A large population study to assess the magnitude of prenatal programming in dairy cattle

C Fouéré, M P Sanchez, M Boussaha, S Fritz, A Vinet, H Kiefer, D Boichard, C Hozé

To cite this version:

C Fouéré, M P Sanchez, M Boussaha, S Fritz, A Vinet, et al.. A large population study to assess the magnitude of prenatal programming in dairy cattle. 2024. hal-04569709

HAL Id: hal-04569709
https://hal.inrae.fr/hal-04569709

Preprint submitted on 6 May 2024

J. Dairy Sci. TBC
https://doi.org/10.3168/jds.2023-24051

A large population study to assess the magnitude of prenatal programming in dairy cattle

C. Fouéré,[1,2]* M. P. Sanchez,[2] M. Boussaha,[2] S. Fritz,[1,2] A. Vinet,[2] H. Kiefer,[3] D. Boichard,[2] and C. Hozé[1,2]*
[1]Eliance, 75012 Paris, France
[2]Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France
[3]Université Paris-Saclay, INRAE, Ecole Nationale Vétérinaire d'Alfort, BREED, 78350 Jouy-en-Josas, France

ABSTRACT

The performance of an adult dairy cow may be influenced by events that occur before her birth. The present study investigated potential effects of 2 prenatal groups of factors, Assisted Reproductive Techniques (ART) and maternal characteristics (e.g., dam parity), on offspring performance during their first lactation, in populations of 2 dairy cow breeds: French Holstein and Montbéliarde. The different ART studied included the type of semen (conventional or X-sorted) used for Artificial Insemination (AI) and the technology of conception used (AI, embryo transfer, or in vitro fertilization). Three maternal characteristics were considered: (1) the dam age at first calving, (2) dam parity number, and (3) indicators of dam udder health during gestation (somatic cell score and events of clinical mastitis). First, we investigated whether heifer survival from 3d to 18 mo old was associated with any of the prenatal factors considered. We then estimated the associations of these prenatal factors with 8 traits of commercial interest: (1) stature, (2–4) milk, fat, and protein yields, (5) somatic cell score, (6) clinical mastitis, and (7–8) heifer and cow conception rate, all measured on genotyped cows. Linear models were used for this study with the prenatal factors as covariates in the model, and for the 8 traits, phenotypes were adjusted for their corresponding genomic estimated breeding value. The results indicated that the survival rate of heifers born from embryo transfer was significantly higher than that of heifers born from AI (probably due to preferential management practices), while the other prenatal factors did not explain differences in heifer survival. Among the Montbéliarde cows born from AI, those born from X-sorted semen showed a lightly but significantly lower milk yield than those born without X-sorting of the semen (−52 kg of milk in the first lactation). Among the Holstein cows, those born from embryo transfer presented significantly lower milk performance than cows born from AI. Regarding the maternal characteristics, none or very weak associations were found between the dam age at first calving and the offspring performance in both breeds. Dam parity, on the other hand, was associated with offspring performance for milk, fat, and protein yield in both breeds, however not in the same direction. In the Holstein breed, an increase in dam parity was favorable for offspring performance for milk, fat, and protein yield, whereas in the Montbéliarde breed, an increase in dam parity was associated with lower milk and protein yield and no association was found for fat yield. The udder health of the dam during gestation was not or only weakly associated with the traits studied in the offspring. Although some significant associations were identified due to the large sample size, the effects were modest, typically less than 1% of the phenotypic mean, and were not consistently observed across the 2 breeds.

Key Words: developmental programming, assisted reproductive technologies, dairy cows

INTRODUCTION

The concept of prenatal programming, first introduced by Barker in 1990 (Barker, 1990) and subsequently extended to the developmental origins of health and disease theory (Barker, 2007), suggests that events that occur before an animal is born can have lasting effects on its future life and performance. While this phenomenon has been observed in various species, including human (Schulz, 2010), the underlying mechanisms in livestock species remain elusive, with previous research often yielding inconsistent results.

As highlighted by Carvalho et al. (2020), the extensive amount of phenotypic records, along with pedigree and genomic information available for thousands of hundreds (or sometimes millions) of animals in dairy

---

Received August 5, 2023.
Accepted March 8, 2024.

*Corresponding authors: Corentin Fouéré, Address: 149 rue de Bercy, 75595 PARIS CEDEX 12, France, E-mail: corentin.fouere@inrae.fr, Chris Hozé, Address: 149 rue de Bercy, 75595 PARIS CEDEX 12, France, E-mail: chris.hoze@eliance.fr

cattle populations, provide an excellent opportunity to study the magnitude of effects from prenatal programming mechanisms. In addition, with genomic selection implemented since 2009 in several dairy breeds, accurate predictions of breeding values are available and can be efficiently used to study systematic deviations from expectations.

In dairy farming, Assisted Reproductive Technologies (ART) are widely deployed, a practice that raises a particular attention for being known to cause fetal disturbances (Siqueira et al., 2020). More specifically ART in dairy farming include Artificial Insemination (AI) with conventional (CONV) or X-Sorted Semen (SS), Embryo Transfer after multiple ovulation (ET) and In Vitro Fertilization (IVF). Studies on both human and cattle semen have shown that various environmental conditions can induce epigenetic changes in male gametes, which affect the health of the offspring (Schagdarsurengin and Steger, 2016; Kiefer et al., 2021). Moreover, the effect of the sorting process to obtain SS on the epigenome of bull spermatozoa remains uncertain (Kumaresan et al., 2020), although so far, no association with offspring reproductive or lactation performance has been reported (Maicas et al., 2020). The effect of IVF in the performance of animals has been the subject of a substantial amount of research. However, as indicated by Beilby et al. (2023) in a review of many studies on multiple species, although the use of IVF was found to be associated with greater size of the offspring, the findings for other traits such as metabolism, behavior, and cardiovascular diseases were not consistent. Bonilla et al. (2014) reported an increased mortality in dairy cattle within the first 20d of a calf's life, when the calves were conceived by ET rather than AI, but no significant difference in adult fertility or Milk Yield (MY) was observed. More recently, Lafontaine et al. (2023) compared the performance of milk traits for cows born from AI, multiple ovulation ET, and IVF, reporting no difference in the performance of the first 3 lactations; however, the authors identified a minor decrease in the conception rate when cows were conceived by multiple ovulation ET or IVF, rather than by AI. In addition, Urrego et al. (2014) reported that bovine IVF embryos presented a reduced developmental capacity, which was partly attributed to alterations in the epigenetic profile of the gametes. Finally, multiple ovulation treatments on heifers have also been reported to be associated to altered gene expression patterns in embryos, however to a lesser extent than IVF (Gad et al., 2011).

Prenatal factors related to maternal characteristics have also been investigated by several studies, in addition to the effects of ART on offspring performance. Swartz et al. (2021) identified a minor negative effect of the Somatic Cell Score (SCS) of the dams on the Fat Yield (FY) of their daughters during the first lactation. Similarly, González-Recio et al. (2012) found an unfavorable association between the occurrence of mastitis during a cow's gestation and the productive life of her daughter (−11d). Carvalho et al. (2020) compared the performance for several traits of daughters from dams that presented or not a clinical disease (e.g., lameness, mastitis, retained placenta) during the gestation. The authors found that, while daughters of dams who presented clinical diseases were less likely to present a clinical disease in their own first lactation, they were more likely to leave the herd in the first lactation. In the same study, Carvalho et al. (2020) observed that daughters of lactating cows during pregnancy presented no significant difference in MY, compared with daughters of nonlactating cows, a result that contrasts with that from González-Recio et al. (2012), who showed that daughters of lactating cows during pregnancy had a lower MY than daughters of nonlactating cows

Our present study aimed to investigate the association between prenatal factors related to ART or maternal characteristics and the adult offspring performance. To investigate such effects, we modeled the observed performance of the daughters adjusting for their Genomic Estimated Breeding Values (GEBV; calculated before performance recording) as a function of the aforementioned prenatal programming effects of our interest. This study was conducted for the 2 major French dairy cattle breeds, Holstein and Montbéliarde, for which large data sets are available.

MATERIALS AND METHODS

Data

Our study used phenotypic, pedigree, and genomic data from the French national cattle database (INRAE, CTIG, Jouy-en-Josas) for both the Holstein and Montbéliarde cows studied, and this data set also included the maternal characteristics and the ART category from which cows were born. Table 1 presents the number of animals with records available for each trait and their descriptive statistics, for both studied breeds. The general outline of the study indicating the prenatal factors and traits analyzed is in Figure 1.

Prenatal factors

Assisted reproductive technologies. We investigated 2 types of prenatal factors related to ART: semen sexing and conception methods. For semen sexing, we selected cows born from either CONV or SS. To avoid confounding effects and to assess the specific effect of SS, the analysis for semen sexing was restricted to fe-

Table 1. Descriptive statistics (numbers of records, means, and standard deviations (s.d.)) of the traits analyzed in this study for genotyped female offspring born after 2015

	Holstein			Montbéliarde		
Trait	Number	Mean	SD	Number	Mean	SD
Stature[1,2]	138,180	6.40	1.54	89,979	145.7	3.90
Milk Yield (kg)[2]	213,923	7875	1475	108,894	6831	1275
Fat Yield (kg)[2]	213,923	315.0	59.1	108,894	264.7	52.4
Protein Yield (kg)[2]	213,923	254.3	48.3	108,894	228.7	44.8
Somatic Cell Score[2]	213,863	2.06	1.21	108,889	2.26	1.29
Clinical Mastitis[2]	163,893	0.10	0.30	80,214	0.08	0.27
Heifer Conception Rate[3]	244,691	0.56	0.49	98,087	0.57	0.49
Cow Conception Rate[4]	105,396	0.46	0.50	30,485	0.53	0.50

[1] Observation unit is dependent on cow breed: score from 1 to 9 in Holstein, in cm in Montbéliarde.

[2] Only the first lactations were retained.

[3] Only the first AI was retained.

[4] Only the first AI of the first lactation was retained.

males born from heifers and not from embryo transfer procedures (i.e., from AI only). For the analysis of conception methods, the performance of cows born from AI, ET, and IVF were compared. Both ET and IVF techniques can be combined with fresh or frozen (F) embryos, defining the 4 categories ET, FET, IVF-ET, and IVF-FET, in which ET and FET indicate fresh and frozen embryos, respectively. Finally, 5 conception methods were compared: AI, ET, FET, IVF-ET, and IVF-FET. The AI conception method was the control group, being it the conventional AI without embryo transfer, and to avoid confounding of effects for the other conception methods, only cows born from heifer surrogates were considered for the analysis. In addition, the control females were born from heifers of the same breed and were from the same herd as the embryo transfer females.

Maternal characteristics. The following 4 maternal characteristics were investigated:

- Parity, with 4 categories (1, 2, 3, > 3);
- Age at First Calving (AFC) for heifer dams (non-lactating during their gestation);
- Average SCS of the lactating dams, computed within 2 specific 120-d windows: 0 to 120d of gestation, and 120 to 240d of gestation; at least 2 dam test-day records were required within each window and the average SCS was calculated from the first and the last test-day records available in each window.
- Clinical Mastitis (CM) of the lactating dams, classified as 0 (no mastitis during gestation) and 1 (at least one event of mastitis during gestation). Only records from herds that reported at least 3 CM cases per year were kept.

Daughter performance. To assess the extent of prenatal programming, we focused on the performance of the daughters that have been exposed to these prenatal factors. Our data set consisted of phenotypic records, pedigree, and genotypes from cows born after 2015. First, to assess any potential bias associated with the selection of genotyped offspring, we examined the association of the prenatal factors of our interest with offspring survival between 3d and 18 mo of age (i.e., the percentage of animals that remained alive during this period) for both genotyped and non-genotyped heifers. Thus, the data consisted of heifers that were either alive at 18 mo of age or had died of natural causes; heifers culled before 18 mo of age were excluded from the study. Then, considering only genotyped cows, 8 traits related to stature, milk production, udder health, and fertility were analyzed:

- Stature (STAT);
- 305-d MY, FY, and Protein Yield (PY): calculated from test-day records using the Fleischmann method (International Committee for Animal Recording, 2014);
- Average SCS over the lactation, computed using the formula: $\text{SCS} = \log_2(\text{somatic cell count}/100{,}000) + 3$;
- CM, defined by the absence (0) or presence (1) of at least one clinical mastitis event in the first 150d of lactation;
- Heifer Conception Rate (HCR) and Cow Conception Rate (CCR), both at first AI.

Except for heifer fertility, all traits were recorded during the first lactation of the cow. To ensure an adequate analysis for every one of the traits, the data filtering was based on each specific trait and factors of interest, resulting in different subsets of data for each analysis.

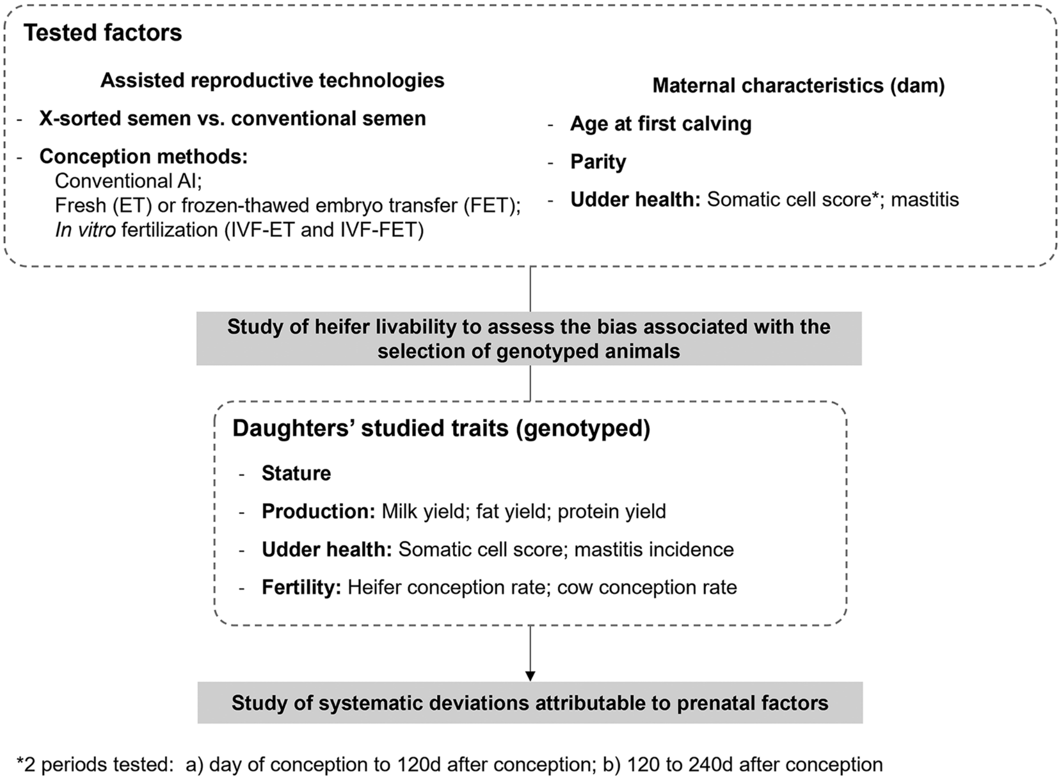

Figure 1. Workflow of the analyses performed. First, we investigated the effect of various prenatal factors on heifer survival (considering both genotyped and non-genotyped females) to assess the potential bias introduced by selecting only genotyped females. Second, we explored the effect of prenatal factors on 8 traits in genotyped females, to identify differences in performance associated with the different modalities of each factor tested.

The descriptive statistics for these 8 traits are presented in Table 1.

Models and statistical analyses. To estimate the effects of the prenatal factors on the offspring survival, the following linear model [1] was used:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{e} \qquad [1]$$

where **y** is the vector of the female survival (1 = female still alive at 18 mo of age; 0 = female died of natural cause between 3d and 18 mo of age); $\boldsymbol{\beta}$ is the vector of the fixed effects to be estimated: (1) the herd-year combinations, (2) the month-year combinations, and (3) the ART or the maternal characteristic of interest, modeled individually; **X** is the corresponding incidence matrix for the listed fixed effects; and **e** is the vector of random residuals, assumed to be independent and identically distributed, following a normal distribution with mean zero and variance σ^2_e.

For all the other traits, the prenatal factors were individually estimated with the following linear model [2]:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathrm{b}\,\mathbf{GEBV} + \mathbf{e} \qquad [2]$$

where **y** is the univariate vector of the cow performance (for the cows born after 2015) for each one of the traits studied; $\boldsymbol{\beta}$ is the vector of the fixed effects to be estimated: (1) the non-genetic effects included in the French routine genetic evaluation (International Bull Evaluation Service official website, https://interbull.org/ib/geforms) (Supplementary Table S1; https://doi.org/10.6084/m9.figshare.25127177); and (2) the ART or the maternal characteristic of interested, modeled individually; **X** is the corresponding incidence matrix for the listed fixed effects; **GEBV** is the vector of genomic estimated breeding values obtained with a prior genetic evaluation for the analyzed trait; b is the corresponding regression coefficient associated to the GEBV; and **e** is the vector of random residual, assumed to be independent and identically distributed, following a normal distribution with mean zero and variance σ^2_e.

The GEBV of the daughters was included in the linear model [2] to account for the genetic potential of these daughters. Those GEBV were obtained from the genetic evaluations performed in March 2018 for both breeds in this study, Holstein and Montbéliarde. Those genetic evaluations performed in March 2018 predicted the GEBV of the daughters without information on their own performance, being therefore uninfluenced by potential effects of the prenatal factors. A side note with respect to the GEBV is that they were obtained from a full genomic model, and the French genomic evaluation does not include any residual polygenic effect, being the GEBV considered in this study the sum of the estimated contributions of each marker (Boichard et al., 2023).

Models [1] and [2] were executed using the PROC GLM procedure of the SAS software version 9.4 (SAS Inst. Inc., Cary, NC). To account for multiple testing (334 independent class-breed-factor-trait combinations), we adjusted P-values for a false discovery rate (FDR) threshold of 0.05, following the Benjamini-Hochberg approach (Benjamini and Hochberg, 1995).

RESULTS

With respect to the multiple testing, out of the 334 tests for the independent class-breed-factor-trait combinations, 85 were significant at the nominal 0.05 P-value. Assuming an FDR threshold of 0.05, the adjusted P-value threshold was 0.008 and only 61 tests remained statistically significant.

Association between prenatal factors and offspring survival

To ensure the absence of bias in selecting only genotyped daughters, we first tested the association of prenatal factors (ART or maternal characteristics) with the survival of the daughters. Overall, average heifer survival was 0.88 in Holstein and 0.85 in Montbéliarde. Out of all the prenatal factors tested, the largest differences in heifer survival were observed for daughters born from different conception methods. For both breeds, animals born from ET presented a higher survival rate compared with their counterparts born from AI, and for Montbéliarde, animals born from FET also presented a higher survival rate compared with those born from AI (Figure 2). For both breeds, daughter mortality also increased, although lightly, with increasing SCS in late gestation of their dams, and for Montbéliarde the same association between mortality rate and SCS in early gestation was found. In this latter breed, mortality was mildly higher for daughters of heifers than for daughters of older dams. For most prenatal factors tested, observed differences in heifer survival were small (Supplementary Table S2; https://doi.org/10.6084/m9.figshare.25127177), suggesting that the selection of genotyped animals generated limited bias in the subsequent analyses.

Performance of cows born from ART

X-sorted semen. Table 2 presents the differences in the performance of cows born from either CONV or SS. We observed only minor differences associated to SS in MY and PY in the Montbéliarde breed. MY of Montbéliarde cows born from SS was 52 kg lower than the MY from Montbéliarde cows born from CONV, representing only 0.8% of the phenotypic mean. No effect of SS was detected for the remaining traits.

Conception technologies. In the investigation of conception technologies, no significant association was found between these methods and the traits studied in the Montbéliarde daughters. Conversely, some significant associations were detected for Holstein. For this latter breed, Holstein cows born from ET and FET had slightly lower MY and PY, compared with those born from AI (−1.3 and −1.8% of the MY phenotypic mean, respectively; and −0.7 and −1.4% of the PY phenotypic mean, respectively). In addition, a lower FY (−1% of the phenotypic mean) was observed for Holstein cows born from FET compared with those born from AI. (Supplementary Table S3; https://doi.org/10.6084/m9.figshare.25127177).

Performance of cows born from dams with different maternal characteristics. Dam age at first calving: For both breeds, the performance of cows born from heifer dams was generally not associated with the AFC of their dams (Supplementary Table S4; https://doi.org/10.6084/m9.figshare.25127177). The only exception was observed for Holstein, for which the cows born from dams aged over 1000d at their first calving presented a lower FY and heifer conception rate, when compared with cows born from dams aged less than 800d at their first calving, representing a reduction of 0.9% (P-value = 0.005) and 3.6% (P-value = 0.003) of the phenotypic mean, respectively.

Dam parity: For both Holstein and Montbéliarde breeds, cows born from second calving dams (i.e., cows gestated during their dams' first lactation) were found to have the lowest height, compared with cows born from heifer dams or dams of later parity. Holstein cows born from heifer dams exhibited reduced MY, FY, and PY during their first lactation compared with those born from lactating dams, corresponding to reductions of up to −1.7% of the phenotypic mean. Conversely, for Montbéliarde the cows born from multiparous dams presented lower MY and PY, compared with cows born from heifer dams. Additionally, for Montbéliarde, the udder health of the daughter was associated to the dam parity, with cows born from dams with at least 3 parities presenting lower SCS than those born from dams of parity 1 and 2. Finally, for the Holstein breed, the heifer conception rate was lower for cows born from a

Table 2. Difference in performance (estimate (SE)) between females born from conventional semen (reference, estimate = 0) and those born from sexed semen on the different traits for Holstein and Montbéliarde breeds

Breed	Holstein		Montbéliarde	
Trait	Number	Estimate (SE)	Number	Estimate (SE)
Stature[1,2]	40329	0.00 (0.01)	9685	0.09 (0.06)
Milk yield[1] (kg)	66077	−10.9 (9.39)	13352	−51.8 (17.6)*
Fat yield[1] (kg)	66077	0.21 (0.36)	13352	−1.45 (0.67)
Protein yield[1] (kg)	66077	−0.35 (0.29)	13352	−1.92 (0.57)*
Somatic cell score[1]	66077	0.00 (0.01)	13352	0.03 (0.02)
Clinical mastitis[1]	41942	0.00 (0.003)	3926	0.00 (0.01)
Heifer conception rate[3]	82265	0.01 (0.004)	12766	0.01 (0.01)
Cow conception rate[4]	28687	0.01 (0.01)	1366	0.04 (0.05)

*Significant differences between means (FDR < 0.05).

[1] Only the first lactations were retained.

[2] Observation unit is dependent on cow breed: score from 1 to 9 in Holstein, in cm in Montbéliarde.

[3] Only the first AI was retained.

[4] Only the first AI of the first lactation was retained.

dam's second calving, compared with those cows born from heifer dams (Table 3).

Dam udder health: The results for the estimated effects of SCS of the dams on their daughters' studied traits are shown in Supplementary Table S5 (https://doi.org/10.6084/m9.figshare.25127177). Modest associations were detected between the dam SCS during gestation and their corresponding daughters′ performance. For Holstein, a low SCS of the dams during the early gestation period (0d to 120d after conception) was associated with a reduced MY in daughters. For this same breed, a low SCS during the period 120d to 240d after conception was associated with lower values of MY, FY, and PY in the offspring. Mastitis incidence was higher for daughters that were gestated by dams with a high SCS during the period between 0d and 120d (both breeds) and between 120d and 240d (Holstein). During both studied periods in the Holstein breed, SCS was lower for the daughters of dams with a low SCS. No association was detected between SCS and the other traits in both breeds.

The results for the estimated effects of CM of the dams on their daughters' studied traits are shown in Supplementary Table S6 (https://doi.org/10.6084/m9.figshare.25127177). For Holstein cows, the occurrence of CM in the dam during gestation was associated with an increase in the daughter conception rate in the first lactation. For Montbéliarde, no association was found between the occurrence of mastitis during the dam's gestation and the traits studied in their daughters.

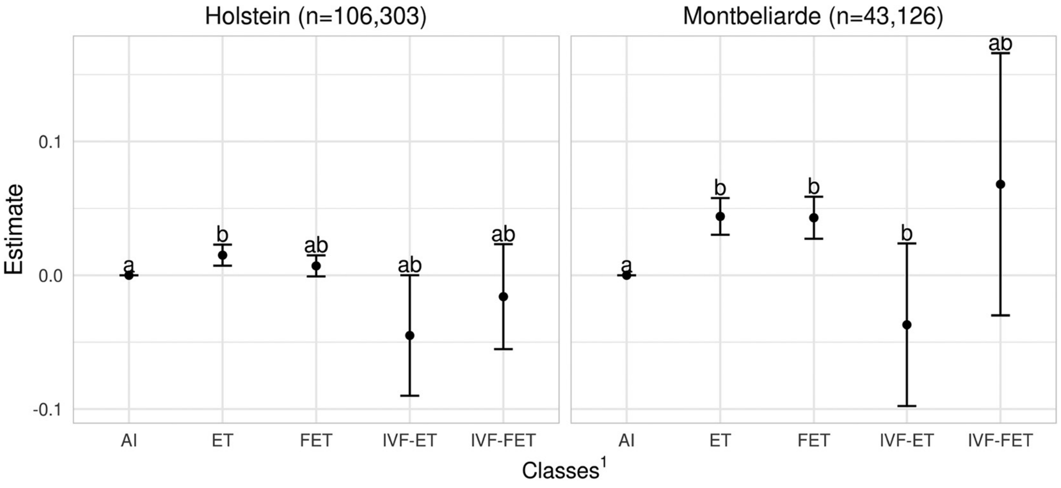

Figure 2. Heifer survival relative to the reference value (heifers derived from conventional AI; AI = 0) depending on the technique of conception for Holstein and Montbéliarde. $^{a-b}$ Different superscripts indicate significant differences between means (FDR < 0.05). [1]Classes: AI: conceived using AI; ET: fresh Embryo Transfer; FET: Frozen Embryo Transfer; IVF-ET: In Vitro Fertilization followed by Fresh Embryo Transfer; IVF-FET: In Vitro Fertilization followed by Frozen Embryo Transfer.

Table 3. Effect (estimate (SE)) of dam parity on stature, lactation, udder health, and fertility traits of daughters for Holstein and Montbéliarde breeds. Results are expressed as contrasts to cows born from primiparous dams (parity 1, nonlactating heifers during gestation)

	Breed	Holstein		Montbéliarde	
Trait	Class	Number	Estimate (SE)	Number	Estimate (SE)
Stature[1,2]	1	49770	0^{a}	17107	0^{a}
	2	26648	−0.04 (0.01)^{b}	12198	−0.08 (0.03)^{b}
	3	15080	0.01 (0.01)^{ac}	8371	0.01 (0.03)^{ab}
	>3	13792	0.04 (0.01)^{c}	12568	0.04 (0.03)^{a}
Milk yield (kg)[1]	1	64280	0^{a}	21500	0^{a}
	2	34620	45 (7)^{b}	15035	−28 (9)^{b}
	3	20222	67 (8)^{b}	10366	−22 (10)^{ab}
	>3	18555	97 (8)^{c}	15099	−35 (9)^{b}
Fat yield (kg)[1]	1	64280	0^{a}	21500	0
	2	34620	4.1 (0.3)^{b}	15035	0.4 (0.3)
	3	20222	4.7 (0.3)^{bc}	10366	0.5 (0.4)
	>3	18555	5.5 (0.3)^{c}	15099	0.1 (0.3)
Protein yield (kg)[1]	1	64280	0^{a}	21500	0^{a}
	2	34620	1.2 (0.2)^{b}	15035	−1.2 (0.3)^{b}
	3	20222	1.8 (0.3)^{b}	10366	−1.3 (0.3)^{b}
	>3	18555	2.7 (0.3)^{c}	15099	−1.8 (0.3)^{b}
Somatic cell score[1]	1	64280	0	21500	0^{a}
	2	34620	0.01 (0.01)	15035	0.00 (0.01)^{a}
	3	20222	0.00 (0.01)	10366	−0.02 (0.01)^{ab}
	>3	18555	0.00 (0.01)	15099	−0.04 (0.01)^{b}
Clinical mastitis[1]	1	40928	0	12245	0^{a}
	2	22048	0.00 (0.002)	8397	0.01 (0.003)^{b}
	3	12831	0.00 (0.003)	5731	0.00 (0.004)^{a}
	>3	11941	0.00 (0.003)	8391	0.00 (0.003)^{a}
Heifer conception rate[3]	1	70185	0^{a}	19478	0
	2	37563	−0.01 (0.003)^{b}	13144	−0.01 (0.01)
	3	22317	−0.01 (0.004)^{ab}	9155	−0.01 (0.01)
	>3	19836	0.00 (0.004)^{ab}	13161	−0.01 (0.01)
Cow conception rate[4]	1	37733	0	2048	0
	2	19659	−0.02 (0.01)	1435	−0.02 (0.02)
	3	11452	−0.01 (0.01)	955	−0.04 (0.02)
	>3	10168	−0.01 (0.01)	1273	−0.01 (0.02)

[a–c] Within a trait, different superscripts indicate significant differences between means (FDR < 0.05).

[1] Only the first lactations were retained.

[2] Observation unit is dependent on cow breed: score from 1 to 9 in Holstein, in cm in Montbéliarde.

[3] Only the first AI was retained.

[4] Only the first AI of the first lactation was retained.

GEBV regression coefficients in Model [2]

To account for genetic differences between the daughters, and to prevent potential confounding effects between genetics and prenatal factors, trait-dependent GEBV were included into the linear model [2]. The estimated regression coefficients associated with the GEBV are shown in Supplementary Table S7 (https://doi.org/10.6084/m9.figshare.25127177). For only 3 out of the 112 prenatal factor-trait model combinations, the GEBV presented no significant effect, being all of these instances associated to the Montbéliarde cow conception rate. This lack of significance of the GEBV effect is likely attributable to the small sample size (<600) and the very low heritability of the trait. Across most prenatal factor-trait model combinations, the estimated GEBV regression coefficients were between 0.8 and 1.2. The largest deviations from 1 were observed for clinical mastitis on both breeds.

DISCUSSION

Research related to fetal programming has received increasing attention in the recent years, particularly in human medicine, however for dairy cattle it remains relatively unexplored (Maicas et al., 2020; Swartz et al., 2021). In the present study, we investigated associations between prenatal factors related to ART or maternal characteristics and the subsequent offspring performance, for the 2 main French dairy cattle breeds, Holstein and Montbéliarde. The traits analyzed for the female offspring were heifer survival rates and 8 commercial traits: stature, milk, fat, and protein yield, SCS, and heifer and cow conception rate, with sample sizes ranging from 281 to 149,901 cows.

In general, for the traits investigated by this present study, our results suggest minor effects of ART or maternal characteristics on the daughters' phenotypes, yet, the results were not consistent between the 2 breeds. Regarding ART, 2 notable results were observed: for Montbéliarde, cows born from SS presented lower milk yields than cows born from conventional AI; for Holstein, cows born from ET or FET presented lower milk performance than cows born from AI. Maternal characteristics revealed interesting findings, such as the lack of association between dam AFC and the performance of the resulting progeny for both breeds. Moreover, higher dam parity was associated with milk performance in the resulting cow in both breeds, however for Holstein this association was positive, while for Montbéliarde this association was negative. The dam udder health during gestation, assessed by SCS or the occurrence of CM was not or weakly associated with the traits studied in the offspring. The observed inconsistencies between breeds were surprising and could be attributed to the multiplicity of tests conducted across breeds and traits, possibly leading to some spurious associations and false positives. The performance of the studied daughters was substantially lower than those reported by other countries with high-yielding dairy cows (e.g., Canada, USA), but their level was in line with the French national reports (Institut de l'Élevage, 2022). This relatively lower performance observed in our data set may have contributed to mitigate the estimated effects, and may not represent the effects for other populations of high yielding cows exposed to the same prenatal factors.

To accurately estimate the non-genetic prenatal factors, GEBV were included as covariates in the linear model [2]. This adjustment was applied only to genotyped cows, excluding those that died before genotyping. To ensure that early death would not affect the results, we first investigated a potential association between heifer survival and the analyzed prenatal factors in the whole population. This 2-step process was crucial to ensure that our results remained unbiased. Conception techniques were associated with heifer survival rates, with the daughters born from embryo transfer procedures exhibiting higher survival rates than those daughters born from conventional AI. This difference most likely results from preferential management practices of these high economic value cows, rather than being a true biological effect. As recommended by Lafontaine et al. (2023), we kept only the herds with animals born from embryo transfer, to minimize variations in management practices, and to reduce potential environmental bias. Negligible associations between survival rate and the other factors were observed for all traits. We concluded that the potential differential mortality associated with the analyzed prenatal factors was an unlikely source of bias in the subsequent analyses that considered only genotyped daughters.

Adjusting the performance for the genetic level of the cows is crucial to accurately estimate non-genetic prenatal factors. However, estimating the breeding values together with the prenatal factor in the model is not recommended, since in a joint model, prenatal effects can be partially or largely captured by the breeding values, thus resulting in potentially reduced or even obliterated prenatal effects. An accurate approach, especially in breeds with large reference populations for genomic evaluation, is to use the animals' GEBV (Carvalho et al., 2020) as a covariate in the model used to study the effects of prenatal factors. In the present study, we used the GEBV computed in the French evaluation from March 2018, i.e., before the recording of phenotypes for the cows included in our study, thus avoiding direct dependencies between an animal GEBV and performance. Finally, we included the GEBV of the animals as a covariate, following an approach similar to that of Carvalho et al. (2020). In their study, the inclusion of GEBV led to changes in the estimated effects compared with the same models without GEBV. If the traits analyzed are the same as those considered in the genomic evaluation, the expected theoretical regression coefficients associated to the GEBV should be equal to 1. While some of our reported coefficients associated to the GEBV deviated from 1, we believe that these deviations are mainly due to differences between the traits included in the evaluation and those traits included in the model to study the effects of prenatal factors. For example, to estimate the effects of prenatal factors on MY, we considered only performance recorded in the first lactation, whereas the performance used for the genomic evaluation considered all lactations, thus expressing GEBV in terms of mature-equivalent MY. Moreover, for some traits (STAT, MY, FY, PY, CM), genomic evaluation models included heterogeneous variances, and the definition of the variance basis may also explain the deviations from 1 obtained with our models to study the effects of prenatal factors. Furthermore, we cannot exclude the potential inflation of the genomic evaluation (before phenotypic recording), which also explains coefficients lower than 1. Finally, for the analyses with limited sample size and/or low heritability, the regression coefficient may be poorly estimated. Nonetheless, even if a regression coefficient associated with the GEBV differs from 1, if it is significant, its inclusion as a covariate in the models to study the effects of prenatal factors ensures that the adjusted performance is independent of the breeding values.

Assisted reproductive technologies and daughter traits

During the sexing process, spermatozoa are exposed to different conditions that may cause molecular alterations (e.g., handling, mechanical stress, staining with a cytotoxic agent), resulting in a reduced success rate of insemination compared with conventional semen (Reese et al., 2021). Sexing techniques have been associated with increased oxidative stress in stallions (Balao da Silva et al., 2016), and altered morphokinetics and embryonic development in cattle (Steele et al., 2020). These alterations have the potential to modify the development of bovine embryos (Siqueira et al., 2017). However, few studies have investigated the associations between SS and offspring phenotypes to assess potential long-term consequences. Maicas et al. (2020) analyzed the performance of 5,179 calves produced from the same ejaculates, born from either conventional AI or SS, and found no significant differences in either milk or reproductive performance. The results of Maicas et al. (2020) are in agreement with our findings in the Holstein breed, although we also observed a mildly negative association between SS and MY and PY in the Montbéliarde breed.

While embryo manipulation is known to induce an increase in birth weights for several species (Walker et al., 1996), the available literature on adult stature is relatively limited. The consensus suggests that the morphological differences observed at birth tend to gradually decrease, although some studies have shown long-term effects on tissue composition (reviewed by Sinclair et al. (2016)). In our study, we found a small negative association between fresh or frozen ET and MY, FY, and PY for Holstein, which contradicts previous reports by Bonilla et al. (2014). As previously mentioned in the introduction, a recent study by Lafontaine et al. (2023) in Holstein cattle found no differences in milk performance and only minor variations in conception rate, which is in agreement with our findings. In line with all the aforementioned studies, our primary conclusion emphasizes the limited magnitude of the observed effects.

Maternal characteristics and daughter traits

For both breeds, most traits measured on daughters were unaffected by the AFC of primiparous dams. It suggested that the long-term effects of a low dam AFC were minimal on the resulting cow performance. Banos et al. (2007) observed that cows born from younger dams showed higher milk yield (+4.5% of the mean) but reduced fertility levels. Carvalho et al. (2020) suggested that the limited size of the still-growing heifer uterus might contribute to lower milk yield, although our results do not support this hypothesis.

Parity is one of the factors frequently considered in studies of fetal programming in dairy cattle. We observed that Holstein cows born from heifer dams presented a lightly reduced MY, compared with cows born from lactating dams. Our findings contradict those of Carvalho et al. (2020) who reported no difference in MY but better reproductive performance. For the same breed, González-Recio et al. (2012) reported that cows born from primiparous dams had a longer lifespan and higher MY than cows born from multiparous dams, with an increase of 52 kg in MY. In this latter study, the authors speculated that their observed results could be due to the potential accumulation of unfavorable epigenetic marks, although this hypothesis remains to be tested. For the Montbéliarde breed, we observed no or a minor positive association between a low parity of the dam and the daughter milk production traits. This could be related to the higher age at first calving of Montbéliarde heifers, thus an increased maturity, compared with their Holstein counterparts.

Mastitis, a common disease in dairy cattle, can be diagnosed by clinical symptoms or indirectly by elevated SCS in milk. Several studies have investigated the transgenerational effects of high SCS or the occurrence of CM. For instance, Swartz et al. (2021) reported a small reduction in FY in first lactation of the offspring (−0.3% of the phenotypic mean) for each unit increase in dam SCS. In contrast, we did not observe such a reduction for any of the milk traits analyzed in our study. Using a smaller data set and a model incorporating GEBV, Carvalho et al. (2020) did not detect any difference in the milk performance of cows born from multiparous dams due to the incidence of clinical diseases (including mastitis) during the previous lactation. However, they did observe a higher culling rate for the daughters of dams diagnosed with clinical disease, although the specific causes of culling were not provided. González-Recio et al. (2012) found that mastitis during embryogenesis were negatively associated with the survival and MY of the resulting offspring. However, their results did not reach statistical significance.

Practical considerations

Overall, our findings align with the existing literature. While the estimated associations between the prenatal factors and the performance of the cows were weak, with effect sizes typically accounting for less than 1% of the phenotypic mean, our results suggest that the long-term effect of adverse prenatal factors is limited, even for factors known to affect fetal programming, such as IVF and ET. While our results do not entirely match some previously published findings for dairy cows (González-Recio et al., 2012; Carvalho et al., 2020; Lafontaine et al., 2023), there is a general consensus is that the effects on milk production and fertility traits are small in their magnitude. Nonetheless, it remains important to consider the implications of certain widely used technologies, such as SS, for which associations reached significance. Although we did not specifically investigate the underlying mechanisms in this study, it is plausible that the observed associations could be related to epigenetic modifications that may be partially transmitted to future generations, and further research is needed to thoroughly investigate the interactions between the prenatal environment and epigenetic marks.

CONCLUSION

This study investigated the associations between several prenatal factors and the performance of dairy cows. Several associations were identified, but the magnitude of their estimated effects was limited, accounting for no more than one percent of the phenotypic mean for most of the traits, a result in line with previous findings. Furthermore, these associations were not always consistent between related factors (e.g., SCS vs. CM) or between the 2 breeds examined (e.g., SS vs. CONV), despite the use of a large database. Given the limited size of these effects, the practical implications may be minimal in most cases. According to our results, fetal programming due to the factors investigated may be a limited reason of concern for the dairy industry.

ACKNOWLEDGMENTS

C. FOUERE is the recipient of a CIFRE PhD grant from the Association Nationale de la Recherche et de la Technologie and APIS-GENE (Paris, France). This work was part of the POLYPHEME project funded by the Agence Nationale de la Recherche (ANR-21-CE20-0021) and APIS-GENE. We thank the companies in the Eliance network for the animal recording data. Supplementary file: https://doi.org/10.6084/m9.figshare.25127177. The authors have not stated any conflicts of interest.

REFERENCES

Balao da Silva, C., C. Ortega-Ferrusola, J. Morrell, H. Rodriguez Martínez, and F. Peña. 2016. Flow Cytometric Chromosomal Sex Sorting of Stallion Spermatozoa Induces Oxidative Stress on Mitochondria and Genomic DNA. Reprod. Domest. Anim. 51:18–25. https://doi.org/10.1111/rda.12640.

Banos, G., S. Brotherstone, and M. P. Coffey. 2007. Prenatal Maternal Effects on Body Condition Score, Female Fertility, and Milk Yield of Dairy Cows. J. Dairy Sci. 90:3490–3499. https://doi.org/10.3168/jds.2006-809.

Barker, D. J. 1990. The fetal and infant origins of adult disease. BMJ 301:1111. https://doi.org/10.1136/bmj.301.6761.1111.

Barker, D. J. P. 2007. The origins of the developmental origins theory. J. Intern. Med. 261:412–417. https://doi.org/10.1111/j.1365-2796.2007.01809.x.

Beilby, K. H., E. Kneebone, T. J. Roseboom, I. M. van Marrewijk, J. G. Thompson, R. J. Norman, R. L. Robker, B. W. J. Mol, and R. Wang. 2023. Offspring physiology following the use of IVM, IVF and ICSI: a systematic review and meta-analysis of animal studies. Hum. Reprod. Update 29:dmac043. https://doi.org/10.1093/humupd/dmac043.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. B 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Boichard, D., S. Fritz, P. Croiseau, V. Ducrocq, T. Tribout, M. Barbat, and B. C. D. Cuyabano. 2023. Methods to estimate erosion factors of genomic breeding values of candidates due to long-distance linkage disequilibrium. Interbull Meeting, August 26–28 2023, Lyon, France. Interbull Bull. 59:96–100.

Bonilla, L., J. Block, A. C. Denicol, and P. J. Hansen. 2014. Consequences of transfer of an in vitro-produced embryo for the dam and resultant calf11Conflict of interest: LB, JB, and PJH have an ownership position in Cooley Biotech LLC, Gainesville, Florida, the licensee of BBH7 medium. J. Dairy Sci. 97:229–239. https://doi.org/10.3168/jds.2013-6943.

Carvalho, M. R., C. Aboujaoude, F. Peñagaricano, J. E. P. Santos, T. J. DeVries, B. W. McBride, and E. S. Ribeiro. 2020. Associations between maternal characteristics and health, survival, and performance of dairy heifers from birth through first lactation. J. Dairy Sci. 103:823–839. https://doi.org/10.3168/jds.2019-17083.

Gad, A., U. Besenfelder, F. Rings, N. Ghanem, D. Salilew-Wondim, M. M. Hossain, D. Tesfaye, P. Lonergan, A. Becker, U. Cinar, K. Schellander, V. Havlicek, and M. Hölker. 2011. Effect of reproductive tract environment following controlled ovarian hyperstimulation treatment on embryo development and global transcriptome profile of blastocysts: implications for animal breeding and human assisted reproduction. Hum. Reprod. 26:1693–1707. https://doi.org/10.1093/humrep/der110.

González-Recio, O., E. Ugarte, and A. Bach. 2012. Trans-Generational Effect of Maternal Lactation during Pregnancy: A Holstein Cow Model. PLoS One 7:e51816. https://doi.org/10.1371/journal.pone.0051816.

Institut de l'Élevage. 2022. Résultat de Contrôle Laitier - Espèce Bovine - France 2021. Résultats.

International Bull Evaluation Service Official Website. Accessed October 25, 2023. https://interbull.org/ib/geforms.

International Committee for Animal Recording. 2014. ICAR Recording Guidelines. ICAR. https://pecuaria.pt/docs/Guidelines_2014.pdf

Kiefer, H., E. Sellem, A. Bonnet-Garnier, M. Pannetier, V. Costes, L. Schibler, and H. Jammes. 2021. The epigenome of male germ cells and the programming of phenotypes in cattle. Anim. Front. 11:28–38. https://doi.org/10.1093/af/vfab062.

Kumaresan, A., M. Das Gupta, T. K. Datta, and J. M. Morrell. 2020. Sperm DNA Integrity and Male Fertility in Farm Animals: A Review. Front. Vet. Sci. 7:7. https://doi.org/10.3389/fvets.2020.00321.

Lafontaine, S., R. Labrecque, P. Blondin, R. I. Cue, and M.-A. Sirard. 2023. Comparison of cattle derived from in vitro fertilization, multiple ovulation embryo transfer, and artificial insemination for milk production and fertility traits. J. Dairy Sci. 0:4380–4396. https://doi.org/10.3168/jds.2022-22736.

Maicas, C., I. A. Hutchinson, A. R. Cromie, P. Lonergan, and S. T. Butler. 2020. Characteristics of offspring derived from conventional and X-sorted bovine sperm. J. Dairy Sci. 103:7509–7520. https://doi.org/10.3168/jds.2020-18178.

Reese, S., M. C. Pirez, H. Steele, and S. Kölle. 2021. The reproductive success of bovine sperm after sex-sorting: a meta-analysis. Sci. Rep. 11:17366. https://doi.org/10.1038/s41598-021-96834-2.

Schagdarsurengin, U., and K. Steger. 2016. Epigenetics in male reproduction: effect of paternal diet on sperm quality and offspring health. Nat. Rev. Urol. 13:584–595. https://doi.org/10.1038/nrurol.2016.157.

Schulz, L. C. 2010. The Dutch Hunger Winter and the developmental origins of health and disease. Proc. Natl. Acad. Sci. USA 107:16757–16758. https://doi.org/10.1073/pnas.1012911107.

Sinclair, K. D., K. M. D. Rutherford, J. M. Wallace, J. M. Brameld, R. Stöger, R. Alberio, D. Sweetman, D. S. Gardner, V. E. A. Perry, C. L. Adam, C. J. Ashworth, J. E. Robinson, and C. M. Dwyer. 2016. Epigenetics and developmental programming of welfare and production traits in farm animals. Reprod. Fertil. Dev. 28:1443–1478. https://doi.org/10.1071/RD16102.

Siqueira, L. G., M. V. G. Silva, J. C. Panetto, J. H. Viana, L. G. Siqueira, M. V. G. Silva, J. C. Panetto, and J. H. Viana. 2020. Consequences of assisted reproductive technologies for offspring function in cattle. Reprod. Fertil. Dev. 32:82–97. https://doi.org/10.1071/RD19278.

Siqueira, L. G. B., S. Dikmen, M. S. Ortega, and P. J. Hansen. 2017. Postnatal phenotype of dairy cows is altered by in vitro embryo production using reverse X-sorted semen. J. Dairy Sci. 100:5899–5908. https://doi.org/10.3168/jds.2016-12539.

Steele, H., D. Makri, W. E. Maalouf, S. Reese, and S. Kölle. 2020. Bovine Sperm Sexing Alters Sperm Morphokinetics and Subsequent Early Embryonic Development. Sci. Rep. 10:6255. https://doi.org/10.1038/s41598-020-63077-6.

Swartz, T. H., B. J. Bradford, and J. S. Clay. 2021. Intergenerational cycle of disease: Maternal mastitis is associated with poorer daughter performance in dairy cattle. J. Dairy Sci. 104:4537–4548. https://doi.org/10.3168/jds.2020-19249.

Urrego, R., N. Rodriguez-Osorio, and H. Niemann. 2014. Epigenetic disorders and altered gene expression after use of Assisted Reproductive Technologies in domestic cattle. Epigenetics 9:803–815. https://doi.org/10.4161/epi.28711.

Walker, S. K., K. M. Hartwich, and R. F. Seamark. 1996. The production of unusually large offspring following embryo manipulation: Concepts and challenges. Theriogenology 45:111–120. https://doi.org/10.1016/0093-691X(95)00360-K.

ORCIDS

C. Fouéré https://orcid.org/0009-0005-8735-2918
M. P. Sanchez https://orcid.org/0000-0002-1371-5342
M. Boussaha https://orcid.org/0000-0002-5432-4604
S. Fritz https://orcid.org/0000-0002-3378-1048
A. Vinet https://orcid.org/0000-0001-7327-6049
H. Kiefer https://orcid.org/0000-0002-6631-741X
D. Boichard https://orcid.org/0000-0003-0361-2961
C. Hozé https://orcid.org/0000-0002-5900-5506