Double vision: management of twinning in dairy cows

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Abstract

Twinning in Holstein dairy cows has increased over time concurrent with increased milk production. More than 95% of twins in Holsteins arise due to double ovulations resulting in dizygotic twins. It is now clear that low progesterone during growth of a preovulatory follicle increases the incidence of double ovulation. Increased hepatic metabolism of progesterone as a result of the increased feed intake associated with high milk production provides a physiological mechanism for decreased progesterone levels in high-producing dairy cows resulting in an increase in dizygotic twinning. Twinning will likely continue to increase with milk production over time, so strategies to effectively manage twinning in dairy cows need to be identified and implemented. Cows carrying unilateral twins had greater pregnancy loss compared to cows carrying bilateral twins, and bilateral twins had increased survival and body weight at birth, a longer gestation length, and less dystocia than unilateral twins. Selective embryo reduction by manual rupture of the amnion followed by progesterone treatment did not increase the risk of pregnancy loss for cows with unilateral twins but increased the risk of loss for cows with bilateral twins. Hormonal synchronization protocols that increase progesterone during development of the preovulatory follicle increase conception rate, decrease pregnancy loss, and decrease double ovulation rate thereby decreasing twinning in high-producing dairy cows. Twinning can be decreased in high-producing dairy cows using a two-pronged approach. First, the incidence of double ovulation and dizygotic twinning can be decreased in high-producing Holstein cows by manipulating ovarian function to increase progesterone during growth of the preovulatory follicle before timed AI. Second, cows identified with bilateral twins using transrectal ultrasonography early during gestation should be allowed to continue gestation with extra assistance provided at calving, whereas selective reduction can be attempted for cows diagnosed with unilateral twins.

Résumé

Le jumelage de vaches laitières Holstein a augmenté au fil du temps parallèlement à l'augmentation de la production de lait. Plus de 95 % des enfants jumeaux dans Holsteins surviennent en raison d'une double ovulations résultant de jumeaux dizygotes jumeaux. Il est maintenant clair que de faibles au cours de la croissance de la progestérone un follicule préovulatoire augmente l'incidence de la double l'ovulation. Augmentation du métabolisme hépatique de la progestérone à la suite de l'augmentation de la consommation d'aliment associé à haute production du lait fournit un mécanisme physiologique pour la diminution de la concentration de progestérone dans les vaches laitières très productives résultant en une augmentation de caractère dizygote. Le jumelage continueront vraisemblablement à augmenter avec la production laitière dans le temps, de sorte que des stratégies pour gérer efficacement les jumelages dans les vaches laitières ont besoin d'être identifiés et mis en oeuvre. Des vaches porteuses de jumeaux unilatérale avait une plus grande perte de grossesse comparativement aux vaches transportant des jumeaux, et bilatéraux bilatéraux augmentent la survie et jumelles avait le poids corporel à la naissance, une plus longue durée de la gestation, et moins d'une dystocie qu'unilatérale des jumeaux. La réduction embryonnaire sélective par le manuel de rupture de l'amnios suivie par traitement à la progestérone n'a pas augmenté le risque de perte de grossesse pour les vaches avec jumeaux unilatérale mais a augmenté le risque de perte pour les vaches avec jumeaux bilatéraux. Protocoles de synchronisation hormonal qui augmentent la progestérone au cours du développement du follicule préovulatoire augmenter le taux de conception, diminution de la grossesse perte, et de diminuer le double taux d'ovulation diminuant ainsi le jumelage dans les vaches laitières très productives. Pairage peut être diminué dans les vaches laitières très productives en utilisant une approche à deux volets. Tout d'abord, l'incidence de la double l'ovulation et caractère dizygote peut être diminuée en haute production de vaches Holstein en manipulant la fonction ovarienne pour augmenter la progestérone durant la croissance du follicule préovulatoire avant EA temporisée. Deuxièmement, les vaches identifié avec jumeaux bilatérale à l'aide de l'échographie transrectale tôt durant la gestation devraient être autorisés à continuer la gestation avec l'aide supplémentaire fournie au vêlage, tandis qu'une réduction sélective peut être tenté pour les vaches diagnostiqués avec jumeaux unilatérale.

Introduction

In 2001, I published a review of the scientific literature on the topic of twinning in dairy cattle.[14] 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 US had increased over time.[28] From an economic perspective in the US, lost revenue due to twinning was estimated to be $55 million per year.[27] 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[14] have only gotten worse and will continue to be a reproductive problem for dairy cows in the future.

Controlled experiments testing strategies to mitigate the negative impacts of twinning in dairy cows were lacking 15 years ago, and I concluded my review by stating that dairy farmers and their consultants were ill prepared to make sound management decisions to mitigate the negative effects of twinning on their operations because of a lack of scientific data on management strategies proven to mitigate the negative effects of twinning.[14] During the past 15 years, many experiments have been published in the area of twinning in dairy cattle that have filled many of the gaps in our knowledge. The purpose of this review is to update and expand upon my initial review on twinning in dairy cattle and determine if we now can make research-based management recommendations to mitigate or even prevent the negative impacts of twinning in dairy cows.

Twinning Trend across Time

Based on an epidemiologic study of twinning published in 1998,[28] twinning in the US 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.[28] 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 US from 1996 to 2004.[50] 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 US. Holstein population from 1983 to 1993 based on data from Kinsel et al[28] and from 1996 to 2004 based on data from Silva del Rio et al.[50] 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.

A novel observation based on our analysis was a significant parity by time interaction on twinning. For primiparous and multiparous cows, the twinning rate increased from 4.0% and 5.2% during the first 12 months of the study to 5.9% and 7.3% during the last 12 months of the study, whereas the twinning rate for nonlactating heifers increased from only 1.1% to 1.3% during this same time period.[50] Although

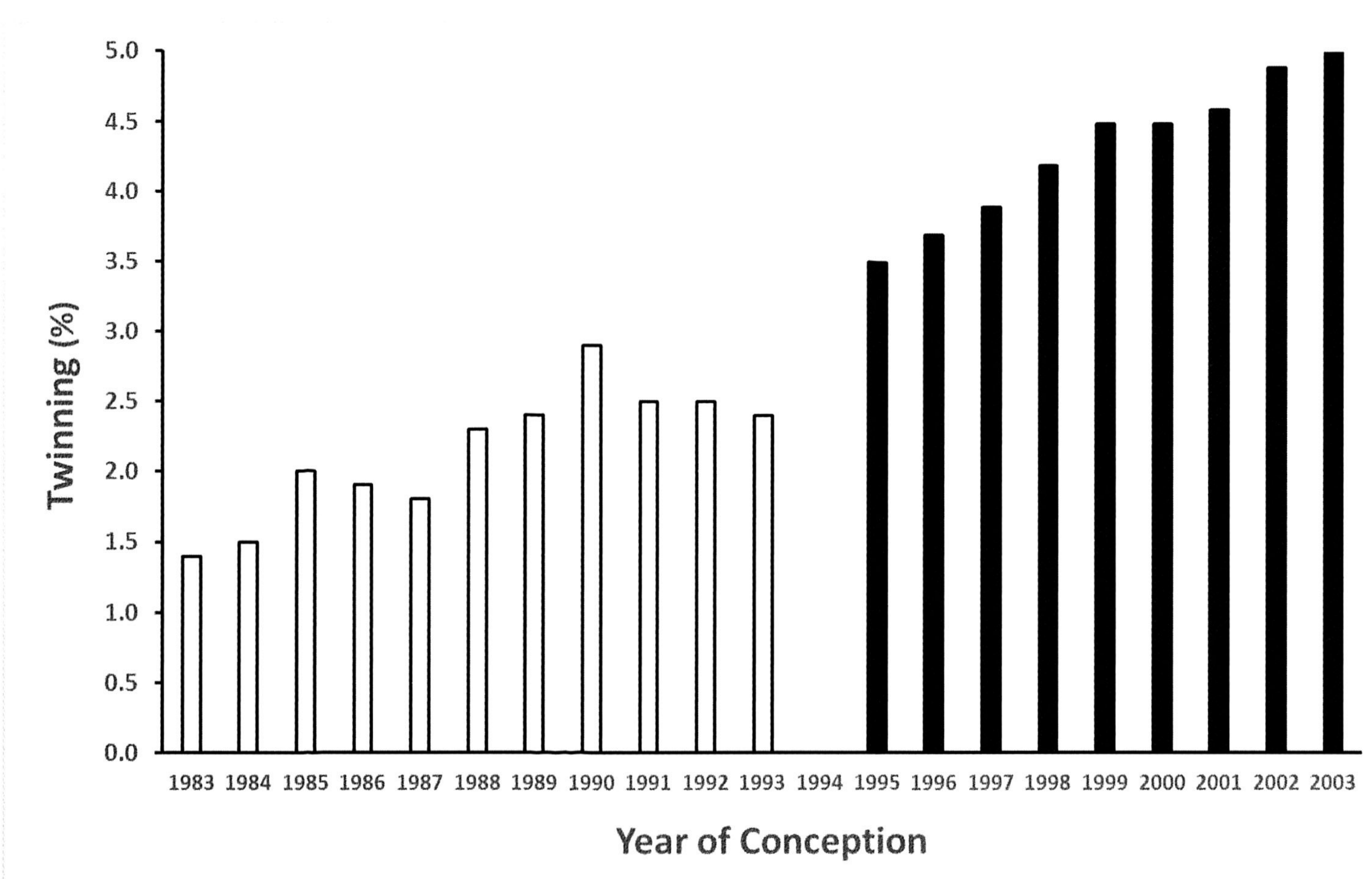

Figure 1. Trend in reported twinning rate in Holstein cows in the US from 1983 to 2003. Data includes nonlactating and lactating Holsteins. Open bars: data adapted from Kinsel et al[28]; Solid bars: data adapted from Silva del Rio et al.[50]

a causal relationship cannot be established based on our epidemiologic approach, the greater increase in twinning across time for lactating cows compared to non-lactating heifers further implicates the concurrent increase in milk production as a causative factor associated with twinning as speculated by Kinsel et al.[28]

Mechanism of Twinning

Dairy cows are a monotocous species meaning that they normally produce only one calf per pregnancy. Occasionally, however, the reproductive process in dairy cows results in twins or even triplets. 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. Mathematical models, including Weinberg's Differential Method[55] and Bonnier's Equation,[5] have been used to estimate the proportion of monozygotic twinning in dairy cows. Because monozygotic twins are always of the same sex, these equations are based on the skewing of the sex ratio away from mixed-sex twins and toward same-sex twins. These mathematical models, however, tend to overestimate the observed proportion of monozygotic twinning in dairy cows.[49] 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[49] (Table 1). Thus, monozygotic twinning occurs infrequently in Holstein cows, and the primary mechanism for twinning in dairy cows is double ovulations resulting in dizygotic twins (Table 1).

Endocrinology of Twinning

Progesterone

Progesterone is the most biologically active progestagen in cattle and is primarily produced and secreted by the corpus luteum during the estrous cycle and the placenta during pregnancy. It is now clear that low progesterone during growth of an ovulatory follicle is associated an increased incidence of double ovulation.[56] Cows in which the preovulatory follicle develops in the absence of progesterone from a corpus luteum have a greater incidence of co-dominant follicles resulting in double ovulation.[22] All dairy cows experience a low progesterone environment during the postpartum anovular period from calving to first ovulation. Postpartum double ovulation rate after a natural estrus was greater for anovular compared to cycling cows.[31] Gumen et al[21] classified cows as ovular vs anovular and submitted to a timed AI after an Ovsynch protocol (Table 2). Incidence of double ovulation to the first GnRH treatment of the Ovsynch protocol was greater for anovular compared to ovular cows, however, incidence of double ovulation to the second GnRH treatment of the Ovsynch protocol was similar between ovular and anovular cows.[21] Thus, the first postpartum ovulation results in a high double ovulation rate due to the lack of progesterone during growth of the preovulatory follicle, and the first exposure to progesterone during the postpartum anovular period reduces the incidence of double ovulation.

Table 1. Frequency of dizygotic and monozygotic twinning in a population of twin Holstein calves determined empirically based on polymorphic microsatellite DNA markers (adapted from Silva del Rio et al[49]).

		Classification	
Classification	**n**	**Dizygotic, % (n)**	**Monozygotic, % (n)**
Same-sex twins	67	92.5 (62)	7.5 (5)
Opposite-sex twins	40	100.0 (40)	-
All twins	107	95.3 (102)	4.7 (5)

Table 2. Ovulatory responses to GnRH of ovular and anovular cows submitted to an Ovsynch protocol (adapted from Gumen et al[21]).

Item	Ovular cows % (n/n)	Anovular cows % (n/n)	P-value
1st GnRH treatment of Ovsynch			
Overall ovulation rate	62 (72/117)	88 (29/33)	0.004
Double ovulation rate	4 (3/72)	41 (12/29)	<0.001
2nd GnRH treatment of Ovsynch			
Overall ovulation rate	97 (114/117)	94 (31/33)	0.323
Double ovulation rate	12 (14/114)	13 (4/31)	0.926
Cows with a short luteal phase	6 (7/114)	23 (7/31)	0.006

Ovarian Cysts

An association between dairy cows diagnosed with cystic ovaries and the incidence of double ovulation has been reported.[30,37,46] Based on a study population of nearly 9,000 cows, Bendixen et al[4] reported a greater incidence of twinning for cows diagnosed with cysts either during the first 40 d postpartum or after the estrus before AI. The odds of double ovulation were 3.3 times greater for cows identified with ovarian cysts and lacking a corpus luteum based on ultrasonography that were submitted to timed AI after an Ovsynch protocol than for cows inseminated after visual observation of estrus or non-cystic cows submitted to an Ovsynch protocol.[47] When cows were diagnosed cystic in the absence of a corpus luteum, the underlying association between the cystic condition and twinning was speculated to be due to the absence of progesterone rather than the presence of a cystic structure.[47]

Milk Production

With the exception of one study,[37] milk production near the time of ovulation has been positively associated with the incidence of double ovulation in dairy cattle. Fricke and Wiltbank[17] reported that the incidence of double ovulation after an Ovsynch protocol was greater for cows with above average milk production near the time of AI (40.7 kg/day) compared to cows with below average milk production (20% vs 7%, respectively), and this relationship was consistent within parity (1, 2 and 3+ lactations). Level of milk production during the 14 d immediately preceding a natural estrus was positively associated with the incidence of double ovulation in Holstein cows.[31] Figure 2 illustrates this relationship in which cows producing >40 kg/d in the 14 d preceding estrus had a dramatically increased incidence of double ovulation compared to cows producing <40 kg/d, with more than half of cows producing >50 kg/d having double ovulations. Compared to cows with a single dominant follicle, cows with co-dominant follicles during the first follicular wave produced more milk, and had decreased serum progesterone concentrations and increased FSH and LH concentrations during the 24 h period before the expected time of deviation of the dominant follicle.[32]

Physiology of Twinning

Taken together, 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. Steady state progesterone concentration in circulation is a balance between progesterone production by the corpus luteum and progesterone catabolism by the liver.[57] Because milk production is highly correlated (r = 0.88) with feed intake,[23] 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.[44] 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

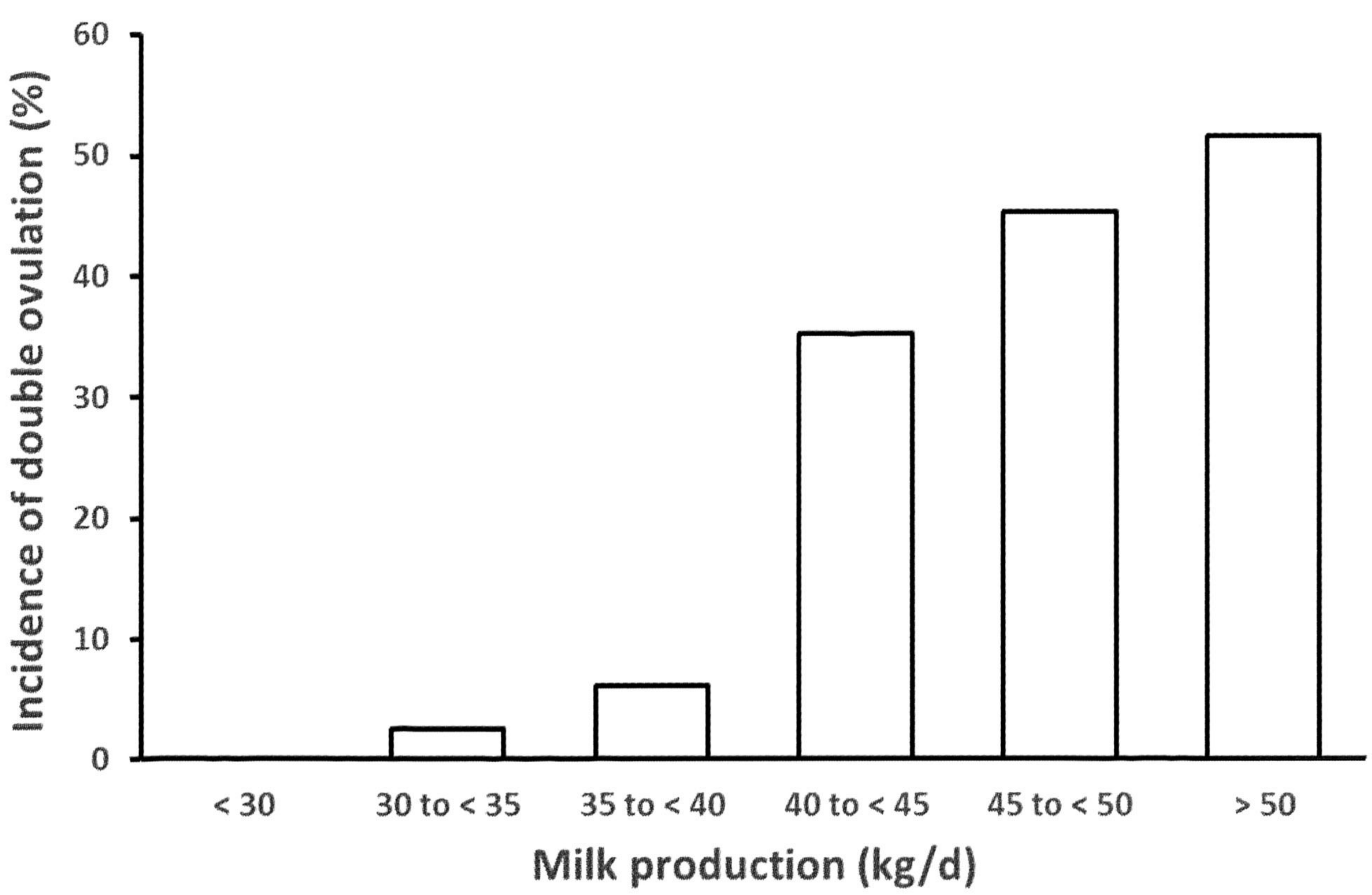

Figure 2. Incidence of double ovulation for Holstein cows based on milk production during the 14 d immediately preceding natural estrus (adapted from Lopez et al., 2005a).

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.[57] Because the primary mechanism for twinning is via double ovulation,[49] 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,[15] 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.[8,9,10] 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,[49] 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.[15] Because ~5% of all twins in Holsteins are monozygotic,[49] the presence of twins early during gestation based on the presence of multiple CL would slightly underestimate the number of twins. Thus, 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.[15]

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[17] (64% vs 45%), 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[47] (Table 3). 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.[36] Interestingly, the rate of pregnancy loss of 8% reported for bilateral twins by Lopez-Gatius and Hunter[36] 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,[45] and the 14% loss for cows with bilateral twins reported by Andreu-Vazquez et al.[2] Laterality of twins also affects the incidence of dystocia as well as calf survival rate. In a long-term experiment in which cows

Table 3. 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)

Table 4. Effect of twinning on gestation length and calf survival in cows genetically selected for multiple ovulations (adapted from Echternkamp et al., 2007).

				Calf survival (%)	
Pregnancy type	**No. of cows**	**Gestation length (d)**	**No. of calves**	**Birth**	**Weaning**
1 Left	300	284.5 ± 0.2	711	97.3 ± 1.1	87.6 ± 1.5
1 Right	360	284.2 ± 0.2	876	97.0 ± 1.0	88.3 ± 1.3
2 Left	96	277.2 ± 0.2	446	83.6 ± 1.4	70.7 ± 1.9
2 Right	167	277.0 ± 0.1	838	82.7 ± 1.0	73.2 ± 1.4
2 Bilateral	259	278.2 ± 0.1	1,158	94.0 ± 0.9	85.4 ± 1.2

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[11] (Table 4). Cows calving twins or triplets, however, had a greater incidence of dystocia than cows calving singletons.[11]

Pregnancy-Associated Glycoproteins

Circulating PAG levels in both blood and milk are associated with stage of gestation, parity, pregnancy loss, and milk production and can be accurately used to diagnose pregnancy status in dairy cows.[43] Cows carrying twin fetuses had greater serum PAG concentrations compared to cows carrying singleton fetuses,[35] and the difference in PAG levels increased as gestation progressed from 35 to 65 d after AI. In another experiment, the difference in PAG levels between cows carrying singleton vs twin fetuses was significant by 27 to 29 d after AI.[19] Several factors, however, may prevent accurate identification of cows carrying twins based on PAG levels. First, pregnancy loss from an initial pregnancy diagnosis to a pregnancy reconfirmation based on transrectal ultrasonography for cows diagnosed with twins was threefold greater compared to cows diagnosed with singletons.[47] In addition, some cows diagnosed with twins (6% to 11%) at an early pregnancy diagnosis undergo spontaneous embryo reduction and go on to calve singletons.[36,47] Thus, some cows diagnosed with twins early in gestation using PAG levels (or any other method) will calve singletons resulting in an overestimation of the true proportion of cows that calve twins. Second, because of the relatively long half-life of PAG levels in circulation, PAG levels did not decrease to levels similar to nonpregnant cows until 9.5 d after induction of pregnancy loss at 39 d in gestation.[19] If PAG levels were to be used as a diagnostic indicator for identification of cows carrying twins, the exact day of gestation would have to be submitted with the blood or milk sample at the time of testing. Even if this could be accomplished accurately, most commercial PAG tests are qualitative rather than quantitative and would not be useful for differentiating PAG levels as a diagnostic indicator of twins.

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.[19] 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[12] to \$300.[18] 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[20,54] (0.08 and 0.09, respectively), a prior incidence of twinning is a risk factor for subsequent twin births.[4,39] 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,[2] whereas pregnancy loss for cows carrying bilateral twins[36] was similar to that reported for Holsteins overall.[45] Finally, bilateral dizygotic twins had increased survival and body weight at birth, a longer gestation length, and less dystocia than unilateral dizygotic twins.[11] 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,[11] 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[25] and mares.[38] Reasonable success has been reported in mares when one twin was manually crushed when the procedure was performed before 30 d in gestation.[13] 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.[2,34]

In the first experiment,[34] 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.[36] 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.[34]

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[2] (Table 5). 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.[2]

Nutritional Management during the Transition Period

Energy demands during gestation are 50% to 70% greater for cows carrying twins compared to singletons,[29,40] yet cows carrying twins have less prepartum DMI than herdmates carrying singletons.[53] In addition, cows carrying twins have a decreased gestation length and therefore are less likely to experience a full three-week exposure to a close-up diet during the dry period.[39] Thus, feeding management strategies may offer an opportunity to mitigate the negative effects of twinning in dairy cattle.[3]

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.[48] 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[48] 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,[48] 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

Use of synchronization protocols for inseminating dairy cows has increased over time.[41] 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.[26] 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.[16] 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[33] 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.[6,52] A Double Ovsynch protocol[24,51] effectively presynchronizes

Table 5. 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)

cows to maximize progesterone during growth of the ovulatory follicle[57] and should thereby decrease double ovulation rate and subsequent dizygotic twinning.

To test the effect of progesterone during growth of the ovulatory follicle on the incidence of double ovulation, Holstein cows were randomly assigned to two presynchronization protocols that manipulated cows into either a high or a low progesterone environment during an Ovsynch protocol[7] (Table 6). Cows in the high progesterone treatment were submitted to a Double Ovsynch protocol[51] and had more progesterone at the first GnRH treatment of the Ovsynch protocol (1.8 vs 0.3 ng/mL, respectively) and at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol (4.4 vs 2.2 ng/mL) compared to cows in the low progesterone treatment.[7] Ovulatory response to the last GnRH treatment of the Ovsynch protocol was similar between treatments; however, cows in the low progesterone treatment, had more double ovulations compared to cows in the high progesterone treatment.[7] Furthermore, fertility was greater (48% vs 33%) and pregnancy loss was less (4% vs 16%) for cows in the high vs the low progesterone treatment.[7] Thus, increasing progesterone during growth of the ovulatory follicle dramatically decreased double ovulation rate while increasing fertility and decreasing subsequent pregnancy loss. Use of a hormonal synchronization protocol such as Double Ovsynch, therefore, decreases double ovulation rate thereby decreasing the dizygotic twinning rate in high-producing dairy cows. Hormonal manipulation may be the best strategy to decrease twinning in dairy cows by decreasing the incidence of double ovulation thereby decreasing conception of dizygotic twins.

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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Table 6. Effect of progesterone during growth of the preovulatory follicle on incidence of double ovulation in Holstein dairy cows (adapted from Cunha et al., 2008).

Double ovulation rate	Low progesterone % (n/n)	High progesterone % (n/n)	P-value
Overall	23 (54/231)	12 (32/261)	<0.001
Primiparous cows	20 (21/105)	10 (11/115)	0.022
Multiparous cows	26 (33/126)	14 (21/146)	0.011

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