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### CONCOMITANT FACTORS

### AFFECTING GESTATION LENGTH AND PERINATAL MORTALITY IN HOLSTEIN-FRIESIAN COWS

#### N. M. Bogas<sup>1</sup>, A. V. Sherasiya<sup>2</sup>, J. C. Simões<sup>3</sup>

- <sup>1</sup> Veterinarian, The Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes e Alto Douro, Vila Real, Portugal, e-mail: neca.smb@gmail.com
- <sup>2</sup> Veterinarian, Ex-Veterinary Officer, Wankaner, India, e-mail: sherasiyaanjum@gmail.com; ORCID ID 0000-0002-1598-1820
- <sup>3</sup> PhD, The Veterinary and Animal Research Centre (CECAV), Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal, e-mail: jsimoes@utad.pt; ORCID ID 0000-0002-4997-3933

#### **SUMMARY**

The objective of this study was to identify and quantify factors presenting simultaneous moderate or strong influence on gestation length and perinatal mortality (until 4 days postpartum) in Holstein-Frisian dams. The overall gestation length least square mean was  $278.2 \pm 0.2$  days (n = 962). According to the multivariate model (p < 0.001), a shorter gestation length was observed in Holstein-Friesian (-2.0 days) and Red Holstein-Friesian (-3.9 days) breed sires whereas Brown Swiss (2.0 days) and Aberdeen-Angus (2.3 days) breed sires showed a longer gestation length. Primiparous cows and twin pregnancies, as well calving in June and July, shortened gestation length in -1.4, -4.0, -1.9 and -1.8 days, respectively. The perinatal mortality incidence was 7.4% (n = 72) and was more likely to occur in twin pregnancies (p < 0.01) than in pregnancies carrying female (odds ratio = 8.1) or male (odds ratio = 7.9) singletons, as well in primiparous (odds ratio = 2.6) than multiparous dams (p < 0.05). In conclusion, parity and twinning were the major factors which influenced simultaneously gestation length of dams and perinatal mortality incidence. Nevertheless, all studied factors had a significant impact on gestation length and should be considered for reproductive management programs of dairy herds.

Key words: breed sire, dairy cows, herd health, perinatal mortality, pregnancy, twinning.

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## СОПУТСТВУЮЩИЕ ФАКТОРЫ,

## ВЛИЯЮЩИЕ НА ПРОДОЛЖИТЕЛЬНОСТЬ СТЕЛЬНОСТИ И ПЕРИНАТАЛЬНУЮ СМЕРТНОСТЬ У КОРОВ ГОЛШТИНО-ФРИЗСКОЙ ПОРОДЫ

#### N. M. Bogas<sup>1</sup>, A. V. Sherasiya<sup>2</sup>, J. C. Simões<sup>3</sup>

<sup>1</sup> Ветеринарный врач, Научно-исследовательский центр ветеринарии и изучения животных (CECAV),

Университет Траз-уж-Монтиш и А́лту-До́ру, Ви́ла-Реа́л, Португалия, e-mail: neca.smb@gmail.com

- <sup>2</sup> Ветеринарный врач, экс-ветеринарный инспектор, Ванканер, Индия, e-mail: sherasiyaanjum@qmail.com; ORCID ID 0000-0002-1598-1820
- <sup>3</sup> PhD, Научно-исследовательский центр ветеринарии и изучения животных (CECAV), факультет ветеринарии,

Университет Траз-уж-Монтиш и А́лту-До́ру, Ви́ла-Реа́л, Португалия, e-mail: jsimoes@utad.pt; ORCID ID 0000-0002-4997-3933

#### **РЕЗЮМЕ**

Цель данного исследования состояла в идентификации и количественном определении факторов, оказывающих умеренное или сильное воздействие одновременно на продолжительность стельности (GL) и перинатальную смертность (в период до 4 суток после рождения) у коров голштино-фризской породы. Общее наименьшее среднеквадратичное значение продолжительности стельности (GL) составило  $278,2\pm0,2$  суток (n=962). В соответствии с многомерной моделью (p<0,001) при оплодотворении быками-производителями голштино-фризской и красной голштино-фризской пород продолжительность стельности коров была короче (-2,0 суток и -3,9 суток соответственно), тогда как при осеменении быками-производителями бурой швицкой и абердин-ангусской пород продолжительность стельности была дольше на 2,0 суток и 2,3 суток соответственно. У первотелок и при двуплодной стельности, а также при отеле в июне и июле продолжительность стельности сокращалась на -1,4; -4,0; -1,9 и -1,8 суток соответственно. Инцидентность перинатальной смертности составляла 7,4% (n=72), и такие случаи чаще наблюдали при двуплодной стельности (p<0,01), чем при одноплодной стельности телочками (относительный риск =8,1) или бычками (относительный риск =7,9), а также у первотелок (относительный риск =2,6) по сравнению с коровами (p<0,05). В заключение необходимо отметить, что количество отелов и двуплодность были основными факторами, значимо влияющими одновременно на продолжительность стельности у коров и на инцидентность перинатальной смертности. Тем не менее все изученные факторы оказывали существенное воздействие на продолжительность стельность стельность стельность стельность стельность стельность стельность остельность стельность и должны учитываться при реализации программ по регулированию репродукции в молочных стадах.

Ключевые слова: племенной бык-производитель, дойные коровы, здоровье стада, перинатальная смертность, стельность, двуплодность.

#### INTRODUCTION

The calving represents an important step in dairy farms due to their influence on the immune status, health and welfare of the dam, calf survival and the following lactation. During the peripartum period, a significant immune suppression of the dam, with emphasis for lymphocytes and neutrophils depression activity, increasing the susceptibility to infectious diseases [24]. Also several herd health management practices, such as vaccination, mastitis control, dry cow procedures, and nutritional assessment in dry cow period need be taken into consideration while estimating the gestation length (GL). The determination of GL is one major key to estimate the date of calving. This GL estimation can increase some days of the lactating period and reduces the dry cow period without negative impact on the next lactation [8], representing an improved revenue and reducing puerperal diseases prevalence of dam and offspring. It has been reported that intermediate GL was related with more productive dairy cows [28] presenting fewer problems during the next lactation [25, 27]. Moreover, A. Kok et al. [8] observed that dairy cows presenting a short dry period (3 to 5 weeks) produce less 1.2 kg of milk per day than in normal dry period (6 to 8 weeks). So, despite the hormonal (e.g., progesterone, estrogens) and immunological (e.g., major histocompatibility complex I, pro-inflammatory cytokines) influences on the pregnancy maintenance and/or term [16, 18, 19, 26], environmental (e.g., sex of calf, parity of dam, climate) and genetic factors (e.g., breed) causing strong GL variation should be evaluated in different worldwide regions according to the herd management and environmental factors.

In other hand, some studies also reported the association between shorter (foetal immaturity) and longer (higher calf birth weight) GL and perinatal mortality incidence [4, 29]. Despite dystocia, GL was considered the most important predictor of stillbirth occurrence [23]. Nevertheless, some phenotypic and genetic factors which affect the GL (e.g., parity, fetal sex and twinning) also have a direct association with perinatal mortality [21, 29] and the risks also should be evaluated and quantified.

Our study aimed to evaluate and quantify the main factors which moderately or strongly affect, in simultaneous, GL and perinatal mortality in (Portuguese) Holstein-Frisian dairy cows.

#### **MATERIAL AND METHODS**

Study area. This retrospective study was carried out in a typical Portuguese small and familiar dairy herd (Lat. 41°32′58.2″ N and Long. 8°33′48.7″ W). According to the Köppen-Geiger climate classification [38], in this region, the climate is considered temperate with dry and mild summer and mild winter with rainfall (Mediterranean Csb).

Animals and dairy herd. All complete records were taken into consideration from June 2000 to April 2017. According the milk yield records for 962 Holstein-Friesian cows in the dairy herd, the 305-day standardized varied from less than 8000 kg (year 2000) to more than 10,000 kg (year 2017; Pearson correlation r = 0.31; n = 962; p < 0.001). The main purpose of this herd was milk production, but economic revenues were complemented with meat production. At present, a total of 105 producing cows (Holstein-Frisian and some Red Holstein-Frisian, Jersey, Montbeliarde, Brown Swiss, and dairy crossbreeding breeds), 65 heifers for self-replacement and 50 steers and heifers for meat production. In 2016 (the last complete studied year), 79 dairy cows were dried in this herd. At national level,

in 2016, a total of 1676 herds presenting an average size of 52 dairy cows (reaching the dry period) per herd were evaluated for official milk recording. The 305-day standardized milk production was, in average, 9450 kg). For artificial insemination purposes, 198 sires from 8 breeds were used throughout the studied period.

Records. The following information was retrieved from the herd database from each calving throughout the studied period: 1) breed sires (Holstein-Friesian, Brown Swiss, Aberdeen-Angus, Jersey, Red Holstein-Friesian, Montbeliarde, Limousine and Belgian Blue); 2) pregnancy type and sex (twin pregnancy, single pregnancy with female singleton and single pregnancy with male singleton); 3) parity (primiparous vs multiparous cows); 4) month of calving, and; 5) year of calving.

The GL was calculated considering the difference between calving and artificial insemination dates.

The perinatal mortality incidence was calculated considering at calf mortality, including stillborn, until 4 days. For twins, the presence of mortality was considered when at least one calf died.

Statistical analysis. A full least squares model was build to test fixed effects on GL by the following formula:

$$Y_{ijk} = \mu + H_i + W_j + S_k + T_m + P_n + E_{ijklmno}$$

where  $Y_{ijk} = GL$ ;

 $\mu = \text{overall mean};$ 

H<sub>i</sub> = fixed effect due to i<sup>th</sup> breed sires;

W = fixed effect due to j<sup>th</sup> pregnancy type and sex;

 $S_{k} =$ fixed effect due to  $k^{th}$  parity;

 $T_m =$ fixed effect due to  $m^{th}$  month;

 $P_n =$ fixed effect due to  $n^{th}$  year;

 $\xi_{ijklmno}$  = random error.

A multivariable logistic model was also made using the Hosmer and Lemeshow method [17] to test the effect of the five dependent variables on perinatal mortality incidence. In a first step, univariate association of each one variable and the perinatal mortality incidence was tested and included on a full model if p-value < 0.25. Nonsignificant variables, at 0.05 level for likelihood ratio tests, were successively removed and the full model was compared with the previous one. Finally, only the significant variables were considered at 0.05 level for Wald test and respective odd ratios were calculated.

The JMP11 package [36] was used for all evaluations.

#### **RESULTS AND DISCUSSION**

The estimated least square mean of GL was  $278.2 \pm 0.2$  ( $\pm$  S.E.) days and 95% interval of confidence from 277.9 to 278.6 days and was influenced by all studied factors (Fig.).

Four breed sires were found to influence significantly the GL on Holstein-Friesian dams. These findings are in agreement with the reported by H. D. Norman et al. [14] for both dairy breed sires. A longer GL on Holstein-Friesian inseminated with Belgian Blue (2.3 days) and Aberdeen Angus (2.6 days) was observed by A. M. Fitzgerald et al. [12] using a gestational age model prediction based on ultrasonography technique. R. Fouz et al. [33] also observed a significant longer GL on Holstein dams when served by Limousine (5.3 days), Belgian Blue (1.6 days) and Galician Blonde (4.5 days) than by Holstein breed sires. Even if non-significant values on GL were observed for Belgian Blue and Limousine breed sires of our study, and probably due to the low samples number of these breeds, the GL profile of crossbreeding between Holstein-Friesian and

	Term	Scaled Estimate	Std Error	Lower 95%	Upper 95%	P value
	Intercept	277.5	0.5	276.6	278.4	<0.001
Breed sire	Aberdeen-Angus (n=28)	2.3	1.0	0.4	4.2	0.02
	Belgian Blue (n=12)	0.5	1.3	-2.1	3.2	0.68
	Holstein-Friesian (n=817)	-2.0	0.4	-2.9	-1.2	<0.001
	Red Holstein-Friesian (n=19)	-3.9	1.1	-6.0	-1.8	<0.001
	Jersey (n=21)	-0.1	1.1	-2.2	2.0	0.92
	Limousine (n=13)	1.7	1.3	-0.9	4.2	0.20
	Montbeliarde (n=16)	-0.4	1.2	-2.7	1.9	0.72
	Brown Swiss (n=36)	2.0	8.0	0.3	3.6	0.02
Pregnancy /sex	Single [female] (n=451)	1.3	0.3	0.7	1.9	<0.001
	Twins (n=42)	-4.0	0.5	-5.1	-3.0	<0.001
	Single [male] (n=469)	2.8	0.3	2.2	3.4	<0.001
Parity	Multiparous (n=278)	1.4	0.2	1.0	1.7	<0.001
	Primiparous (n=675)	-1.4	0.2	-1.7	-1.0	<0.001
Month	Jan (n=96)	1.3	0.5	0.4	2.3	0.01
	Fev (n=76)	0.1	0.6	-1.0	1.1	0.92
	Mar (n=83)	0.2	0.5	-0.8	1.3	0.69
	Apr (n=90)	1.2	0.5	0.2	2.2	0.02
	Mai (n=79)	-0.5	0.5	-1.5	0.6	0.39
	Jun (n=62)	-1.9	0.6	-3.0	-0.7	0.002
	Jul (n=75)	-1.8	0.6	-2.9	-0.7	0.001
	Aug (n=93)	-0.7	0.5	-1.7	0.3	0.19
	Set (n=71)	0.2	0.6	-0.9	1.3	0.71
	Oct (n=84)	-0.3	0.5	-1.4	0.7	0.53
	Nov (n=76)	1.1	0.6	0.0	2.2	0.05
	Dec (n=77)	1.0	0.5	-0.1	2.1	0.07
Year	2000 (n=26)	0.1	0.9	-1.7	2.0	0.88
	2001 (n=49)	1.7	0.7	0.3	3.1	0.01
	2002 (n=65)	2.2	0.6	1.0	3.5	<0.001
	2003 (n=62)	0.4	0.6	-0.8	1.7	0.48
	2004 (n=54)	-0.2	0.7	-1.5	1.2	0.82
	2005 (n=49)	1.9	0.7	0.6	3.3	0.005
	2006 (n=49)	0.2	0.7	-1.1	1.6	0.74
	2007 (n=57)	0.8	0.7	-0.5	2.1	0.20
	2008 (n=61)	-0.4	0.7	-1.7	0.9	0.51
	2009 (n=59)	-1.3	0.6	-2.5	0.0	0.05
	2010 (n=50)	-0.9	0.7	-2.2	0.5	0.21
		-0.3				0.64
	2011 (n=56) 2012 (n=49)	-0.6	0.7 0.7	-1.6 -2.0	1.0 0.7	0.84
		-0.6		-2.0 -1.5		0.36
	2013 (n=53)		0.7		1.2	
	2014 (n=63)	-0.5	0.6	-1.7	0.7	0.38
	2015 (n=67)	-0.7	0.6	-1.9	0.5	0.25
	2016 (n=77)	-1.2	0.6	-2.3	-0.1	0.04
	2017 (n=16)	-1.3	1.2	-3.7	1.2	0.31

Fig. Effects of major factors on gestation length of Holstein-Friesian cows

Table
Risk factors (odds ratios) for perinatal mortality in Holstein-Friesian cows

Groups		Odds ratio	<i>p-</i> value	
level 1 (reference)	level 2	(95% CI)		
Single pregnancy carrying female singleton	Twinning	8.1 (2.5–26.0)	0.001	
Single pregnancy carrying male singleton	Twinning	7.9 (2.5–25.2)	0.002	
Multiparous cows	Primiparous	2.6 (1.2–5.9)	0.02	
Twinning X parity interaction			0.78	

95% CI: 95% interval confidence

meat breed sires are in consonance that meat breed sires increase the GL in overall. All these results indicate a strong influence of genetic breed sire on GL.

A strong shorter GL was also observed in twins pregnancies and indicated that these two effects are the major effects which should be considered to estimate the GL. Shorter GL of twins than single pregnancies were also observed by H. D. Norman et al. [14] (-4.6 days), R. Tomasek et al. [34] (-5.2 days) and S. E. Echternkamp et al. [7] (-5.7 days). Moreover, significant differences between twins pregnancies carrying two males (277.3  $\pm$  0.6 days) than carrying two females (274.3  $\pm$  0.7 days) or one male and one female (273.6  $\pm$  0.5 days) were also observed by A. Vieira-Neto et al. [2]. In our study, the GL was improved by single pregnancies carrying males (2.8 days) and females (1.3 days) and agree with longer GL (1.1 to 1.8 days) observed in other studies [25, 31, 34]. It is known that the median litter size is negatively correlated with GL in species carrying multiple foetuses [10, 11, 31] due to several factors, such as endocrine and physical patterns. In cows, A. Vieira-Neto et al. [2] also suggested a slowest development of hypothalamic-pituitary-adrenal axis activity in males during the last days of pregnancy.

In our study, primiparous showed shorter GL than multiparous cows, in agreement with other studies [2, 14, 25]. The shorter GL observed in primiparous cows are probably related to the calf weight [15] which increase quickly in the last two weeks of gestation [37].

The heat stress seems to play an important role in GL in temperate and hot climates. In our study, a shorter GL was observed in June (–1.9) and July (–1.8) whereas in several months in fall and winter the GL was longer. Similar profile findings were observed in other studies [2, 25, 30, 32, 34]. Probably, the heat stress improves the foetal stress [9, 35], a key step to induce the natural calving.

The present study evidenced a consistent GL decrease throughout the study period. This observation can be related, at least in part, with the improvement of milk production observed in the farm. The GL profile is in agreement with the observed by A. Kumar et al. [13], which observed a difference of 3.3 days between 1978 and 2013.

The overall perinatal mortality incidence was 7.38% (71/962). Regarding the final multivariable logistic model (p < 0.001; Table) of our study, only the twining and parity of females showed significant effects on perinatal mortality incidence. Some studies [2, 27] reported that perinatal mortality incidence was higher for shorter and longer GL than for median GL. However, primary factors such as parity and twinning can partially explain these variations. Even if longer GL can be related with high calf weight, greater

dystocia incidence and stillbirth occurrence [23], twins have more chances to present dystocia and consequently, perinatal mortality [29]. Although J. F. Mee et al. [22] classified parity and twining as non-modifiable risk factors, twin reduction can be induced to mitigate their adverse effects in dairy farms [1, 20].

In our study, other than month and year effects, no significant effects of breed sires on perinatal mortality incidence were also observed. Nevertheless, these results should be interpreted with caution due to the low number samples of crossbreeding involving non Holstein-Friesian sires, and further research is warranted. In fact, calf birth weight, as well dystocia occurrence can be influenced by crossbreeding [3, 5, 6].

#### CONCLUSION

The breed sire, twinning, parity of cows and month of calving in temperate climate are factors that should be taken into account for GL estimation in Holstein–Friesian cows.

Mainly twinning, but also parity, concomitantly play a major role in perinatal mortality incidence and should be considered for the herd health management program of dairy farms. We also suggest the inclusion of early ultrasonographic diagnosis of twins in reproductive programs at individual level.

**Conflict of interest.** The authors declare that there is no conflict of interest.

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