**Title:** Lactation performances in primiparous Holstein cows following short and normal gestation lengths

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**ABSTRACT**

The aim of this study was to compare the lactation performances in primiparous Holstein cows after a short gestation length (GL) or abortion to those after a normal gestation length. The data were collected using an automated data collection system. The herds evaluated were located in Belgium, France, Italy, the Netherlands and Germany. Data from a wide range of physiological cow-life events including birth and calving events, reproduction events (insemination, pregnancy checks, abortions), milking events were collected and combined into one dataset. The GL was defined as the interval between the last insemination and the subsequent calving (or abortion) within a range of 150-279 d. Animals were categorized to one of 4 categories based on quantiles 0-1%, 1-5%, 5-25% and 25-75% of GL. Lactation curve parameters including the scale, ramp and decay were estimated using the Milkbot model. Then, derived 305-d milk yield (M305), peak yield, and time to peak were compared between different GL categories. Of the 10,698 lactations, 15 (0.14%) were found with a gestation period shorter than 210 d (min. 158 - max. 208 d). Six out of 15 animals had a natural service, eight had an artificial insemination and one heifer became pregnant after an embryo transfer. The M305 production was significantly lower in the 0-1% and 1-5% group (7,519 ± 189 and 7,795 ± 136 kg), compared to the 5-25% (8,140 ± 119 kg) and 25-75% (8,238 ± 115 kg) groups. The same trend was found for the scale and peak yield of the lactation; the lowest scale and peak yield were found for the 0-1% and 1-5% group and the highest were found for the 5-25% and 25-75% group. Peak yield increased significantly from 0-1% and 1-5% group to the 5-25% and further to the 25-75% groups. Moreover, primiparous cows of 0-1% and 5-25% GL categories showed a higher milk yield persistency compared to those belonged to 5-25% and 25-75% groups. In conclusion, results showed an effect of the GL on the lactation performances of primiparous Holstein. The primiparous cows with a shorter GL (0-1% and 1-5%) produced significantly less M305 and peak yield, had a higher lactation persistency, and showed a lower upward slope of the lactation curve compared to those with a normal GL.

**KEYWORDS:** short gestation length, primiparous, milk production

# INTRODUCTION

In many countries, milk yield per cow has more than doubled in the last 40 years, mainly due to the rapid progress in management and genetics selection [1]. Looking at the literature, it appears that many of the fundamentals of milking process for a successful lactation have been understood [2]; moreover, the advent of sensitive assays to measure concentrations of mammogenic, lactogenic, and galactopoietic hormones and, subsequently, growth factors in the blood, milk, and tissues, has allowed creation of multiple hypotheses to explain mammary cell proliferation and regulation of functions [2]. However, some of the principles that had been identified when cows produced markedly less milk may not be still valid for the high-producing cows of today [2], and some mechanisms regarding physiology of lactation are still unexplored. The initiation of milk secretion in cattle is usually thought to follow the termination of pregnancy; still, it has long been known that cows may begin to secrete milk also previous to the time of parturition [3], so that the practice of pre-partum milking in dairy cows has been investigated as a means to shorten calving intervals and enhance milk production [4-8]. For decades, researchers also focused on the hormonal induction of lactation, from the first successful induction in goat [9] until the development of a short-term protocol that ensures induction of lactation in most treated cows and heifers [10-12]. Induced lactation of non-pregnant cows can represent an alternative management to avoid culling of high-producing cows with low fertility thus increasing profits [12], while the induction of lactation in young heifers can also provide a tool to collect milk before a normal lactation for early testing of transgenic animals as possible mammary bioreactors [13, 14]. Nevertheless, the average milk yield per lactation hormonally-induced is about 90% in multiparous cows [15], and 60-70% in primiparous cows [16] of an equivalent post calving lactation. The use of hormones for lactation induction is legally forbidden in most of the countries because of the concerns regarding consumer safety and presence of residues in the milk [17]. Rearing heifers represents about 20% of the total milk production costs [18, 19], and the return on the investment allocated from the birth to the first lactation is commonly not fully recovered until at least the end of the first lactation [20]. As consequence, productive life of heifers is an important factor in determining profits of dairy enterprises [20]. Pregnancy losses would still allow heifers to start their first lactation if they are sufficiently far advanced in pregnancy, but the exact time point when this is possible is unknown. Scattered through the earlier literature on the milk secretion are reports on lactation in suckled virgin heifers [3] and in heifers milked as early as 120 d of first pregnancy [21]. The secretory activity of the mammary gland during the first pregnancy in heifers is of considerable interest, as the growth of the mammary glands during the first pregnancy is remarkable [3]. Early studies on the mammary development in cattle showed that the histological development of the mammary gland from early gestation to near parturition is a progressively continuous process, more nearly exponential than linear, with marked developmental changes only in late pregnancy [22, 23]; most of the rapid increase in udder weight and in growth of the duct system occurs after the fifth month of pregnancy [24], particularly during the last 35 d pre-partum [25]. In pregnant heifers, serum concentrations of α-lactalbumin (i.e., a whey protein that plays a central role in milk production) become detectable only in the last trimester of the gestation, with modest increases until just before calving, when concentrations markedly increase [2]. This pattern mirrors a 2-stage onset of lactogenesis, with a modest increase in milk component biosynthesis in the last month before calving followed by a marked increase just before and after calving [26]. Despite decades of research, little is known regarding physiologic temporal limits for initiation of lactation in pregnant non-lactating cattle. In research from Swanson (27), lactations of cows following abnormal mean gestation lengths (GL) of 263, 246 and 242 d were compared with normal lactations by the same cows, and reductions to 87, 73 and 68% of the normal mature-equivalent lactations were registered, respectively. Recently, Keshavarzi et al (28) reported that abortion reduced the milk yield by 19.4% for cows that were able to start a new lactation; Atashi and Asaadi (29) found that primiparous cow with a short GL (250 d as minimum duration) had less lactation performances compared to those with a longer GL. To the best of our knowledge, the effect of a very short gestation on lactation performances in cows is unknown. Therefore, the objective of this study was to evaluate the lactation performances in primiparous cows following a short gestation or an abortion, by comparison with lactation in primiparous cows after a normal GL.

**MATERIALS AND METHODS**

## Observational dataset

The observational data were collected using an automated data collection system using a wide variety of herd management software programs as described by [30]. The herds included were located in Belgium, France, Italy, the Netherlands and Germany. Data from a wide range of physiological cow-life events including birth and calving events, reproduction events (insemination, pregnancy checks, abortions), milking events were collected and combined into one dataset.

## Definition of gestation length

Throughout the study, a pregnancy was defined as the last insemination and subsequent calving within a range of 150-279 d. The upper limit was set as mean + 2 times the SD after initial exploration of the GL. Given the focus of the current study, no further exploration of long GL was done. The included animals were categorized to one of 4 gestation length categories based on quantiles 0-1%, 1-5%, 5-25% and 25-75%. Then, lactation curve parameters including the scale, ramp and decay were estimated using the Milkbot model. [31]. The MilkBot function is as follows:

In which, *a* is the scale parameter, representing the theoretical maximum daily yield; *b* is the ramp parameter, controlling the rate of rise in milk production in early lactation; *c* is the offset parameter, describing the offset in time between parturition and the start of lactation; and *d* is the decay parameter, representing the rate of senescence of production capacity. The time at which peak lactation occurred (tpeak) was defined as: , and peak yield was calculated by substitution tpeak in the MilkBot equation. The 305-d milk, the cumulative milk yield between calving and day 305 of the lactation, was calculated as:

The calculated 305-d milk (M305), peak yield and time to peak were compared between different GL categories. For each of the outcome variables, a multi-level mixed model was built taking into account a random effect of the herd, fixed effects of month and year of calving, and age at first calving (AFC) as covariates. Least square means and contrasts were computed for each category of the pregnancy length. Significance and tendency levels were determined at *P* < 0.05 and 0.10 < *P* ≥ 0.05, respectively. All statistical analyses were carried out in R [32]. The data analysis was made publicly available through a central code repository at <https://github.com/Bovi-analytics/probo-et-al-2019>.

# RESULTS

## Descriptive data analysis

The dataset consisted of 8,175,067 milkings on 100 herds on which data was collected from 26,448 animals calving between January 2013 until December 2018. An average of 192 calvings per year was recorded (IQR 83 – 215). After filtering out all first lactation animals, 2,135,210 milkings from 10,698 animals on 94 farms remained for further analysis.

## Individual lactation curves

In order to identify extreme short GL, the original dataset was mined using a cut-off of maximum 210 d GL. Next, a minimum of 10 d in milk was required for the individual lactation curve exploration. Of the 10,698 lactations, 15 lactations (animal prevalence - 15 / 10,698: 0.14%) on 12 herds (herd prevalence - 12/94: 12.8%) were found with a GL shorter than 210 d and with a minimum of 10 d in milk. Each of these 15 individual lactation curves were individually plotted (Figure 1) and their reproductive history was illustrated in Table 1. Six out of 15 animals had a natural service, eight had an artificial insemination, and one heifer became pregnant after embryo transfer.

## Lactation curve parameters

The result of the lactation curve analysis is reported in Table 2. The M305 production was lowest in the 0-1% and 1-5% group (7,519 ± 189 and 7,795 ± 136 kg), followed by the 5-25% (8,140 ± 119 kg) and 25-75% (8,238 ± 115 kg) group (Table 2). The same trend was found for the scale and peak yield of the lactation, while the lowest scale and peak yield were found for 0-1% and 1-5% group and the highest were found for 5-25% and 25-75% group. The animals belonging to 0-1% and 1-5% group showed a lower upward slope of the lactation curve, reached their peaks later, and had a higher level of lactation persistency (lower downward slope of the lactation curve) than those belonging to the 5-25% and 25-75% group (Table 2).

**DISCUSSION**

Of the 94 farms, the 12,8% had one or more cases of very short gestation or abortion in primiparous cows, but total incidence was low (0.14%), and the number of animals involved per farm was barely more than one, although the requirement of a minimum of 10 d in milk for the individual lactation curve exploration probably leads to an underestimation of the real incidence within the herd. In general, in the present study the aim was to investigate the effect of the GL on lactation performances of Holstein primiparous cows. The results showed that primiparous cows with a very short GL produced less M305 and peak yield, tended to reach their peaks later, had a higher lactation persistency and showed a lower upward slope of the lactation curve compared to those with a normal gestation length. All results are in the line with previous studies regarding the effect of GL on milk production [29, 33]. Atashi and Asaadi (29) reported that Holstein heifers with a short GL produced less partial and 305-d lactation performance than those with an average or long GL. Norman et al (33) found that heifers with a longer GL produced more milk, fat, and protein. Most of the differences in the parameters of the lactation curve in the present study can be observed between the categories 0-1%, 1-5% on the one hand and 5-25%, and 25-75% on the other hand. A difference of 718 ± 154 kg was detected in the production of M305 d between the categories 0-1% and 25-75%, and it can mainly be attributed to a minor peak yield in 0-1% animals (27.2 ± 0.66 kg milk / animal vs 30.1 ± 0.40 kg milk / animal). The same differences were found regarding the scale values. This can be partially explained by the fact that, since nutrients in primiparous cows are prioritized not only for lactation but also for the continued growth of the animal, milk production is generally lower but lactation persistency higher in primiparous than multiparous cows, and the same can occur when comparing primiparous cows calving at different stage of the pregnancy and thus at different ages and body development. Comparison with previous studies highlights a greater decrease in production of M305 d in the short GL animals of the present study, but the present dataset was analyzed for milk production after an extremely short gestation period. The maximum gestation period was set at 210 d, and the GL ranged between 158 and 208 d, while in previous studies short gestations were generally considered as those less than 271 d [33] or ranging between 250-272 d [29]. Milk production losses in this study were significantly lower (maximum 90%) than previous observations by [27], who found losses of around 68% in multiparous cows after 242 d of gestation. This is somehow surprising, considering the remarkable shorter GL examined in the present study compared to that in Swanson (27). Nevertheless, it is well known that lactation curve in multiparous cows differs from that in primiparous, as it is characterized by a higher M305 and peak milk production, which amplifies the magnitude of production losses. Moreover, heifers do not require a dry period, and therefore the impact of a shorter gestation or an early abortion in primiparous cows is possibly lower than in multiparous cows. Shorter dry periods (0 to 35 d and 36 to 50 d) have been associated with a lower initial milk yield, steeper inclining, and declining slopes of the lactation curve, and a higher milk persistency compared with dry period length of 51 to 60 d [34]. Peak lactation is achieved later in cows with 0- to 35-d and 36- to 50-d dry period length than in those with dry period length of 51 to 6 d. Therefore, the effects of a short gestation in heifers and those induced by a short dry period in cows are comparable, although in this study differences in time to peak were only a tendency.

It has been reported that reducing the DPL resulted in a reduction in milk yield in the next lactation and a reduction in mammary cells renew rate [25]. This results in a large concentration of renewed mammary cells at the moment of calving which explains the high peak milk yield in the next lactation after a traditional dry period [35, 36]. In heifers, no renewal of mammary cells occurs since the mammary gland cells are still developing, and it is known that bovine mammary gland during the first gestation follows a continuous exponential form of growth [37], but it is reported that the majority of mammary growth occurs during the latter part of the gestation [38]. Thus, the effect of a short gestation length on the lactation performances is unavoidable. Lactation from animals of 0-1% and 1-5% category in this study showed a higher level of persistency compared to 5-25 and 25-75% group. Atashi and Asaadi (29) also found that the average milk yield persistency in primiparous cows with a short GL was higher than in those with an average or long GL. The association between GL and lactation performance may be, at least in a part, explained by this fact that the greatest increase in the mass of parenchymal tissue occurs in late pregnancy [39]; therefore, shorter the GL, less the mammary cells, and subsequently less the milk yield. Atashi and Asaadi (29) reported that Holstein primiparous with a short GL produced less milk at the beginning of lactation and at the peak than those with an average or long GL. However, inverse trends were found for milk yield persistency, upward and downward slopes of the lactation curve. Persistency of lactation is considered as a very important feature of the lactation. The shape of the lactation curve differs between cows in the first and in later parities, as primiparous cows have a lower initial and peak milk yield but a higher persistency compared to multiparous cows [40]. In other words, persistency decreases with increasing parity number. One explanation is that the heifer mammary cell population undergoes less apoptosis as lactation progresses due to the entirely new generation of cells, and besides, heifers are still growing, and so most probably is their mammary gland. In the present study, a difference in the level of persistency was found also within the same parity. This may relay on the favorite genetic relationship existing between persistency and various reproduction traits, such as age at calving [41]; as persistency decreases with increasing age at first calving, the very short GL in this study may have concurred to the greater persistency in 0-1% animals compared to 25-75% group. A higher lactation persistency is more profitable when yield and lactation persistency are correlated [42], but in the present study, the greater persistency in 0.1% animals was counterbalanced by the minor milk yield compared to 25-75% animals. To the best of the authors' knowledge, this is the first study to demonstrate the actual lactation performances in primiparous cows after a GL less than 210 d.

The negative effects registered on M305, peak yield, and scale were at least partially offset by a greater persistency; moreover, the loss of milk production was minor than what expected based on previous reports in cows. However, natural mating was registered as the last insemination in 6 of the 15 lactations encountered with extremely short gestation periods; one possibility is also that some of these were mistakenly considered by the breeder as mating, while the animals were already pregnant from a previous insemination or mating.

**CONCLUSIONS**

In conclusion, results showed an effect of the GL on the lactation performances of Holstein primiparous cows. The animals with shorter GL (0-1% and 1-5%) produced significantly less M305 production and peak yield, but had a higher lactation persistency and showed a lower upward slope of the lactation curve compared to those with a normal GL.

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| **Table 1.** List of reproduction events from 15 animals with short gestation lengths (<210 d). | | | | | | | | | |
| Animal | Birth Date | Number of Services | Type of  Service | Insemination Date | Pregnancy Check Date | Reported Calving Date | Expected Calving  Date | Gestation Length (d) | Remarks |
| 1 | 3/17/2014 | 1 | NS1 | 7/16/2015 | 10/21/2015 | 12/22/2015 | 3/15/2015 | 159 |  |
| 2 | 6/1/2011 | 1 | NS | 2/19/2013 |  | 8/1/2013 | 10/23/2012 | 163 |  |
| 3 | 10/13/2013 | 3 | NS | 9/4/2015 | 10/2/2016 | 3/29/2016 | 6/21/2015 | 207 |  |
| 4 | 7/22/2013 | 1 | AI2 | 10/1/2014 | 12/5/2014 | 3/16/2015 | 6/7/2014 | 166 |  |
| 5 | 8/3/2014 | 1 | AI | 9/30/2015 | 10/30/2015 | 3/11/2016 | 6/3/2015 | 163 |  |
| 6 | 3/17/2014 | 1 | NS | 7/16/2015 | 10/21/2015 | 12/22/2015 | 3/15/2015 | 159 |  |
| 7 | 9/18/2013 | 1 | NS | 5/25/2016 | 11/30/2016 | 12/4/2016 | 2/26/2016 | 193 |  |
| 8 | 10/30/2013 | 1 | NS | 8/12/2015 | 1/28/2016 | 2/8/2016 | 5/2/2015 | 180 |  |
| 9 | 3/17/2016 | 1 | AI | 4/13/2017 | 6/13/2017 | 11/7/2017 | 1/29/2017 | 208 | Abort - 11/7/2017 |
| 10 | 6/26/2015 | 1 | AI | 11/8/2016 |  | 5/11/2017 | 8/2/2016 | 184 |  |
| 11 | 4/2/2017 | 1 | ET3 | 7/3/2018 | 8/1/2018 | 12/20/2018 | 3/13/2018 | 170 |  |
| 12 | 6/23/2016 | 1 | AI | 12/18/2017 | 4/30/2018 | 5/28/2018 | 8/19/2017 | 161 | Heat - 12/17/17 |
| 13 | 5/11/2015 | 1 | AI | 12/21/2016 |  | 5/28/2017 | 8/19/2016 | 158 |  |
| 14 | 1/9/2016 | 1 | AI | 7/18/2017 | 10/13/2017 | 1/31/2018 | 4/24/2017 | 197 |  |
| 15 | 7/31/2017 | 1 | AI | 6/15/2018 | 11/16/2018 | 12/9/2018 | 3/2/2018 | 177 |  |
| NS1 = Natural service, AI2 = Artificial insemination, ET3 = Embryo transfer | | | | | | | | | |

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| --- | --- | --- | --- | --- |
| **Table 2.** The effect of the length of the gestation on the Milkbot lactation curve parameters1 in the first parity cows split by quantile of gestation length. | | | | |
|  | Gestation length categories | | | |
| Parameter | 0-1%  [150-242]  N=129 | 1-5%  [243-266]  N=501 | 5-25%  [267-274]  N=8019 | 25-75%  [275-297]  N=2231 |
| N | 129 |  |  |  |
| M305 (kg) | 7519 ± 189a1 | 7795 ± 136a | 8140 ± 119b | 8238 ± 115bc |
| Scale | 30.7 ± 0.75a | 32.3 ± 0.53a | 34.0 ± 0.46b | 34.4 ± 0.44bc |
| Ramp | 29.2 ± 0.35ab | 29.2 ± 0.21a | 28.9 ± 0.13ab | 28.7 ± 0.14b |
| Decay | 0.00123 ± 0.0000582a | 0.00130 ± 0.0000355a | 0.00137 ± 0.0000270b | 0.00138 ± 0.0000248b |
| Time to peak (d) | 81.5 ± 1.96a | 80.7 ± 1.18ab | 77.9 ± 0.87ac | 77.4 ± 0.78ac |
| Peak yield (kg) | 27.2 ± 0.66a | 28.3 ± 0.47a | 29.7 ± 0.41b | 30.1 ± 0.40c |
| 1 | | | | |

The MilkBot function is as follows:

In this function, *a* is the scale parameter, representing the theoretical maximum daily yield; *b* is the ramp parameter, controlling the rate of rise in milk production in early lactation; *c* is the offset parameter, describing the offset in time between parturition and the start of lactation; and *d* is the decay parameter, representing the rate of senescence of production capacity. The time at which peak lactation occurred (tpeak) was defined as: , and peak yield was calculated by substitution tpeak in the MilkBot equation. The 305-d milk, the cumulative milk yield between calving and day 305 of the lactation, was calculated as:

a, b, c Different superscripts indicate significant differences between gestation length categories at *P* < 0.05.

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| **Figure 1.** Individual lactation curves of the first parity animals with gestation length less than 210 d and minimum of 10 d in milk (blue lines = Artificial insemination, red lines = Natural service, and orange = Embryo transfer). |

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