program. In either case, these 4 animals plus the other 123 showed synchronization programs can induce follicular activity and ovulation in cattle that were otherwise unable to develop cyclic activity and ovulate follicles in the post partum period Thus, producers should understand post partum anovulatory cattle can be rendered cyclic and ovular with synchronization programs as TAI programs will resolve pre-existing anovular conditions. Whether or not anovular cattle rendered ovulatory by synchronization programs are fertile, conceive and retain pregnancies similar to ovulatory cattle submitted for TAI is doubtful. Synchronization of anovulatory cattle with Ovsynch produces pregnancies ranging between 22-26% of the anovular cows submitted for TAI. (Sterry et al, 2009).

In a recent report of 127 anestrous cows placed into a (Sterry et al.,2009) Presynch, Ovsynch program, 30% were confirmed pregnant 31 days after timed insemination. Of the other 96 anovular cow not pregnant in the TAI program, it was unknown how many simply failed to conceive and how many conceived but developed EED prior to pregnancy confirmation on day 31. Poor quality embryos as early as 5 days post conception fail to achieve rapid rates of growth by day 16. Small, slow growing embryos send a weak fetal- derived signal of pregnancy that may not stop the dam from recycling. As a result embryonic mortality may occur before day 16 after conception in otherwise anovular cattle. Indeed EED may be evolving into serious cause of reproductive inefficiency in most cows and especially anovular cattle (Sartori et al., 2002). When pregnancy confirmation was performed at 28 days and 50-60 days after insemination of ovular and anovular cattle, loss of early pregnancies between these two periods ranges between 10-20%. The loss of pregnancies 30 to 50 days post TAI was 31% in anovular cattle compared with a much lower 16% in ovular cattle (Stevenson et al., 2006).

Clearly, anovulatory cows produce lower than desirable pregnancy rates in TAI programs. EED may become the primary cause for low pregnancy rates in anovular cows (as well as normally cycling, ovular) after TAI. Significant amounts of data suggest there may be problems with the embryo itself. Oocyte growth, development and maturation appears to be less than optimal with overly small or large follicles on the ovaries of anovular cattle. Since synchronization programs force follicles with poorer quality oocytes to ovulate, fertilization of poor quality oocytes at the time of TAI may produce the lower grade embryos noted to exist as early as 5 days post insemination. Some evidence exists to support the contention that poor quality oocytes may be an important element in the infertility problems of anovular cattle.

An association has also established between pre-insemination levels of progesterone, post fertilization progesterone levels and pregnancy. The higher the level of maternal progesterone prior to and 4 and 11 days after insemination, the greater the likelihood of pregnancy. Heavily lactating anovular cattle possess slower growing, smaller follicles than ovular cattle. Steroidogenisis (particularly estrogen synthesis and secretion) by small follicles on ovaries of anovular cattle can be lower than normal. As a result these cattle are often anestrus or show weak estrous behavior. More importantly, lower than normal estrogen synthesis could result in an ill prepared uterine endometrium that lacks sufficient ability to synthesize and secrete nutrients and growth factors that sustain fetal growth. Poor quality, slow growing fetal tissues fail to signal pregnancy and promote EED. In addition, forced ovulation of the smaller, slower growing follicles during synchronization of anovular cattle could result in insufficient luteal cell mass in the post ovulatory corpus luteal body. Moreover since anovular cattle (by definition) have not cycled they lack corpus luteal tissue and therefore enter synchronization programs with low serum progesterone levels. Even ovular, cycling cows can present to TAI with lower than desirable progesterone levels because livers of heavily lactating animals clear steroids (progesterone) from the blood more rapidly than livers of moderately producing animals. Low progesterone levels prior to follicular ovulation and insemination trigger problems with follicular development and ovulation. In fact, acyclic cattle lacking progesterone prior to ovulation have increased frequency of multiple ovulations with twinning and ovulation of small, underdeveloped follicles. These problems are associated with increased failure of fertility. Smaller than normal luteal mass is accompanied by lower than normal progesterone levels in the blood shortly after TAI. Lower progesterone levels post TAI in anovular cattle could compound pre-existing, hostile intrauterine problems established with low estrogen production by further limiting uterine gland secretion of nutrients and growth factors supporting fetal growth. Anovular cows with very low progesterone levels tend to destroy corpus luteal bodies more readily than cows with higher progesterone levels. A high percent of anovular cows therefore show shortened estrus cycles (after TAI) characterized by severely shortened luteal phases (11 day) in comparison with longer luteal phases (16 days) of normally, cyclic, ovulatory cattle (Gumen et al., 2003). The increased susceptibility of the luteal body to destruction 11 days post conception in TAI of anovular cattle would result in increased incidence of early embryonic death.

Several approaches have been designed in an attempt to reverse problems with anovulatory cows entering TAI programs. Synchronization protocols such as Ovsynch and Presynch, Ovsynch successfully trigger ovulation in a majority (but not all) anovulatory or ovulatory cows submitted for TAI. Ovulation is forced through the exogenous administration of pharmacologic doses of GnRH rather than depending on the physiologic release of endogenous GnRH that depends upon the presence of progesterone and estrogen. Thus synchronization programs over ride the failure of pre-ovulatory GnRH and LH secretion that occurs in anovulatory cattle. Never the less, the outstanding problem with anovular cattle in TAI programs is fewer pregnancies per timed insemination with higher rates of pregnancy loss occurring 6-60 days after timed insemination. These observations, in light of the relatively high prevalence of poor quality embryos 5 days post TAI imply pregnancy loss rather than ovulation and oocyte fertilization per se underlie low pregnancy rates in anovulatory cattle entering TAI programs. Accordingly, recent efforts have been directed at attaining higher retention of pregnancies after TAI. One approach involved the administration GnRH shortly after insemination in TIA. In trials involving the administration of GnRH day 4, day 5 or day 11 to 14 post insemination, the desired effect was to induce a second ovulation following the first ovulation induced by the second GnRH of Ovsych. The goal was to generate a second ancillary corpus luteum and enhance post TAI progesterone production. Elevated progesterone production should supplement progesterone production by the corpus luteum ovulated at the time of TAI and sustain pregnancy. As stated earlier, the corpus luteum formed by the follicle ovulated during Ovsynch may be smaller than desirable and produce insufficient progesterone to support pregnancy and optimal fetal growth in the first 16 days of pregnancy. An accessory corpus luteum would be expected to correct insufficient amounts of progesterone production in these animals. In addition, since corpus luteal bodies of anovulatory cattle are more susceptible to early destruction and shortened life spans, an accessory corpus luteum might insure some level of luteal tissue would persist on the ovarian surface to sustain the pregnancy after TAI. Collectively, the different trials resulted in very mixed effects on pregnancy rates. More work is required before this procedure can be recommended for use in commercial dairy cattle.

Intra-vaginal progesterone releasing inserts (CIDR) containing 1.38g of progesterone (P4) have been employed pre-or post TAI insemination in an attempt to improve conception and pregnancy rates in anovulatory cattle. Progesterone inserts are associated with resumption of cyclicity and ovulation in anovular cattle presumably because the exogenous progesterone replaces the progesterone that is absent in acyclic cattle lacking a corpus luteal body. Progesterone inserts have been placed during Presynch as well as Ovsynch protocols in synchronization programs. Again, the effect on pregnancy rates has been inconsistent. In one large multi-centered study cows received Ovsynch alone or received Ovsynch and a CIDR insert at time of the first GnRH dose of Ovsynch. The insert was removed within 2 hours of the second dose of PGF2α of Ovsynch. Pregnancy rates across all centers were greater in cows synchronized by Ovsynch + CIDR compared to Ovsynch only. In this particular study the proportion of non-cycling, anovular cows varied from 6% to as high as 41% across different herds. CIDR insert improved pregnancy rates by 5-10% in Ovsynch cows that received a CIDR (50%) compared to Ovsynch cows not receiving a CIDR (40%).However, the CIDR effect was not universal across all non cycling cows or cycling cows that received a CIDR. Rather CIDR associated improvement in 56 day pregnancy rates occurred in cycling and non-cycling, anovular cows *that showed no evidence of a functioning corpus luteum at the time of the second dose of PGF2α of Ovsynch.* CIDR inserts did not benefit non-cycling, anovular cows induced to cycle with the first dose of GnRH in Ovsynch or cycling cows that had been set up by the first dose of PGF2α and GnRH to respond to the second doses of PGF2α and GnRH in Ovsynch with ovulation (Stevenson et al., 2006). Since these events occur across any Ovsynch program in commercial herds with cycling as well as anovular, non-cycling cows, producers can expect supplementing Ovsynch with CIDR inserts to improve pregnancy rates in some but never 100% of cows entering an Ovsynch program supplemented with CIDR inserts. The amount of pregnancy rate improvement with CIDR inserts could be expected to fall off with higher percentages of anovular transitional cows in the herd. Anovular, acyclic transitional cows do not respond nearly as well as cycling cows to the first dose of GnRH expected to induce and support corpus luteal formation in Ovsynch cows. Part of the improvement in cyclic and acyclic cow fertility induced by pre-insemination CIDR inserts could be attributed to a reduction in EED specifically in the two groups of animal cited above that lacked corpus luteal function by the second Ovsynch dose of prostaglandin. Supplemental progesterone from CIDR inserts pre-insemination of acyclic, anovular cows actually enhanced follicular growth before ovulation and TAI. This could improve corpus luteal growth and function, resulting in higher production and more sustained levels of progesterone in support of uterine functions sustaining the post TAI pregnancy. The take home message from this type of data is that 30 and 60 day pregnancy rates are almost always lower for non-cycling transition cows compared to cycling cows. EED induced erosion of pregnancy rates during the 6-30 days and 30-60 days of pregnancy is greater in acyclic, anovular cows compared to cycling cows. CIDR supplemented Ovsynch programs may of benefit in herds with high numbers of acyclic, anovular cows but can be expected to benefit only a portion of the population of acyclic, anovular cows and cyclic cows entering the Ovsynch program.

The insertion of progesterone inserts during the Presynch protocol of a Presynch, Ovsynch program increase cyclicity in anovulatory cattle (Chebel et al., 2006). Never the less, 30 and 60 day pregnancy rates were not improved over those cattle not receiving the progesterone inserts during Presynch. Several studies recorded improved pregnancy rates in Resynch cattle receiving progesterone inserts 14-21 and even as late as 28 days post insemination (Chebel et al., 2006). Clearly the effect of supplemental progesterone after insemination was to reduce the high incidence of EED in anovulatory cattle. Insert mediated progesterone supplementation appears to improve fetal survival in heavily lactating animals that have developed anovular problems. Thus progesterone inserts may be beneficial pre-insemination because they initiate cyclicity and can improve fertility in acyclic and cyclic cows lacking corpus luteal bodies at the time of PGF2α in Ovsynch. The post insemination use of inserts may also benefit pregnancy rates by reducing the erosive effect of EED on reproductive efficiency in acyclic cattle.

In conclusion, entry of anovular and anestrous cattle into synchronization programs can force ovulation and even cyclicity in cattle that would not otherwise ovulate. Therefore submitting anestrus, transition cows to synchronization and TAI programs results in some pregnancies that otherwise would not occur in the absence of synchronization. Never the less, the infertility and low pregnancy rates associated with anovular cows are incompletely resolved even in synchronization as fertility and 30 to 60 day pregnancy rates can remain low even though synchronization induces ovulation. Strategic use of GnRH or progesterone inserts have been employed to improve the reproductive efficiency of anovular cattle in TAI. Different strategies have met with variable success at improving reproductive efficiency. Collectively, results from many of these investigations suggest fertility and improved pregnancy rates are largely dependent upon some level of spontaneous cyclicity and sustained endocrine events governing follicular growth, ovulation and luteal tissue activity. Anovulation and anestrus conditions negatively impact each of the areas of reproductive function resulting in increased frequency of abnormal cycle lengths, multiple ovulations per cycle, nonexistent heats, and errors in heat detection and costly increases in EED. Synchronization and TAI is a tool offering partial recovery from anovulatory problems but avoiding anovulation and anestrus conditions in transition cows by careful management of pre-and postpartum health, rations, feed bunks and comfort is likely to be an equally or even more productive approach to this problem.

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