



REVIEW: Twinning in Dairy Cattle

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Abstract

Twinning in dairy cattle is an unavoidable outcome of reproduction and is undesirable because it reduces the overall profitability of a dairy operation through negative effects on cows calving twins as well as on calves born as twins. Cows calving twins are at greater risk for many periparturient reproductive and metabolic disorders than nontwinning herdmates, and incidences of abortion, stillbirth, neonatal calf mortality, and reduced birth weight are greater among twin compared with singleton calves. Twinning is a complex trait with multiple causative factors, and empirical evidence supports a role for both genetic and environmental risk factors in cattle. Risk factors for twinning include genetics, season, parity, ovulation rate, and milk production. The observation that twinning has increased in the dairy cattle population over time suggests a concurrent change in one or more of these causative factors during this same period. At present, dairy farmers and their consultants are ill prepared to make sound-management decisions to mitigate the negative effects of twinning on their operations because of a lack of applied scientific data on management strategies

for periparturient dairy cows carrying twin fetuses. A clear understanding of the factors responsible for twinning is essential for future development of strategies to manage twinning in dairy operations.

(Key Words: Twinning, Dairy Cattle, Milk Production, Double Ovulation, rbST.)

Introduction

Cattle are a monotocous species, meaning that, under most circumstances, a successful pregnancy results in the birth of one calf. Occasionally, however, the reproductive process in cattle, as with many other monotocous species, results in the birth of twins. In some beef-cattle production systems, twinning is considered a desirable trait that can enhance the overall profitability of the production enterprise by increasing weaned calf weight produced per cow (12, 28). By contrast, twinning in dairy cattle is an undesirable trait that reduces the overall profitability of a dairy operation through negative effects on cows calving twins as well as on calves born as twins.

Twinning in the dairy cattle population appears to be increasing over time (34). If this trend continues, the dairy industry must be prepared

to cope with the negative effects associated with twinning. At present, dairy farmers and their consultants are ill prepared to make sound-management decisions to mitigate the negative effects of twinning on their operations because of a lack of basic and applied scientific data on twinning in dairy cattle. Understanding the factors associated with twinning and their physiologic or genetic basis may allow for the development of management systems that minimize or eliminate the negative effects of twinning in dairy-production systems.

Discussion

Negative Impacts of Twinning in Dairy Cattle. Recent estimates calculating the economic impact of twinning on dairy farm profitability have not been published. However, economic analyses conducted 10 yr ago in the United States (2) and Great Britain (18) estimate that the occurrence of a twin birth on a dairy incurs a \$100 to \$250 economic loss to the dairy enterprise. The transition period is a challenge for dairy cows, twinning and nontwinning alike, and the metabolic and physiologic changes that occur during this time represent the greatest health risk for the cow

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during her productive life. Cows calving twins as well as the calves born as twins are at higher risk for health-related problems compared to nontwinning herdmates.

Impact of Twinning on Cows.

Twinning reduces reproductive performance by increasing average days open and services per conception during the subsequent lactation (46). Cows calving twins are at greater risk for many reproductive disorders including retained placenta, dystocia, and metritis (17, 26, 38, 46), as well as metabolic disorders including displaced abomasum, and ketosis (38, 46, 49). Not surprisingly, cows calving twins are culled at a greater rate than nontwinning herdmates (18).

Impact of Twinning on Calves.

Incidence of abortion, stillbirth, neonatal calf mortality, and reduced birth weight are greater among twin compared with singleton calves, probably because of reduced gestation length and increased incidence of dystocia among cows calving twins (11, 17, 19, 21, 40, 46, 49). Several studies in which the number of replacement heifers per pregnancy was calculated for both twinning and nontwinning dairy cattle showed that twinning decreases the number of replacement heifers available on a dairy, with 0.42 to 0.48 replacement heifers per pregnancy for calves born as singletons compared with 0.29 to 0.42 for calves born as twins (11, 19, 46). That this decrease in the number of replacement heifers occurs as a result of freemartinism is a common misconception. Rather, the decrease in replacement heifers per twin pregnancy arises from increased neonatal calf mortality for calves born as twins and a skewed gender ratio resulting in more homozygous male twins than homozygous female or heterozygous twins (51).

Freemartinism. Freemartinism in heifers occurs when embryonic membranes of a male and a female conceptus occupying the same uterus fuse during gestation, resulting in exchange of blood between the fetuses. Endocrine factors or cells from the male fetus cause abnormal

development of the reproductive organs of the female fetus resulting in infertility (45). The earliest developmental abnormalities of the female reproductive tract resulting in freemartinism occur between 49 to 52 d post-fertilization (32). Freemartinism has been documented in singleton female calves when loss of a male twin occurs after embryonic membrane fusion but before parturition (55). About 92% of heifer calves born co-twin with bull calves are freemartins (5). Thus, about 8% of heifers from heterosexual twin pregnancies are fertile, presumably because the fetal membranes fail to fuse or because membrane fusion occurs after the critical period of reproductive organ differentiation during embryonic development (5).

Mechanisms of Twinning.

Twinning in cattle can be classified into two types: monozygous twinning and dizygous twinning. Each ovarian follicle contains a single oocyte or egg that is expelled from the follicle into the oviduct after ovulation, where it awaits fertilization. A zygote is the single cell that forms after an oocyte is fertilized by a sperm. Thus, twins that arise from fertilization of one oocyte that subsequently cleaves and forms two embryos during development *in utero* are termed monozygous twins, whereas twins that arise from fertilization of two oocytes during the same estrous cycle are termed dizygous twins.

Monozygous Twinning. Monozygous twins, commonly called identical twins, are genetically and phenotypically identical and therefore are always of the same sex. Although mathematical estimates of monozygous twinning rates in dairy cattle range from 7.4% (19) to 13.6% (51) of all twin births or less than 0.3% of all births, these estimates seem high considering the frequency of double ovulation (which would result in dizygous twins) in the dairy cattle population (23, 51). Thus, monozygous twinning occurs infrequently in cattle and likely accounts for relatively few twin births in the dairy cattle population.

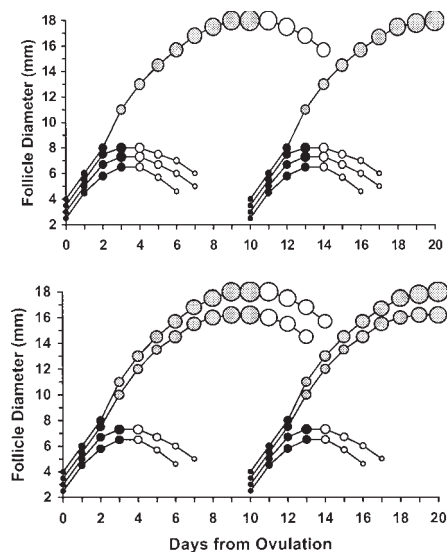


Figure 1. Schematic diagram depicting two-wave patterns of follicular growth during the bovine estrous cycle with the occurrence of a single dominant follicle (upper panel) or codominant follicles (lower panel) during each wave. Black circles = growing follicles before selection of the dominant follicle(s); gray circles = dominant follicles; open circles = atretic follicles.

Dizygous Twinning. Dizygous twinning accounts for most twin births in dairy cattle (19, 31, 51). Likewise, twinning and ovulation rate in cattle are strongly associated traits (44, 52) with the incidence of double ovulation in the dairy cattle population reported to be 14% (22, 23). Dizygous twins can be the same or opposite in sex and are no more alike phenotypically or genetically than siblings from the same sire and dam born during separate gestations. Because dizygous twins arise from ovulation of two follicles during the estrous cycle, understanding the nature of dizygous twinning requires an understanding of follicular dynamics in cattle and the mechanism by which a single follicle is selected to ovulate at estrus.

Follicular Growth in Cattle.

Follicular growth during the estrous cycle in cattle occurs as regular periods of follicular growth, dominance and atresia, termed "follicular waves" (Figure 1, A). Normally, only one follicle within each wave is selected to become dominant and acquire ovulatory capacity through a

process termed “deviation” (24). Deviation, therefore, provides an intrinsic mechanism that restricts the number of ovulatory follicles present on the ovaries at any given time during the estrous cycle to one follicle. Unless monozygous twinning occurs, fertilization of the oocyte from one follicle normally results in the birth of one offspring.

Codominance. Occasionally, however, the follicular selection mechanism is abrogated and two follicles within the same follicular wave undergo deviation resulting in codominance (Figure 1, B). Cattle normally exhibit either two or three follicular waves during a cycle, and the relationship between the number of follicular waves and the incidence of codominance and/or twinning in cattle has not been established. Because both codominant follicles have undergone deviation and thereby have acquired dominance and ovulatory capacity, an endogenous or exogenous ovulatory stimulus that occurs when codominant follicles are present will result in a double ovulation and the release of two oocytes. If the developmental events from fertilization to parturition occur without interruption for both oocytes, dizygous twins will result. Although double ovulation must precede the occurrence of dizygous twins, double ovulation does not consistently result in twinning because cows carrying twins experience high rates of embryonic loss and abortion during gestation (11).

Risk Factors for Twinning

Twinning in cattle is a complex trait with multiple causative factors that include physiologic as well as genetic components. That twinning is increasing in the dairy cattle population over time suggests a concurrent change in one or more of these causative factors during this same period. A clear understanding of the factors responsible for twinning is essential for development of strategies to manage twinning in dairy operations.

TABLE 1. Effect of parity on twinning rate (%) in dairy cattle^a.

| Reference | No. of calvings | Parity | | | | | |
|-----------|-----------------|--------|-----|------------------|-----|------------------|------------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| (49) | 937 | 0.7 | 5.0 | 4.2 | 5.0 | 7.0 | 6.7 ^b |
| (19) | 7,387 | 1.3 | 4.4 | 5.3 | 4.6 | 5.8 | 6.0 |
| (46) | 11,951 | 0.8 | 2.7 | 4.1 | 4.5 | 4.9 | 4.8 ^b |
| (18) | 19,755 | 0.9 | 2.1 | 3.5 | 3.4 | 3.7 | 3.2 |
| (51) | 24,843 | 1.0 | 7.0 | 7.5 | 7.9 | 9.1 ^b | – |
| (4) | 19,497 | 1.3 | 6.0 | 9.4 ^b | – | – | – |
| (34) | 52,362 | 1.0 | 2.9 | 3.2 | 3.9 | 3.3 | 4.1 ^b |

^aData adapted from Wiltbank et al. (56).

^bIncludes all cows greater than or equal to the parity listed.

Genetics. Genetic selection for twinning in cattle has been demonstrated through a long-term experiment conducted at the USDA Meat Animal Research Center (MARC) in Clay Center, Nebraska. Genetic selection of cattle based on twin births and later combined with ovulation rate increased twinning rate from 4% in 1984 to 31% in 1995 (27). By 1997, over 35% of all births in this experimental herd resulted in twins (17). The experimental herd at MARC comprises several breeds of beef cattle and is managed as a seasonally calved beef herd; however, many of the foundation animals selected to initiate the experiment were Holstein cows purchased from local dairy farms based on their previous history of twinning (25). Although heritability and repeatability estimates for twinning rate are low [0.08 and 0.09, respectively; (52, 27)], previous incidence of twinning is a risk factor for subsequent twin births (3, 46).

Breed. In general, the twinning rate for most beef breeds of cattle is less than 1% (50). By contrast, epidemiologic estimates of twinning in the dairy cattle population are greater than those of beef cattle and range from 2.5 to 5.8% (Table 1). In addition to the variation reported among studies, twinning rate also varies widely among individual herds within these studies. For example, data from Dutch Friesians and Dutch

Friesian-Holstein crosses in Holland exhibited an overall twinning rate of 3.2% with a range among herds of 0 to 7.1% (46). Similarly, the average twinning rate in a sample of North American Holsteins was 2.4% with a range among herds of 0 to 9.6% (34). In studies comparing the five major dairy breeds of cattle, Holsteins consistently exhibit the highest incidence of twinning (7, 21). Assessment of twinning rates in non-Holstein dairy breeds is difficult, however, because few recorded calving outcomes for these breeds are reported in these studies. Assessment of twinning within a dairy herd must take into account the variation associated with this trait. Twinning is a bimodal outcome; that is, a cow either calves a singleton calf or she calves more than one calf. Because bimodal response variables require large sample sizes to accurately estimate a population mean, assessing the incidence of twinning on an individual dairy requires many calving observations. Thus, estimation of twinning rates is difficult for smaller farms where the number of calving observations per unit time is limited.

Season. Although many studies have shown seasonal effects on the incidence of twinning, others have failed to show such a trend (30). For example, seasonal increases in twinning have been reported to occur

TABLE 2. Effect of parity and milk production (mean \pm SEM) on the incidence of double ovulation (%) after a synchronized ovulation^a.

| Item | Milk production group | | Overall (number/total) |
|------------------------|--------------------------|---------------------------|---------------------------|
| | Low (\leq 40 kg/d) | High ($>$ 40 kg/d) | |
| Parity | 17.4 | 22.2 | 9.5 ^b (6/63) |
| Parity | 24.0 | 14.3 | 10.8 ^c (8/74) |
| Parity 3+ | 8.7 | 27.8 | 20.3 ^d (12/59) |
| Overall (number/total) | 6.9 ^e (7/102) | 20.2 ^f (19/94) | 13.3 (26/196) |

^aData adapted from Fricke and Wiltbank (23).

^{b,c,d}Linear increase ($P=0.09$) in incidence of double ovulation with increasing parity.

^{e,f}Proportions differ by chi square analysis ($P<0.05$).

from April to September in Holland (46) and from May through June in Saudi Arabia (51), whereas no seasonal effect was detected in a study of dairy cattle in North America (34). Although speculative at present, the tendency toward increased twin births during summer months has been attributed to an increased plane of nutrition during the fall when cows calving during the summer would have conceived, a decreasing photoperiod, or a decrease in the viability of early-stage embryos conceived during summer months compared with those conceived during cooler fall months (7, 46). Further studies are required to substantiate the seasonal effect on twinning as well as the factors responsible for seasonal fluctuations in the incidence of twinning.

Parity. Twinning in dairy cattle increases with parity, ranging from 1% for first parity to nearly 10% during later parities (Table 1). The greatest increase in twinning occurs between parity 1 (e.g., virgin heifers) and parity 2; twinning continues to increase during later parities but at a lesser rate. The effect of parity on twinning rate is not clearly understood but may be explained by an increased ability of older cows to support twins throughout gestation, an increase in the incidence of double ovulation, or an interaction between these factors. Although increased uterine capacity of cows calving twins has been reported (51), several studies

support increased ovulation rate as the primary factor explaining the effect of parity on twinning. Because monozygous twinning appears to be independent of parity, the effect of parity on twinning rate likely results from an increased frequency of dizygous twinning (31), and hence the incidence of double ovulation. Indeed, the incidence of double ovulation increases with parity in lactating dairy cows (23, 37; Table 2). Furthermore, other risk factors for twinning such as milk production are confounded with parity and may explain changes due to parity.

Ovulation Rate. Twinning and ovulation rate in cattle are strongly associated traits (44, 51). The incidence of double ovulation in lactating dairy cows is around 14% (22, 23), and, as with the incidence of twinning, increases with parity (23, 37). Few studies have examined factors influencing double ovulation in dairy cattle; however, high milk production near the time of ovulation has been associated with a greater frequency of double ovulation (23). Kinsel et al. (34) speculated that feeding higher energy diets to high-producing cows may be increasing the incidence of double ovulation and hence the rate of twinning in dairy cattle. Although this nutritional effect may be similar to the practice of "flushing" in ewes (15), this mechanism remains unsubstantiated in dairy cattle. These results must be interpreted with caution, however,

because although double ovulation must precede the occurrence of dizygous twins, double ovulation may not consistently result in twinning. More research is needed to fully understand the relationship between double ovulation and twinning and the mechanisms responsible for double ovulation in dairy cattle.

Milk Production. The practical implication of the relationship between milk production and twinning in dairy cattle is important because current dairy-management strategies aim to maximize milk production per cow in the dairy industry. If twinning is related to milk production, this increase would not be unexpected considering the annual increases in milk production per cow that have occurred during the past several decades as a result of genetic selection and artificial insemination.

A positive association between milk production and twinning in dairy cattle has been observed in some studies (34, 46), but not in others (13, 33). However, effects of milk production have been reported when comparing dams carrying or calving twins with their nontwinning herdmates. Cows calving twins produce less milk during the subsequent lactation compared with cows calving singletons (46). This reduction in milk production may result from increased incidences of metabolic disorders experienced by cows calving twins during the early stages of lactation. By contrast, milk production at 100 d in milk was greater during the lactation when cows were carrying twin fetuses compared with cows carrying singletons; however, no difference in milk production was detected at later points in lactation (46). Similarly, cows calving twins produced 2.7 kg more milk at peak production than cows calving singletons, although total production for the lactation did not differ between groups (34). Kinsel et al. (34) concluded that the single largest contributor to the increase in twinning over a 10-yr period (1983 to 1993) was the concurrent increase in peak

milk production during that time. Although the direct relationship between the incidence of double ovulation and twinning is unclear, cows with greater than average milk production near the time of artificial insemination after a synchronized ovulation exhibited a three-fold greater incidence of double ovulation than cows with less than average milk production (Table 2).

At present, data supporting a relationship between twinning, double ovulation, and milk production are associative rather than causative, and physiologic mechanisms by which milk production may affect twinning remain unclear.

Increasing levels of dietary bypass protein can increase ovulation rate and twinning rate in ewes (47). Although unproven, high levels of bypass protein fed to lactating cows may partially account for the increased twinning rate. Recent data (53) suggest that feed intake may increase hepatic metabolism of ovarian steroids in lactating dairy cows similar to that reported previously in ewes (41, 48). Increased steroid metabolism may subsequently alter the endocrine environment sufficiently to allow for deviation of two follicles during the selection period of a follicular wave, thereby resulting in codominance (56). Future studies should continue to investigate the relationships between milk production, ovulation rate, and twinning in dairy cattle.

Recombinant Bovine Somatotropin (rbST). In the United States, commercial sales of the prolonged-release formulation of rbST (POSILAC[®]; Monsanto Co., St. Louis, MO) began in February 1994 upon FDA approval (1). Since its introduction, the relationship between use of rbST and twinning in dairy cattle has generated controversy within the dairy industry. At initial FDA approval, the POSILAC[®] label stated that cows treated with rbST might exhibit increased twinning rates. Inclusion of this warning was based on pre-approval studies in which cows receiving intramuscular injections of

TABLE 3. Estimated intervals and cumulative days in milk associated with events after a management decision to terminate the pregnancy of a cow carrying twins.

| Item | Interval (d) | Cumulative days in milk |
|---|------------------|-------------------------|
| Mean interval from | | |
| Calving to twin diagnosis and induction of abortion | 184 ^a | 184 |
| Induction of abortion to second conception | 84 ^b | 268 |
| Second conception to dry off | 232 ^c | 500 |

^aAverage days open (144 d; voluntary waiting period = 60 d, conception rate = 40%, and service rate = 40%) + day of gestation at diagnosis of twins (40 d).

^bMedian days to second conception using AI breeding (84 d; conception rate = 40%, and service rate = 40%).

^cAverage gestation length (282 d) – average dry period (50 d).

rbST exhibited a greater incidence of twinning compared with cows receiving subcutaneous injections of rbST (9). However, upon completion and review of data from the post-approval monitoring program in which rbST was administered to cows subcutaneously, the FDA removed the warning regarding twinning from the POSILAC[®] label based on a lack of a detectable effect of POSILAC[®] on twinning rate (9). The mechanism by which route of rbST administration affected twinning rates in these studies is not known.

High milk yield has a small but significant negative effect on general measures of reproductive efficiency, with increases in days to first breeding, days to last breeding, days open, and number of total breedings for each 100-kg increase in 180-d yield for fat-corrected milk (36). As discussed previously, high milk production has been associated with increased frequency of double ovulation and twinning in dairy cattle, and POSILAC[®] is one management factor among many that increases milk production. Comparisons between the reproductive performance of cows of similar milk-production levels within control and rbST-supplemented groups showed that level of milk production was more often a significant factor affecting reproductive parameters than was rbST administration (8). Thus, a rbST-supple-

mented herd should have a similar incidence of twinning to that of a nonsupplemented herd of equal milk production level. Although negative effects of rbST on fertility in lactating cows have been reported (6, 20, 29, 42, 57), rbST has recently been shown to improve conception rates in lactating dairy cows when administered in conjunction with a hormonal protocol for synchronization of ovulation and timed artificial insemination (43). Further research is needed to clarify the effects of rbST and increased milk production on reproductive efficiency and twinning in dairy cattle.

Strategies for Managing Twinning

At present, information on twinning management in dairy cattle is inadequate, and further research is needed to make sound management decisions regarding twinning. Development of practical management strategies to cope with twinning on dairies is critical, especially if the trend toward increased twinning in the dairy cattle population continues. Despite this lack of data, several management strategies could be considered when a dairy is experiencing significant levels of twinning.

Early Identification of Twins. Management of twinning in a dairy

operation begins with early identification of cows carrying twins because management intervention cannot occur unless cows carrying twins are accurately and efficiently identified. Transrectal ultrasonography can be used to identify cows carrying twin fetuses at 40 to 55 d post-AI (10, 14, 17). Palpation per rectum between 50 to 70 d post-AI also results in an acceptable degree of accuracy among experienced veterinarians (11); however, few veterinarians routinely examine cows for twin fetuses during rectal examinations for early-pregnancy diagnosis. Systematic identification of cows carrying twin fetuses allows for differential management of these cows later during gestation, especially during dry and transition periods.

Elective Abortion and Culling.

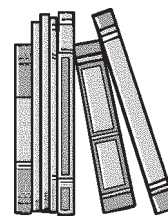
Several management scenarios could be considered upon diagnosis of a twin pregnancy. Continued management of the cow carrying twins could be avoided either by culling the cow or by aborting the pregnancy during the first trimester of gestation, usually through administration of an ecbolic agent such as prostaglandin F_{2a} . Several factors should be considered before electing to abort a twin pregnancy with the intent of rebreeding the cow. First, the estimated average lactation length of cows subjected to induced abortion and rebreeding would approach 500 d (~18.5-mo calving interval) based on average reproductive performance and management indices for lactating cows (Table 3). Second, the risk for a twin pregnancy during the subsequent gestation is increased because cows calving twins are at greater risk for subsequent twinning (3, 46). Third, establishing pregnancy in lactating dairy cows is difficult, and a pregnancy represents an inherent value to the dairy operation that is forfeited by electively aborting the pregnancy. Finally, cows carrying twins experience greater rates of early embryonic loss than cows carrying singletons and, on occasion, lose one fetus while maintaining the other (11). Elective

abortion of a twin pregnancy early during gestation that may result in the birth of a singleton calf at parturition is not a sound management practice. Based on these considerations and depending on the value of the dam and calf, culling to avoid continued management of a cow carrying twins is a better alternative than aborting the pregnancy.

Management of Cows Carrying Twins. If a cow carrying twins is to be maintained in the herd until parturition, several management strategies should be considered. Based on research in beef cows (35, 54), feeding dairy cows carrying twin fetuses a higher plane of nutrition, especially during the last trimester of gestation may be beneficial (11, 46). Furthermore, because gestation length of cows calving twins is reduced by 6 to 10 d (17, 21, 46, 49, 51), most cows carrying twins miss at minimum a portion of the 2- to 3-wk transition diet feeding period if calculated based on the estimated calving date of nontwinning cows. Thus, earlier dry off and feeding of a transition diet may reduce the incidence of physiologic and metabolic disorders associated with cows calving twins (51). Finally, providing assistance at calving for cows carrying twins may reduce complications associated with dystocia and may reduce economic losses by reducing the incidence of neonatal calf mortality.

Implications

Twinning in the dairy cattle population has increased over time and, if this trend continues, the dairy industry must be prepared to cope with the negative effects associated with twinning. Few studies have dealt directly with management strategies directed specifically for cows carrying twins. Further research is needed to investigate the factors responsible for twinning in dairy cattle and to develop and test the efficacy of practical strategies for identifying and managing cows carrying twins.



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