

Maximizing Embryonic and Early Fetal Survival in Dairy Cattle

E. Keith Inskeep and Robert A. Dailey

Division of Animal and Nutritional Sciences, West Virginia University, Morgantown WV
26506-6108

Email: einskeep@wvu.edu

■ Take Home Messages

- ▶ Reproductive failure in lactating dairy cows is a significant limitation to the dairy industry. Embryonic and early fetal losses are major contributors to that limitation.
- ▶ Tweaking regimens for synchronization of estrus is not the answer; simpler breeding protocols with less intensive handling are needed.
- ▶ Attention must be paid to multiple traits in selection programs; selection for milk production only has contributed to the problem. Measures of fertility, health and longevity must be emphasized in future selection programs.
- ▶ Because fertility does not reach a stable point until day 60 and many cows are not cycling earlier, breeding should not be planned until cows are at least 70 days in milk.

■ Introduction

Components of female fertility include ovulation, fertilization and survival of the conceptus through embryonic and fetal development and parturition. Embryonic and early fetal losses contribute significantly to reproductive wastage or inefficiency in dairy cattle. The extent of these losses, which constitute the majority of pregnancy failures in most herds, has been estimated in various ways (e.g., Thatcher et al., 1994; Peters, 1996; Lucy, 2001; Inskeep and Dailey, 2005; Diskin et al., 2006; Rodriguez-Martinez 2008). Inskeep and Dailey (2005) summarized the distribution of pregnancy failures as depicted in Table 1. Numerous authors have looked for causes and means of prevention of these losses. However, solutions offered to date have addressed mainly the methods used for synchronization of estrus and ovulation. Alterations in those methods have involved additional treatments and labor and increased costs of breeding, and have achieved very little or no

increase in conception or pregnancy rates (e.g., Pursley, 2007). In 1980, Allaire and Cunningham estimated that maximum profits for a dairy herd occurred when culling was less than 26%. Evans et al. (2006) reported that only milk price exceeded replacement rate among factors affecting farm profit. In a recent computer simulation, reduced culling rate was preferential to reduced days open (Meadows et al., 2006). That conclusion translates into a requirement for timely and sustained pregnancies.

Table 1. Distribution of Pregnancy Failures in Cattle^a

Timing or Cause	Percentage of Losses
Fertilization failure	
Male factors	10
Ovum transport, etc.	8
Embryonic	57
Lethal genes	5
Rebred by mistake	1
Placentation	
Early	4
Late	12
Fetal	3

^aFrom Inskeep and Dailey (2005).

There has been an increasing recognition that answers are not found in the modification of regimens for synchronization of estrus or ovulation since Pursley raised that question in his talk to this group in 2007. At the Roy Wallace Memorial Symposium at Select Sires in Columbus this past fall (2009), at least four presenters called for simpler schemes for breeding the lactating cow. Further, Rodriguez-Martinez et al. (2008), Kent Weigel of the University of Wisconsin and P. M. Van Raden of the US Dept. of Agriculture have recognized the need for inclusion of measures of reproductive success and fitness as a component of the genetic selection program. They presented data illustrating success of giving greater weight to fertility, health and longevity (productive herd life) in selection strategies.

To set the stage to consider how simpler management and genetic selection might ameliorate some of the problems, this review will first illustrate some of the factors involved in losses. There are four susceptible periods after breeding: (a) the early stages of embryonic cleavage (days 2 to 3), (b) the

morula to blastocyst transition (days 5 through 8), (c) during maternal recognition of pregnancy (days 14 through 18) and (e) during placentation (days 28 to 45).

■ Importance of Progesterone

One causative factor, which is common to each of the four stages mentioned above, is lower concentrations of progesterone at a critical time before or during that stage. Lactating cows had lower progesterone and ovulated larger follicles more frequently than virgin heifers when treated with intravaginal devices containing progesterone (Cooperative Regional Research Project, 1996). Two logical possibilities to explain why progesterone is lower in the lactating dairy cow than in the heifer are that secretion by the corpus luteum is reduced or that metabolism of progesterone is increased. Rhinehart et al. (2008) found no difference in luteal secretion of progesterone in cows with low or high progesterone, but metabolism of injected progesterone was greater in the cows with low progesterone. Feed intake, milk yield, and route of administration of exogenous progesterone influenced metabolism and excretion of progesterone in some studies in lactating dairy cows, but neither feed intake nor metabolizable energy had an effect in others (reviewed by Inskeep, 2004). Metabolic clearance of progesterone was correlated highly with liver blood flow ($r = 0.92$). Based upon the work at Wisconsin (Wiltbank, 2009) and in New Zealand (reviewed by Inskeep, 2004), the elevation in liver blood flow as a result of greater feed intake is the major factor. Liver enzymes involved in steroid metabolism likely are increased as well (Lemley et al., 2008).

Embryonic Losses during Early Cleavage

As proposed originally by Rajakoski (1960), follicular growth occurs in waves, predominantly in two waves per estrous cycle (Townson et al., 2002), as a result of growth of a cohort of follicles. One (occasionally two) follicles in a wave will become the dominant follicle, while the others cease growing and degenerate (Ginther et al., 1996).

Losses Due to Aged Follicles/Oocytes

As early as 1951, Ulberg et al. recognized the larger size of follicles in animals completing treatment with low dosages of progesterone. Frequency of secretion of pulses of luteinizing hormone (LH) from the anterior pituitary is regulated principally by concentrations of progesterone in the blood. When progesterone is low, a high frequency of pulses of LH stimulates continued growth of the dominant follicle, which in turn secretes more estradiol. When progesterone is high, LH pulses are less frequent and fail to support the

dominant follicle; it stops producing estradiol and degenerates. Thus, the dominant follicle cannot ovulate until the corpus luteum regresses. When it stops growing, an increase in secretion of follicle stimulating hormone (FSH) stimulates development of a new cohort of follicles (Adams et al., 1992). The largest follicle that is present when regression of the corpus luteum occurs is the one that ovulates at estrus.

With extensive studies of follicular growth by transrectal ultrasonography, the relationship of lower fertility to larger, older follicles began to be recognized. Breuel et al. (1993b) observed that cows with larger dominant follicles 5 days before the surge of LH had greater pre-ovulatory concentrations of estradiol and a lower conception rate (36%) than cows with smaller follicles at that time (91% conception). Oocytes from larger follicles were likely to be at a more advanced stage of maturation than those from follicles of normal age and size (Mihm et al., 1999; reviewed by Inskeep, 2004) and fertility declined linearly as age of follicle increased (Bleach et al., 2004). Conception rate was lower in lactating dairy cows inseminated when ovulating from the second follicular wave (63%) than in those inseminated when ovulating from the third follicular wave (81%), which might be expected because follicles from the second wave were 1.5 days older at estrus (Townson et al., 2002).

Oocytes from the larger, older follicles were fertilizable, but early development was retarded, and embryonic death usually occurred before the 16-cell stage (Wishart, 1977; Ahmad et al., 1995). The sequence of relationships of more LH, more estradiol, and aged oocytes provided an explanation for lowered fertility when progesterone was low during the natural estrous cycle before breeding (Meisterling and Dailey, 1987) or low dosages of progestogens were used to synchronize estrus. This pattern has been overcome in part by short-term treatment with progesterone in combination with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

Unlike lactating dairy cows, heifers have not lost fertility as shown by Canadian (Bousquet et al., 2004) and Ohio (Rajala-Schultz and Frazer, 2003) studies, likely because they have not been on the high feed intake associated with lactation and have not experienced the increase in metabolism of progesterone and reduced circulating concentrations of progesterone seen in lactating cows. When high feed intake decreases progesterone, then the changes described above lead to ovulation of aged oocytes. Nutritional management will be addressed by others in this seminar, but avoiding metabolic disorders that can delay conception or induce embryonic or fetal loss is very important. Cows bred during the stress of negative energy balance in early lactation will have less success in establishing and maintaining pregnancy.

Losses Due to Premature Ovulation of Follicles

Several authors have found that premature ovulation of follicles is probably as detrimental to fertility as ovulation of aged or persistent follicles. Again, as with delayed ovulation, development to the blastocyst stage was compromised (Mermillod et al., 1999). Perry et al. (2005) observed that follicular diameter affected conception in timed-inseminated beef cows treated with gonadotropin releasing hormone (GnRH), but not in control cows bred in relation to observed estrus. Conception rate, measured at days 25 to 39 after insemination, averaged 72% in control cows compared with 45% in timed-inseminated cows. Much of the difference in fertility was accounted for by the cows with follicles 12 mm in diameter or less in one study, or 11 mm or less in another study. Mussard et al. (2007) examined data from beef cows that were induced to ovulate by treatment with GnRH after withdrawal of progesterone, according to the duration of proestrus (the interval from the drop in progesterone to the induced surge of LH). Conception rates increased from only 4 to 8% when duration of proestrus was only 1 day, to 57 to 76% when proestrus lasted 2 to 3 days. Thus, follicular maturity may be the important variable, and diameter may not reflect maturity as effectively as duration of proestrus.

Other Factors

Finally, health or disease may be important factors in early losses. Both Dailey (2008) and Rodriguez-Martinez et al. (2008) pointed to factors such as dystocia, stillbirth and uterine infection in delays of breeding and early embryonic losses. Mastitis reduced pregnancy rate in cows infected soon after insemination (Barker et al., 1998). Stewart et al. (2003) attempted to determine the specific cause in experimentally-infected ewes. Neither increased body temperature, increased secretion of PGF₂ α , LH, estrogen, or cortisol, nor decreased progesterone could be shown individually to account for the effects, so it is likely a combination of these factors. Conception rates of ewes decreased with increasing exposure to the pathogens in the study by Holásková et al. (2004).

Embryonic Death Associated with Short Luteal Phases: the Morula to Blastocyst Transition

Two situations have been reported in which embryonic death is related to the occurrence of shortened luteal phases. The first is in the postpartum cow or newly pubertal heifer, in which the first ovulation is followed usually by a short luteal phase. If the cow showed estrus and was inseminated, fertilization rate was near normal, but the embryos died during luteal regression on days 5 through 9 in a series of studies at West Virginia, Missouri and Tennessee, (reviewed by Inskeep, 2004). Replacement therapy with progesterone was

ineffective without further treatment. Additional experimentation revealed that an early increase in secretion of $\text{PGF}_2\alpha$ not only caused regression of the corpus luteum, but also $\text{PGF}_2\alpha$ was toxic to the embryo. Treatment with an inhibitor of prostaglandin synthesis together with removal of the regressing corpus luteum saved a significant portion of the embryos in animals receiving progesterone. The toxic effect of $\text{PGF}_2\alpha$ was limited to a specific window of time after breeding, the period of morula to blastocyst transition. It did not occur in cows treated with $\text{PGF}_2\alpha$ at later stages after insemination. At later stages, progesterone protected the embryo even though the corpus luteum regressed.

The second situation was in cows in which duration of proestrus was shortened by treatment with GnRH. Ohio workers found that life span of the corpus luteum in beef cows was less than 12 days in 74% of 38 animals in which duration of proestrus averaged only 1.3 days before induced ovulation, compared with only 30% of 40 animals in which duration of proestrus averaged 2.3 days (Mussard et al., 2007; Bridges et al, 2010). Cordoba and Fricke (2002) had found similar short cycles in 51% of 49 lactating dairy cows that returned to service after timed insemination on an OvSynch program. Based upon studies at Missouri (Kieborz-Loos et al., 2003), prevention of the early secretion of $\text{PGF}_2\alpha$ in response to the post-ovulatory rise in progesterone required a sequence of pre-ovulatory exposure to progesterone and estrogen. Thus, if follicular maturation, and hence exposure of the oocyte to estrogen, was inadequate, premature secretion of $\text{PGF}_2\alpha$ could have been involved in this case as well.

The extent of losses due to embryotoxic effects of $\text{PGF}_2\alpha$ is not clear. But considering that oxytocin is released during milking or by uterine manipulation and is injected to cause milk letdown in lactating dairy cows, and that secretion of $\text{PGF}_2\alpha$ is increased by oxytocin or by uterine manipulation (Roberts et al., 1975), the potential for loss by this mechanism is significant.

Practical Application

Knowledge gained from the basic studies of embryotoxic effects of $\text{PGF}_2\alpha$ has led to development of applications in the embryo transfer industry. Direct embryotoxic effects of $\text{PGF}_2\alpha$ had been observed *in vitro* in rat (Breuel et al., 1993a) and bovine (Fazio and Schrick, 1997) embryos. Hockett et al. (2004) found that treatment with $\text{PGF}_2\alpha$ on days 5 through 8 delayed or stopped development of bovine embryos recovered on day 8. Whereas embryos from control cows were expanded blastocysts (80%) or blastocysts (20%) on day 8, only 29% of embryos from $\text{PGF}_2\alpha$ -treated animals reached the expanded blastocyst stage, and 64% were still in the morula stage.

Manipulation of the reproductive tract during embryo transfer increased

secretion of $\text{PGF}_2\alpha$ (Scenna et al., 2005). These workers examined the concept that inhibition of uterine secretion of $\text{PGF}_2\alpha$ could increase success of embryo transfers in a large study involving 2097 cattle. A single embryo was transferred non-surgically seven days after synchronized estrus; 1300 animals received flunixin meglumine treatment and 797 received no treatment. Overall pregnancy rates of treated cattle (65%) were greater than in controls (60%; $p < 0.02$), but the beneficial effect was seen mainly in animals that received embryos of quality grade 2 (fair) with pregnancy rates of 65 vs 54% in treated vs control groups. Pregnancy rates for both morula- and blastocyst-stage embryos were greater in treated than in control cows. Subsequently, Scenna et al. (2006) showed that there are receptors for $\text{PGF}_2\alpha$ (both mRNA transcripts and protein) on bovine embryos at the morula and blastocyst stages, explaining the observed effects of $\text{PGF}_2\alpha$ at those stages.

The discovery of receptors for $\text{PGF}_2\alpha$ on the embryo opened the possibility for new strategies to prevent detrimental effects of $\text{PGF}_2\alpha$ during early embryonic development. Indeed, Scenna et al. (2008a,b) showed that an antagonist to the $\text{PGF}_2\alpha$ receptor inhibited detrimental effects of $\text{PGF}_2\alpha$ on development of embryos and improved development of morulae *in vitro* to the blastocyst stage in the presence of $\text{PGF}_2\alpha$ by 13 percentage points. Finally, in two small trials, embryos were collected in a medium containing the receptor antagonist or a control medium and transferred either fresh or as frozen/thawed embryos (Scenna et al., 2008b). In each case, pregnancy rates were improved (frozen; 45% vs. 34%; $P < 0.01$) or tended to be improved (fresh; 76% vs. 66%; $P < 0.09$). Thus, a method has been developed to incorporate protection into the embryo transfer process without the need for systemic injections into the recipients. Improvement of pregnancy rates to embryo transfer procedures has significant economic implications for that industry. Similar measures to control inappropriate secretion of $\text{PGF}_2\alpha$ might be effective in cows inseminated artificially.

Maternal Recognition of Pregnancy

The ratio of estrogen to progesterone during maternal recognition of pregnancy and the ability of the embryo to secrete interferon tau, which is critical to maintenance of the corpus luteum, are important factors in embryonic survival during the period beginning around day 14 after breeding. The concept that an excess of estrogen interfered with maternal recognition of the embryo was originally presented by Macmillan et al. (1986). In subsequent studies (e.g. Thatcher et al. 1989), ovulation or atresia of the largest follicle during the mid-luteal phase sometimes increased pregnancy rate. Our attention was directed to the maternal recognition period during studies of survival of embryos when transferred into postpartum beef cows with short luteal phases that were supplemented with progestogens (Butcher et al., 1992). Low dosages of progestogen allowed large follicles to develop in

the ovaries during days 12 to 20, and embryos transferred into these cows on day 7 failed to survive. With higher dosages in subsequent studies, the large follicles did not develop and embryonic survival was increased. Even short periods of deprivation of progesterone can decrease embryo survival during the maternal recognition period (reviewed by Inskeep, 2004).

Concentrations of progesterone rose at a slower rate in cows with smaller follicles at induced ovulation than in cows with larger follicles. Thus, embryos might have been less advanced (Garrett et al., 1988) and produced less interferon tau (Kerbler et al., 1997; Mann et al., 1999) in the cows that ovulated small follicles. In addition, luteal function was lower on days 8 through 14 in cows with normal luteal life span, when proestrus was shortened by earlier preovulatory treatment with GnRH (Mussard et al., 2007; Bridges et al., 2010), as is done in the OvSynch protocol.

Pritchard et al. (1994) studied the association of embryonic loss with excessive secretion of estrogen during maternal recognition of pregnancy in 100 lactating beef cows. Conception rate to first service by artificial insemination declined from 77 to 42%, as concentration of estradiol increased during days 14 to 17 after breeding. Ahmad et al. (1997) found that fewer beef animals conceived among those that had two (70%) rather than three (96%; $P < 0.05$) waves of follicular development during the equivalent of one estrous cycle after insemination. However, concentrations of estradiol in peripheral blood were not greater on day 14 after estrus in animals with two waves than in those with three waves.

A clear association of pregnancy loss during maternal recognition with greater circulating estradiol exists in some cases. However, neither the exact timing of an estradiol effect nor the mechanism by which estradiol may interfere with the maternal recognition process or the developing embryo has been established. Either GnRH or human chorionic gonadotropin has been used to ovulate or luteinize large follicles during this stage after breeding. However, response has varied considerably, and one cannot conclude that these treatments are routinely valuable (Lewis et al., 1990; Hernandez-Cerón et al., 2001).

Late Embryonic/Early Fetal Mortality in Lactating Dairy Cows during Placentation

Embryonic mortality after maternal recognition of pregnancy, during and immediately after placentation is a significant problem in the dairy cow. With some protocols for synchronization of estrus designed to regulate follicular development, it has been possible to time artificial insemination, without detection of estrus, and obtain small improvements in initial rates of pregnancy (viable embryos imaged ultrasonographically at 25 to 32 d after timed insemination) compared to previous systems. However, high

frequencies of late embryonic and early fetal losses have been observed in lactating dairy cows during attachment of the embryo in the uterus and placentation, between days 28 and 45 (reviewed by Inskeep and Dailey, 2005). Estimates of late embryonic or early fetal death ranged as high as 40%, but averaged between 10 and 15%. An exception is the greater rates of loss in heat stressed lactating dairy cows in Florida, 65% in cows inseminated artificially and 41% in cows with transferred embryos (Drost et al., 1999).

The observations by Perry et al. (2003, 2005) in beef cows led to consideration of the possibility that follicle size in cows timed-inseminated after GnRH might affect late embryonic losses, as well as early losses discussed above. Thirteen of 43 timed-inseminated cows with small follicles (≤ 11 mm) were pregnant at day 27, but 5 of the 13 (39%) lost pregnancy by day 68, whereas no late losses occurred in 57 pregnant cows with larger follicles (overall loss 6.7%). In cows inseminated at estrus, 3 of 127 (2.4%) lost pregnancies from first to second diagnosis, but their follicle sizes were ≥ 12 mm. These data illustrate the necessity to sample large numbers of cows to elucidate causes of late embryonic mortality. In contrast to lactating dairy cows, late embryonic/early fetal losses in beef cows and heifers usually have been low (2 to 4%) and have not exceeded 8% (Inskeep and Dailey, 2005).

Reports discussed above were based upon dairy cows inseminated at predetermined times, without detection of estrus. Starbuck et al. (2004) studied lactating dairy cows and heifers that were inseminated approximately 12 h after observed onset of estrus or bred naturally. Eleven percent (11%) of 211 animals that were pregnant at ultrasonography during the fifth week after breeding (day 28 to 36) had lost the pregnancy by week 9, with 65% of those losses having occurred by week 7. In lactating Holstein cows in California, Cerri et al. (2003) found no difference in loss rates from 30 to 58 days of gestation between timed-inseminated cows (11%) and those inseminated at detection of estrus (12.4%). Losses in heifers were consistently lower. The problem appears to be most prevalent or of greater magnitude in lactating dairy cows.

In reports reviewed by Inskeep and Dailey (2005), late embryonic death usually preceded luteal regression. However, the possibility that luteal function was compromised before embryos were lost was not ruled out by that observation. They also reviewed a series of studies in which it was shown that low progesterone during days 28 to 37 of gestation was predictive of greater late embryonic and early fetal losses. Any association with estradiol was equivocal from one study to another. Starbuck et al. (2004) found that retention of pregnancy from weeks 5 to 7 was associated positively with concentrations of estradiol at week 5, but retention to week 9 showed no association. In cows with replacement corpora lutea induced during placentation (days 28 to 31 of gestation; Bridges et al., 2000), there was a tendency for more pregnancies to continue when concentrations of estradiol

on days 31 through 35 were lower.

■ Heritability of Fertility Traits

Estimates of heritability of various measures of fertility in cattle have been low (10% or less). In most cases, this has meant that fertility has been ignored in selection programs. In fact, most Holstein herds have been selected largely for the single trait, milk production, with occasional emphasis on classification score. However, one of the authors completed his M.S. degree program with a study of the inheritance of conception rate at first service in Holsteins bred to high fertility AI sires (Inskeep et al., 1961). In that study, conception was defined as birth of a live calf and averaged 67%. A high fertility sire had a 60- to 90-day non-return rate in the upper $\frac{2}{3}$ of the bulls in the study, and the non-return rate for the selected service sires averaged 73%. Heritability of conception rate to first service, estimated from intra-sire correlations of paternal half-sib groups, was approximately 8.5%. Collins et al. (1962) completed a similar study in Guernseys, and depending upon method of estimation, heritability estimates ranged from 2 to 10%.

Inskeep et al. (1961) went on to estimate expected gains in conception rate of future daughters of selected sires whose previous daughters had high conception rates compared to daughters of all sires. If one were to select daughters of bulls in the upper 25% of sires for daughter's conception rate, based upon 20 previous daughters, the expected advantage would be 4.7%. If the difference in conception rate of 20 previous daughters of each of two bulls was 20%, their future daughters could be expected to differ in conception rate to first service by 6%. Selection programs based upon conception rate to first service have not been implemented, but some schemes now utilize daughter pregnancy rate.

In a recent review, Rodriguez-Martinez et al. (2008) have examined causes and effects of low reproductive performance in dairy cattle. They looked at relationships to milk yield, and trends over time in fertility and calving interval. They noted that daughter fertility index for Holsteins in Sweden had declined since 1985, but in imported Holsteins, that index was already low in 1985. They pointed to higher conception rates in Swedish Red cattle than in Swedish Holsteins.

Bamber et al. (2009) summarized studies in 9 herds utilizing OvSynch protocols with a total of 3,775 cows. In those herds, there was an average pregnancy loss of 14.4% between about 30 and 60 days after timed AI. Losses within herds varied from 7.6 to 21.6%. They estimated the heritability of pregnancy loss (from a sire-maternal grandsire model) as 0.49. Thus selection for survival after 30 days should be an effective tool.

Finally, Khatib et al. (2008) have found that a specific gene, known as signal transducer and activator of transcription 5A (*STAT5A*), was associated with sperm factors that cause low fertilization rate *in vitro*, and with death rates of *in vitro* fertilized embryos, as well as with milk composition in Holstein dairy cattle. It appeared that selection for heterozygotic females could promote both successful pregnancy and production.

■ Other Factors

Rodriguez-Martinez et al. (2008) identified several other risk factors for fertility, including negative energy balance, dystocia, retained placenta, twinning, stillbirths, and endometritis. Negative energy balance was exacerbated by overfeeding pre-partum and they attributed much of the variation in body condition to the feeding of total mixed rations during the dry period. Inappropriate body condition at calving contributed to metabolic disorders that can affect not only the proportion of anestrous cows, but also conception rate and embryonic loss. The combination of high milk yield and high feed intake during lactation has been shown to increase steroid metabolism, as discussed above in relation to persistent follicles and aged oocytes. Rodriguez-Martinez et al. (2008) emphasized that the male contributes to the ability of the embryo to survive throughout pregnancy, as well as to fertilization rate.

■ Conclusions: Possible Solutions to Low Fertility and Specifically to Reducing Embryonic Losses – Recommendations

Multiple and complex factors are involved in embryonic and early fetal losses, therefore no single simple solution will suffice. However, possibly helpful approaches can be identified from the literature reviewed and other studies.

Selection

The time has come to include more or direct measures of fertility traits in selection programs for dairy cattle. Rodriguez-Martinez et al. (2008) emphasized this need in their review, stating “despite the low heritability of fertility and health traits, there is yet a significant genetic variation and thus a chance to balance the genetic gain for production and animal health and reproduction.” They illustrated gains made by increasing the weight given to fertility traits in the selection program in Scandinavian data. Conception rate trends in Swedish Red cattle were less negative than those in Swedish Holsteins, apparently at least in part because of fewer imports and greater

emphases on health and reproduction in the Swedish Red. Based upon their summary and the earlier reports from Wisconsin on conception rate to first service cited above, the first recommendation is: ***Institute a selection program that includes measures of fertility and health traits and give significant weight to these traits.*** Importation of Swedish semen might be a way to achieve progress more rapidly, because bulls with superior daughter fertility index have been identified in their selection scheme. The bulls used as service sires should have high fertility as well.

Estrus

Rodriguez-Martinez et al. (2008) compiled data on changes over time in duration of estrus; more recent values showed a shorter duration, but not as short in Swedish Holsteins as reported in American Holsteins. Although studies in American Holsteins were limited to a single institution (Dransfield et al., 1998), it is not surprising that duration of estrus would change if ignored in selection programs. Rottensten and Touchberry (1957) reported a heritability of 21% for that trait in Red Danish, Black and White, and Jersey breeds studied in Denmark. Animals must exhibit a detectable estrus; this trait not only allows proper timing of insemination, but also indicates appropriate maturation of the oocyte and readiness of the reproductive tract to establish pregnancy. Thus the second recommendation is: ***Include strength and duration of estrus as a criterion in the selection program.***

The third recommendation is: ***Complex protocols for synchronization of estrus and ovulation, with multiple injections of a plethora of drugs or hormones, must be abandoned.*** They can be replaced by simpler systems that require less frequent handling and less labor, but have the potential to reduce embryonic and early fetal losses. A scheme has been developed, using only progesterone (via CIDR) and PGF₂α in an approved combination, in which cows are inseminated only in relation to detected estrus. Groups of cows rather than individuals are treated, so that observation for estrus is required for only 3 weekdays in each 3-week period and all treatments and inseminations occur on weekdays (Table 2). Inseminated cows are resynchronized with CIDR only. Preliminary tests of the scheme in two herds showed promise for increases in cows inseminated, and in conception and pregnancy rates. This scheme avoids premature ovulation of follicles that occurs with timed breeding protocols, and minimizes occurrence of persistent follicles by keeping duration of progesterone treatment to 7 days.

Timing of Breeding Season

The fourth recommendation is: ***Do not initiate breeding until cows are at least 70 days in milk.*** The literature shows that conception rates in lactating dairy cows do not reach maximum until 60 to 90 days postpartum (Table 3). That recommendation is utilized in the simple program described above and avoids wasting semen in cows expected to have lower fertility.

Table 2. Simple synchrony and re-synchrony program

Week	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1			Insert CIDR				
2		Inject PGF	Pull CIDR	Heat detect and Breed	Heat detect and Breed	Heat detect and Breed	
3							
4			Insert CIDR				
5			Pull CIDR	Heat detect and Breed	Heat detect and Breed	Heat detect and Breed	

Expected heat response after first CIDR should be ~70% or greater.

1. *Recommend that cows should be at least 70 days in milk before start of the program.* Cows earlier than that can show heat, but conception rate does not reach a maximum until 60-90 days. Thus, many more cows inseminated earlier than 70 days will not conceive as compared to cows first inseminated later.
2. *Breed only cows that show heat.* Use tail paint and numerous checks for heat. Heavy milking cows show shorter heat periods.
3. *Breed within 12 hours of observing heat.* It is better to breed before the 12 hours have ended than to wait on strictly the am-pm rule. For example, if you are breeding at 6 am and 6 pm, breed any cow in heat during the night at 6 am and any cow in heat during the day at 6 pm that day.
4. *Fertility will be same as normal rate without synchronization treatment.* For example, if conception rate in the herd is 35%, it will be similar to that.

Expected heat response after second CIDR should be ~45%.

1. *Cows that were not in heat after first CIDR should show heat.* If a cow does not show heat after either CIDR, evaluate her for potential culling.
2. *Cows not pregnant to the breeding after the first CIDR should show heat.* A proportion of the inseminated cows will have conceived but lose the pregnancy within the length of a normal estrous cycle.
3. *Cows pregnant to the first insemination should not show heat.* However, a proportion of these cows will not remain pregnant until pregnancy check.
4. *Fertility will be same as normal rate without synchronization treatment.*

Overall, ~85% of the cows should have been inseminated at least once in this program.

Table 3. Unweighted means for conception rates (CR%) to first service following parturition in dairy cattle, summarized by 30-day periods.

	Intervals following parturition (days)					
	<30	31-60	61-90	91-120	121-150	151-180
CR%	39	53	62	62	65	64

¹Compiled by Casida et al. (1968) from five published studies.

■ Acknowledgements

The authors' personal research in this area has been supported by a series of grants from USDA-NRICGP and by Hatch, Hatch Multistate and CSREES Animal Health Funds of the West Virginia Agricultural and Forestry Experiment Station. We thank numerous graduate students, postdoctoral trainees, and cooperating producers for assistance in the conduct of these studies.

■ References

- Adams, G.P., R.L. Matteri and O.J. Ginther. (1992) Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. *J. Reprod. Fertil.* 96:627–640.
- Ahmad, N., F.N. Schrick, R.L. Butcher, and E.K. Inskeep. (1995) Effect of persistent follicles on early embryonic losses in beef cows. *Biol. Reprod.* 52:1129–1135.
- Ahmad, N., E.C. Townsend, R.A. Dailey and E.K. Inskeep, (1997) Relationships of hormonal patterns and fertility to occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. *Animal Reprod Sci.*49: 13-28,.
- Allaire, F.R. and E.P. Cunningham. (1980) Culling on low milk yields and its economic consequences for the dairy herd. *Livestock Prod. Sci.* 7:349-359.
- Bamber, R.L., G.E. Shook, M.C. Wiltbank, J.E.P. Santos, and P.M. Fricke. (2009) Genetic parameters for anovulation and pregnancy loss in cattle. *J Dairy Sci.* 92:5739–5753.
- Barker, A.R., F.N. Schrick, M.J. Lewis, H.H. Dowlen and S.P. Oliver. (1998) Influence of clinical mastitis during early lactation on reproductive performance of Jersey cows. *J. Dairy Sci.* 81:1285-1290.
- Bleach, E.C.L., R.G. Glencross and P.G. Knight. (2004) Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous oestrous cycles. *Reproduction*;127:621–629.

- Bousquet, D., E. Bouchard and D. Dutremblay. 2004. Decreasing Fertility in Dairy Cows: Myth or Reality? Proceedings: 23rd World Buiatrics Congress, Quebec, Canada July 11-16, 2004. Available on line <http://www.ivos.org/proceedings/wbc/wbc2004/WBC2004-Bousquet-ang-simple1.pdf>. Accessed August 26, 2008.
- Breuel, K.F., A. Fukuda, and F.N. Schrick. (1993a). Effects of prostaglandin $F_2\alpha$ on development of 8-cell rat embryos *in vitro*. Biol. Reprod. 48(Suppl. 1):173 (Abstr.).
- Breuel, K.F., P.E. Lewis, F.N. Schrick, A.W. Lishman, E.K. Inskeep and R.L. Butcher. (1993b) Factors affecting fertility in the postpartum cow: role of the oocyte and follicle in conception rate. Biol. Reprod. 48:655–661.
- Bridges, G.A., M.L. Mussard, C.R. Burke and M.L. Day. (2010) Influence of the length of proestrus on fertility and endocrine function in female cattle. Anim. Reprod. Sci. 117:208-215.
- Bridges, P.J., D.J. Wright, W.I. Buford, N. Ahmad, H. Hernandez-Fonseca, M.L. McCormick, F.N. Schrick, R.A. Dailey, P.E. Lewis and E.K. Inskeep. (2000) Ability of induced corpora lutea to maintain pregnancy in beef cows. J. Anim. Sci. 78:2942-2949.
- Butcher, R.L., J.E. Reber, A.W. Lishman, K.F. Breuel, F.N. Schrick, J.C. Spitzer and E.K. Inskeep. (1992) Maintenance of pregnancy in postpartum beef cows that have short-lived corpora lutea. J. Anim. Sci. 70:3831-3837.
- Casida, L.E., W.E. Graves, E.R. Hauser, J.W. Lauderdale, J.W. Riesen, S. Saiduddin and W.J. Tyler. (1968) Studies on the postpartum cow. Res. Bull. 270, Univ. Wisconsin, Madison.
- Cerri, R.L.A., K.N. Galvao, S.O. Juchem, R.C. Chebel, and J.E.P. Santos. (2003) Timed AI (TAI) with estradiol cypionate (ECP) or insemination at detected estrus in lactating dairy cows. J. Anim. Sci. 81(Suppl.1):181.
- Collins, W.E., E.K. Inskeep, W.J. Tyler and L.E. Casida. (1962) Variation in conception rates of Guernsey cattle. J. Dairy Sci. 45:1234-1236.
- Cooperative Regional Research Project, NE-161. (1996) Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. J. Anim. Sci. 74:1943–1952.
- Cordoba, M.C., and P.M. Fricke. (2002) Initiation of the breeding season in a grazing-based dairy by synchronization of ovulation. J. Dairy Sci. 85:5739-5753.
- Dailey, R.A.(2008) Insemination success and the problem of early embryonic death. Proceedings NAAB 22nd Technical Conference on Artificial Insemination and Reproduction. pp 94-102.
- Diskin, M.G., J.J. Murphy and J.M. Sreenan. (2006) Embryo survival in dairy cows managed under pastoral conditions. Anim. Reprod. Sci. 96: 297-311.
- Dransfield, M.B.G., R. L.Nebel, R.E. Pearson and L.D. Warnick (1998) Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system, J. Dairy Sci. 81:1874-1882.

- Drost M., J.D. Ambrose, M.J. Thatcher, C.K. Cantrell, K.E. Wolfsdorf, J.F. Hasler and W.W. Thatcher. (1999) Conception rate after artificial insemination or embryo transfer in lactating dairy cows during summer in Florida. *Theriogenology* 52:1161-1167.
- Evans, R.D., M. Wallace, L. Shalloo, D.J. Garrick and P. Dillon. (2006) Financial implications of recent declines in reproduction and survival of Holstein-Friesian cows in spring-calving Irish dairy herds. *Agricultural Systems* 89:165-183.
- Fazio R. and F N. Schrick. (1997) Effect of elevated prostaglandin $F_{2\alpha}$ during *in vitro* culture of blastocyst stage bovine embryos. *Biol. Reprod.* 56(Suppl 1):187. (Abstr.).
- Garrett, J.E., R.D. Geisert, M.T. Zavy, L.K. Gries, R.P. Wettemann and D.S. Buchanan. (1988) Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J Reprod Fertil* 84:437-446.
- Ginther, O.J., M.C. Wiltbank, P.M. Fricke, J.R. Gibbons and K. Kot. (1996) Selection of the dominant follicle in cattle. *Biol. Reprod.* 55:1187-1194.
- Hernandez-Cerón, J. and J.S. Morales-Roura. (2001) Falla en la concepción en el ganado lechero: Evaluación de teapias hormomales. *Vet. Mex.* 32:279-287.
- Hockett, M.E., N.R. Rohrbach and F.N. Schrick. 2004. Alterations in embryo development in progestogen-supplemented cows administered prostaglandin $F_{2\alpha}$. *Prostaglandins Other Lipid Mediat.* 73:227-236.
- Holásková, I., G.S. Lewis, M. Elliott, K.P. Blemings and R.A. Dailey. (2004) Effect of peptidoglycan-polysaccharide complex on reproductive efficiency in sheep. *J. Reprod. Immunol.* 52:197-203.
- Inskeep E.K. (2004) Preovulatory, postovulatory, and post-maternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *J. Anim. Sci.* 82(Suppl E):E24-39.
- Inskeep, E.K. and R.A. Dailey. (2005) Embryonic death in cattle. *Vet. Clin. Food Anim.* 21:437-461.
- Inskeep, E.K., W.J. Tyler and L.E. Casida. (1961) Hereditary variation in conception rate of Holstein-Friesian cattle. *J. Dairy Sci.* 44:1857-1862.
- Kerbler, T.L., M.M. Buhr, L.T. Jordan, K.E. Leslie and J.S. Walton. (1997) Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. *Theriogenology* 47:703-714.
- Khatib, H., R.L. Monson, V. Schutzkus, D.M. Kohl, G.J.M. Rosa, and J.J. Rutledge (2008) Mutations in the *STAT5A* gene are associated with embryonic survival and milk composition in cattle. *J. Dairy Sci.* 91:784-793.
- Kieborz-Loos, K.R., H.A. Garverick, D.H. Keisler, S.A. Hamilton, B.E. Salfen, R.S. Youngquist and M.F. Smith. (2003) Oxytocin-induced secretion of prostaglandin $F_{2\alpha}$ in postpartum beef cows: Effects of progesterone and estradiol-17 β treatment. *J. Anim. Sci.* 81:1830-1836.

- Lemley C.O., S.T. Butler, W.R. Butler and M.E. Wilson. (2008) Short communication: insulin alters hepatic progesterone catabolic enzymes cytochrome P450 2C and 3A in dairy cows. *J. Dairy Sci.* 91: 641-645.
- Lewis, G.S., D.W. Caldwell, C.E. Rexroad, Jr., H.H. Dowlen and J.R. Owen. (1990) Effects of gonadotropin-releasing hormone and human chorionic gonadotropin on pregnancy rate in dairy cattle. *J. Dairy Sci.* 73:66-72.
- Lucy, M.C. (2001) Reproductive loss in high producing dairy cattle: where will it end? *J. Dairy Sci.* 84(6):1277-93.
- Mann G.E., G.E. Lamming, R.S. Robinson and D.C. Wathes. (1999) The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. *J. Reprod. Fertil. (Suppl 54):*317-328.
- Macmillan, K.L., V.K. Taufa and A.M. Day. (1986) Effects of an agonist of gonadotropin releasing hormone (Buserelin) in cattle. III. Pregnancy rates after a post-insemination injection during metoestrus or dioestrus. *Anim. Reprod. Sci.* 11:1-10.
- Meadows, C., P.J. Rajala-Schultz and G.S. Frazer. 2005. A spreadsheet-based model demonstrating the nonuniform economic effects of varying reproductive performance in Ohio dairy herds. *J. Dairy Sci.* 88:1244-1254.
- Meisterling, E.M. and R.A. Dailey. (1987) Use of concentrations of progesterone and estradiol-17 β in milk in monitoring postpartum ovarian function in dairy cows. *J. Dairy Sci.* 70:2154-2161.
- Mermillod, P., B. Oussaid, and Y. Cognie. (1999) Aspects of follicular and oocyte maturation that affect the developmental potential of embryos. *J. Reprod. Fertil. Suppl.* 54:449-460.
- Mihm, M., N. Curran, P. Hyttel, P.G. Knight, M.P. Boland and J.F. Roche. (1999) Effect of dominant follicle persistence on follicular fluid oestradiol and inhibin and on oocyte maturation in beef heifers. *J. Reprod. Fertil.* 116:293-304.
- Mussard, M.L., C.R. Burke, E.J. Behlke, C.L. Gasser and M.L. Day (2007) Influence of premature induction of a luteinizing hormone surge with gonadotropin-releasing hormone on ovulation, luteal function and fertility in cattle. *J. Anim. Sci.* 85: 937-943.
- Perry, G.A., M.F. Smith, M.C. Lucy, A.J. Roberts, M.D. MacNeil and T.W. Geary. (2003) Effect of ovulatory follicle size at time of GnRH injection or standing estrus on pregnancy rates and embryonic/fetal mortality in beef cattle. *Proceedings, Western Section, American Society of Animal Science* 54:281-284.
- Perry, G.A., M.F. Smith, M.C. Lucy, J.A. Green, T.E. Parks, M.D. McNeil, A.J. Roberts and T.W. Geary. (2005) Relationship between follicle size at insemination and pregnancy success. *Proc. Nat. Acad. Sci.* 102:5268-5273.
- Peters, A.R. (1996) Embryo mortality in the cow. *Animal Breeding Abstracts* 64: 587-598.

- Pritchard, J.Y., F.N. Schrick and E.K. Inskeep. (1994) Relationship of pregnancy rate to peripheral concentrations of progesterone and estradiol in beef cows. *Theriogenology* 42:247–259.
- Pursley, R. (2007) Profitable reproductive management of high producing dairy herds. *WCDS Advances in Dairy Technology* 19:187-200. Accessed at: <http://www.wcds.afns.ualberta.ca/Proceedings/2007/Manuscripts/Richard2.pdf>
- Rajakoski, E. (1960) The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical, and left-right variations. *Acta Endocrinol. Suppl.* 52:7–68.
- Rajala-Schultz, P.J. and G.S. Frazer. 2003. Reproductive performance in Ohio dairy herds in the 1990s. *Anim. Reprod. Sci.* 76:127-142.
- Rhinehart, J.D., M.J. Starbuck-Clemmer, J.A. Flores, R.A. Milvae, J. Yao, D.H. Poole and E.K. Inskeep. (2008) Low peripheral progesterone and late embryonic/early fetal loss in suckled beef and lactating dairy cows. *Theriogenology* 71:480–490.
- Roberts, J.S., B. Barcikowski, L. Wilson, Jr., R.C. Skarnes and J.A. McCracken. (1975) Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus. *J. Steroid Biochem.* 6:1091-1097.
- Rodriguez-Martinez H., J. Hultgren, R. Båge, A-S. Bergqvist, C. Svensson, C. Bergsten, L. Lidfors, S. Gunnarsson, B. Algers, U. Emanuelson, B. Berglund, G. Andersson, M. Håård, B. Lindhé, H. Stålhammar and H. Gustafsson (2008). Reproductive performance in high-producing dairy cows: can we sustain it under current practice? In: *IVIS Reviews in Veterinary Medicine*, I.V.I.S. (Ed.). International Veterinary Information Service, Ithaca NY (www.ivis.org), Last updated: 12-Dec-2008; R0108.1208 (Open Journal).
- Rottensten, K. and R.W. Touchberry. (1957) Observations on the degree of expression of estrus in cattle. *J. Dairy Sci.* 40:1457-1465.
- Scenna, F.N., J.L. Edwards, G.M. Pighetti and F.N. Schrick. (2006) Presence of prostaglandin $F_{2\alpha}$ receptor in *in vitro*-derived morula and blastocyst stage bovine embryos. *Reprod., Fertil. Dev.* 18:180 (Abstr.).
- Scenna, F.N., J.L. Edwards and F.N. Schrick. (2008a). Detrimental effects of prostaglandin $F_{2\alpha}$ on *in vitro* embryo development in bovine are inhibited by a receptor antagonist. *Reprod. Fertil. Dev.* 20:153-4 (Abstr.).
- Scenna, F.N., J.L. Edwards, G.M. Schuenemann, D.A. Roper and F.N. Schrick. (2008b). Pregnancy rates of recipient animals following application of a selective prostaglandin $f_{2\alpha}$ receptor antagonist during Starbuck embryo recovery. *Reprod., Fertil. Dev.* 20:154 (Abstr.).
- Scenna, F.N., M.E. Hockett, T.M. Towns and F.N. Schrick. (2005) Influence of a prostaglandin synthesis inhibitor administered at embryo transfer on pregnancy rates of recipient cows. *Prostaglandins Other Lipid Mediat.* 78:38-45.

- Starbuck, M.J., R.A. Dailey and E.K. Inskeep. (2004) Factors affecting retention of early pregnancy in cattle. *Anim. Reprod. Sci.* 84:27-39.
- Stewart, A.B., E.K. Inskeep, E.C. Townsend and R.A. Dailey. (2003) Effects of gram-positive bacterial pathogens on reproductive efficiency of ewes: Peptidoglycan as a potential mediator of interruption of early pregnancy. *Reproduction* 125: 295-299.
- Thatcher, W.W., K.L. Macmillan, P.J. Hansen, and M. Drost. (1989) Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology* 31:149-164.
- Thatcher, W.W., C.R. Staples, G. Danet-Desnoyers, B. Oldick, and E-P. Schmitt. (1994) Embryo health and mortality in sheep and cattle. *J. Anim. Sci.* 72(Suppl. 3):16-30.
- Townson, D.H., P.C.W. Tsang, W.R. Butler, M. Frajblat, L.C. Griel, Jr., C.J. Johnson, R.A. Milvae, G.M. Niksic, and J.L. Pate. (2002) Relationship of fertility to ovarian follicular waves before breeding in dairy cows. *J. Anim. Sci.* 80:1053–1058.
- Ulberg, L.C., R.E. Christian, and L.E. Casida. (1951) Ovarian response of heifers to progesterone injections. *J. Anim. Sci.* 10:752–759.
- Wiltbank, M.C. (2009) Interaction of hormones and nutrition on reproductive efficiency of dairy cows. Proceedings 2009 Dairy Cattle Nutrition Workshop Nov 11-12 Grantville, PA. <http://www.das.psu.edu/research-extension/dairy/nutrition/pdf/nutrition-workshop-proceedings-2009.pdf>. accessed Dec 28, 2009.
- Wishart, D.F. (1977) Synchronization of oestrus in heifers using steroid (SC 5914, SC 9880 and SC 21009) treatment for 21 days: The effect of treatment on the ovum collection and fertilization rate and the development of the early embryo. *Theriogenology* 8:249–269.

