

Positive and Negative Effects of High Energy Consumption on Reproduction in Lactating Dairy Cows

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Introduction

Seven years after graduating from the University of Wisconsin-Madison, John Smith (name changed by request) had found that the management headaches associated with running a 500 cow dairy were becoming overwhelming. He had built a newer freestall facility using a loan from his banker and was able to stock it with the 100 dairy cows purchased from his father's operation and many other cows purchased from surrounding operations. He now had the operation filled for the last 3 years. He had one problem with drainage into his lagoon that had resulted in one end of the barn being flooded on some occasions, but other than that, he was happy with the new facilities. The new double-8 parallel parlor was extremely efficient, and he had gone to 3-times per day milking to increase milk production and to utilize the parlor to greater capacity. His diet was balanced by a local nutrition professional, and he had been fairly pleased with the results. Milk production had climbed to where it now averaged over 85 lb/cow/day with 3.51% fat and 3.01% protein. His somatic cell count had dropped to 142,000 cells/ml and so he was pleased with his milk quality. His problem was that he continually had to buy springing heifers to keep the facility full. He had purchased 45 springing heifers during the last year, and it seemed that he would have to purchase about the same number during the coming year. Even though it had been over 3 years since he had first filled the facility with cows, he just could not generate enough heifers from his own operation to keep the facility full.

He knew he had reproduction problems but was not able to determine how to resolve them. He felt that part of the problem was the extremely high energy ration that his nutritionist had recommended about 11 months earlier. He had detected a lot of lame cows since that time. He did all of the artificial insemination (AI) in his herd and felt that he had a good technique; however, he did split straws (breed 2 cows with 1 straw of semen) in more than half of the breedings. Our group from the University of Wisconsin-Madison was asked to come in and do an evaluation of his operation as part of a class for our advanced undergraduate and graduate students. In this class, the students work with faculty members to diagnose any problems and help to design practical solutions for the participating dairy producers.

On the first visit, the students found out that the producer split straws of semen and felt that this was probably a primary problem. I asked them to continue to dig into the records to obtain data related to this potential problem or other problems. After 2 weeks of analysis of computer records and on-farm analysis, they had a different conclusion.

Although John said that he was using Ovsynch for timed AI of cows, he did not have any cows that were bred after Ovsynch before 150 days in milk. In other words, he only started using Ovsynch if he had not caught the cow in heat after more than 100 days of heat detection. In fact, some of these cows had not been bred for the first time

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by 200 days in milk. The average days to first breeding were 169 days. The students were definitely finding the real problem of reproduction on John's dairy. We asked John what was happening to his heat detection program, and he felt that the lameness issue had really decreased his heat detection efficiency. However, the students evaluated his current lameness scores and did not find that it was abnormally high. John told us that he was responsible for the heat detection, but that as herd manager, he found many other things that took a lot of his time. He did try to do heat detection at least 2 times per day for about 15 minutes at each time but was not always consistent with this time. We asked why he did not use Ovsynch on cows earlier in lactation, but he said that he did not have the time to set up the hormonal injections. He was in charge of all aspects of reproduction, as well as overall management of the dairy and there were only so many hours in the day. In addition, he did not want to spend money on the hormones if he could possibly catch the cow in a natural heat.

The students evaluated the conception rate on the lactating dairy cows and found a 41% rate. This was higher than most of the farms that we evaluate, particularly given the high level of milk production. They also found no difference between the cows bred with a full straw of semen or cows bred with a half straw of semen. So much for straw splitting providing an explanation for poor reproduction.

What had changed the reproduction on John Smith's dairy? Probably a combination of factors had converged to cause "the perfect storm" in reproduction on his dairy. On his family's smaller dairy operation, he could do a fine job with reproduction because he could completely focus on this one job. Now, his many management responsibilities took precedence, and he did not have enough time to do the job the way it needed to be done. So, in other words, he had not begun to delegate out this critical responsibility to other

people (perhaps an AI organization could be contracted to help with reproduction). Second, he had not been aggressive enough in using new reproductive management technology at an early enough time in lactation to receive maximum benefits from these new technologies. Third, the milk production in his cows had been rapidly increasing, and this increase had dramatically changed the reproduction in his dairy cows. He needed to make management changes to counter these changes in reproductive efficiency. Unfortunately, he was spending over \$100,000 per year on buying springing heifers, and there was no end in sight because of too few replacement heifers being produced by his herd. He also had so many cows that were not pregnant in late lactation that he would have to cull many non-pregnant cows in the next few months. This was a problem that would not be solved overnight but could be solved in the next 2 to 3 years by immediately implementing some aggressive reproductive management strategies.

Is the problem on John Smith's dairy the genetics of the cows? Maybe partially, but the major reproductive problems become apparent in these cows only when milk production increases to higher levels. Is the problem nutritional? Maybe partially, but there is probably not a major problem with nutritional deficiencies as much as nutrition becoming focused on and succeeding in increasing milk production. Is the problem with John Smith's management? This is definitely an important part of the problem. He could hire a reproductive management specialist for a fairly large salary and still make a good return on the investment. He clearly must delegate reproductive management responsibilities to others. In particular, he should hire someone to help with heat detection and hormonal injections. He also needs to change his management strategy to incorporate timed AI programs into an earlier stage of lactation (probably before 100 days in milk). These 2 changes would help him more efficiently catch cows in heat and to breed any cows at an early stage of lactation if they

were not caught in heat. He also needs to design an early pregnancy diagnosis and resynchronization strategy into his management strategy.

Unfortunately, the reproductive problems on John Smith's farm are not unique. Each farm is challenged to efficiently use labor and control costs to improve reproduction. This review will discuss the major changes that are occurring in reproduction in high producing lactating dairy cows and some reproductive management strategies to deal with these changes.

Changes in Some Reproductive Measures in Lactating Dairy Cows

Time to first ovulation

Use of ultrasonography, combined with hormonal assays has allowed a greater understanding of ovarian function during the period from parturition to first ovulation. Following parturition, there is a surge in circulating follicle stimulating hormone (**FSH**) during the first week (Ginther et al., 1996), probably due to the decrease in circulating estradiol after calving. There is subsequent emergence of the first follicular wave on average at 4 (Ginther et al., 1996) to 12 (Savio et al., 1990; Haughian et al., 2002) days postpartum. Some cows ovulate this first follicular wave; however, first ovulation is delayed in many lactating dairy cows with time to first ovulation averaging 33.3 ± 2.1 days in Holstein cows in the U.S.A. (compilation of 10 studies reported in Ferguson, 1996). Pasture-fed dairy cattle had, on average, 4.2 waves of follicle growth before first ovulation with maximal size of the largest follicle increasing as first ovulation approached (McDougall et al., 1995). This delay in first ovulation is generally attributed to the period of negative energy balance during the early postpartum period in dairy cattle, and a reduction in the pulsatile luteinizing hormone (**LH**) secretion needed to stimulate the final stages of follicle growth and estradiol production (Savio

et al., 1990; Staples et al., 1990; Beam and Butler, 1997; Lamming and Darwash, 1998; Roche et al., 2000; Butler, 2001).

Although delayed first ovulation is associated with negative energy balance during early lactation, it is not as clearly associated with level of milk production. Cattle that have been selected for high milk production compared to control lines (lower genetic merit for milk yield) have been reported to have a longer interval to first postpartum ovulation (+14 days reviewed by Lucy, 2001); +8 days [Gong et al., 2002]) and the first postpartum detected estrus (+4.5 days, [Hageman et al., 1991]). However, in our recent study, there was no relationship between level of milk production and percentage of cows that were "anovular" at 71 days in milk (Lopez et al., 2005a) with about 25% of cows being "anovular" irrespective of milk production (55 to 132 lb/day average production from 50 to 71 days postpartum). The surprisingly high rate of anovulation in this study is somewhat higher but not inconsistent with other recent studies (Moreira et al., 2001; Gumen et al., 2003; Santos et al., 2004). Unfortunately, these studies did not report any potential relationship between level of milk production and anovulation. Erb (1984) reviewed four North American studies in which they contrasted the levels of milk production, or genetic potential for milk production, between ovulatory and anovulatory cows. He concluded that high milk production did not cause anovulation in Holstein dairy cows, but anovulatory cows produced more milk than their herdmates (Erb, 1984).

Recently, we have suggested that anovulation be classified into physiological categories based on maximal size of the largest growing follicle and circulating estradiol (Wiltbank et al., 2002). Consistent with this classification, cows with lower body condition scores (BCS of 2.5 or lower out of 5) had a greater likelihood of anovulation and had smaller maximal size of anovulatory follicles. Nevertheless, the majority of

anovulatory cows (63%) had adequate BCS and average follicular sizes greater than ovulatory size (> 20 mm). For example, 20% of cows with a BCS of 3.25 were found to be anovulatory. Thus, negative energy balance and inadequate follicular growth can explain a portion of anovulatory dairy cows but does not seem to be an adequate explanation for all anovulatory dairy cows. Similarly, a simple relationship between anovulation and level of milk production does not appear to exist, and therefore, more complex physiological models are needed to fully explain anovulation in dairy cows (Gumen and Wiltbank, 2002; Wiltbank et al., 2002).

Conception rate

Most studies of reproduction in dairy cattle have focused on conception rate and pregnancy loss because of the economic implications for commercial dairy operations of these reproductive measures (for reviews see Lucy, 2001; Lopez-Gatius, 2003). Nevertheless, the relationship between various measures of fertility (conception rate) and level of milk production remains controversial. Washburn et al. (2002) analyzed the relationship of conception rate and milk production over more than a 20-year time period (1976 to 1999) in dairy herds in southeastern U.S.A. Conception rates decreased from ~ 55 to 35% during this time period as milk production dramatically increased. However, differences in recording of data could also be at least partially responsible for these changes (Lucy, 2001). Faust et al. (1988) showed a clear relationship between level of milk production and conception rate in primiparous Holstein dairy cattle. In contrast, Peters and Pursley (2002) reported that higher producing cows had higher conception rates following the use of Ovsynch protocols than lower producing cows. Most large data sets have demonstrated an antagonistic relationship between milk production and fertility, but the size of the effect has been questioned (Gröhn and Rajala-Schultz, 2000; Hansen, 2000; Lucy, 2001). Nevertheless, it seems

clear that high producing, lactating dairy cows have much lower conception rates than heifers (Xu and Burton, 1999; Royal et al., 2000; Lucy, 2001; Peters and Pursley, 2002; Washburn et al., 2002; Gumen et al., 2003; Lopez-Gatius, 2003). For example, Pursley et al. (1997) reported much higher conception rates in heifers (74.4%) than in lactating cows (38.9%). In a recent study, we compared embryo quality on day 5 after ovulation from normally-ovulating, lactating dairy cows versus similar age and size non-lactating dairy cows (Sartori et al., 2002). Although fertilization rate was similar (88 to 90%), the percentage of embryos that were viable was much lower in lactating cows (52.8%) than in non-lactating cows (82.3%) (Sartori et al., 2002). This study was done during the cool time of the year so that heat stress was not a problem. The collection of embryos from heat-stressed, lactating dairy cows resulted in a reduced fertilization rate (55.3%) and even a greater reduction in percentage of viable embryos (33.3%) (Sartori et al., 2002). A major effect of milk production on fertility is found during heat stress and may not be present during cooler times of the year (Lopez-Gatius, 2003). This is probably due to a greater increase in body temperature in higher than lower producing dairy cows exposed to the same environmental temperatures (Sartori et al., 2002). However, the lower conception rates in lactating dairy cows, even during cool times of the year, suggest that not all the reduction in fertility can be explained by greater heat stress. Obviously, fertility is a complex trait and is likely to be related to numerous factors, including uterine infection, negative energy balance, urea concentrations in the blood, vitamins, fertility of sire, accuracy of estrous detection, insemination technique, etc. (Faust et al., 1988; Staples et al., 1990; Ferguson, 1996; Lamming and Darwash, 1998; Gröhn and Rajala-Schultz, 2000; Roche et al., 2000; Royal et al., 2000; Butler, 2001; Lucy, 2001; Moreira et al., 2001; Gong et al., 2002; Washburn et al., 2002; Lopez-Gatius, 2003; Santos et al., 2004a; Santos et al., 2004b). For example, an increase in double ovulation rate in high-

producing dairy cows (illustrated below) would increase the chances for pregnancy, even though possible negative effects of high milk production could decrease the percentage of ovulated oocytes that produce a pregnancy. Thus, a simple relationship between milk production and conception rates seems unlikely.

Duration of estrus

It is clear that low rates of estrous detection are reducing reproductive efficiency on commercial dairy farms. Indeed, Washburn et al. (2002) reported a decrease from 50.9% in 1985 to 41.5% in 1999 for estrous detection rates in Holstein dairy herds in southeastern U.S.A. However, studies have reported both a negative relationship between level of milk production (Harrison et al., 1989; Harrison et al., 1990) or no relationship (Fonseca et al., 1983; Van Eerdenburg et al., 2002) using visual observation twice daily to measure expression of estrus. We have recently completed a study in which we evaluated the duration of estrus in a group of lactating dairy cows using the HeatWatch system (Lopez et al., 2004). This system allowed continuous monitoring of all mounts 24 h per day and can be used to calculate the duration of estrus in individual dairy cows. Cows with milk production above the herd average (~ 88 lb/day) had shorter ($P < 0.001$) duration of estrus (6.2 ± 0.5 h) than cows with lower milk production (10.9 ± 0.7 h). This effect was not due to a parity effect because separate analysis of primiparous and multiparous cows showed a similar effect. Figure 1 shows the relationship between level of milk production and duration of estrus. In order to consistently observe this strong negative relationship between level of milk production and duration of estrus, it is critical that milk production data be collected close to the time of estrus, only data from ovulations after the first postpartum ovulation be utilized (first ovulation has low expression of estrus), all ovulations be consistently monitored throughout the observation period (to avoid false estrus or missing data from ovulations),

and that duration of estrus be monitored on a continuous basis with an electronic heat monitoring system.

In a subset of these cows ($n = 71$), we analyzed maximal follicular size and circulating estradiol concentrations on the day of estrus (Lopez et al., 2004). High producing cows (103 lb/day) had larger follicles (18.6 ± 0.3 versus 17.4 ± 0.2 mm diameter; $P < 0.01$) but lower circulating estradiol (6.8 ± 0.5 versus 8.6 ± 0.5 pg/ml; $P < 0.01$) compared to lower producing cows (71 ± 1.3 lb/day). Correlations were evaluated between a number of different values. Surprisingly, there was no detectable relationship between maximal follicular size and peak estradiol concentrations ($r = -0.17$; $P = 0.15$). As expected, duration of estrus was positively correlated with peak estradiol concentrations ($r = 0.57$; $P < 0.0001$) and negatively with milk production ($r = -0.51$; $P < 0.0001$). Level of milk production was also negatively correlated with follicular size ($r = -0.45$; $P < 0.0001$). As discussed below, we theorize that high milk production leads to decreased circulating estradiol concentrations, resulting in decreased duration of estrus. Decreased estradiol could also cause increased follicular size by delaying the time to estradiol-induction of estrus, gonadotrophin releasing hormone (**GnRH**)/LH surge, and ovulation in high-producing cows.

Double ovulation rate

Another reproductive trait that has been directly linked to milk production is double ovulation rate (for a more complete review see Wiltbank et al., 2000; Lopez et al., 2005a). From a practical standpoint, double ovulation rate appears to be the underlying cause of increased twinning rate in lactating dairy cows, with 93% of twins being non-identical (Silvia Del Rio et al., 2004). Numerous factors have been recognized as possible regulators of twinning rates, including age of dam, season, genetics, use of reproductive hormones or

antibiotics, ovarian cysts, days open, and peak milk production [reviewed in Wiltbank et al., 2000]. In a large study on risk factors for twinning, Kinsel et al. (1998) concluded, “the single largest contributor (> 50%) to the recent increase in the rate of twinning is the increase in peak milk production”. We performed a study in which we evaluated double ovulation rate in 240 dairy cows Fricke and Wiltbank, 1999) that had ovulation synchronized with the Ovsynch protocol (Pursley et al., 1995; Pursley et al., 1997). Ovulation was determined by transrectal ultrasonography at the time of the second GnRH injection and 48 h later. The mean milk production, determined 3 d before ovulation, was 80.5 ± 1.8 lb/day and cows were segregated by whether they were below or above the mean value. Double ovulation rate in cows that were above average production was 20.2% compared to 6.9% in those below average ($P < 0.05$) (Fricke and Wiltbank, 1999). This difference was similar regardless of lactation number. Recently, we reported results of a study (Lopez et al., 2005a) that evaluated naturally ovulating dairy cattle and found a similar relationship between milk production and double ovulation rate (Figure 2). Cows that produced less than 88 lb/day had a very low double ovulation rate, whereas, cows producing above 110 lb/day had more than a 50% double ovulation rate. It is surprising that there is such a dramatic inflection point in double ovulation rate as milk production increases above 88 lb/day, and it is still unclear what physiological changes occur as milk production increases above this critical value. This increase in double ovulation rate is likely to continue to increase twinning rate in dairy herds as milk production increases. It is also clear that this effect of milk production is most related to the level of production within the 2 weeks before the cow ovulates and not to total milk production during the entire lactation. This effect was also similar when a more extensive regression model was used for analysis, and when multiparous and primiparous cows were analyzed separately (Lopez et al., 2005a). As with duration of estrus, the first postpartum ovulation differed from

other ovulations, showing a high double ovulation rate that was unrelated to milk production (Lopez et al., 2005a).

Circulating Steroids and Steroid Metabolism in Lactating Dairy Cows

A number of studies have evaluated circulating hormone concentrations in lactating dairy cows. As discussed above, cows with higher milk production ovulate larger follicles but have lower circulating estradiol concentrations (Lopez et al., 2004). In addition, higher producing dairy cows have a larger volume of luteal tissue but reduced circulating progesterone (Lopez et al., 2005a). Table 1 shows a comparison of dairy heifers and lactating dairy cows that were monitored by daily ovarian ultrasonography and hormonal analyses (Sartori et al., 2004). It is clear that cows ovulated larger follicles but had reduced circulating estradiol-17 β concentrations. This is somewhat surprising because it would be expected that cows with larger follicles would tend to have greater follicular estradiol-17 β production. Again paradoxically, lactating cows had a much larger volume of luteal tissue but reduced circulating progesterone. This study also shows the much higher multiple ovulation rate in lactating cows. Other studies have also reported changes in circulating hormones and size of ovarian structures in lactating cows (Ahmad et al., 1995; Inbar et al., 2001).

There appear to be two reasonable explanations for the disconnection between circulating steroid hormones and size of follicles and corpus luteum (CL). The first possible explanation is that follicles and CL are less steroidogenically active in lactating dairy cows. This could be due to inadequate circulating stimulatory hormones, substrate for steroidogenesis, or intracellular steroidogenic pathways. There were more LH pulses in lactating than similar size non-lactating cows (Vasconcelos et al., 2003), suggesting that LH is not likely to be the cause of reduced

steroidogenic output. In addition, the primary substrate for bovine ovarian steroidogenesis is high-density lipoprotein, and this is particularly elevated in lactating dairy cows (Grummer and Carroll, 1988). There is a reduction in circulating insulin-like growth factor-1 in lactating dairy cows, and this could be related to reduced steroidogenic capacity (Lucy, 2000). Nevertheless, the hypothesis that ovarian structures in lactating dairy cows have reduced steroidogenic output has not yet been adequately investigated and therefore, cannot be disregarded or advocated at this time.

A more likely explanation is that lactating dairy cows have increased metabolism of steroid hormones as milk production increases. Circulating hormone concentrations are determined by rates of production and metabolism of the hormone. Increased feed consumption, such as during lactation, has been shown to alter circulating progesterone and excretion of progesterone metabolites during continuous delivery of progesterone (Parr et al., 1993a; Parr et al., 1993b; Rabiee et al., 2001a; Rabiee et al., 2001b). Increased steroid metabolism due to high feed consumption could alter the reproductive physiology of any species but may particularly alter reproduction in species with extreme increases in feed intake, such as lactating dairy cows. We propose that some of the reproductive changes in lactating dairy cows are caused by dramatic increases in steroid metabolism due to elevations in feed consumption and liver blood flow.

In recent experiments, we tested the hypothesis that increased liver blood flow as a result of elevated feed intake in lactating dairy cows would increase steroid metabolism (Sangritavong et al., 2002). We found that prior to feeding, liver blood flow was greater in lactating (1561 ± 57 L/h) than similar size and age non-lactating (747 ± 47 L/h) cows. The liver blood flow and metabolism of progesterone and estrogen increased immediately after any amount of feed consumption in both

lactating and non-lactating cows (Sangritavong et al., 2002). The metabolism of estrogen and progesterone was much greater (2.3 X) in lactating than in non-lactating cows (Sangritavong, 2002; Sangritavong et al., 2002). Thus, the changes in metabolism of estrogen and progesterone in response to feeding are immediate and appear to be related to acute changes in liver blood flow. In lactating cows, a continuous high plane of nutrition appears to chronically elevate liver blood flow and metabolism of steroid hormones to approximately double the amount observed in similar size and age non-lactating cows. These results indicate that even with a similar level of hormone production, there would be lower circulating hormone concentrations in lactating dairy cows.

Can elevated steroid metabolism explain the paradox of reduced circulating steroids in spite of larger follicular and luteal sizes? If we use the data in Table 1 to calculate a rough index of circulating progesterone concentration divided by luteal volume, we find that heifers have roughly twice the value that is calculated for lactating cows (1.0 versus 0.5 ng/ml of progesterone per cm^3 of luteal volume). A similar calculation for circulating estradiol and follicular volumes also yields about a 2-fold greater value in heifers than cows (6.5 versus 3.2 pg/ml circulating estradiol per cm^3 of follicular volume). These values correspond closely to the roughly 2-fold elevation in metabolism of estrogen and progesterone that we have found in lactating vs non-lactating cows (Sangritavong, 2002; Sangritavong et al., 2002). A recent analysis of a larger group of individual lactating cows using this index showed a closer relationship of this index (circulating hormone/volume of tissue) to milk production ($R^2 = 0.44$ to 0.47 ; $P < 0.01$) than found when comparing either circulating hormones or follicular or luteal volume alone to milk production (Lopez et al., 2005a). Thus, although we cannot rule out the importance of changes in steroidogenic production by luteal and follicular tissue, it seems reasonable that the changes in circulating estradiol and progesterone can be

accounted for by increased rates of steroid metabolism in lactating cows.

We have synthesized this information into a simplified working model (Figure 3). Lactating cows have greater energy requirements than non-lactating cows (for example, a cow producing 110 lb/day of milk will require 53 Mcal/day of net energy vs 12.5 Mcal/day for a non-lactating cow; NRC, 2001). The high feed consumption required to meet these energy requirements leads to a dramatic increase in liver blood flow (Sangsritavong, 2002; Sangsritavong et al., 2002) which leads to elevated metabolism of both estrogen and progesterone. This would cause a reduction in circulating estrogen and progesterone concentrations, even in the midst of high production of steroid hormones by the follicle or CL.

This simple model could potentially explain some of the results described in the sections above. Figure 4 provides a model that focuses on changes in circulating hormones and follicular and luteal sizes that occur due to the elevated steroid metabolism in lactating cows with elevated milk production. In high-producing, lactating dairy cows, follicle growth rate may be similar to lower producing cows, but circulating estradiol would increase at a slower rate due to elevated steroid metabolism. Thus, estradiol would continue to rise until eventually circulating estradiol is sufficiently elevated for a sufficient length of time to induce a GnRH/LH surge. The LH surge is likely to be induced at a larger follicular size in high producing dairy cows and probably at a lower circulating estradiol concentration (based on our previous results). In addition to lower estradiol concentrations at the start of estrus, there is also likely to be a more rapid decrease in circulating estradiol after the LH surge due to elevated estradiol metabolism. Therefore, it makes sense that a higher producing cow would have a shorter duration of estrus because of increased steroid metabolism. Thus, this model provides a logical and likely explanation for the changes in duration of estrus,

and for the paradox of lower circulating steroids but larger ovarian structures occurring in lactating dairy cows. In addition, it provides scenarios for how elevated steroid metabolism due to high milk production could reduce fertility. The preovulatory follicle and oocyte would be exposed to a longer period of elevated LH pulses that could lead to ovulation of an overstimulated oocyte and reduced fertility (Ahmad et al., 1995; Ahmad et al., 1996; Revah and Butler, 1996). Alternatively, a reduced rate of progesterone rise following ovulation could also reduce fertility, as has been suggested by others (Folman et al., 1973; Ahmad et al., 1996; Dunne et al., 1999; Mann, 2001). Nevertheless, this model does not yet explain how very high milk production (> 88 lb/day) can produce the dramatic increase in double ovulation rate. Our recent intensive study of hormonal changes associated with selection of single, double, or triple dominant follicles in lactating dairy cows demonstrates that reduced circulating estradiol near follicle selection is not responsible for multiple dominant follicles (Lopez et al., 2005b), as we originally proposed (Wiltbank et al., 2000). Nevertheless, circulating progesterone is decreased and LH and FSH are increased near the time of selection, making it possible that changes in hormonal metabolism may still have a role in this process.

The critical involvement of estrogen and progesterone in almost every aspect of reproductive physiology makes changes in steroid metabolism an attractive explanation for the numerous changes in reproduction that have been observed in lactating dairy cows. The elevation in steroid metabolism is a logical extension of elevated metabolic activity in lactating dairy cows. Nevertheless, more definitive data are needed to link any particular reproductive change to elevated metabolism of a particular reproductive hormone. The physiological relevance of the models in Figures 3 and 4 can be tested by timely supplementation of estradiol and/or progesterone, as well as potentially decreasing activity of specific steroid-metabolizing liver

enzymes. Another review is needed to correlate these models and other physiological models with the numerous older, recent, and on-going scientific investigations of steroid hormone supplementation in cattle. Future practical manipulations of reproductive function in lactating dairy cows can be more rationally designed as the precise effects of elevated steroid metabolism on reproductive physiology in lactating dairy cows continue to be more fully defined.

Practical Reproductive Management Implications

The next section will briefly suggest some practical implications and reproductive management strategies for each of these areas.

Decreased duration of estrus due to high milk production

What does this practically mean for a dairy farm? We used the data on duration of estrus versus milk production to analyze what would happen to heat detection efficiency for cows with different levels of milk production. In Figure 5, the probability of detecting a cow in heat with different frequency of heat detection is shown. If a cow is producing about 70 lb/day, a 4-times per day heat detection program will detect about 90% of cows that are in estrus. However, this same program (4 times/day) will only detect about 50% of cows in heat if they are producing above 100 lb/day. This result gets even worse if heat detection is done only twice per day or once per day. It should be noted that all of the probabilities in this analysis were based on actual ovulation by the cows (detected by ultrasound). Some producers will say that the high producing cows are not cycling, but they are cycling normally. They do not detect them in heat because they have so short of a time that they are in heat. Increasing number of times that cows are checked for heat can help to solve this problem. Many producers are using heat detection aids, such as tail

chalk, to help find cows that are in showing heat at a time that they are not present. This can be critical because high producing cows are showing heat for only 4 hours or less in many cases. Most dairy producers in the United States are incorporating timed AI programs, such as Ovsynch, into their reproductive management programs to allow high-producing cows to be bred in a timely manner.

Treating anovular cows

Although level of milk production is not normally associated with incidence of anovulation, dairy producers still need to design programs to treat anovular cows. Generally, 20% of dairy cows will not be cycling by 70 days after calving. This percentage will increase if there are a high percentage of cows with low BCS (2.5 or less). These cows need to be quickly assigned to a hormonal program (and possibly nutritional program if they have low BCS) that will start the cows cycling. An Ovsynch program alone is not the ideal treatment for anovular dairy cows. Use of a CIDR® (Pfizer, Inc., New York, NY) or estradiol should be incorporated into these programs to be optimal treatments for non-cycling dairy cows.

Increasing double ovulation rate (and twinning rate) with increasing milk production

From a practical standpoint, it appears that there may be little that we can do to change this trend. Using Ovsynch does not seem to increase or decrease double ovulation, with double ovulation related to milk production whether we look after a hormonal synchronization program or a natural estrus. Obviously, not all double ovulations result in twins, but increasing double ovulation rate will almost surely result in increased twinning rates on higher producing farms. It seems clear that the main increase occurs after cows are producing about 90 lb/day. Thus, we must anticipate that we will have a dramatic increase in double ovulation rate in cows producing over 90 lb/day, and this will result in an

increase in twinning rate in cows that conceive during this time of high milk production. We must align our management procedures to deal with this increasing twinning rate if we are increasing milk production into this range on our dairy farms. First, we must set a program to diagnose twins. Second, we should set up procedures to manage cows that are likely to have twin births. Twinning cows will calve earlier (10 to 14 days on average) and are likely to have more problems during the calving process. These twin calving cows were, on average, our highest producing cows during the previous lactation; therefore, we must carefully design our calving and early lactation procedures with these twinning cows in mind.

Decreasing conception rate due to higher milk production

As discussed above, there are many different factors that impact conception rate in lactating dairy cows and higher milk production is just one of these factors, and on many farms, it may be a fairly minor factor. The effect of milk production on fertility is dramatically amplified during hotter times of the year. This is because there is a greater increase in body temperature as cows increase milk production. This increase in body temperature leads to decreased reproductive success, particularly death of the early embryo.

From a practical viewpoint, we have tried to utilize the information that many of the problems with fertility in dairy cows appear to occur during the first week after breeding. We hypothesized that we could improve reproduction just by transferring a good quality embryo at 7 days after expected time of AI. So in a fairly large experiment, we compared conception rate (**CR**) in our herd when cows were bred either by AI or by embryo transfer (**ET**). During 365 days, 550 potential breedings were used from 243 lactating Holstein cows (77 lb/day of milk). Cows were synchronized (GnRH-7days-PGF_{2a}-3days-GnRH) and randomly

assigned to receive AI immediately after the second GnRH injection (day 0) or to receive transfer of one embryo 7 days later. Circulating progesterone and follicular and luteal sizes were determined on days 0 and 7. Pregnancy diagnosis was performed on days 25 or 32, and pregnant cows were reevaluated on days 60 to 66. Synchronized cows with single ovulation had similar ($P > 0.30$) CR on days 25 to 32 with ET ($n = 176$; 40.3%) and AI ($n = 160$; 35.6%). Pregnancy loss between days 25 to 32 and 60 to 66 also did not differ ($P = 0.38$) between ET (26.2%) and AI (18.6%). When single ($n = 34$) and multiple ($n = 57$) ovulators were compared, independent of treatment, multiple ovulators had greater ($P < 0.01$) circulating progesterone on day 7 (2.7 versus 1.9 ng/ml), and there was a tendency ($P = 0.10$) for greater CR in multiple ovulators (50.9 versus 38.1%). However, there was no difference in CR between AI and ET cows with multiple ovulation (50.0 versus 51.7%). The CR tended to be lower for AI than ET in single-ovulatory cows ovulating smaller (< 15 mm; 23.7 vs. 42.3%; $P = 0.06$) but not average (16 to 19 mm; 41.2 versus 37.3%; $P = 0.81$) or larger (> 20 mm; 34.3 versus 51.0%; $P = 0.36$) follicles. Thus, ET did not improve overall CR in lactating cows but size and number of ovulating follicles may determine success with these procedures. We obviously have a large number of future experiments to do in order to resolve the problems with fertility in lactating dairy cows.

Many laboratories are currently experimenting with a number of changes in timed AI programs that may increase conception rates in high producing dairy cows. There are numerous intriguing possibilities, but they still lack sufficient data to allow recommendation at this time.

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Table 1. Comparisons (means $\pm$ SEM and percentages) between all (single- and multiple-ovulating) heifers (n = 27) and lactating cows (n = 14) with typical interovulatory intervals (Sartori et al., 2004).

	Heifers	Lactating Cows	P-value
Interovulatory interval (days)	22.0 $\pm$ 0.4	22.9 $\pm$ 0.7	0.28
Day of luteolysis	18.5 $\pm$ 0.4	18.9 $\pm$ 0.6	0.53
Cycles with two waves, % (no./no.)	55.6 (15/27)	78.6 (11/14)	0.15
Cycles with three waves, % (no./no.)	33.3 (9/27)	14.3 (2/14)	0.19
Cycles with four waves, % (no./no.)	11.1 (3/27)	7.1 (1/14)	0.68
Day of emergence of second follicular wave	8.9 $\pm$ 0.3	11.1 $\pm$ 0.6	<0.01
Interval (days) from emergence of last wave and ovulation	10.1 $\pm$ 0.5	10.9 $\pm$ 0.5	0.29
Days from luteolysis to ovulation	4.6 $\pm$ 0.1	5.2 $\pm$ 0.2	<0.01
Incidence of co-dominant follicles during first wave, % (no./no.)	3.7 (1/27)	35.7 (5/14)	0.01
Multiple ovulation rate, % (no./no.)	1.9 (1/54)	17.9 (5/28)	0.02
Maximal size of largest ovulatory follicle (mm)	14.9 $\pm$ 0.2	16.8 $\pm$ 0.5	<0.01
Estradiol peak preceding ovulation (pg/ml)	11.3 $\pm$ 0.6	7.9 $\pm$ 0.8	<0.01
Maximal luteal tissue volume (mm ³)	7303 $\pm$ 308	11120 $\pm$ 678	<0.01
Progesterone peak (ng/ml)	7.3 $\pm$ 0.4	5.6 $\pm$ 0.5	0.01

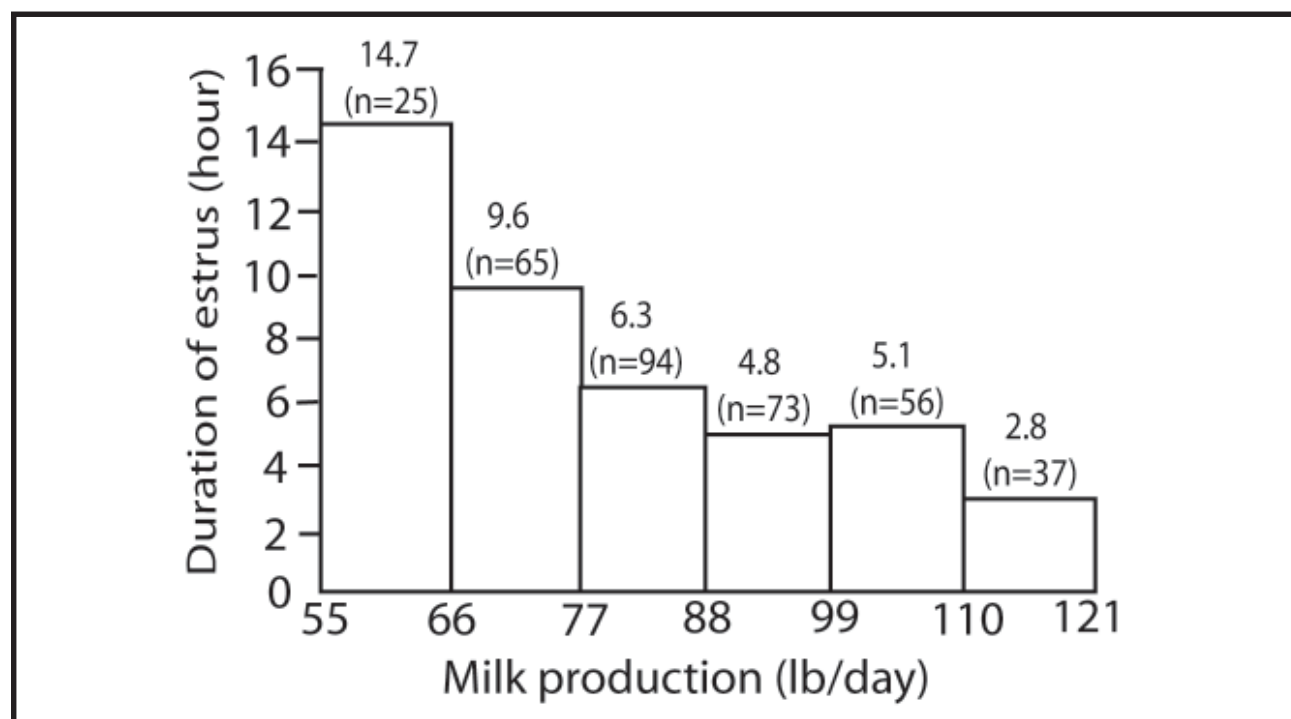


Figure 1. Relationship between level of milk production and duration of estrus. Analysis included all single ovulations (n = 350) except first post-partum ovulations. Average milk production is for the 10 days before estrus (Lopez et al., 2004).

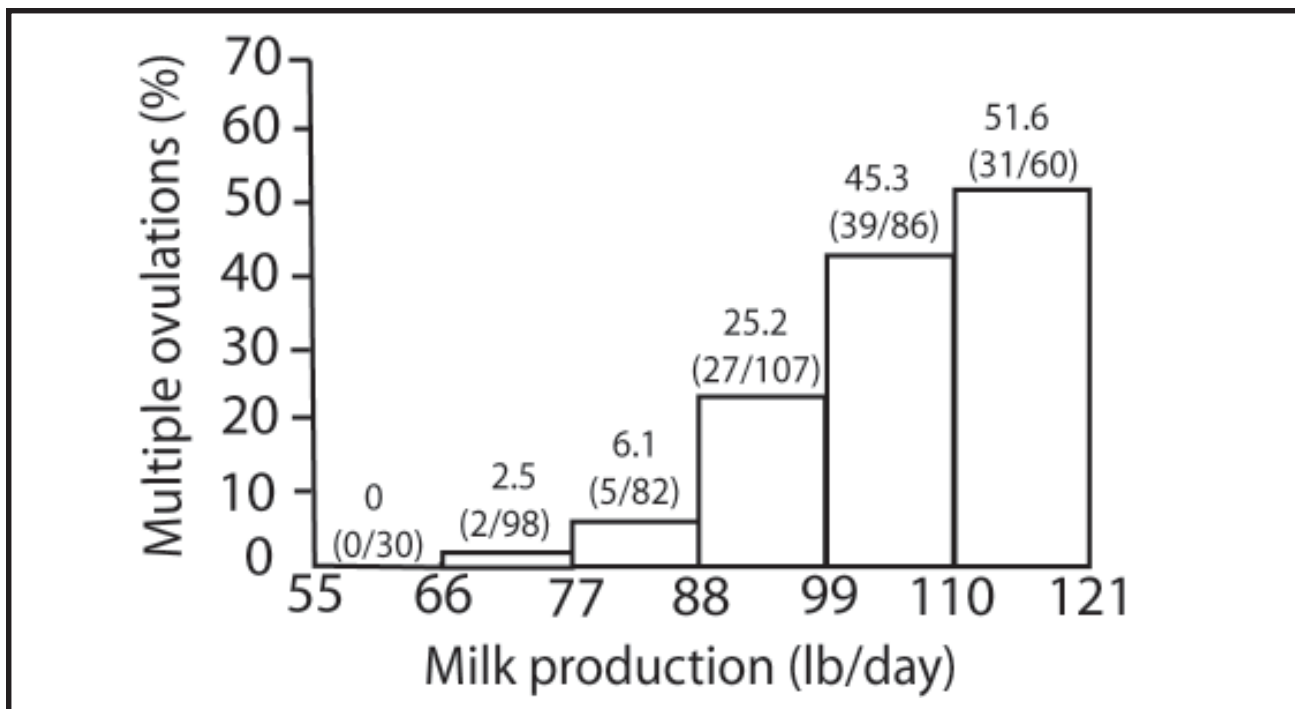


Figure 2. Relationship between incidence of multiple ovulation and milk production. Analysis included all ovulations ($n = 463$) except first post-partum ovulations. Average milk production was for the 14 days before estrus (Lopez et al., 2005a).

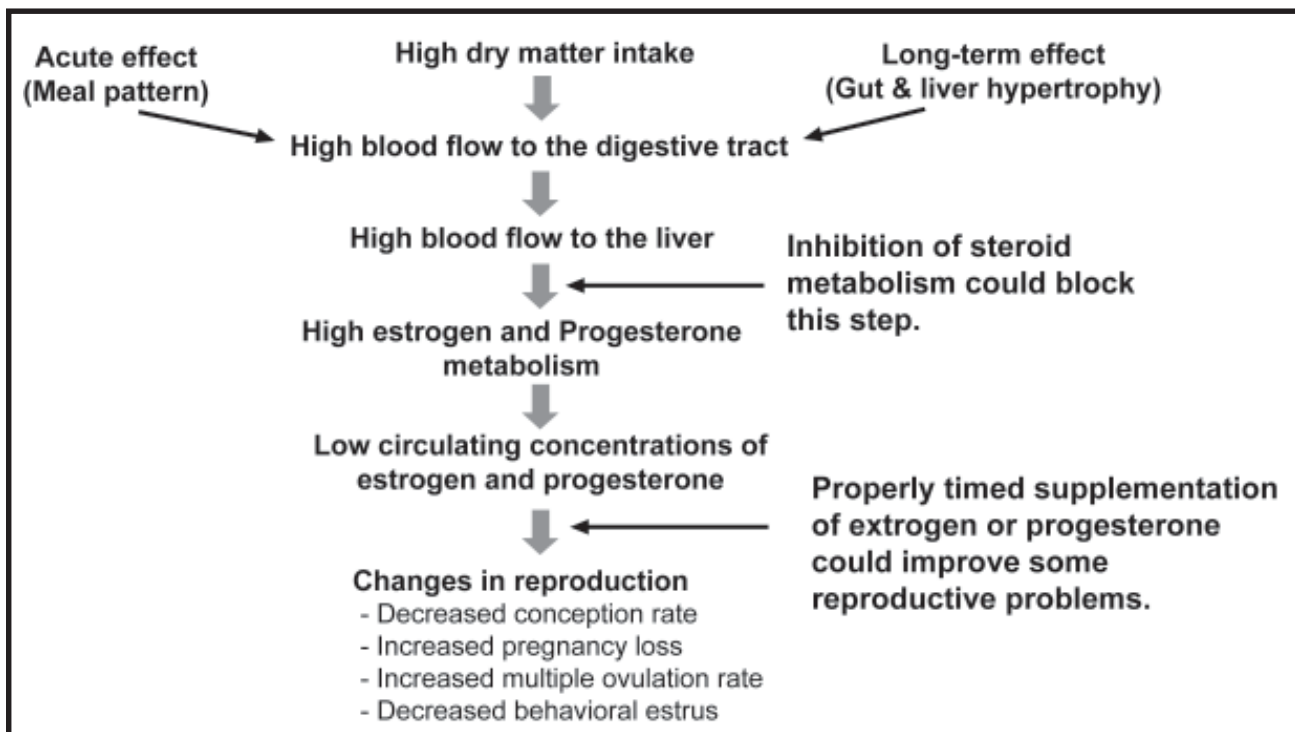


Figure 3. Schematic of the potential physiological pathway that may produce the changes in reproductive physiology observed in high-producing lactating dairy cows

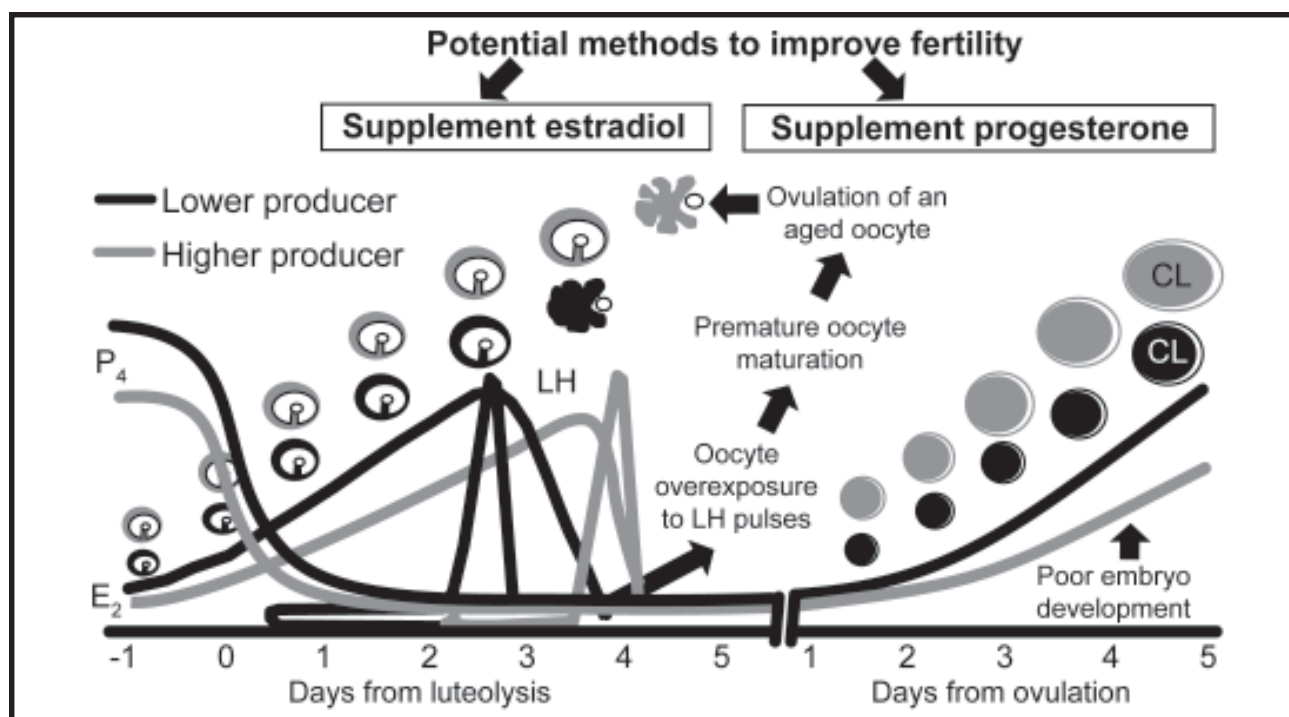


Figure 4. A physiological model showing the changes in circulating estradiol, luteinizing hormone (LH), and progesterone, as well as the growth patterns of the preovulatory follicle and corpus luteum (CL) in lactating dairy cows with higher or lower milk production. Possible reasons and potential treatments for lower fertility in higher producing lactating dairy cows, based on this model, are also shown.

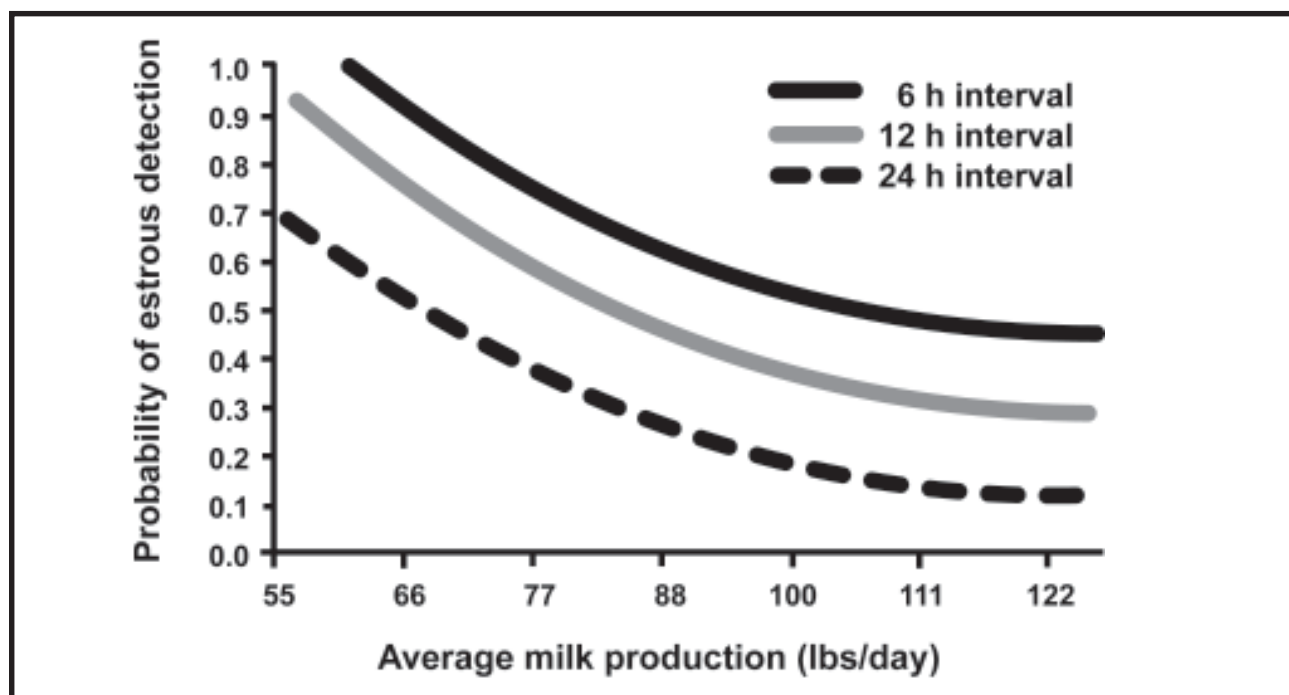


Figure 5. How the probability of heat detection changes with different frequencies of heat detection and different levels of milk production.