

Hidden Factors Affecting Fertility

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■ Take Home Message

- ▶ Reproduction has a genetic component and fertility can be improved by selection for reproductive traits or through use of crossbreeding
- ▶ Reducing mastitis will pay dividends in terms of reproductive performance because mastitis leads to reduced conception rates and increased abortions
- ▶ Cow rations can be monitored to ensure that specific dietary ingredients that compromise fertility are kept at safe levels – crude protein should not exceed 19% of DM and cottonseed can be fed at levels up to 2.7-3.6 kg/head/day (upland varieties) or 1.8-2.7 kg/head/day (unprocessed pima varieties)
- ▶ Increases in milk yield have meant that cows are becoming increasingly sensitive to heat stress so that fertility declines in the summer even in Alberta – use of timed AI and cooling cows during hot weather can reduce effects of heat stress

■ Introduction – The Problem of Infertility

By now, most dairy farmers and dairy scientists know that the modern, intensively-managed dairy cow is both an incredibly productive cow in terms of milk yield and an infertile cow in terms of reproductive function. While conception rates in beef cows and dairy heifers routinely exceed 60%, values of 25-40% are the norm in lactating dairy cows. The problem of infertility is increasing: conception rates today are lower than they were 20 or 40 years ago.

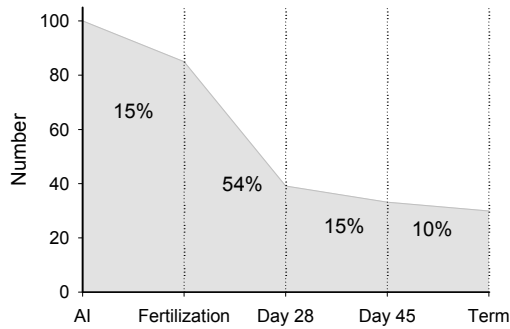


Figure 1. Losses of pregnancies at different stages of gestation in a hypothetical 100-cow herd with a calving rate of 30%. Pregnancy is initiated by fertilization of the oocyte with one of the sperm cells deposited in the reproductive tract by natural or artificial insemination. Successful fertilization occurs only about 85% of the time. Following fertilization, large numbers of embryos die before pregnancy diagnosis by ultrasound at Day 28. Other losses occur between Day 28 and Day 45 (about 15% of pregnancies), when pregnancy is diagnosed by ultrasound, and Day 45 and term (about 10% of pregnancies present at Day 45).

Figure 1 is provided to indicate the timing and scope of embryonic and fetal wastage that occurs in the lactating cow. Most embryonic losses occur some time between fertilization and Day 28, when pregnancy is first detectable by ultrasonic examination. In addition, however, in about 15% of cases, the ovulated oocyte (what the mammalian egg is called) is not successfully fertilized so that no pregnancy results. Also, a significant proportion of cows that are pregnant at Day 28 subsequently lose their pregnancy, with about 15% loss between Day 28 and Day 45, when pregnancy diagnosis by rectal palpation is usually performed, and 10% loss between Day 45 of pregnancy and term. Survival of the embryo is thus a risky proposition.

To a large extent, the embryo's chances of survival have already been partly determined even before it is formed by fertilization of oocyte and sperm cell. This is so because events affecting the development of the oocyte can influence the embryo's potential for development. Embryonic survival is reduced if embryos are derived from oocytes from cows with aged follicles, fed a diet generating high production of ammonia, having high genetic merit for milk yield, low body condition score, or exposed to heat stress (see Hansen, 2002 for review). A follicle that ovulates today started growing over 100 days previously (Webb et al., 2004). This fact means that events taking place weeks before insemination can affect embryonic survival. Another major factor determining whether the embryo will survive is the characteristics of the

reproductive tract that maintains the embryo after fertilization.

Optimal fertility is difficult to achieve and depends upon achieving several goals including 1) using cows that are inherently fertile, 2) minimizing occurrence and severity of diseases affecting reproduction, 3) providing the cow with an environment that promotes reproductive function and 4) implementing reproductive technologies such as timed artificial insemination in ways that are maximally effective. The purpose of this article is to identify a few of the causes of infertility in dairy cattle that are both actionable (you can do something about it) and which are likely to be less obvious, at least to some.

■ Genetics

In any course on livestock genetics, the point is made that reproduction is not a heritable trait. Heritability estimates for reproductive traits are usually around 0.05 or less. A low heritability does not mean, however, that a trait is not controlled genetically. It only means that most of the variation between cows is not due to genetics. Given all the environmental factors affecting reproduction (nutrition, disease, weather, inseminator skill, to name just a few), it makes sense that heritability estimates for reproduction are low. Low heritability means that the accuracy of identifying genetically-superior animals for reproduction is low. There are genes that control reproduction, though, and progress can be made in selecting for them.

Beginning in 2003, daughter pregnancy rate has been used in the United States to calculate differences between bulls in genetic ability of their daughters for reproductive function. Daughter Pregnancy Rate (DPR) is calculated from days open and is the proportion of females eligible to become pregnant in a 21-day period that actually become pregnant. In Canada, Daughter Fertility (DF) is calculated based on four variables. Two traits are measured on each daughter when she receives her first insemination as a heifer, the age at that first insemination and the 56-day non-return rate for that first insemination. The other two traits are measured on daughters at first service for each lactation – the interval between calving and insemination and the 56-day non-return rate for that first insemination. The correlation between DPR and DF is 0.50 (Jorjani, 2005).

One problem with estimates of sire daughter fertility is that the heritability of reproductive traits is low so that genetic progress will be reduced. The heritability of DPR is 0.04 and for DF is 0.05 (Jorjani, 2005). However, work is underway to control for some factors affecting days open to improve the accuracy of the fertility estimates. There are distinct differences between sires in daughter pregnancy rate. The Canadian Dairy Network reports that the 56-day non-return rate for cows in the top 10% of bulls is 66% vs. 61% for the

average bull and 54% for bulls in the lower 10% of bulls (Canadian Dairy Network, 2006a).

One limitation of placing a large amount of emphasis on DF is that genetic estimates of daughter fertility are negatively correlated with milk yield. However, bulls can be identified that have positive estimated breeding values for milk yield and fertility traits. The Canadian index for total merit includes a 5% weighting for DF and the US index includes a 7% weighting for DPR.

Crossbreeding has also been put forward as a method for improving reproductive function as well as improvement in health traits and longevity traits. Crossbreeding will eliminate inbreeding (estimated at 5.6% for Canadian Holsteins in 2005; Canadian Dairy Network, 2006b). In addition, there has been more emphasis on selection for health and reproductive traits in some European breeds than for the breeds used in North America. As can be seen in Table 1, crossbreeding can indeed improve fertility although this improvement comes with some loss in milk yield.

Table 1. Reproduction and lactation traits in first lactation for purebred Holsteins and Holstein crossbreds^{a,b}

Trait	Holstein	Normande x Holstein	Montebeliarde x Holstein	Scandinavian Red x Holstein ^c
Days to first breeding	69	62**	65*	66
Conception rate at first breeding	22	35*	31**	30
Days open	150	123**	131**	129**
305-day milk yield, kg	9,757	8,530**	9,161**	9,281**

^a Data are from Heins et al. (2006ab)

^b Number of observations varies from 245-536

^c Scandinavian Red is a term to describe both Swedish Red and Norwegian Red sires.

Differences with Holsteins indicated by * (P<0.05) and ** (P<0.01).

A cow's genetic ability for reproduction depends on the actions of many individual genes. The science of molecular genetics is offering new opportunities for genetic selection. Variants of specific genes are being identified that affect reproductive function. Recently, a mutation in the calpastatin gene was found that was related to DPR (Garcia et al., 2006). The predicted transmitting ability for DPR was found to be +0.13 for the wild type genotype, -0.44 for the heterozygous genotype and -0.69 for animals that were homozygous for the mutant genotype. Identification of gene mutations such as this allows direct selection for animals possessing gene variants that are superior for reproduction.

The take-home message here is that genetic strategies can be used to improve reproduction in dairy cattle. Crossbreeding can be an effective quick fix for reproductive traits as well as other traits such as longevity, calf mortality, and mastitis resistance. The problem is that the maximal response is seen in the F1 population and further improvement in subsequent generations is limited. It is also possible to select for genes controlling reproduction. Progress will be slower and inbreeding problems are not solved but continuous progress occurs from one generation to the next.

■ Mastitis

It is well known that mastitis is the most costly disease affecting dairy cattle. It is perhaps less well known that losses caused by mastitis include reduced conception rates and increased early and late fetal losses. Reduced conception rate occurs for subclinical mastitis as well as for clinical mastitis and for mastitis caused by gram-negative and gram-positive organisms.

The magnitude of the fertility problems associated with mastitis is illustrated in Table 2. Data in this table were obtained on 1001 lactating cows on two commercial dairies in California (Santos et al., 2004). Mastitis was diagnosed by herdsmen at each milking based on inflammation of the udder or abnormal milk. Subsequently, cows were classified as not having mastitis, being diagnosed for mastitis before first service, being diagnosed between first service and pregnancy diagnosis at 35-58 days post artificial insemination (AI), or being diagnosed with mastitis after pregnancy diagnosis. Note that first service conception rate was reduced if mastitis occurred before AI or some time between AI and pregnancy diagnosis. In addition, abortion after pregnancy diagnosis was more likely to occur if cows were diagnosed with mastitis at any time, even if cows were diagnosed with mastitis before AI.

Table 2. Effect of timing of clinical mastitis on lactation and reproduction traits of lactating cows in California.^a

Trait	Control	Mastitis before AI	Mastitis between AI and pregnancy diagnosis	Mastitis after pregnancy diagnosis
Days in milk at first AI (mean \pm SEM)	64.0 \pm 1.4 ^{bc}	68.0 \pm 2.9 ^c	58.5 \pm 2.3 ^b	62.3 \pm 2.6 ^{bc}
Conception rate at first AI (%)	29 ^b	22 ^c	10 ^d	38 ^b
Abortion incidence (%)	6 ^b	12 ^c	12 ^c	10 ^c

^a From Santos et al. (2004)

^{b,c,d} Numbers with different subscripts within a row differ ($P < 0.05$).

The finding that mastitis is associated with infertility does not lead to recommendations for additional management practices but rather just illustrates the importance of developing effective mastitis management procedures on the farm.

■ Dietary Ingredients

It is well known that diet is an important factor in cow fertility – cows in negative energy balance take longer to get pregnant than cows in positive energy balance. Specific components of the diet can also play a role in reducing fertility.

Protein itself can inhibit fertility when fed at high levels. The amino acids in excess protein are converted to the waste product urea. Urea is toxic to oocytes. In addition, urea can cause lowered uterine pH, which in turn reduces embryo survival. One other consequence of feeding excess protein is reduced energy balance. This effect occurs because excretion of excess protein nitrogen requires energy. As a practical matter, effects of excess protein on fertility can be reduced by limiting the crude protein content of the diet to less than 19% and limiting the ruminal degradable protein to less than 10% of diet DM. It is possible to monitor protein status of cows by measuring urea concentrations in milk. It is recommended that milk urea nitrogen concentrations not exceed 20 mg/dl.

Another toxic molecule in feedstuffs is the yellow pigment gossypol found in the pigment glands of cotton seeds. Gossypol evolved to protect the cotton plant from insects but is also unfortunately a toxin for mammals too. Feeding

too much cottonseed can cause acute poisoning characterized by difficulty in breathing, weakness, diarrhea and death. Young animals are particularly sensitive to gossypol toxicity. Also, it is recommended that bulls not be fed cottonseed because sperm production is very sensitive to gossypol toxicity.

Cottonseed meal is in many ways an excellent feed that provides protein, fat, energy, and fiber. It is the most abundant plant protein feedstuff for dairy cattle in the United States after soybean meal. Most of the fiber is in the lint so that linted cottonseed products contain more fiber and less protein and fat than a delinted cottonseed like that derived from pima cotton. The lint slows down passage through the rumen so that pima cottonseed meal, which passes through the rumen more quickly, has lower digestibility. As a result, pima cottonseed is often processed for cattle feed by cracking to slow down rumen passage.

It is difficult to predict gossypol content of cottonseed meal. Gossypol exists in two chemical forms (or isomers) called (+) and (-) gossypol. The (-) gossypol is the more toxic form. In addition, gossypol that becomes bound to protein (either in the cotton seed or in the rumen) is inactive – only the free gossypol is toxic. Pima cottonseed meal is generally more toxic than the linted upland cotton seed varieties because there is more (-) gossypol in the seeds and passage through the rumen (unless retarded by cracking or other processing) is more rapid so that less gossypol is detoxified in the rumen. A rule of thumb is to feed no more than 6-8 lb (2.7 - 3.6 kg) of upland cottonseed and 4-6 lb (1.8 – 2.7 kg) of unprocessed pima cottonseed. There are a few laboratories that will measure the free gossypol content of cottonseed – the California Veterinary Diagnostic Laboratories is one organization that will do so. The recommendation is to feed a maximum of 0.1-0.2% free gossypol or 20 grams of free gossypol per day.

The effects of feeding excess gossypol on fertility in lactating cows are shown in Table 3. These data are from an experiment in which lactating cows were fed a diet containing 10% cottonseed on a dry matter basis. In one group, the cottonseed was an upland variety (estimated amount of free gossypol=717 mg/kg dry matter) while the other group received a 1:2 mixture of upland and pima (about 951 mg free gossypol/kg dry matter). Cows started the cottonseed after calving. Note that there was no effect of cottonseed type on conception rate at first service but that conception rate for other services was lower in the group receiving the upland:pima mixture. Gossypol concentrations in blood accumulated over time and thus only later services were compromised by the increased level of gossypol feeding. Cows fed the higher gossypol diet also experienced a greater incidence of abortions.

Table 3. Effect of cottonseed type on reproductive performance of lactating dairy cows when fed at 10% on a dry-matter basis.^a

Trait	Upland cotton	Upland: cracked pima (1:2)
Conception at first service (%)	28.2	29.3
Conception at other services (%)	36.8	30.7*
Abortions (%)	3.3	7.9**
Cows pregnant by 170 days in milk (%)	79.1	70.6**

^a from Santos et al. (2003) * P<0.05 ** P<0.01

■ Heat Stress

It may sound strange to be thinking of the Canadian dairy cow as being susceptible to heat stress. In fact, however, there are times during the summer throughout large parts of Canada when cows are at risk from heat stress. This is especially true for high-producing cows.

Many of the effects of heat stress on lactation and reproduction occur because body temperature of the cow rises. Such a cow is said to be experiencing hyperthermia. The rise in body temperature during heat stress occurs because the cow cannot lose all of its heat of metabolism to the surrounding air. Lactation increases the cow's heat production and makes it more difficult to maintain normal body temperature in warm weather. The more milk a cow produces – the more heat she produces and the more sensitive she is to heat stress.

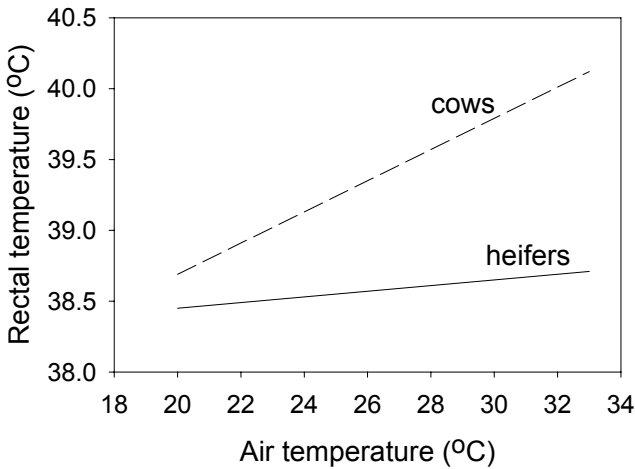


Figure 2. The relationship between air temperature and rectal temperature for non-lactating heifers and lactating cows. Data were collected in Wisconsin and are from Sartori et al. (2002).

The effect of lactation on body temperature is shown in Figure 2 for non-lactating heifers and lactating cows in Wisconsin. Note that at an air temperature of 30°C, the predicted rectal temperature of a heifer is 38.7°C, only slightly above the typical body temperature of 38.5°C. For lactating cows at an air temperature of 30°C, the predicted body temperature is 39.8°C. A good ballpark figure is that reproduction starts to suffer when cows have a body temperature of 39.5°C. Accordingly, the lactating cow at an air temperature of 30°C is at risk of infertility because of heat stress.

Actual body temperatures will vary somewhat from the example given above depending on features of the cow (foremost of which is milk yield), other meteorological variables (wind speed and solar radiation), as well as cow housing. Nonetheless, it becomes apparent from looking at Figure 2 that there will be many times during the summer that cows, particularly-high producing cows, will be hyperthermic.

There are two major effects of heat stress on reproduction. First, heat-stressed cows show estrus less intensely than other cows. As a result, heat detection is more difficult. Secondly, conception rate is reduced during heat stress. In Florida, where heat stress is severe, estrus detection rates during the summer can be as low as 20% and conception rates 15%.

Recently, a reduction in conception rate during the summer has been documented in Alberta. In this study, Divakar Ambrose and colleagues analyzed data on over 1500 inseminations from a single dairy near Edmonton

(Ambrose et al., 2006). Conception rate was lower ($P < 0.01$) for cows inseminated during the summer than for other seasons (23 vs. 32%).

It is easy to determine whether heat stress affects cows at your operation. The first thing to be done is to evaluate herd records to see if fertility declines in the summer. Secondly, measure rectal temperature in selected cows during the summer - the higher the body temperature, the greater the reduction in fertility that can be expected. The cow's normal body temperature is about 38.5°C. A cow with a rectal temperature of 39.5°C in the afternoon is likely to be heat stressed (if she does not have mastitis or is otherwise sick).

Effects of heat stress on estrus detection can be solved by incorporation of a timed AI program into the reproductive management system. Timed AI can increase the number of cows pregnant during the summer because it increases the number of cows inseminated (Aréchiga et al., 1998). Timed AI does not improve conception rate, however.

Enhancing conception rate during heat stress has proven difficult. The best approach is to alter animal housing to keep cows cool during heat stress. The simplest structures for providing cooling are shade structures. These can be inexpensive structures based on use of shade cloth or more permanent structures. By itself, shade is not very effective at preventing elevated body temperature in lactating cows if heat stress is moderate or severe. A common and fairly effective system for cooling cows housed in freestall or loose housing is to install sprinklers and fans. Sprinklers can be relatively inexpensive systems using PVC tubing and sprinkler heads used for lawn irrigation systems. See Bucklin et al. (1992) for more details on dairy cow housing to reduce heat stress.

Keep in mind that heat stress compromises fertility by altering many aspects of the cow's reproductive physiology. The follicle that ovulates today started growing months before and there is evidence that heat stress at least 20-26 days before estrus can compromise the follicle (Roth et al., 2001). What that means is you should not expect an increase in fertility just by cooling cows on the day of insemination or by limiting inseminations to the morning during heat stress.

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