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Photoperiod length and the estrus synchronization protocol used before AI affect the twin pregnancy rate in dairy cattle

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Abstract

This study addresses potential management risk factors affecting the incidence of twin pregnancies in high-producing dairy cows. Special attention was paid to the estrus synchronization protocol used before the AI resulting in pregnancy. Possible factors affecting the twin pregnancy rate were analyzed through binary logistic regression procedures on 2015 pregnant cows from July 2010 to July 2011. Twin pregnancy was recorded in 361 of the 2015 pregnancy diagnoses made (17.9%). Twin pregnancy rates differed among herds ($P < 0.001$) and ranged from 12.4% to 23.9%. Based on the odds ratios, the risk of twin pregnancy was reduced by factors of 0.65 or 0.71 when AI was performed during the warm season or an increasing photoperiod, respectively and increased by a factor of 1.11 for each unit increase in lactation number; by factors of 4.57 or 6.33 in cows that received a progesterone-releasing intravaginal device (PRID) plus 500 or 750 IU of equine chorionic gonadotropin (eCG) 28 days before the pregnancy AI, respectively; by a factor of 2.39 in cows with an ovarian cyst diagnosed in the 14 days prior to AI and treated with prostaglandins (PG); by factors of 1.94 or 3.91 in cows that received two PG doses during the 14 days prior to AI or cows that following failed PRID treatment had received PG started over the 28 days prior to AI, respectively; and by a factor of 2.58 in cows that had previously delivered twins compared to cows delivering singletons. Our results indicate that cow factors, such as lactation number and previous twining, as well as environmental factors, such as photoperiod and season and management related to synchronization protocols affect significantly the incidence of twin pregnancies.

Keywords: Multiple pregnancies; Environmental factors; Hormones; Pregnancy diagnosis; Cattle

1. Introduction

Interest in controlling the twinning rate in dairy herds has varied over the past decades. Early studies sought to increase the twinning rate by genetic selection and hormone administration to improve milk production and progeny per cow [1–4]. However, most authors today agree that twin pregnancies are undesirable in a dairy herd, and any anticipated benefits of twin calving are insufficient to outweigh such undesirable effects as a higher risk of pregnancy loss [5–7], abortion, retained placenta, dystocia, stillbirths and freemartins [8,9], or a longer calving to conception interval and higher culling rate for cows delivering twins [10–12]. In effect, the few studies assessing the economic consequences of twinning have described that twin pregnancies reduce herd profitability, with an average loss of income attributed to cows delivering twins estimated at $74 to $108 [13,14]. In addition, the real economic impacts of twinning are probably on the rise since the twinning rate has considerable increased over the past 20 yrs and

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estimates run at 9%, and even exceed 12% in some herds [15].

Prior work has addressed the management factors affecting the prevalence of twinning and double ovulation [16–21]. However, it should be noted that neither the incidence of twins at calving nor double ovulation are a true reflection of the rate of twin pregnancy. Thus, a vast difference exists between the incidence of double ovulation and the twinning rate [22]. Recording twins on pregnancy diagnosis (PD) is a more appropriate end point to consider when examining possible factors related to the incidence of twin pregnancies. Given the high risk of pregnancy loss and abortion, the actual occurrence of twin pregnancies may have been underestimated in studies designed to examine twin births. In addition, while the twinning rate (i.e., incidence of twin birth calvings) has been steadily rising, fertility has drastically declined in high producing cows [23,24], and this decline has prompted the development of estrus synchronization protocols based on several combinations of different hormones. However, as far as we are aware no study has yet explored how these protocols could affect the incidence of twin pregnancies. The present study was designed to assess potential cow, environmental and management risk factors affecting the incidence of twin pregnancies in high-producing dairy cows with special emphasis placed on the estrus synchronization protocols applied before insemination.

2. Materials and methods

2.1. Animals

The data analyzed were derived from 2015 cows that became pregnant over a one-yr period (July 2010-July 2011) belonging to three commercial dairy herds in northeastern Spain. Mean annual milk production for the three herds, comprising 1600, 640 and 1091 lactating cows, were 11 797, 11 350 and 11 150 kg, respectively. Cows were housed in free stalls with concrete slatted floors and cubicles, milked three times daily and fed complete rations. Feeds consisted of cotton-seed hulls, barley, corn, soybean and bran, and roughage, primarily corn, barley and alfalfa silages and alfalfa hay. Rations were in line with NRC recommendations [25]. The culling rates for the three herds during the study period were 27%, 27% and 34%, respectively.

2.2. Reproductive management

Herds were maintained on a weekly reproductive health program. Daily checks were performed during the first two wks after calving to diagnose and treat any puerperal disease. Cows were examined at least once within 35 to 50 days postpartum to check for ovarian and uterine structures. Reproductive disorders, such as incomplete uterine involution, endometritis, pyometra, and ovarian cysts, diagnosed either in a gynecologic postpartum examination or at any subsequent time, were treated until resolved. The voluntary waiting period for the herds was 50 days. Only cows free of detectable reproductive disorders were inseminated. Cows more than 60 days in milk showing no estrus signs within the previous 21 days were examined weekly until specific estrus synchronization or until AI during a natural estrus [26]. At the weekly visit, ovarian structures were recorded. If a cow had a corpus luteum (CL) estimated to be at least 15 mm (mean of the maximum and minimum diameters), the animal was synchronized for estrus with a single prostaglandin dose (PG; 25 mg dinoprost i.m.; Enzaprost, CEVA Salud Animal, Barcelona, Spain). Cows with persistent follicles (a follicular structure of at least 8–15 mm detected in two consecutive exams 7 days apart in the absence of a corpus luteum and no estrous signs [27]) were fitted with a progesterone releasing intravaginal device (PRID, containing 1.55 g of progesterone; CEVA Salud Animal, Barcelona, Spain) for 9 days and given the PG dose 24 h before PRID removal [26,28]. A subset of cows with CL also received a PRID for 9 days. At the time of PRID removal, some cows received randomly a dose of 500 or 750 IU of equine chorionic gonadotropin (eCG; i.m.; Syncrostim, CEVA Salud Animal, Barcelona, Spain). In one of the herds, PRIDs were removed on Day 5 followed by two doses of PG given 24 h apart. Ovarian cysts (a follicular structure larger than 15 mm detected in either one or both ovaries in the absence of CL and uterine tone [29]) were ruptured and treated either with PG [26,29] or PRID [29] following rupture. Under a reproductive control on a weekly basis, cyst treatment with manual rupture favors reproductive efficiency of the cow and does not impair her productive life [29]. All cows not showing estrus signs during the 14 days after a treatment based on PG or PRID (considered as a failed synchronization protocol) were returned to the reproductive control program. Cows were inseminated after a time-fixed schedule or at estrus detection based on walking activity [30] (pedometer system: Afifarm; SAE Afikim) using the frozen sperm of bulls of proven fertility. A dose of gonadotropin releasing hormone (GnRH; 100 μg i.m.; Cystoreline, CEVA Salud Animal, Barcelona, Spain) was given 12 or 24 h before AI

to all cows under a time-fixed protocol or at the time of AI to cows showing natural estrus.

The PD was performed by ultrasonography 28 to 34 days post AI. Scanning was performed along the dorso/lateral surface of each uterine horn. The presence of twins was recorded after the observation of two embryos in different positions within one uterine horn on two screen scans, two embryos simultaneously present on the screen or one embryo in each uterine horn. Cows diagnosed as not pregnant were either returned to the reproductive program or registered for culling. All gynecologic exams and pregnancy diagnoses were performed by the same operator.

2.3. Data collection and analysis

The following data were recorded for each cow: number of embryos observed on PD (twins/triplets or singletons), cow factors: lactation number, milk production and milk protein and fat contents (average for the month of AI), days in milk at AI, insemination number and date, ovarian structures recorded in the 14 days prior to AI, walking activity on the day of AI, twinning and occurrence of stillbirth at previous calving, pregnancy loss (before or after Day 90 of gestation) over the current lactation and prior to the analyzed positive PD, and number of embryos/fetuses aborted, and management factors: herd, inseminating bull, estrus synchronization protocol in the 28 days prior to AI, type of AI (time-fixed or at estrus detection) and GnRH administration and time before AI (0, 12 or 24 h).

In our geographic area of study there are two clearly differentiated weather periods: warm (May to September) and cold (October to April) [31]. Reproductive variables are impaired during the warm season [24,32]. For this reason, insemination dates were used to analyze the effect of AI season (environmental factor, warm vs. cold period) on the occurrence of a twin PD. Additionally, since endocrine patterns of gestation are affected by photoperiod length [33], insemination dates were also used to analyze the effect of the photoperiod (environmental factor, increasing day length, December 21: sunrise at 8: 20 h and sunset at 17: 29 h, to June 20, vs. decreasing day length, June 21: sunrise at 5: 23 h and sunset at 20: 35 h, to December 20) on the occurrence of twin pregnancies. For the analyses, data on ovarian structures, the estrus synchronization protocol and type of AI were grouped and coded (Table 1).

A binary logistic regression analysis was performed to determine the relative contribution of each variable to the probability of a twin pregnancy. Triplet pregnancies (n = 43) were considered as twin pregnancies. The dependent variable was twin pregnancy; the categorical and continuous variables possibly affecting twin PD are listed in Table 2. Regression analysis (PASW Statistics 18, SPSS, Inc., Chicago, IL) was performed according to the method of Hosmer and Lemeshow [34]. Basically, this method consists of six steps as follows: preliminary screening of all variables for univariate associations; construction of a full model using all the significant variables resulting from the univariate analysis; stepwise removal of non-significant variables from the full model; comparison of the reduced model with the previous model for model fit and confounding; evaluation of interactions among variables; and assessment of model fit using Hosmer-Lemeshow statistics. Variables with univariate associations showing P values <0.25 were included in the initial model. Modeling was continued until all the main effects or interaction terms were significant according to the Wald statistic at $P < 0.05$.

Regression coefficients from the logistic regression were exponentiated to obtain the odds ratio and corresponding 95% confidence interval associated with each factor. An odds ratio significantly higher (or lower) than 1 indicates an increased (or reduced) risk of twin PD if the factor is present and is a dichotomous variable. For continuous variables, an odds ratio significantly higher (or lower) than 1 implies an increased (or reduced) risk of twin PD with each 1 unit increase in the value of this factor. For class variables, one class of each variable was considered as the reference, and an odds ratio significantly higher (or lower) than 1 for any other class of this variable was taken to indicate an increased (or reduced) risk of twin PD when compared to the reference class. Mean values are expressed as the mean ± standard deviation (SD).

3. Results

Twin PD was recorded in 361 of the 2015 pregnancies analyzed (17.9%). In 208 of the 361 pregnancies (57.6%) the twin embryos were located in the same uterine horn (152 and 56 in the right and the left uterine horns, respectively) and 153 were bilateral twin pregnancies. The incidence of twin pregnancies ranged from 12.4% to 23.9% among the three herds included in the study. Twin PD was recorded in 132 out of 885 (14.9%), 90 out of 483 (18.7%), 71 out of 348 (20.4%), 41 out of 187 (21.9%) and 27 out of 112 (24.1%) cows in their first, second, third, fourth and fifth or higher lactation, respectively. The odds ratios for the factors found to significantly affect twin pregnancies are

Table 1
Hormonal treatments.

Hormonal treatment code	Ovarian structure recorded over the 14 days prior to AI	Estrus synchonization protocol Applied over the 28 days prior to AI	Type of AI	n of cows enrolled
0 natural estrus	Unknown or follicle	Absence of treatment	Estrus detection	1241
1 PRID_FOL	Persistent follicle	Prid[ab] during 9 days	Estrus detection	171
2 PRID_FOL5d	Persistent follicle	Prid[ac] during 5 days	Time-fixed	97
3 PRID_FOL500eCG	Persistent follicle	Prid[ab] during 9 days and 500 UI of eCG[d] at the time of PRID removal	Estrus detection or time-fixed	23
4 PRID_FOL750eCG	Persistent follicle	Prid[ab] during 9 days and 750 UI of eCG[d] at the time of PRID removal	Estrus detection or time-fixed	15
5 PRID_CL	Corpus luteum	Prid[ab] during 9 days	Estrus detection or time-fixed	29
6 PRID_CL500eCG	Corpus luteum	Prid[ab] during 9 days and 500 UI of eCG[d] at the time of PRID removal	Estrus detection or time-fixed	20
7 PRID_CL750eCG	Corpus luteum	Prid[ab] during 9 days and 750 UI of eCG[d] at the time of PRID removal	Estrus detection or time-fixed	21
8 PG	Corpus luteum	PG[e] (with no previous protocol Over the 28 days prior to AI)	Estrus detection	304
9 PRID_Cyst	Ovarian cyst	Prid[a] during 9 days	Estrus detection	55
10 PG_Cyst	Ovarian cyst	PG[e]	Estrus detection	28
11 failed PG then PG	Corpus luteum	PG[e] (with a failed[f] PG given Over the 14 days prior to AI)	Estrus detection	116
12 failed PRID then PG	Corpus luteum	PG[e] (with a failed[f] PRID[ab] given Over the 28 days prior to AI)	Estrus detection	10

[a] Progesterone releasing intravaginal device (containing 1.55 g of progesterone; CEVA Salud Animal, Barcelona, Spain).
[b] The protocol includes a prostaglandin G2α given 24 h before PRID removal.
[c] The protocol includes two prostaglandins G2α given at the time of PRID removal and 24 h later.
[d] Equine chorionic gonadotropin (i.m.; Syncrostim, CEVA Salud Animal, Barcelona, Spain).
[e] Prostaglandin G2α (25 mg dinoprost i.m.; Enzaprost, CEVA Salud Animal, Barcelona, Spain).
[f] A synchronization treatment based on PG or PRID was considered as failed when a cow did not show estrus signs during the 14 days after treatment, was returned to the reproductive control program and with a corpus luteum (CL) present received a PG dosis.

shown in Table 2. No significant effects were observed of days in milk at AI, milk production, milk fat and protein contents, insemination number, inseminating bull, walking activity recorded on the day of AI, GnRH administration before AI, the occurrence of stillbirth in previous calvings and previous single or twin pregnancy losses during the current lactation. Plausible interactions, such as season-photoperiod and lactation number-synchronized/natural estrus were not detected.

4. Discussion

The overall incidence of twins was 17.9%, a figure closer to the lower end of the range of 15 to 37% reported for double ovulation in high producing dairy cows [21,35,36] than the 9% currently reported for twin births [15]. This expected result reinforces the idea that neither the incidence of twins at calving nor double ovulation following insemination are a true reflection of the twin pregnancy rate. The high incidence of pregnancy loss in twin pregnancies and, no less important, the fact that natural twin reduction usually occurs following a positive pregnancy diagnosis [37–39], will logically reduce the number of twins recorded at parturition. On the contrary, since double ovulation may occur in cows conceiving a single embryo [6,7], the rate of twin pregnancy will be lower than that of double ovulation.

The twin pregnancy rate differed significantly among the three herds. This could reflect differences in management practices not considered in this study. However, irrespective of the herd, our results still reveal how management factors can intensely contribute to the incidence of twin pregnancies.

The estrus synchronization protocol used before AI affected the twin pregnancy rate. Among the 12 estrus synchronization protocols investigated here, six were found to significantly increase the incidence of twins compared to cows conceiving at natural estrus. The risk of twin pregnancy was not significantly greater in the cows subjected to a progesterone-based protocol for 5 or 9 days (PRID). However, although calculated on a small study population, the odds ratio reached figures of 4.6 or 6.3 for the subset of anestrous cows that were

Table 2
Cow, environmental and management factors assessed and odds ratios for the factors included in the final binary logistic regression model for twin pregnancy (TP).

Factor	Mean ± SD (ranges)	Class description	N of pregnancies	TP (%)	Odds ratio	95% CI	P value
Environmental factors							
Season		Cold	1560	19.3	Reference	—	—
		Warm	455	13.2	0.646	0.47–0.90	0.009
Photoperiod		Decreasing	1058	17.7	Reference	—	—
		Increasing	957	18.2	0.705	0.54–092	0.010
Management factors							
Herd		1	925	12.4	Reference	—	—
		2	429	20.5	—	—	NS
		3	661	23.9	2.413	1.78–3.26	<0.001
Inseminating bull[a]		30	2015	17.9	—	—	NS
Hormonal treatment[b]		0 natural estrus	1241	16.1	Reference	—	—
		1 PRID_FOL	131	20.6	—	—	NS
		2 PRID_FOL5d	22	13.6	—	—	NS
		3 PRID_FOL500eCG	23	43.5	4.572	1.9–11.08	0.001
		4 PRID_FOL750eCG	15	53.3	6.329	2.17–18.5	0.001
		5 PRID_CL	29	20.7	—	—	NS
		6 PRID_CL500eCG	20	15.0	—	—	NS
		7 PRID_CL750eCG	21	38.1	3.045	1.17–7.94	0.023
		8 PG	304	14.1	—	—	NS
		9 PRID_Cyst	55	14.5	—	—	NS
		10 PG_Cyst	28	35.7	2.390	1.06–5.38	0.035
		11 failed PG then PG	116	26.7	1.937	1.23–3.06	0.005
		12 failed PRID then PG	10	40.6	3.913	1.06–14.51	0.041
GnRH[c]		Absence	656	23.8	Reference	—	—
		At AI	1311	15.1	—	—	NS
		12 h prior to AI	22	13.6	—	—	NS
		24 h prior to AI	26	23.1	—	—	NS
Cow factors							
Previous calving		Singleton	1941	17.4	Reference	—	—
		Twins	74	32.4	2.572	1.52–4.36	<0.001
Stillbirth/s previous calving		Absence	1831	17.7	Reference	—	—
		Presence	184	19.6	—	—	NS
Previous pregnancy loss		Absence	1867	17.7	Reference	—	—
		Single pregnancy loss	103	17.5	—	—	NS
		Twin pregnancy loss	45	26.7	—	—	NS
Lactation number	2.12 ± 1.33 (1–10)	Continuous	2015	17.9	1.106	1.01–1.21	0.028
Days in milk at conception	125.16 ± 71.11 (34–635)	Continuous	2015	17.9	—	—	NS
Insemination number	2.40 ± 1.84 (1–14)	Continuous	2015	17.9	—	—	NS
Milk production (kg)[d]	41.41 ± 9.18 (15.0–78.2)	Continuous	2015	17.9	—	—	NS
Protein content (%)[d]	3.34 ± 0.28 (1.94–4.67)	Continuous	2015	17.9	—	—	NS
Fat content (%)[d]	3.32 ± 0.75 (1.20–6.68)	Continuous	2015	17.9	—	—	NS
Walking activity at estrus	562.27 ± 221 (43–1498)	Continuous	2015	17.9	—	—	NS

Likelihood-ratio test=102.711; 18 df; $P < 0.001$. Hosmer Lemeshow goodness-of-fit test = 5.695; 7 df; $P = 0.576$ (the model fits). Nagelkerke $R^2 = 0.084$.

NS, not significant; PRID, progesterone-releasing intravaginal device; TP, twin pregnancy.

[a] Bulls with less than 10 AI recorded (n 38) were group together.

[b] List of abbreviations for the hormonal treatments: PRID, Progesterone releasing intravaginal device (containing 1.55 g of progesterone; CEVA Salud Animal, Barcelona, Spain); eCG, Equine chorionic gonadotropin (i.m.; Syncrostim, CEVA Salud Animal, Barcelona, Spain); PG, Prostaglandin G2α (25 mg dinoprost i.m.; Enzaprost, CEVA Salud Animal, Barcelona, Spain).

[c] Cystoreline (CEVA Salud Animal, Barcelona, Spain) 100 μg i.m.

[d] Mean at the month of pregnant AI.

administered 500 or 750 IU of eCG at the time of PRID removal. Because of its LH- and FSH like activity and its long half-life [40], eCG has been widely used to induce multiple ovulation in small ruminants [41], beef and dairy cattle [42]. Notwithstanding, the mechanisms whereby eCG exerts its effects remain to be determined. Thus, if eCG promotes double ovulation, it could be that in cows producing two oocytes, short exposure to exogenous elevated progesterone concentrations immediately prior to insemination will enhance embryo survival [43].

The condition of ovarian cysts has been linked to subsequent double ovulation and twinning [18,44–45]. With regard to twinning, Kinsel, et al. [18] stated that the probability of delivering twins was increased by a factor of 2.52 in cows with an untreated ovarian cyst, but was lowered when the cows had been treated with PG or GnRH. The authors of this study failed to mention the time interval between ovarian cyst diagnosis and pregnancy AI. In our study, all cows diagnosed with an ovarian cyst over the 14 days prior to AI received treatment. The risk of twins on PD was 2.39 times higher in cows treated with PG, whereas PRID treatment did not increase the twin pregnancy rate. Differences between treatments could be attributed to the time from treatment to AI. Thus, while cows receiving a PRID were not inseminated during the following 9 days, cows treated with PG that showed signs of estrus within the following 24 to 48 h were inseminated. In the latter cows, the presence of the ovarian cyst could be related to double ovulation [44]. Similarly, cows that received 2 doses of PG over the 14 days prior to AI (indicating a possible persistent CL) and cows that received a PG dose following a failed PRID protocol over the 28 days prior to AI had a 1.94 and 3.91 times higher risk of twin PD, respectively, than cows conceiving after a natural estrus. In these cows ovulating after a failed estrus synchronization protocol, a certain degree of ovarian dysfunction might be assumed and this could be responsible for the higher risk of double ovulation. The scenario might be similar to that of anovular cows spontaneously recovering, in which the multiple ovulation rate at first ovulation reaches 46.3% [20]. On the other hand, the incidence of twin pregnancy was unaffected by synchronization with a single dose of PG or application of GnRH immediately before or at AI, in agreement with previous studies in which PG, human chorionic gonadotropin and estradiol were included in the synchronization protocol 21, nor by the administration of GnRH at AI [46]. In contrast, some authors noted an increased risk of twinning when cows had received PG alone [18] or in combination with FSH/LH or GnRH [17]. The authors of these studies also reported a significant effect of the administration of GnRH prior to AI [17,18]. The timing of these hormones was not mentioned in the latter studies and this might be crucial for the outcome of the subsequent AI.

The duration of estrus has been reported to be shorter in cows experiencing multiple ovulations [20], and walking activity has been strongly correlated to fertility [30]. Thus, we should expect some link between walking activity during the day of AI and the number of embryos at pregnancy diagnosis. However, differences were not found in walking activity in cows with single or twin pregnancies. This could be explained by the inclusion of cows conceiving after a time-fixed insemination protocol in which an increase in activity prior to AI might have not occurred. Further, eCG treatment on PRID removal seems to reduce estrous behavior [47].

As anticipated, the likelihood of twin pregnancy increased with parity (a unit increase in lactation number led to a 1.11-fold increased risk of twin pregnancy). Likewise, older cows have been described to be more likely to deliver twins [13,16–18] and to experience double ovulation [19–21]. Further, cows that had delivered twins in the previous calving showed an increased risk of twin PD. This is in agreement with the findings of previous studies [17] and, besides increased parity, probably reflects a maternal trait.

Cows inseminated during the cold season were more likely to conceive twins. This is in agreement with studies describing a higher rate of double ovulation during the cold season [21] and the fact that most twins are born during the summer [16,17]. Some authors attribute seasonality of twinning to feed supplementation during the fall [16]. However, in the current intensive-production setting, disturbed ovarian activity due to heat stress [21] seems to be a more plausible explanation.

Regardless of season, decreasing day length at the time of conception was found to increase the likelihood of twin pregnancy. Whether to do with seasonality [33] or with what remains of an ancient strategy in cows and other mammals increasing the chances of parturition when feed avalilabilty is higher, warrants further investigation.

Although high milk production has been traditionally linked to the incidence of double ovulation [19,20] and is thought to be the single largest contributor to the recent increase in the twinning rate [18], we observed

no effect of milk production, milk fat and protein contents or days in milk at AI on the risk of twin pregnancy. Expedited liver metabolism and hormonal clearance in high-producing dairy cows have been linked to impaired follicular development favoring follicular codominance and double ovulation [19]. Our results however do not support this hypothesis. In fact, our univariate analyses performed prior to logistic regression (data not shown) indicated that milk production was lower for cows carrying twins than cows carrying singletons (40.5 ± 9.3 kg vs. 41.7 ± 9.1 kg, twins vs. single PD, respectively, P = 0.032). However, this effect was not detectable after adjusting for other risk factors in the logistic regression analysis. This finding reinforces the results of a study conducted in our geographic area in which a 1 kg increase in milk yield led to a 0.97-fold reduced risk of double ovulation [21].

Collectively, our results indicate that cow factors, such as lactation number and previous twining, as well as environmental factors, such as photoperiod and season and management related to synchronization protocols affect significantly the incidence of twin pregnancies. Attention should be focused on implementing palliative practices, such as embryo reduction in twin pregnant cows [39,48], in combination to reduce estrous synchronization protocols to prevent increasing twinning rates in the herds.

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References

[1] Rutledge JJ. Twinning in cattle. J Anim Sci 1975;40:803–15.
[2] Echternkamp SE, Gregory KE, Dickerson GE, Cundiff LV, Koch RM, Van Vleck LD. Twinning in cattle: II. Genetic and environmental effects on ovulation rate in puerperal heifers and postpartum cows and the effects of ovulation rate on embryonic survival. J Anim Sci 1990;68:1877–88.
[3] Echternkamp SE. Fetal development in cattle with multiple ovulations. J Anim Sci 1992;70:2309–21.
[4] Van Vleck LD, Gregory KE, Echternkamp SE. Ovulation rate and twinning rate in cattle: heritabilities and genetic correlation. J Anim Sci 1991;69:3213–9.
[5] López-Gatius F, Santolaria P, Yániz J, Rutllant J, López-Béjar M. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. Theriogenology 2002;57:1251–61.
[6] Lopez-Gatius F, Szenci O, Bech-Sàbat G, García-Ispierto I, Serrano B, Santolaria P, et al. Factors of non-infectious nature affecting late embryonic and early foetal loss in high producing dairy herds in north-eastern Spain. Magyar Allatorvosok Lapja 2009;131:515–31.
[7] Lopez-Gatius F, García-Ispierto I. Ultrasound and endocrine findings that help to assess the risk of late embryo/early foetal loss by non-infectious cause in dairy cattle. Reprod Domest Anim 2010;45(Suppl 3):15–24.
[8] Echternkamp SE, Gregory KE. Effects of twinning on gestation length, retained placenta, and dystocia. J Anim Sci 1999;77:39–47.
[9] Echternkamp SE, Gregory KE. Effects of twinning on postpartum reproductive performance in cattle selected for twin births. J Anim Sci 1999;77:48–60.
[10] Chapin CA, Van Vleck LD. Effects of twinning on lactation and days open in Holsteins. J Dairy Sci 1980;63:1881–6.
[11] Gregory KE, Echternkamp SE, Dickerson GE, Cundiff LV, Koch RM, Van Vleck LD. Twinning in cattle: III. Effects of twinning on dystocia, reproductive traits, calf survival, calf growth and cow productivity. J Anim Sci 1990;68:3133–44.
[12] Bicalho RC, Cheong SH, Galvão KN, Warnick LD, Guard CL. Effect of twin birth calvings on milk production, reproductive performance, and survival of lactating cows. J Am Vet Med Assoc 2007;231:1390–7.
[13] Eddy RG, Davies O, David C. An economic assessment of twin births in British dairy herds. Vet Rec 1991;129:526–9.
[14] Beerepoot GM, Dykhuizen AA, Nielen M, Schukken YH. The economics of naturally occurring twinning in dairy cattle. J Dairy Sci 1992;75:1044–51.
[15] Silva del Río N, Stewart S, Rapnicki P, Chang YM, Fricke PM. An observational analysis of twin births, calf sex ratio, and calf mortality in Holstein dairy cattle. J Dairy Sci 2007;90:1255–64.
[16] Cady RA, Van Vleck LD. Factors affecting twinning and effects twinning of in Holstein dairy cattle. J Anim Sci 1978;46:950–6.
[17] Nielen M, Schukken YH, Scholl DT, Wilbrink HJ, Brand A. Twinning in dairy cattle: A study of risk factors and effects. Theriogenology 1989;32:845–62.
[18] Kinsel ML, Marsh WE, Ruegg PL, Etherington WG. Risk factors for twinning in dairy cows. J Dairy Sci 1998;81:989–93.
[19] Fricke PM, Wiltbank MC. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology 1999;52:1133–43.
[20] Lopez H, Caraviello DZ, Satter LD, Fricke PM, Wiltbank MC. Relationship between level of milk production and multiple ovulations in lactating Dairy cows. J Dairy Sci 2005;88:2783–93.
[21] López-Gatius F, López-Béjar M, Fenech M, Hunter RH. Ovulation failure and double ovulation in dairy cattle: risk factors and effects. Theriogenology 2005;63:1298–307.
[22] Kidder HE, Barrett GR, Casida LE. A study of ovulations in six families of Holstein-Friesians. J Dairy Sci 1952;35:436–44.
[23] Lucy MC. Reproductive loss in high producing dairy cattle: where will it end? J Dairy Sci 2001;84:1277–93.
[24] Lopez-Gatius F. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. Theriogenology 2003;60:89–99.
[25] National Research Council. Nutrient Requirements of Dairy Cattle, 7th rev. ed. Washington, DC: National Academic Science; 2001.
[26] López-Gatius F, Mirzaei A, Santolaria P, Bech-Sàbat G, Nogareda C, García-Ispierto I, et al. Factors affecting the response to

the specific treatment of several forms of clinical anestrus in high producing dairy cows. Theriogenology 2008;69:1095–103.
[27] López-Gatius F, Santolaria P, Yániz J, Rutllant J, López-Béjar M. Persistent ovarian follicles in dairy cows: A therapeutic approach. Theriogenology 2001;56:649–59.
[28] Garcia-Ispierto I, López-Gatius F, Bech-Sàbat G, Yániz JL, Angulo E, Maris C, et al. Effects of a progesterone-based oestrous synchronization protocol 6 in 51- to 57-day postpartum high-producing dairy cows. Reprod Domest Anim 2010;45: e168–73.
[29] Hanzen Ch, Bascon F, Theron L, López-Gatius F. Ovarian cysts in bovine. III, in Therapeutic Aspects. Ann Med Vet 2008;152: 103–15.
[30] López-Gatius F, Santolaria P, Mundet I, Yániz JL. Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology 2005;63:1419–29.
[31] Labèrnia J, López-Gatius F, Santolaria P, Hanzen C, Laurent Y, et al. Influence of calving season on the interactions among reproductive disorders of dairy cows. Anim Sci 1998;67:387–93.
[32] García-Ispierto I, López-Gatius F, Bech-Sabat G, Santolaria P, Yániz JL, Nogareda C, et al. Climate factors affecting conception rate in high producing dairy cows in northeasterm Spain. Theriogenology 2007;67:1379–85.
[33] García-Ispierto I, López-Gatius F, Almería S, Yáñiz J, Santolaria O, Serrano B, et al. Factors affecting plasma prolactin concentrations throughout gestation in high producing dairy cows. Domest Anim Endocrinol 2009;36:57–66.
[34] Hosmer DW, Lemeshow S. Applied logistic regression. Wiley; 1989.
[35] Santos JE, Huber TH, Theurer CB, Nussio CB, Nussio LG, Tarazon M, et al. Effects of grain processing and bovine somatotropin on metabolism and ovarian activity of dairy cows during early lactation. J Dairy Sci 2000;83:1004–15.
[36] Sartori R, Haughian JM, Shaver RD, Rosa GJ, Wiltbank MC. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J Dairy Sci 2004;87:905–20.
[37] López-Gatius F, Hunter RH. Spontaneous reduction of advanced twin embryos: its occurrence and clinical relevance in dairy cattle. Theriogenology 2005;63:118–25.
[38] López-Gatius F, García-Ispierto I, Hunter RH. Factors affecting spontaneous reduction of corpora lutea and twin embryos during the late embryonic/early fetal period in multiple-ovulating dairy cows. Theriogenology 2010;73:293–9.
[39] Andreu-Vázquez C, Garcia-Ispierto I, López-Béjar M, de Sousa NM, Beckers JF, López-Gatius F. Clinical implications of induced twin reduction in dairy cattle. Theriogenology 2011;76: 512–21.
[40] Bevers MM, Dieleman SJ, Van Tol HTM, Blankenstein DM, Van den Broek J. Changes in pulsatile secretion patterns of LH, FSH, progesterone, androstenedione andoestradiol in cows after superovulation with PMSG. J Reprod Fert 1989;87:745–54.
[41] Rahman ANMA, Addullah RB, Wan-Khadijah WE. Estrus synchronization and superovulation in goats: a review. J Biol Sci 2008;8:1129–37.
[42] Mapletoft RJ, Bennett SK, Adams GP. Recent advances in the superovulation in cattle. Reprod Nutr Dev 2002;42:601–11.
[43] Diskin MG, Murphy JJ, Sreenan JM. Embryo survival in dairy cows managed under pastoral conditions. Anim Reprod Sci 2006;96:297–311.
[44] Labhsetwar AP, Tyler WJ, Casida LE. Analysis of variation in some factors affecting multiple ovulations in Holstein cattle. J Dairy Sci 1963;46:840–2.
[45] Kesler DJ, Garverick HA. Ovarian cysts in dairy cattle: a review. J Dairy Sci 1982;55:1147–59.
[46] López-Gatius F, Santolaria P, Martino A, Delétang F, De Rensis F. The effects of GnRH treatment at the time of AI and 12 days later on reproductive performance of high producing dairy cows during the warm season in northeastern Spain. Theriogenology 2006;65:820–30.
[47] Garcia-Ispierto I, López-Helguera I, Martino A, López-Gatius F. Reproductive performance of anoestrous high-producing dairy cows improved by adding equine chorionic gonadotrophin to a progesterone-based oestrous synchronizing protocol. Reprod Domest Anim. 2011 [10.1111/j.1439-0531.2011.01954.x].
[48] López-Gatius F. The effect on pregnancy rate of progesterone administration after manual reduction of twin embryos in dairy cattle. J Vet Med A Physiol Pathol Clin Med 2005;52: 199–201.