

## COMPARISON OF SERVICE PERIOD AND LACTATION MILK YIELDS IN DAIRY COWS WITH SINGLE- AND TWIN-CALVING

DÖRSTELMANN H.K. Maximiliane<sup>1</sup>, ARI Melinda<sup>2</sup>, BECSKEI Zsolt<sup>3</sup>, GULYÁS László<sup>4</sup>, GÁSPÁRDY András<sup>1\*</sup>

<sup>1</sup>University of Veterinary Medicine Budapest, Department for Animal Breeding, Nutrition and Laboratory Animal Science, 1078 Budapest, István utca 2, Hungary, maxi-doe@gmx.de, gaspardy.andras@univet.hu; <sup>2</sup>National Association of Hungarian Holstein Friesian Breeders, 1134 Budapest, Lőportár u. 16, Hungary, arime@holstein.hu; <sup>3</sup>University of Belgrade, Faculty of Veterinary Medicine, Department for Animal Breeding, 11000 Belgrade, Bulevar Oslobođenja 18, Serbia, beckeizolt@gmail.com; <sup>4</sup>Széchenyi István University, Faculty of Agricultural and Food Science, Institute of Animal Science, 9200 Mosonmagyaróvár, Vár tér 2, Hungary, gulyas.laszlo@sze.hu

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### Abstract

*Introduction.* Twin-calving occurs in 3 to 5 % of Holstein Friesian cattle, and it is mostly followed by reproductive and economic problems. This evaluation will compare the service period and the milk yield in cows with single and twin births at a Hungarian large-scale farm.

*Materials and Methods.* The data were collected from 4223 cows between 2000 and 2010. In Cox's regression model (service period) and general linear model (milk production traits), the type of calving (single/twin), the construction code (referring to the Holstein Friesian blood proportion), the season and number of calvings were fixed effects, and furthermore, the year of calving was a covariate.

*Results and Conclusions.* This analysis showed there was no significant difference in the length of service period (SP) between the two groups of cows. The total lactation milk yield produced a meaningful difference in performance: single-calving cows had greater milk yield ( $p=0.013$ ), fat yield ( $p=0.030$ ), and protein yield ( $p=0.028$ ) than cows with twins. The standard lactation milk yield showed the same tendency at a stronger significance and a lower level of production. This unexpected and contradictory result in service period could be explained by the much longer period of open days in twinning

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\*Corresponding author – e-mail: [gaspardy.andras@univet.hu](mailto:gaspardy.andras@univet.hu)

cows, known from previous research. Regarding milk yield, an involuntary decrease for cows after twin calving was confirmed.

**Key Words:** dairy cattle, twin-calving, lactation performance, service period

## INTRODUCTION

Twin-calving in dairy cows seems to be on the rise worldwide during recent years, although scientific research on twin-calving commenced early in the 20<sup>th</sup> century. Cows, as well as horses, are uniparous animals, even though for cows there is about a 3.1 % chance of giving birth to twins (Roffeis & Krehl, 2016).

Economic considerations are very important factors for each farmer. Although there is a higher total number of calves produced due to twin-calving, there is an increase in costs and management, seen for example, in decreased milk yields and higher needs for medical treatments after parturition (Szelényi et al., 2009; Cincović et al., 2017). Furthermore, the higher risks of health issues for the mother cow, specifically the higher risk of abortion (Nielen et al., 1989) and dystocia (Cady & Van Vleck, 1978), are pronounced.

Considering twin pregnancy, the question about the gender of the offspring is also important for the farmer more than it is in beef cattle production or in other species. In cows, both siblings share the same blood supply for their placentas. Due to this, there is a degree of cell exchange between both embryos. In about 98 % of mixed sex twins (male and female), the female offspring will be infertile and cannot be used for further breeding. We call this phenomenon freemartinism (Fésüs, 2004).

The aim of this study is to investigate our hypothesis that cows with a twin pregnancy will have an extended service period and a higher total and standard lactation milk yield in comparison to single-calving cows after calving.

## MATERIALS AND METHODS

**Ethical approval:** No ethical approval was obtained because this study did not involve laboratory animals and only involved non-invasive procedures (statistical data).

For the research, data were collected from 4223 cows from one large size dairy operation in North Hungary. The database was put together for 10 years, between 2000 and 2010 and contains information about a population upgraded to Holstein Friesian (HF). We analyzed the length of service period (SP), and the milk yield (MY), fat yield (FY) and protein yield (PY) in both the total and standard lactations. The average number of records per cow for SP and milk production was 1.3 and 2.5, respectively.

In this study, SP was defined as the period from the date of beginning insemination to the date of the last (successful) insemination, calculated in days. Basic statistics are

given for comparing the SP in the two cow groups (non-twinning and twinning cows), then a survival analysis (Cox's regression) was used taking the same effects as were applied in the general linear model (GLM) statistics (see below) into consideration. Days of SP over 200 days were considered as censored data (17 records (0.32%) from the total of 5383 records). Wald statistics and a graphical presentation of cumulative percentages of groups of cows after single- and twin-calving which became pregnant by time are given. We present the number of days in the SP by which the half of the cows in the two groups became pregnant.

In the next statistical processing, raw milk production data were analyzed by GLM. In both statistical procedures (also in the Cox's regression described above), we chose the following effects: type of calving (fixed effect); construction code based on HF blood proportion (fixed effect); season of calving (fixed effect); number of calvings (fixed effect); year of calving (covariate).

The type of calving differentiated between single- and twin-calving. The construction code was based on HF blood proportion. In our database, the following construction codes with a given range of HF proportion were distinguished: 221/  $\geq 96.88\%$ ; 222/  $\geq 93.75\%$  and  $< 96.88\%$ ; 223/  $\geq 87.5\%$  and  $< 93.75\%$ ; 224/  $\geq 75\%$  and  $< 87.5\%$ ; 225/  $\geq 50\%$  and  $< 75\%$ . For the season of calving, we divided the 12 months of a year into four seasons. Winter, spring, summer and autumn (coded 1-4, respectively) were considered as being from December to February, from March to May, from June to August and from September until November, respectively. The number of calvings is shown in our tables as lactations one to five. The last group includes the 5<sup>th</sup> lactation as well as all later ones. As the only covariate effect, which means continuous effect in this study, the year of calving is given in the 10-year study span.

Variance components were calculated for production traits. To measure differences, the Tukey's post hoc method was used for production traits. All statistical evaluations were performed with the Statistica Computer Software, version 13 (StatSoft Inc., 2015).

## **RESULTS AND DISCUSSION**

### **Service Period**

From 5,366 collected non-censored records, Table 1 shows the basic statistics of SP. The cows with single births had shorter SP (44.4 days) than cows with twin calving (51.2 days); however, this difference was not statistically significant ( $p = 0.111$ ). The optimal length of SP should be as short as possible. For cows, it is normally 60 to 90 days, but should not exceed 100 days. If this time period is extended, the next calving will be temporally shifted. As is well known, the length of the SP is multifactorial. It depends on hormonal status (Répási et al, 2014), level of milk production (Szelényi et

al, 2015), involution disorders (Szenci et al, 2015), body condition (Pivko et al, 2016), metabolic status (Cicnović et al, 2017) and other reproductive features (Keary, 2017).

**Table 1.** The basic statistics of service period (days; in cases where SP<200 days) in the investigated groups of cows (N = number of records)

Type of calving	N	Mean	Median	SEM	Lower quartile 25%	Upper quartile 75%
Cows with single birth	5222	44.4	24.0	0.696	1.0	77.0
Cows with twinning	144	51.2	46.5	4.058	1.0	90.0
Total and overall mean	5366	44.6	24.1	0.687	1.0	77.0

SEM – standard error of mean

Table 2 presents results of Cox’s proportional hazards regression model showing the impact of the investigated effects on the chance of being pregnant sooner along with the Wald statistics and the p-values for those statistics.

**Table 2.** Cox proportional hazards regression model showing the impact of investigated effects on the chance of pregnancy (N = number of records)

Effects N = 5383	Beta <sup>1</sup>	Standard error <sup>2</sup>	Wald Statistic <sup>3</sup>	p-value <sup>4</sup>	Risk ratio <sup>5</sup>	95% CI <sup>6</sup>
Type of calving (single=0, twin=1)	-0.038	0.085	0.202	0.653	0.962	0.814 to 1.137
Construction code (221 to 225 with decreasing HF blood proportion)	-0.006	0.003	4.012	0.045	0.994	0.988 to 1.000
Season (winter=1, spring=2, summer=3, autumn=4)	0.007	0.012	0.382	0.537	1.007	0.984 to 1.031
Number of calving	0.002	0.011	0.042	0.838	1.002	0.980 to 1.025
Year of calving (2000 to 2010)	-0.025	0.003	74.5917	< 0.001	0.975	0.970 to 0.981

<sup>1</sup>Beta – regression coefficient

<sup>2</sup>Standard error – variability of each of the estimated regression coefficients

<sup>3</sup>Wald Chi<sup>2</sup> – the test statistics computed from the data and from which p-values are determined

<sup>4</sup>p-values – the probability of observing the results

<sup>5</sup>Risk ratio – the degree of risk associated with each effect while controlling for all other effects

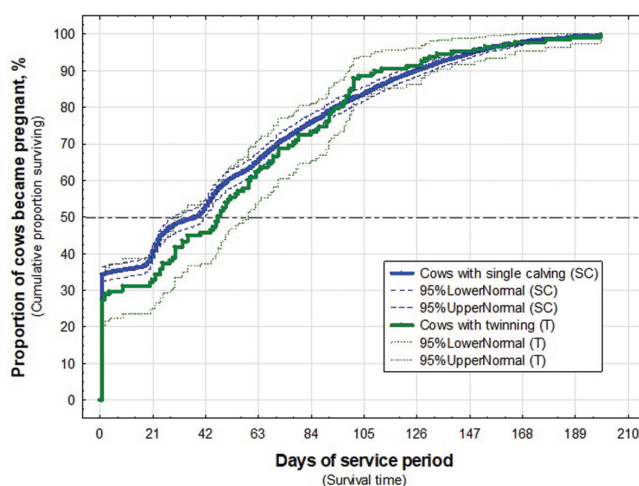
<sup>6</sup>95% CI – the precision of estimated risk ratio

The beta values (Table 2) are regression coefficients. A negative regression coefficient for an explanatory effect means that the hazard is higher, and thus, the prognosis is better with a lower value. This is the case for the type of calving (-0.038), the construction code (-0.006) and the year of calving (-0.025). The negative beta value for type of calving means that the chance of becoming pregnant earlier was higher for cows who had single births, although this was not significantly different between the

cow groups ( $p = 0.653$ ). In contrast to this, for construction code and year of calving, the negative beta values had significant  $p$ -values (0.045 and  $< 0.001$ , respectively), indicating that the higher the HF blood proportion, the longer the insemination period. The SP showed a statistically significant year-by-year increase.

The standard error (Table 2) characterizes the variability of each of the estimated regression coefficients, and correlates well with level of significance ( $p$ -value). Wald  $\chi^2$  is the test statistic computed from the data and from which  $p$ -values are determined. For binary variables, a risk ratio of around 1 indicates that having the characteristic of the effect neither promotes nor hinders the event. In other words, it indicates that the type of calving (single or twin) was not significantly associated with the chance of becoming pregnant sooner or later. The confidence interval (95% CI; Table 2) is the precision of estimated risk ratio; the narrower the confidence intervals, the more precise are the estimates.

Figure 1 shows the cumulative proportion of cows which became pregnant during the course of the insemination period. Approximately one third of the cows became pregnant right after the very first insemination. The graded increase in cumulative proportion of pregnant cows, especially at the beginning of the SP, is in good concordance with the reproductive cycles of cows. Half (50 %) of cows with single- and twin-calving became pregnant by the 40<sup>th</sup> and 48<sup>th</sup> day of SP, respectively, a time difference which was not statistically significant ( $p = 0.653$ ), and tells us that both cow groups in our study responded similarly to the re-breeding. However, from the investigation of Keary (2017), the significantly longer period of open days (including a longer calving to service interval) in twinning cows was responsible for delaying their consecutive calving. Open days were understood as the period of time between calving and the next gestation (this period of time integrates the calving to service interval and SP).



**Figure 1.** Cumulative proportion of cows which became pregnant in the course of the insemination period

### Total lactation performance

Table 3 presents the total lactation performances (kg) with variance components (VC) obtained from 10,666 records. The much lower average number of records per cow in SP (1.3) than in lactation performance (2.5) reveals a high proportion of cows did not become pregnant in their last lactation.

**Table 3.** Total lactation performances (kg) with variance components (VC)

Effect	Number of records	Milk yield (kg)		Fat yield (kg)		Protein yield (kg)	
		p-value		p-value		p-value	
		VC <sup>1</sup>	SE <sup>3</sup>	VC <sup>1</sup>	SE <sup>3</sup>	VC <sup>1</sup>	SE <sup>3</sup>
Type of calving		p = 0.013 5.40%		p = 0.030 3.94%		p = 0.028 4.57%	
- Non-twinning cows	10314	7390	69.0	277	2.7	245	2.3
- Twinning cows	352	6890	202.8	260	7.9	230	6.9
Construction code		p= 0.433 0.84%		p= 0.643 0.53%		p= 0.232 1.31%	
221	6784	7110	107.3	266	4.2	235	3.6
222	1972	6970	124.7	264	4.8	230	4.2
223	1074	7190	147.3	270	5.7	238	5.0
224	589	7200	181.1	272	7.0	240	6.1
225	247	7240	253.9	270	9.9	243	8.6
Calving season		p< 0.001 10.51%		p< 0.001 7.11%		p< 0.001 7.34%	
Winter	2808	7350 <sup>c</sup>	129.3	275 <sup>b</sup>	5.0	243 <sup>b</sup>	4.4
Spring	2124	7290 <sup>bc</sup>	134.1	272 <sup>b</sup>	5.2	241 <sup>b</sup>	4.5
Summer	2758	6800 <sup>a</sup>	127.1	257 <sup>a</sup>	4.9	228 <sup>a</sup>	4.3
Autumn	2976	7120 <sup>b</sup>	127.3	269 <sup>b</sup>	4.9	239 <sup>b</sup>	4.3
Lactation		p< 0.001 53.95%		p< 0.001 42.53%		p< 0.001 58.59%	
1 <sup>st</sup>	4103	8130 <sup>c</sup>	130.1	303 <sup>d</sup>	5.1	271 <sup>d</sup>	4.4
2 <sup>nd</sup>	2973	7230 <sup>b</sup>	128.2	268 <sup>bc</sup>	5.0	242 <sup>b</sup>	4.3
3 <sup>rd</sup>	1779	6940 <sup>ab</sup>	135.9	261 <sup>ac</sup>	5.3	231 <sup>c</sup>	4.6
4 <sup>th</sup>	936	6810 <sup>a</sup>	156.9	260 <sup>ac</sup>	6.1	226 <sup>ac</sup>	5.3
5 <sup>th</sup> and more	875	6610 <sup>a</sup>	157.2	250 <sup>a</sup>	6.1	218 <sup>a</sup>	5.3
Year of calving	10666	p< 0.001 28.42% 38.391*		p< 0.001 45.06% 1.962*		p< 0.001 27.25% 1.170*	
Error		0.88%		0.84%		0.94%	
Total and overall mean	10666	7140	141.6	268	5.50	237	4.78

<sup>1</sup>VC – variance components in %; <sup>2</sup>LSM – least squares means; <sup>3</sup>SE – standard error; a, b, c – different letters mean significant (p< 0.05) differences (Tukey's post-hoc test); \* – regression coefficient

### Standard lactation performance

The lactation performances corrected for 305 days (kg) are shown in Table 4.

**Table 4.** Standard lactation performances (kg) with variance components (VC)

Effect	Number of observations	Milk yield (kg)		Fat yield (kg)		Protein yield (kg)	
		p-value VC <sup>1</sup> LSM <sup>2</sup>	SE <sup>3</sup>	p-value VC <sup>1</sup> LSM <sup>2</sup>	SE <sup>3</sup>	p-value VC <sup>1</sup> LSM <sup>2</sup>	SE <sup>3</sup>
Type of calving		p= 0.002 3.34%		p= 0.001 2.85%		p= 0.004 2.98%	
- Non-twinning cows	10314	6890	44.4	255	1.6	226	1.4
- Twinning cows	352	6480	130.4	240	4.7	214	4.2
Construction code		p= 0.212 0.49%		p= 0.719 0.15%		p= 0.042 0.90%	
221	6784	6664	69.0	246	2.5	219 <sup>bc</sup>	2.2
222	1972	6562	80.2	245	2.9	215 <sup>a</sup>	2.6
223	1074	6697	94.7	248	3.5	220 <sup>a</sup>	3.0
224	589	6621	116.6	246	4.2	218 <sup>a</sup>	3.7
225	247	6880	163.2	252	5.9	229 <sup>ac</sup>	5.2
Calving season		p< 0.001 7.90%		p< 0.001 4.21%		p< 0.001 5.77%	
Winter	2808	6890 <sup>c</sup>	83.2	253 <sup>c</sup>	3.0	224 <sup>b</sup>	2.7
Spring	2124	6737 <sup>b</sup>	86.2	247 <sup>b</sup>	3.1	220 <sup>b</sup>	2.8
Summer	2758	6376 <sup>a</sup>	81.7	238 <sup>a</sup>	3.0	212 <sup>a</sup>	2.6
Autumn	2976	6735 <sup>b</sup>	81.8	250 <sup>bc</sup>	3.0	224 <sup>b</sup>	2.6
Lactation		p< 0.001 4.22%		p= 0.009 0.94%		p< 0.001 4.59%	
1 <sup>st</sup>	4103	6923 <sup>b</sup>	83.6	249 <sup>b</sup>	3.0	226 <sup>b</sup>	2.7
2 <sup>nd</sup>	2973	6830 <sup>b</sup>	82.4	249 <sup>b</sup>	3.0	226 <sup>b</sup>	2.6
3 <sup>rd</sup>	1779	6690 <sup>b</sup>	87.4	249 <sup>b</sup>	3.2	221 <sup>b</sup>	2.8
4 <sup>th</sup>	936	6644 <sup>b</sup>	100.9	250 <sup>b</sup>	3.7	219 <sup>b</sup>	3.2
5 <sup>th</sup> and more	875	6336 <sup>a</sup>	101.1	238 <sup>a</sup>	3.7	208 <sup>a</sup>	3.2
Year of calving	10666	p< 0.001 83.71% 84.847*		p< 0.001 91.56% 3.521*		p< 0.001 85.39% 2.642*	
Error		0.34%		0.28%		0.36%	
Overall mean	10666	6685	91.07	247	3.30	220	2.92

<sup>1</sup>VC – variance components in %; <sup>2</sup>LSM – least squares means; <sup>3</sup>SE – standard error; a, b, c – different letters mean significant (p< 0.05) differences (Tukey's post-hoc test); \* – regression coefficient

If we consider the MY ( $p=0.002$ ), FY ( $p=0.001$ ) and PY ( $p=0.004$ ), they all had significant differences according to the type of calving. Twinning cows produced, on average, 410 kg less milk than single-calving ones. There was no meaningful distinction for the MY ( $p=0.212$ ) nor for the FY ( $p=0.719$ ) according to the construction code. All other effects had a significant impact on standard lactation performances ( $p<0.05$ ). Cows that calved in summer achieved lower lactation performances ( $p<0.05$ ). Cows in their 5<sup>th</sup> or later lactation had decreased MY, FY and PY compared to cows with fewer lactation cycles. For the year of calving, all production traits underwent significant increases ( $p<0.05$ ), based on positive regression coefficients; this was a manifestation of improving genetic trends in the herd studied.

## CONCLUSION

Our investigation contributes to the exploration of post-partum reproductive circumstances in cows with twinning. Since there was no difference in length of SP between cows giving birth to single or twin calves, the mothers can be considered to have had similar body condition and involution stage, and to have responded equally to service.

However, previous research on the same database reported that cows that give birth to twins were rebred later, shown as longer total days open than other cows in the herd. The explanation of this seeming contradiction is the length of the post-partum interval and how early cows became ready for rebreeding when they formerly produced single or twin calves. Twinning cows responded well to insemination done at an appropriate time but they required more time spent in regeneration.

The higher load on cows' bodies caused by twin pregnancy and calving was manifested in significant drops in standard lactation performances (MY, FY and PY). The long-lasting impact of twinning was also detectable in the total lactation performance. While compensation in the twinning cows' SP seemed to be complete but time dependent, cows which had produced twins were not able to match the milk production performance of single-calving cows.

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## UPOREĐIVANJE DUŽINE SERVIS PERIODA I PROIZVODNJE MLEKA U LAKTACIJI IZMEĐU KRAVA SA JEDNIM TELETOM I KRAVA SA BLIZANCIMA

DÖRSTELMANN H.K. Maximiliane<sup>1</sup>, ARI Melinda<sup>2</sup>, BECSKEI Zsolt<sup>3</sup>, GULYÁS László<sup>4</sup>, GÁSPÁRDY András<sup>\*</sup>

### Kratak sadržaj

*Uvod.* Blizanački graviditet kod krava holštajfrizijske rase se javlja u 3-5% slučajeva i uglavnom je praćen reproduktivnim poremećajima i ekonomskim gubicima. Ispitivanja u ovom radu se odnose na evaluaciju i upoređivanje servis perioda i prinosa mleka kod grupe krava sa jednim teletom i grupe krava sa blizancima, na komercijalnim farmama u Mađarskoj.

*Materijal i metode.* Podaci su sakupljeni u periodu od 2000 do 2010. godine od ukupno 4223 krava. U Coxovom regresionom modelu (za servis period) i generalnom linearnom modelu (osobine proizvodnje mleka) tip teljenja (jedno tele ili blizanci), kao i sezona i broj teljenja su bili fiksni efekti, dok je godina teljenja bila kovarijat.

*Rezultati i zaključak.* Analizom dužine servis perioda nije ustanovljena signifikantna razlika između ispitanih grupa krava. Količina ukupno proizvedenog mleka u laktaciji

je pokazala značajne razlike između krava sa jednim teletom i dva teleta, u korist krava sa jednim teletom: prinos mleka ( $p=0.013$ ), količina mlečne masti ( $p=0.030$ ) i proteina ( $p=0.028$ ). Neželjeni pad u prinosu mleka je zabeležen kod krava sa bližnjijima. Mlečnost je bila značajno veća kod krava sa jednim teletom čak i u slučajevima kada su one imale relativno nisku mlečnost. Ovi oprečni rezultati bi se mogli objasniti činjenicom da su krave koje su se bliznile imale mnogo veći broj otvorenih dana, kao što je to opisano u brojnim predhodnim ispitivanjima.

**Ključne reči:** mlečna goveda, blizanačko teljenje, mlečnost, servis period