Management of Twinning in Dairy Cows

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Introduction

In 2001, I published a review of the scientific literature on the topic of twinning in dairy cattle (Fricke, 2001). My purpose was to overview the causative factors associated with twinning and to identify management strategies based on data from controlled experiments that might mitigate the negative effects of twinning. At the time of this review, epidemiologic data supported that the incidence of twinning in Holstein dairy cows in the U.S. had increased over time (Kinsel et al., 1998). From an economic perspective in the U.S., lost revenue due to twinning was estimated to be $55 million per year (Johanson et al., 2001). If twinning has continued to increase over time, the negative impacts of twinning on calves born as twins, cows calving twins, and the resulting decrease in dairy farm profitability (Fricke et al., 2001) have only gotten worse and will continue to be a reproductive problem for dairy cows in the future.

Twinning Trend across Time

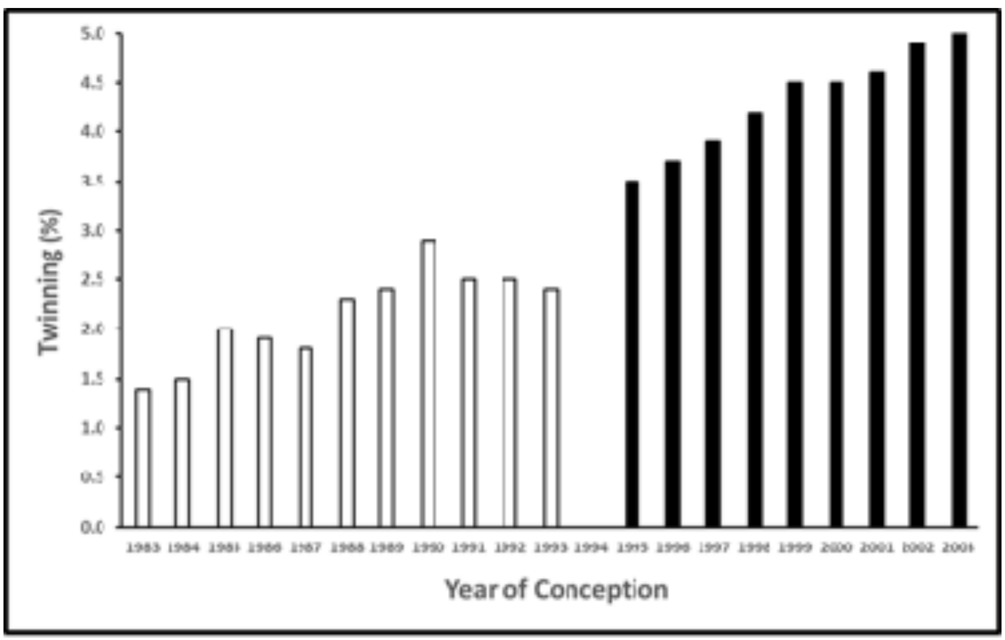

Figure 1. Trend in reported twinning rate in Holstein cows in the U.S. from 1983 to 2003. Data includes nonlactating and lactating Holsteins. Open bars: data adapted from Kinsel et al., 1998; Solid bars: data adapted from Silva del Rio et al., 2007.

Based on an epidemiologic study of twinning published in 1998 (Kinsel et al., 1998), twinning in the U.S. increased over a 10-year period from 1983 to 1993. The authors implicated the concurrent increase in milk production during this period as the single most important factor associated with the increase in twinning (Kinsel et al., 1998). To determine if this trend for an increase in twinning has continued over time, we analyzed and published an observational analysis of twin births in Holstein cows in the Upper Midwest region of the U.S. from 1996 to 2004 (Silva del Rio et al., 2007). A data set of Holstein calving records from January 1996 to September 2004 comprising 4,103 herds with 2,304,278 calving events representing 1,164,233 cows and 96,069 twin births was extracted from Minnesota DHIA archives to assess reported twinning trends across time. Overall, the reported twinning rate averaged 4.2%, and twinning increased with parity (1.2% for nulliparous vs. 5.8% for multiparous cows) and with time (3.4% in 1996 to 4.8% in 2004). Figure 1 shows the trend for increased twinning across time in the U.S. Holstein population from 1983 to 1993 based on data from Kinsel et al. (1998) and from 1996 to 2004 based on data from Silva del Rio et al. (2007). Clearly, twinning in Holsteins has consistently increased from 1983 to 2004 (Figure 1). Based on this long-term trend, we may expect twinning in Holsteins to continue to increase.

Mechanism of Twinning

Twinning can be classified into two types: monozygotic and dizygotic. Monozygotic twins (i.e., identical twins), result from the *in vivo* cleavage of one fertilized oocyte during early embryonic development. By contrast, dizygotic twins (i.e., fraternal twins), result from fertilization of oocytes from two follicles that ovulate during the same estrous cycle. We conducted an observational experiment to determine the incidence of monozygotic twins in Holstein cows and reported a monozygotic twinning frequency of 7.5% of same-sex twins and 4.7% of all twins (Silva del Rio et al., 2006). Thus, monozygotic twinning occurs infrequently in Holstein cows, and the primary mechanism for twinning in dairy cows is double ovulations resulting in dizygotic twins.

Physiology of Twinning

A physiological link can now be made between increasing milk production, the increased rate of double ovulation and dizygotic twinning, and decreased circulating progesterone levels in lactating dairy cows (Wiltbank et al., 2000). Steady state progesterone concentration in circulation is a balance between progesterone production by the corpus luteum and progesterone catabolism by the liver (Wiltbank et al., 2014). Because milk production is highly correlated (r = 0.88) with feed intake (Harrison et al., 1990), hepatic blood flow increases as milk production and feed intake increases. Hepatic metabolism of progesterone increases as feed intake associated with high milk production increases thereby providing a physiological mechanism for decreased circulating progesterone concentrations in high-producing dairy cows (Sangsritavong et al., 2002). Thus, as milk production has increased over time in dairy cows, circulating progesterone has decreased resulting in an increase in the incidence of double ovulation and dizygotic twinning. Decreased progesterone concentrations near the time of deviation of the dominant follicle may cause a delay in the FSH nadir and increase LH pulses resulting in selection of two or more dominant follicles during a wave (Wiltbank et al., 2014). Because the primary mechanism for twinning is via double ovulation (Silva del Rio et al., 2006), an increase in the double ovulation rate would best explain the increase in twinning in dairy cows over time.

Identification of Cows Carrying Twins

Transrectal Ultrasonography. Management of cows carrying twins depends on accurate identification of cows carrying twins early during gestation. As predicted (Fricke, 2002), the adoption of transrectal ultrasonography by bovine practitioners as a reproductive management tool has increased over the past 15 years. Cows carrying twins can be accurately identified using transrectal ultrasonography by 40 to 55 d after AI (Echternkamp and Gregory, 1991; Davis and Heibel, 1993; Dobson et al., 1993). Because of the advances in the resolution of ultrasound scanners since publication of these studies, and because the majority of twinning in dairy cows is dizygotic (Silva del Rio et al., 2006), the presence of two or more CL on the ovaries at the time of an early pregnancy diagnosis conducted 32 to 39 d after AI can be used to identify cows carrying twins (Fricke, 2002). In addition to examining both ovaries, a thorough examination of the entire length of both uterine horns during a pregnancy examination should be performed to accurately diagnose twins using transrectal ultrasonography (Fricke, 2002).

Table 1. Embryo viability, pregnancy loss, and spontaneous embryo reduction based on transrectal ultrasonography between a first pregnancy examination (FPE; 25 to 40 d after AI) to a second pregnancy examination (SPE; 48 to 82 d after AI) in Holstein cows (adapted from Silva del Rio et al., 2009).

	Pregnancy type

Item	Singleton	Twin
Cows with embryos at FPE, n	518	98
Cows with dead embryos at FPE, % (n)	3.7 (19)	-
Cows with live embryos at FPE, n	499	98
Cows undergoing pregnancy loss by SPE, % (n)	4.6 (23)	13.3 (13)
Cows with twins undergoing reduction by SPE, % (n)	-	11.2 (11)
Cows maintaining pregnancy by SPE, % (n)	91.9 (476)	75.5 (74)

Pregnancy Loss. Pregnancy loss confounds accurate identification of cows that eventually go on to calve twins because cows carrying twins have a greater incidence of pregnancy loss compared to cows carrying singletons. Although cows with double ovulations tended to have a greater conception rate at a first pregnancy diagnosis compared to cows with single ovulations (64% vs. 45%; Fricke and Wiltbank, 1999), pregnancy loss from an initial pregnancy diagnosis to a pregnancy reconfirmation based on transrectal ultrasonography was three-fold greater for cows diagnosed with twins compared to cows diagnosed with singletons resulting in an embryo survival rate of 92% for cows diagnosed with singletons compared to 76% for cows diagnosed with twins (Silva del Rio et al., 2009; Table 1).

Laterality of twin pregnancies also affects pregnancy loss as well as spontaneous embryo reduction. Pregnancy loss before 90 d in gestation was greater for cows with unilateral twins (35%) compared to bilateral twins (8%) in one experiment (Lopez-Gatius and Hunter, 2005). Interestingly, the rate of pregnancy loss of 8% reported for bilateral twins by Lopez-Gatius and Hunter (2005) is slightly less than the 13% loss reported to occur in Holstein cows from 27 to 31 d and 38 to 50 d of gestation based on transrectal ultrasonography in a summary of 14 studies (Santos et al., 2004), and the 14% loss for cows with bilateral twins reported by Andreu-Vazquez et al. (2011). Laterality of twins also affects the incidence of dystocia as well as calf survival rate. In a long-term experiment in which cows were genetically selected for multiple ovulations, bilateral dizygotic twins had increased survival and body weight at birth, a longer gestation length, and less dystocia than unilateral dizygotic twins (Echternkamp et al., 2007). Cows calving twins or triplets, however, had a greater incidence of dystocia than cows calving singletons (Echternkamp et al., 2007).

Strategies for Managing Twinning

Pregnancy Termination. One method to dramatically reduce or eliminate twinning in a dairy herd is to identify cows carrying twins and induce pregnancy loss by administration of a luteolytic agent such as prostaglandin $F_{2\alpha}$. For singleton pregnancies, treatment with a luteolytic dose of prostaglandin $F_{2\alpha}$ at 39 d in gestation decreased progesterone within 24 h and caused expulsion of the conceptus in all cows within 48 h (Giordano et al., 2012). There are however several arguments against proactive termination of all twin pregnancies identified early in gestation. First, the economic loss incurred due to pregnancy loss has been estimated to range from $46 (Ferguson and Galligan, 2011) to $300 (Galligan et al., 2009). Because the incidence of twinning increases with increasing milk production, cows diagnosed with twins often are the highest producing cows in the herd that incur the greatest economic loss associated with pregnancy loss. Second, although heritability and repeatability estimates for twinning in dairy cows are low (0.08 and 0.09, respectively; Van Vleck et al., 1991; Gregory et al., 1997), a prior incidence of twinning is a risk factor for subsequent twin births (Bendixen et al., 1989; Nielen et al., 1989). Third, pregnancy loss before 90 d in gestation for cows with unilateral twins did not differ between control cows and cows subjected to manual amnion rupture followed by progesterone treatment for 21 d (Andreu-Vazquez et al., 2011), whereas pregnancy loss for cows carrying bilateral twins (Lopez-Gatius and Hunter, 2005) was similar to that reported for Holsteins overall (Santos et al., 2004). Finally, bilateral dizygotic twins had increased survival and body weight at birth, a longer gestation length, and less dystocia than unilateral dizygotic twins (Echternkamp et al., 2007). Based on these data, a possible strategy would be to allow cows diagnosed with bilateral twins to continue gestation whereas selective reduction could be attempted for cows identified with unilateral twins. Because, twin and triplet births had a greater incidence of dystocia than single births (Echternkamp et al., 2007), cows diagnosed pregnant with bilateral twins should be provided extra assistance at calving.

Selective Reduction. Selective embryo reduction early during gestation has been used to mitigate potentially dangerous maternal effects of multiple births in both women (Iberico et al., 2000) and mares (Macpherson and Reimer, 2000). Reasonable success has been reported in mares when one twin was manually crushed when the procedure was performed before 30 d in gestation (Frazer, 2003). Two controlled experiments reported the efficacy of using manual crushing of the amnion of one of the embryos in an attempt to maintain a viable singleton pregnancy (Lopez-Gatius, 2005; Andreu-Vazquez et al., 2011).

In the first experiment (Lopez-Gatius, 2005), 33 cows identified with unilateral twins were randomly assigned to one of three treatments: 1) untreated controls; 2) manual amnion rupture; and 3) manual amnion rupture plus progesterone treatment (PRID containing 1.55 g progesterone) for 28 d. Embryo reduction was attempted between 35 to 40 d in gestation because most cows undergo spontaneous reduction of twins at this time (Lopez-Gatius and Hunter, 2005). Pregnancy loss for control cows (i.e., spontaneous loss of both twins) was 27% (3/11), whereas pregnancy loss for cows undergoing manual amnion rupture was 100% (11/11). Interestingly, pregnancy loss for cows treated with progesterone for 28 d after amnion rupture was 55% (6/11). One embryo from a cow treated with progesterone after manual amnion rupture survived and the cow calved twins, whereas the remaining five cows calved singletons (Lopez-Gatius, 2005).

Table 2. Effect of laterality of twin pregnancy on rates of pregnancy loss before Day 90 for control cows (no manipulation) and cows subjected to manual twin reduction followed by progesterone treatment for 21 d (adapted from Andrieu-Vazquez et al., 2011).

Item	n	Loss rate before 90 d % (n/n)
Unilateral twin pregnancy	27	
Control	14	64 (9/14)
Manual twin reduction	13	54 (7/13)
Bilateral twin pregnancy	28	
Control	14	0 (0/14)
Manual twin reduction	14	29 (4/14)

A follow-up experiment was conducted to evaluate the effect on pregnancy maintenance of embryo reduction via manual rupture of the amnion in Holstein cows diagnosed with both unilateral and bilateral twin pregnancies (Andreu-Vazquez et al., 2011; Table 2). At 35 to 41 d of gestation, 55 cows identified with live twins using transrectal ultrasonography were blocked by laterality and randomly assigned to manual reduction followed by treatment with progesterone (PRID containing 1.55 g progesterone) for 21 d or served as untreated controls in which no manipulation was done. Pregnancy loss before 90 d in gestation did not differ between treatments and occurred in 32% of control cows and 41% of cows after manual amnion rupture followed by progesterone for 21 d. Independent of treatment, risk of pregnancy loss was 8.7 times greater for unilateral compared to bilateral twin pregnancies, yet pregnancy loss did not differ between control cows with unilateral twins and unilateral twin reduction cows (62% vs 54%, respectively). By contrast, 29% of cows with bilateral twin pregnancies subjected to twin reduction lost their pregnancies, whereas no losses occurred in control cows with bilateral twin pregnancies. Overall, 44% (12/27) of cows subjected to manual amnion rupture went on to calve singletons compared to 54% (15/28) of control cows which went on to calve twins. The authors concluded that embryo reduction by manual amnion rupture followed by progesterone treatment did not experience an additional risk of pregnancy loss for unilateral twin pregnancies, whereas it increased the risk of pregnancy loss in bilateral twin pregnancies (Andreu-Vazquez et al., 2011).

Nutritional Management during the Transition Period. We conducted an experiment to evaluate the effect of dry period feeding management on metabolic status and lactation performance in Holstein cows carrying singleton vs. twin pregnancies (Silva del Rio et al., 2010). Dry period feeding management consisted of feeding a moderate-energy close up diet throughout the entire dry period (8-week close-up) versus feeding a far-off diet from 60 to 21 d before expected calving date followed by a close-up diet until calving (3-week close-up). Treatments were arranged in a 2 × 2 factorial design with a randomized block design that included 47 Holstein cows. Our hypothesis was that increasing the duration of feeding a close-up diet during the dry period would improve metabolic status and lactation performance for cows carrying twins, but not for cows carrying singletons. Contrary to our hypothesis, metabolic response to dry period feeding strategy was independent of twin status (Silva del Rio et al., 2010) indicating that altering nutritional management to increase energy during the dry period did not improve metabolic status for cows carrying twins. Interestingly, there was an effect of diet in which cows fed a moderate energy diet throughout the entire dry period had greater milk production compared to cows fed according to the NRC (2001) energy requirements for the entire dry period (i.e., a far-off diet followed by a close-up diet for 3 weeks). Based on our results (Silva del Rio et al., 2010), differential management of cows carrying twins during the dry period did not improve metabolic status of cows carrying twins.

Hormonal Manipulation before AI to Reduce Double Ovulation. Dairy farmers have adopted hormonal synchronization protocols for use in reproductive management programs for dairy cows (Carievello et al., 2006), and the use of synchronization protocols for inseminating dairy cows has increased over time (Norman et al., 2009). Twinning also has increased in women over time, and this increase has been attributed to the increased use of exogenous hormones and in vitro fertilization during assisted fertility treatments (Imaizumi, 2003). Although the endocrine physiology differs dramatically between these situations, dairy farmers often associate the increased use of hormonal synchronization protocols with increased twinning. A common strategy to submit cows for first insemination on farms is to use a Presynch Ovsynch protocol and combine AI to detection of estrus after the Presynch portion of the protocol with submission of cows failing to be detected in estrus to timed AI after an Ovsynch protocol (Fricke et al., 2014). Under this management system, cows receiving timed AI after an Ovsynch protocol differ from cows inseminated to estrus in that they are likely the highest producing cows that express estrus for a shorter duration (Lopez et al., 2004) and anovular cows that have low progesterone at the onset of the Ovsynch protocol. Both of these conditions are risk factors for double ovulation and twinning as discussed previously. Contrary to the idea that hormonal synchronization protocols increase twinning, hormonal manipulation of cows so that progesterone was increased during development of the ovulatory follicle resulted in a decreased incidence of double ovulation (Stevenson et al., 2007; Cerri et al., 2011). A Double Ovsynch protocol (Souza et al., 2008; Herlihy et al., 2012) effectively presynchronizes cows to maximize progesterone during growth of the ovulatory follicle (Wiltbank et al., 2014) and should thereby decrease double ovulation rate and subsequent dizygotic twinning.

Conclusion

Based on data in this review, we can now formulate a two-pronged approach to decrease the negative impact of twinning in dairy cows. First, the incidence of double ovulation and dizygotic twinning can be decreased in high-producing Holstein cows by hormonally manipulating ovarian function to increase progesterone during growth of the preovulatory follicle before AI. The best strategy to accomplish this is to submit cows to first timed AI after a Double Ovsynch protocol. Second, because bilateral dizygotic twins had increased survival and body weight at birth, a longer gestation length, and less dystocia than unilateral dizygotic twins, cows identified with bilateral twins early during gestation should be allowed to continue gestation with extra assistance provided to these cows at calving. Because selective reduction of unilateral twins resulted in similar pregnancy loss compared to control cows with unilateral twins, selective reduction can be attempted for cows diagnosed with unilateral twins with the realization that overall pregnancy losses for unilateral twins exceeded 50%. Overall, this strategy should decrease the incidence of twinning in a dairy herd while minimizing the negative impacts of twinning for the cows that do conceive twins.

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