

An Overview of Strategies to Improve Reproductive Efficiency

Divakar J. Ambrose

Beef and Dairy Group, Alberta Agriculture Food and Rural Development, 9th Floor, 6909 - 116 Street,
Edmonton, Alberta, T6H 4P2, Canada
E-mail: divakar.ambrose@agric.gov.ab.ca

▪ Take Home Messages

- Inefficient heat detection is the single largest reason for infertility. Spend more time detecting heat and do it more frequently.
- Take advantage of estrus synchronization. Inducing estrus in groups of three or more cows helps in enhancing estrus behaviour and heat detection rate. Choose a simple, effective protocol and use it aggressively.
- If the heat detection rate is poor, try synchronization of ovulation (OvSynch). It eliminates heat detection. About 40% pregnancy rates can be expected.
- Revisit semen handling procedures and insemination techniques.
- Reconsider managerial factors. Set voluntary waiting period at 60 days. Cows in poor body condition at breeding are less likely to conceive. Use body condition scores for reproductive management.
- Consult your veterinarian on methods to minimize embryonic loss and manage repeat breeders.
- High-protein rations may have adverse effects on reproduction but fat supplementation may improve fertility.
- Maximize cow comfort and minimize environmental effects.

▪ Introduction

Traditionally, dairy cattle have been selected for milk production. Genetic selection and improved management practices have dramatically increased milk yield over the past 35 years. However, reproductive efficiency in dairy cattle has been declining over the same period. For instance, the average pregnancy rate to first insemination among dairy cows in North America has

dropped from more than 65% in the early 50s to about 45% in the 80s (8) and it has declined further in the 90s. Today, 20 to 30% of dairy cattle culled each year are for reasons of infertility. Since fertility is not a heritable trait (22), little can be gained through genetic selection favouring efficiency of reproduction. Further, there is a negative relationship between milk production and reproduction (8, 15). Therefore, as we strive for even higher levels of milk production, reproductive efficiency may be further affected. Larger herds, fewer personnel and increased milk production demands placed on cows, all account for this decrease in fertility. A recent study in Ontario (18) suggests that a 1-day increase in calving interval, exceeding 12.5 months leads to a mean reduction in revenue of \$ 4.70 per cow. Thus, high reproductive efficiency is vital to a successful dairy operation.

▪ **Factors affecting reproductive performance**

The key factors affecting reproductive performance in dairy cattle are outlined in Figure 1. As presented, the four major factors are of Human, Animal, Nutritional and Environmental origin.

▪ **Strategies to improve reproductive efficiency**

The main focus of this paper is to suggest strategies to improved reproductive efficiency through the manipulation of some critical factors that can be controlled to a certain extent. Thus, of the nine strategies presented, five will relate to human factors. Strategies for the minimization of embryonic loss and management of repeat breeders will be presented next, and finally, strategies to manipulate nutritional and environmental effects on reproduction and the importance of cow comfort will be discussed briefly.

Strategy 1: Focus on heat detection - do more not less.

Among the many human factors that affect reproductive performance, inefficient heat detection is the most important as about 50% of heats go undetected on an average dairy farm. Conception rate is defined as the percent cows pregnant per insemination, whereas pregnancy rate is the percent cows pregnant considering all eligible open cows in the herd intended to be inseminated. Let's say, a producer starts with ten cows for estrus synchronization. If seven cows showed estrus and were inseminated, the heat detection rate is 70% (7/10). If four of the seven cows inseminated were confirmed pregnant, then the conception rate is 57% (4/7) whereas the

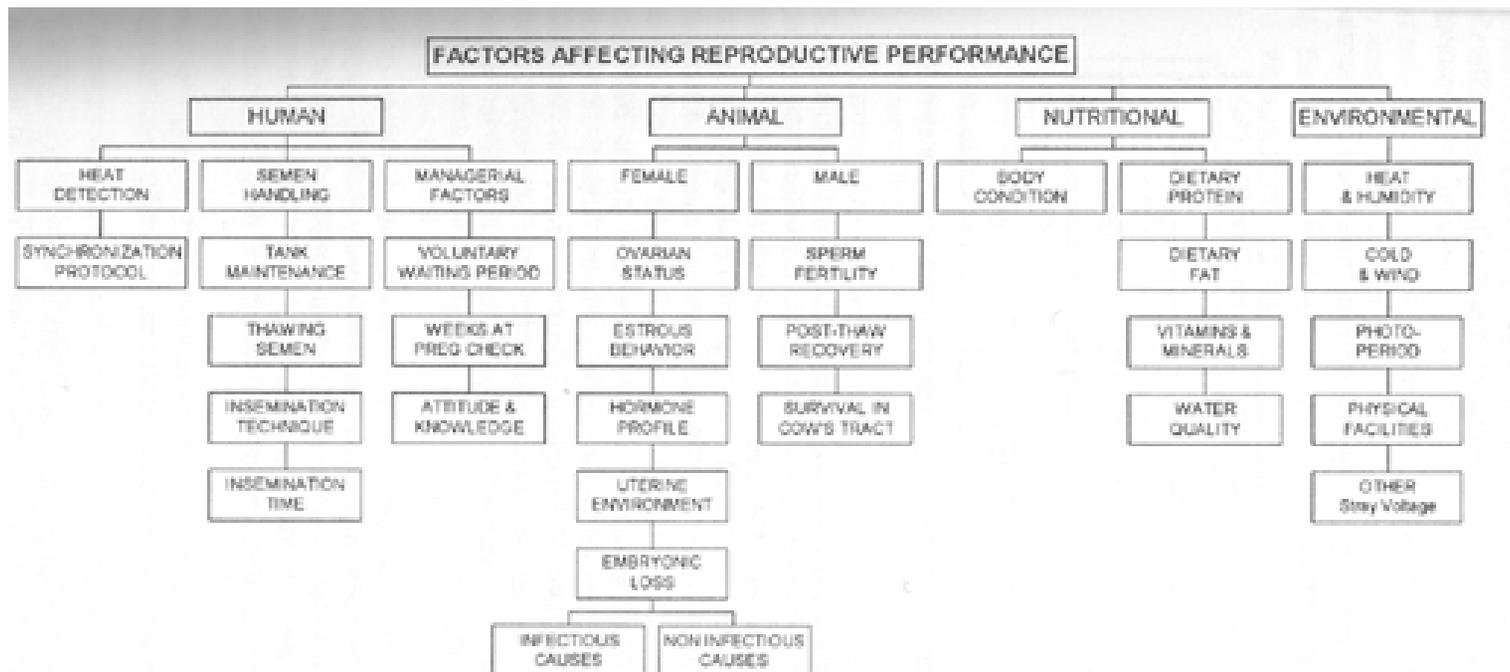


Figure 1. Factors affecting reproductive performance in dairy cattle

pregnancy rate is only 40% (4/10). Thus, pregnancy rate (PR) is a product of head detection rate (HDR) and conception rate (CR). See Table 1 for an example of how poor heat detection rate can severely affect pregnancy rate, even though conception rate remains constant at 60%.

Table 1. Example of how pregnancy rate can be improved by increasing the heat detection rate.

CR (%)	HDR (%)	PR=CRxHDR (%)
60	30	18
60	50	30
60	70	42
60	100	60

CR=conception rate; HDR=heat detection rate; PR=pregnancy rate

Several studies, based on blood or milk progesterone concentrations, confirm that five to 30% of cows are routinely inseminated when not in heat. That is, they are inseminated too soon, too late or at a time when they are not even close to being in estrus (eg., mid-cycle). This must be borne in mind when checking for heats. Five to 10% of all pregnant cows exhibit signs of heat, usually from mid to late gestation. This is normal as ovarian follicular growth continues during pregnancy. Large follicles (a follicle is a fluid-filled sac containing the egg) develop just as in normal estrous cycles but they do not ovulate. The presence of an active follicle on the ovary increases blood estrogen levels and occasionally triggers estrus behaviour in pregnant cattle. Therefore, it is essential that farm personnel concerned with heat detection and A.I. are familiar with the breeding history of cows to minimize chances for erroneous inseminations.

Colour-coded identification schemes (e.g. tail chalk, paint, ribbon or adhesive tape) can be used to mark cows that have recently calved, those due for insemination, that have been inseminated but not palpated for pregnancy and that are confirmed pregnant. Such a system can help to identify the status of cows in heat quickly without having to look through the records each time.

Allow cows to interact in open area with natural (dirt) footing (not concrete). This is very important as the estrus behaviour of cows differ depending on type of flooring (Table 2).

Use estrus detection aids such a tail head chalk, paint or Kamar heat mount detector. Applying chalk or paint to the tail head of cows at the time of PG injection will enhance estrus detection efficiency. Record paint scores (0 to 5) daily for 7 days following PG. No change in colour = 5; complete disappearance of colour = 0. Cows receiving a paint score of 2 or less may have been in

standing estrus after the last observation period. Such cows must be watched carefully and bred upon confirming estrus.

Table 2. Estrus activity (mounts and stands) of Holstein cows on dirt versus concrete flooring.

Activity	Dirt	Concrete
Hours in heat	13.8	9.4
No. of total mounts	7	3.2
No. of total stands	6.3	2.9

Source: Britt et al. (6)

In larger herds where heat detection is poor, investing in an electronic heat detection system such as HeatWatch7 may prove economical in the long run. Studies from Virginia Tech University report that HeatWatch is 94% efficient in detecting estrus and 95% accurate (4). Days open decreased by about 30 days, pregnancy rates increased 20% and services per pregnancy decreased by 0.5. Data from herds using HeatWatch indicate that the duration of estrus in today's Holstein cows is much shorter than previously documented. For instance, in one study, more than 70% of cows remained in estrus for less than 12 h (30% were in heat for less than 6 h), with an average heat period of 8.5 h and approximately 10 mounts during that period. Some cows had less than 3 mounts, each lasting about 2 seconds. Most of the estrus activity occurred when cows were on dirt surfaces, travelling to and from the milking parlour regardless of the time of day.

Heat detection matters. So invest time detecting estrus. Increase observation time and frequency. Recommended frequency is 4 times a day, at least 20 min each observation. Do not perform any other activity such as cleaning, mixing feed, giving shots, etc. while observing for heat; stay calm and focus on watching the cows.

Strategy 2: Be aggressive in estrus synchronization.

Take advantage of estrus synchronization. If possible group at least 3 cows together every time estrus is induced, to facilitate the forming of Asexually active groups[≡]. This makes heat detection easier. Cows that do not normally show overt signs of estrus are encouraged to exhibit estrus behaviour in the presence of other animals in heat.

Progesterone devices or prostaglandin F₂ alpha (PG) injections can be used for synchronization of estrus. Progesterone devices are not available in Canada for use in dairy cows. Therefore, PG is the drug of choice for synchronization

of estrus in dairy cattle. What PG does is terminate the CL present on the ovary. When PG is administered, blood progesterone declines (due to the demise of CL). The CL is the primary source of progesterone in cows. Progesterone prevents cows from coming into estrus prematurely, and it is also the chief hormone of pregnancy. When progesterone declines, a dominant follicle present on the ovary grows rapidly. Estrogen is a hormone present in high concentrations in the fluid of an actively growing dominant follicle. Associated with a decline in progesterone, blood estrogen concentrations increase near the time of estrus. This affects centres in the brain leading to estrus behaviour.

For a cow to show heat in response to PG treatment, it is essential that a healthy follicle and a mature CL be present on her ovary at the time of PG injection. Response to PG is maximal between days 6 and 16 when the CL is active. Giving PG from day 1 to 5 may not be effective as the CL is too young. Even if the CL regresses in response to PG, synchronized estrus cannot be guaranteed as it largely depends on the stage of the follicle present on the ovary at that time. In most cows follicles grow in a wave-like pattern (usually two waves) during each estrous cycle. Associated with each wave is one healthy (dominant) follicle. Normally, 5 days after ovulation a dominant follicle is present on the ovary. This follicle has a healthy life of about 5 days. Cows receiving PG on day 6, 7, 8 or 9 are more likely to respond normally and come into heat within 72 hours as the first dominant follicle is active, ready to grow and ovulate. However, if PG is given on day 10, 11, 12 or 13, the time to estrus could vary as the first dominant follicle present on the ovary on these days is either inactive or regressing and the second dominant follicle is too young and needs time to grow and ovulate. This process could take up to 5 or more days depending on the stage of follicular growth. In some cases an old dominant (now inactive) follicle may ovulate but the quality of the egg released is likely compromised due to ageing. This may lead to fertilization failure or early embryonic death. As the second dominant follicle is active after day 14, injecting PG to cows on days 14 to 17 should evoke an optimal response. Beyond day 17 PG is unlikely to be effective as CL regression may have begun naturally by this time.

Numerous protocols are available for estrus synchronization. One of the common protocols is a single injection of PG given any day (with or without palpation for CL) and A.I. at detected estrus. Another protocol is two injections of PG given either 11 or 14 days apart with A.I. either after first injection (if estrus detection is done) or timed insemination 72 to 80 h after the second PG injection (if estrus detection is not done). Several deviations from these standard protocols have been tested with varying degrees of success.

It is important to choose a PG protocol that works best in your situation. It must be easy to understand and implement, and yield consistent results. Choose a simple yet effective protocol in consultation with your veterinarian, and use it aggressively.

Strategy 3: Ovulation synchronization works - try it.

If heat detection rate is low, but conception rate is high, consider synchronization of ovulation (OvSynch) as it allows for cows to be inseminated without heat detection. For a simplified explanation of how this system works, refer to Alberta Dairy Herd Management Fact Sheet No. 2T1:1. Do not modify the protocol. The sequential injection of GnRH, PG, GnRH is critical. Understand the significance of each injection. Conception rates are satisfactory even if the second GnRH is given at the time of AI rather than 16 hours earlier. However, to maximize conception rates (21), stick with the standard protocol and give second shot of GnRH 16 h before A.I.

Conception rates to first A.I. following synchronization of ovulation are not different from that of control cows inseminated at detected estrus (Table 3). Interval to first conception is significantly reduced following OvSynch, thus reducing days open. There is a general consensus among researchers that OvSynch is beneficial in herd situations where heat detection rate is poor. It may not be advantageous in herds where the heat detection rate exceeds 70%.

Table 3. Pregnancy rate to first timed A.I. after synchronization of ovulation in lactating dairy cows - a review of four reports.

No. of cows	% pregnant to 1st A.I.		Source Reference #
	Control	OvSynch	
298	37	39	19
299	30.5	29	7
310	38.9	37.8	20
67	25.7	37.3	28

Preliminary results from an ongoing research project in Alberta and BC indicate that an average pregnancy rate of 42% is achievable with OvSynch (Table 4). The multi-location study includes research herds and producer herds. All cows are treated with OvSynch protocol and inseminated 16 h after the second injection of GnRH. The objective of the study is to compare the effect of additional GnRH treatments at 7 and/or 14 days after A.I. on pregnancy rates. Though this project does not directly compare A.I. at OvSynch to A.I. at detected estrus, the overall pregnancy rate (pooled regardless of post A.I. treatment) suggests that OvSynch is effective.

Table 4. Pregnancy results from 142 Holstein cows after OvSynch and A.I.

Herd	Number of cows		Pregnancy rate (%)
	All	Pregnant	
A	16	6	37.5
B	16	6	37.5
C	22	9	40.9
D	36	18	50
E	20	5	25
F	56	25	44.6
Total	166	69	41.6

Strategy 4: Revisit semen handling and insemination techniques - do it right every time.

Seemingly simple tasks such as semen tank maintenance and thawing techniques are vital to herd reproductive efficiency. How often do we stop to think if these things are done correctly? It is a simple fact that even excellent quality semen from a *Atop* sire used to inseminate the best cow at the most ideal time cannot assure a pregnancy if the semen was improperly stored, incorrectly thawed, or deposited in the wrong site.

Semen tank

Check liquid nitrogen level periodically. Maintain a log book (keep it attached to the tank) to show dates of last filling, and record removal or addition of semen straws.

Minimize evaporation of liquid nitrogen. Narrow-mouthed tanks help reduce evaporation. Replace lid soon after each use. Store semen tank in a cool, dry, well-ventilated area. Persistent frost around the mouth and the neck region of the tank is an indication of rapid evaporation likely due to poor insulation. If this happens, have your tank inspected as soon as possible and replace if necessary.

Handle the tank with care. Avoid dragging or violent movements; lift and carry or place on a wooden platform fitted with casters and transport gently. If transporting in a vehicle, make sure tank is secured firmly.

It is often assumed that the temperature within a semen tank is constant. This is not true. The temperature of liquid nitrogen is -196°C . When semen straws are held immersed in liquid nitrogen, all straws are at this constant temperature but there is a wide range of temperature zones from the surface of the liquid nitrogen upwards reaching as high as $+7^{\circ}\text{C}$ near the mouth of the tank (Figure 2). Ampules that monitor temperature are available commercially. Contents of ampules placed in canes will thaw and change colour if the temperature rose to levels that may damage sperm.

Removing a straw from the cane should not take more than 6 sec. Remember that even minor fluctuations in temperature severely damage sperm. Exposing straws to the warm zone (closer to the mouth of the tank) for 10 to 12 sec could cause substantial damage to sperm.

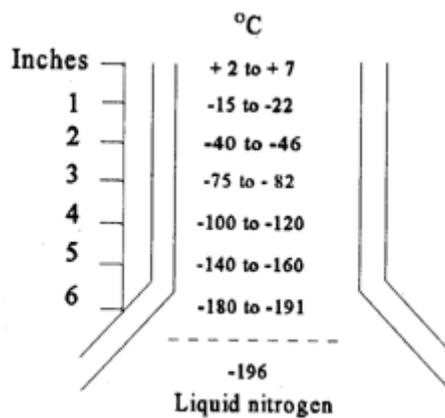


Figure 2. Temperature range in a 6 inch (15 cm) neck of semen tank (adapted from reference #23)

Thawing

Do not thaw straws in the air or in the cow. The recommended procedure is to thaw straws in a water bath at 35 to 37°C for 40 seconds. If a thermostat-controlled water bath is not available, use a simple thermometer to set water temperature. Do not rely on your fingers to check temperature, particularly during winter months. Verify the temperature of thermostat-controlled water bath occasionally (eg., once every 3 months). Avoid thawing straws in batches. If batch thawing is unavoidable for reasons beyond control, leave thawed straws in water bath till insemination. Upon removing from water bath wipe the straw thoroughly. Even if tiny drops of water get mixed with semen, drastic osmotic changes could occur causing sperm damage.

Never thaw straws in an open, windy area especially in winter. Recommended thawing location is a heated room protected from drafts.

Pre-warm A.I. rod before loading the straw. Once loaded, the A.I. rod must be wrapped in clean paper towels and tucked under clothing and held close to body to minimize further drops in temperature. Drying and loading the straw in the A.I. rod must be performed quickly. In the hands of an efficient operator, approximately 1 minute lapses from the time a straw is picked up from water bath to the time it is loaded in A.I. rod and tucked under clothing. There is a rapid drop in semen temperature during this period. The lower the ambient temperature, the greater the drop in semen temperature (see Table 5).

Table 5. Post-thaw temperature change occurring in semen packaged in 0.5 ml straws during the time taken to load a straw after removing from warm water bath.

Ambient temp. (°C)	Semen temperature (°C)		
	Initial*	Final	Drop
21	35	30	5
4	35	20	15
-16	35	13	22

* Average time lapsed between initial and final temperature = 1 min.;
Adapted from Ref #24

Use a clean pair of scissors or a straw-cutter. If the cutting instrument is cold, warm it before cutting. Wash the cutting instrument at end of each day with mild detergent, rinse thoroughly, disinfect and dry. Once thawed, inseminate as soon as possible.

Discuss semen handling and insemination procedures with your semen supplier or sales agent. Make sure you do it right every time.

Insemination technique

Entrust insemination to a trained, skilled inseminator. Always wipe the cow's external genitalia with a clean paper towel. If too dirty, wash with soap and water before insemination. Under any circumstance avoid touching faecal matter or other contaminants with the A.I. rod (sheath) while inseminating. Deposit semen in body of the uterus anterior to the cervix. Be as quick as possible.

Timing of insemination

The timing of insemination affects fertility. Sperm must undergo a series of surface changes before acquiring the ability to fertilize an egg. These changes, termed *Acapacitation*≡ take 4 to 8 h in bull sperm that has been frozen and thawed. Unfrozen sperm take a little longer to complete the capacitation process. About 8 to 10 h after insemination sperm reach the site of fertilization in the fallopian tube. Capacitation takes place during transport. Some sperm may live in the reproductive tract of a cow longer than 48 h. However, since sperm cells age with the progression of time, those living beyond 24 h are likely to have reduced vigour and fertilizing ability. The fertile life of an ovulated egg is about 8 h.

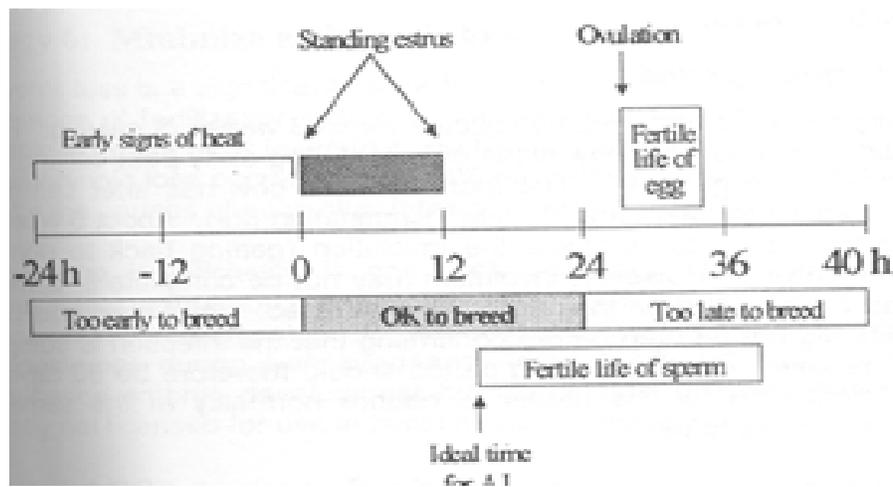


Figure 3. Diagram showing the ideal time for breeding in relation to the onset of standing estrus (0 h). To maximize the chances for conception, the time of A.I. chosen must allow the fertile periods of sperm and egg to overlap.

A standing heat coincides with a sharp increase (surge) in luteinizing hormone (LH; also known as the ovulatory hormone) essential for the final maturation process of the egg and ovulation. Ovulation occurs 24 to 30 h after the first standing heat (see Figure 3). If a cow is inseminated much earlier than a standing heat (that is, more than 24 h before ovulation), only aged sperm will be available to fertilize the egg. On the other hand, if insemination is performed later than 24 h after standing heat (that is, after ovulation has taken place), chances are that sperm reach the site of fertilization well past the fertile life of the egg. Thus, insemination must be timed in such a manner that fertile sperm reach the site of fertilization prior to ovulation and are waiting for the arrival of the egg. In other words, insemination should occur 8 to 12 h prior to ovulation, the best time for A.I. being 15 h after the onset of standing estrus (Figure 3). Satisfactory conception rates, however, can be expected from the onset of standing heat up to 12 h after the end of standing heat. The AM-PM rule holds true. So if a cow was seen a standing at 6 am, breed her at 6 pm. If seen a standing at 6 pm, breed at 6 am next morning. If in doubt, breed at standing heat.

Kits to check progesterone in milk or plasma, or probes to check vaginal mucus conductivity can be used to determine the ideal time for breeding, but such tools may not be practical or cost effective at all times.

Strategy 5: Reconsider your managerial factors and implement changes where needed.

Voluntary waiting period

In most dairy cows the first ovulation occurs within 3 weeks of calving. Though cycling, many cows do not show signs of heat during early post calving period but likely do ovulate normally. The more cycles a cow has after calving, the higher the chances for pregnancy to first insemination (29). About 5 weeks are needed for the uterus to complete the involution (getting back to normalcy) process after calving. However, involution may not be complete in 5 weeks if calving was complicated or the uterus was infected. Cows that had uterine infections should not be bred before confirming that the infection is completely cleared. The ideal voluntary waiting period should therefore be 60 days as it allows sufficient time for the uterus to restore normalcy at the same time allowing 2 to 3 cycles to pass.

Cows not detected in estrus for 6 weeks after calving must be submitted for examination by a veterinarian to assess their reproductive status.

Consider body condition

Cows in a negative energy state often do not conceive. Poor body condition is associated with lower blood progesterone concentrations and significantly lower pregnancy rates (8). One study involving heat-stressed dairy cattle projected a 37% increase in pregnancy rate when body condition score (BCS) increased from 2.25 to 3.25 (1). The rate at which body condition loss occurs has a

greater detrimental effect on fertility rather than the BCS itself. For example, a loss of condition from 3.25 to 2.25 (1.0 loss) during the first five weeks after calving is likely to have a more damaging effect on fertility than a loss of condition from 2.50 to 2.25 (0.25 loss). Considering these, monitoring the relative change in BCS will be useful for optimal reproductive management.

If desired, cows that are in good body condition (eg., BCS \geq 3.0) can be selectively bred earlier than 60 days. In any case, breeding before 50 days is not recommended. Incorporate condition scoring into your breeding management. Condition score at weekly intervals beginning 3 wk before the expected calving date. When practical, segregate cows in poor condition and place on a high energy diet to help restore condition and breed when BCS improves.

Motivate employees

If using hired hands for heat detection, help them stay motivated. A good attitude and knowledge are extremely important. Casual employees may not realize the importance of reproductive management and show little interest in heat detection. Make sure they know what they are doing, if not, do it yourself.

Strategy 6: Minimize embryonic loss

Embryonic loss is a significant cause for reproductive wastage. While only a 15 % incidence of fertilization failure is documented, more than 35% of embryos die within 40 days after insemination (16). A recent report (21) suggests that 22% embryonic loss occurs between 25 days after A.I. and calving time. What could be the causes of such high rates of embryonic death?

Progesterone insufficiency is one likely reason. This can be treated by supplementing exogenous progesterone or by inducing the cow's ovary to produce additional progesterone. Intramuscular injection or intravaginal inserts of progesterone during early pregnancy (days 1,2,3 and 4 after insemination) can enhance embryo development but intravaginal progesterone devices are currently not licensed for use in lactating dairy cows in Canada.

GnRH or hCG injections after insemination

Administration of human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH) 5 to 7 days after insemination will induce the ovulation of a secondary follicle that normally does not ovulate. This induced ovulation causes the development of a secondary CL which secretes additional progesterone. The increased progesterone may benefit embryo survival. Some studies have reported an increase in pregnancy rate while others have not.

Injection of GnRH or hCG on day 14 to 15 after A.I. is another approach. Recent research findings support the view that estrogen secreted by the dominant follicle present on the ovary at this time is largely responsible for

inducing PG secretion from the uterus causing CL regression and termination of the estrous cycle. Estrogen, oxytocin and PG are the hormones involved in orchestrating the demise of the CL. In pregnant cows, the developing embryo sends out strong signals (proteins secreted by the embryo) to the mother indicating its presence. It is essential that these signals are received in a timely fashion to prevent the demise of CL and maintain pregnancy. Injecting GnRH or hCG around day 15 eliminates the estrogenic influence of the follicle, leading to an extension in the lifespan of the CL. Since not all embryos develop at the same rate, this Δ window of opportunity may allow slow-growing embryos some extra time to signal their presence. This concept is currently under extensive research.

Use of a subcutaneous GnRH implant

This novel concept has been tested and reported recently (2). Cows were subjected to OvSynch protocol. Instead of the second GnRH injection, a GnRH implant was placed subcutaneously in one group of cows. A second group of cows received GnRH injection. A third group of cows was allowed to ovulate spontaneously. In the presence of the implant, the growth of large follicles was restricted on the ovaries and the establishment of a dominant follicle was delayed substantially (see Figure 4). At the same time, CL function was enhanced as determined by a significant increase in progesterone concentrations. The absence of the influence of a large follicle may delay CL regression and help embryo survival. In a follow-up experiment, the influence of GnRH implant on pregnancy rates in lactating dairy cows was tested. Cows that were in poor body condition (2.25 BCS) were assigned to the study. All cows were synchronized using the OvSynch protocol. Of the 16 cows in the study, 8 received the GnRH implant 16 h prior to insemination (instead of the second GnRH injection) while the 8 control cows received an injection of GnRH as in a standard OvSynch protocol. Only 1 (12.5%) of the 8 control cows became pregnant while 5 (62.5%) of the 8 cows treated with GnRH implant were confirmed pregnant. The delayed emergence of a dominant follicle on ovaries of GnRH implant treated cows may have contributed to the increased rate of embryo survival. These results, suggesting that the use of GnRH may increase pregnancy rates even in cows of poor body condition, are very exciting, but this data must be interpreted cautiously as these findings are preliminary and must be confirmed with a larger number of animals.

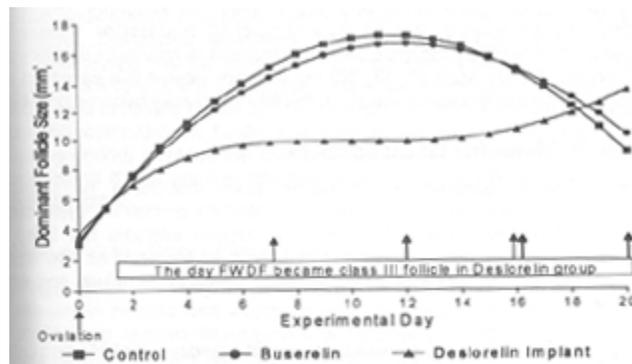


Figure 4. The establishment of first wave dominant follicle (FEDF) as a Class III (>9mm) follicle was delayed ($P<0.01$; arrows) in the GnRH (Deslorelin) implant (14.2 ± 1.3 d) compared with untreated controls (4.6 ± 1.3 d) and GnRH (Buserelin) injection (5.0 ± 1.5 d). 5 cows/group. Pooled SEM at the mean experimental day = ± 0.40 . (Source: Reference #2)

Figure 4. The establishment of first wave dominant follicle (FEDF) as a Class III (>9mm) follicle was delayed ($P<0.01$; arrows) in the GnRH (Deslorelin) implant (14.2 ± 1.3 d) compared with untreated controls (4.6 ± 1.3 d) and GnRH (Buserelin) injection (5.0 ± 1.5 d). 5 cows/group. Pooled SEM at the mean experimental day = ± 0.40 . (Source: Reference #2)

Pregnancy diagnosis

Many dairy practitioners can accurately diagnose pregnancy by rectal palpation before six weeks. However, there is a small risk of embryonic loss when palpation is performed very early in pregnancy (10). Pregnancy can be confirmed with 100% accuracy by 28 days after A.I. using transrectal ultrasonography. Though this technique carries a lower risk of embryonic loss, it has some practical limitations for routine on-farm application. Progesterone kits can be used to detect open cows early. While results from a single sample of milk or blood taken on day 21 are not highly reliable, open cows can be identified with more than 90% accuracy when two consecutive samples are collected 21 and 24 days after A.I. Since a high percentage of early embryonic loss is being reported (10, 21) during early pregnancy stages, it may be a good idea to wait till about 6 weeks to confirm pregnancy by rectal palpation. Even if pregnancy diagnosis is performed early, it is best to reconfirm around day 70 as embryonic losses beyond day 70 are negligible except in cases of infectious abortions.

Bovine Interferon

Bovine interferon-tau, a protein complex secreted by the developing conceptus (embryo and its components) around day 15 plays an important role in the establishment of pregnancy through prevention of CL regression. Intrauterine administration of bovine conceptus secretory proteins in natural or recombinant forms extends CL life span (5, 13, 30) but it is not clear if this approach will consistently increase embryo survival. In the future this may become possible.

Strategy 7: Managing repeat breeders

The term *Repeat breeder* is applied to cows that have no palpable abnormalities and no clinical evidence of an infection or ovarian dysfunction. They must have been detected in estrus at regular intervals but failed to conceive despite four or more inseminations. Animals identified as true repeat breeders have greater rates of defective eggs, fertilization failure and early embryonic loss.

Ovulation failure is one possible reason for repeat breeding. GnRH given either 12 to 16 hours prior to (or) at the time of insemination is one method by which ovulation can be induced. This method is likely to help cows that do not spontaneously ovulate. It must be remembered though that GnRH injections do not always ensure ovulation and ovulation failure is not the only cause for repeat breeding. Since GnRH is relatively inexpensive and likely to help at least some repeat-breeder cows, this is a recommended procedure.

Repeat breeder cows that ovulate normally but fail to conceive could have inadequate progesterone secretion. In such cases, administration of hCG (3000 IU) or GnRH (100 ug) 5 days after A.I. may be tried as a possible remedy. It has been shown that hCG is more effective than GnRH in increasing progesterone concentration (25).

If the tubal (fallopian tube) environment is not conducive to fertilization or early embryonic development, embryo transfer may be helpful, but do not invest in an expensive donor embryo.

Transferring an embryo to the uterus of a repeat breeder cow seven days after insemination is another potential method to increase the chances for pregnancy. In some parts of Ontario, in vitro produced beef cattle embryos are transferred routinely to repeat breeder dairy cows seven days after A.I. In vitro-produced beef cattle embryos are affordable, and allow for the easy differentiation between a calf born through A.I. (dairy breed) versus E.T (beef breed). The success rate of such an approach is not known as published results are unavailable at this time.

Strategy 8: Nutrition management - Fat supplementation may improve fertility.

Good body condition at calving is very important to minimize negative energy balance. Develop an effective dry cow feeding program in cooperation with your nutritionist. At the time of dry-off, aim for a BCS of at least 3.0 and at calving, 3.5 to 4.0, but be cautious not to over-condition. Obese cows (BCS >4.0) tend to have a higher incidence of calving difficulty, retained placenta and possibly compromised health and reproductive parameters. During the post calving period, increase the energy intake (by feeding higher levels of bypass protein) so that a positive energy balance is achieved with minimum loss of body condition.

High protein intake affects fertility

A high intake of rumen degradable protein increases concentrations of urea nitrogen in plasma and vaginal mucus resulting in lowered fertility. Sperm viability and embryo development is reportedly affected by the presence of excessive urea in the reproductive tract of cows. To reduce the possibility of these negative effects, do not supply rumen degradable proteins in excess of the needs of the cow.

Supplemental Fat

From what is currently known, fat supplementation exerts beneficial effects on reproduction (27). Long chain polyunsaturated fatty acids such as linoleic acid are known to stimulate reproductive function. Other fatty acids that are known to influence reproductive function are linolenic acid, eicosapentaenoic acid and docosahexaenoic acid. Fat supplements that have improved fertility of lactating dairy cows include fish meal, tallow and calcium soaps of palm oil. A recent study (17) reported significantly ($P < 0.05$) higher (89%) non return rates to first insemination in dairy cows that were fed formaldehyde-treated flaxseed compared to cows receiving a diet supplemented with Megalac (59%). Treating oil seeds with formaldehyde is known to protect fatty acids from the rumen environment, thereby allowing an increased delivery of fatty acids in a protected form to the lower gut.

How does fat influence reproduction? Firstly, fat supplementation increases the overall number of ovarian follicles and size of the dominant follicle. Secondly, an increase in plasma cholesterol often occurs following dietary fat supplementation. Since cholesterol is a precursor for progesterone, the increased availability of cholesterol may contribute to an increased concentration of progesterone in plasma. The third mechanism by which dietary fats may enhance reproductive efficiency is via a reduction in endometrial PG secretion. In a recent experiment we found that mean PG secretion in response to estradiol-oxytocin challenge was significantly ($P < 0.01$) lower (93.4 ± 26.3 pg/ml) in lactating Holstein cows receiving Menhaden fish meal diet compared to control (151.4 ± 24.7) cows (9). The peak PG concentrations for the two groups were 170 and 380 pg/ml, respectively. A

decrease in PG secretion will delay CL regression and could potentially increase embryo survival.

Thus, supplementation of fat in the diet of lactating dairy cows holds promise for improving reproductive performance. Other nutritional strategies for improved reproduction have been described recently (26). Copper, magnesium, selenium, phosphorus and calcium, and the Vitamins A, D and E play important roles in regulating normal reproductive function. The role of vitamins and minerals in reproduction has been described in detail by Hurley and Doane (14).

Strategy 9: Maximize cow comfort and minimize environmental effects.

It is needless to say how important the general well-being and comfort of cows are for maximizing reproductive efficiency. From providing an unrestricted quantity of clean drinking water to dry bedding, everything counts. Photoperiod plays a role in the physiological processes associated with the onset of puberty and possibly initiation of cyclicity during the postpartum period (12). Longer photoperiods may hasten uterine involution, reduce postpartum anestrus and shorten calving intervals in cows. While heat stress severely impairs fertility in dairy cows (12), other environmental stressors like extreme cold weather (11, 31), poor air-quality, and stray voltage (3) can affect reproductive performance directly or indirectly. Protect cows from excessive solar radiation by providing adequate shade during summer, and provide shelter from extreme cold during winter months. Stray voltage is known to bring about changes in hormone profiles. Intermittent electrical stimulation amplifies peak oxytocin response. Since oxytocin, a pituitary hormone responsible for milk ejection, also plays an important role in the events leading to PG secretion and CL regression, chronic stray voltage may affect reproduction possibly through premature CL regression. Conscious efforts must be taken to minimize the effects of these stressors as they could significantly affect overall production.

▪ References

1. Ambrose, J.D., M. Drost, R.L. Monson, J.J. Rutledge, M.L. Leibfried-Rutledge, M.-J. Thatcher, T. Kassa, M. Binelli, P.J. Hansen, P.J. Chenoweth and W.W. Thatcher (1997) Timed embryo transfer in heat-stressed dairy cattle: A field trial with IVF-derived embryos. *Journal of Dairy Science* 80 (Suppl 1):239, abstr.
2. Ambrose, J.D., M.F.A. Pires, F. Moreira, T. Diaz, M. Binelli and W.W. Thatcher (1998) Influence of Deslorelin (GnRH-agonist) implant on plasma progesterone, first wave dominant follicle and pregnancy in dairy cattle. *Theriogenology* 50:1157-1170.
3. Appleman R.D., Gustafson R.J. (1985) Source of stray voltage and effect on cow health and performance. *J Dairy Sci* 68:1554-1557.

4. Bailey T. (1997) Strategies for estrus detection to improve dairy reproductive performance. *Proc Soc Theriogenology* 264-273.
5. Bleach EC, Peoros ID, Grewal TS, Shepherd DA, Savva D (1998) Effect of administration of a novel recombinant bovine interferon on length of estrous cycle in cattle. *Res Vet Sci* 64:73-77.
6. Britt J.H., Scott R.G., Armstrong J.D., Whitacre M.D. (1986) Determinants of estrous behavior in lactating Holstein cows. *J Dairy Sci* 69:2195-2202.
7. Burke JM, De La Sota RL, Risco CA, Staples CR, Schmitt E.J-P, Thatcher WW. (1996) Evaluation of timed insemination using a gonadotropin releasing hormone agonist in lactating dairy cows. *J Dairy Sci* 79:1385-1393.
8. Butler W.R., Smith R.D. (1989) Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J Dairy Sci* 72:767-783.
9. Coelho S, J.D. Ambrose, M. Binelli, J. Burke, C.R. Staples, M-J. Thatcher and W.W. Thatcher (1997) Menhaden fish meal attenuates estradiol- and oxytocin-induced uterine secretion of PGF_{2α} in lactating dairy cattle. *Theriogenology* 47:143, abstr.
10. Franco OJ, Drost, M, Thatcher MJ, Shille VM, Thatcher WW (1987) Fetal survival in the cow after pregnancy diagnosis by palpation per rectum. *Theriogenology* 27:631-644.
11. Gwazdauskas F.C. (1985) Effects of Climate on reproduction in cattle. *J Dairy Sci* 68:1568-1578.
12. Hansen PJ (1997) Effects of environment on bovine reproduction *In: Current Therapy in Large Animal Theriogenology*, Ed. R.S. Youngquist. W.B. Saunders Co., Philadelphia, PA, pp 403-415.
13. Heyman Y, Camous S, Fevre J, Meziou W, Martal J (1984) Maintenance of the corpus luteum after uterine transfer of trophoblastic vesicles to cyclic cows and ewes. *J Reprod Fertil* 70:533-540.
14. Hurley W.L., Doane R.M. (1989) Recent developments in the roles of vitamins and minerals in reproduction. *J Dairy Sci* 72:784-804.
15. Nebel RL, McGilliard ML (1993) Interactions of high milk yield and reproductive performance in dairy cows. *J Dairy Sci* 76:3257-3268.
16. Peters and Ball (1995) Reproductive problems in the bull and cow *In Reproduction in cattle* 2nd ed. Blackwell Science, Oxford, p 172.
17. Petit H.V., R.J. Dewhurst, J.G. Proulx, M. Khalid, W. Haresign (1998) Milk yield and reproduction of dairy cows fed saturated or unsaturated fat. *J Dairy Sci* 81 (Suppl 1):302.
18. Plaizier J.C.B., King G.J., Dekkers J.C.M., Lissemore K. (1997) Estimation of economic values of indices for reproductive performance in dairy herds using computer stimulation. *J Dairy Sci* 80:2775-2783.
19. Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL (1997) Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J Dairy Sci* 80:295-300.
20. Pursley JR, Kosorok MR, Wiltbank MC (1997a) Reproductive management of lactating dairy cows using synchronization of ovulation. *J Dairy Sci* 80:301-306.

21. Pursley JR, Silcox RW, Wiltbank MC (1998) Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J Dairy Sci* 81:2139-2144.
22. Raheja K.L., Burnside E.B., Schaeffer L.R. (1989) Relationships between fertility and production in Holstein dairy cattle in different lactations. *J Dairy Sci* 72:2670-2678.
23. Saacke RG (1974) Concepts in semen package and use. *Proc 8th Conf AI Beef Cattle, NAAB*, p15.
24. Saacke RG (1977) Procedures for handling frozen semen. *Proc 12th Conf A.I. Beef Cattle, NAAB*, p 55.
25. Schmitt EJ, Diaz T, Drost M, Thatcher WW (1996) Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. *J Anim Sci* 74:1074-83.
26. Spain J (1998) Reproductive management - a nutritionist=s perspective. *Advances in dairy technology. Proc Western Canadian Dairy Seminar 10*: 57-66.
27. Staples C.R., Thatcher W.W. (1997) Fat supplementation influences reproduction of lactating dairy cows. *Proc 8th Ann Florida Ruminant Nutr Symp, Jan 16-17, Gainesville. P 127-144.*
28. Stevenson JS, Kobayashi Y, Thompson KE (1997) Conception and pregnancy rates in dairy cattle after various programmed breeding systems. *J Dairy Sci* 80 (Suppl 1): 238.
29. Thatcher WW, Wilcox CJ (1973) Postpartum estrus as an indicator of reproductive status in the dairy cow. *J Dairy Sci* 56:608-610.
30. Thatcher WW, Macmillan KL, Hansen PJ, Bazer FW (1994) Embryonic losses: cause and prevention *In: Factors affecting calf crop*, Eds Fields MJ, Sand RS, CRC Press Inc., Boca Raton, FL, pp 135-153.
31. Young B.A. (1983) Ruminant cold stress: effect of production. *J Anim Sci* 57:1601-1607.

