Progesterone and betahydroxybutyrate in line measurements for a better description and understanding of Holstein cows fertility in field conditions

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Abstract

PLF devices allow to follow up large populations of animals in a non-invasive way over long periods of time and under field conditions. The objective of this work was to describe the ovarian activity and metabolic status during the postpartum period in dairy cows and their impact on further fertility in commercial herds. Data from 760 cows housed in 21 French farms were collected over the first 140 days postpartum (dpp). Milk progesterone was assayed on average every 2.7 days from 20 to 140 dpp whereas BHB was assayed every 3- 4 days during the first 21 dpp. Date and success of artificial insemination (AI) were recorded. Overall, 22.9% (174/760) cows resumed cyclicity (progesterone > 5 ng/ml) after 45 dpp (prolonged anovulatory interval postpartum), ovulation was delayed (follicular phase > 14 days) in 27.9% (212/760), luteolysis was delayed (a luteal phase > 20 days) for 26.1% of the cows (199/760) whereas 34.3% (261/760) suffered from an early luteolysis (a short luteal phase < 10 days). A total of 74.3% (565/760) cows showed at least one atypical pattern of cyclicity. The prevalence of biochemical ketosis (BHB> 0.15 mmol/L) is higher in cows with at least one cyclicity abnormality (p-value=0.02). In cows without any atypical pattern, a shortening in calving to first insemination interval (83.2 dpp vs 97.1 dpp; p-value<0.001) and calving to conception interval (120.7 dpp vs 143.6 dpp; p-value<0.001) were observed. Atypical patterns of ovarian activity are thus of high frequency in Holstein cows, associated to metabolic issues and significantly impact on fertility.

Keywords: Dairy cow, progesterone, betahydroxybutyrate, fertility,

Introduction

During the last month of pregnancy, ovarian activity decreases with a progressive follicular wave spacing,and even a complete stop of wave patterns during the last 21 days of pregnancy (Driancourt, 2001). Normal follicular waves appear again within one week after calving (Roche *et al.*, 1991). However, ovulation, that effectively characterizes cyclicity recovery, occurs at very variable postpartum delays (Royal *et al.*, 2000; Petersson *et al.*, 2006b). Moreover, after this first ovulation, ovarian cycles do not necessarily follow in a regular way, that is a succession of a follicular phase being 3 to 14 days long and a luteal phase 10 to 19 days long. Other durations of the two phases together with first ovulation occurring later than 45 days postpartum (dpp) characterize atypical patterns of ovarian cyclicity (Royal *et al.*, 2000; Opsomer *et al.*, 2000). In addition to the frequency of the different abnormalities, the question of their subsequent consequences on reproductive performances arises.

The aim of this project was to describe the ovarian cyclicity resumption in dairy cows under field breeding conditions in France, to evaluate the impact of atypical patterns on fertility. Since the onset of lactation implies a huge increase in cow energy requirements, dairy cows are at high risk of ketosis during the postpartum period: this study also aimed to evaluate the frequence of ketosis in the field, together with its association with atypical patterns of ovarian cyclicity and further reproductive performances Available data (especially those in French breeding conditions) were often collected from experimental herds and/or from a small number of farms, on a limited number of animals, and/or with a low frequency of sampling, limiting the accuracy of the description (Cutullic *et al.*, 2011; Disenhaus *et al.*, 2008; Ledoux *et al.*, 2011 ; Opsomer *et al.*, 2000 ; Petersson *et al.* 2006a).

Material and methods

Data collection

Twenty-one French commercial herds equipped with Voluntary Milking System (VMS, DeLaval International, Tumba, Sweden) were included in the study. The mean number of cows by herd was 109 [min-max: 64-203] with 1.8 VMS per farm. Their milking robots were coupled to Herd Navigator system (HN, DeLaval International, Tumba, Sweden), repeatedly assaying milk progesterone (P4) and beta hydroxybutyrate (BHB) concentrations. Biomodels control HN automatic in-line sampling and measuring at varying intervals (Nielsen *et al.*, 2005; Friggens *et al.*, 2008). BHB is measured during the first three months of lactation. Progesterone assays begin at 20 days postpartum until the confirmation of pregnancy. A total of 153 715 milk P4 records and 78 362 milk BHB records were collected between April 2014 and July 2015 during 4 029 Holstein cow's lactations. Data on calving date, parity, milk production, insemination dates and pregnancy check were also registered.

The study focused on the period between the calving date and 140 days postpartum ("daily milk production filter"; table 1). Data were then selected as follows. In case of gaps between two consecutive progesterone assays longer than 10 days, the whole lactation was deleted from the study ("Progesterone filter", table 1). For the 760 lactations retained, progesterone was assayed every 2.8 ± 0.5 days (mean ± sd) between day 20 and day 140 postpartum. For the analysis of BHB risk factor, only lactations with a minimum of three BHB measurements during the 21 days postpartum were kept ("BHB filter", table 1):740 lactations were retained with on average a BHB assay every 1.4 ± 0.5 days. Table 1 describes the number of assays and cows after application of each filter.

Table 1: Number of progesterone (P4), β-hydroxybutyrate records (BHB) lactations, and cows retained by filtering criteria.

Criteria	P4	BHB	Lactations
Raw database	153 715	78 362	4029
Daily milk production filter [day 0- day 140 pp]	46 753	40 302	1102
Progesterone filter	38 539	28 837	760
BHB filter	37 632	28 343	740

Data smoothing

After applying daily milk production and progesterone filters (Table 1), the remaining progesterone data were processed. Periods during which progesterone was higher than 5 ng / mL were called “luteal phases” and periods during which progesterone is lower than 5 ng/mL , “follicular phases”.

The first step consisted of estimating true onset and end date of each phase (time when the progesterone crosses the threshold value of 5 ng/mL) by the application of a linear interpolation method. Then, in the second step, fake follicular and luteal phases were deleted (luteal phase duration less than 3 days and follicular phase less than 2 days because biologically meaningless).

Definitions

The various patterns of abnormal cyclicity resumption are defined in Table 2. Cows with BHB concentration higher than 0.15 mmol/L at least once during the first 21 days of lactation were considered as affected by biochemical ketosis (BHB + group). The others were considered non affected (BHB – group).

Table 2: Definitions of atypical patterns (modified from Royal *et al.*, 2000, Opsomer *et al.*, 2000 and Petersson *et al.*, 2006a).

Delayed cyclicity (DC)	Progesterone levels ≤ 5 ng/mL during the first 45 days postpartum (included)
Delayed ovulation (DO)	Progesterone levels ≤ 5 ng/mL during more than 14 days (included) between two luteal phases
Delayed luteolysis (DL)	Progesterone levels ≥ 5 ng/mL during more than 20 days (included)
Early luteolysis (EL)	Progesterone levels ≥ 5 ng/mL during less than 10 days (included)

Statistical analysis

Data editing, filtering, trait definition and statistical analysis were carried out in R software version 3.3.2. (R Core Team, 2016). The normality was evaluated with the Shapiro–Wilk test. Data were analyzed using univariate tests (chi square and t-tests).

Results and discussion

Prevalence of atypical patterns

A total of 74.3% (565/760) cows exhibited at least one atypical pattern of cyclicity over the first 140 days of lactation.

The prevalences of the different patterns observed in the present study (Table 3) are in part different from those described in literature. Reported prevalence of delayed ovulation ranges between 3.7 and 12% (Opsomer *et al.*, 2000; Royal *et al.*, 2000; Cutullic *et al.*, 2008; Shrestha *et al.*, 2004) versus 28.7% in our study. The most stricking difference is for early luteolysis, whose prevalence was 34.3% in our study whereas very rare (0.5% to 3.7%) according to Opsomer *et al.*, (2000), Shrestha *et al.*, (2004), Cuttulic *et al.*, (2012) and Ranasinghe *et al.*, (2011); in those studies, the first luteal phase was always excluded, in contrast with the present work. In some other studies dealing with patterns of ovarian resumption in cows, short luteal phases were even not looked for (Disenhaus *et al.*, 2008; Cuttulic *et al.*, (2012); Ledoux *et al.*, 2011). Conversely, prevalences of delayed luteolysis (26.1%) and delayed cyclicity (22.9%) are coherent with those reported in earlier studies, ranging respectively from 11.9 to 35% and from 13 to 21% (Opsomer *et al.*, 2000; Royal *et al.*, 2000; Disenhaus *et al.*, 2008; Cuttulic *et al.*, 2012; Ledoux *et al.*, 2011; Shrestha *et al.*, (2004); Ranasinghe *et al.*, 2011)

Table 3 Prevalence of the different types of atypical patterns of ovarian cyclicity resumption (n=760 cows). One cow can have developed several types of abnormalities within one postpartum period.

Pattern	Cows	%
Delayed cyclicity	174	22.9
Delayed ovulation	212	29.7
Delayed luteolysis	199	26.1
Early luteolysis	261	34.3

<u>Ketosis as a risk factor for atypical pattern of ovarian cyclicity</u>

Overall, 17% of the cows developed biochemical ketosis over the first 21 days postpartum in our population. This figure is coherent with the observation of Philippe and Raboisson (2012) in French dairy herds, with 19% of cows affected based on BHB milk concentration. Ketosis has also a significant negative effect on the risk of cyclicity abnormality: only 11.5% of the cows with normal pattern vs 19.0% of females with at least one atypical pattern were BHB+ (p-value=0.02). Cows with at least on atypical pattern represent 83% of BHB+ group against 72% of BHB- group. However, no significant difference was evidenced when each anomaly was considered separately.

This result, based on ketosis diagnosed based on biochemical criteria is consistent with those of Walsh et al (2007) and Shin et al (2015) who described an impact of subclinical ketosis on the time of ovarian resumption. Considering clinical ketosis, Opsomer et al (2000) measured a 11-fold increase of the risk of delayed ovulation.

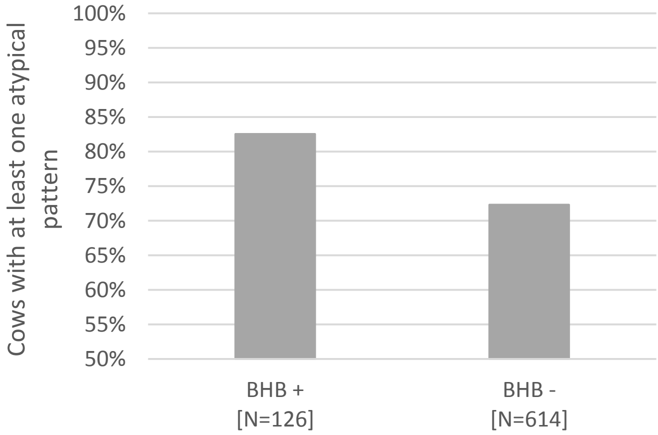

Figure 1: Percentage of cows with at least on atypical pattern in BHB + and BHB – groups (p-value=0.02)

Impact on fertility

Calving to first insemination interval was shorter for cows that resumed ovarian cyclicity postpartum following a normal pattern compared to cows suffering from at least one abnormality (83.2 dpp vs 97.1 dpp; p-value < 0.001; figure 2A). A significant increase was also observed for calving to conception interval (120.7 dpp vs 143.6 dpp; p-value<0.001; figure 2B).
Few studies evaluated the impact of the pattern of postpartum ovarian resumption on further fertility. A negative impact of atypicity was evidenced in Great-Britain twenty years ago with a + 7.5 and + 12.5 days increase of calving to first insemination (Lamming and Darwash (1998), Royal et al (2000) respectively) and a +18 days increase in calving to conception interval (Lamming and Darwash 1998). Ranasinghe et al (2011) also observed an increase in these intervals in Holstein dairy cows in case of different postpartum anomaly resumptions.

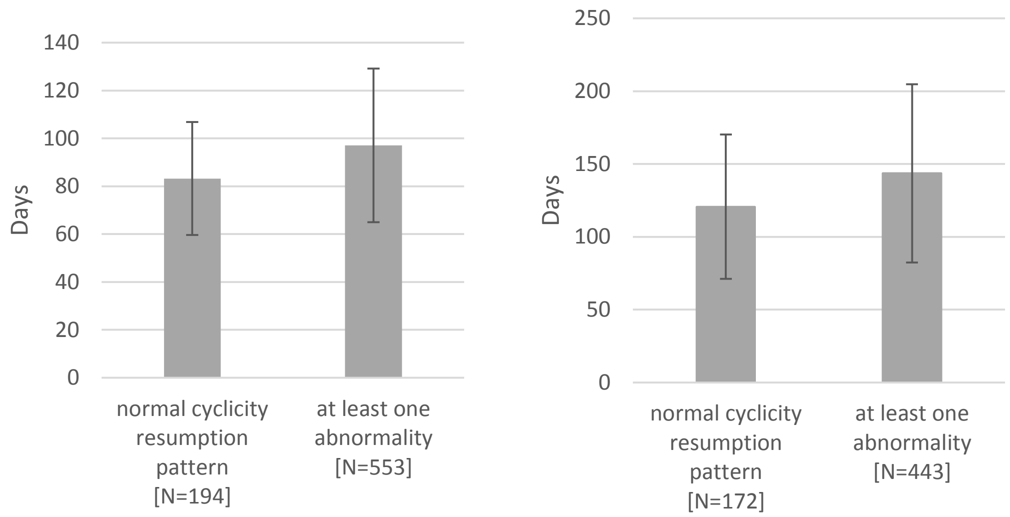

Figure 2 : Calving to first insemination (at left) and calving to conception (at right) intervals depending on the normality of ovarian resumption pattern (p<0.001)

Conclusions

This study evidenced not only the high prevalence of abnormalities in ovarian cyclicity resumption in modern Holstein dairy cows in field conditions in France, but also downstream their significant impact on further fertility and upstream ketosis as a risk factor. In that context, PLF tools contribute to a precise phenotyping of cows postpartum, allowing an early detection of cows at higher risk of infertility, and in consequence a precocious and appropriate care.

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References

Cutullic E., Delaby L., Gallard Y. and Disenhaus C. 2011. Dairy cows' reproductive response to feeding level differs according to the reproductive stage and the breed. *Animal* **5**(5):731-40

Disenhaus C., Cutullic E., Blanc F., Gatien J., Agabriel J., Hetreau T., Michel G., Paccard P., Badinand F., Egal D. and Ponsart C. 2008. Caractéristiques comparées de la cyclicité après vêlage de différentes races bovines. *Rencontres Recherches Ruminants* **15**, 383-386

Driancourt M.A. 2001. Regulation of ovarian follicular dynamics in farm animals. Implications for manipulation of reproduction. *Theriogenology*.**1;55**(6):1211-39.

Friggens N.C., Bjerring M., Ridder C., Hojsgaard S. and Larsen T. 2008. Improved Detection of Reproductive Status in Dairy Cows using Milk Progesterone Measurements. *Reproduction in Domestic Animals* **43** (Suppl 2), 113-121.

Lamming G.E. and Darwash A.O. 1998 The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. *Animal Reproduction Science*. **11;52**(3):175-90.

Ledoux D., Touze J.L., Richard C., Ponter A.A., Bosc M.J. and Grimard B. 2011. Abnormal patterns of resumption of cyclicity after calving in Holstein cows : risk factors, relationships with the ultrasound appearance of the ovaries and with gestation failure after AI. *Revue Medecine Vétérinaire* **162**, 98-106

Nielsen N.I., Friggens N.C., Chagunda M.G.G. and Ingvartsen K.L. 2005. Predicting risk of ketosis in dairy cows using in-line measurements of beta-hydroxybutyrate: A biological model. *Journal of Dairy Science* **88**, 2441-2453

Opsomer G., Gröhn Y.T., Hertl J., Coryn M., Deluyker H. and De Kruif A. 2000. Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology* **53**(4):841-57

Petersson K.J., Gustafsson H., Strandberg E. and Berglund B. 2006a. Atypical progesterone profiles and fertility in Swedish dairy cows. *Journal of Dairy Science* **89**(7):2529-38.

Petersson K.J., Strandberg E., Gustafsson H. and Berglund B. 2006b. Environmental effects on progesterone profile measures of dairy cow fertility. *Animal Reproduction Science* **91**, 201–214

Philippe P. and Raboisson D. 2012. Prévalence de la cétose subclinique dans les troupeaux bovins laitiers de l'Ouest de la France. *Renc. Rech. Ruminants* **19**

Ranasinghe R.M., Nakao T., Yamada K., Koike K. and Hayashi A., Dematawewa C.M. 2011. Characteristics of prolonged luteal phase identified by milk progesterone concentrations and its effects on reproductive performance in Holstein cows. *Journal of Dairy Science*. **94**(1):116-27

Roche J.;F., Boland M.P. 1991. Turnover of dominant follicles in cattle of different reproductive states. *Theriogenology* **35**:81-90.

Royal M.D., Darwash A.O., Flint A.P.F., Webb R., Wooliams J.A. and Lamming G.E. 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. *Animal Science* **70**, 487-501

Shin E.K., Jeong J.K., Choi I.S., Kang H.G., Hur T.Y., Jung Y.H. and Kim I.H. 2015. Relationships among ketosis, serum metabolites, body condition, and reproductive outcomes in dairy cows. *Theriogenology*. **15;84**(2):252-60.

Shrestha H.K., Nakao T., Higaki T., Suzuki T. and Akita M. 2004. Resumption of postpartum ovarian cyclicity in high-producing Holstein cows. *Theriogenology*. **61**(4):637-49.

Walsh R.B., Kelton D.F., Duffield T.F., Leslie K.E., Walton J.S. and LeBlanc S.J. 2007. Prevalence and risk factors for postpartum anovulatory condition in dairy cows. *Journal of Dairy Science* **90**(1):315-24.