Aptitude des vaches laitières à se reproduire en systèmes pâturants contrastés : Quelle vache pour quel système ?

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Bedere, N., L. Delaby, V. Ducrocq, S. Leurent-Colette, C. Disenhaus. 2016. Towards improved post-partum cyclicity of primiparous dairy cows: effects of genetic merit for production traits under contrasted feeding systems. Journal of Dairy Science 99:1266-1276.

Bedere, N., C. Disenhaus, V. Ducrocq, S. Leurent-Colette, L. Delaby. Ability of dairy cows to be inseminated according to breed and genetic merit for production traits under contrasting pasture based feeding systems. Manuscript accepted the 12/09/2016 for publication in Animal.

Bedere, N., C. Disenhaus, V. Ducrocq, S. Leurent-Colette, L. Delaby. Ability of dairy cows to ensure pregnancy according to breed and genetic merit for production traits under contrasting pasture based systems. Manuscript accepted the 12/12/2016 for publication in the Journal of Dairy Science.

International conference proceedings

Oral communications

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2016. The cow for the system: limiting milk yield and body condition loss to ensure reproduction on time, in: 67th Annual Meeting of the European Federation of Animal Science. Belfast, Ireland.

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Posters

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2016. Selecting on fat and protein content instead of milk yield would not improve dairy cows' fertility, in: 67th Annual Meeting of the European Federation of Animal Science. Belfast, Ireland.

National conference proceedings

Oral communications

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2015. Les vaches capables de limiter leur production laitière et leur amaigrissement lors d'une restriction alimentaire sont celles qui se reproduisent le mieux, in: Rencontres Recherche Ruminants. Paris, France.

Posters

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2016. L'aptitude des vaches laitières à assurer chaque étape de la reproduction dépend de leurs caractéristiques génétiques, in: Rencontres Recherche Ruminants. Paris, France.

Popular science

Posters

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2016. Sélectionner pour la production laitière via les taux, quelles conséquences sur la reproduction des vaches ? Affiche à la Journée Des Doctorants. Saint-Gilles, France.

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2015. Faciliter la reproduction des vaches en temps limité. Affiche aux Prairiales Normandie du Pin, journée professionnelle. Exmes, France.

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2015. Se reproduire en temps limité : effets génétiques. Affiche aux Prairiales Normandie du Pin, journée professionnelle. Exmes, France.

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List of Abbreviations

ACTH: adrenocorticotropic hormone
AFC: age at first calving
BTA: Bos taurus autosome
BW: body weight
CLA or C-LA: commencement of luteal activity
d: days
DGAT1: diacylglycerol acyltransferase
DMI: dry matter intake
dpp: days postpartum
E2: 17β-estradiol
EB: energy balance
EBV: estimated breeding values
EEM: early embryo mortality
ELISA: enzyme-linked immunosorbent assay
FD: foetal death
FSH: follicle-stimulating hormone
GEBV: genomic estimated breeding values
GH: growth hormone
GnRH: gonadotropin releasing hormone
GWAS: genome wide association study
h: hour
IFNτ: interferon tau
IGF: insulin-like growth factors
ILI: inter-luteal interval
IOI: inter-ovulatory interval
LEM: late embryo mortality
LH: luteinizing hormone
LUT: luteal phase length
mRNA: messenger ribonucleic acid
NEB: negative energy balance
NF: non-fertilisation of the oocyte
ODM: once a day milking
OT: oxytocin
P4: progesterone
PAGs: pregnancy-associated glycoproteins
PDI: protéines digestibles dans l'intestin
PGE: luteinizing prostaglandins
PGF2α: luteolysing prostaglandins
PLP: prolonged luteal phase
QTL: quantitative trait loci
RIA: radioimmunoassay
RUP: rumen undegradable protein
UFL: unité fourragère lait

Introduction

Chez les mammifères, la reproduction conditionne la lactation, car cette dernière permet la survie et le développement du jeune notamment en assurant son alimentation et la transmission d'éléments immunitaires. Chez la vache laitière, ces deux fonctions sont concomitantes et en concurrence car elles partagent les mêmes ressources. Parallèlement à l'augmentation de leurs performances de production, les performances de reproduction des vaches laitières se sont même dégradées. La production laitière a augmenté grâce à l'amélioration des connaissances en génétiques, en nutrition et grâce aux techniques d'élevages (notamment de traite). Le déclin de la fonction reproduction des vaches laitières s'explique par des corrélations génétiques défavorables, certes faibles mais suffisantes du fait de la forte pression de sélection appliquée sur la fonction de lactation (Veerkamp *et al.*, 2000; Boichard *et al.*, 2002). Depuis les années 2000, des index de fertilité sont inclus dans les programmes de sélection ce qui a permis de freiner voire de stopper le déclin de l'aptitude à se reproduire associé à la sélection génétique. La recherche sur l'alimentation des vaches laitières s'est consacrée à couvrir leurs besoins, notamment pour permettre aux animaux d'exprimer au mieux leur potentiel de production. Les effets de l'alimentation sur la reproduction ont été explorés et documentés. Ces effets passent notamment par l'état nutritionnel des animaux (bilan apports/besoins). Les relations directes entre lactation et reproduction ont été moins étudiées mais depuis quelques années font l'objet de plus de recherches.

L'objectif politique au lendemain de la 2ème guerre mondiale était d'assurer l'autonomie alimentaire de la France. Beaucoup d'efforts ont été investis pour s'affranchir des contraintes associées au milieu en vue d'augmenter la productivité agricole. Depuis les années 1950, l'arrivée de l'insémination artificielle a permis l'organisation et le développement des schémas de sélection en bovins laitiers (Gérard *et al.*, 2008). La sélection s'est alors focalisée sur les aptitudes de production des vaches laitières afin d'augmenter la productivité des animaux et la compétitivité des élevages. La diversité génétique des races de bovins laitiers s'est alors considérablement réduite à la race Holstein haute productrice (70 % du cheptel français), pour 10 % de vaches de race Montbéliarde (modeste laitière), 10 % de Normande (race mixte lait/viande) et 10 % d'autres races locales (e.g. Brune Suisse). Pour répondre aux besoins nutritionnels de cet animal à haut potentiel, des pratiques d'élevages adaptées ont été mises en place telles que l'alimentation en bâtiment basée sur les stocks, la complémentation en concentrés de production. Or le contexte des productions agricoles a changé et les systèmes d'élevage doivent tenir compte de (i) l'augmentation de la demande mondiale en denrées alimentaires et de la limite des ressources disponibles sur la planète, de (ii) l'évolution de la demande en qualité des produits animaux, du (ii) respect de l'environnement et des évolutions des demandes sociétales. Il apparait de plus en plus probable qu'afin de répondre à ce contexte changeant, il faut maintenir voire accroitre la diversité des systèmes de production en France et en Europe. Ces différents systèmes vont avoir différents avantages, inconvénients, et vont générer des contraintes différentes pour les animaux. Le maintien de la diversité des types génétiques est alors également crucial car en fonction de leurs caractéristiques génétiques les vaches accordent des priorités différentes à chaque fonction et au cours de la lactation. Leur capacité à s'adapter à un environnement contraignant (ressource nutritive ou temps imparti limités) et à assurer les fonctions biologiques conditionne leur plage de robustesse et donc l'adéquation entre les types génétiques et les systèmes (Phocas *et al.*, 2016a; b).

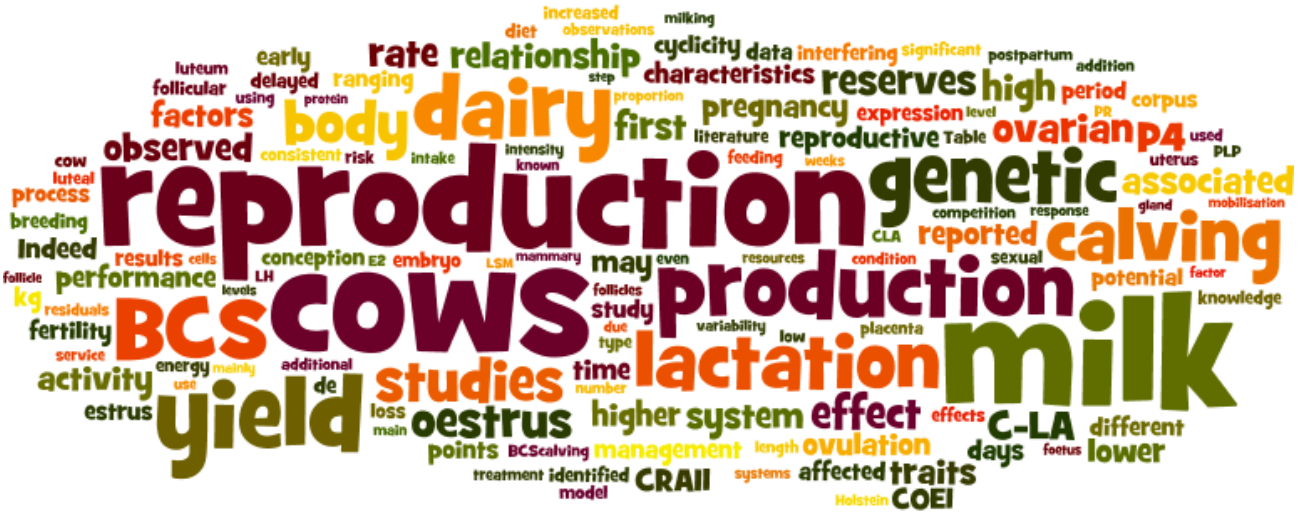
C'est dans ce contexte que nous tentons de répondre à la question **« Quelle vache laitière pour quel système ? »** au sein de l'Unité Mixte de Recherche de Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Élevage entre l'Institut National de la Recherche Agronomique et Agrocampus Ouest, dans le Grand-Ouest Français. Situés dans le contexte pédoclimatique de l'Europe du Nord-Ouest favorable à la pousse de l'herbe, nous nous sommes focalisés sur la diversité des systèmes herbagers. Ces systèmes semblent pertinents dans le contexte changeant décrit en amont. En effet, ils sont plus autonomes, économes, enrichissent les produits en éléments d'intérêt (e.g. oméga 3) et sont plus respectueux de l'environnement. Une des contraintes générées par ces systèmes est la saisonnalité de la pousse de l'herbe. Afin de faire correspondre le pic de production d'herbe par les prairies au pic de lactation qui représente le moment où la vache laitière a le plus de besoins nutritifs dans sa carrière, les animaux sont conduits en vêlages groupés sur 3 mois. Dans ces systèmes, les vaches doivent obtenir un veau par an. Chez le bovin, la gestation dure 9 mois ce qui rend cet objectif réalisable. Cependant, cette contrainte temporelle est d'autant plus difficile à respecter aujourd'hui que les performances de reproduction se sont dégradées. De plus, garder un troupeau en vêlages groupés sur 3 mois est un vrai challenge.

Les objectifs de ce projet de recherche sont :

- **d'étudier comment différents types génétiques s'adaptent à des niveaux d'apports nutritifs contrastés, dans des systèmes herbagers en vêlages groupés sur 3 mois ;**
- **et d'identifier les caractéristiques génétiques des animaux et les leviers d'actions à l'échelle du système qui permettent de piloter les stratégies d'adaptation des animaux afin de préserver leurs performances de production et de reproduction.**

Le travail présenté dans ce manuscrit s'inscrit pleinement dans ce projet de recherche et est centré sur les aptitudes à se reproduire dans un environnement contraignant des vaches laitières. Le manuscrit est structuré en 6 chapitres. Le premier chapitre contient (i) une synthèse bibliographique qualitative sur les étapes du processus de reproduction *postpartum* chez la vache laitière et de ses facteurs de variations, et (ii) une méta-analyse sur la compétition entre la lactation et la reproduction. Le second chapitre présente la démarche scientifique adoptée durant le projet, les questions de recherches et les hypothèses testées. Puis viennent 3 chapitres de résultats (chapitres 4, 5 et 6) qui traitent respectivement de la cyclicité *postpartum* chez les primipares, de l'aptitude à être inséminée et de l'aptitude à être gestante quel que soit le rang de lactation. Le dernier chapitre est une discussion générale autour de la hiérarchie des effets de la production laitière et de la gestion des réserves corporelles à chaque étape du processus de reproduction, des stratégies génétiques et du système pour accompagner les vaches laitières vers le succès de la reproduction et de la lactation avant de donner des éléments de conclusions à la question « Quelle vache laitière pour quel système ? ».

CHAPTER 1: Literature review of the reproduction of dairy cows from calving to re-calving (or failure) and its interfering factors.



Objectifs

Ce premier chapitre traite de la capacité des vaches laitières à assurer la reproduction. Les fondamentaux de la physiologie de la reproduction de ces animaux sont d'abord exposés. Une seconde partie fait un état des lieux des problèmes de reproduction rencontrés aujourd'hui. Enfin les facteurs de variation connus ainsi que les actuels manques de connaissances sont présentés.

L'essentiel

Chez la plupart des mammifères, la reproduction se décline en 2 grandes phases : donner naissance à un jeune (par la fonction de reproduction au sens strict), puis assurer sa survie (par la fonction de lactation). Il faut donc un jeune né pour qu'une femelle mammifère produise du lait. Chez la vache laitière, la fonction de reproduction est concomitante de celle de lactation. Cette concomitance met en concurrence les 2 fonctions et les animaux face à un dilemme d'allocation des ressources disponibles : que prioriser ? La lactation ou la reproduction ?

Depuis des dizaines d'années, l'homme a amélioré la productivité des vaches laitières grâce à des progrès génétiques, à l'alimentation et à l'amélioration technique et technologique de la conduite d'élevage (traite, santé…). Or, les performances de reproduction de ces animaux se sont détériorées. L'étude bibliographique a permis de mettre en avant le fait que chaque étape de la reproduction est concernée par cette dégradation : acquisition de la cyclicité *postpartum*, qualité de la cyclicité rétablie, durée et intensité des chaleurs, fécondité et capacité à assurer la gestation.

Les facteurs de variation de ces performances sont identifiés : à l'échelle individuelle il s'agit de l'âge, des problèmes sanitaires, de caractéristiques génétiques ; à l'échelle du troupeau il s'agit des facteurs qui ont permis l'amélioration des performances de reproduction (génétique, alimentation et fréquence de traite). Est-il possible de quantifier ces effets ? Peut-on déterminer des lois de réponse entre lactation et reproduction ? Quelles sont les zones d'ombre à investir pour mieux comprendre ces mécanismes biologiques et leur variabilité ?

Valorisation

Article dans une revue internationale à comité de relecture :

Bedere, N., E. Cutullic, L. Delaby, F. Garcia-Launay, C. Disenhaus. Meta-analysis about the competition between reproduction and production performance in dairy cows. Manuscript under review in Journal of Dairy Science.

Affiche de vulgarisation scientifique à des journées techniques :

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2015. Faciliter la reproduction des vaches en temps limité. Affiche aux Prairiales Normandie du Pin, journée professionnelle. Exmes, France.

1 From calving to re-calving: basics of the physiology of the series of reproductive steps.

The reproductive process of mammals is a series of interconnected steps. The first step is commencement of ovarian activity at puberty or resumption after calving. This activity consists in the development of the follicles, ovulation and production of the gonadal steroids. Ovarian activity is cyclic and repeats over time. Steroids have an effect on the brain and are responsible for sexual behaviours occurring prior to ovulation. These signs are meant to tell the male that the female is standing mating and this period is called oestrus. The following steps after insemination are: fertilization of the ovule, initiation and maintenance of pregnancy. Then, the female gives birth to a young and lactation is initiated and maintained until weaning of the offspring. However, some mammals like dairy cows resume ovarian activity and ensure the subsequent steps of the reproductive process while producing milk (Figure 1). Consequently they have to ensure 2 steps of the reproductive process at the same time (producing milk for the new born calf and also investing in the future offspring).

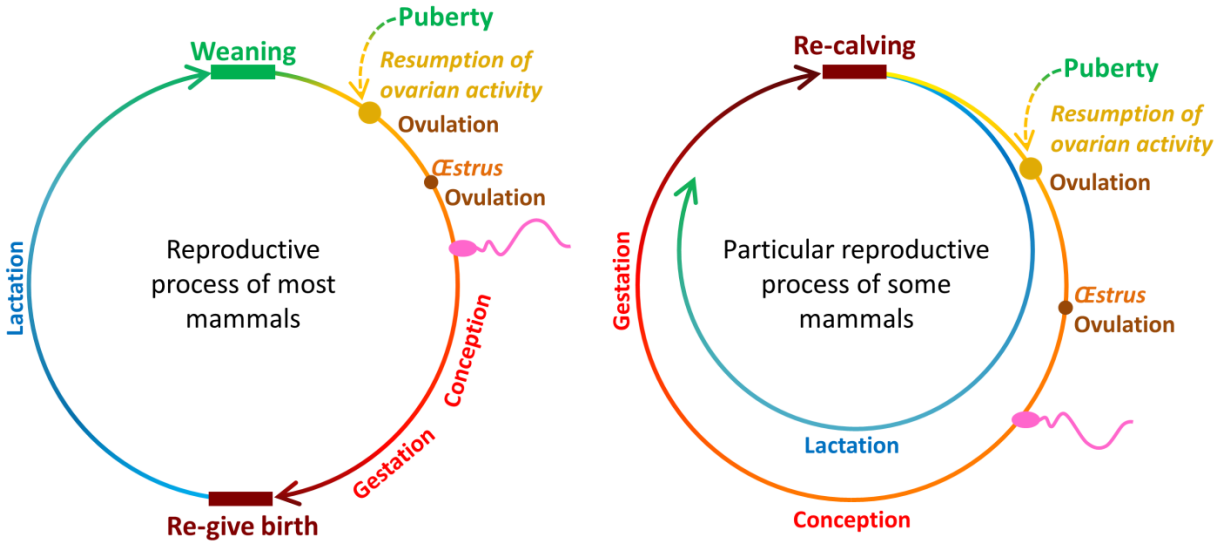


Figure 1: Comparison of most mammals' reproductive process (left graph) and the particular case of some of them (e.g. cattle; right graph).

Each reproductive step depends on previous reproductive events. Consequently, the ability of dairy cows to re-calve is affected by calving problems, impaired cyclicity, estrus expression and detection (Darwash *et al.*, 1997; Opsomer *et al.*, 2000; Gautam *et al.*, 2010). Calving rate has been declining at 1% per year since 1970' (Royal *et al.*, 2000b; Lucy, 2001; Barbat *et al.*, 2005; Rodriguez-Martinez *et al.*, 2008).

1.1 Ovarian activity[1]

The ovary is an organ made of an innervated and vascularized medulla, and a cortex where

[1] When no literature is cited, the information was found in Driancourt *et al.* (2001), Mermillod (2001), Robel (2001), Inskeep (2004), Schams and Berisha (2004), Webb *et al.* (2004), Cutullic (2010), Forde *et al.* (2011), Martin *et al.* (2013), Valour (2013)

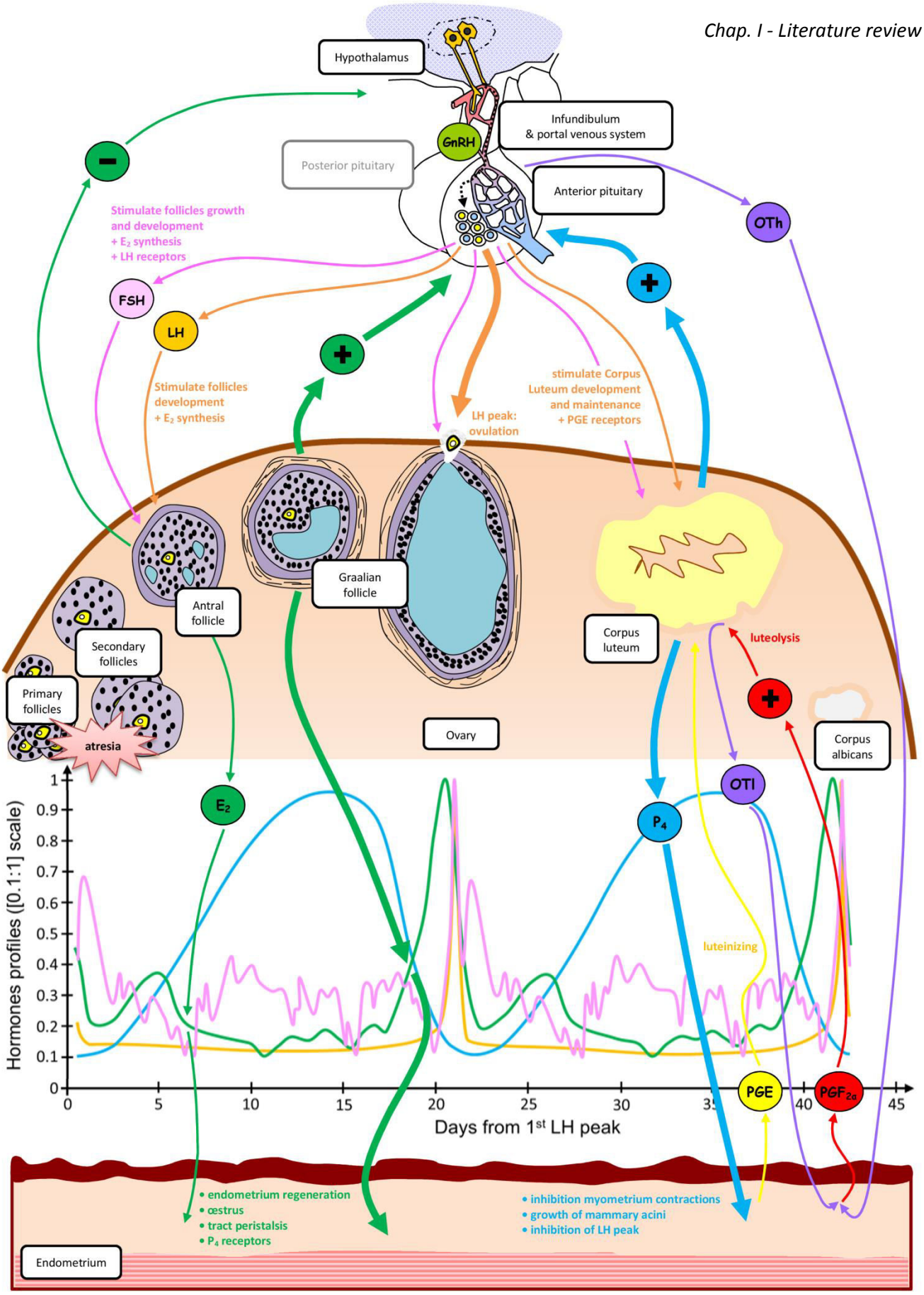


Figure 2: Representation of dairy cows oestrus cycle based on a 21-d rythme, with the follicle-stimulating hormone (FSH; pink), the luteinizing hormone (LH; orange), the gonadotropin releasing hormone (GnRH; light green), the 17β-estradiol (E_2; green), the progesterone (P_4; blue), the oxytocin (OTh for the pituitary one and OTl for the ovarian one; purple) and the luteinizing prostaglandins (PGE; yellow) and luteolysing prostaglandins ($PGF_{2\alpha}$; red).

ovarian activity takes place. The ovary ensures 2 main functions: the production of the female germ cells and the synthesis of the ovarian steroids (oestrogens and progesterone). The oogenesis begins during foetal life: the oocytes initiate the meiosis process and stop it in prophase I. At this stage, each oocyte is surrounded by a layer of somatic granulosa cells forming the primordial follicle. At birth, cows have a reserve of about 235,000 primordial follicles to have offspring.

The oestrus cycle is made of 3 phases: a basic follicular growth, a follicular period and a luteal period. Figure 2 synthetises the evolution of follicles across these 3 periods and the endocrine control of the oestrus cycle in dairy cows. During the basic follicular growth, a wave of 80 primordial follicles are recruited daily to develop firstly into primary follicles (when the shape of granulosa cells becomes cubic) and then into secondary follicles (when the granulosa is made of at least 2 layers of somatic cells). This process takes about 3 months to be realised. The mechanisms involved remain unclear and may involve paracrine factors (e.g. insulin-like growth factors; IGF). However, it is known that primordial and primary follicles are not sensitive to gonadotrophins (follicle-stimulating hormone: FSH; luteinizing hormone: LH).

Then, the follicular period starts: a vascularized layer of endocrine cells called the *theca interna* appears around the follicle that is now a pre-antral follicle. This layer responds to the recurrent increase in FSH plasmatic concentration (every 7 to 10 days). With this increase of FSH, only 2 to 3 follicles are selected from the cohort, their granulosa develops and their *theca interna* starts producing 17β-estradiol (E_2). Cavities are formed in the granulosa filled with follicular fluid and the follicle is now antral. The other recruited but not selected follicles are resorbed. The E_2 production of the selected follicles applies a negative feed-back on the pituitary gland, stimulating the production of LH. In the same time, FSH stimulates the secretion of inhibin B by the *granulosa* which, together with E_2, applies a positive feed-back on the hypothalamus which is inhibiting the secretion of FSH itself. While being exposed to FSH one of the selected follicles acquires LH receptors and becomes dominant. The dominant follicle finishes its development under LH and growth factors control, the others selected follicles are resorbed because of decreasing levels of FSH. The increased magnitude and frequency of LH pulses until the LH peak induce important changes in the dominant follicle: meiosis of the oocyte (that was locked in prophase I) proceeds again (until metaphase II), and the LH surge induces the ovulation. With the FSH cycles, there are follicular waves leading to the emergence of a dominant follicle every 7 to 10 days. However, the standard ovarian cycle of dairy cows is considered to last 21 d. This means that for 1 ovulation, 2 to 3 follicular waves occur. From the recruitment of primordial follicles to ovulation, 5 months have passed and 99.9 % of the primordial follicles have degenerated.

The presented endocrine changes during oestrus cycles also affect other organs like the uterus. The endometrium develops under the influence of E_2 in order to prepare gestation. The epithelial cells proliferate and acquire progesterone receptors and mucus glands appear. The tract peristalsis intensifies and the cervix dilates until ovulation to enable sperm cells to enter the uterus and fertilize the oocyte.

Nonetheless, a large variability in these biological processes is observed. There is increasing evidences that the standard length of ovarian cycles of dairy cows does not always last 21 d and can range from 19 to 26 days. In Holstein cows, it has been shown that the median cycle length

changed from 21 d in 1980' to 23 d 20 years later (Royal *et al.*, 2000a; Sakaguchi *et al.*, 2004; Sartori *et al.*, 2004; Disenhaus *et al.*, 2008). Two days in 20 years may not appear significant but it can disturb the farmers' management and working plan for the reproduction of their animals (detection of ovulations, monitoring of pregnancy…). Although there are 2 follicular waves in most ovarian cycles (71 %), there is also a substantial occurrence of cycles with 3 follicular waves (26 %; Townson *et al.*, 2002; Bleach *et al.*, 2004; Sakaguchi *et al.*, 2004). It was reported that cycles made of 3 follicular waves lasts longer than those of 2 waves (24.1 *vs* 21.6 d). Because they occur only in 26 % of the cycles, it is also possible that the length of the follicular wave itself has increased. Interestingly, the chances of conception may be higher in cycles with 3 short follicular waves compared with cycles with 2, certainly because the oocyte would be "younger" and exposed to different patterns of hormones (higher P_4 levels, lower LH frequency, lower E_2 levels…; Inskeep, 2004; Cerri *et al.*, 2009). Further studies are still required on these aspects. Another source of variability of cyclicity performance of dairy cows is that normally only 1 follicle becomes dominant and ovulates. However, it has been reported that high levels of FSH during the recruiting process induces multiple (double or triple) ovulations (Lopez *et al.*, 2005; López-Gatius *et al.*, 2005). Further studies on actual risks of twinning or other complication for the next steps of the reproductive process are still required.

Ovulation signals the end of the follicular period and the beginning of the following luteal period. The exposure to LH transforms the cells from the *granulosa* and *theca* of the follicle that ovulated in luteal cells. The ex-follicle is now a new transitory endocrine gland: the *corpus luteum*. These cells no longer produce E_2 but instead produce progesterone (P_4). P_4 has antagonist effects of E_2: increased level of plasmatic P_4 inhibits the reproductive tract peristalsis, which enables the implantation of the potential embryo, and the endometrium produces E prostaglandins (PGE; luteotrophic) to maintain the *corpus luteum*. The elevating plasmatic level of P_4 applies a positive feed-back on the hypothalamic-pituitary-adrenal system which causes LH and FSH drops and the production of pituitary oxytocin. In response to this oxytocin, the endometrium starts producing $F_{2\alpha}$ prostaglandins ($PGF_{2\alpha}$; luteolytic). $PGF_{2\alpha}$ induce the luteolysis that is the degeneration of the luteal cells and resorption of the *corpus luteum*. During luteolysis, the *corpus luteum* starts producing luteal oxytocin to maintain the production of $PGF_{2\alpha}$ by the endometrium and the luteolysis itself. Once the *corpus luteum* has totally disappeared, a new follicular period starts. However, a persistent *corpus luteum* (luteolysis was incomplete or did not occur) can be frequently observed: from 3 to 43 % of dairy cows experience this condition (Cutullic; 2010). Persistent *corpus luteum* is an actual threat for the success of reproduction: no ovulation can occur as long as the P_4 level is high.

Checking on ovarian activity can be realized by monitoring P_4 concentrations. Milk and plasmatic concentrations of P_4 are highly correlated (ranging from 0.88 to 0.95; Dobson and Fitzpatrick, 1976; Meisterling and Dailey, 1987). This enables the use of milk P_4 profiles to monitor ovarian activity, which is time-effective and non-invasive for the cow. Roelofs *et al.* (2006) described the limitations to milk P_4 use: they found a moderate overall correlation between milk and plasmatic concentrations of P_4 ($r = 0.43$). They suggest because milk P_4 drops last longer than

plasmatic P_4 ones, determining ovulation time based on milk P_4 profile only is not accurate. Still, the profile pattern is a valuable piece of information. This limit also highly relies on the material and methods used: fresh/preserved/frozen milk, radioimmunoassay (RIA)/enzyme-linked immunosorbent assay (ELISA), sampling frequency, etc. Nevertheless, there is a consensus in the scientific literature on the use of P_4 profiles to estimate parameters of ovarian cycles (Bulman and Wood, 1980; Darwash *et al.*, 1997; Lamming and Darwash, 1998; Opsomer *et al.*, 1998; Horan *et al.*, 2005; McCoy *et al.*, 2006; Pollott and Coffey, 2008; Windig *et al.*, 2008; Cutullic *et al.*, 2011; Gilmore *et al.*, 2011; Tenghe *et al.*, 2015; etc.). Figure 3 illustrates the calculation of these parameters.

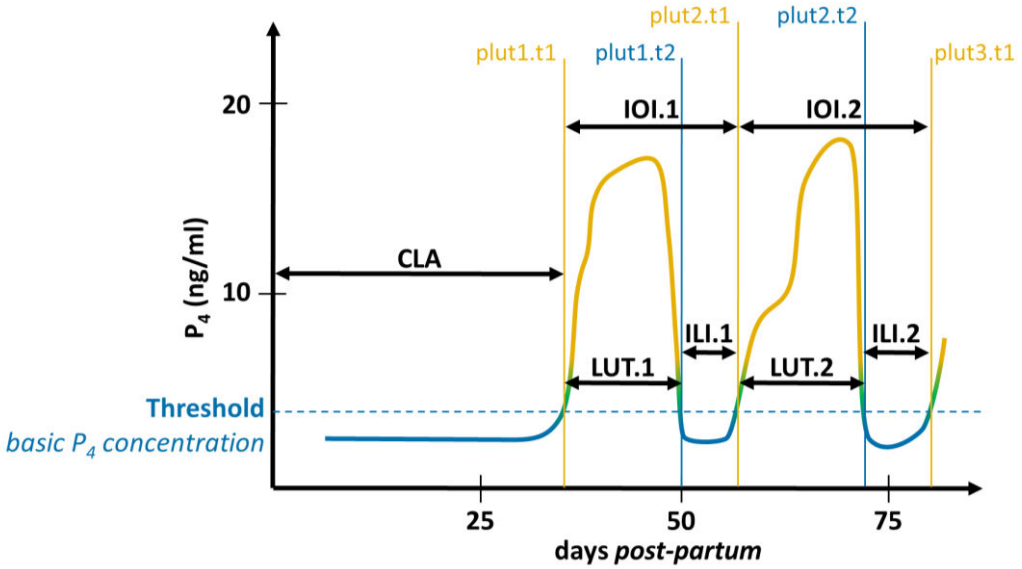


Figure 3: Ovarian parameters determined from milk progesterone monitoring. The Xth luteal phase begins when the P_4 concentration rises above the basic level and is thus produced by a *corpus luteum* at plutX.t1 and ends up at plutX.t2. From these time marks, commencement of luteal activity (CLA), luteal phase lengths (LUT), inter-luteal intervals (ILI) and inter-ovulatory intervals (IOI) are computed as: CLA = plut1.t1, LUT.X = plutX.t2 - plutX.t1, ILI.X = plut(X+1).t1 - plutX.t2, IOI.X = LUT.X + ILI.X = plut(X+1).t1 - plutX.t1.

The value of the threshold to separate basic concentrations of P_4 to levels induced by the presence of a *corpus luteum* is not fixed because it depends on the method used. When using fresh or preserved milk, thresholds of 3 ng/ml (Horan *et al.*, 2005b; McCoy *et al.*, 2006; Gilmore *et al.*, 2011) or 5 ng/ml (Gautam *et al.*, 2010; Ranasinghe *et al.*, 2010; Tenghe *et al.*, 2015) are often used. With preserved and frozen milk, determination based on quantile method is also used. This method is further explained in chapters III to V (Petersson *et al.*, 2006a; b; Cutullic *et al.*, 2011).

1.2 Oestrus and sexual behaviours

The high level of sexual hormones before ovulation induces physiological changes, involving the brain, and one of the consequences is a behavioural change (Figure 4). Indeed, high levels of plasmatic E2 at the end of the follicular phase and high levels of plasmatic P4 during the preceding luteal phase are both associated with the occurrence of sexual behaviour before ovulation (Vailes *et al.*, 1992; Lyimo *et al.*, 2000; Lopez *et al.*, 2004; Roelofs *et al.*, 2004; Walker *et al.*, 2008a). This period is called oestrus and is defined by the acceptance by the female of the male for mating. In

dairy cattle, artificial insemination is widely used which means that most dairy herds are composed of females only. Bulls may be introduced according to the moment of the breeding season, breeding systems, etc. Therefore, a cow is considered to be in true oestrus when she is standing to be mounted by another cow. During oestrus, cows can show other sexual behaviours: mounting, chin resting, sniffing/licking the vulva of other cows. Other signs are also known to be associated with oestrus: restlessness (increased physical activity, decreased time spent lying down), aggressiveness, mooing, mucous vaginal discharge, or milk yield drop (Van Eerdenburg *et al.*, 1996; Kerbrat and Disenhaus, 2004; Roelofs *et al.*, 2005; Løvendahl and Chagunda, 2010; Sveberg *et al.*, 2011).

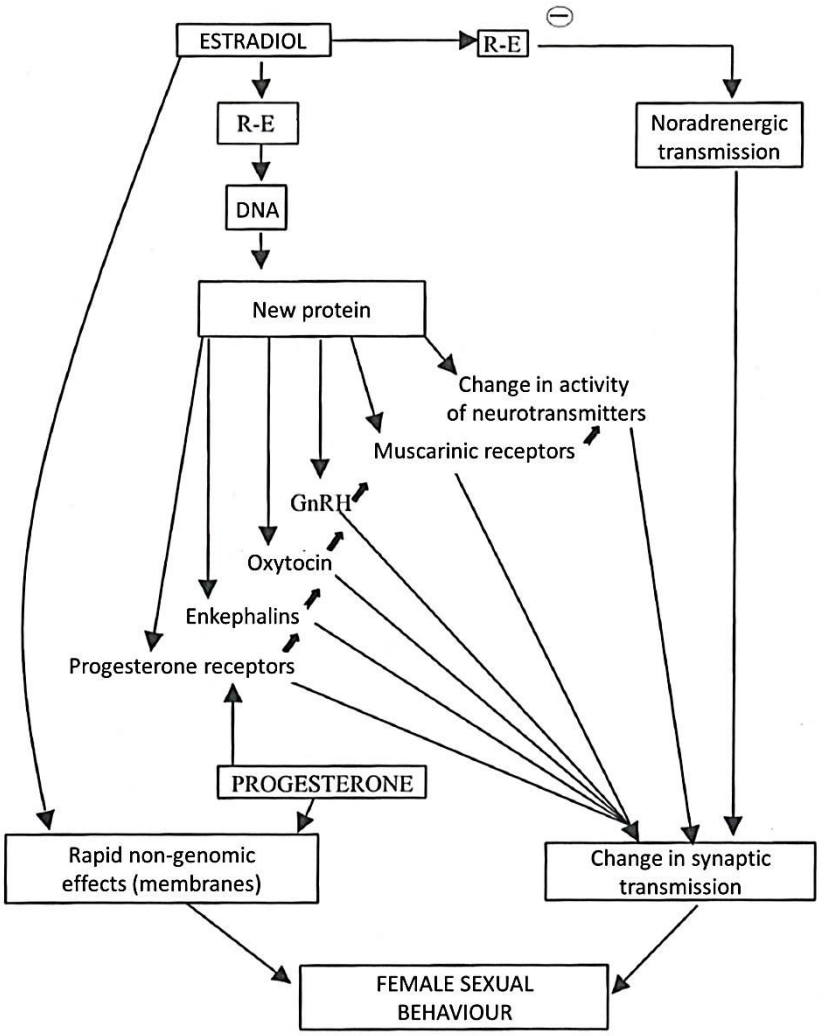


Figure 4: Schematic representation of the implication of E2 and P4 in the expression of sexual behaviours in female mammals (R-E = E2 receptors; reprinted from Balthazart and Fabre-Nys, 2001)

The number of standing behaviours has dramatically decreased during the past decades, certainly because of a decrease in both duration and intensity of oestrus. Forty years ago, the oestrus of dairy cows lasted 15 hours (h) and cows would stand to be mounted 56 times per oestrus (Hurnik *et al.*, 1975; Esslemont and Bryant, 1976). Nowadays, oestrus lasts 4 to 8 h and only 35 to 60 % of ovulations are preceded by oestrus when standing behaviour is observed (Lyimo *et al.*, 2000; Kerbrat and Disenhaus, 2004; Roelofs *et al.*, 2005a). Figure 5 illustrates the reduction in

oestrus duration when defined as the period between the first and last stand to be mounted behaviour.

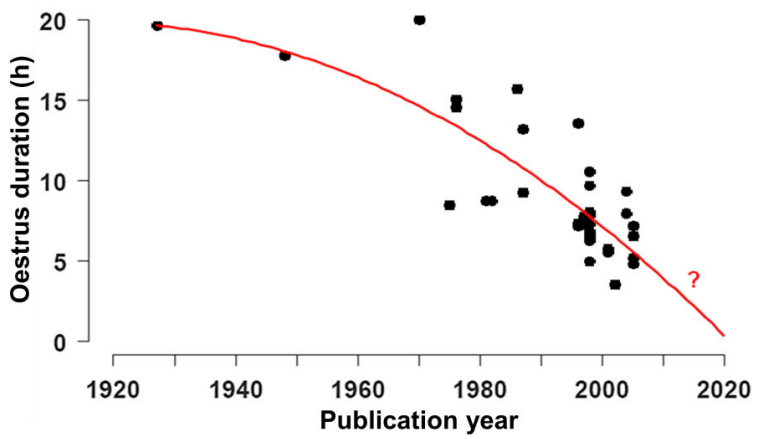


Figure 5: Duration of the oestrus in dairy cattle through time (quantitative review of 39 records from 25 studies between 1927 and 2005; reprinted from Cutullic, 2010)

With this, it was concluded that standing behaviour could not be the only evidence of oestrus in dairy cows. Van Eerdenburg *et al.* (1996) had the idea to rank the different oestrus behaviours and to give them a score according to their specificity (Table 1). Cows are considered to be in oestrus when a certain cumulative score is reached (of 100 points if observed 12 times or 50 if observed 3 times over 24 h). Through video recording, Kerbrat and Disenhaus (2004) found that even though other sexual behaviours than standing to be mounted were not specific (they are expressed in other periods than oestrus), their increased frequency is specific. Indeed, 100 % of the cows in oestrus expressed 4 non-specific behaviours within a 15 min period while only 3 % of the cows were doing so during the luteal phase. The decline in oestrus duration and expression even results

Table 1: Oestrus behaviours and the scoring scale of Van Eerdenburg *et al.* (1996) and the detection categories of Cutullic *et al.* (2009).

Signs	Points[1]	Category
Standing to be mounted	100	Standing
Mounting head side of other cow	45	Standing
Mounting (or attempting) other cows	35	Mounting
Resting with chin on other cow	15	Slight sign
Sniffing vagina of other cow	10	Slight sign
Being mounted but not standing	10	Slight sign
Restlessness	5	Slight sign
Cajoling	3	Slight sign
Mucous vaginal discharge	3	Slight sign
Aggressiveness		Slight sign
Mooing		Slight sign
Milk yield drop		Slight sign

[1]This scoring system is cumulative during a 24 hour period. When observed 12 times per day for 30 minutes, a score of 100 points is reached, the animal is considered to be in heat and can be inseminated if desired. When the cows are observed 2 or 3 times per day for 30 minutes, a threshold of 50 points can be applied.

in 8 to 15 % of "silent" ovulations (no behavioural change at all; Kerbrat and Disenhaus, 2004; Palmer *et al.*, 2010; Ranasinghe *et al.*, 2010).

The ability of dairy cows to express oestrus is crucial to enable insemination on time. The study of Kerbrat and Disenhaus (2004) also showed that sexual behaviours were mostly expressed from 1 to 7 o'clock in the morning. Fortunately, herd movements such as from pasture to the milking parlour are also periods during which dairy cows show oestrus behaviour (Britt *et al.*, 1986). These are elements to take into account in oestrus detection: the farmers' possibilities to detect oestrus are a key in the success of the reproduction of dairy cows.

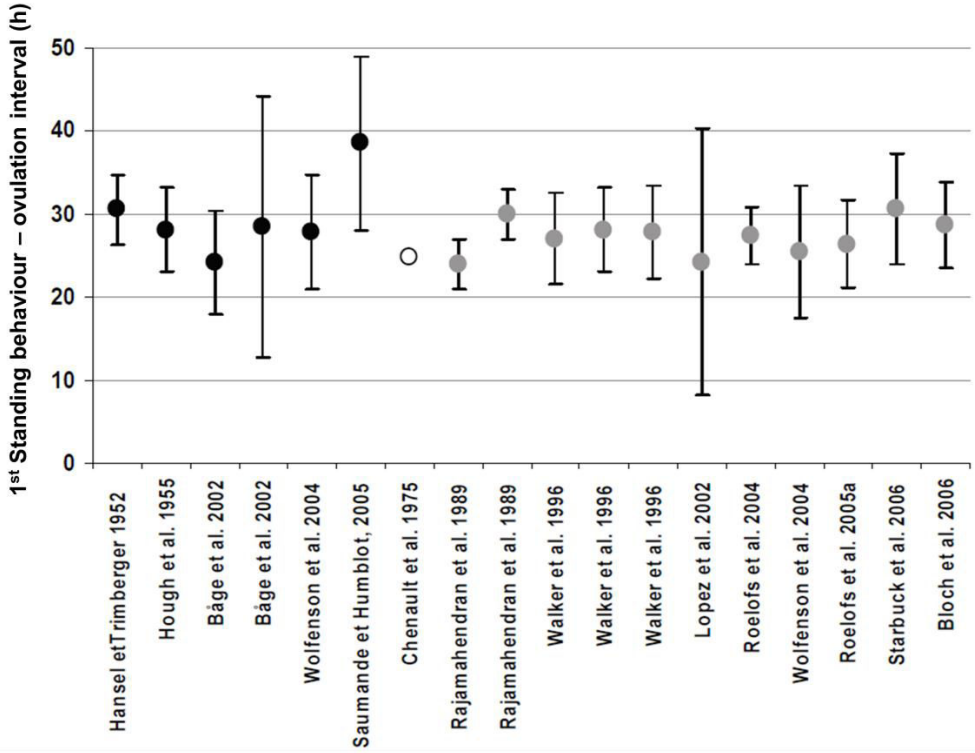


Figure 6: 1st standing to be mounted – ovulation interval (means and standard deviations) of 18 populations of either heifers (●), cows (●) or both (○) reported in 18 studies (reprinted from Cutullic, 2010).

The interval between the beginning of the oestrus and the ovulation is very variable between studies (Figure 6). The best timing for insemination is between 2 and 18 h after the start of the oestrus (Maatje *et al.*, 1997; Roelofs *et al.*, 2005a; b). In practice, when oestrus is detected, the cow is inseminated 12h later. Another source of variation is that 8 to 22 % of dairy cows have a delayed ovulation relative to the start of oestrus (> 36 h; Walker *et al.*, 1996; Roelofs *et al.*, 2005a; Saumande and Humblot, 2005; Bloch *et al.*, 2006). This is likely due to low levels of gonadal steroids and consequently to a delayed LH surge.

Behavioural traits are difficult to measure, study and use. In experimental conditions, 10 to 30 % of false-detected ovulations were recorded (during luteal phase). It was even reported that 6 % of pregnant dairy cows were expressing sexual behaviours which can lead to erroneous inseminations (Dijkhuizen and van Eerdenburg, 1997; Disenhaus *et al.*, 2010; Roelofs *et al.*, 2010).

1.3 Conception and pregnancy

After insemination, the oocyte is fertilized in the oviduct and starts its development while migrating towards the uterus (Figure 7). The embryo is made of 3 tissues: the trophoblast (future chorion part of the placenta), the primitive endoderm (future yolk sac) and the primitive ectoderm. This ectoderm is itself made of the future endoderm (digestive tract, respiratory tract, liver), the future mesoderm (placenta: chorion, allantois, amnion) and the future ectoderm (somatic and gonadic tissues of the foetus; Guillomot, 2001). The embryo enters the uterus around 4 days after ovulation and floats free in the uterine fluid which provides the necessary resources for its development (Spencer, 2013). Deprived of contact with the uterus, the embryo must rapidly send a signal to survive, otherwise the *corpus luteum* will degenerate and it will be expulsed. Around 10 days after ovulation, the embryo begins to produce the interferon tau (IFN_τ). The IFN_τ stimulates the production of PGE and inhibits the production of $PGF_{2\alpha}$ by the endometrium which maintain the structure and function of the *corpus luteum* (Guillomot, 2001; Bazer *et al.*, 2010; Sandra *et al.*, 2014). It also reduces the number of oxytocyn receptors and inhibits the action of E_2 receptors of the endometrium, which reinforces the inhibition of $PGF_{2\alpha}$ production. Around 19 days after ovulation, the implantation of the embryo occurs. The conceptus also produces pregnancy-associated glycoproteins (PAGs), they affect concentrations of LH and prolactin and contribute to the conceptus survival (Ayad *et al.*, 2007; Thompson *et al.*, 2012).

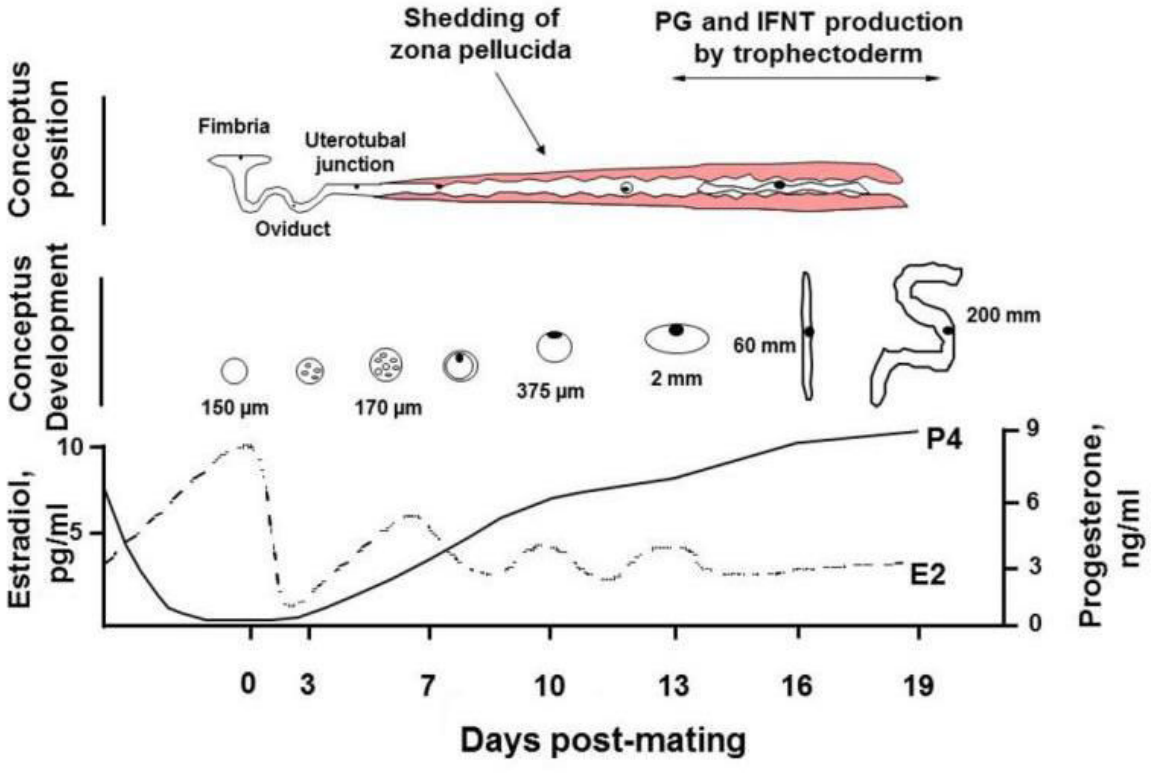


Figure 7: Early pregnancy events in cattle. This schematic summarizes the relative changes in embryo/blastocyst/conceptus development after fertilization in relation to position in the female reproductive tract and circulating concentrations of ovarian steroid hormones. PG = prostaglandins; IFNT = interferon tau; E2 = estrogen; P4 = progesterone (reprinted from Spencer, 2013).

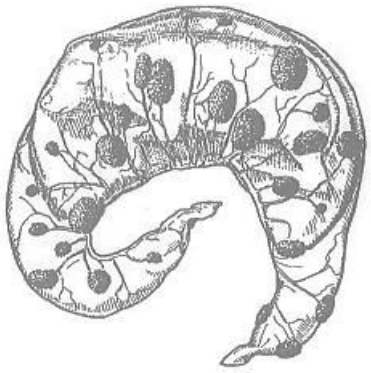


Figure 8: Cotyledonary placenta of dairy cows (reprinted from Guillomot, 2001)

As shown in Figure 8, cows have a cotyledonary type of placenta. Cotyledons are the zone of the placenta that joins the uterus to form a button like stucture. Cotyledons are the exchange zone of resources and wastes, essentially gas, nutrient and steroidic hormones, between the cow and her foetus (Martal and Haddad, 2001; Tarrade *et al.*, 2014). The glucose is the principle energy ressource for the foetus. The placenta plays a crucial metabolic role by capturing and storing a part of the glucose in glycogen, and supporting foetus metabolism by producing lactate and fructose from the maternal glucose. The placenta prioritizes the oxydation of lipids for its own energy source and is capable of synthetizing specific fatty acid required by the foetus. Finally it is capable of metabolizing the amino-acids in order to provide an adequate proportion of each to the foetus.
The placenta is a good barrier: the embryo does not receive any protein (peptidic hormones, immunoglobulin…) from the cow. Few pathogen agents can be transmitted through the placenta which provides a good protection to the foetus. However, potentially toxic soluble elements for the foetus such as heavy metals and drugs are transmitted to the foetus.
The placenta is also an important endocrine gland. Its early production of E_2 is important because it stimulates the growth of the endometrium and myometrium (the uterus of a gravide cows weighs about 10 kg compared to 0.9 kg for non-gravid cows; Gier and Marion, 1968). It stimulates the appearance of P_4 receptors in the uterus, vasodilation, development of the mammary gland and production of prolactin at parturition. The placenta also produces P_4 which is essential to maintain pregnancy. Indeed, the embryo produces IFN_τ in order to maintain the *corpus luteum* to survive for only 16 to 23 days. The production of P_4 by the placenta plays an important local role by inhibiting myometrium and endometrium activity (mitosis, contractions and production of $PGF_{2\alpha}$). In other species, the placenta can rapidly ensure the required production of P_4, but not in cows because a fetus would survive the regression of the *corpus luteum* from 200 days of gestation (compared to 50 days in humans). The placenta produces most of the pituitary hormones or analogues, neurotransmitters, growth factors, interleukins and leptine. They all play important roles either in the maintenance of pregnancy or in the development of the foetus, uterus and mammary gland.

The average gestation length ranges from 282 to 291 d among cattle breeds (Guillomot, 2001; Guerrier *et al.*, 2007). The parturition is the expulsion of the foetus and placenta out of the maternal uterus. This process induces an oxytocin and prolactin surge (also called Ferguson reflex). The prolactin is inducing the start of lactation while the oxytocin is further stimulating the expulsion

of the calf and appendices (Maltier *et al.*, 2001; Breuiller-Fouché *et al.*, 2014). The physiology of lactation is not explained in this manuscript. More information can be found on the physiology of lactation in the following references: Delouis *et al.* (2001), Martinet and Houdebine (2006), Lollivier *et al.* (2014).

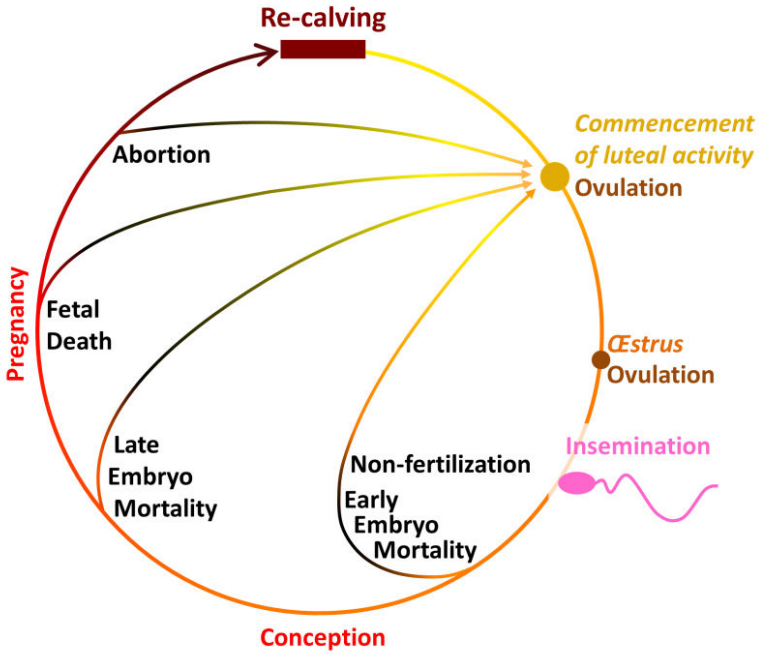


Figure 9: Depiction of the reproductive process (cyclicity, oestrus, and fertility), and source of fertility failure from calving to re-calving.

As represented in Figure 9, there are a lot of pitfalls in the way from insemination to re-calving. The earliest possible failure is non-fertilisation (NF) of the oocyte, despite of an insemination on time. Oocyte fertilization rate is very high but decreasing: 95 % in 1980' to 83 % in 2010' (Walsh *et al.*, 2011). There are among these conceptuses 55 to 88 % of viable embryos (Santos *et al.*, 2004b). Embryo mortality is one of the main sources of infertility; two different embryo deaths are distinguished based on P_4 concentrations and pregnancy monitoring (Table 2). Early embryo mortality (EEM) occurs when an embryo dies before 16 d after conception, its production of IFN_τ will not delay luteolysis, consequently it is not possible to distinguish NF and EEM by monitoring P_4 concentrations. Late embryo mortality (LEM) occurs after 16 d, and luteolysis is delayed (which can be observed on P_4 profiles from 25 d). Death occurring from 42 d of life is called foetal death (from 50 d on P_4 profiles); and if a cow does not re-calving while diagnosed pregnant at 70 d of gestation, it is considered to be abortion. Most of studies on pregnancy failures report occurrences calculated as the number of cows that experienced each type of outcome divided by the total number of inseminated cows (only first or pooled first and second services). The distribution of this outcomes in dairy cattle is ranging from 21 to 44 % for NF/EEM, 6.6 to 20.2 % for MET, 3 to 5 % for FD/abortion and consequently 26 to 64 % for calving (Humblot, 2001; Michel *et al.*, 2003; Santos *et al.*, 2004; Horan *et al.*, 2005; Freret *et al.*, 2006; Grimard *et al.*, 2006; Ponsart *et al.*, 2007; Cutullic *et al.*, 2011; Ledoux *et al.*, 2011, 2015).

Table 2: Decision rules to classify inseminations outcomes by combining information from P_4 concentrations (luteal phase length) and pregnancy diagnosis (by either PAGs or ultrasonography; adapted from Humblot, 2001).

Insemination Outcome[1]	luteal phase length	Pregnancy diagnosis[2]	Other
NF/EEM	< 25 d		
LEM	≥ 25 d & < 50 d	NP or P 35 d	
FD	≥ 50 d	P 35 d & NP 70 d	
Abortion	≥ 50 d	P 35 d & 70 d	(aborted fetus)
Calving	≥ 50 d	P 35 d & 70 d	Calf

[1]Non-fertilization/early embryo mortality (NF/EEM), late embryo mortality (LEM), fetal death (FD), abortion and calving
[2]Ultrasonography examination or determination of PAGs maternal plasmatic concentrations about 35 and 70 days after service; outcome = P (pregnant)/NP (not pregnant).

1.4 Resumption of *postpartum* reproduction activity

After calving, a series of complex anatomic, histologic, immunologic and bacteriologic changes reshape the entire uterus (stroma, endometrium, and myometrium) to resume the ability to ensure it role (Sheldon and Dobson, 2004). This process is called uterine involution and is under control of $PGF_{2\alpha}$ and PGE. After calving, the uterus of a cow weighs 10 kg and the horns are measuring 1 m length and 40 cm of diameter (Figure 10). In about 30 days, the uterus weight decreases to 0.9 kg, the horns length to 20 cm and diameter to 5 cm (Gier and Marion, 1968). During the 48 first hours after calving, lochia (placental liquid + blood from cotyledonary placenta removing + endometrium desquamation remains) are eliminated thanks to myometrium contraction. Cell size also diminishes resulting in a return to normal status. This phase plays an important role of decontamination: bacteria are expelled with lochia. Indeed, bacteria contaminate the uterus of 90% of cows after calving (Sheldon and Dobson, 2004). However, this is not always sufficient because 40 % of cows suffer endometritis (inflammation of the endometrium) during the firsts 2 weeks after calving, 15 % are persistent up until 6 weeks and require treatment.

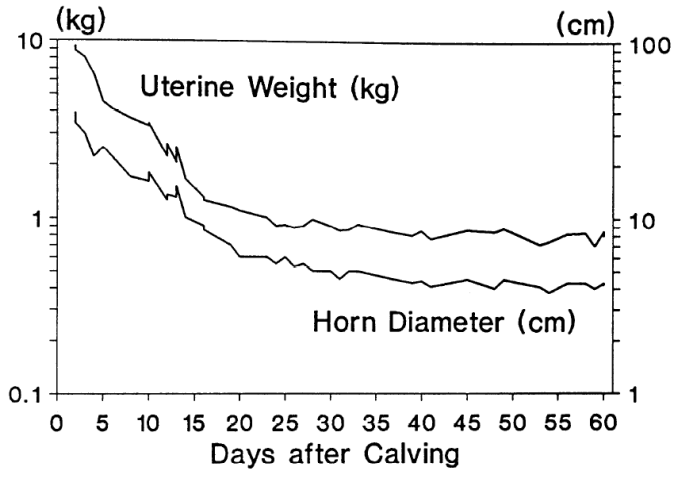


Figure 10: Rate of uterine involution as measured by weight (kg) and diameter of previously pregnant horn (cm; reprinted from Gier and Marion, 1968).

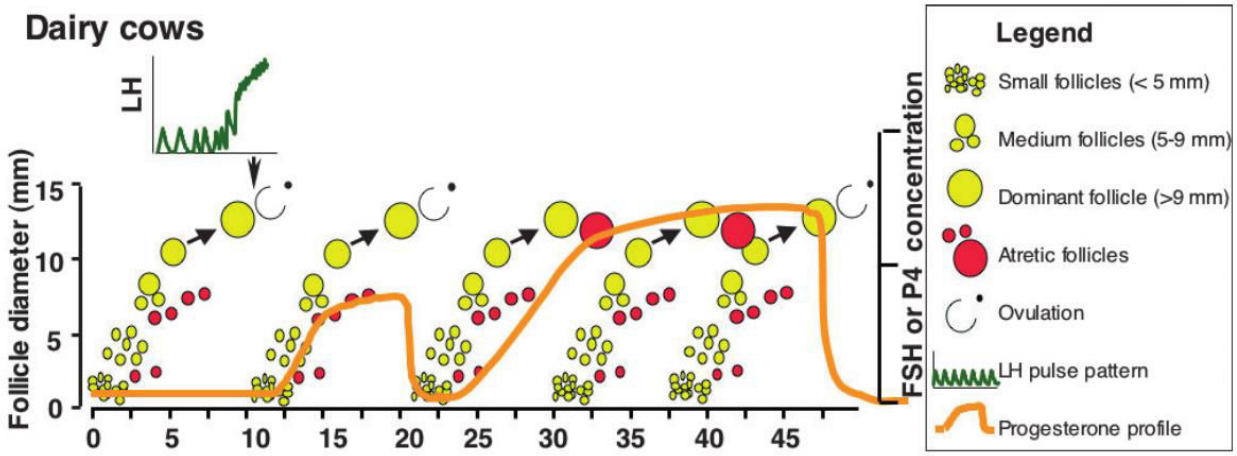


Figure 11: Diagrammatic scheme of resumption of dominant follicles and ovarian cycles during the postpartum period (in days) in dairy cows not nutritionally stressed. LH pulse frequency is that occurring during an 8-h window where cows are blood sampled every 15min. Short cycles occur in most (70%), but not all cows after first ovulation (reprinted from Crowe, 2008).

The physiology of resumption of *postpartum* ovarian activity is explained using the information found in the reviews of Crowe (2008) and Forde *et al.* (2011) and is illustrated in Figure 11. During the firsts 6 months of pregnancy follicular growth continues: a recruited wave stops its development waiting for parturition. The parturition process induces changes in endo/paracrine factors concentrations (E_2, P_4, $PGF_{2\alpha}$, IGF-I…). Then, the plasmatic concentrations of these return to basal levels. Rapidly (3-5 days), plasmatic FSH and LH levels enable recruited follicles to start the selection and dominance process. The first dominant follicle ovulates for 30 to 80% of dairy cows, otherwise it degenerates (15 to 60%) or results in ovarian cysts (1 to 5%). Most of the time no oestrus precedes the first ovulation (for more than 70% of dairy cows) and the following luteal phase is short (about 10 days, with a single follicular wave). Oxytocin levels in early lactation may be responsible for the early production of $PGF_{2\alpha}$ during this first cycle resulting in early regression of the *corpus luteum*. As illustrated in Figure 11, monitoring progesterone profiles enables ovarian activity to be studied.

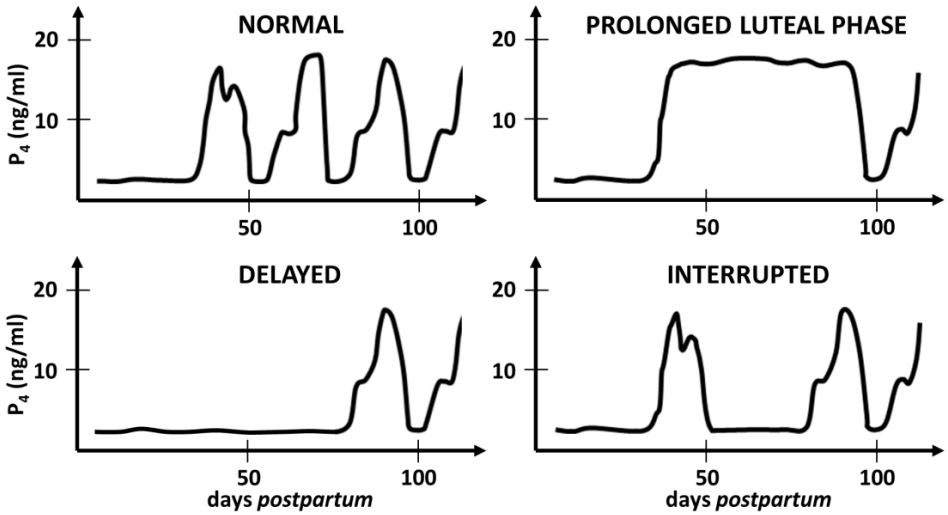


Figure 12: Main patterns of P_4 profiles as commonly classified in the literature (e.g. Lamming and Darwash, 1998; Kerbrat and Disenhaus, 2000; Royal *et al.*, 2000a; Horan *et al.*, 2005b; Petersson *et al.*, 2006a)

Different patterns are observed (Figure 12) and with the parameters derived from the P_4 profile, they can be classified as follows:

- **Normal**, if CLA occurres before 50 days *postpartum* (dpp) and the pattern is made of regular inter-ovulatory interval (IOI) ranging from 20 to 25 days (d);
- **Prolonged luteal phase** (PLP), also known as persistent *corpus luteum* if a LUT lasts more than 25 d;
- **Delayed**, when CLA occurs after 50 dpp;
- **Interrupted**, if an inter-luteal interval (ILI) lasts more than 12 d;
- **Unclassifiable**, when estimated ovarian parameters could not be calculated.

Regrettably, abnormal ovarian activity is common in modern dairy cattle. The distribution of these patterns shows an important variability in the population (Table 3). Indeed, the proportion of cows exhibiting a normal ovarian cycle is about 59 %, 22 % for PLP, 16 % for the delayed type of P_4 profile, and 10 % for the interrupted type of P_4 profile. The proportion of unclassifiable P_4 profiles is rarely reported and not very substantial (1 % in the study of Cutullic *et al.*, 2011).

Table 3: Distribution of P_4 profiles (Normal/PLP/Delayed/Interrupted) in the modern population of dairy cows (84 % of the animals involved in the 17 studies were Holstein cows; reprinted from Cutullic, 2010)

	Normal	PLP[4]	Delayed[5]	Interrupted[6]
Number of studies[1]	16	17	16	15
Number of treatments[2]	32	32	29	27
primiparous cows (average proportion)	44	50	46	52
type of P_4 profile (%; mean ± s.d.)	53 ± 10	22 ± 10	19 ± 8	8 ± 7
[min - max]	[35 - 73]	[3 - 43]	[0 - 36]	[0 - 24]
Number of profiles	4,825	4,708	4,728	4,593
primiparous cows (weighed average proportion)[3]	34	36	34	36
type of P_4 profile (weighed average proportion)	59	22	16	10

[1]Studies used: Senatore *et al.* (1996), Smith and Wallace (1998), Opsomer *et al.* (2000), Royal *et al.* (2000), Pushpakumara *et al.* (2003), Taylor *et al.* (2003), Kerbrat and Disenhaus (2004), Shrestha *et al.* (2004), Gümen *et al.* (2005), Horan *et al.* (2005), Mann *et al.* (2005), Shrestha *et al.* (2005), McCoy *et al.* (2006), Petersson *et al.* (2006a), Pedernera *et al.* (2008), Pollott and Coffey (2008), Windig *et al.* (2008)
[2]treatments were either breed, genetic line, parity, feeding system and milking frequency
[3]when the proportion of primiparous cows was not reported in the study, it was fixed at 33%
[4]PLP defined as LUT > 21 to 28 days depending on studies
[5]Delayed defined as CLA > 45 to 65 days depending on studies
[6]Interrupted defined as ILI > 12 to 14 days depending on studies

1.5 Trade-offs

The concomitance of the different life functions such as lactation and reproduction can lead to competition between them. Figure 13 illustrates that requirements in energy and material (here with the example of protein) of the main expenses: lactation, maintenance (tissues turnover, metabolism, heat production…), and reproduction (maternal reproductive system and foetus) for a standard dairy cows. This cow is producing 8,600 kg of milk throughout lactation, which means 590 kg of milk solids (320 kg of fat, 270 kg of protein) and 410 kg of lactose. This explains why milk

production is the main energy and material expense of a dairy cow. The reproduction requirements exist from calving but are almost unsubstantial until the second half of gestation (i.e. during the first 8 months of lactation). Even though it is almost not substantial at the animal scale, the maternal ovaries and uterus also need energy and materials (protein, lipids, water, vitamins, minerals…) to function as well as for the embryo to develop. Later, the development of the foetus and uterus from the second half of gestation represents substantial energy and material expense to the cow.

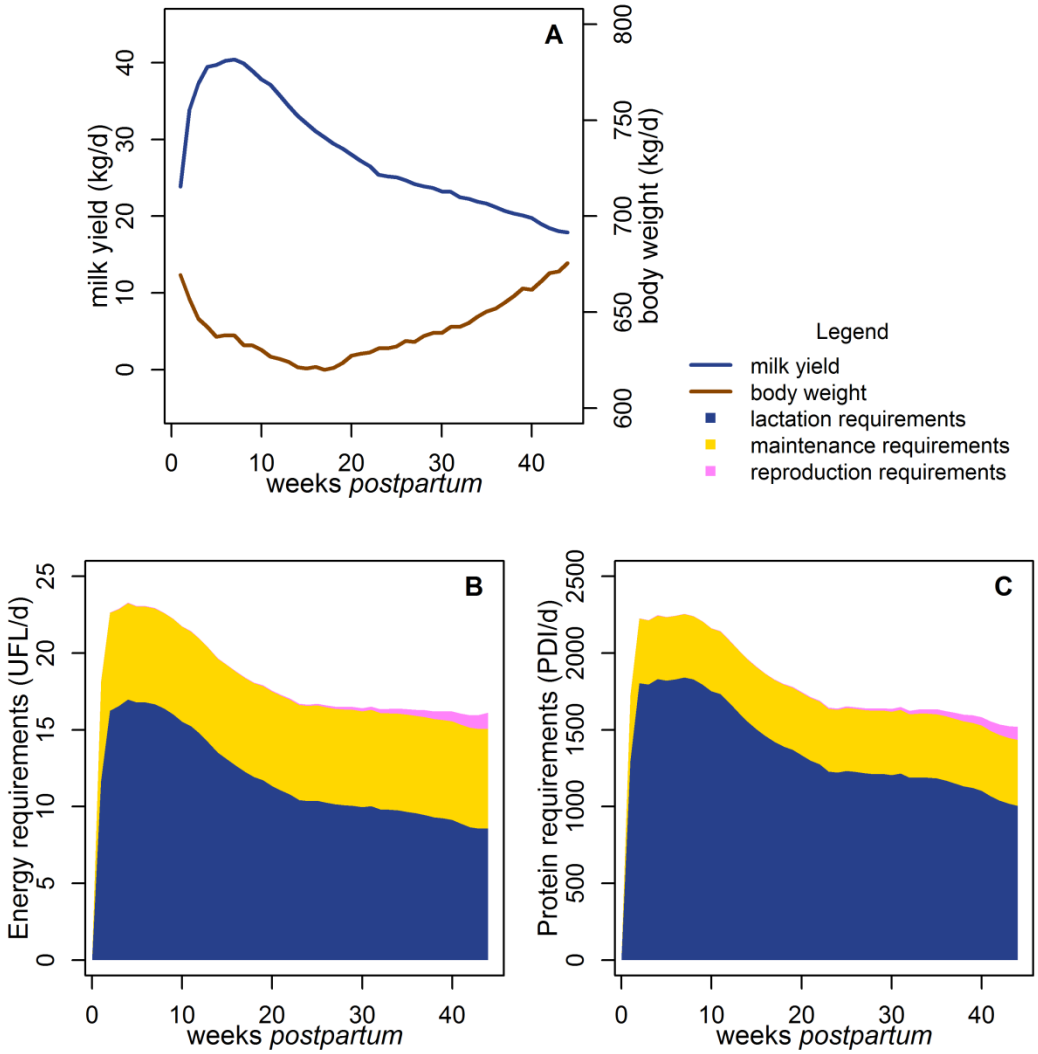


Figure 13: Typical evolution of milk yield and body weight of a Holstein cow calving at 670 kg and producing 8,600 kg of milk during 44 weeks of lactation under a "standard" grazing-based system (A) with gestation initiated around 90 days *postpartum*. The estimated requirements in energy (in UFL, B) and protein (in PDI, C) according to the equations of Faverdin *et al.* (2007).

For many reasons, resources are often limited (insufficient intake capacity, restrictive, or unbalanced diets…). One of the major problems in dairy cows is that their digestive tract is not sufficiently developed and their intake capacity is limited in early lactation. Indeed, the peak milk yield occurs during the 9 first weeks of lactation while the maximum intake capacity is reached between 15 and 20 weeks *postpartum*. The energy and protein requirements exceed intake and

dairy cows experience a condition called negative energy balance (NEB). A healthy dairy cows resumes positive energy balance between 40 and 60 dpp (Friggens *et al.*, 2007). During NEB, they rely on their body fat and protein reserves as energy and material resources to provide the missing material to fulfil the requirements of their life functions.

The genetic characteristics of the animals monitors priorities and nutrient allocation to support survival of the young and the species (homeorhesis phenomena; Sauvant, 1994; Friggens *et al.*, 2013). For instance, glucose is primarily partitioned towards the mammary gland. In early lactation, despite regular plasma insulin concentrations, the glycaemia is below regular standard. In such situations, the uptake of glucose by organs by using insulin is not possible. This phenomenon is called insulin resistance and occurs in all tissues except in the mammary gland (Kahn, 1978). Indeed, glucose uptake by the mammary gland is insulin independent (controlled by GLUT-1 transporters; Rose *et al.*, 1997). In other words, this evolutionary process makes the glucose more available for the mammary gland than other organs in early lactation to ensure milk production and thus survival of the new-born calf (homeorhesis; Chagas *et al.*, 2009).

However, both lactation and reproduction are homeorhetic phenomena which lead to trade-offs between these functions. Cows have to adapt and prioritise through investment in the current calf (lactation) and the future calf (reproduction; Friggens, 2003). Martin and Sauvant (2010a; b) illustrated this concept through successful modelling of the trajectories of priorities and thus trade-offs in dairy cows (Figure 14). The management of these priorities define adaptive pathways and thus the robustness of each individual to the constraints of the environment.

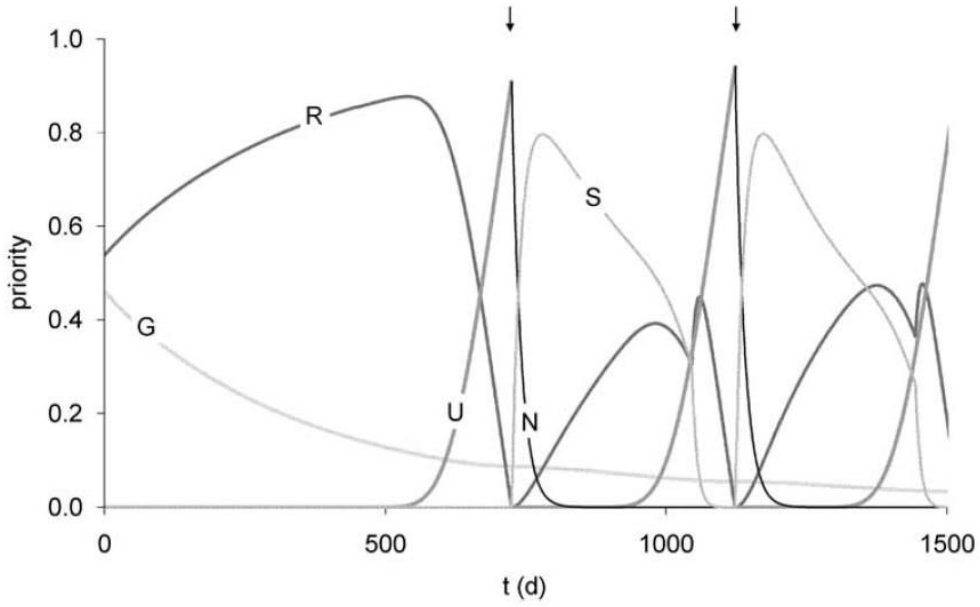


Figure 14: Trajectories of priorities over 1500 days of life G: growth, R: balance of body reserve, U: ensuring survival of the unborn calf, N: ensuring survival of the newborn calf and S: ensuring survival of the suckling calf. Arrows indicate parturition times of two successive reproductive cycles (reprinted from Martin and Sauvant, 2010a).

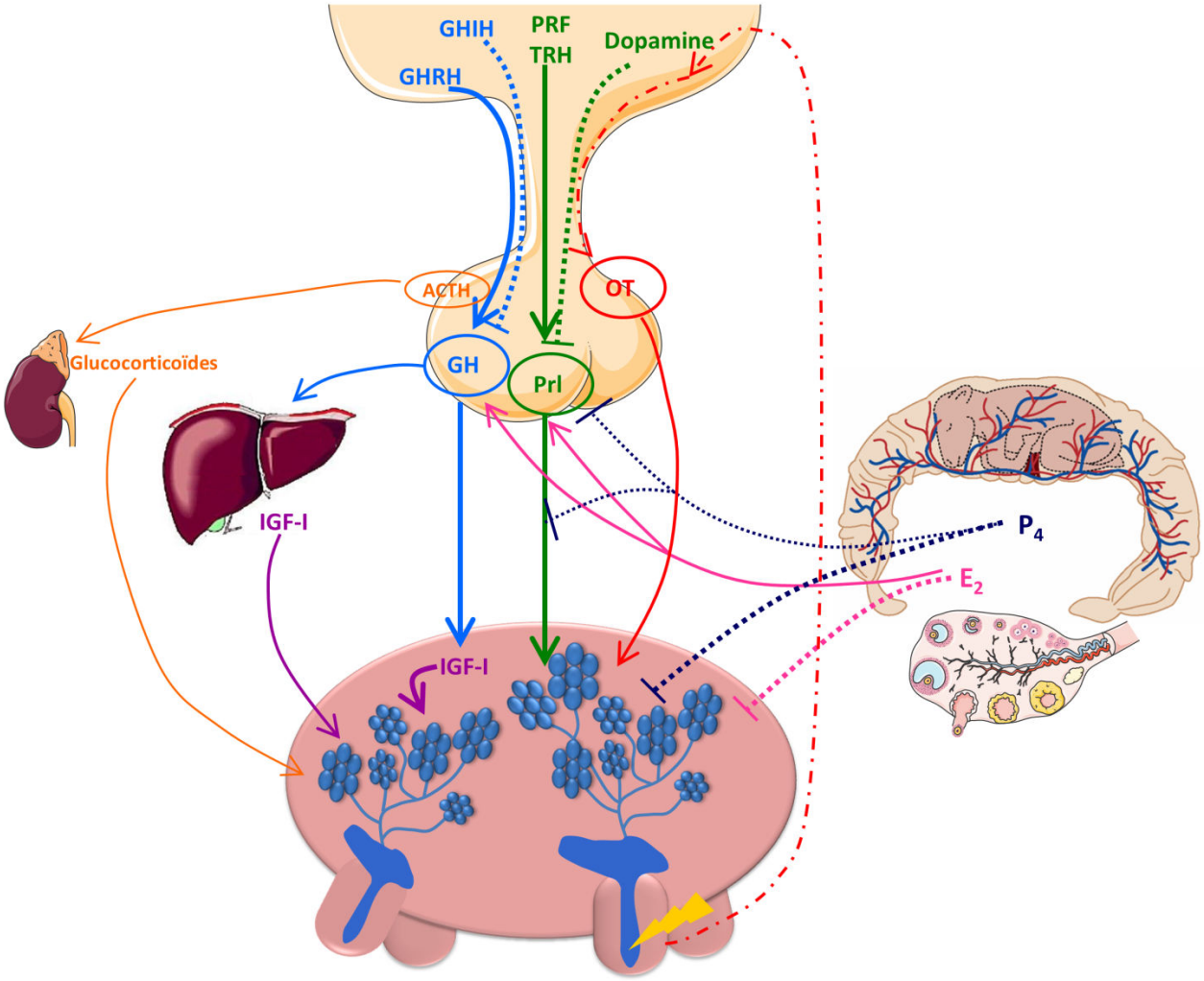


Figure 15: Endocrine controls of lactation, the solid arrows represent inducing/enhancing effects and the dotted segments limiting/inhibiting actions. GH: Growth Hormone; GHRH: GH Releasing Hormone; GHIH: GH Inhibiting Hormone; Prl: Prolactin; PRF: Prolactin Releasing Factor; TRH: Thyrotropin Releasing Hormone; ACTH: AdrenoCorticoTropic Hormone; IGF-I: Insulin-like Growth Factor I, OT: Oxytocin; E_2: estradiol; P_4: progesterone (review and depiction by L. Yart, F. Dessauge and V. Lollivier)

Lactation and reproduction may not be in competition for nutrient only, but also for other resources like hormones[2]. Indeed, both functions are sharing common endocrine signals (Figure 2; Figure 15). Oxytocin, prolactin and GH are the 3 main endocrine factors that control lactation are pituitary hormones, as LH and FSH. Indeed, oxytocin is involved in the control of lactation by stimulating milk expulsion out of the *acini* and cistern of the mammary gland. This hormone also affects ovarian activity by maintaining the luteolysis process, the expression of sexual behaviour during oestrus, and parturition. Prolactin which induces lactation at calving, is also involved in the manifestation of sexual behaviour and in the maintenance of the *corpus luteum* in ewes, and may play a role in the conceptus survival during gestation. GH is mainly involved in nutrient mammary supply (regulation of blood flow) and uptake, mammary epithelial cell activity (synthesis of milk)

[2] The information in this subsection was found in Bauman (1999); Balthazart and Fabre-Nys (2001); Driancourt et al. (2001); Guillomot (2001); Martinet and Houdebine (2006); Lucy et al. (2009); Breuiller-Fouché et al. (2014); Lollivier et al. (2014); Yart et al. (2014); Herve et al. (2016).

and survival. GH is also involved in many biological processes concerning nutrient partitioning (mobilisation/accretion of body reserve, hepatic gluconeogenesis…). GH may negatively affect reproduction because it strongly drives the trade-off to support milk production. Leptin as well is mainly involved in the management (constitution and mobilization) of the adipose tissue, and may also affect sexual behaviour. Sexual steroid hormones also affect lactation: P_4 is also involved in the development of mammary *acini* and both ovarian steroids (P_4 and E_2) are known to be key regulators of the apoptosis of mammary epithelial cells and thus reduction of the alveolar size and secretory tissue, resulting in the decline in persistency at the end of lactation.

The resource can be limited and the cow will experience a trade-off in hormone availability and allocation. Hormones can be a limiting factor either because of a limited synthesis or an exacerbated catabolism or both. As explained, because of milk production or insulin resistance, plasma glucose availability may be limited for the ovaries, and thus sexual steroid synthesis impaired. In addition, milk production is related with increased intake capacity, and consequently increased liver blood flows. This would result in a high catabolism of sexual steroids. Both mechanisms might be participating to the trade-off between lactation and reproduction (Wiltbank *et al.*, 2006).

In this first section of the literature review, the basics of the physiology of the reproduction of dairy cows and their variability were presented. The elements to remember are:

- Lactation and reproduction are concomitant and in competition for resources: they partly share the same endocrine signals, energy and biological material. This leads to trade-off: if a cow invests most of her resources in lactation, there would be almost nothing left for reproduction.
- Reproduction of dairy cows is a succession of inter-connected steps. Regrettably, each step of the process is declining :
    - Only 60 % of dairy cows have a normal ovarian cyclicity (*postpartum* anovulation shorter than 50 d and regular ovarian cycles of 20-25 d);
    - The oestrus has dramatically shortened from 15 h to 4-8 h during the past decades, and the intensity of expression of sexual behaviours has decreased to the point that there are even 8-15 % of ovulations without any behavioural change;
    - 83 % of the oocytes are fertilized but only 26 to 64 % of inseminated cows are calving. Pregnancy losses can be due to distinct clinical situations: early embryonic death, late embryonic death, foetal death and abortion.

Due to the importance of the threats identified at each reproductive step and their constant amplification for decades, it is unlikely that this variability is only due to chance. The next section presents known impacting factors, and knowledge gaps that lead to the question of this research project.

Dans cette première partie, les fondamentaux de la physiologie du système reproductif des vaches laitières et la variabilité observée ont été présentés. Les éléments à retenir sont :

- *La lactation et la reproduction sont concomitantes et en concurrence sur les ressources : elles partagent en partie les mêmes signaux hormonaux, l'énergie et le matériel biologique (protéines, lipides…) fournis par l'animal. Cette situation mène à des compromis : si une vache investit presque toutes ses ressources dans l'une des deux fonctions, il en restera très peu pour l'autre.*
- *La reproduction des vaches laitières est une succession d'étapes qui s'enchaînent :*
    - *Seulement 60 % des vaches laitières présentent une activité ovarienne normale (reprise de cyclicité postpartum inférieure à 50 jours avec des cycles réguliers de 20 à 25 jours) ;*
    - *L'œstrus s'est fortement raccourci ces dernières années passant de 15 h à 4-8 h, l'intensité des comportements sexuels a tant diminué que 8-15 % des ovulations ne sont accompagnées d'aucune modification comportementale ;*
    - *83 % des ovules sont fécondés mais seulement 26 à 64 % des vaches inséminées vêlent. Les pertes de gestation sont de différents types : on observe des mortalités embryonnaires précoces, tardives, fœtales, ou des avortements.*

L'importance des échecs constatés à chaque étape du processus de reproduction et leur constante amplification depuis des années écarte la piste du hasard. Dans la partie suivante, les facteurs interférents et les manques de connaissance menant à la problématique de ce projet de recherche sont présentés.

2 Factors impacting reproductive performance of dairy cows

Both the variability observed at each step of the reproductive process and the competition between lactation and reproduction are related to characteristics of the animal and of the farming systems. In this section, the association of reproduction with some factors at the animal scale are detailed in a first sub-section. In a second one, factors of the farming system affecting reproduction are presented. In the last subsection, a quantitative approach of the competition between lactation and reproduction to measure the biological responses and leverages to manage them is described.

2.1 Animal characteristics

2.1.1 Genetics: a structural source of variation

2.1.1.1 Strong selection on production traits resulted in poor reproduction performance

During past decades, a strong emphasis on production traits was used in breeding goals in order to improve production performance of dairy cows. Reproductive performance has been declining in the same time so that strong selection on production traits is held responsible for the declined ability of dairy cows to ensure reproduction. Indeed, high genetic merit for milk yield is associated with delayed CLA (Fulkerson *et al.*, 2001, 2008; Pollott and Coffey, 2008; Windig *et al.*, 2008), decreased oestrus intensity (Westwood *et al.*, 2000; Pollott and Coffey, 2008), lower submission rate (proportion of cows inseminated; Kolver *et al.*, 2005), lower conception and pregnancy rates (Buckley *et al.*, 2000; Fulkerson *et al.*, 2001; Snijders *et al.*, 2001; Horan *et al.*, 2004, 2005b; Kolver *et al.*, 2005; Macdonald *et al.*, 2008; Fulkerson *et al.*, 2008). This is consistent with the fact that the genetic correlations between milk yield and reproduction traits are unfavourable. Table 4, derived from the review of Berry *et al.* (2014), presents the genetic correlation between production or functional traits and traditional fertility traits. On average, these correlations are substantial. Increasing milk yield results in lengthened reproduction intervals (calving to first service, calving to conception, and calving interval) and decreased pregnancy rates. Surprisingly, submission rate and non-return rate (proportion of cows not inseminated again after service and therefore supposed pregnant) were exceptions: they had favourable genetic correlation with production traits. These “traditional” traits can potentially be biased by management decisions and actions (e.g. observing oestrus behaviour) in opposition to “physiological” traits that are derived from progesterone data (Royal *et al.*, 2000b). Milk yield is also unfavourably correlated with physiological traits: the genetic correlation ranges from 0.18 to 0.36 with CLA (Veerkamp *et al.*, 2000; Royal *et al.*, 2002b; Tenghe *et al.*, 2015).

However, these effects are controversial in the literature: many studies could not find any relationship between genetic merit for milk yield and reproduction performance (Barnes *et al.*, 1990; Silke *et al.*, 2002; Kennedy *et al.*, 2003; Patton *et al.*, 2007; Chagas *et al.*, 2009). It was even reported that the occurrence of PLP at first ovulation is negatively, thus favourably genetically correlated with milk yield (-0.31; Royal *et al.*, 2002b), and with energy-corrected milk yield (-0.60; Nyman *et al.*, 2014). Nonetheless, phenotypically the higher the milk yield, the greater the risk for

Table 4: Pooled genetic correlations (pooled standard error in parenthesis) as well as the range in genetic correlations (in square parenthesis) between female productive traits and a selection of performance traits in different dairy populations[†‡¥] (reprinted from Berry *et al.*, 2014).

Trait	Calving to first service	Number of services	Pregnant/conception to first service	Pregnant within a given time period	Calving interval	Days open/calving to conception interval	Interval from first to last service	Non-return rate	Submission rate
Milk yield	0.14 (0.005)[13] [−0.45 to 0.67]	0.02 (0.007)[8] [0.00 to 0.61]	−0.35 (0.07)[4] [−0.49 to −0.16]	0.27 (0.065)[3] [−0.22 to 0.67]	0.46 (0.018)[4] [−0.45 to 0.74]	0.27 (0.212)[2] [0 to 0.27]	0.38 (0.039)[3] [0.31 to 0.44]	−0.01 (0.007)[6] [−0.59 to 0.31]	0.22 (0.098)[2] [0.03 to 0.45]
Fat yield	0.24 (0.056)[5] [−0.08 to 0.42]	0.28 (0.077)[4] [0.23 to 0.39]	−0.29 (0.065)[3] [−0.48 to −0.11]	−0.31 (0.13)[1]	0.48 (0.032)[8] [−0.3 to 0.68]	0.32 (0.192)[1]	0.45 (0.041)[2] [0.20 to 0.46]	−0.08 (0.107)[1]	
Protein yield	0.42 (0.026)[6] [−0.09 to 0.51]	0.35 (0.072)[4] [0.21 to 0.54]	−0.37 (0.073)[3] [−0.51 to −0.13]	−0.17 (0.15)[1]	0.50 (0.032)[8] [−0.39 to 0.67]	0.29 (0.201)[1]	0.41 (0.051)[2] [0.27 to 0.42]	−0.23 (0.04)[2] [−0.24 to −0.13]	
Body condition score	−0.51 (0.032)[7] [−0.63 to −0.37]	−0.01 (0.052)[4] [−0.42 to 0.29]	0.09 (0.074)[3] [−0.14 to 0.34]	0.35 (0.051)[2] [0.35 to 0.35]	−0.44 (0.022)[6] [−0.51 to −0.14]	−0.43 (0.054)[2] [−0.46 to −0.31]	−0.16 (0.075)[3] [−0.30 to 0.02]	−0.30 (0.097)[1]	
Live weight	−0.25 (0.090)[1]	0.15 (0.130)[1]	−0.22 (0.230)[1]	−0.24 (0.110)[1]			0.37 (0.170)[1]		
Somatic cell score	0.12 (0.03)[2] [0.08 to 0.32]	0.15 (0.083)[1]		−0.15 (0.026)[1]	0.20 (0.041)[3] [−0.05 to 0.22]			−0.13 (0.04)[2] [−0.14 to −0.07]	
Survival	0.09 (0.034)[3] [−0.36 to 0.15]	−0.32 (0.176)[1]		0.63 (0.158)[1]	−0.34 (0.038)[3] [−0.74 to −0.26]	−0.78 (0.040)[1]		−0.04 (0.05)[1]	0.15 (0.218)[1]

[†]Superscript is the number of populations included in the range; this may not necessarily be also the number of studies included in the pooled estimate due to a lack of standard errors provided for some population.
[‡]No literature estimates could be found for empty cells of pair-wise correlations.
[¥]Studies contributing to the correlations are listed in Supplementary material S3.

PLP (Royal *et al.*,2002b; Petersson *et al.*, 2007; Kafi *et al.*, 2012), the lower oestrus duration and intensity (Friggens *et al.*, 2010; Ranasinghe *et al.*, 2010), and the more non-fertilization and pregnancy loss (Humblot, 2001; Grimard *et al.*, 2006). This suggests that other genetic characteristics (e.g. genetic merit for reproduction traits) and the environment (e.g. nutrition) play key roles in the relationship between lactation and reproduction.

As explained in section 1.5, there is much evidence that priorities of the cows are informed by their genetic characteristics. With selection, it is most likely that these adaptive abilities have been changed (Friggens *et al.*, 2013). Indeed, milk yield is also unfavourably correlated with live weight (genetic correlation ranging from -0.26 to 0.09; Veerkamp *et al.*, 2000; Berry *et al.*, 2003), body condition score (genetic correlation ranging from -0.63 to -0.12; Berry *et al.*, 2003; Bastin and Gengler, 2013), and weight or body condition losses (genetic correlation respectively ranging from -0.59 to -0.38; Veerkamp *et al.*, 2000; and-0.46 to -027 Berry *et al.*, 2003). This means that dairy cows with high genetic merit for milk yield are partitioning their resources towards milk yield rather than body reserve constitution and that these reserves are more predisposed to be mobilised in periods of nutrient scarcity. Cows from high yielding breeds or genetic lines mobilise their body reserve to support milk production, all the more since nutritive supply is very limiting (Roche *et al.*, 2006; Delaby *et al.*, 2009; Cutullic *et al.*, 2011).

Physiologically, in early lactation plasma growth hormone (GH) concentrations rise up which results in this nutrient partitioning from the liver and body reserve to support milk production as intake does not fulfil lactation requirements. But during this period of NEB, insulin levels are low and thus liver GH-receptor decline, resulting in decreased production of IGF-I by the liver. The negative feedback of IGF-I on the pituitary gland is insufficient and the cow further partitions nutrient toward the mammary gland. This is called GH - IGF-I axis uncoupling, and the somatotropic axis is recoupling during lactation with increasing nutrient intake and decreasing requirements for milk production (see review of Walsh *et al.*, 2011). This is an indirect effect of high genetic merit for milk yield. This could in turn negatively impact reproduction, as insulin and IGF-I are involved in ovarian function (steroids synthesis and ovulation of the dominant follicle; see section 1). Other indirect genetic effects of the prioritization of lactation may also contribute to the declining fertility of dairy cows. Inskeep (2004) suggested that because of both reduced steroid synthesis and increased metabolic catabolism, high yielding cows would have lower plasma P_4 concentrations, resulting in lower oocyte quality and thus more pregnancy failures (Figure 16).

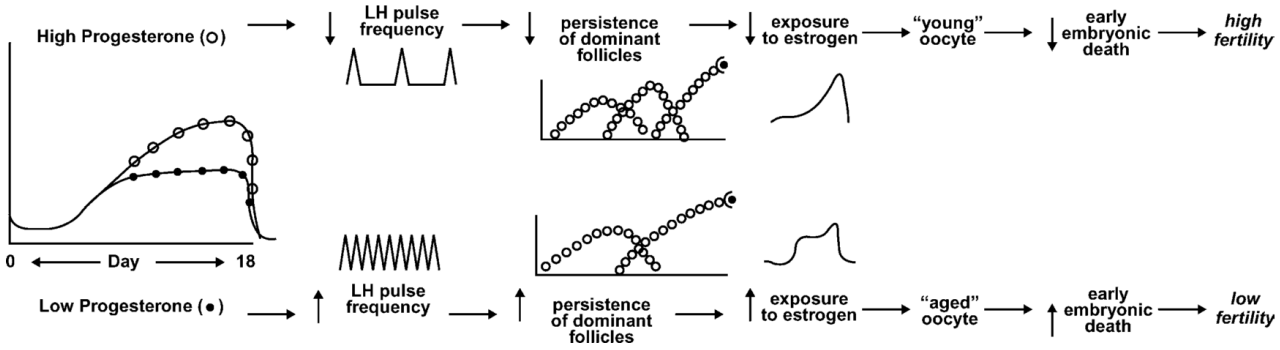


Figure 16: Effects of follicular development patterns on fertility in the cow (Inskeep, 2004).

2.1.1.2 Genetics and genomics of reproduction traits

Some cows may simply have poor genetic predisposition to reproduce. Indeed, part of the variability observed in reproductive performance is due to variability in genetic characteristics (i.e. heritability). However, these heritability estimates are quite low (below 0.05) for traditional reproduction traits (Figure 17) and for oestrus intensity (ranging from 0.01 to 0.04; Roxström *et al.*, 2001; Gernand *et al.*, 2012; Carthy *et al.*, 2016). However, physiological traits have a more substantial heritability such as CLA with estimates ranging from 0.12 to 0.30 (Veerkamp *et al.*, 2000; Royal *et al.*, 2002; Petersson *et al.*, 2007; Tenghe *et al.*, 2015), the proportion of P_4 samples on a weekly basis considered to be at a concentration induced by a *corpus luteum* before 60 days in milk (PLA, highly correlated with PLP) with estimates also ranging from 0.12 to 0.30 (Petersson *et al.*, 2007; Tenghe *et al.*, 2015).

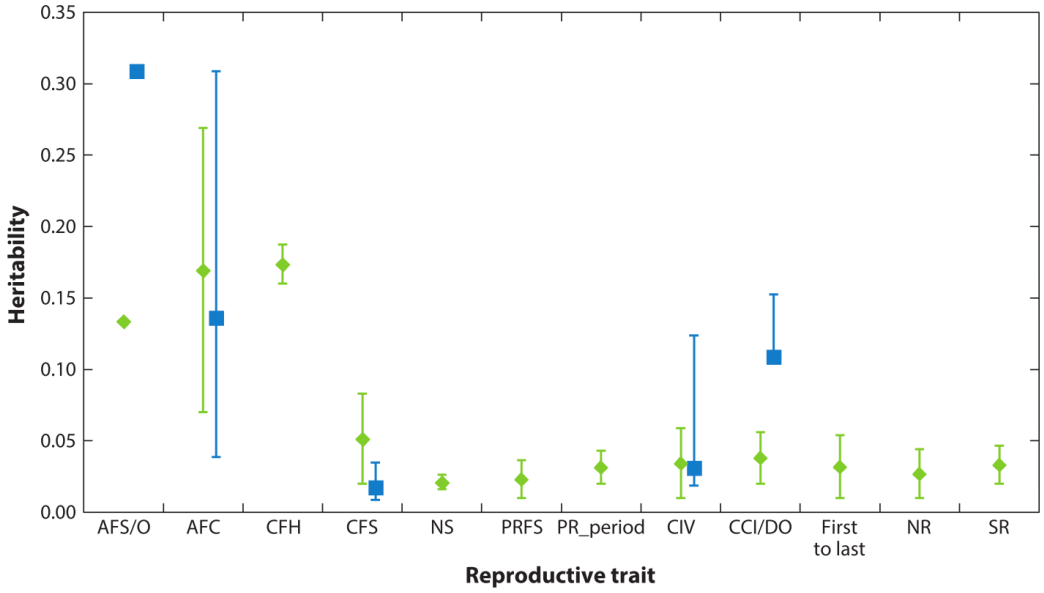


Figure 17: Mean heritability estimates as well as minimum and maximum heritability estimates per study from a meta- analysis (39) of Holstein-Friesian (green diamonds) and beef (blue squares) cattle for the fertility traits age at first service or ovulation (AFS/O), age at first calving (AFC), interval from calving to first heat (CFH), interval from calving to first service (CFS), number of services (NS), pregnant to first service (PRFS), pregnant in a given period of time relative to the start of a breeding season (PR_period), calving interval (CIV), calving to conception interval of days open (CCI/DO), interval from first to last insemination (First to last), non-return rate (NR), and submission rate (SR; reprinted from Berry *et al.*, 2014, 2016)

Because of the physiology or time series of the reproductive steps, failure of one of them compromises the success in the others (Darwash *et al.*, 1997; Gautam *et al.*, 2010). In addition, reproductive traits are moderately to strongly genetically correlated to each other (Table 5, derived from the review of Berry *et al.*, 2014). However, Table 4 and 5 also clearly show that knowledge on genetic correlation between some traits are lacking. The authors also point to the fact that in some cases few estimates could be found in the literature and that the precision of some of them can be improved. Also, heritability estimates of reproductive traits are globally low and very few populations are phenotyped for physiological traits. This gap of knowledge is a threat to not repeat the same error that lead to the reproduction decline. For instance very little information is available about the genetic link between reproductive and other health traits.

Table 5: Pooled genetic correlations (pooled standard error in parenthesis) as well as the range in genetic correlations (in square parenthesis) between female reproductive traits and across different dairy populations[†‡¥] (reprinted from Berry *et al.*, 2014).

Trait	Age at first calving	Calving to first service interval	Number of services	Pregnant/ conception to first service	Pregnant in a given time period	Calving interval	Days open/calving to conception interval	Interval from first to last service	Non-return rate
Calving to first service interval	0.37 (0.10)[1]								
Number of services	−0.11 (0.15)[1]	0.08 (0.04)[7] [−0.18 to 0.30]							
Pregnant/conception to first service	−0.27 (0.06)[1]	−0.53 (0.03)[3] [−0.60 to −0.11]	−0.85 (0.03)[2] [−0.92 to −0.78]						
Pregnant in a given time period	−0.41 (0.13)[1]	−0.70 (0.06)[3] [−0.84 to −0.32]	−0.56 (0.08)[2] [−0.66 to −0.41]						
Calving interval	0.16 (0.13)[2] [0.05 to 0.16]	0.82 (0.02)[9] [0.00 to 0.92]	0.86 (0.02)[5] [0.36 to 0.89]	−0.61 (0.01)[3] [−0.84 to −0.59]	−0.86 (0.05)[3] [−0.91 to −0.57]				
Days open/calving to conception interval	0.15 (0.05)[2] [−0.55 to 0.24]	0.82 (0.03)[3] [0.00 to 0.84]	0.93 (0.01)[4] [0.44 to 0.94]	−0.94 (0.002)[2] [−0.94 to −0.74]	−0.92 (0.05)[1]	0.98 (0.004)[3] [0.11 to 0.99]			
Interval from first to last service		0.41 (0.04)[4] [0.24 to 0.50]	0.91 (0.02)[6] [0.67 to 0.96]	−0.93 (0.06)[1]	−0.79 (0.08)[1]	0.97 (0.01)[3] [0.80 to 0.98]	0.99 (0.01)[3] [0.73 to 0.99]		
Non-return rate		−0.04 (0.01)[5] [−0.69 to 0.24]	−0.90 (0.02)[4] [−0.94 to −0.46]		0.79 (0.25)[1]	−0.51 (0.06)[2] [−0.89 to −0.21]	−0.86 (0.12)[1]	−0.76 (0.04)[2] [−0.78 to −0.66]	
Submission rate	−0.36 (0.15)[1]	−0.94 (0.04) [−0.95 to −0.64]	0.04 (0.20)[1]		0.29 (0.005)[2] [0.29 to 0.74]	−0.62 (0.12)[2] [−0.74 to −0.38]			0.47 (0.29)[1]

[†]Superscript is the number of populations included in the range; this may not necessarily be also the number of studies included in the pooled estimate due to a lack of standard errors provided for some population.
[‡]No literature estimates could be found for empty cells of pair-wise correlations.
[#]Studies contributing to the correlations are listed in Supplementary material S1.

Nowadays, breeding goals in the different countries consider functional traits (including reproduction) that have arrested the genetic decline of reproduction. For instance, estimated breeding value (EBV) for fertility in France (conception rate and calving to first service interval) is included in breeding goal since 1998 (Boichard and Barbat, 1998). The relative emphasis on reproduction in breeding goals from regions that wished to halt the reproductive decline due to reproduction ranged from 17% to 20 % (Australia, Ireland, France, UK; Boichard and Barbat, 1998; Berry *et al.*, 2014).

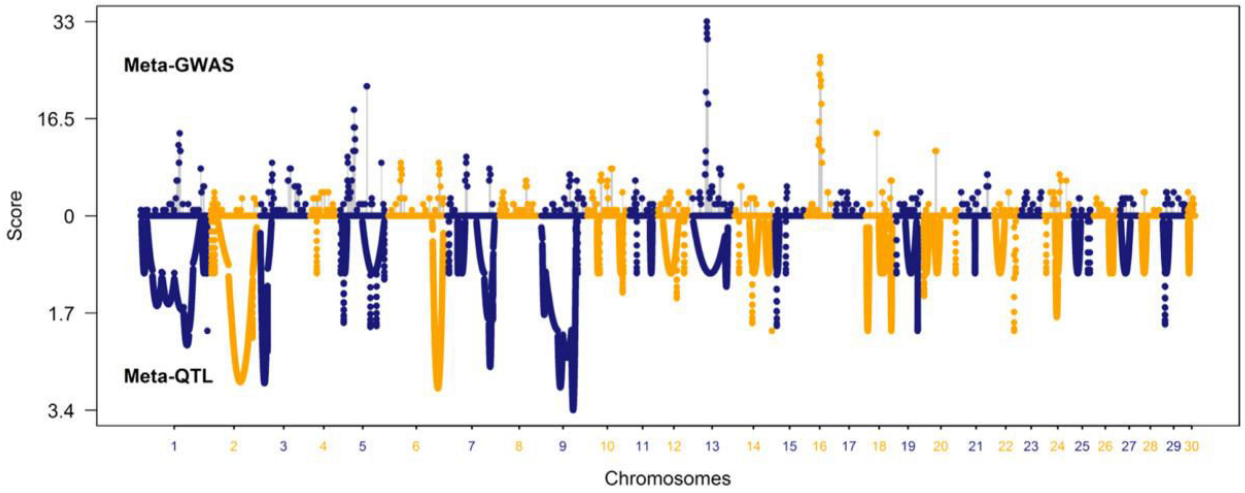


Figure 18:Genome-wide meta-QTL and meta-GWAS scores for fertility trait-class computed using published data (reprinted from Khatkar *et al.*, 2014).

Genomic information is a way to improve our knowledge on the genetic make-up of reproduction traits. A recent meta-assembly of quantitative trait loci (QTL) and genome-wide association studies (GWAS) summarises the genomic knowledge to date (Khatkar *et al.*, 2014). The authors compiled the information from 35 studies covering the full genome and reporting QTL for a combination of 11 traditional fertility traits. They report scores, the peak of QTL was set to 1 and overlapping scores were summed up. A similar method was done with 23 GWAS with 48 traditional and physiological fertility traits, the score was the sum of the number of significant SNP within and across studies (within 2.5 Mb intervals, Figure 18). All chromosomes of dairy cows contained regions associated with reproduction performances. This illustrates the complexity of traits and the need for both refining phenotypes and large scale phenotyping. *Bos taurus* autosome (BTA) 1 had high signals in both QTL and GWAS analyses. BTA5, 13 and 18 also contained regions identified through both methods. BTA 2, 3, 6, 7, and 9 also had strong QTL for fertility and BTA 16 a high signal in GWAS studies. In addition, haplotypes and candidate genes studies specifically identified genomic regions associated with embryonic lethal mutations. There was even a causative deletion found to have a strong and positive effect on milk yield but lethal to embryos in Viking red cattle (Khatkar *et al.*, 2014).

Despite these recent advances due to technology progress, there are still many gaps in knowledge today on (i) the genetic make-up of reproduction in *Bos Taurus* (genetic correlations among all production and functional traits, causative mutations, representative population in different environments, epigenetics, gene expression and regulation…) and on (ii) the use of precision

livestock farming data or other in-line phenotypes for improved genomic selection. In addition, the update of traditional breeding goals by considering other weighing than economic values such as integrating benefits for both environment and society must be scientifically addressed. This lack of knowledge currently delays the genetic improvement of reproduction and will require further research combining "traditional" and "modern" methods.

2.1.2 Health problems associated with reproduction failure

Early *postpartum* health status is known to be a major risk for reproductive failure (Walsh *et al.*, 2011). As explained in section 1.4, *postpartum* endometritis and persistent metritis are frequent in dairy cows. Their occurrence is associated with delayed cyclicity and PLP (Opsomer *et al.*, 2000; Petersson *et al.*, 2006a; Royal *et al.*, 2002b). Indeed, lipopolysaccharides produced by pathogens such as Escherichia coli impair the production of messenger ribonucleic acid (mRNA) coding for the aromatase in *granulosa* cells (enzyme essential to the production of E_2). This results in both impaired growth of the dominant follicle and low plasma concentration of E_2. Histamines and endotoxins released during the infection affect the central nervous system and consequently impair LH synthesis (Sheldon *et al.*, 2009). Moreover, during infection the endometrial cells are damaged which impairs secretion of $PGF_{2\alpha}$. It was even suggested that endotoxins would enhance the production of PGE (luteotrophic). In case of ovulation, both phenomena would increase the risk of developing a PLP (Sheldon *et al.*, 2009). As a result, endometritis are also associated with later conception (+15 d compared to healthy cows), but also with lower conception rate (-20 percentage units) and pregnancy rate (-16 percentage units; meta-analysis of Fourichon *et al.*, 2000). Problems at calving such as calving unease and retained placenta are associated with delayed cyclicity and PLP (Opsomer *et al.*, 2000; McCoy *et al.*, 2006). For 10 years, interest in the implication of mastitis in fertility failure has been growing. Indeed, occurrence of clinical mastitis was found to be associated with later resumption of ovarian activity (onset of oestrus behaviour; Huszenicza *et al.*, 2005), later conception (+0.5 service and +60 d empty; Ahmadzadeh *et al.*, 2009), and higher risk of pregnancy loss (2.80 times more likely to undergo LEM; Santos *et al.*, 2004a). Lameness is also associated with delayed cyclicity (Garbarino *et al.*, 2004); as well as higher risk of ovarian cyst (follicular or luteal; Melendez *et al.*, 2003); lower oestrus intensity (Walker *et al.*, 2008b; a, 2010); and lower conception rates (Melendez *et al.*, 2003).

2.1.3 Effect of time as age, parity and lactation stage on reproduction

As explained with Figure 14 in section 1.5, priorities and thereby trade-offs are changing through time which includes age and therefore parity. Indeed, dairy cows are usually calving for the first time between 24 and 30 months. Modern dairy cows are considered to reach mature size during the third lactation meaning that they are still growing during their first two lactations (Le Cozler *et al.*, 2008). Adrien *et al.* (2012) proved that nutrient partitioning between lactation and reproduction was different in primiparous and multiparous cows. Primiparous dairy cows are known to be lighter and to have lower milk production than multiparous ones (Faverdin *et al.*, 2007; Le Cozler *et al.*, 2008). However, there are discrepancies in the literature. In many studies, the major problem of primiparous cows is that they resume ovarian activity or oestrus cyclicity later than multiparous ones (Darwash *et al.*, 1997; Meikle *et al.*, 2004; Horan *et al.*, 2005; Tanaka *et al.*,

2008; Cutullic *et al.*, 2012). In those studies, primiparous dairy cows mobilise more body reserve in early lactation. A possible confounding effect between parity and energy balance is possible. This is consistent with the fact that body condition at calving is higher for first lactation cows than for second lactation ones. It is also supported by the fact that in studies where primiparous and multiparous cows had similar body condition at calving and similar mobilisation , there was no difference in resumption of ovarian activity (Canfield *et al.*, 1990; Barton *et al.*, 1996; Friggens and Labouriau, 2010). Nonetheless, after growth, aging may also impair reproduction and it was reported that from the 3rd lactation, the risk of pregnancy loss was increased by 1.7 to 2 fold compared to 2nd and 1st lactation respectively (Lee and Kim, 2007). As mentioned in section 1.5, the priorities of the different life functions in dairy cows evolve with time. Thus, lactation stage is an important interfering factor (e.g. oestrus, see section1.2)

2.2 Farming systems characteristics

2.2.1 Reproduction management

There are, among farming systems, all intermediates from compact calving systems (mostly with 12 weeks of breeding season like in Ireland) to year-round calving systems. Compact calving systems are often chosen for working plan organisation or to match the nutritional requirements of dairy cows with the resources supply (e.g. pasture-based systems). This inevitably results in trade-off on a new limiting resource: time. Indeed, for a successful compact-calving system (on a yearly basis), 90 % of the cows in the herd should be calving within a 12 weeks period (between February and April in pasture-based systems; Butler, 2014). To do so, at least 70 % of the cows must have resumed ovarian activity when the breeding season starts (usually between April and June), more than 90 % should have been submitted to a first service within the first 3 weeks of the breeding season and 70 % should be pregnant within the firsts 6 weeks of the breeding season. In such systems, the ability of dairy cows to resume ovarian activity early, express oestrus, and to ensure pregnancy is crucial. Fortunately, if the herd size is substantial, this system can also be beneficial to reproduction: the first risk factor for oestrus expression and detection in dairy cows is the presence of another herdmate in oestrus (Cutullic *et al.*, 2009; Disenhaus *et al.*, 2010; Sveberg *et al.*, 2011).
On the other hand, extending lactation and delaying the breeding season may enable high yielding dairy cows to cope with the competition between lactation and reproduction. As time passes, milk yield decreases and dairy cows regain condition. It was recently reported that the 8th oestrus was expressed more intensively than the 1st one (Gaillard *et al.*, 2016). Moreover, in a 2 year calving interval system, high genetic merit (North American strain) Holstein cows had similar reproduction performance than moderate genetic merit ones (New-Zeeland strain; Kolver *et al.*, 2007). Although the use of extended lactation in pasture-based system is not impossible, their profitability may be questionable (Washburn and Mullen, 2014).

2.2.2 Nutritional strategies to pilot trade-off

Genetically the higher the milk yield the larger the body reserve mobilisation after calving. Because of the rapid increase in energy exported in milk and the slow increase and thus insufficient nutrient intake, dairy cows experience NEB in early lactation. The induced body reserve

mobilisation is often considered to be responsible for reproduction failure. Thus, moderating and shortening NEB and body reserve mobilisation should improve reproduction performance of dairy cows (Royal *et al.*, 2000b; Friggens *et al.*, 2010; Walsh *et al.*, 2011; Butler, 2014). Indeed, there is a positive relationship between CLA and intake (Burke and Roche, 2007), energy balance (Chagas *et al.*, 2008; Pollott and Coffey, 2008) or protein balance (Bruckental *et al.*, 2000; Law *et al.*, 2009). The occurrence of PLP increases with milk yield (Royal *et al.*, 2002a; Kafi *et al.*, 2012) or with too high BCS at calving and important loss (Friggens *et al.*, 2010). Low submission rate is related to low BCS (Buckley *et al.*, 2003) or severe BCS loss (Roche *et al.*, 2007). Days to conception and conception rate are positively associated with BCS at calving (López-Gatius *et al.*, 2003; Cardoso *et al.*, 2013). Pregnancy rates are positively associated with BCS and negatively associated with lactation persistency (Silke *et al.*, 2002; Buckley *et al.*, 2003; Grimard *et al.*, 2006; Santos *et al.*, 2009). All these effects associated with either milk yield or body reserve management are rather consequences of adaptive strategies to cope with nutrient scarcity rather than a direct effect of nutrient supply (and thus feeding systems). Although apparently obvious, improving reproduction through nutritional strategy is not straightforward.

Figure 19 illustrates the fact that when supplementing dairy cows with more concentrates (energy and protein resources), milk yield is improved, body reserve mobilisation is limited and reconstitution is faster and greater (Friggens *et al.*, 1998; Roche *et al.*, 2006; Delaby *et al.*, 2009). Paradoxically, the effect of feeding system on reproduction was either not significant (Friggens *et al.*, 1998; Delaby *et al.*, 2009) or not reported (Roche *et al.*, 2006). Indeed, even in other studies there was no effect of feeding system (defined by contrasted nutrient supplies) on CLA (Walsh *et al.*, 2008; Vance *et al.*, 2013), occurrence of PLP (Cutullic *et al.*, 2011), ovarian cycles length (Pollott and Coffey, 2008; Windig *et al.*, 2008; Gilmore *et al.*, 2011), submission rate (Kennedy *et al.*, 2003), conception rate (Horan *et al.*, 2004), pregnancy rate (Walsh *et al.*, 2008; Vance *et al.*, 2012). However, cows under restrictive nutrient supply express more intense oestrus (Cutullic *et al.*, 2009, 2011). But this effect was confounded and explained by differences in milk yield. In their study, Cutullic *et al.* (2012) proposed that the effect of body reserve management and milk yield were differently hierarchized for each step of the reproductive process: cyclicity (CLA and type of P_4 profile) would be mainly affected by body reserve management, oestrus detection by milk yield, NF/EEM by body reserve management and LEM by milk yield. As shown in Figure 18, the additional resource offered to the cows was invested in milk production. There is from 0.7 to 1 kg of additional milk produced per kg of supplemented dry matter (Butler, 2014). This variability depends on genetic characteristics (breed, strain…; Delaby *et al.*, 2009; Horan *et al.*, 2005) and is probably related to different priorities and adaptive strategies. This illustrates why breed or strain by nutrition interactions are expected on production and reproduction performance.

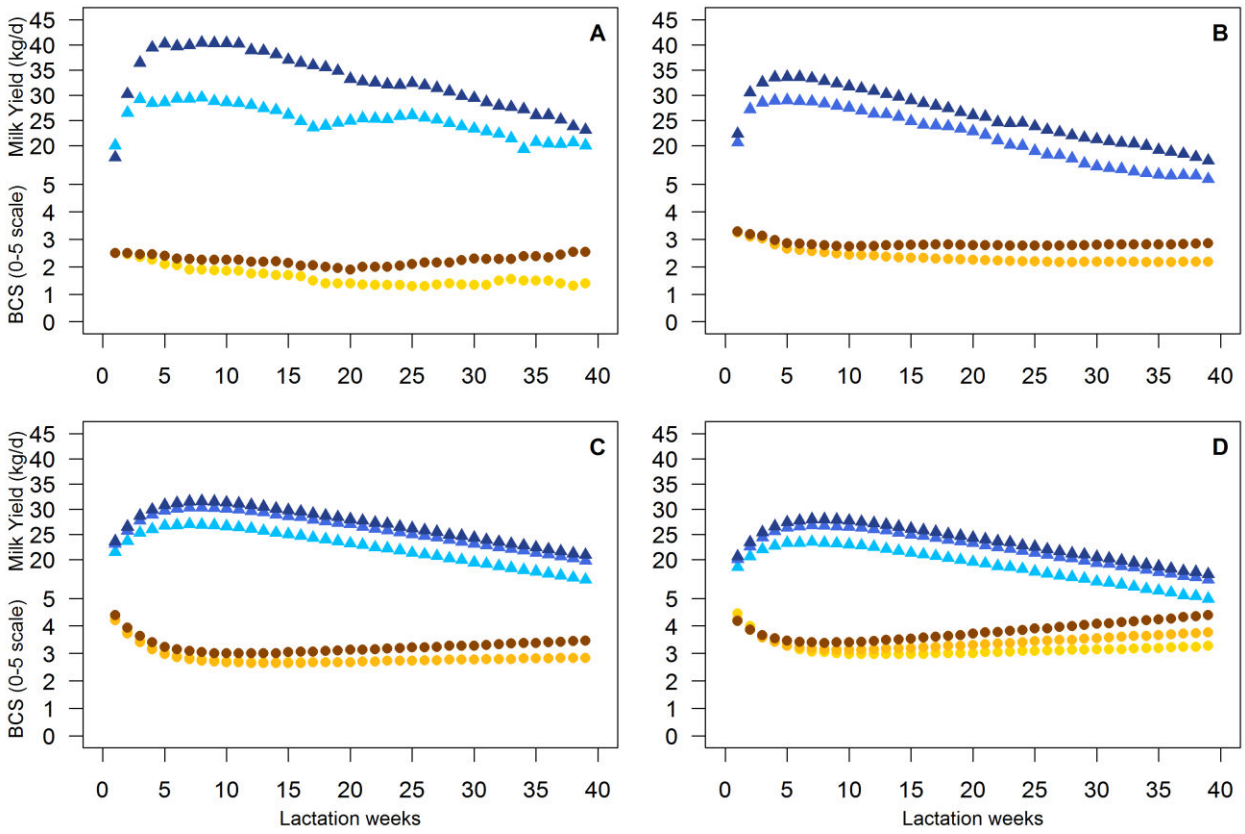


Figure 19: Milk yield (blue triangles) and body condition score (orange dots; 0-5 scale of Bazin *et al.*, 1984) throughout lactation of dairy cows fed with grass based diets with almost no supplementation (ranging from 0 to 1.5 kg/cow/d, light color), with moderate supplementation (ranging from 2.4 to 3 kg/cow/day, medium color) or with high concentrate level (6kg/cow/d, dark colors) throughout lactation (A: of British Holstein cows in Friggens *et al.*, 1998; B: of combined French Normande and Holstein cows in Delaby *et al.*, 2009; C: of North American Holstein and D: New-Zeeland Holstein cows in Roche *et al.*, 2006).

Indeed, high supplementation is helping to recouple the GH - IGF-I axis, so that it would be possible to find an effect of feeding systems on ovarian activity when the genetic characteristics are conversely enhancing the decoupling of the somatotropic axis (Lucy *et al.*, 2009). In addition, the lack of effect of feeding systems on reproduction could also be due to a "forgotten" homeostatic priority: in addition to ensure the survival of the current calf and investing in unborn offspring, dairy cows must ensure their own survival (Berry *et al.*, 2016).

There is still room for nutritional strategies and transition management to improve reproduction. During the last decade, scientific interest towards gluco-lipogenic diets or fatty acids/antioxidants supplementations has grown (Friggens *et al.*, 2010 and Butler, 2014). Rumen fermentation of glucogenic diets is resulting in the production of propionate while that of lipogenic diets in acetate and butyrate. As a consequence, plasma insulin and glucose concentrations are higher with glucogenic diets than with lipogenic ones. Thus, glucogenic diets result in little body reserve mobilisation (effect of insulin) while lipogenic diets do not limit the genetically programmed mobilisation. Effects on reproduction are expected, however there are no clear effects reported in the current literature. This may be due to a paradoxical positive (early CLA) and then deleterious effect (altered oocyte quality) of insulin on reproduction across time. Further studies are required

but promising results from the study of Garnsworthy *et al.* (2009) show that switching from a glucogenic to a lipogenic diet at resumption of luteal activity would enable to shorten the anoestrus period without impairing fertility.

Supplementing dairy cows in omega 3 has been reported to improve ovarian and uterine functions, fertility (specifically embryo survival), to enhance immune function, and to limit the synthesis of $PGF_{2\alpha}$ (Friggens *et al.*, 2010 and Butler, 2014). Supplementing with antioxidants would enhance the production of enzymes to balance the free radicals produced by the mammary gland. This has been reported to be associated with better reproduction (Friggens *et al.*, 2010 and Butler, 2014).

During the lasts weeks of gestation, dairy cows are usually dried-off. This period could be beneficial to reproduction since it is supressing the resources expenditure towards lactation. However, the length of this period is associated with the decrease in intake capacity, with increased subsequent peak milk yield, and with over-conditioned status. It was reported that the longer the dry period, the greater the NEB (Rastani *et al.*, 2005; Roche, 2006; Watters *et al.*, 2008), the more delayed P_4 profile (Opsomer *et al.*, 2000; Watters *et al.*, 2009), and the lower the pregnancy rate (in multiparous cows only, Watters *et al.*, 2009). *Prepartum* diet composition can also help to reach an ideal BCS at calving and even ideal mobilisation pattern *postpartum* by keeping under control NEB (Roche, 2006; Chagas *et al.*, 2007). Maintaining rumen activity and intake capacity could be reached by the use of diets enriched in fibres during the dry-period (Roche, 2006). However, studies failed to prove the beneficial effects of different composition dry-period diets on reproduction (McNamara *et al.*, 2003; Pushpakumara *et al.*, 2003). In their study, Burke and Roche (2007) reported that resumption of ovarian activity tended to be earlier with high intake *prepartum* but the number of animals was very limited. In their study, Adrien *et al.* (2012) tried to nutritionally manage BCS 1 month before calving, they found that cows that gained condition between 100 d and 30 d *prepartum* had improved endocrine stratus, earlier cyclicity and higher milk yield. However, 37% of the animals used did not respond to the dietary treatment as expected and were removed from the analyses.

2.2.3 Milking practice

Milking frequency is obviously a major impacting factor on milk yield. Cows milked once daily instead of the regular twice have lower milk yield (Rémond and Pomiès, 2005). The effect of milking dairy cows only once a day during the first weeks of lactation was more intensively studied, because it would not impair DMI and consequently limit the extent of NEB and body reserve mobilisation (Amos *et al.*, 1985; Barnes *et al.*, 1990; Rémond and Pomiès, 2005; Blevins *et al.*, 2006; Clark *et al.*, 2006; McNamara *et al.*, 2008; Patton *et al.*, 2006; Windig *et al.*, 2008; Kay *et al.*, 2013). Some studies reported that milking dairy cows once daily during the first four weeks is associated with earlier CLA (Patton *et al.*, 2006), higher proportion of normal P_4 profile (Disenhaus *et al.*, 2002), and shorter interval from calving to conception and higher pregnancy rate (Clark *et al.*, 2006). However, the effect on conception rate and pregnancy rate in the study of Clark *et al.* (2006) was not always significant according to the time from service. In their study, McNamara *et al.* (2008) found no effect of milking frequency on reproduction and cows milked twice daily had a significantly higher intake than those milked once-daily. And even with a quantitative range of milking frequencies using voluntary milking system, no effect on reproduction could be clearly

identified (Gaillard *et al.*, 2016). Milking frequency could be a way to limit milk yield and body reserve mobilisation in early lactation, that would change trade-offs. Once daily milking can be applied in early, mid, late or even overall lactation and substantially reduces milk yield (Pomiès *et al.*, 2004; Rémond and Pomiès, 2005). Further studies are required on the biology behind it to elucidate the apparent discrepancies in the literature and to study the economical durability of such management tools. Clark *et al.* (2006) concluded that it could be an economical valuable choice for New-Zeeland farmers to opt for once daily milking, given the milk solids yield loss exchange for increased time for non-milking tasks.

2.3 Genetic by environment interactions: adaptive strategies and reproduction

Depending on their genetic characteristics, dairy cows adopt different adaptive strategies to cope with the constraints of their environment. For instance, under restrictive diets dual purpose cows such as Normande cows reduce milk yield to a higher extent than Holstein cows in the same environment. However, they have limited body reserve mobilisation whereas Holstein cows are mobilising to reduce milk yield loss (Dillon *et al.*, 2003a; Walsh *et al.*, 2008; Delaby *et al.*, 2009; Cutullic *et al.*, 2011). This is related to differences in intake capacity, nutrient partitioning, uncoupling of the IGF-I - GH axis, and insulin resistance (Yan *et al.*, 2006; Chagas *et al.*, 2009; Lucy *et al.*, 2009). It is often reported that no genetic by feeding system interaction is substantial on reproduction traits (Pryce *et al.*, 1999; Horan *et al.*, 2004, 2005b; Walsh *et al.*, 2008; Coleman *et al.*, 2009, 2010; Vance *et al.*, 2012). Studying reproduction already requires large number of animal, thus it is not surprising that very few study reported significant genetic by environment interaction on reproduction performance. In their experiment, Cutullic *et al.* (2011) observed that there was no substantial effect of feeding system on fertility of Normande cows. However, depending on the feeding system, Holstein cows experienced different pregnancy failures. Indeed, in a high feeding system Holstein cows had more LEM that was associated with a greater peak milk yield and lactation persistency; in a low feeding system, Holstein cows had more NF/EEM and had a very low condition score at nadir. All this suggests that some genetic characteristics are best suited for a given system and that the cow for system A may not be the one for system B. For instance, the Normande cows seems to be the cow for the low input system with compact calving management since they rapidly resume ovarian activity and they safeguard their body reserve under a restrictive system, which is beneficial to reproduction. On the other hand, Holstein cows seems to be more suited for high input systems with year-round calving management since they invest most of the energy intake in milk production, they benefit from having more time than a year to ensure reproduction. It is often possible to adapt farming system to give the cows more time if resumption of normal ovarian cyclicity is the main issue. However, the results of Cutullic *et al.* (2011) suggest that there is more: genetic by feeding system interactions are involved in fertility, which is a problem that time cannot solve. Further studies on genetic by feeding systems interactions on reproduction traits are required, as well as other elements of farming systems such as dry period length or milking frequency that are poorly documented.

In this second section, the major impacting factors of reproduction, response laws between lactation and reproduction and recommendations were presented. The elements to remember are:

- There are strong and negative genetic correlation between milk yield and many reproductive traits. Despite globally low heritability, there are a lot of genomic regions associated with reproduction all along their genome.
- The environment plays a major role in the observed variance of reproduction, still using this information as leverage for building strategies to cope with the reproduction decline is not straightforward (no clear-cut answer for using feeding strategies, milking frequencies…).
- The effects of body reserve management and milk yield are differently hierarchized according to the reproductive steps:
  - Cyclicity is impaired if cows are too thin or too fat at calving and greatly mobilising;
  - Oestrus intensity is unfavourably associated with milk yield during the ovulation week;
  - There is a greater risk of NF/EEM when BCS at calving is low; andof LEM when peak milk yield is great and persistency poor .

Further studies are required to fill the gaps of knowledge concerning the genetics, genomics, epigenetics, and transcriptomics of reproduction and in various reference populations. And there is another gap of knowledge on the genericity of the biological response laws between reproduction, milk production and body reserve management and thus on the targets to reach for successful production and reproduction.

Dans cette seconde partie, les principaux facteurs de variation, les lois de réponses entre lactation et reproduction et les recommandations ont été présentées. Les éléments à retenir sont :

- *Les corrélations génétiques entre la production laitière et les caractères de reproduction sont fortes et défavorables. Malgré de faibles héritabilités, beaucoup de zones du génome sont liées à la reproduction.*
- *L'environnement joue un rôle majeur dans la variabilité observée des performances de reproduction. Néanmoins il n'est pas évident d'utiliser cette information dans la mise en place de stratégies visant à améliorer la reproduction (alimentation, fréquence de traites…).*
- *Les effets de la gestion des réserves corporelles et du niveau de production laitière sont hiérarchisés différemment à chaque étape de la reproduction :*
  - *La cyclicité est dégradée si les vaches vêlent trop maigres ou trop grasses et mobilisent ;*
  - *L'expression des chaleurs est affectée par le niveau de production laitière ;*
  - *Le risque de non-fécondation/mortalité embryonnaire précoce est accru si l'état minimum est bas ; celui de mortalité embryonnaire tardive si le pic de lactation est élevé et la persistance mauvaise.*

D'autres études sont nécessaires pour approfondir les connaissances et pallier aux manques concernant la génétique, la génomique, l'épigénétique et la transcriptomique de la reproduction et ce dans plusieurs populations de référence. La généricité des lois de réponses et recommandations associées doit aussi être éprouvée pour identifier les stratégies vers la réussite de la lactation et de la reproduction.

3 Quantitative approach of the competition between lactation and reproduction to identify leverages

In addition to the genetic improvements of fertility to be made, the farming tools presented in the preceding subsection (nutrition, dry period, milking frequency, calving system…) aim to drive trade-offs towards the success of both lactation and reproduction. To do so, some targets are recommended in the literature based on the current knowledge on the biological response of each reproductive step to milk yield, body condition and mobilisation.

Cutullic *et al.* (2012) proposed a different hierarchy between milk yield and body reserve management at each step of the reproductive process. Cyclicity (CLA, proportion of normal P_4 profile and occurrence of PLP) is rather influenced by body reserve management than milk yield. Indeed, cows that are too thin or too fat at calving have delayed CLA. It was suggested that the relationship between CLA and BCS at calving was quadratic, with an optimal ranging from 3.0 to 3.5 points (1-5 scale of Wildman *et al.*, 1982) for an early CLA (Roche *et al.*, 2009). However, it is also known that too early CLA is a risk factor for PLP (Petersson *et al.*, 2006a). Over-conditioned cows at calving that mobilise a lot of body reserve in early lactation are at risk of PLP (Cutullic *et al.*, 2012). Figure 20 summarises this information and represents how the relationship between cyclicity and BCS at calving is believed to be in dairy cows. Ovulation detection rate, and thus oestrus duration and intensity, are mainly affected by milk yield at ovulation (Cutullic *et al.*, 2012). Figure 21 shows that the higher the milk yield during the ovulation week, the lower the chance to detect oestrus on both mounting behaviour and all sexual behaviour together (Cutullic, 2010). Fertility failures are associated with both milk yield and body reserve management. Indeed, the lower the BCS at nadir, the higher the risk of NF/EEM. On the other hand, the higher the peak milk

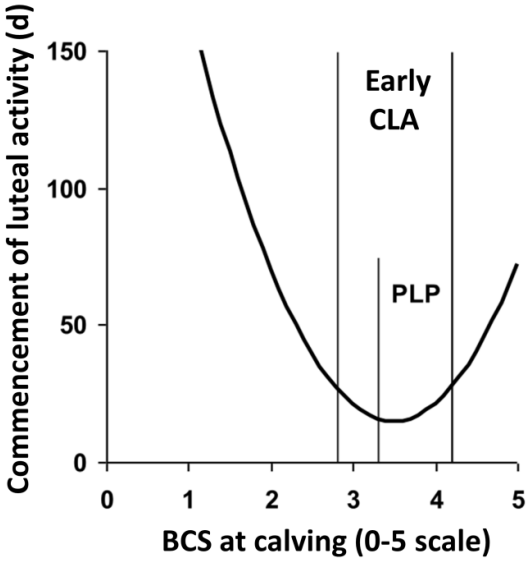


Figure 20: Schematic representation of the quadratic relationship between CLA and the occurrence of PLP with BCS at calving in dairy cows (reprinted from Cutullic, 2010)

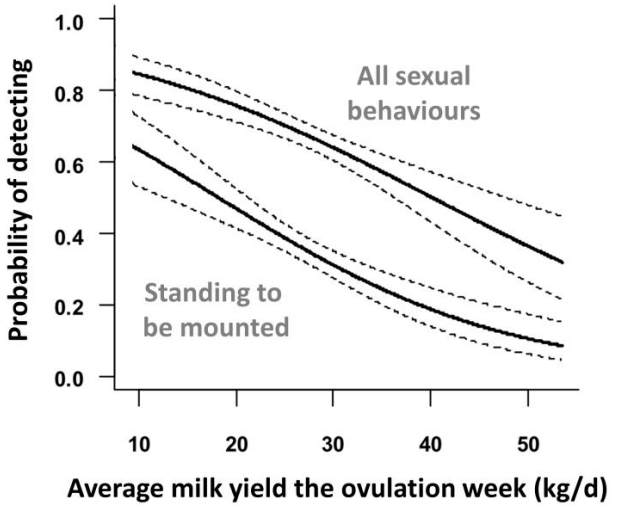


Figure 21: Response of the probability of detecting ovulation according to the type of oestrus expression (all sexual behaviour and standing to be mounted behaviour) to milk yield the ovulation week (data on 587, 110 and 87 ovulation from 3 INRA experimental farms, respectively: Le Pin-au-Haras, Méjusseaume, Nouzilly; Coyral-Castel, personal communication; reprinted from Cutullic, 2010)

yield followed by a poor lactation persistency, the higher the risk of LEM (Buckley *et al.*, 2003; Cutullic *et al.*, 2012). All these biological response laws from experimental data could be confirmed and further refined through mechanistic modelling (Brun-Lafleur *et al.*, 2013). Both the response laws and results of the model are consistent with excellent qualitative reviews on the topic (Royal *et al.*, 2000b; Santos *et al.*, 2004b; Diskin and Morris, 2008; Friggens *et al.*, 2010; Walsh *et al.*, 2011).

From knowledge on response laws, recommendation can be formulated to lead dairy cows towards successful reproduction. In their review, Chagas *et al.* (2007) proposed the ideal body reserve profile throughout lactation (Figure 22) based on the data reported in the studies of Waltner *et al.* (1993), Buckley *et al.* (2003), and Roche *et al.* (2007). However, further studies are still required to confirm such concepts based on very few data. In addition, the fact that the steps of the reproductive process are firstly impaired either by milk yield or body reserve management suggest a possible uncoupling and compensatory phenomena. This means that managing body reserve only may not fully improve reproduction.

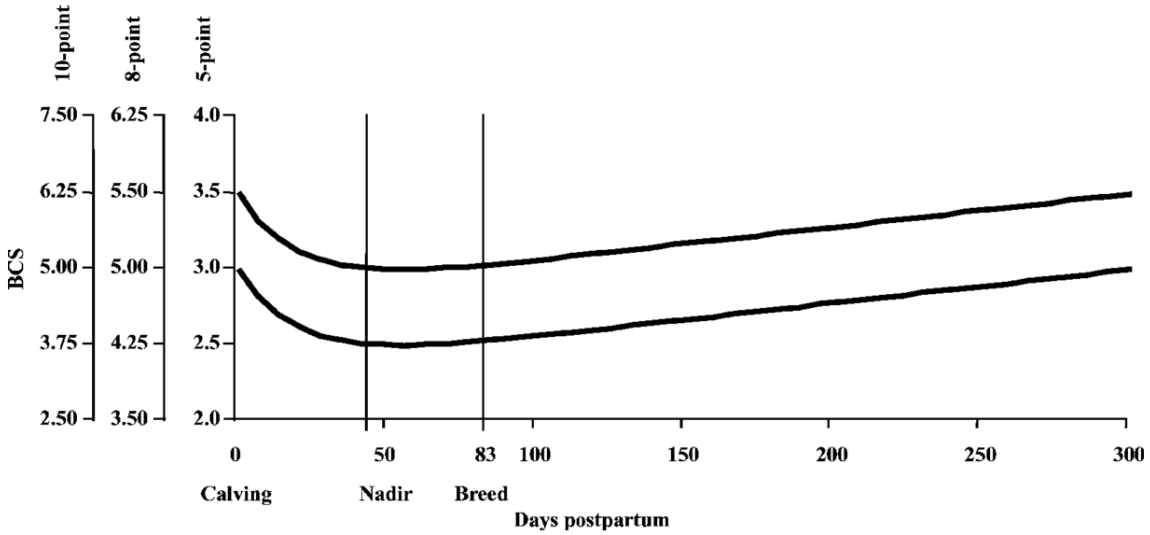


Figure 22: Proposed "ideal body condition score profile" for dairy cows to minimize the effect of energy balance on reproductive failure. Body condition score is presented for the 5-, 8-, and 10-point scales (reprinted from Chagas *et al.*, 2007).

Limiting milk yield, particularly in early lactation would result in higher ovulation detection rates, lower risk of LEM, and in limited body reserve mobilisation (and thus improved reproduction performance mainly affected by BCS). However, milk is the main income for dairy farmers so lowering milk yield to improve reproduction should be economically worthy. Pregnancy loss represent a substantial cost because of the consequent lengthening of calving interval and increased involuntary culling rate (Lee and Kim, 2007). However, the profitability improvement is not linear and more substantial if the reproductive performance is initially poor (Meadows *et al.*, 2005). Still profitability of limiting milk production and improving reproduction highly relies on feed cost, farming systems, local breeding goals and milk price. This is the reason why there is no clear-cut answer to such strategies yet, even though this option should be considered.

COMPETITION LACTATION-REPRODUCTION

Meta-analysis of the competition between reproduction and lactation performance in dairy cows.

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Interpretive summary
This meta-analysis explored associations between milk production, body condition score and reproductive outcomes. We hypothesized that there would be competition between lactation and reproduction performance for resources. The results suggest that the effects of milk yield and body reserve are differ in importance according to cyclicity, estrus expression and fertility. The biological responses identified can be used for mechanistic modelling and recommendations in the field to successfully manage milk production and reproduction of dairy cows.

ABSTRACT

Lactation and reproduction are concomitant functions in dairy cows and in competition for resources. The present study aimed to quantitatively review the existing literature to clarify the implication of milk production and body reserve at each step of the reproduction process. Inclusion criteria for the studies were: comparison of at least 2 treatments and reporting of both reproduction and production performance. The final database consisted of 275 treatment groups from 75 articles. Data investigation showed that the only investigable relationships were between commencement of luteal activity (C-LA), days from calving to first observed estrus (COE1), conception rate at first service (CRAI1), overall pregnancy rate (PR), milk yield and body condition scores (BCS; converted to the 0-5 points scale). The results showed that C-LA was not related to milk yield and that the relationship between C-LA and BCS at calving was quadratic. Although COE1 is an indicator of C-LA, no relationship was identified between any of the BCS parameters and COE1. However, for each additional kg of milk yield produced at both peak and over the initial 14 weeks of lactation, COE1 was delayed by 1.1 days. In this meta-analysis, CRAI1 was affected by both milk yield and BCS. In addition, CRAI1 was reduced by 2.0 % (of inseminations) and 2.2 % for each additional kg of milk yield at peak and at service, respectively. CRAI1 was increased by 38.2 % and 22.0 % for each additional unit of BCS at service and at nadir, respectively. Finally, no relationship between milk yield and PR was identified. PR was increased by 42.8 % (of cows) and 16.8 % for each additional unit of BCS at calving and at nadir respectively. Postpartum cyclicity of dairy cows is mainly affected by BCS at calving, whereas estrus expression is mainly affected by milk yield and fertility is affected by both BCS and milk yield. Strategies adjusting feeding level, milking frequency and dry period length to target a BCS of 3.10 and limiting BCS loss and peak milk yield could be an effective way to improve reproduction. Even when target BCS is achieved, a high milk yield strategy will require strong attention on estrus expression to detect ovulations and ensure that high PR is achieved. On the other hand, mitigating the strong genetic selection on milk yield and selecting dairy cows for functional traits such as fertility and higher BCS would enable genetic improvement of reproduction performance.

KEY WORDS: reproduction, milk production, body reserve management, BCS, meta-analysis

INTRODUCTION

Dairy cows are challenged concerning resources partitioning (nutrients and hormones): if most of them are invested in milk production, there may not be enough left to ensure other functions. Indeed, reproduction performance has been declining over the past decades while milk production has been improving (Lucy, 2001; Friggens et al., 2010).

Reproduction of dairy cow is a succession of interconnected steps: establishment and maintenance of ovarian cyclicity, expression of sexual behaviors (estrus), and establishment and maintenance of pregnancy. However, abnormal ovarian activity is common in the current population: only 60% of Holstein cows have regular cycles of 20 to 25 days. Delayed commencement of luteal activity and prolonged luteal phases (PLP) are the most commonly abnormalities reported (Petersson et al., 2006; Windig et al., 2008). Thirteen to 30% of the variability in the commencement of luteal activity (C-LA) is due to genetic characteristics and C-LA is unfavorably genetically correlated to milk production (Royal et al., 2002; Petersson et al., 2007). However, the deleterious effect of high genetic merit for milk production on cyclicity is not always clear (Royal et al., 2002; Windig et al., 2008). Low body reserve at calving or large mobilization at the beginning of lactation are known to be risk factors for delayed C-LA. On the other hand, cows that are too fat at calving experience more abnormal cyclicity patterns (Cutullic et al., 2012). The hierarchy of the effect of body reserve and milk production on C-LA needs to be studied in order to identify opportunities to improve ovarian cyclicity of dairy cows.

Once cyclicity is established, the next step is estrus expression (and therefore ovulation detection) to enable insemination on time. However, there is large variability in both duration and intensity of estrus in dairy cows (Kerbrat and Disenhaus, 2004; Sveberg et al., 2015). Very few studies exist on estrus expression due to the difficulty to accurately measure this trait. Estrus intensity and duration are known to be unfavorably associated with milk production, low body reserve in early lactation or large mobilization (Madureira et al., 2015). Studies on estrus expression between breeds are not in concordance. Some studies show that Holstein cows express less specific estrus behavior than dual purpose cows (Cutullic et al., 2009; Sveberg et al., 2015), whereas in other studies, the opposite was observed (Løvendahl and Chagunda, 2010). There is a lack of information concerning the hierarchy of impacting factors (milk production and body reserve) on estrus expression and apparently conflicting results in the literature.

The last step of the reproduction process is to conceive and maintain a pregnancy. Fertility is known to be negatively associated with milk production. This is mostly due to high selection intensity on production traits and a negative genetic correlation between milk production and fertility (Pryce et al., 2004; Grimard et al., 2006). Body reserve also play a key role in fertility and are positively associated with re-calving rates (López-Gatius et al., 2003; Santos et al., 2004; Grimard et al., 2006). Further knowledge concerning the different importance of milk production and body reserve on fertility performance is required to identify opportunities for improvements.

Conducting a meta-analysis is an appropriate approach to understand discrepancies in the literature, improve knowledge and establish recommendations to cope with declining reproduction performance of dairy cows. The present study aimed to quantitatively review the existing literature to clarify the competition between lactation and reproduction functions in dairy cows. Our

hypothesis is that (1) parameters of lactation (production and management of body reserve) differ in importance for each reproduction step; and (2) these effects can be genetically or nutritionally managed.

MATERIALS AND METHODS

Database creation

Literature search A database was created with studies published between 1985 and 2015 dealing with production and reproduction performance. The literature search was done using electronic databases ISI Web of Knowledge (https://apps.webofknowledge.com) and Google Scholar (https://scholar.google.com). The literature cited in articles and reviews were also checked. The languages accepted were English, French, German, or Spanish. Inclusion criteria into the database included: the reporting of both production and reproduction performances for at least 2 treatments. Each observation corresponds to the mean or the overall proportion of cows in a given condition of a treatment group. The database included 102 articles, adding to 300 observations, with 97 different reproduction variables reported, 248 different production variables (milk yield, fat and protein content), and 169 different body measurements (body condition score, body weight). The database was refined by keeping only observations from treatments based on diet and genetic characteristics (breed or genetic line) because too few studies dealt with other experimental factors such as milking frequency and parity. Consequently, the final database included 275 observations from 75 articles.

Calculations When not reported in the studies, variables were calculated in order to compare the results. Peak milk yield, fat and protein contents at nadir, and body weight variations were computed. In addition, averages of different production traits (milk yield, fat and protein contents, and body weight) were estimated over the "n" first weeks of lactation (n ranged from 1 to 44; for example if n is the 14th week the mean for milk production was calculated from week 1 to week 14 of lactation).

Body condition was scored on different scales in different studies. The scores were translated in the 0-5 scale with 0.25 increments of Bazin et al. (1984) using the equations adapted from Roche et al. (2004) and Banos and Coffey (2010), assuming a linear conversion between the scales of Bazin et al. (1984) and Lowman et al (1976) or Edmonson et al. (1989):

$$BCS_{0\text{-}5\ (Bazin\ et\ al.,\ 1984)} = BCS_{1\text{-}5\ (Lowman\ et\ al.,\ 1976;\ Edmonson\ et\ al.,\ 1989)} \times 6/5 - 1$$
$$= \{[BCS_{1\text{-}5\ (Wildman\ et\ al.,\ 1982)} - 1.5] \times 1.25 + 0.81\} \times 6/5 - 1$$
$$= [BCS_{1\text{-}8\ (Earle,\ 1976)} \times 0.74 - 1.39] \times 6/5 - 1$$
$$= \{[BCS_{1\text{-}9\ (Aalseth\ et\ al.,\ 1983)} - 1] \times 0.50 + 1\} \times 6/5 - 1$$
$$= [BCS_{1\text{-}10\ (Macdonald\ and\ Roche,\ 2004)} \times 0.40 + 0.81] \times 6/5 - 1$$

BCS loss was estimated by subtracting the BCS at nadir from BCS at calving.

In regard to reproductive measures, only C-LA needed adjustment for analysis. The time period observed between ovulation and progesterone concentration rise range is about 5 days (Inskeep, 2004; Forde et al., 2011). Consequently, 5 days were added to calving to first ovulation intervals in

order to estimate C-LA. In addition, log-transformed least square means of C-LA were reported in 2 studies; therefore an exponential transformation was used to estimate C-LA.

Table 1. Description of the variables used in the meta-analyses1

	Numbers[2]				Description[3]		
	n_s	$n_{o\text{-}all}$	$n_{o\text{-}diet}$	$n_{o\text{-}gen}$	Minimum	Mean ± SD	Maximum
Reproduction							
C-LA (d)[4]	42	162	134	· · ·	16.2	34.5 ± 9.56	90.0
COE1 (d)[5]	20	92	· · ·	71	29.0	49.1 ± 11.53	74.0
CRAI1 (%)[6]	34	138	· · ·	74	11.6	46.0 ± 12.97	65.8
PR (%)[7]	22	98	· · ·	59	70.0	83.6 ± 7.21	98.0
Lactation							
MY_{14wk} (kg/d)[8]	22	97	· · ·	48	17.5	26.6 ± 4.47	40.2
MY_{peak} (kg/d)[8]	28	117	· · ·	59	21.3	31.1 ± 5.62	44.2
MY_{AI} (kg/d)[8]	5	28	· · ·	22	21.3	29.4 ± 4.53	39.6
$BCS_{calving}$[9]	37	150	114	· · ·	1.66	2.69 ± 0.405	3.52
BCS_{nadir}[9]	20	76	· · ·	37	1.45	2.13 ± 0.358	2.97
BCS loss[9]	20	75	· · ·	37	-1.45	-0.81 ± 0.284	-0.31
BCS_{AI}[9]	3	21	· · ·	18	2.31	2.52 ± 0.156	2.84

[1]Publication used: Adrien et al. (2012), Barnes et al. (1990), Barton et al. (1996), Beam and Butler (1997, 1998), Beerda et al. (2007), Boken et al. (2005), Bruckental et al. (2000), Burke et al. (2005), Burke and Roche (2007), Canfield et al. (1990), Cavestany et al. (2009), Chagas et al. (2006, 2008, 2009), Clark et al. (2006), Coulon et al. (1987), Cutullic et al. (2009, 2011), Delaby et al. (2009, 2010), Dillon et al. (2003a, 2003b), Disenhaus et al. (2002), Ferris (2003), Ferris et al. (2014), Fulkerson et al. (2001, 2008), Garnsworthy et al. (2009), Gilmore et al. (2011), Gruber et al. (1995), Gümen et al. (2005), Harrison et al. (1990), Heins et al. (2008), Horan et al. (2004, 2005), Keady et al. (2005), Kennedy et al. (2002, 2003), Kolver et al. (2000, 2002, 2005), Law et al. (2009), Macdonald et al. (2008), McGowan et al. (1996), Meier et al. (2006), Patton et al. (2006, 2007), Pedernera et al. (2008), Petersson et al. (2006a, 2006b), Piccand et al. (2011, 2013), Pleasants et al. (2005), Pollott and Coffey (2008), Pushpakumara et al. (2003), Rastani et al. (2005), Roche (2007), Rukkwamsuk et al. (1999), Sklan et al. (1991), Spicer et al. (1993), Vance et al. (2012, 2013), Verkerk et al. (2000), Walsh et al. (2007, 2008), Washburn et al. (2002), Watters et al. (2008, 2009), Westwood et al. (2000, 2002), White et al. (2002), Windig et al. (2008).

[2] n_s = number of studies selected in the model, $n_{o\text{-}all}$ = number of all the observations collected, $n_{o\text{-}diet}$ = number of the observations in the diet data subset, $n_{o\text{-}gen}$ = number of the observations collected in the genetic data subset

[3]Descriptive statistics of the variables in the selected studies (with diet or genetic characteristics as experimental factor)

[4]Commencement of luteal activity

[5]Calving to first observed estrus interval

[6]Conception rate at first service, in % of inseminations

[7]Overall pregnancy rate, in % of cows

[8]Milk yield over the 14 firsts weeks of lactation, at peak, and at service

[9]Body condition score at calving, nadir, service, and loss from calving to nadir rescaled to the 6 points scale (0-5; 0.25 increments; Bazin et al., 1984)

Data coding In order to observe contrasting responses of production and reproduction performance, the selected studies used dietary treatment or studied distinct genetic groups or both. The responses to a dietary treatment or caused by different genetic characteristics cannot be compared. In order to select relevant observations for the meta-analyses, they were coded

according to a combination of the unique article code and the unique experimental group within article code (as recommended by Sauvant et al., 2008). Therefore, there were 2 different subsets of data according to either diet or genetic characteristics (see the illustration of the interest to distinguish the treatment with coding in the Figure A1 A-B of the Appendix).

Meta-analyses

Data investigation Plots of the relationships among all variables collected, with observations from the same experiment linked, enabled the determination of (i) correlations among potential explanatory variables, (ii) relationships between reproduction and lactation parameters. From these graphical representations of the relationships, it was decided to focus only on the relationships between reproduction parameters (C-LA, calving to first observed estrus interval: COE1, conception rate at first service: CRAI1, and overall pregnancy rate: PR) and either milk yield (average milk yield over the first 14 lactation weeks: MY_{14wk}, peak milk yield: MY_{peak}, and milk yield at service: MY_{AI}) or BCS (BCS at calving: $BCS_{calving}$, BCS at nadir: BCS_{nadir}, BCS loss from calving to nadir: BCS loss, and BCS at service: BCS_{AI}). Descriptive statistics for these variables in the selected database are presented in Table 1.

Minimum variation of Independent Variables Between Observations Within Treatments

Determination of reliable responses of dependent variables (Y) to independent ones (X) relies on a minimum of variation of the independent variables within treatments. Therefore a threshold of minimum acceptable variation of X (ΔX_{min}) was calculated for each independent variable using the following calculations (Loncke et al., 2009):

$$\Delta X_{min} = \mu(\Delta X_{ij}) - 2 \times SD(\Delta X_{ij}) \text{ where: } \Delta X_{ij} = | X_i - X_j |$$

X_i and X_j are the values of X for the *i*th and *j*th treatment within code. The number of observations removed according to this criterion was small, leading to the elimination of 0 to 2 treatments collected (0-10%) depending on the models. Indeed, the ΔX_{min} was 2.04 kg for MY_{14wk}, 1.13 kg and 1.28 kg for MY_{peak} in studies also reporting COE1 and CRAI1 respectively, 2.93 kg for MY_{AI}, 0.10 units for $BCS_{calving}$, 0.05 units for BCS_{nadir}, 0.10 points for BCS loss, and 0.14 unitd for BCS_{AI}. All these minimum variations of milk yield or body condition between treatments within experiment are biologically acceptable.

Statistical Analyses In the present studies, conception and pregnancy rates are quantitative variables, distributed among the studies. Relationships between reproduction dependent variables (C-LA, COE1, CRAI1, and PR) and independent variables were studied using the following initial linear mixed model:

$$Y_{ij} = \alpha + \alpha_i + \beta_1 X_{ij} (+ \beta_2 X^2_{ij}) + \beta_i X_{ij} + e_{ij}$$

where Y_{ij} was the dependent variable of the *j*th treatment in the *i*th code, α was the overall mean of the dependent variable (inter-study intercept), α_i was the random effect of the *i*th code, β_1 was the fixed overall regression coefficient of Y on X, β_2 was the fixed overall quadratic coefficient of Y on X^2 (fitted only if appropriate), β_i was the random effect of the *i*th code on the regression coefficient of Y on X, and e_{ij} the residual error. The random effects were assumed to be distributed as $N(0,\sigma^2_\alpha)$,

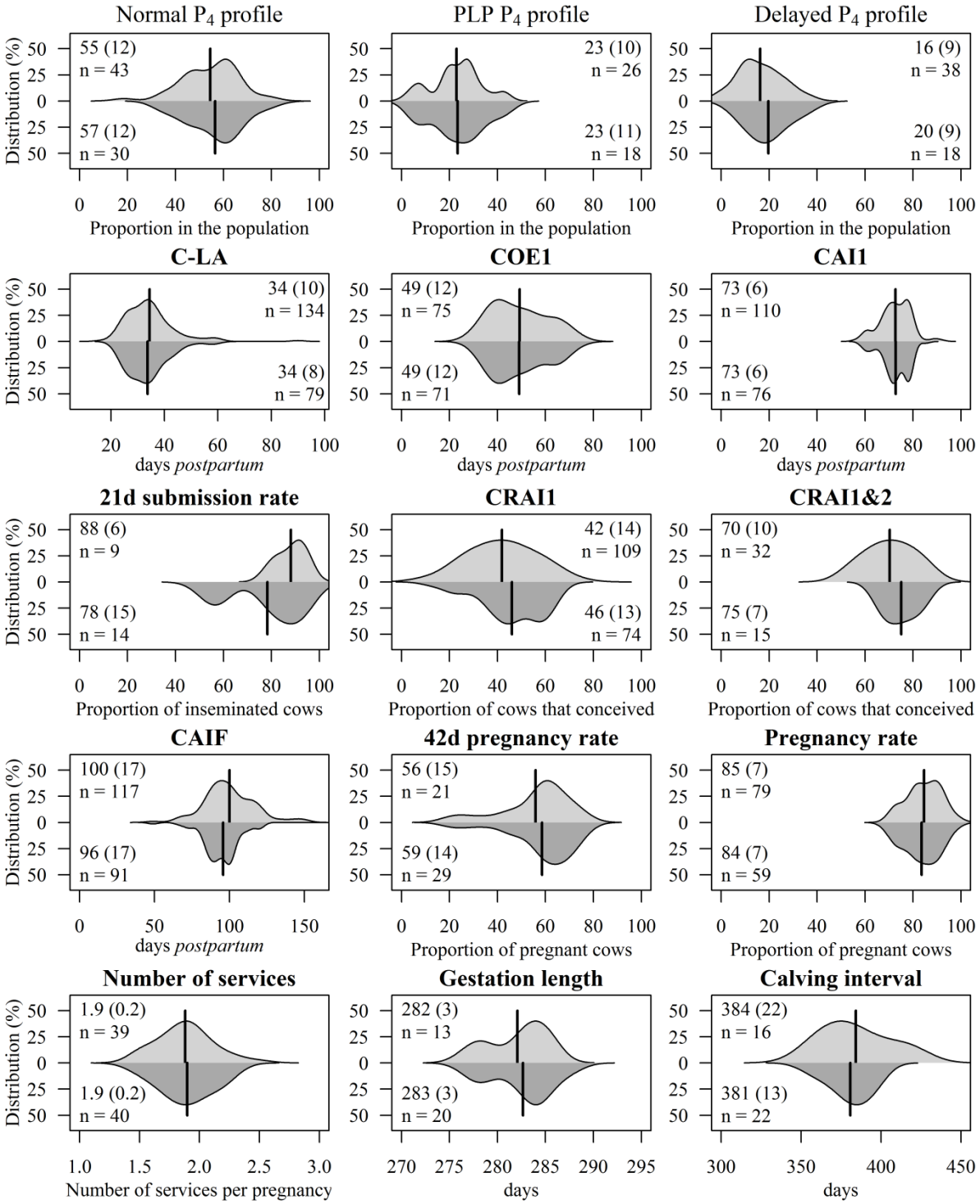

Figure 1: Mean, standard deviation within parenthesis, and number of treatments used to describe the distribution of reproductive traits: proportion of normal P4 profiles, prolonged luteal phases profiles (PLP), and delayed type of P4 profiles, commencement of luteal activity (C-LA), calving to first observed estrus interval (COE1), calving to first service interval (CAI1), 21-d submission rate, conception rate at first service (CRAI1) and at combined first and second services (CRAI1&2), calving to conception interval (CAIF), 42-d pregnancy rate, final pregnancy rate, number of services per pregnancy, gestation length and calving interval. The upper distribution is the one observed in the genetic data subset and the lower distribution in the diet data subset.

$N(0,\sigma^2_\beta)$ and $N(0,\sigma^2_e)$ for treatment intercept, treatment coefficient and error respectively. Also, observations were weighed by the number of animals in the treatment. Goodness of fit of the models was assessed by examining the Studentized residuals of the model and checking if $e \sim N(0,\sigma^2_e)$. Outliers were identified using different indicators: distribution of residuals, Cook's distance, and externally Studentized residuals (as recommended by Sauvant et al., 2008). With these techniques, 1 observation was removed from the analyses, in both the association of C-LA with $BCS_{calving}$ and of COE1 with MY_{peak}, and 6 observations were removed in the association of PR with $BCS_{calving}$. In some cases, the estimation of variance components failed, probably because of a too limited number of records (Sauvant et al., 2008). In such cases, the model was fitted with the random effect of the *i*th code (α_i) only (i.e. β_i , the random effect of the *i*th code on the regression coefficient of Y on X was not included in the model). All linear mixed model analyses were performed using the *lmer* procedure of the R statistical package (R Core Team, 2016). The procedures *plotresid*, *influence*, *cooks.distance*, *dfbetas* and *romr.fnc* were used to assess goodness of fit of the models and to identify outliers.

Some factors may interfere in the relationships between Y and X. Among the studies used in this meta-analysis, the checked potential interfering factors were type of genetics of dairy cows (Gen = American Holstein/British Holstein/Continental Holstein/Southern Holstein/Holstein crossbreed/Other dairy breed/Dual purpose breed); type of concentrates supplementation (Conc = high/medium/low/high-low succession/low-high succession); type of main forage in the diet (Forage = grass/maize/maize and grazed grass); the proportion of primiparous cows in the group (Parity = 0-25/25-50/50-75/75-100); use of inseminations synchronization protocol (Sync = yes/no); and type of calving system (Sys = compact/year round). These factors may influence between-code differences and affect the response laws among them. We followed the approach of Loncke et al. (2009): an ANOVA was run on both residuals and LSM of the models to test the influence of the interfering factors. When significant ($P<0.05$), it was checked if their inclusion in the models improved the fit based on the comparison of the AIC, RMSE and adj-R^2 of the models with and without the interfering factors. The relationship between the dependent and explanatory variables can be determined either in the diet data subset (Figure A1 A of the Appendix) or the genetic data subset (Figure A1 B of the Appendix) with the example of CRAI1 and MY_{peak}. The statistical terminology and definitions are represented as well as the modalities of the factor Gen to show why it can be a major interfering factor in Figure A1 C of the Appendix.

RESULTS

Description of Reproductive Performance

The reported C-LA in the body of literature is 34 d on average (Figure 1). The proportion of cows in the herds with normal cyclicity pattern ranged between 55 and 57 % depending on the subsets (genetics or diets). Concerning abnormal [DEFINE] cyclicity patterns, there is about 23 % of prolonged luteal phase (PLP) in both subsets; and between 16 % and 20 % of delayed progesterone (P_4) profiles. On average, COE1 is about 49 d but first service is usually performed an ovarian cycle later with CAI1 of about 73 d. Submission rate 21 d after the start of the breeding ranged from 78 to 88 % of the cows in the herd. Conception rate to first service ranged from 42 to 46 % of the

Table 2. Response laws of commencement of luteal activity (C-LA), calving to first observed estrus (COE1), conception rate at first service (CRAI1) and overall pregnancy rate (PR) to milk yield, body condition score, and body condition loss

		Numbers[3]				Intercept		Slope		Quadratic		Model[4]		Interfering factors[5]	
Y[1]	X[2]	n_s	n_t	n_o	n_r	β_0	SE	β_1	SE	β_2	SE	RMSE	adj-R^2	residuals	LSM
C-LA	$BCS_{calving}$	5	8	18	3	188.4***	41.04	-106.1***	29.17	17.0***	5.75	10.42	1.00	Conc*	Sys*, Gen**, Parity***
COE1	MY_{peak}	5	12	26	1	6.7^{ns}	7.98	1.1***	0.32	···	···	9.80	0.95	None	Sys*, Gen*, Parity*, Conc*
	MY_{14wk}[6]	3	9	14	0	10.1^{ns}	10.79	1.1**	0.35	···	···	10.23	0.79	Gen***	None
CRAI1	MY_{peak}[6]	5	15	30	0	112.2***	10.36	-2.0***	0.29	···	···	13.85	0.89	Gen**	Sync*, Gen**
	MY_{AI}[6]	3	7	14	0	119.6***	8.59	-2.2***	0.26	···	···	8.30	0.97	None	Parity†, Forage**, Conc**
	BCS_{AI}[6]	3	7	14	0	-44.7***	10.13	38.2***	3.97	···	···	4.03	0.94	None	Forage†, Conc**
	BCS_{nadir}[6]	5	13	26	0	-2.4^{ns}	5.59	22.0***	2.25	···	···	8.95	0.91	None	Sync***, Gen**, Parity*, Forage*, Conc*
	BCS loss[6]	5	13	24	0	75.0***	4.10	33.9***	4.32	···	···	6.89	0.88	None	Conc***
PR	$BCS_{calving}$[6]	4	12	20	6	-48.4***	11.75	42.8***	3.69	···	···	9.66	0.98	None	Sync***, Gen***, Forage**
	BCS_{nadir}[6]	3	10	20	0	45.4***	6.14	16.8***	2.70	···	···	4.64	0.79	None	Sync*, Conc**

[1]Dependent variables: C-LA = commencement of luteal activity, COE1 = calving to first observed estrus interval, CRAI1 = conception rate at first service (% of inseminations), PR = overall pregnancy rate (% of cows)

[2]Independent variables: MY_{14wk} = milk yield over the 14 firsts weeks of lactation, MY_{peak} = peak milk yield, MY_{AI} = milk yield at service, $BCS_{calving}$ = body condition score at calving (0-5 scale), BCS_{nadir} = body condition score at nadir, BCS_{AI} = body condition score at service, BCS loss = body condition loss from calving to nadir

[3]n_s = number of studies selected in the model, n_t = number of experimental groups selected in the model, n_o = number of observations selected in the model, n_r = number of outliers removed from the model

[4]RMSE = residual mean square errors, and adj-R^2 = adjusted coefficient of determination of the final model

[5]Interfering factors: Gen = type of genetics of dairy cows (American Holstein/British Holstein/Continental Holstein/Southern Holstein/Holstein crossbreed/Other dairy breed/Dual purpose breed), Conc = type of concentrates supplementation (high/medium/low/high-low/low-high), Forage = type of main forage (grass based/maize based/maize and grazing), Parity = class of the proportion of primiparous cows in the group (0-25/25-50/50-75/75-100), Sync = use of inseminations synchronization protocol (yes/no), and Sys = type of calving system (compact/year round).

[6]Estimation of variance components failed, the model was fitted with the random effect of the *i*th treatment on the intercept only and not on the slope.

Significant levels: *** P<0.001, ** P<0.01, * P<0.05, † P<0.10, ^{ns} P>0.10

inseminated cows and from 70 to 75 % at combined first and second service. It takes 1.9 services for a dairy cow to be pregnant which is consistent with the average reported calving to conception interval (CAIF) ranging from 96 to 100 d (i.e. approximately an ovarian cycle after CAI1). The reported pregnancy rate 42 d after the start of the breeding ranged from 56 to 59 % of the cows and overall from 84 to 85 %. The gestation length ranged from 282 to 283 d and calving interval from 381 to 384 d. With 21 d submission rate, there seems to be 2 populations when comparing genetic characteristics (Figure 1). Indeed, the New-Zealand and Swiss Holstein strains in the studies of Piccand et al. (2011, 2013) had lower 21 d submission rate, ranging from 53 to 59 % in the New-Zealand strain and 56 to 58 % in the Swiss strain. This is explained by their later C-LA in the case of the New-Zealand strain and lower estrus expression and lower conception rates for the Swiss strain (Piccand et al., 2011). If the results of the New-Zealand and Swiss Holstein strains in Piccand et al. (2011, 2013) are not included in the calculation, 21 d submission rate is about 87 % (SD = 6, n = 10). Among the remaining studies, there is one reporting 21 d submission ranging between 79 and 90 % using the New-Zealand strain (Clark et al., 2006). However, the cows involved had COE1 ranging from 51 to 54 d, so they did not have delayed C-LA, and a postponed start of breeding about a month later than the other studies. There seems to be 2 populations also with gestation length. The studies in the body of literature that reported gestation lengths were these of Horan et al. (2004, 2005) and Pollot and Coffey (2008). Gestation length was lower for New-Zealand strain (about 278 d) compared to others (about 284 d), probably because gestation length is one of the traits included in the fertility index.

Body Condition Score at Calving has a strong effect on Cyclicity

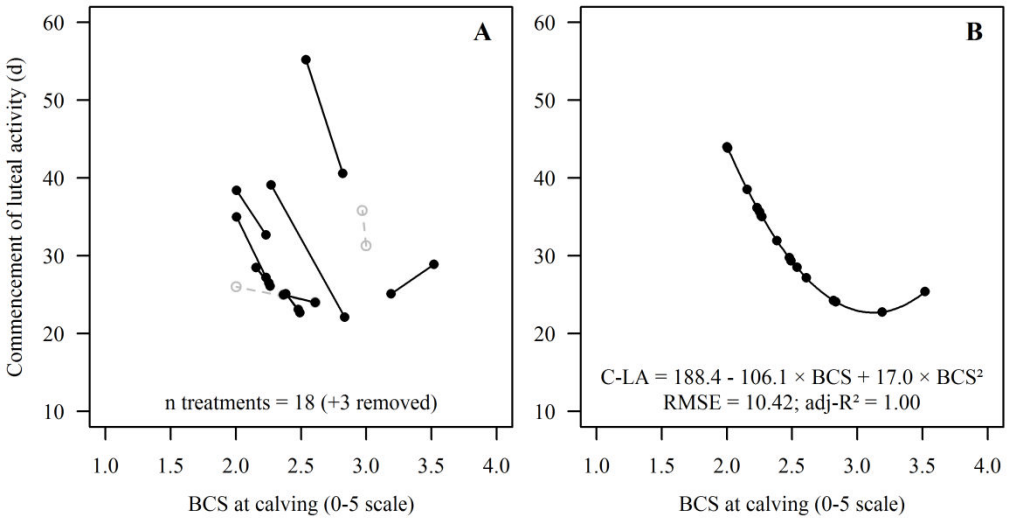

Figure 2: Within-experiment relationships between C-LA and BCScalving (A) in the diet data subset. The prepartum diet characteristics (long run studies or drying off diets) caused the differences between treatments. Values of the same experiment are linked. Outliers of the model are represented with grey dotted lines. Adjusted model (B): the line represents the predicted values and the points represent the values corresponding to the sum of predicted values and residuals.

No relationship between any of the milk yield parameters and C-LA could be identified during data investigation. However, data collected showed a clear curvilinear relationship between C-LA and $BCS_{calving}$ (Figure 2 A-B; Table 2). This relationship could be observed only when using the diet subset. In the particular case of $BCS_{calving}$, only long run (at least 2 consecutive years) studies or with different prepartum diets were considered. Otherwise differences in $BCS_{calving}$ are not induced by the treatment and the results may be biased. Three of the observations (14 %) were identified as outliers and removed from the analyses. The model included a significant and quadratic response ($P < 0.001$). This model explained almost 100 % of the variability (adj-R^2), also considering inter-experiments variation, with a residual mean square error (RMSE) of 10.42. According to this model, there is an optimal $BCS_{calving}$ around 3.10 units for an early resumption of luteal activity (about 23 d) that can be obtained through prepartum nutritional management. Level of concentrates supplementation influenced the residuals of the model and was therefore an interfering factor. This is consistent with the fact that this relationship was observed in the diet data subset. Consequently, their effect could not be included in the model to avoid bias in the results. The type of calving system, the genetic characteristics, and the proportion of first lactation cows in the treatment significantly altered LSM.

Milk Yield impacts interval to first observed Estrus

Very few studies from the selected literature reported data on estrus expression. The indicator of estrus expression used in these studies is COE1. Interestingly, COE1 is an indicator including C-LA and no relationship between any of the BCS parameters and COE1 could be identified during data investigation. However, a significant and linear relationship between COE1 and MY_{peak} was observed (Figure 3 A-B; Table 2). In addition, COE1 was delayed by 1.1 days for each additional kg of MY_{peak} produced by a higher production potential (breeds or strains; $P < 0.001$; adj-$R^2 = 0.95$; RMSE = 9.80). This relationship was also only observed in the genetic subset. There was no effect of potential interfering factors on the residuals. However, the type of calving system, genetics, parity and level of concentrates supplementation significantly affected LSM. One (4 %) observation was identified as outliers and removed from the analyses. Furthermore, for each additional kg of MY_{14wk} permitted by a higher production potential (Holstein *vs* Jersey or Holstein × Jersey crossbred cows), COE1 was delayed by 1.1 days ($P < 0.01$; adj-$R^2 = 0.79$; RMSE = 10.23; Figure 3 C-D; Table 2). The Gen factor was identified as a potential interfering factor on the residuals, consistent with the fact that this relationship was observed in the genetic data subset. There was no effect of potential interfering factors on the LSM.

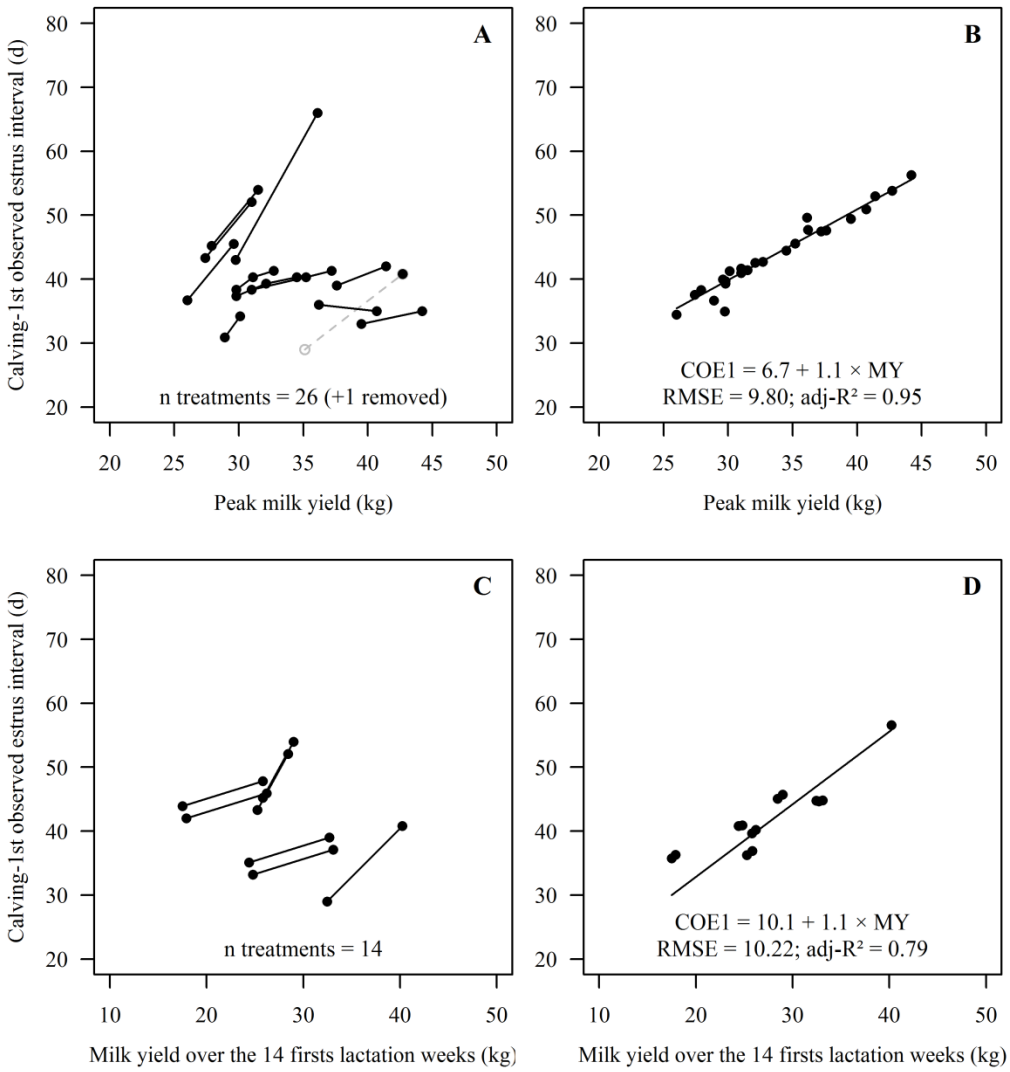

Figure 3: Within-experiment relationships between COE1 and MYpeak (A) or MY14wk (C) in the genetic data subset. Values of the same experiment are linked. Outliers of the model are represented with grey dotted lines. Adjusted models (B, D): the lines represent the predicted values and the points represent the values corresponding to the sum of predicted values and residuals.

Milk Yield impacts Fertility

Data exploration showed that milk yield and CRAI1 were related, when genetic characteristics made the difference between observations within treatments (i.e. in the genetic subset). A significant and linear relationship between CRAI1 and MY_{peak} was observed (Figure 4 A-B; Table 2). CRAI1 was decreased by 2.0 % (of insemination) for each additional kg of MY_{peak} permitted by a higher production potential (breeds or strains; $P < 0.001$; adj-R^2 = 0.89; RMSE = 13.85). The Gen factor was identified as a potential interfering factor on the residuals, consistent with the fact that this relationship was observed in the genetic data subset. In addition, the use of synchronization protocol and genetic characteristics significantly affected LSM. Another significant and linear relationship was observed between CRAI1 and MY_{AI} (Figure 4 C-D; Table 2). CRAI1 was decreased by 2.2 % for each additional kg of MY_{AI} permitted by a higher production potential (strains; $P < 0.001$; adj-R^2 = 0.97; RMSE = 8.30). There was no effect of potential interfering factors on the residuals.

However, the type of the main forage in the diet and the level of concentrates significantly affected LSM, and the proportion of primiparous cows tended to do so.

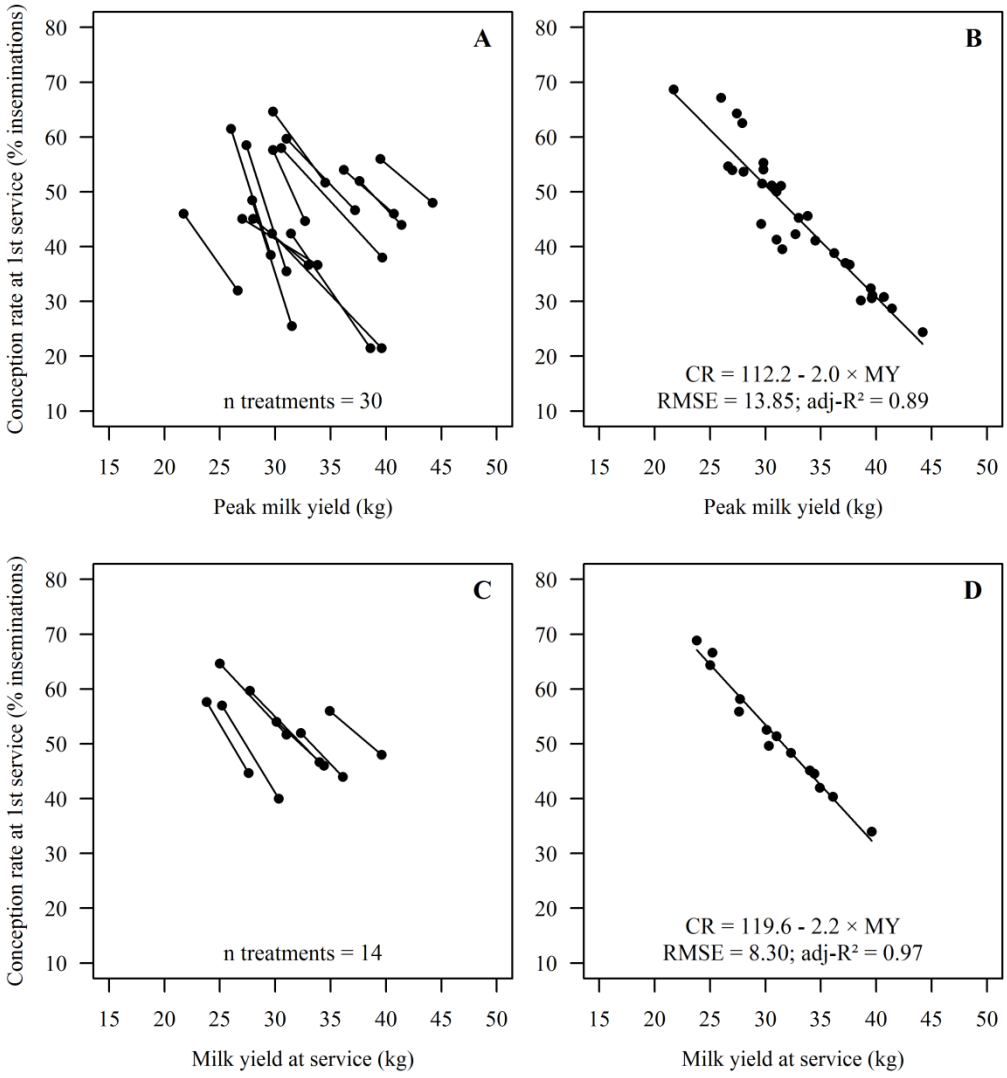

Figure 4: Within-experiment relationships between CRAI1 and MYpeak (A) or MYAI (C) in the genetic data subset. Values of the same experiment are linked. Adjusted models (B, D): the lines represent the predicted values and the points represent the values corresponding to the sum of predicted values and residuals.

Body Condition Score impacts Fertility

Data exploration showed that BCS and CRAI1 were related, when genetic characteristics made the difference between observations within treatments (i.e. in the genetic subset). A significant and linear relationship between CRAI1 and BCS_{AI} was observed (Figure 5 A-B; Table 2). CRAI1 was increased by 38.2 % for each additional BCS_{AI} unit permitted by a lower production potential (strains; $P < 0.001$; adj-$R^2 = 0.94$; RMSE = 4.03). There was no effect of potential interfering factors on the residuals. However, the level of concentrates significantly affected LSM and the type of the main forage in the diet tended to do so. A significant and linear relationship between CRAI1 and BCS_{nadir} was observed (Figure 5 C-D; Table 2). CRAI1 was increased by 22.0 % for each additional unit of BCS_{nadir} permitted by a lower production potential (breeds or strains; $P < 0.001$; adj-$R^2 = 0.91$;

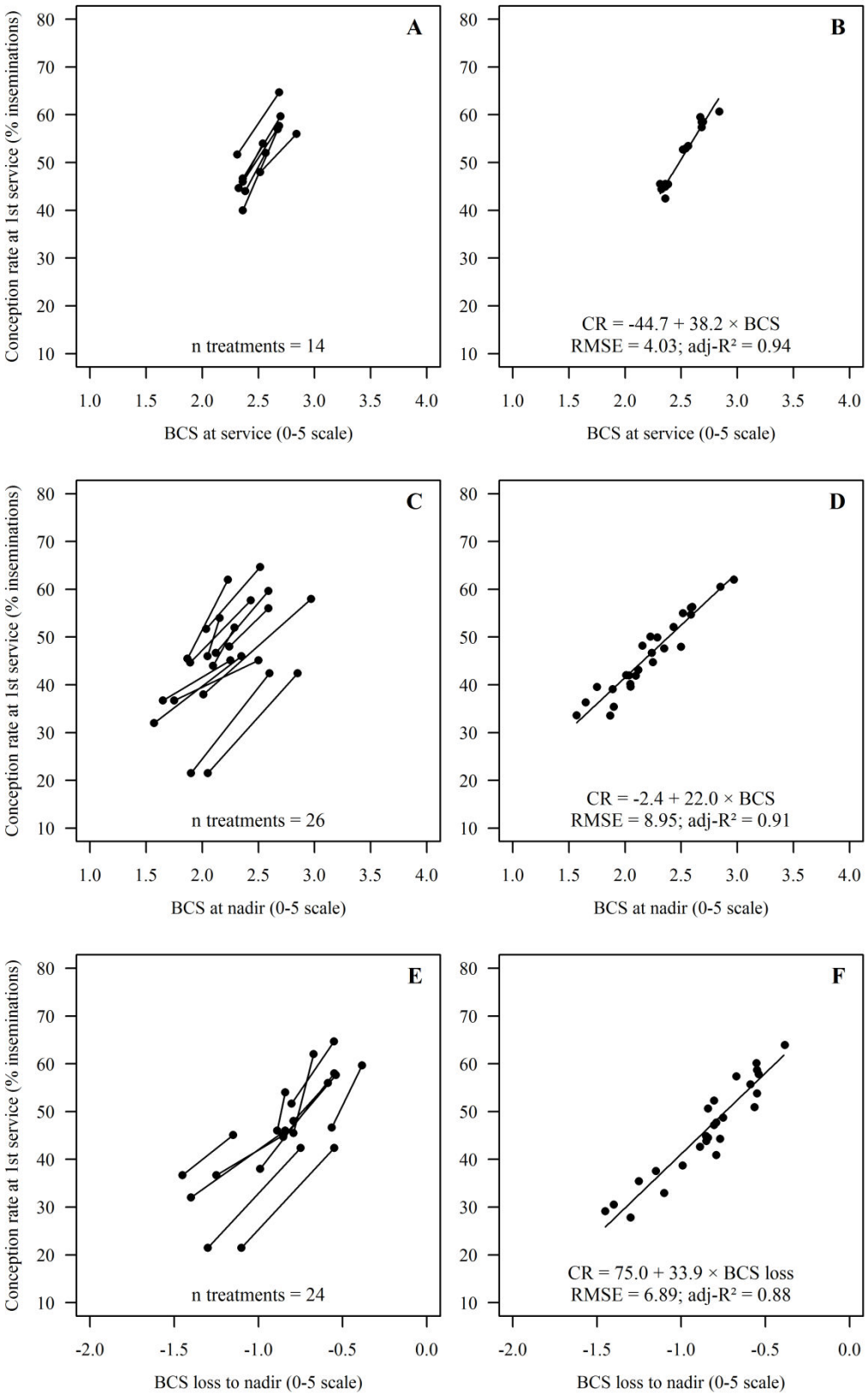

Figure 5: Within-experiment relationships between CRAI1 and BCSAI (A), BCSnadir (C), and BCS loss (E) in the genetic data subset. Values of the same experiment are linked. Adjusted models (B, D, F): the lines represent the predicted values and the points represent the values corresponding to the sum of predicted values and residuals.

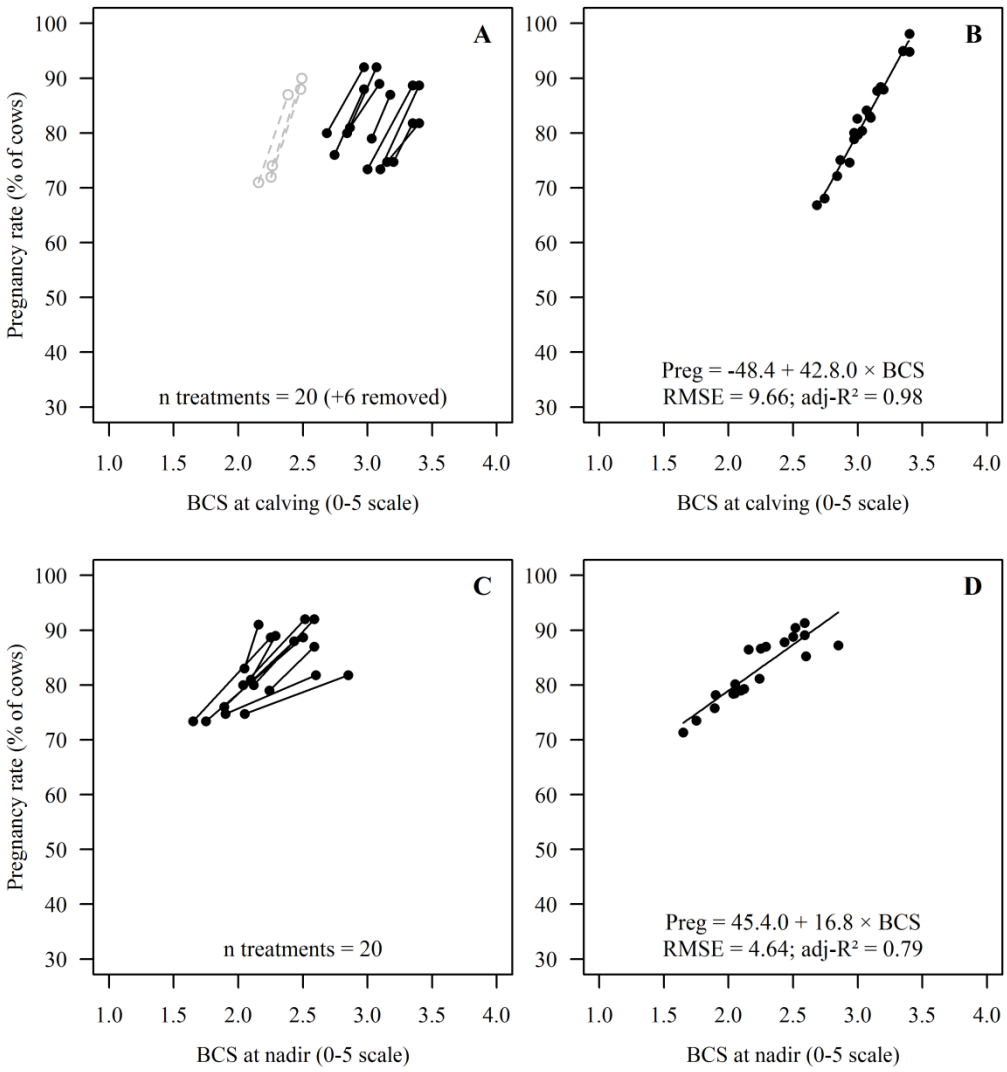

Figure 6: Within-experiment relationships between PR and BCScalving (A) or BCSnadir (C) in the genetic data subset. Values of the same experiment are linked. Outliers of the model are represented with grey dotted lines. Adjusted model (B, D): the line represents the predicted values and the points represent the values corresponding to the sum of predicted values and residuals.

RMSE = 8.95). There was no effect of potential interfering factors on the residuals. However, the use of synchronization protocol, genetic characteristics, the proportion of primiparous cows, the type of the main forage in the diet, and the level of concentrates significantly affected LSM. A significant and linear relationship between CRAI1 and BCS loss was also observed (Figure 5 E-F; Table 2). CRAI1 was decreased by 33.9 % for each additional point of BCS loss between calving and nadir caused by a higher production potential (breeds or strains; $P < 0.01$; adj-R^2 = 0.88; RMSE = 6.89). There was no effect of potential interfering factors on the residuals. However, the level of concentrates significantly affected LSM. Higher MY_{peak} and lower BCS_{nadir} or substantial loss are associated with lower CRAI1. These relationship were identified by using the genetic subset. Genetically, the higher the milk yield the more important the BCS loss (more information on the biological response law between MY_{peak}, BCS_{nadir} or BCS loss in Figure A2 of the Appendix). These effects may be confounded, and the model with the higher adj-R^2 was with MY_{AI} (adj-R^2 = 0.97).

No relationship between any of the milk yield parameters and PR was identified during data investigation. However, data exploration showed that BCS and PR were related, when genetic characteristics made the difference between observations within treatments (i.e. in the genetic subset). A significant and linear relationship between PR and $BCS_{calving}$ was observed (Figure 6 A-B; Table 2). PR was increased by 42.8 % (of cows) for each additional point of $BCS_{calving}$ ($P < 0.001$; adj-$R^2 = 0.98$; RMSE = 9.66). There was no effect of potential interfering factors on the residuals. However, the use of synchronization protocol, genetic characteristics and the type of main forage in the diet significantly affected LSM. All observations from the study of Vance et al. (2013) were identified as outliers which led to the elimination of 6 observations (23% of the data). The reported $BCS_{calving}$ in this particular study were 0.5 to 0.8 BCS units below the other studies (Kennedy et al., 2003; Horan et al., 2004; Delaby et al., 2009; $P<0.001$). Another significant and linear relationship was observed between PR and BCS_{nadir} (Figure 6 C-D; Table 2). PR was increased by 16.8 % for each additional point of BCS_{nadir} permitted by lower production potential (breed or strains; $P < 0.001$; adj-$R^2 = 0.79$; RMSE = 4.64). There was no effect of potential interfering factors on the residuals. However, the use of synchronization protocol and the level of concentrates significantly affected LSM.

DISCUSSION

Meta-analyses

Results from research are difficult to promote on field through recommendation or decision tools because they highly depend on experimental conditions and limitations. Meta-analyses are interesting techniques because they enable the determination of a biological response through empirical modeling from a body of studies. They can consolidate knowledge, help identify new hypotheses, and help explain discrepancies in the literature. The responses are also useful to build or evaluate mechanistic models (Sauvant et al., 2008; Lean et al., 2009). Another benefit of these quantitative reviews, compared to qualitative ones, is that several protocols exist, like those of Sauvant et al. (2008) or Lean et al. (2009), to guide authors to have a scientific and impartial approach in selecting and analyzing the data. The main limitation of meta-analyses is to identify most of the relevant existing studies (published and unpublished articles, reports, theses, in many languages…). Unfortunately, failure to find most of existing data can lead to erroneous conclusions. Another limitation of meta-analyses is that, due to missing values, it is almost impossible to use multidimensional approaches, even though they may help in identifying new hypotheses.

In our study, the selected number of studies included in the meta-analyses is small. Several experts in the field helped us to identify most of data available, therefore we can conclude that few studies are reporting both reproduction and production performance. In addition, they were not all comparable, because of experimental factors: some used genetic characteristics, others feeding system, milking frequency, parity or a combination of them. This reduced the number of comparable data to explore relationships between reproduction and production variables. Consequently, testing of all hypotheses was not possible through this meta-analysis. Only three models had an interfering factor on residuals: level of concentrates supplementation in the model with C-LA and $BCS_{calving}$ and type of genetics in the model with COE1 and MY_{14wk} and with CRAI1 and MY_{peak}. These are consistent with the subset used (respectively diet and genetic). A certain number

of interfering factors affected LSM. By definition, LSM are the prediction of the observed Y mean on X average using the coefficients of final models; therefore they may be affected by the effect of the study and interfering factors. In addition, apart from genetic characteristics, the other potential interfering factors were not included in the final models because of sample size. Still, our results are consistent with mechanistic modeling (Brun-Lafleur et al., 2013) and qualitative reviews (Royal et al., 2000; Friggens et al., 2010).

The effects of Body Condition Score and Milk Yield differs in importance for Reproduction Steps

This meta-analysis showed that C-LA was firstly affected by BCS at calving. This is consistent with previous studies (Cutullic et al., 2012; Bedere et al., 2016). The relationship between C-LA and BCS at calving was quadratic, which supports the idea of Roche et al. (2009) who suggested a Gaussian relationship between BCS and postpartum anestrus. This is also consistent with the model of Brun-Lafleur et al. (2013) that includes a quadratic effect of BCS at 30 days postpartum. Roche et al. (2009) recommend a BCS at calving ranging from 3.0 to 3.5 points on the 1-5 scale (Wildman et al., 1982). If we apply the conversion equation, it results in an optimal BCS at calving between 2.2 and 3.0 in the 0-5 scale (Bazin et al., 1984). The present meta-analysis pointed an optimal BCS of 3.10. Very few recent studies reported the effect of prepartum diet on $BCS_{calving}$ and reproduction. Contrasting dry period diets only managed to create a difference of $BCS_{calving}$ of less than 0.50 score (in reported units) that was either significant (Burke et al., 2007; Adrien et al., 2012) or not (Pushpakumara et al., 2003; McNamara et al., 2008; Cavestany et al., 2009). On the other hand, the residual effect of dietary treatment postpartum of the preceding lactation can also create up to 0.50 points of BCS unit difference (Kolver et al., 2005; Walsh et al., 2008; Delaby et al., 2009; Cutullic et al., 2011; Vance et al., 2012, 2013). The results of this meta-analysis suggest that prepartum diet is a key opportunity to increase $BCS_{calving}$ and improve postpartum cyclicity of modern dairy cows although further research is required in this area. In addition, the relationship between BCS and C-LA is influenced by a strong genetic correlation of -0.84 (Bastin and Gengler, 2013). As expected, we found no relationship between milk yield and C-LA (Friggens et al., 2010; Cutullic et al., 2012). Studies in which C-LA was found to be associated to milk yield may have observed a confounded effect with BCS. Even though heritability estimates for C-LA are moderate (ranging from 0.13 to 0.30; Veerkamp et al., 2000; Royal et al., 2002; Petersson et al., 2007), those of BCS are relatively high (from 0.20 to 0.50; Bastin and Gengler, 2013). This suggests that selecting for higher BCS would also select for earlier C-LA. BCS and milk yield are also genetically correlated (-0.37; Bastin and Gengler, 2013), which can explain why intense selection on milk yield resulted in dairy cows with low BCS and delayed resumption of ovarian activity. Further investigations are still needed to better understand the genetic structure of cyclicity and the biology of requirements and supply of resources for the ovaries.

As expected, these meta-analyses also showed that milk yield was positively associated with COE1 (Roche, 2006; Friggens et al., 2010; Cutullic et al., 2012). This is also consistent with the result of the mechanistic model of Brun-Lafleur et al. (2013); where COE1 was lengthened by 0.9 day per kg of milk because of a reduced estrus expression and by 0.4 day per missing point of BCS at calving because of longer C-LA. Indeed, Fulkerson et al. (2001) determined a conversion equation: COE1 =

48.5 + 0.48 × C-LA. This may indeed explain why COE1 is associated with BCS in some studies. However, the proportion of variation of COE1 explained by C-LA is very low (adj-R^2=0.05; Fulkerson et al., 2001) and the one explained by energy balance also (adj-R^2=0.04; de Vries et al., 1999). We found no relationship between COE1 and BCS, which supports the idea that estrus intensity and duration are mainly related to milk yield (Roche, 2006; Friggens et al., 2010). Once again, a possible explanation is that high milk yield is associated with high intake, and consequently to high liver blood flow and thus high clearance of sexual steroids (Wiltbank et al., 2006). Even though heritability estimates for estrus intensity are low (from 0.01 to 0.04; Roxström et al., 2001; Carthy et al., 2016); there remains some breed specificities (Friggens and Labouriau, 2010; Sveberg et al., 2015). The link between milk yield and COE1 could only be observed when diet characteristics made the difference between observations in these meta-analyses. MY_{peak} seemed to be a better predictor of COE1 than MY_{14wk} given the goodness of fit of the models (comparison based on RMSE, adj-R^2, and interfering factors). There are very few studies on estrus, mainly because of the difficulty to accurately measure behaviors. The emergence of activity meters systems may help to improve knowledge but also needs to be augmented with visual detection and behavioral studies. Biological hypotheses on the drivers of the competition between milk production and estrus expression still need to be investigated.

In these meta-analyses, CRAI1 was affected by both milk yield and BCS. BCS_{AI} seemed to be the best predictor for CRAI1, even though MY_{AI} was also a good predictor according to the goodness of fit of the 5 models determined for CRAI1. This is consistent with the fact that lower BCS at calving is associated with lower conception rates and longer days to conception (López-Gatius et al., 2003; Cardoso et al., 2013). This may be explained by different sources of fertility failure: in a previous study, we found that non-fertilization or early embryo mortality (before 25 days of life) is associated with BCS at nadir (Cutullic et al., 2012). There are strong genetic correlations between BCS and CRAI1 (0.60), pregnancy rate 63 d from start of the breeding (0.37) and re-calving rate (0.43; Bastin and Gengler, 2013). The meta-analyses showed that pregnancy rate was associated with BCS, which is consistent with these genetic correlations and other studies (Buckley et al., 2003; Santos et al., 2009). Also, mobilization results in high plasma concentrations of non-esterified fatty acids that are damaging oocytes and endometrium, causing embryonic death (Santos et al., 2004; Friggens et al., 2010; Wathes et al., 2013). The other source of fertility failure is late embryo mortality (between 25 and 50 d of life). Late embryo mortality is more frequent in high yielding dairy cows (Grimard et al., 2006) and affected by lower lactation persistency (Buckley et al., 2003; Cutullic et al., 2012). All this is also in agreement with the results of the mechanistic model of Brun-Lafleur et al. (2013) with re-calving rate decreased by 1.4 % per kg of milk and 0.6 % per missing point of BCS at calving. Further investigation on the genetic implications and biological causes of non-fertilization and embryo mortality are still required to better understand fertility failures due to the competition with lactation in dairy cows. Nonetheless, the effects of body condition on reproductive performance found in this meta-analysis on dairy cows are also consistent with studies on suckling cows (Blanc and Agabriel, 2008; Recoules et al., 2013). In their mechanistic modelling approach, Blanc and Agabriel (2008) proved that BCS at calving was a good predictor for C-LA, and consequently involved in the prediction of COE1, CRAI1 and PR (because their prediction

depends upon C-LA). The study of Recoules et al. (2013) identified a threshold of body fatness, below which C-LA is delayed and above which no relationship was observed. This is consistent with the quadratic effect reported in the present meta-analysis. Interestingly, in this last study feeding levels impaired estrus expression but milk yield was not responsible for this (suckling cows). This highlights the need to better understand tradeoffs and priorities of the different life functions across time in cattle.

CONCLUSIONS

This meta-analysis of the competition between lactation and reproduction showed that the effects of milk yield and BCS differ in importance according to the step of the reproduction process. Cyclicity is mainly affected by BCS at calving, estrus expression is mainly affected by milk yield and fertility is modified by both BCS and milk yield (probably rather by BCS concerning non-fertilization and milk yield concerning embryo losses). Our results suggest that targeting BCS at calving close to 3.0 (0-5 scale) would contribute to keep C-LA below 25 d postpartum. In addition, nutritional management that limits BCS loss and peak milk yield could be an effective way to improve conception rate and pregnancy rate. On the other hand, mitigating the strong genetic selection on milk yield and selecting dairy cows for higher BCS would enable genetic improvement of reproduction performances. Our results suggest that genetically improving peak milk yield by 10 kg would result in lengthening COE1 by 11 d, lowering CRAI1 by 20 %, and probably lowering BCS at nadir by 0.8 units (0-5 scale). Nevertheless, genetically lowering BCS at nadir by 1 unit (0-5 scale) would result in lowering by 22 % CRAI1 and 17 % the final pregnancy rate. And there are still gaps in knowledge on ways to reverse the trend for sustainable dairy systems. Questions remain around the biological mechanisms underlying those trade-offs, especially around the determination of the actual requirements, flows and effects for glucose, non-esterified fatty acids, and hormones that structure the competition between lactation and reproduction. There are too few recent studies on other promising opportunities to improve reproduction such as milk frequency, prepartum diet and dry period length.

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APPENDIX

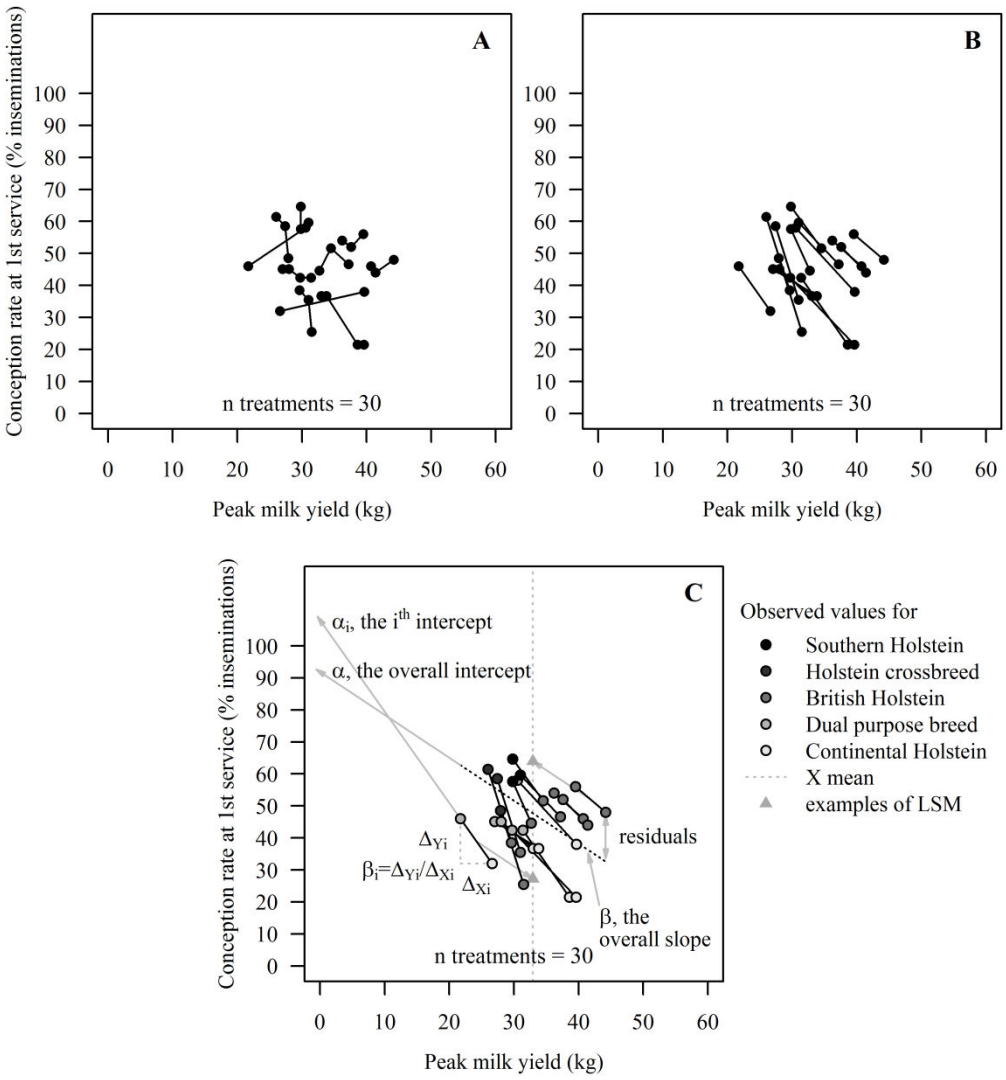


Figure A1: Depiction of the overall meta-analytic approach with the example of the relationship between CRAI1 and MY_{peak}. Examination of the within experimental relationship between Y and X according to either the dietary treatment (A) or the genetic characteristics (B). Representation of the terms used in meta-analyses, the response law and the Gen interfering factor (C).

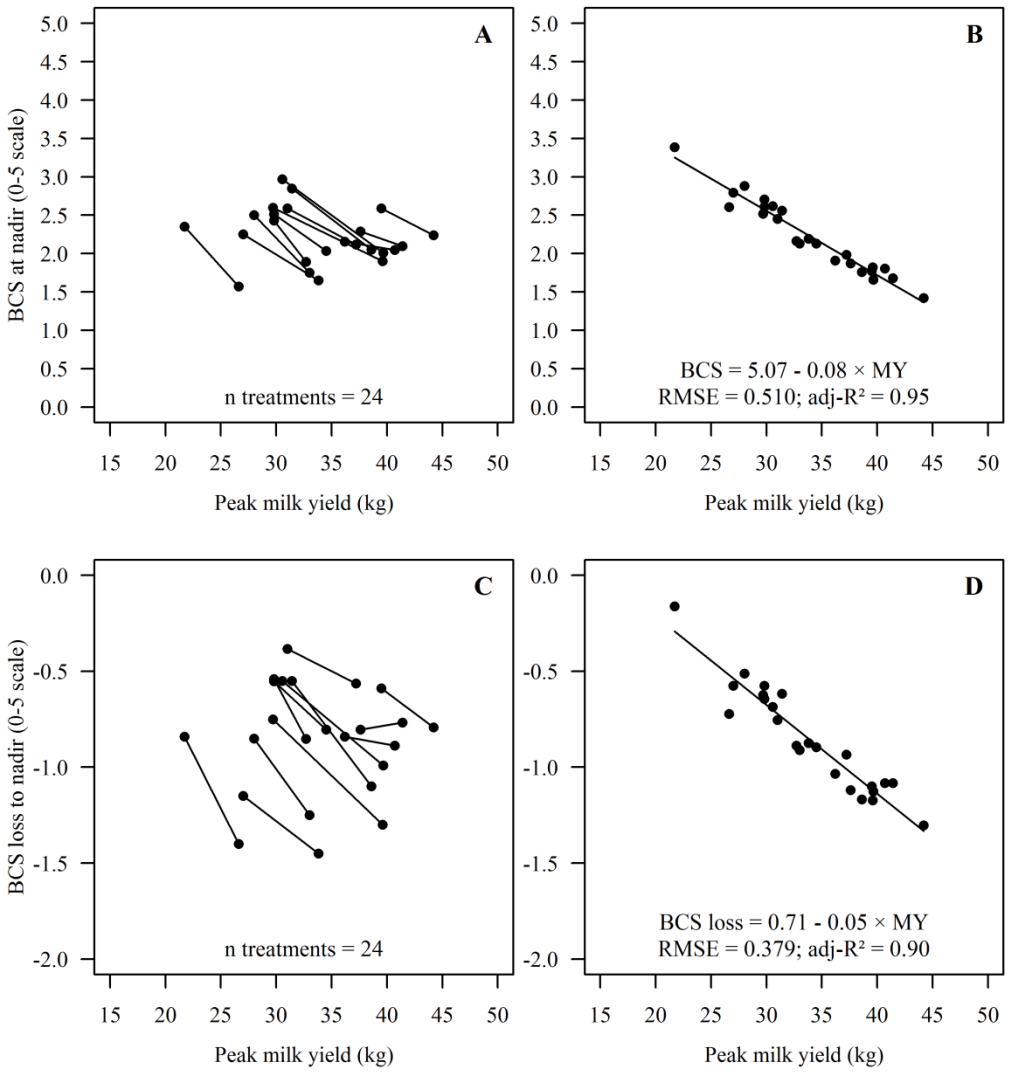
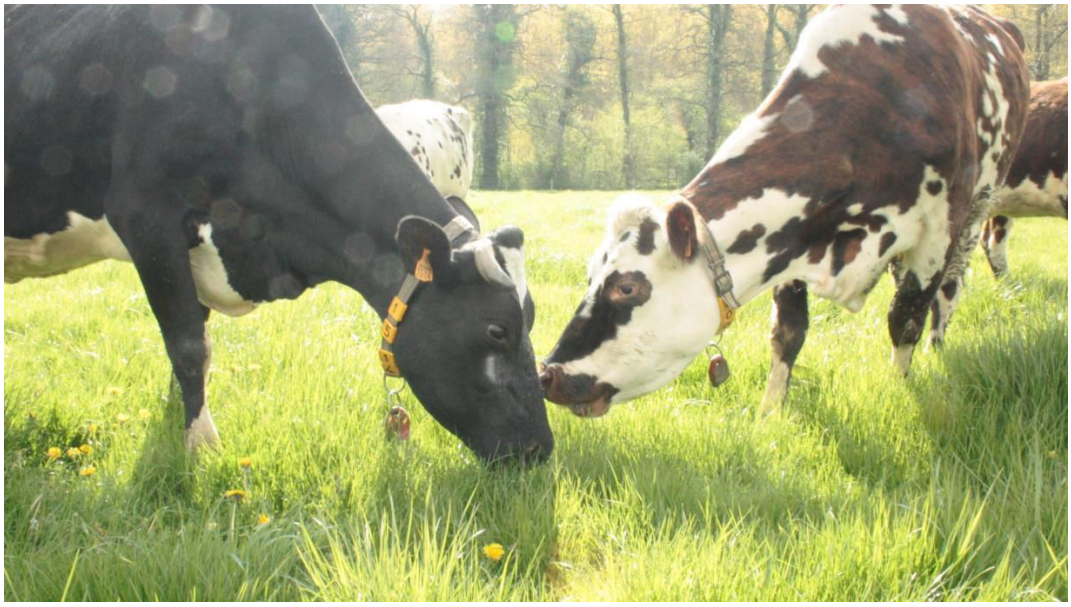


Figure A2: Within-experiment relationships between MY_{peak} and BCS_{nadir} (A) or BCS loss (C) in the genetic data subset used to explore the relationship between CRAI1 and either milk yield or body condition variables. Information on milk yield and body condition was available for 24 treatments. Values of the same experiment are linked. Adjusted models (B, D): the lines represent the predicted values and the points represent the values corresponding to the sum of predicted values and residuals.

CHAPTER 2: Scientific Approach



Objectifs

Ce court chapitre a pour objectif d'exposer la question de recherche et les hypothèses issues de l'étude bibliographique ainsi que la stratégie expérimentale utilisée pour tester ces hypothèses.

L'essentiel

L'étude de la bibliographie a montré que le processus de reproduction des vaches laitières est une succession d'étapes interconnectées : cyclicité, œstrus et fertilité. Les caractéristiques de l'animal (e.g. génétique) et du système (e.g. alimentation) affectent les performances de production et de reproduction des animaux. Elles influent également sur la gestion de la compétition entre ces fonctions biologique chez l'animal.

De nombreuses études comparent les performances de reproduction entre des animaux à forts *vs* faibles index génétiques de production laitière ou de matières utiles. A notre connaissance, il n'y a pas d'étude qui compare les performances de reproduction de vaches à fort index génétique de production laitière *vs* à forts index génétiques de taux butyreux et protéique, à même index génétique de production de matières utiles. Or, les vaches produisant un lait avec des taux butyreux et protéique plus élevés exporteraient moins d'énergie dans le lait, utiliseraient moins de glucose (pour la synthèse du lactose), auraient un débit sanguin plus faible et donc un moindre catabolisme hépatique des hormones. D'après la bibliographie, ces vaches seraient donc dans de meilleures conditions pour assurer leur reproduction grâce à une meilleure capacité de synthèse des stéroïdes sexuels, une meilleure capacité des ovocytes à poursuivre leur méiose, et un meilleur environnement utérin.

Cette thèse a pour objectif de répondre aux questions suivantes :

1. Quelles sont les **stratégies d'adaptation** de vache à forts index génétiques de taux butyreux et protéique ou à fort index génétique de production laitière ?
2. Est-ce que sélectionner des vaches laitières sur les taux butyreux et protéique au lieu de la production laitière est une bonne alternative pour **améliorer leurs performances de reproduction** tout en préservant une production intéressante ?

Ce projet de recherche vise à tester 2 hypothèses :
A production de matières utiles identique, par rapport aux vaches à fort index génétique de production laitière, celles à forts index génétiques de taux butyreux et protéique :

- **exportent moins d'énergie dans le lait, elles préservent ainsi leurs réserves corporelles ;**
- **sont plus aptes à se reproduire (la reprise d'activité ovarienne postpartum est plus précoce, l'expression de comportements d'œstrus est plus intense, l'aptitude à assurer la gestation est meilleure).**

Pour tester ces hypothèses, nous nous sommes appuyés sur l'expérimentation appelée **« Quelle vache laitière pour quel système ? »** qui a été mise en place en 2006 sur **le domaine expérimental INRA du Pin-au-Haras** (48.448N, 0.098E, Normandie). Chaque année, environ 30 vaches de race **Holstein** (laitière) et 30 de race **Normande** (mixte) participent à l'étude et sont réparties dans deux systèmes alimentaires pâturants. Le système alimentaire **« Haut »** permet un niveau de production laitière élevé et une perte d'état corporel modérée. Le système **« Bas »**, limite la production laitière et provoque une forte mobilisation des réserves corporelles des animaux. Nous avons besoin de comparer des groupes distincts selon leurs index génétiques de production laitière, production de matières utiles, taux butyreux et protéiques. **Dans chaque race**, les vaches sont classées dans 2 groupes génétiques à index génétique de production de matières utiles identiques. Celles avec un fort index de production laitière sont classées dans le groupe **« Lait »**, celles avec de forts index de taux butyreux et protéique dans le groupe **« Taux »**.

1 Scientific background

The context of animal production systems is changing and challenging. Global food demand is increasing and the planet resources are limited. Consumers are now looking for safe and healthy animal products. Animal production systems also have to get more environmental friendly. In addition, they have to adapt to changing societal preferences which are more concerned about animal welfare, local food, food identity and labelled production, etc. The most appropriate strategy to cope with these new challenges is to keep or increase diversity in both types of genetic resources and farming systems (Phocas *et al.*, 2016a; b). According to their genetic characteristics, animals will experience different trade-offs and use different adaptive strategies to cope with the challenges of the environment. This defines the limits of their adaptive capacity and thus the match between animals and systems (Phocas *et al.*, 2016a; b). Animals are suited to systems when they successfully ensure biological functions: milk production, reproduction, maintenance, health, heat production…

The literature review showed that lactation and reproduction are concomitant biological functions in dairy cows and in competition for resources. This leads to a trade-off: if most of the resources available are invested in production, there is little left to ensure other functions. Reproduction of dairy cows is a succession of interconnected steps. And regrettably, each step of the reproductive process has been declining while milk yield increased. Some characteristics at the animal scale (e.g. genetics) and at the farming system scale (e.g. nutrition) affect both production and reproduction performance of dairy cows. These characteristics also impact trade-offs between lactation and reproduction. Therefore there is a need to study different types of animals (breeds and others genetic characteristics) in contrasted farming systems to answer the question **"The dairy cow for the system?"**

Many studies investigated the differences between high and low genetic merit for milk yield (Barnes *et al.*, 1990; Snijders *et al.*, 2001; Kennedy *et al.*, 2002, 2003; Windig *et al.*, 2008); or milk solids (Fulkerson *et al.*, 2001, 2008); or the differences between Continental/American and New-Zealand strains of Holstein cows (Horan *et al.*, 2004, 2005a; b; Kolver *et al.*, 2005; Macdonald *et al.*, 2008). **To our knowledge, there was no study on production and reproduction performance of cows with high genetic merit for milk yield *vs* high genetic merit for fat and protein content, at identical genetic merit for milk solids.**

Energy in milk is contained in the fat, protein and lactose. Consequently there are two ways of exporting the same amount of energy in milk: either through high milk yield or through high fat, protein and lactose contents. Holstein cows are known to have a lower lactose content than other breeds (Dillon *et al.*, 2003a; Walsh *et al.*, 2008). However, variations in lactose content within breeds are less substantial than fat and protein contents because it is highly related to milk osmotic pressure. At similar amount of fat and protein yield, cows producing milk with higher fat and protein contents are supposed to have lower lactose yield than those producing higher milk yield. In such cows, the mammary gland would require less glucose for lactose production and glucose would be more available for other tissues. This glucose can support ovarian activity and thus

production of ovarian steroids and development of the oocytes. By having lower milk yield, cows with high fat and protein contents would have a lower liver blood flow and thus lower sexual hormone catabolism. All these effects could be beneficial to the reproductive process.

2 Research questions and hypotheses

This research project aimed to answer the following questions:

- What are the adaptive strategies of cows with either high genetic merit for fat and protein contents or high genetic merit for milk yield in contrasted farming systems?
- Is selecting cows for fat and protein contents instead of milk yield a good alternative to ensure both lactation and reproduction?

Thus, the hypotheses to be tested are that:
At similar genetic merit for milk solids yield, compared to cows with high genetic merit for milk yield, dairy cows with high genetic merit for fat and protein contents:

- **export less energy in milk and are consequently safeguarding their body reserve;**
- **have better reproductive performance (earlier resumption of ovarian activity, more intense oestrus, better ability to ensure pregnancy).**

3 Strategy to address these questions and test the hypotheses

In order to test these hypotheses, an experiment called **"The cow for the system?"** was conducted from 2006 to 2014 at the **INRA experimental dairy farm of Le Pin-au-Haras** (48.724986N, 0.185428E, Normandy, France). The experimental farm was headed by Yves Gallard. The experiment was under the supervision of Luc Delaby, and locally managed by Ségolène Leurent-Colette. The experimental farm is located in a pedoclimatic context with favourable grass growth. Grass-based systems are part of the systems diversity that we referred to earlier and there is a large diversity within such systems. Compact calving grass-based systems aim decrease inputs and thus to maximise the use of the resources on farm. In these systems, milk production is seasonal to synchronise nutrient requirements for lactation to grass production. This means that all cows from the herd have to calve in a 3 month period (January-March here), are dried-off during the winter period when grass is scarce. Although, gestation length is about 9 months in cattle and reaching the goal of 1 calf/year/cow seems feasible, it is real challenge for the cow, especially given current reproduction performance.

In this experiment, cows were approximately equally distributed between 2 breeds and 2 grass-based feeding systems each year. A total of 296 lactations from 132 **Normande** cows (dual purpose cows) and 240 lactations from 128 **Holstein** cows (dairy cows) were recorded throughout the trial. In both feeding systems, cows were fed *ad libitum* but with contrasting nutrient supply. The **"High"** feeding system enabled high milk yield while limiting body condition loss. The **"Low"** feeding system limited milk yield while inducing a large body condition loss. **Within breed**, cows were

classified into 2 groups according to their estimated breeding values (EBV) for milk yield, fat and protein contents as 2 groups globally producing the same milk solids quantity with different genetic characteristics: cows with high EBV for milk yield were included in a **"Milk-Group"** and those with high EBV for fat and protein contents were included in a **"Content-Group"** (more details in the following "focus" sub-section).

The monitoring of production performance was regular with records on individual milk yield, 3 times a week fat and protein contents determination, weekly weighing and monthly BCS estimation. The monitoring of reproduction was intense. Morning milk samples were taken 3 times a week and milk P_4 concentration was determined on frozen samples. Milk P_4 information was used to monitor ovarian activity. Oestrus signs were recorded 5 times a day. Ultrasonography examinations were performed at 35 and 60 d after service. The pregnancy diagnosis was combined with milk P4 information in order to identify different sources of pregnancy failures (Humblot, 2001; adapted by Cutullic *et al.*, 2011).

Thus, it was possible to compare the milk production traits, management of body reserve and the performance at each step of the reproductive process of cows from the Milk- or Content-Group within and between the two feeding systems. This enabled an evaluation of the adaptive strategies of both genetic groups in contrasted environment.

Focus on the constitution of the genetic groups

The experimental farm is characterized by very heterogeneous and unusual management conditions. Consequently, recorded performance of the cows are not routinely included in the national data base and no national EBV are available for these cows. Furthermore, genomic evaluations were not available for the oldest cows of this study. Therefore, specific EBV had to be computed. This was performed combining within herd information with national sire and maternal grand sire EBV. Based on these results, cows were classified within breeds into 2 groups according to their customized EBV for milk yield (MY), fat and protein contents (respectively FC and PC) as 2 groups capable of producing similar milk solids (MS) quantity in different ways. The EBV for each trait was evaluated by combining within herd information analysed with a BLUP animal model with national EBV of the sires and grandsires. The model of analysis of cow performances over three lactations included the usual fixed environmental effects (year, lactation number, calving age, calving month, drying off period length, and permanent environment effect) and the feeding system (H. Larroque, D. Boichard and R. Lefebvre, personal communication). Within breed and experimental year, nulliparous cows with EBV for milk yield higher than average and EBV for fat and protein contents lower than average constituted a **"Milk-Group"**. Nulliparous cows with EBV for milk yield lower than average and EBV for fat and protein contents higher than average constituted a **"Content-Group"**. The others nulliparous cows (with high EBV for milk yield and high EBV for fat and protein contents or low EBV for milk yield and low EBV for fat and protein contents) did not enter the experiment. EBV were expressed in deviation from a base population, whose average EBV were set to 0. Table 6 shows a breakdown of EBV according to breeds and genetic groups. In order to further understand the link between genetic merit for production traits and the actual performance, regression of adjusted production performances (predicted outcomes of the model used in the study) on EBV are presented in Figure 23.

Table 6: Distribution of the customized Estimated Breeding Values for production traits (milk yield, fat content, protein content, fat yield, protein yield and milk solids yield) for Holstein and Normande cows, in the Milk- or the Content-Group

	Holstein		Normande	
	Milk-Group	Content-Group	Milk-Group	Content-Group
EBV for milk yield (kg)	+308	-303	+290	-264
EBV for fat content (g/kg)	-1.7	+1.9	-1.9	+1.5
EBV for protein content (g/kg)	-0.5	+0.5	-0.9	+0.8
EBV for fat yield (kg)	-1.8	+1.1	+1.1	-2.3
EBV for protein yield (kg)	+6.3	-5.5	+4.7	-4.4
EBV for milk solids (kg)	+4.4	-4.4	+5.8	-6.8

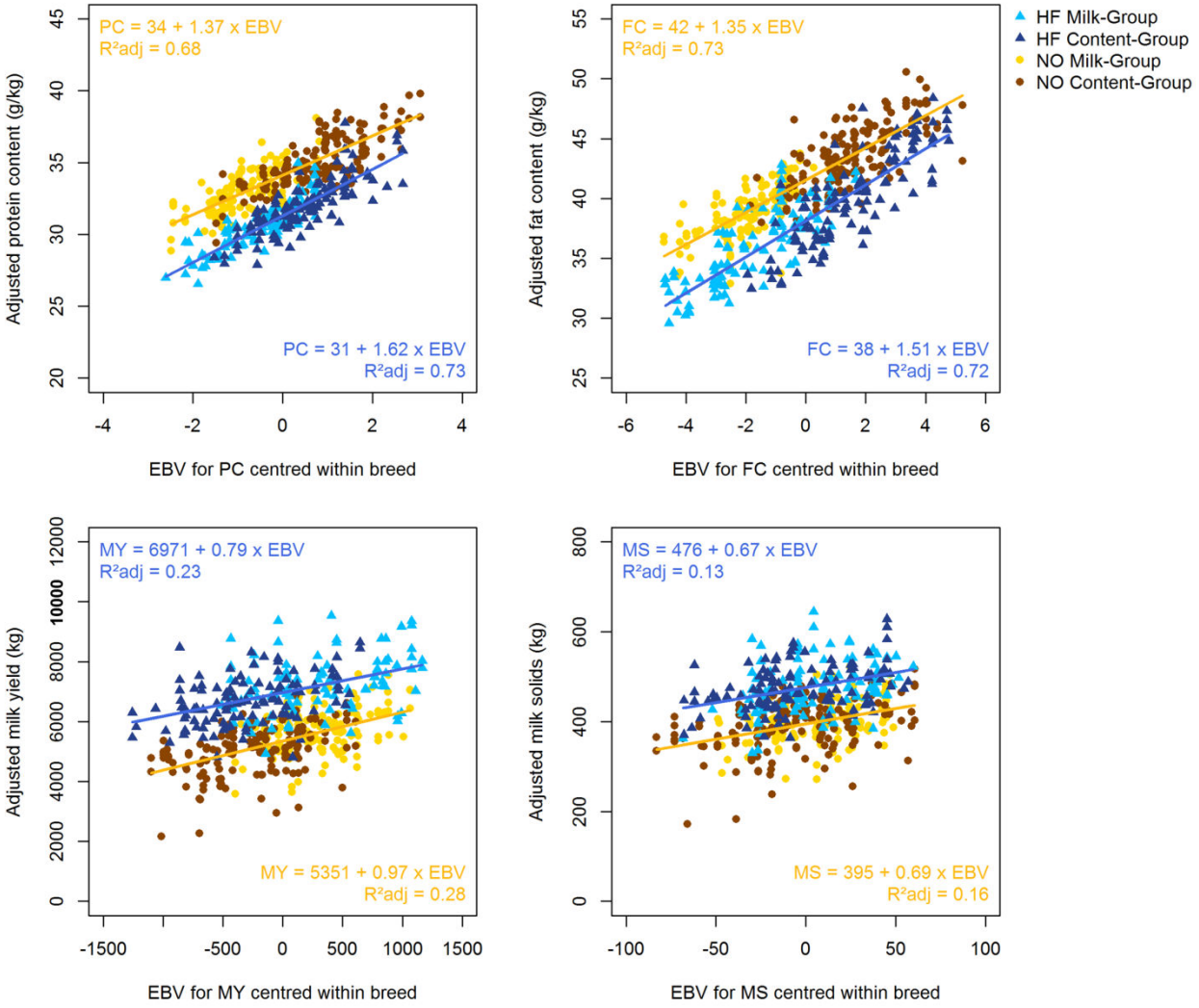
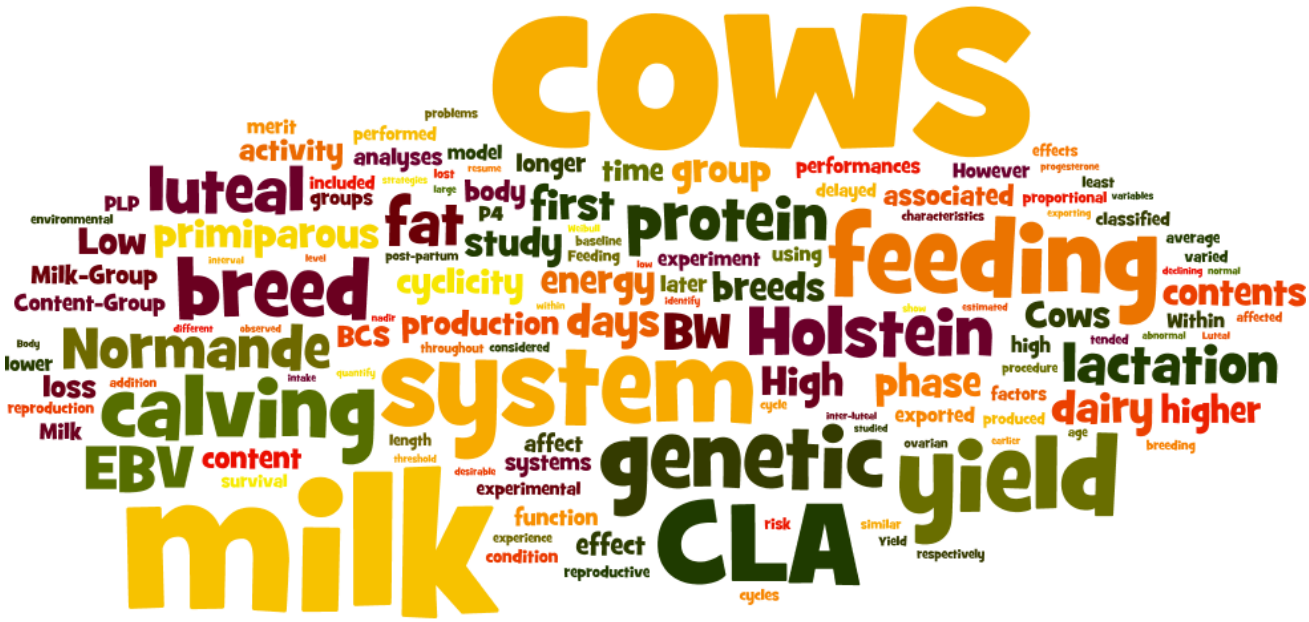


Figure 23: Regression of production traits (protein content, fat content, milk yield and milk solids yield) on associated EBV. Production traits are adjusted from the effect of year, parity, age at first calving, feeding system and lactation length. Holstein cows are indicated by blue triangles (each triangle represents a cow/year) and Normande by orange circles (each circle represents a cow/year). In each breed, cows from the Milk-Group are represented by points with lighter colors whereas those from the Content-Group with a darker color. Regression line of prediction for the 217 Holstein and 283 Normande lactations recorded are reported.

CHAPTER 3: Postpartum cyclicity of primiparous dairy cows according to genetics and breeding system.



Objectifs

Ce premier chapitre de résultats a pour objectif d'identifier et de quantifier les effets de la race, du type génétique et du régime alimentaire sur la cyclicité postpartum de vache en première lactation. Les hypothèses testées sont (1) les vaches ayant des caractéristiques génétiques (race et type) favorables à la production laitière ont une cyclicité postpartum dégradée ; (2) le régime alimentaire affecte la production laitière et la gestion des réserves corporelles, ce qui est lié à la cyclicité.

L'étude des vaches en première lactation uniquement permet de comparer les types génétiques en éliminant des sources de biais. En effet les index génétiques des animaux évoluent avec la prise en compte des lactations réalisées par ces animaux et de leurs parents (père, mère, fratrie et descendance). De plus, les vaches réalisant plusieurs lactations ont un index unique et même si la prise en compte des données appariées est possible par des méthodes statistiques, le facteur de répétition est déséquilibré (toutes les vaches ne réalisent pas le même nombre de lactations).

L'essentiel

Les vaches de race Holstein ont produit plus de lait que les vaches Normande (+1 810 kg dans le système Haut et +1 120 kg dans le système Bas). Elles ont aussi perdu plus de poids durant le premier tiers de lactation (-1,4 kg/sem). Les vaches Normande ont un retour de cyclicité plus précoce que les vaches Holstein. Elles ont aussi des cycles ovariens plus courts (-1,7 j) que les vaches Holstein. La proportion de PLP n'est pas différente entre les 2 races.

Dans les 2 races, les vaches du type Lait ont produit plus de lait que celles du type Taux (+408 kg pour les vaches Holstein et +350 kg pour les vaches Normande). En race Holstein, le type génétique n'a pas eu d'effet sur l'état des réserves corporelles au vêlage ni sur leur mobilisation. En race Normande, les 2 types génétiques ont un état corporel identique au vêlage et les vaches de type Lait mobilisent plus de réserves que celles du type Taux. Les vaches du type Lait sont cyclées plus tardivement que celles du type Taux. Aucun effet du type génétique sur l'occurrence de PLP n'a été observé.

Les vaches du système Haut ont produit plus de lait que celles du système Bas (+2 040 kg pour les vaches Holstein et +1 350 kg pour les vaches Normande). Elles ont aussi perdu moins de poids durant le premier tiers de la lactation (+3,8 kg/sem). Très peu d'effets du système ont été mis en évidence : le délai de reprise de cyclicité n'est pas affecté par l'alimentation, la proportion de PLP non plus, seule la longueur du premier cycle était supérieure dans le système Haut que dans le Bas. Cependant, la gestion des réserves corporelle est associée à la reprise de cyclicité : les vaches plus lourdes au vêlage et perdant du poids en début de lactation ont une reprise de cyclicité plus tardive.

Cette première étude a permis de montrer que la reprise de cyclicité postpartum chez la vache en première lactation est principalement influencée par ses caractéristiques génétiques de production. A potentiel de production de matières utiles donné, il semble prometteur de sélectionner les vaches pour les taux butyreux et protéique plutôt que pour la production laitière afin d'améliorer la reproduction. Ces conclusions concernent la cyclicité, ces résultats doivent être confirmés sur les autres étapes de la reproduction : expression des chaleurs et fertilité. Concernant leurs trajectoires adaptatives : les vaches du type Lait produisent plus de lait et mobilisent autant que celles du groupe Taux. L'énergie mobilisée par le groupe Taux, combinée aux effets d'un moindre investissement dans la production laitière (flux sanguins, moins de glucose consommé par la mamelle) ont-ils permis à ces animaux d'investir plus tôt dans la fonction de reproduction ?

Valorisation

Article dans une revue internationale à comité de relecture :

Bedere, N., L. Delaby, V. Ducrocq, S. Leurent-Colette, C. Disenhaus. 2016. Towards improved post-partum cyclicity of primiparous dairy cows: effects of genetic merit for production traits under contrasted feeding systems. Journal of Dairy Science 99:1266-1276.

Actes de conférences internationales - Présentations orales

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2015. Towards a better understanding of the effect of genetic merit for milk production on post-partum cyclicity of first lactation dairy cows., in: ADSA-ASAS Joint Annual Meeting. Journal of Animal Science, vol. 93, Suppl.s3 / Journal of Dairy Science, vol. 98, Suppl. 2, Orlando, Florida.

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Toward improved postpartum cyclicity of primiparous dairy cows: Effects of genetic merit for production traits under contrasting feeding systems

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ABSTRACT

Milk genetic merit is known to affect commencement of luteal activity (C-LA) in dairy cows. This effect is considered to be due to energy exported in milk production. The present study aimed to identify and quantify the effects of genetic characteristics [breed and estimated breeding value (EBV) for milk yield and fat and protein contents] and feeding system on C-LA of primiparous cows. From 2006 to 2013, an experiment was conducted on 97 primiparous dairy (Holstein) and 97 primiparous dual-purpose (Normande) cows. Within breed, cows were classified into 2 groups: cows with high EBV for milk yield were included in a "milk group" and those with high EBV for fat and protein contents were included in a "content group." Within breed, exported energy in milk and body weight (BW) loss were similar for both genetic groups. Two grazing-based strategies were used, a high feeding system (maize silage in winter and grazing plus concentrate) and a low feeding system (grass silage in winter and grazing with no concentrate). Interval from calving to C-LA was studied performing survival analyses. Milk progesterone profile, milk yield, and body condition were analyzed using χ^2-test and analysis of covariance. Holstein cows produced more milk (+1,810 kg in the high feeding system and +1,120 kg in the low feeding system) and lost more BW from wk 1 to 14 of lactation (−1.4 kg/wk) than Normande cows, whereas Normande cows had earlier C-LA than Holstein cows. Within breed, cows in the content group had earlier C-LA (associated hazard ratio = 2.0) than cows in the milk group. Body weight at calving and loss from wk 1 to 14 of lactation tended to be associated with later C-LA. Cows in the high feeding system produced more milk (+2,040 kg for the Holstein cows and +1,350 kg for Normande cows) and lost less BW from wk 1 to 14 of lactation (+3.8 kg/wk) than cows in the low feeding system. No effect of feeding system or milk yield was observed on C-LA. Prolonged luteal phases were frequent (18% of cows) and were not associated with either breed or genetic group. Ovarian cycles were longer for Holstein than for Normande cows (+1.7 d) because of a longer luteal phase and a longer interluteal interval. Results of the study could be useful to establish strategies to manage declining reproductive performances at genetic and environmental levels. This study showed that cows with a genetic predisposition to export milk energy through fat and protein contents had earlier C-LA than predisposed to export milk energy through yield.

Key words: dairy cow, cyclicity, genetic merit, primiparous

INTRODUCTION

In dairy cows, milk production and reproduction are concomitant. Reproductive performance has been declining while milk production has been increasing (Friggens et al., 2010). Failure in reproduction causes economic losses, disturbs the working plan (e.g. breeding calendar), and represents a mental load (i.e., the stress of missing estruses) for farmers.

In spring-calving pasture-based systems, cows are under the constraint of a breeding period because feed demand needs to be adjusted to grass supply. The ability of cows to resume normal ovarian cyclicity on time is required and it affects the subsequent steps of the reproductive process (Darwash et al., 1997; Gautam et al., 2010). Abnormal ovarian activity is common in the current dairy cow population: only 60% of Holstein cows have normal cyclicity, and the major abnormal cyclicity pattern is delayed commencement of luteal activity (**C-LA**; Petersson et al., 2006; Windig et al., 2008; Cutullic et al., 2011). In most countries, dairy cattle include a large proportion of primiparous cows because the replacement rate is high (from 20 to 40%; Le Cozler et al., 2008). Lactation number is one of the factors influencing milk production and reproduction. Primiparous cows have lower milk yield and experience

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delayed C-LA compared with multiparous cows (Opsomer et al., 1998; Taylor et al., 2003). When cows are still growing, they have different metabolic status, endocrine responses, and partitioning of nutrients toward organs during their first lactation than during subsequent ones (Taylor et al., 2003; Coffey et al., 2006).

Thirteen to 30% of the observed variance of C-LA is due to genetics and C-LA is unfavorably genetically correlated with milk yield (Veerkamp et al., 2000; Royal et al., 2002b; Petersson et al., 2007). For instance, breeds selected for milk yield have later C-LA than dual-purpose (milk and meat production) breeds. Holstein cows show more abnormal cyclicity profiles than others, especially delayed first ovulation (Petersson et al., 2006; Cutullic et al., 2011; Piccand et al., 2013). However, the deleterious effect of high genetic merit for milk yield on cyclicity is not always clear (Horan et al., 2004; Pollott and Coffey, 2008; Windig et al., 2008).

A key environmental factor affecting dairy cow milk production and reproduction is nutrition (Canfield et al., 1990; Burke and Roche, 2007; Chagas et al., 2008). In early lactation, dairy cows require a large amount of nutrients to ensure milk production. Their intake capacity is too low to fulfill these requirements and they experience a negative energy or protein balance. To cope with this deficit, cows mobilize body reserves. Low body reserves at calving or high mobilization at the beginning of lactation are risk factors for later C-LA. In addition, cows that are too fat at calving experience more abnormal cyclicity patterns (Cutullic et al., 2012). Even though body condition and body condition loss influence C-LA, milk yield per se does not. However, the occurrence of prolonged luteal phase (**PLP**) may be related to milk yield (Royal et al., 2002b; Kafi et al., 2012).

The present study aimed to identify and quantify the effect of breed, genetic merit for milk yield, and feeding system on postpartum cyclicity of primiparous dairy cows. Our hypotheses were that (1) cows with genetic characteristics (breed and genetic merit) in favor of milk yield have deteriorated cyclicity; (2) feeding systems affect milk production and body condition (reserves) and mobilization, which are related to cyclicity. Results of the study could be useful to establish strategies to cope with declining reproductive performances at genetic and environmental levels.

MATERIALS AND METHODS

Animals and Experimental Design

Starting in 2006, an experiment was conducted at the dairy research farm of Le Pin-au-Haras (Normandy, France). At the beginning of the experiment, animals were equally distributed among genetic characteristics (2 breeds, 2 genetic groups) and feeding strategies (2 feeding systems).

A total of 97 primiparous Normande (dual-purpose cows) and 97 primiparous Holstein (dairy cows) were involved in the trial. Within breed, cows were classified into 2 groups according to their EBV for milk yield and fat and protein contents as 2 groups capable of producing the same solid milk quantity in a different manner. The EBV for each trait was evaluated by using a BLUP animal model that included the sire and grandsire's genetic evaluation, the dam's performance over 3 lactations, the classical fixed environmental effects (year, lactation number, calving age, calving month, length of drying off period, and permanent environmental effect) and the feeding system (H. Larroque, INRA UMR 1388 GenPhySE, Toulouse, France; personal communication). For Holstein cows, EBV for milk yield varied between +1,726 and +3,966 kg, EBV for fat content varied between −6.9 and +3.5 g/kg, and EBV for protein content varied between −0.8 and +3.8 g/kg. For Normande cows, EBV for milk yield varied between +948 and +2,815 kg, EBV for fat content varied between −4.2 and +5.7 g/kg, and EBV for protein content varied between −0.8 and +4.7 g/kg. Within breeds and experimental years, nulliparous cows with higher EBV for milk yield than the average and lower EBV for fat and protein contents than the average were classified in the milk group (**MG**). Nulliparous cows with lower EBV for milk yield than the average and higher EBV for fat and protein contents than the average were classified in the content group (**CG**). The other nulliparous animals (high EBV for milk yield and high EBV for fat and protein contents or low EBV for milk yield and low EBV for fat and protein contents) did not enter the experiment. Fifty-two Holstein and 44 Normande were classified in MG and 45 Holstein and 53 Normandewere classified in CG. Table 1 describes the diet fed to cows in each feeding system. In both breeds, 44 cows were managed under a "high" feeding system that enabled high milk yield while limiting body condition loss; and 53 cows were managed under a "low" feeding system that limited milk yield while inducing high body condition loss. Cows remained in their feeding system until they were culled due to lack of pregnancy, severe health problem, or accidental death. Among the 231 primiparous cows that participated in this study, 25 heifers failed to conceive on time to fit the compact calving system, 7 could not be milked, and 5 had severe health problems. Finally, 194 primiparous cows were included in the analyses of the present study.

Table 1. Composition of the diets of the high and low feeding systems during stock, pasture, and dry periods

Feedstuff	Feeding system	
	High	Low
Stock diet		
Maize silage (% of DM)	55	—
Dehydrated alfalfa pellets (% of DM)	15	—
Grass silage (% of DM)	—	50
Haylage (% of DM)	—	48
Concentrates + minerals and vitamins (% of DM)	30	—
Minerals and vitamins (% of DM)	—	2
Pasture feeding		
Rotational grazing (ha/cow)	0.35	0.60
Concentrates (kg)	4.0	—
Minerals and vitamins (kg)	0.25	0.50
Dry period		
Grass silage (% of DM)	100	100

Reproductive Management

The herd was managed under a 3-mo compact calving system (January to March). After calving, uterine involution was checked by rectal palpation 25 to 30 d postpartum. When involution was achieved, AI were performed on spontaneous estruses, if expressed at least 40 d postpartum and during the breeding period (April to June). If cows were expressing new estrus in the 35 d following a service, AI was performed again. Otherwise, ultrasonography was conducted to diagnose pregnancy status. If the first diagnosis was positive, a second diagnosis was performed 60 d after last service.

Health events were recorded throughout the lactation with special attention to reproductive problems (calving difficulties, cesarean, retained placenta, metritis). For cesarean, retained placenta, or abnormal involution, cows were injected with a prostaglandin analog (cloprostenol, Estrumate, Schering-Plough, France). For severe metritis, intrauterine infusion of penicillin G and dihydrostreptomycin (Metrijet, Intervet, Beaucouzé, France) was also used to complete benefits from cloprostenol injection. Anestrus cows did not receive prostaglandin analog to resume ovarian activity.

Sampling and Measurements

Cows were milked twice daily at 0630 and 1600 h. Individual milk yields were recorded by flow meters (Metatron, Westfalia, Germany). Three times a week, fat and protein contents were determined by infrared analyzer (MilkoScan, Foss Electric, Hillerød, Denmark), from individual a.m. and p.m. milk samples. From calving to either 2 wk after the service inducing pregnancy or to 5 wk after the end of the breeding season (i.e., July), morning milk samples were collected on Monday, Wednesday, and Friday, and stored at −20°C for progesterone determination by commercial ELISA kits (Milk Progesterone ELISA, Ridgeway Science Ltd., Lydney, UK). The coefficients of variation between assays for ELISA on 5 ng/mL control samples ranged between 8 and 14% among experimental years.

Body condition score (0–5 scale with 0.25 increments) was evaluated monthly by the same 2 trained assessors, as described by Bazin et al. (1984). Body weight at calving was evaluated during the first 2 d of the first lactation week.

Variables and Computation

Estimation of Energy Exported in Milk. Energy exported in milk was estimated according to Faverdin et al. (2007):

$$\begin{aligned}\text{Milk energy (kJ)} = 7{,}115 \times \text{milk yield} \\ \times \{0.44 + [0.0055\ (\text{fat content} - 40)] \\ + [0.0033\ (\text{protein content} - 31)]\}.\end{aligned}$$

Determination of Luteal Activity. Two progesterone (**P4**) milk concentration thresholds were defined as in Petersson et al. (2006) adapted by Cutullic et al. (2011) to distinguish (1) the baseline level of progesterone in milk from the luteal phase level (threshold 1), and (2) a low luteal phase level from a high luteal phase level (threshold 2). Progesterone values were qualified as follows: negative (<threshold 1), positive (>threshold 2), and intermediate. In short, increases of milk P4 concentrations were considered to be induced by corpus luteum activity if at least 2 consecutive values were not negative and at least 1 was positive. Decreases in milk P4 concentrations were considered to result from luteolysis of the corpus luteum when at least 1 value became negative. These definitions enabled us to identify and distinguish luteal phases from interluteal phases.

Qualification of Progesterone Profiles. For each luteal phase, physiological intervals were computed: C-LA, cycle length [interovulatory interval (**IOI**), luteal phase length (**LUT**), and interluteal interval (**ILI**); for details, see Cutullic et al., 2011]. Ovulation was considered to induce a PLP if the luteal phase lasted longer than 25 d. Ovulation was considered to be delayed if ILI was longer than 12 d. Based on these definitions, P4 profiles were classified as (1) normal, (2) PLP profile (if at least one PLP was observed), (3) delayed (if C-LA >60 d), (4) interrupted (if at least one ovulation of rank >2 was delayed), or (5) disordered

(if luteal activity appeared irregular but could not be included in any abnormality class).

Statistical Analyses

Zootechnical Performance. Effects of experimental factors (breed, genetic group, feeding system, and calving age) on zootechnical performance were studied through analysis of covariance (ANCOVA) using the *lm* procedure of the R statistical package (R Core Team, 2014). Post hoc tests were performed through Tukey's pairwise comparison of the means (*lsmeans* procedure in R). The initial model included associated EBV when the dependent variables were total milk yield over the lactation, average daily milk yield, fat content, and protein content during the first 14 wk in milk, total energy exported in milk over the lactation, and average daily energy exported in milk during the first 14 wk in milk. Year of experiment, calving age group, feeding system, breed, genetic group, and first-order interactions between breed and other experimental factors were also included in the initial model. The final model was determined using a stepwise selection procedure.

Type of P4 Profile and Cycle Length. Chi-squared analyses were performed to test the significance of the difference of the number of animals in each breed, feeding system, calving age, genetic group (global and within each breed), per type of P4 profile. Effects of experimental factors (breed, genetic group, feeding system, and calving age) on IOI, LUT, and ILI were studied by ANOVA (*lm* procedure in R) for the first cycles. For the second to the fourth cycles, similar analyses were performed with the addition of the random (genetic and nongenetic) effect of the cow, using mixed models (*lmer* procedure in R).

C-LA. Commencement of luteal activity was defined as the time between calving and C-LA in days. When no luteal phase was observed before the end of the breeding season (day t of lactation), C-LA was set to t and treated as censored (i.e., the true C-LA was only known to be larger than t). Time variables such as C-LA are classically studied using survival techniques modeling the hazard function. The hazard function $h(t)$ for C-LA is the limiting probability of starting luteal activity at time t given it had not started yet just before t (Kalbfleisch and Prentice, 2002). A proportional hazard model (**PHM**) was assumed: the hazard at t was described as the product of a baseline hazard function $h_0(t)$ describing the overall C-LA and a positive term $\exp\{\mathbf{x}'\beta\}$ describing the influence of explanatory variables on the hazard at time t (Kalbfleisch and Prentice, 2002; Cox, 2007).

To test the validity of the PHM assumption, the raw (Kaplan-Meier) estimate of $S(t)$, the survival function for C-LA, was computed separately for different groups; that is, the 2 breeds (Kaplan and Meier, 1958). The PHM assumption is valid when a plot of the logarithm of $S(t)$ against the $\log(t)$ for different groups leads to parallel lines. From these plots, we concluded there was a need for stratification according to breeds (i.e., a distinct baseline hazard had to be assumed for each breed) and that the within-breed baseline hazard could be well approximated by a Weibull hazard function. The Weibull hazard function is a parametric description of $h_0(t)$ using only 2 parameters (ρ and λ), where $h_0(t) = \lambda\rho(\lambda t)^{\rho-1}$, with distinct Weibull parameters for each breed.

Among the explanatory variables, some are qualitative (e.g., genetic group), some are quantitative and remain constant over time (e.g., EBV), and others are quantitative and time-dependent (e.g., BW). The final stratified Weibull model was determined by stepwise selection:

$$h(t;\, \mathbf{x},\, \mathbf{z}) = h_{0,\text{breed}}(t)\, \exp\{\mathbf{x}'\beta + \mathbf{z}'(t)\varphi\},$$

where $h_{0,\text{breed}}(t)$ is the baseline hazard function for each breed as described above; $\mathbf{x}$ is an incidence vector relating the hazard function to a set of time independent effects, β, including the year of experiment, the presence/absence of calving problems (caesarian, vagina displacement, infectious vaginitis, metritis of grade 3/3, non-delivery), the genetic group, the EBV for milk yield and BW at calving; $\mathbf{z}$ is an incidence vector relating the hazard function to the time-dependent effect of changes in BW. Parameters were estimated by maximum likelihood.

Survival functions for a specific individual with given characteristics specified in the $\mathbf{x}$ and $\mathbf{z}$ vectors were predicted using the following expression:

$$\hat{S}\left(t;\mathbf{x},\mathbf{z}\right) = \int_0^t \hat{h}_{0,\text{breed}}\left(u\right) \exp\left\{\mathbf{x}'\hat{\beta} + \mathbf{z}'\left(u\right)\hat{\varphi}\right\},$$

where $\hat{h}_{0,\text{breed}}$, $\hat{\beta}$, and $\hat{\varphi}$ are the estimated parameters. All survival analyses were performed using the Survival Kit statistical package (Mészáros et al., 2013).

RESULTS

During this experiment, 50 cows experienced calving problems; their production performance was similar to that of healthy cows. However, they had later CLA and

the effect of calving problems was accounted for in the analyses.

Production Performance

Higher Milk Yield and Greater Body Condition Loss for Cows with High Genetic Merit for Milk Yield. Holstein cows produced more milk throughout their lactation (+1,810 kg in the high feeding system and +1,120 kg in the low feeding system, $P < 0.001$; Table 2) than Normande cows. In both breeds, cows in MG produced more milk throughout their lactation than cows in CG (+408 kg, $P < 0.05$, and +350 kg, $P < 0.1$, respectively, for Holstein and Normande cows). Holstein cows had similar fat content ($P = 0.77$) and lower protein content (−2.5 g/kg; $P < 0.001$) over the first 14 wk of lactation than Normande cows. Within breed, cows in MG had lower fat content (−1.8 g/kg, $P < 0.01$, and −2.6 g/kg, $P < 0.001$, respectively, for Holstein and Normande cows) and lower protein content over the first 14 wk of lactation (−1.1 g/kg, $P < 0.01$ for both breeds). Holstein cows exported more energy in milk over the lactation than did Normande cows (+4,430 MJ in the high feeding system and +2,790 MJ in the low feeding system; $P < 0.001$). Cows in MG exported similar amounts of energy as those in CG throughout lactation ($P = 0.84$).

Breed did not affect BCS at calving or BW at calving ($P = 0.36$ and $P = 0.91$, respectively). However, Holstein cows lost more body condition to nadir (−0.8, $P < 0.001$) and experienced greater BW loss over the first 14 wk of lactation (−1.4 kg/wk, $P < 0.01$). Within breed, genetic group did not affect BCS at calving ($P = 0.17$). Holstein cows in MG were heavier at calving than cows in CG (+32 kg, $P < 0.05$), whereas for Normande cows, genetic group did not affect BW at calving ($P = 0.99$). For Holstein cows, genetic group did not affect BCS loss to nadir ($P = 0.82$) whereas for Normande, cows in MG tended to lose more BCS to nadir than cows in CG (−0.25, $P < 0.1$). Within breed, genetic group did not affect BW loss over the first 14 wk of lactation ($P = 0.40$).

Higher Milk Yield and Lower Body Condition Loss for Cows in the High Feeding System. Cows in the high feeding system produced more milk than cows in the low feeding system (+2,040 kg for the Holstein cows and +1,350 kg for Normande cows, $P < 0.001$). They also had higher protein content (+3.5 g/kg, $P < 0.001$) and tended to have higher fat content (+0.6 g/kg, $P < 0.1$). Cows in the high feeding system exported more energy in milk throughout lactation than did cows in the low system (+6,200 MJ for the Holstein cows and + 4,560 MJ for the Normande cows, $P < 0.001$). Feeding system did not affect BCS at calving ($P = 0.24$). However, cows in the high system had greater BW at calving than cows in the low system (15 kg, $P < 0.05$). Cows in the high system lost less BCS to nadir (+0.58, $P < 0.001$) and BW over the first 14 wk of lactation (+3.8 kg/wk, $P < 0.001$) than cows in the low system.

Cyclicity

Later C-LA for Cows with High Genetic Merit for Milk Yield. Commencement of luteal activity ranged from 10 to 205 d postpartum, with a median of 30 d. Two cows had censored C-LA. Breeds were treated as population strata and Figure 1 shows that Holstein cows had later C-LA than Normande cows. In both breeds, cows in CG were 2 times more likely to show C-LA than those in MG ($P < 0.001$; Table 3). In addition, within breed and genetic group, the higher the EBV for milk yield, the lower the chance (or, from a survival analysis point of view, the risk), to show C-LA ($P < 0.05$).

Later C-LA for Cows with Greater BW at Calving and BW Changes. In all breeds and genetic groups, cows with greater BW at calving tended to have a higher risk to show C-LA than those with lesser BW at calving ($P < 0.1$; Table 3). In addition, greater BW losses tended to be associated with lower risk of showing C-LA ($P < 0.1$). However, neither milk yield nor fat and protein contents were associated with C-LA ($P = 0.37$, $P = 0.89$, and $P = 0.95$, respectively; data not shown). Feeding system did not affect the risk to resume luteal activity ($P = 0.80$).

Genetic and Feeding System Affect Ovarian Cycle Length. The length of the first cycle was not affected by breed or genetic group in this study (Figure 2; Table 4). Subsequent cycles were longer for Holstein than for Normande cows (+1.7 d, $P < 0.001$) because of a combination of longer luteal phase (+0.9 d, $P < 0.05$) and longer interluteal phase intervals (+0.6 d, $P < 0.05$). Cows in the low feeding system had shorter first luteal phase (−2.6 d, $P < 0.05$) and shorter first cycles (−2 d, $P < 0.05$) than cows in the high feeding system.

Occurrence of PLP Was Not Affected by Genetics or Feeding System. The proportion of primiparous cows experiencing PLP (18%) was not affected by breed ($P = 0.30$), genetic group ($P = 0.75$ and $P = 0.21$ for Holstein and Normande, respectively), or feeding system ($P = 0.29$).

DISCUSSION

Our results confirmed that primiparous cows have delayed C-LA, consistent with the literature (Opsomer

Table 2. Adjusted milk yield, milk contents, energy in milk, BCS (0–5 scale), and BW over the first 14 wk of lactation or over the lactation (44 wk) for primiparous Holstein and Normande cows, in the milk or the content group, under either the high or low feeding system

	Holstein				Normande										
	Milk		Content		Milk		Content		Model[1]		Significance level[2]				
Item	High	Low	High	Low	High	Low	High	Low	RSE	R^2	B	G	B × G	FS	B × FS
No. of cows	22	30	22	23	20	24	24	29							
Over first 14 wk															
Milk yield (kg/d)	30.9^{c}	21.7^{b}	29.6^{c}	20.4^{b}	21.8^{b}	16.4^{a}	21.5^{b}	16.1^{a}	3.70	0.69	***	†	0.32	***	***
Fat content (g/kg)	37.4^{a}	36.8^{a}	39.2^{b}	38.6^{b}	37.2^{a}	36.6^{a}	39.8^{b}	39.2^{b}	2.54	0.44	0.77	***	0.27	†	0.44
Protein content (g/kg)	30.6^{cd}	27.0^{a}	31.6^{ef}	28.1^{b}	33.1^{g}	29.5^{c}	34.2^{h}	30.7^{de}	1.47	0.75	***	***	0.91	***	0.24
Energy in milk (MJ/d)	91.8^{c}	62.2^{b}	92.0^{c}	62.4^{b}	67.8^{b}	48.9^{a}	68.0^{b}	49.1^{a}	10.44	0.73	***	0.84	0.29	***	***
BCS at calving	3.46^{abc}	3.38^{abc}	3.25^{ab}	3.17^{a}	3.54^{c}	3.46^{bc}	3.57^{c}	3.49^{bc}	0.442	0.35	0.36	*	†	0.24	†
BCS at nadir	2.17^{b}	1.55^{a}	2.11^{b}	1.49^{a}	2.79^{c}	2.16^{b}	3.03^{c}	2.4^{b}	0.496	0.57	***	0.55	*	***	0.58
BC change calving to nadir	-1.27^{b}	-1.85^{a}	-1.21^{b}	-1.79^{a}	-0.70^{c}	-1.28^{b}	-0.47^{c}	-1.05^{b}	0.442	0.53	***	0.53	0.18	***	0.79
BW (kg) in wk 1	619^{b}	604^{bc}	587^{ac}	571^{a}	620^{b}	605^{bc}	622^{b}	607^{bc}	53.0	0.43	0.91	**	*	*	0.60
BW change (kg/wk)	-2.1^{c}	-5.9^{a}	-2.1^{c}	-5.9^{a}	-1.1^{cd}	-4.9^{ab}	-0.4^{d}	-4.2^{b}	2.90	0.42	**	0.40	0.37	***	0.28
Over 44 wk															
Total milk yield (kg)	$7{,}534^{e}$	$5{,}495^{cd}$	$7{,}126^{e}$	$5{,}087^{b}$	$5{,}694^{cd}$	$4{,}346^{a}$	$5{,}343^{bc}$	$3{,}996^{a}$	716.0	0.77	***	**	0.78	***	**
Total energy in milk (MJ)	$22{,}288^{d}$	$16{,}071^{b}$	$22{,}017^{d}$	$15{,}800^{b}$	$17{,}879^{c}$	$13{,}317^{a}$	$17{,}608^{c}$	$13{,}046^{a}$	1,967.0	0.78	***	0.40	0.73	***	**
Total BW change (kg)	35.5^{b}	-22.7^{a}	28.9^{b}	-29.2^{a}	68.1^{c}	9.9^{b}	80.8^{c}	22.6^{b}	51.0	0.37	***	0.54	0.25	***	0.50

[a–g]Adjusted means with different superscript letters are different between breeds, genetic groups, and feeding systems ($P < 0.05$, Tukey's pairwise comparison).

[1]Relative standard error.

[2]Effects of breed (B), genetic group (G), breed × genetic group (B × G), feeding system (FS), breed × feeding system (B × FS).

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; †$P < 0.1$.

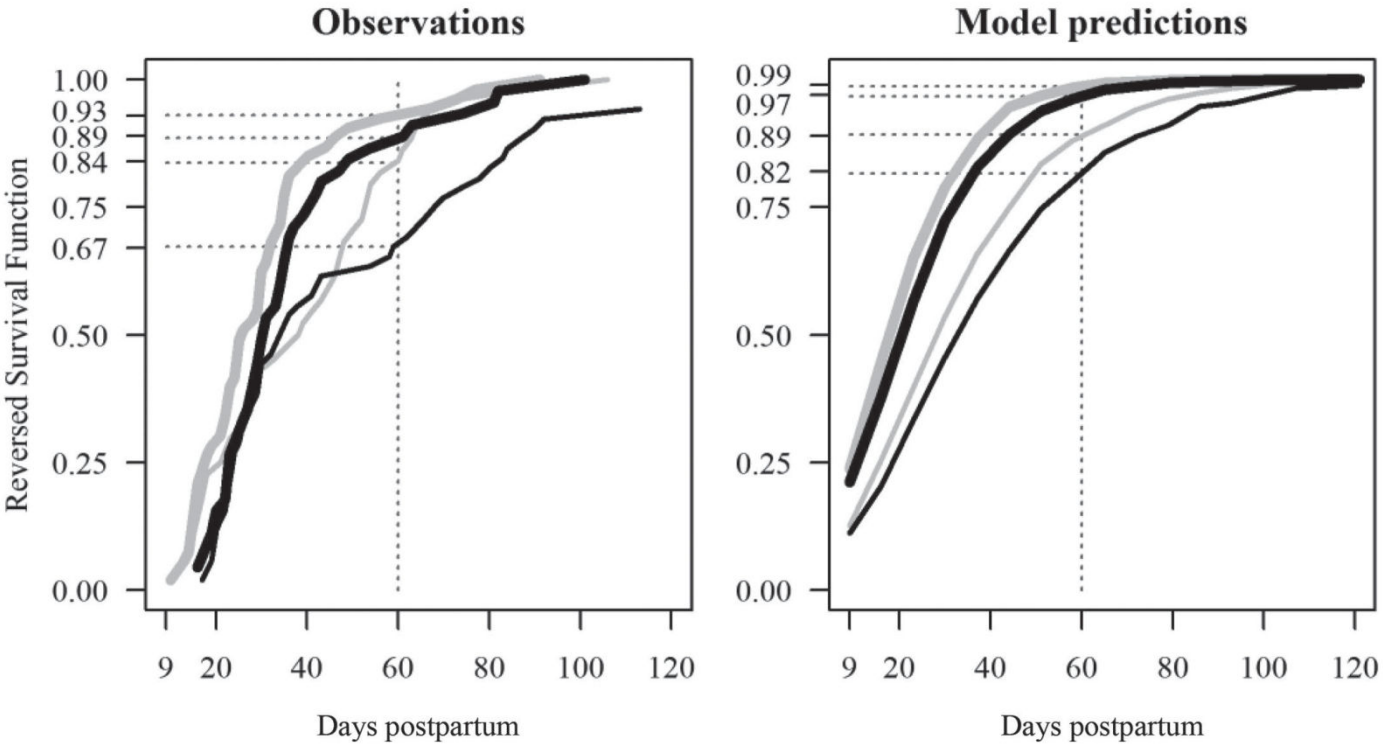

Figure 1. Observed reversed survival function (cumulative proportions) of cows that resumed luteal activity, over the first 120 d postpartum of Holstein (n = 97, black) or Normande (n = 97, gray) cows, belonging either to the milk genetic group (n = 96, thin lines) or to the content genetic group (n = 98, thick lines).

et al., 1998; Taylor et al., 2003; Disenhaus et al., 2009). In this study, we used survival analyses to study time to C-LA. Survival analyses were developed in biomedical and epidemiological statistics and proved suited for small experimental data sets. They allow an assessment of the effect of experimental factors and covariates (Gröhn et al., 1998; Mandonnet et al., 2003). Among survival analyses, we used a proportional hazards model. Simple proportional hazards models have 2 major potential limits: it is not always correct to assume hazards

Table 3. Estimated effect sizes $\left(\hat{\beta}\right)$, associated hazard ratios $\left[HR = \exp\left(\hat{\beta}\right)\right]$, and 95% CI of factors influencing time to commencement of luteal activity (C-LA; n = 194, of which 2 are censored)

Risk factor	Category (%)	Estimate	SE	HR	95% CI	*P*-value
Calving problems						**
No	74	0.54	0.183	1.7	1.2–2.5	
Yes	26	0	—	1.0	—	
Genetic group						***
Milk	49	0	—	1.0	—	
Content	51	0.70	0.216	2.0	1.3–3.1	
Breed × genetic group						0.62
Holstein × Milk	26	−0.15	0.306	0.9	—	
Holstein × Content	23	0	—	1.0	—	
Normande × Milk	23	0.15	—	1.2	—	
Normande × Content	28	0	—	1.0	—	
	(Mean ± SD)					
EBV (milk yield)[1]	0 ± 402	−0.0005	0.00026	—	—	*
BW in wk 1[2]	0 ± 55	0.003	0.0015	—	—	†
BW change	−2 ± 15	0.01	0.005	—	—	†

[1]EBV has a null mean because it was centered within breed and genetic group.

[2]BW has a null mean because it was centered within breed, genetic group, and calving age.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; †$P < 0.1$.

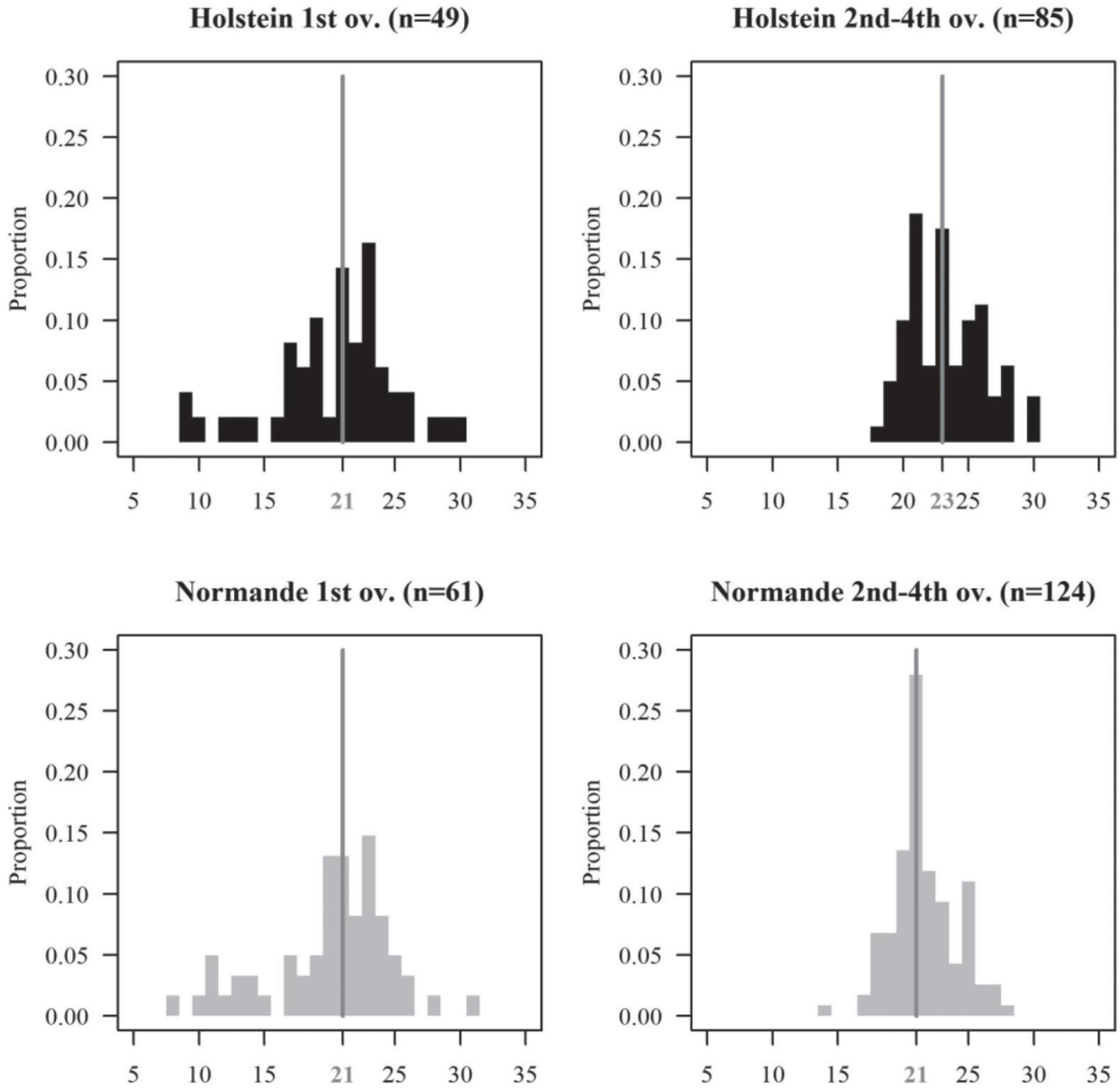

Figure 2. Distributions of cycle length (interovulatory interval, IOI) of the first ovulation (ov.) or for second to fourth ovulations not associated with a service, a prolonged luteal phase, or a delayed ovulation for Holstein and Normande primiparous cows.

to be proportional among individuals and across time. We identified a need for stratification in this study, considering each breed as a sub-population. Hazards were always proportional within breed but not across breeds. In addition, we had time-dependent covariates (e.g., BW) and we used an extension of the PHM that considers covariate changes over time. With these, we made the most complete use of the information available to study risk factors associated with C-LA and were able to quantify their effects.

As expected, Holstein cows had later C-LA than Normande cows. In other studies, C-LA occurred later for breeds selected for milk yield compared with dual-purpose breeds (Petersson et al., 2006; Disenhaus et al., 2009; Piccand et al., 2013). Commencement of luteal activity was mainly affected by genetic characteristics,

Table 4. Adjusted cycle lengths (interovulatory interval, IOI; luteal phase length, LUT; interluteal phase interval, ILI) for first or second to fourth ovulations with a normal type of progesterone pattern and not associated with a service for primiparous Holstein and Normande cows, in the milk or content group, under either the high or low feeding system

	Holstein				Normande											
	Milk		Content		Milk		Content		Model[1]			Significance level[2]				
Item	High	Low	High	Low	High	Low	High	Low	RSE	R^2	σ_a^2	σ_e^2	B	G	B × G	FS	B × FS
No. of first ovulations (ov)[3]	11	11	15	12	10	15	14	22									
IOI: first ov	20.5	19.4	21.6	20.5	20.6	19.6	20.9	19.9	4.42	0.10	—	—	0.76	0.41	0.66	*	0.24
LUT: first ov	15	12.4	15.8	13.1	13.8	14.1	13.9	14.2	4.03	0.12	—	—	0.17	0.52	0.68	*	†
ILI: first ov	6.2	6.7	6.3	6.8	6.2	6.1	6.4	6.3	—	—	—	—	0.49	0.98	0.76	0.7	0.45
No. of second to fourth ov[3]	13	20	29	23	25	31	28	40									
IOI: second to fourth ov	24.2	23.4	23.6	22.8	22.3	21.8	21.8	21.3	—	—	0.98	5.94	***	0.39	0.95	0.15	0.72
LUT: second to fourth ov	17.8	16.5	17.2	15.9	16	15.9	15.7	15.7	—	—	2.75	4.55	*	0.39	0.71	†	0.18
ILI: second to fourth ov	6.3	6.9	6.3	6.9	6.3	5.9	6	5.6	—	—	1.11	1.69	*	0.93	0.56	0.87	†

[1]Relative standard error.

[2]Effects of breed (B), genetic group (G), breed × genetic group (B × G), feeding system (FS), breed × feeding system (B × FS).

[3]For first ovulations, ANOVA were used; for second to fourth ovulations, mixed models were used with animal as random variable.

***$P < 0.001$; *$P < 0.05$; †$P < 0.1$.

which is consistent with the fact that C-LA has a moderate heritability, ranging from 0.13 to 0.30 (Veerkamp et al., 2000; Royal et al., 2002b; Petersson et al., 2007). As expected, cows with the highest EBV for milk yield had later C-LA (Pollott and Coffey, 2008; Windig et al., 2008; Garmo et al., 2009). In addition, exporting milk energy through higher fat and protein contents was associated with earlier C-LA. To our knowledge, the relationship between the way of exporting energy in milk and reproduction performance of primiparous cows has not previously been investigated. These findings reinforce the genetic and phenotypic relationships between milk yield and C-LA. Further investigations are needed to describe the genetic and biological differences between cows exporting energy in milk through higher yield compared with through higher fat and protein contents. We could hypothesize that selecting on milk fat and protein yield is desirable for animals' robustness and durability of dairy systems.

In this experiment, cows were fed ad libitum even though the 2 feeding systems differed in nutrient intake. The low feeding system was very restrictive, causing insufficient energy intake, associated with a large BCS loss and a limited milk yield. In addition, this contrast between the high and low feeding systems partly disentangled the concomitant effects of milk yield and body reserve mobilization. Cows in the high feeding system produced greater milk yield and lost less BCS than did those in the low feeding system. We found no effect of feeding system on cyclicity, even with the contrast created by these 2 feeding systems. This is consistent with most studies, in which authors also found a relationship between cyclicity and intake (Burke and Roche, 2007), energy balance (Chagas et al., 2008; Pollott and Coffey, 2008), or protein balance (Bruckental et al., 2000; Law et al., 2009). As expected, we found that later C-LA was associated with greater BW at calving and greater BW loss. However, we found no association with milk yield (Friggens et al., 2010).

In this study, cycle length, luteal phase length, and interluteal phase interval of the first cycle were not affected by genetics and feeding system, which is consistent with the literature (Pollott and Coffey, 2008; Windig et al., 2008; Gilmore et al., 2011). However, second to fourth ovulation cycles were longer in Holstein cows than in Normande cows. Previous studies also reported that Holstein cows have longer ovarian cycles than other breeds (Disenhaus et al., 2009; Piccand et al., 2013). Feeding system did not affect the proportion of cows with a PLP type of P4 profiles. However, PLP are known to be associated with milk yield (Royal et al., 2002a; Kafi et al., 2012) and to BCS at calving and loss (Friggens et al., 2010). Further investigations are

needed to clarify the relationship between the occurrence of PLP and milk yield or BCS.

To conclude, this study on primiparous dairy cows confirmed that C-LA was strongly related to genetic merit for milk yield. Our results suggest it could be desirable to select cows for production traits through higher fat and protein contents rather than higher milk yield to reach durable systems. Cows with higher fat and protein contents produce enriched milk, which can be cost effective for the dairy industry, and they are more likely to reproduce on time, which is desirable for their robustness to farming system constraints.

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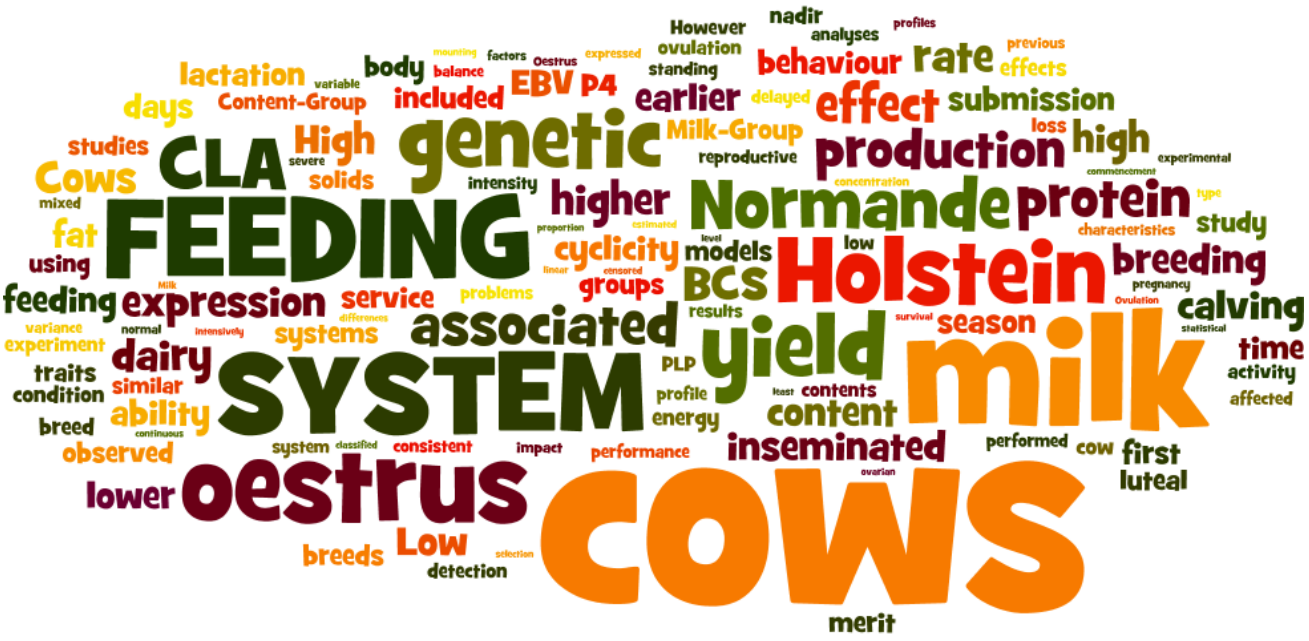
CHAPTER 4: Ability of dairy cows to be inseminated according to genetics and breeding system.



Objectifs

Ce second chapitre de résultats a pour objectif de confirmer sur l'ensemble des données les effets de la race, du type génétique et du régime alimentaire sur la cyclicité observés chez les primipares. Il a aussi pour but de présenter les effets de ces facteurs sur l'intensité des chaleurs observées et l'intervalle de la mise à la reproduction à la première insémination artificielle (IA). Les hypothèses testées sont (1) les vaches ayant des caractéristiques génétiques (race, type et index génétique) favorables à la production laitière ont une aptitude à être inséminée dégradée (cyclicité postpartum, expression des chaleurs, intervalle mise à la reproduction – 1ère IA) ; (2) le régime alimentaire affecte la production laitière et la gestion des réserves corporelles, ce qui est lié à cette aptitude.

L'essentiel

Les vaches de race Holstein ont produit plus de lait que les vaches Normande (+2 294 kg dans le système Haut et +1 280 kg dans le système Bas). Elles ont aussi perdu plus d'état (-1,00 point dans le système Haut et -0,80 point dans le système Bas). Les vaches Normande ont une meilleure aptitude à être inséminée grâce à un retour de cyclicité plus précoce, une moindre occurrence de PLP, et un délai mise à la reproduction - 1ère IA plus court que les vaches Holstein.

Dans les 2 races, les vaches du type Lait ont produit plus de lait que celles du type Taux (+764 kg pour les vaches Holstein et +649 kg pour les vaches Normande). En race Holstein, le type

génétique n'a pas eu d'effet sur l'état des réserves corporelles au vêlage ni sur leur mobilisation. En race Normande, les 2 types génétiques ont un état corporel identique au vêlage et les types Lait mobilisent plus de réserves que les types Taux. Les vaches du type Lait sont cyclées plus tardivement que celles du type Taux. L'occurrence de PLP et le taux de détection des ovulations sont similaires entre les deux types génétiques. Le type génétique n'a pas eu d'effet sur l'intervalle mise à la reproduction - 1ère IA, cependant plus l'index génétique de production laitière est élevé, plus cet intervalle est allongé.

Les vaches du système Haut ont produit plus de lait que celles du système Bas (+2 495 kg pour les vaches Holstein et +1 481 kg pour les vaches Normande). Le système alimentaire n'a pas eu d'effet sur l'état des réserves corporelles au vêlage chez les vaches Holstein. Par contre, les Normande du système Haut ont un état corporel supérieur à celles du système Bas (+0,40 points). Dans les 2 races, les vaches du système Haut ont un état corporel minimum plus élevé que celles du système Bas (+0,40 points chez les vaches Holstein et +0,60 points chez les vaches Normandes). Le système alimentaire n'a pas eu d'effet sur le délai de reprise de cyclicité, ni sur l'occurrence de PLP, ni sur l'intervalle entre la mise à la reproduction et la 1ère IA. Les vaches du système Bas ont exprimé plus intensément leurs chaleurs que celles du système Haut. Cet effet s'explique par la différence de production laitière au moment des chaleurs. Néanmoins, les vaches avec un taux protéique plus élevé, et vraisemblablement un meilleur bilan énergétique, ont une cyclicité plus précoce, et un intervalle mise à la reproduction - 1ère IA plus court.

Cette étude montre clairement que les vaches au potentiel de production le plus élevé (vaches Holstein de type Lait) sont celles dont l'aptitude à être inséminée est la plus dégradée. Cet effet est aggravé par un régime restrictif. Une acquisition précoce et de bonne qualité de la cyclicité postpartum est le principal déterminant de l'aptitude à être inséminée. A potentiel de production de matières utiles identique, il semble prometteur de sélectionner les vaches pour les taux butyreux et protéique plutôt que pour la production laitière afin d'améliorer l'aptitude à être inséminée. Cela conduirait à une acquisition de cyclicité plus précoce sans augmenter l'occurrence de PLP et sans altérer l'expression des chaleurs. Ces conclusions concernent l'aptitude à être inséminée des vaches laitières, les effets de ces facteurs sur l'aptitude à assurer la gestation doivent aussi être étudiés. Concernant leurs trajectoires adaptatives : les vaches du type Lait produisent plus de lait et mobilisent autant que celles du groupe Taux. Les avantages du type Taux ne sont pas systématiques, ce qui suggère un potentiel découplage des étapes de la reproduction. Ces caractéristiques génétiques de production en faveur de la cyclicité et sans effet sur les chaleurs sont-elles bénéfiques à la fertilité ?

Valorisation

Article dans une revue internationale à comité de relecture :

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animal

Ability of dairy cows to be inseminated according to breed and genetic merit for production traits under contrasting pasture-based feeding systems

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Strong genetic selection on production traits is considered to be responsible for the declined ability of dairy cows to ensure reproduction. The present study aimed to quantify the effect of genetic characteristics (breeds and genetic merit for production traits) and feeding systems (FS) on the ability of dairy cows to be inseminated. An experiment was conducted during 9 years on Normande and Holstein cows assigned to contrasted pasture-based FS. Diets were based on maize silage in winter and grazing plus concentrate in spring in the High FS; and on grass silage in winter and grazing with no concentrate during spring in the low FS. Within breed, cows were classified into two genetic groups with similar estimated breeding values (EBV) for milk solids: cows with high EBV for milk yield were included in a Milk-Group and those with high EBV for fat and protein contents were included in a Content-Group. Holstein produced more milk throughout lactation than Normande cows (+2294 kg in the High FS and +1280 kg in the Low FS, $P < 0.001$*) and lost more body condition to nadir (−1.00 point in the High FS and −0.80 kg in the Low FS,* $P < 0.001$*). They also showed a poorer ability to be inseminated because of both a delayed commencement of luteal activity (CLA) and delayed first service (more days from start of the breeding season to first service, DAI1). Cows in the Milk-Group produced more milk than cows in the Content-Group, but milk solids production was similar. Cows in the Content-Group had earlier CLA than cows in the Milk-Group (*$P < 0.01$*). Genetic group neither affected ovulation detection rate nor DAI1. Within breed and FS, cows with high genetic merit for milk yield had later CLA and DAI1. Cows in the High FS produced more milk and lost less condition to nadir than cows in the Low FS. FS did not affect dairy cows' ability to be inseminated. However, cows with higher milk protein content, and presumably better energy balance, had earlier CLA (*$P < 0.01$*) and DAI1 (*$P < 0.10$*). In addition, higher milk yield was associated with poorer ovulation detection rate and oestrus intensity (*$P < 0.05$*). The study showed that at similar EBV level for milk solids, selection for increased milk fat and protein content resulted in improved cyclicity and similar oestrous expression and submission rates compared with selection for increased milk yield.*

Keywords: cyclicity, oestrus, first service, genetic merit, energy status

Implications

During the past decades, reproductive performance of dairy cows has been declining, whereas milk production genetic level was increasing. Our study aimed at improving our understanding of the effect of breed, genetic merit for production traits and feeding system (FS) on their ability to be inseminated. Our results suggest that cows selected for production through higher fat and protein contents would resume ovarian cyclicity on time, express the same oestrus behaviour and achieve the same submission rate than cows selected for milk production. If their fertility is preserved, these cows would be more robust to more durable systems.

Introduction

The ability of cows to resume normal ovarian cyclicity on time impacts the subsequent steps of the reproductive process (Gautam *et al.*, 2010). Abnormal ovarian activity is common in the current dairy cows' population (40% of the Holstein cows), with delayed commencement of luteal activity (CLA) and prolonged luteal phases (PLP) commonly reported (Petersson *et al.*, 2006; Windig *et al.*, 2008). In total, 13% to 30% of the observed variance in the CLA is due

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to genetic factors and CLA is unfavourably genetically correlated to milk yield. Also, the occurrence of PLP may be related to high milk yield (Royal *et al.*, 2002; Petersson *et al.*, 2007; Kafi *et al.*, 2012). However, the deleterious effect of high genetic merit for milk yield on cyclicity is not always clear (Royal *et al.*, 2002; Windig *et al.*, 2008). Low body reserves at calving or large mobilisation at the beginning of lactation are risk factors for CLA. Also, cows that are too fat at calving experience more abnormal cyclicity patterns (Cutullic *et al.*, 2012).

Once cyclicity is established, the ability of dairy cows to express oestrus is crucial to enable insemination and pregnancy. However, there is a large variability in both duration and intensity of oestrus (Kerbrat and Disenhaus, 2004; Sveberg *et al.*, 2015). Very few studies exist on oestrus expression in dairy cows due to the difficulty to accurately measure this trait. Oestrus expression is unfavourably associated with milk yield, low body condition score (BCS) in early lactation or large body condition (BC) loss (Cutullic *et al.*, 2012; Madureira *et al.*, 2015). Studies on oestrus expression between breeds are not in concordance. Some studies show that Holstein cows express less specific oestrus behaviour than dual-purpose cows (Cutullic *et al.*, 2009; Sveberg *et al.*, 2015), whereas in other studies the opposite was observed (Løvendahl and Chagunda, 2010). FS are known to affect oestrus intensity and duration, because of their effect on milk yield rather than on energy balance (Cutullic *et al.*, 2011; Gilmore *et al.*, 2011).

In compact calving systems, the ability to be inseminated can be studied as days from the start of the breeding season to first service or submission rate (proportion of inseminated cows) at a given time after the start of the breeding season. Genetic characteristics are known to impact submission rate: it is lower for Holstein cows than for dual-purpose breeds (Dillon *et al.*, 2003; Walsh *et al.*, 2008). High genetic merit for production traits is associated with low submission rate (Fulkerson *et al.*, 2001). Submission rate is also affected by low BCS and severe BCS loss (Buckley *et al.*, 2003; Roche *et al.*, 2007).

The present study aimed to assess and measure the effects of breed, genetic merit for production traits, and FS on the ability of dairy cows to be inseminated through its components (cyclicity, oestrus, submission to first service). Our hypotheses were that (i) high estimated breeding values (EBV) for production traits is unfavourably associated with this ability; (ii) FS, through their impacts on milk yield and BC, affect this ability. The results of this study should enhance our understanding on how to improve reproductive performance of dairy cows by identifying genetic and environmental strategies that could cope with their potential decline.

Material and methods

Experimental design

From 2006 to 2014, an experiment was conducted at the dairy research farm of Le Pin-au-Haras (48.448N, 0.098E, Normandy, France). Dairy cows were equally distributed among genetic characteristics (two breeds, two genetic groups) and two FS. A total of 296 Normande (dual-purpose cows) and 240 Holstein (dairy cows) were involved in the trial. Within breeds cows were classified into two groups according to their EBV for milk yield, fat and protein contents as two groups globally producing the same milk solids quantity with different genetic characteristics: cows with high EBV for milk yield were included in a 'Milk-Group' and those with high EBV for fat and protein contents were included in a 'Content-Group' (more details in Supplementary Material S1). There were 116 Holstein and 147 Normande managed under a 'High' FS that enabled high milk yield while limiting BC loss; and 124 Holstein and 149 Normande managed under a 'Low' FS that limited milk yield while inducing a large BC loss (further information on diets can be found in Supplementary Table S1). Cows were randomly assigned to their FS and remained in it until they were culled due to lack of pregnancy, severe health problem or accidental death. Among the 536 cows involved in this study, 15 could not be milked and 21 had severe health problems. As they were not related to any experimental factor, they were removed from the study. Finally, 500 cows including 207 primiparous cows were included in the analyses of the present study.

Sampling and measurements

Cows were milked twice daily at 0630 and 1600 h. Individual milk yields were recorded by flow meters (Metatron, Westfalia, Germany). Fat and protein contents were determined three times a week by IR analyser (MilkoScan™; Foss Electric, Hillerød, Denmark) from individually preserved milk samples from the two time points. Milk solids were calculated by multiplying milk yield to fat content plus protein content. Milk progesterone (P4) concentration was determined using commercial ELISA kits (Ridgeway Science Ltd., Gloucestershire, England). Morning milk samples were taken from calving to either 2 weeks after service inducing pregnancy or to the end of July, on Monday, Wednesday and Friday and stored at −20°C. The coefficients of variation between assays on 5 ng/ml control samples ranged between 8% and 14% among experimental years. BCS (0 to 5 scale with 0.25 increments) was evaluated monthly by the same three trained assessors as described by Bazin *et al.* (1984).

Cyclicity parameters

Milk P4 concentration was used to compute ovarian physiological intervals: concentration CLA, cycle length, luteal phase length (LUT) and inter-luteal interval (ILI; for details, see Cutullic *et al.*, 2011).

PLP was diagnosed if LUT lasted longer than 25 days. Ovulation was considered to be delayed if ILI lasted longer than 12 days. Based on these definitions, P4 profiles were classified as normal, PLP (if at least one PLP was observed), delayed (if CLA >50 days), interrupted (if at least one ovulation of rank >2 was delayed) or disordered (if luteal activity appeared irregular, but could not be included in any other previous abnormality class).

Oestrus parameters

Oestrus signs were recorded from April to June, by experienced staff observing the cows five times a day during quiet periods (0620, 1000, 1330, 1550 and 2100 h). A standardised recording procedure was used: it classified oestrus behaviours in standing behaviour (standing to be mounted and mounting head-side on), mounting behaviour and discrete behaviour (sniffing/licking the vulva of another cow, chin resting and caressing, restless, mooing and milk yield drop). Tail paint was used as an aid to oestrus detection. Using the P4 profiles, it was possible to separate observed oestrus into true (observed during ovulatory phases) or false oestrus (observed during luteal phases) and to identify undetected ovulation in order to estimate ovulation detection rate. The presence of another cow in oestrus is a well-known risk factor of oestrus expression. To take it into account, we considered there was another cow (0/1) in oestrus if there was at least one cow in oestrus (detected or expected) on the same or previous day.

Reproductive management

The herd was managed under a 3 months compact calving system (January to March). After calving, uterine involution was checked by rectal palpation 25 to 30 days *postpartum*. When involution was achieved, artificial inseminations were performed on spontaneous oestrus, if expressed at least 40 days *postpartum* and during the breeding period (April to June). If cows were not expressing new oestrus in the 35 days following a service, ultrasonography was conducted to diagnose pregnancy status. If the first one was positive, a second diagnosis was performed 60 days after last service.

Health events were recorded throughout the lactation with emphasis on reproductive problems (calving difficulties, caesarean, retained placenta and/or metritis). During this experiment, 107 cows (21%) experienced reproductive problems. Due to their potential effects on subsequent reproductive performance, the occurrence of calving problems was accounted for in the analyses.

Statistical analyses

All initial models included the fixed effects of year of experiment, parity (as 1, 2 or $\geqslant$3), FS, breed, genetic group within breed, first-order interactions between experimental factors, and the random (genetic and non-genetic) effect of the cow. Effects were included in the final models if their *P*-value was <0.1.

Production performance. Production performance was studied using linear mixed models. In this case, final models also included the effect of the associated EBV as a continuous variable when the dependent variables were milk yield, fat and protein content or yield. All linear mixed model analyses were performed using the *lmer* procedure of the R statistical package (R Core Team, 2016).

Type of P4 profile. Types of P4 profile were studied using generalised (logistic) linear mixed models. A dichotomous variable was created for each type of P4 profile (e.g. Normal profile: 0/1). All generalised mixed models were performed using the *glmer* procedure and the *logit* link function of the R statistical package (R Core Team, 2016).

Ovulation detection. Ovulation detection and oestrus expression were studied using generalised (logistic) linear mixed models. A dichotomous variable was created for each type of oestrus expression (e.g. standing behaviour: 0/1). Final models included the fixed effects of the presence of another cow in oestrus (0/1), observation during the breeding season (0/1) and the effect of milk yield production centred within FS. The estimation of variance components failed presumably because of lack of information or over-parametrisation. As an alternative, an empirical Bayesian approach was implemented and values were estimated using uninformative Wishart priors. The robustness of the other estimated effects to this choice of animal variance were checked by looking at their value when this prior was multiplied or divided by 2. All Bayesian mixed models were performed using the *blmer* procedure of the R statistical package (R Core Team, 2016).

Commencement of luteal activity and days from start of the breeding season to first service. Effects of experimental factors on the time from calving to CLA in days, and on the time from the start of the breeding season to first service in days (DAI1) were studied using survival analyses (Weibull hazard functions). Breeds needed to be treated as population strata for all survival analyses (more details in Bedere *et al*., 2016). When no luteal phase was observed before the end of the breeding season (day t_1 of lactation), CLA was set to t_1 and treated as censored. The final model for CLA included time independent fixed effects of significant experimental factors, year of experiment, parity, calving problems indicators, EBV for milk yield as a continuous variable, and days in milk at turnout as continuous variable; the time-dependent effect of protein content (changing weekly), and the random effect of the cow. When no artificial insemination (AI) was performed before the end of the breeding season (day t_2 from possible AI), DAI1 was set to t_2 and treated as censored. The final model for DAI1 included time independent fixed effects (year of experiment, parity, calving problems, EBV for milk yield, type of P4 profile and type of oestrus behaviour), the time-dependent effect of protein content and the random effect of the cow. For DAI1, the estimation of variance components failed, probably because of a too limited number of records. Animal variance was fixed at 0.49 assuming a loggamma distribution for this effect (a common practice in frailty models) with parameter γ equal to 0.4. The robustness of the models was tested by comparing the results with an animal variance equal to 0.22 ($\gamma = 0.2$) and 1.64 ($\gamma = 0.8$), and the other results were similar. All survival analyses were performed using the *Survival Kit* statistical package (Mészáros *et al*., 2013).

Results

Production performance

According to genetic characteristics. Holstein produced more milk (+2294 kg in the High FS and +1280 kg in the Low FS, $P < 0.001$, Table 1) and milk solids throughout lactation (+120 kg in the High FS and +62 kg in the Low FS, $P < 0.001$) than Normande cows. Over the 14 1st weeks of lactation, Holstein had lower fat (−1.0 g/kg, $P < 0.01$) and protein content (−2.5 g/kg, $P < 0.001$) than Normande. In both breeds, cows in the Milk-Group produced more milk than cows in the Content-Group (+764 kg for Holstein and +649 kg for Normande cows, $P < 0.001$). Milk solids production was not different between genetic groups within breed ($P = 0.17$ for Holstein and $P = 0.12$ Normande cows). Cows in the Milk-Group had lower fat content (−2.1 g/kg for Holstein and −2.7 g/kg for Normande cows, $P < 0.001$) and lower protein content (−1.3 g/kg for Holstein, $P < 0.01$, and −1.5 g/kg for Normande, $P < 0.001$).

Holstein cows had a lower BCS than Normande cows at calving (−0.70 points in the High FS, $P < 0.001$, and −0.35 in the Low FS, $P < 0.01$), at nadir (−1.00 points in the High FS and −0.80 points in the Low FS, $P < 0.001$) and at the end of lactation (−1.05 in the High FS and −0.75 in the Low FS, $P < 0.001$). Normande cows in the Milk-Group had a lower BCS at nadir than cows in the Content-Group (−0.25 points, $P < 0.01$) because of a larger loss to nadir (−0.20 points, $P < 0.01$). They also had a lower BCS at the end of lactation (−0.20 points, $P < 0.05$) despite a similar gain from nadir ($P = 0.15$). For Holstein cows, BCS was not affected by genetic groups ($P = 0.99$ at nadir, $P = 0.97$ for loss to nadir, $P = 0.83$ at the end of lactation).

According to feeding system. Cows in the High FS produced more milk (+2495 kg for Holstein and +1481 kg for Normande, $P < 0.001$) and more milk solids (+168 kg for Holstein and +109 kg for Normande, $P < 0.001$) than those in the Low FS. Holstein cows had similar BCS at calving in the two FS ($P = 0.53$) and Normande cows in the High FS had higher BC at calving than in the Low FS (+0.40 points, $P < 0.001$). Cows in the High FS had a higher BCS at nadir (+0.40 for Holstein and +0.60 for Normande, $P < 0.001$) and at the end of lactation (+0.45 for Holstein and +0.70 for Normande, $P < 0.001$). More information on BW and plasma concentration of glucose, non-esterified fatty acids and urea can be found in Supplementary Table S2.

Cyclicity

Commencement of luteal activity. CLA ranged from 10 to 205 days, with a median of 30 days. Three cows had censored CLA. Holstein cows had later CLA than Normande cows (Figure 1). In both breeds, cows in the Content-Group were 1.7 times more likely to show CLA at a given time than those in the Milk-Group ($P < 0.01$; Table 2). In other words, cows in the Content-Group had earlier CLA than cows in the Milk-Group. Within breed and genetic groups, higher EBV for milk yield was associated with later CLA ($P < 0.01$). Time to CLA was not affected by FS ($P = 0.15$). However, higher milk protein content was associated to earlier CLA ($P < 0.01$). Second lactation cows had earlier CLA than primiparous and older cows ($P < 0.001$). Cows that had later day to turnout had earlier CLA than others ($P < 0.001$).

Type of P4 profile. Holstein cows had a lower proportion of normal P4 profiles than Normande cows (−22 points, $P < 0.001$; Table 3) because they had more PLP (+8 points, $P < 0.05$). Holstein cows seemed to have more delayed profiles (+13 points, $P = 0.26$), but this effect was due to individual deviation. The proportion of cows with normal P4 profiles was neither affected by genetic groups ($P = 0.14$) nor by FS (0.31).

Oestrus expression and detection

More ovulations occurred in Normande cows than in Holstein cows and for both breeds there were more ovulations in the Content-Group than in the Milk-Group (Table 4). Ovulation detection rate was about 70% and was similar between breeds ($P = 0.15$), genetic groups ($P = 0.14$) and FS ($P = 0.12$). However, Holstein cows expressed more intensively oestrus than Normande cows (+7 points of standing behaviour, $P < 0.001$, balanced by −5 points of mounting behaviour, $P < 0.05$). Cows in the Low FS expressed more intensively oestrus than cows in the High FS (+13 points of standing behaviour, $P < 0.001$). Interestingly, when milk yield of the ovulation week was added to the model, there was no residual FS effect (0.53; data not shown). This proved that observed differences in oestrus intensity between FS was mainly due to differences in milk yield. Consistently, we observed a continuous effect of milk yield within FS: the greater milk yield associated with week of ovulation reduced the chances to detect the proportion of standing oestrus behaviour and ovulation ($P < 0.05$).

Days to first service

DAI1 ranged from 1 to 97 days, with a median of 20 days. In total, 31 cows had censored DAI1 with an averaged censoring time of 73 days. Holstein cows had later DAI1 than Normande cows. Submission rate at 21 days was 55% for Holstein and 59% for Normande cows (insemination on true oestrus) and 92% and 96%, respectively, at the end of the breeding season (Figure 2). DAI1 was similar in both genetic groups ($P = 0.79$) and FS ($P = 0.51$). However, higher EBV for milk yield was associated with later DAI1 ($P < 0.05$; Table 5). Cows with higher milk protein content during the breeding season tended to be inseminated earlier ($P < 0.10$). Primiparous cows were inseminated earlier in the breeding season than multiparous cows (associated hazard ratio (HR) = 1.7; $P < 0.01$). Cows without calving problems were inseminated earlier than others (associated HR = 1.6; $P < 0.05$). Cows with normal cyclicity were inseminated earlier than cows with any type of abnormal cyclicity pattern (associated HR = 3.6; $P < 0.001$). Cows expressing standing or mounting behaviour were inseminated earlier than cows

Table 1 *Adjusted production performance over the first 14 lactation weeks or over the whole lactation (44 weeks) for Holstein and Normande cows, in the milk- or the content-group, under either the high or low feeding system*

	Holstein				Normande				Model		Significance levels[2]			
	Milk-Group		Content-Group		Milk-Group		Content-Group							
	High	Low	High	Low	High	Low	High	Low	σ_a[1]	σ_e[1]	B	B:G	FS	B × FS
Number of lactations	41	61	60	55	73	57	67	86						
Over first 14 weeks														
MY (kg/day)	37.6^{f}	25.6^{d}	35.4^{f}	23.5^{c}	28.1^{e}	20.6^{b}	26.1^{d}	18.7^{a}	1.62	3.16	***	***	***	***
Fat content (g/kg)	36.8^{a}	37.3^{a}	38.9^{bc}	39.5^{cd}	37.5^{a}	38.0^{ab}	40.2^{de}	40.7^{e}	1.36	2.03	**	***	*	0.87
Protein content (g/kg)	29.5^{bc}	27.8^{a}	31.0^{de}	29.0^{b}	31.9^{f}	30.2^{cd}	33.9^{g}	31.2^{ef}	0.60	1.27	***	***	***	0.28
Milk solids (g/day)	2480^{d}	1664^{b}	2436^{d}	1620^{b}	1938^{c}	1392^{a}	1902^{c}	1356^{a}	111.4	207.4	***	0.26	***	***
BCS at calving	3.05^{abc}	2.90^{ab}	2.95^{ab}	2.80^{a}	3.60^{d}	3.20^{bc}	3.70^{d}	3.30^{c}	0.258	0.402	***	0.13	***	**
BCS at nadir	1.95^{bc}	1.50^{a}	1.90^{b}	1.50^{a}	2.80^{e}	2.20^{c}	3.05^{f}	2.45^{d}	0.298	0.323	***	**	***	+
BC loss (nadir-calving)	-1.20^{b}	-1.55^{a}	-1.20^{b}	-1.55^{a}	-0.65^{c}	-1.05^{b}	-0.50^{d}	-0.85^{c}	0.195	0.320	***	**	***	0.73
Over 44 weeks														
Total MY (kg)	8857^{f}	6362^{d}	8093^{e}	5598^{c}	6506^{d}	5024^{b}	5857^{c}	4376^{a}	356.7	620.2	***	***	***	***
Total milk solids (kg)	584^{d}	416^{b}	568^{d}	401^{b}	463^{c}	354^{a}	448^{c}	338^{a}	23.1	43.3	***	**	***	***
BCS end of lactation	2.40^{b}	2.00^{a}	2.35^{b}	1.90^{a}	3.30^{d}	2.60^{bc}	3.55^{d}	2.80^{c}	0.293	0.340	***	**	***	**
BC gain (end-nadir)	+0.50	+0.50	+0.50	+0.45	+0.45	+0.40	+0.45	+0.45	0.080	0.325	0.21	0.78	0.36	0.31

BCS = body condition score; BC = body condition; MY = milk yield.
[a,b,c,d,e,f]Distinguish adjusted means that are different between breeds, genetic groups, and feeding systems ($P < 0.05$, Tukey's pairwise comparison).
[1]Standard deviation of the random terms, animal genetic and non-genetic effect (σ_a; assuming uncorrelated animal effects) and error (σ_e).
[2]Effects of breed (B), genetic group within breed (B:G), feeding system (FS), breed × Feeding system (B × FS).
Significant levels: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$; $^{+}P < 0.1$.

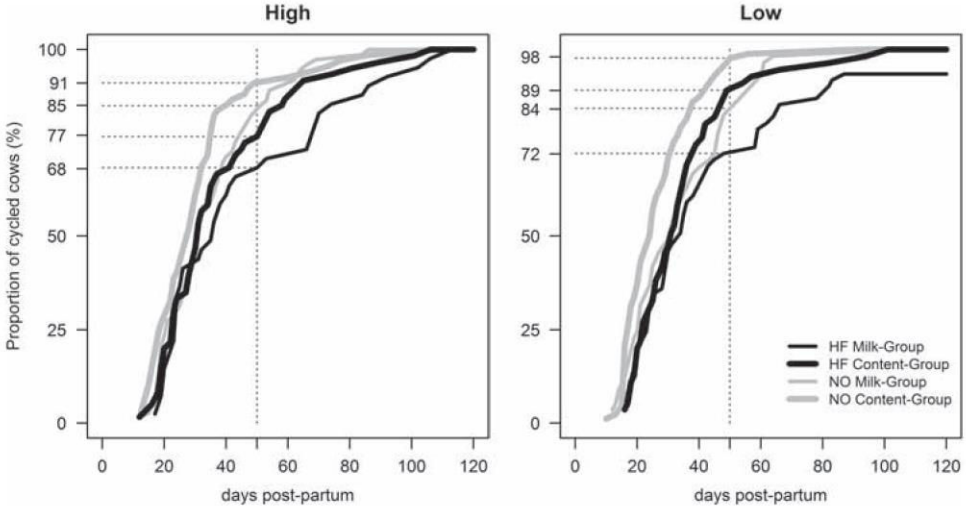

Figure 1 Observed reversed survival function (cumulative proportions) of Holstein cows (HF) and Normande cows (NO) that resumed luteal activity over the first 120 days *postpartum* by feeding system.

Table 2 *Estimated effect sizes* $(\widehat{\beta})$*, associated hazard ratios* $(HR=exp(\widehat{\beta}))$ *and confidence intervals (CI) of factors influencing time to commencement of luteal activity (CLA;* n *= 500 of which three are censored) for Holstein and Normande cows*

Risk factors	Category (%) (mean ± SD)	Estimate	*Sy.x*	HR	95% CI	*P*-value
Calving problems						0.15
No	79	0.24	0.165	1.3	0.9–1.8	
Yes	21	0.00	…	1.0	…	
Genetic group						
Milk	46	0.00	…	1.0	…	**
Content	54	0.50	0.185	1.7	1.2–2.4	
Parity						***
1st lactation	41	− 0.45	0.145	0.6	0.5–0.9	
2nd lactation	28	0.00	…	1.0	…	
⩾3rd lactation	31	− 0.61	0.173	0.5	0.4–0.8	
EBV (MY)[1]	0 ± 413	− 0.0008	0.00029	…	…	**
Tournout[2]	61 ± 32	0.007	0.0024	…	…	**
Protein content (g/kg)	31.4 ± 3.8	0.06	0.021	…	…	**

EBV = estimated breeding values, MY = milk yield.
[1]EBV has a null mean because it was centred within breed and genetic groups.
[2]Tournout is the number of days in milk at turnout (start of grazing).
Significant levels: $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$, $^{+}P<0.1$.

expressing discrete behaviour (associated HR = 1.9; $P<0.001$).

Discussion

The ability to be inseminated is impacted by genetic characteristics

The differences in production performances between Holstein and Normande cows are consistent with our previous studies (Cutullic *et al.*, 2011; Bedere *et al.*, 2016). Our results confirmed that Holstein cows have later CLA than other dairy breeds (Petersson *et al.*, 2006; Piccand *et al.*, 2013). Holstein cows had more PLP than Normande cows, which is consistent with the idea that PLP may be a characteristic of Holstein cows (Royal *et al.*, 2000). However, Holstein cows expressed oestrus more intensively than Normande cows. This difference was consistent with observations between Holstein and Jersey cows observed by Løvendahl and Chagunda (2010). In our experiment, Holstein cows had later DAI1 than Normande cows as well as a lower submission rate at the end of the breeding season. This result is consistent with other studies where the 21-day submission rate of Holstein cows was lower than for dual-purpose breeds (Dillon *et al.*, 2003; Piccand *et al.*, 2013).

As previously observed, producing milk solids through higher fat and protein contents is associated with earlier CLA (Bedere *et al.*, 2016). Interestingly, genetic groups neither affected *postpartum* P4 profile, oestrus expression nor DAI1 in this study. To our knowledge, the relationship between the source of high milk fat plus protein production (high yield or

Table 3 *Distribution of type of progesterone profile for Holstein and Normande cows, in the milk- or the content-group, under either the high or low feeding system*

	Holstein				Normande								
	Milk-Group		Content-Group		Milk-Group		Content-Group		Model	Significance levels[2]			
	High	Low	High	Low	High	Low	High	Low	σ_a[1]	B	B:G	FS	B × FS
Number of lactations	41	61	60	55	73	57	67	86					
Normal (%)	32	46	57	49	64	68	63	77	0.6	***	0.14	0.31	0.26
PLP (%)	27	21	15	31	12	9	24	14	1.1	*	0.34	0.89	+
Delayed (%)	34	26	25	11	17	16	9	4	16.0	0.40	0.91	0.62	0.63
Interrupted (%)	5	4	3	9	6	7	3	3	…	…	…	…	…
Disordered (%)	2	3	0	0	1	0	1	2	…	…	…	…	…

PLP = prolonged luteal phases.
[1]Standard deviation of the random animal genetic and non-genetic effect (σ_a; assuming uncorrelated animal effects). No error term (σ_e) is estimated with logistic regression.
[2]Effects of breed (B), genetic group within breed (B:G), feeding system (FS), breed × feeding system (B × FS).
Significant levels: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{+}P < 0.1$.

Table 4 *Proportion of ovulation detected by oestrus observations and proportion of expressed behaviours among detected ovulations for Holstein and Normande cows, in the milk- or the content-group, under either the high or low feeding system*

	Holstein				Normande									
	Milk-Group		Content-Group		Milk-Group		Content-Group		Model	Significance levels[2]				
	High	Low	High	Low	High	Low	High	Low	σ_a[1]	B	B:G	FS	B × FS	MY
Number of ovulations	100	137	179	164	206	170	209	253						
Detection rate (%)	72	74	65	73	72	71	66	68	0.54	0.15	0.14	0.12	0.81	*
Standing behaviour[3] (%)	57	66	48	67	47	61	47	57	0.29	***	0.51	***	0.87	*
Mounting behaviour[3] (%)	22	17	28	19	32	24	24	27	0.28	**	0.58	+	0.60	+
Discrete behaviour[3] (%)	21	17	24	14	21	15	29	16	0.40	0.47	0.57	***	0.92	0.98

[1]Standard deviation of the random animal genetic and non-genetic effect (σ_a; assuming uncorrelated animal effects). No error term (σ_e) is estimated with logistic regression.
[2]Effects of breed (B), genetic group within breed (B:G), feeding system (FS), breed × feeding system (B × FS), milk yield centred within feeding systems (MY).
[3]The estimation of variance components failed. As an alternative, an empirical Bayesian approach was implemented. The robustness of the other estimated was checked by looking at their value when the chosen prior was multiplied or divided by 2.
Significant levels: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{+}P < 0.1$.

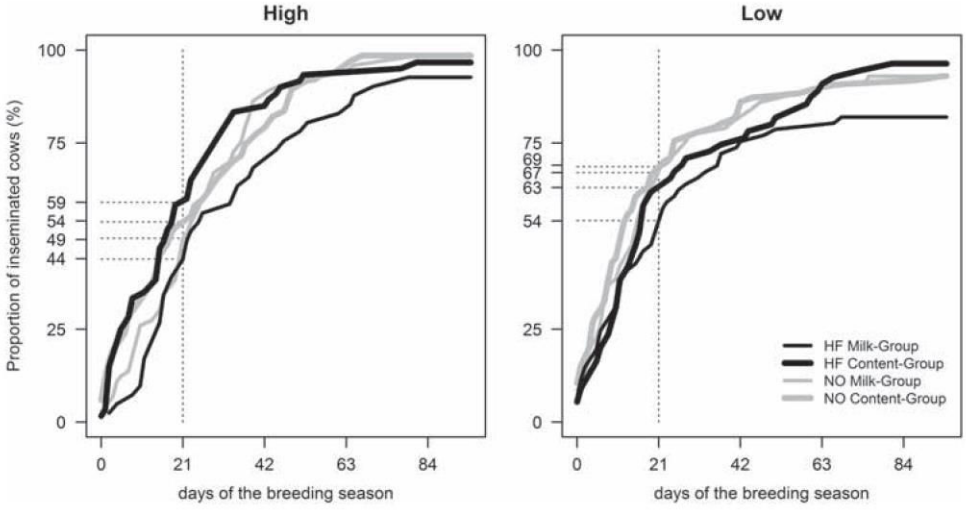

Figure 2 Observed reversed survival function (cumulative proportions) of Holstein cows (HF) and Normande cows (NO) that were inseminated over the breeding season by feeding system.

Table 5 *Estimated effect sizes ($\widehat{\beta}$), associated hazard ratios ($HR=exp(\widehat{\beta})$) and confidence intervals (CI) of factors influencing days from start of the breeding season to first service (DAI1;* n = *500 of which 31 are censored) for Holstein and Normande cows*

Risk factors	Category (%) (mean ± SD)	Estimate	*Sy.x*	HR	95% CI	*P*-value
Calving problems						*
No	80	0.48	0.214	1.6	1.1–2.5	
Yes	20	0.00	…	1.0	…	
Parity						**
1st lactation	42	0.51	0.167	1.7	1.2–2.3	
2nd lactation	28	0.00	…	1.0	…	
⩾3rd lactation	30	0.18	0.187	1.2	0.8–1.7	
Cyclicity profile						***
Normal	63	0.00	…	1.0	…	
PLP	17	−1.26	0.216	0.3	0.2–0.4	
Delayed	14	−1.31	0.263	0.3	0.2–0.5	
Interrupted	5	−1.17	0.324	0.3	0.2–0.6	
Disordered	1	−1.32	0.641	0.3	0.1–0.9	
Oestrus						***
Standing behaviour	51	0.00	…	1.0	…	
Mounting behaviour	24	−0.02	0.179	1.0	0.7–1.4	
Discrete behaviour	25	−0.68	0.188	0.5	0.4–0.7	
Not detected	0	…	…	…	…	
EBV (MY)[1]	0 ± 504	−0.0006	0.00030	…	…	*
Protein Content	30.4 ± 3.0	0.055	0.0301	…	…	+

PLP = prolonged luteal phases; EBV = estimated breeding values, MY = milk yield.
[1]EBV has a null mean because it was centred within breed.
Significant levels: $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$, $^{+}P<0.1$.

high milk fat and protein contents) and reproductive performance has not been investigated previously. Relatively little is known about the genetic control of oestrus expression, due to the difficulty to measure and quantify oestrus intensity. Oestrus expression is often associated with production traits, suggesting the existence of an animal (and thus a genetic) component in the variability of oestrus expression. Yet, published heritability estimates for oestrus intensity are low, ranging from 0.01 to 0.04 (Roxström *et al.*, 2001; Carthy *et al.*, 2016).

As reported before, EBV for milk yield was negatively associated with CLA (Royal *et al.*, 2002; Windig *et al.*, 2008). We also found that higher genetic merit for milk yield was associated with later DAI1. Fulkerson *et al.* (2001) found that cows with high genetic merit for production traits had lower submission rate than cows with low genetic merit, which is consistent with our results. A recent review by Washburn and Mullen (2014) indicates that 21-day submission rate is also genetically correlated with BCS (about 0.50).

Restrictive feeding system enhances oestrus expression but had no effect on cyclicity and time to first service

In this experiment, cows were fed *ad libitum* even though the 2 FS differed in nutrient intake. The Low FS was very restrictive, causing an insufficient energy intake associated with a large BCS loss. Cows adjusted their performances by limiting their milk production according to this nutritive constraint in order to reduce the severity of negative energy balance. Cows in the Low FS expressed oestrus more intensively than cows in the High FS, as in our previous studies (Cutullic *et al.*, 2009 and 2011). The effect of FS is largely explained by differences in milk yield. As expected, higher milk yield was associated with lower oestrus intensity (Friggens *et al.*, 2010). A possible reason suggested in the literature is that high yielding dairy cows have a higher blood flow and consequently a higher hepatic catabolism of steroids that leads to decreased plasma oestradiol concentration (Wiltbank *et al.*, 2006). Some studies suggested that low BCS in early lactation was associated with reduced oestrus expression (Løvendahl and Chagunda, 2010; Madureira *et al.*, 2015). But in these studies, effects of level and dynamics of milk yield and BC may be confounded. Our previous work showed that oestrus expression was more associated with milk yield than BCS, even though a threshold of low BCS might exist beneath which both BCS and milk yield impact oestrus expression (Cutullic *et al.*, 2012).

As expected, feeding strategies that partly disentangle milk production and body reserves mobilisation do not impact cyclicity (Walsh *et al.*, 2008; Vance *et al.*, 2013). However, higher protein content was associated with earlier CLA suggesting a residual effect of energy status after considering genetic effects. Protein content is known to be lower for cows in severe and prolonged negative energy balance (Fulkerson *et al.*, 2001). This result is consistent with previous studies where earlier CLA was associated with better energy balance (Royal *et al.*, 2002; Chagas *et al.*, 2008).

DAI1 was not affected by FS. Kennedy *et al.* (2003) reported that the level of concentrates supplementation on

pasture-based FS did not impact submission rate in the first 3 weeks of the breeding season. Cows with higher protein content had earlier DAI1 in our study. Considering that cows with higher protein content had a better energy balance, this finding is consistent with other studies where cows that had too low BCS (Buckley *et al.*, 2003) or too severe BCS loss (Roche *et al.*, 2007) had lower 21 days submission rate.

Conclusion

This study clearly showed that high yielding dairy cows (Holstein in the Milk-Group) had the poorer ability to be inseminated on time and this effect was also exacerbated by restricted diet. This effect was mostly due to a poor cyclicity. Our results suggest that selecting cows on production traits differently (high milk fat and protein contents) would not change milk solids production, would improve cyclicity, with no impact on oestrus expression or submission rate. Given the impact of CLA on re-calving rate and cow survival in compact calving systems, such selection strategy would lead to more robust cows, if their fertility is preserved. Investigations are still required on the ability to ensure pregnancy of such dairy cows.

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Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731116002111

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Supplementary Material S1

The experimental farm is characterized by very heterogeneous and unusual management conditions. Consequently, recorded cow performances are not routinely included in the national data base and no national EBV are available for these cows. Furthermore, genomic evaluations were not available for the oldest cows of this study. Therefore, specific EBV had to be computed. This was performed combining within herd information with national sire and maternal grand sire estimated breeding values (EBV). Based on these results, cows were classified within breeds into 2 groups according to their customized estimated breeding value (EBV) for milk yield (MY), fat and protein contents(respectively FC and PC) as 2 groups capable of producing similar milk solids (MS) quantity in different ways. Here, we present these customized EBV.

Estimation of customized Breeding values

The EBV for each trait was evaluated combining within herd information analysed with a BLUP animal model with national EBV of the sires and grandsires. The model of analysis of cow performances over three lactations included usual fixed environmental effects (year, lactation number, calving age, calving month, drying off period length, and permanent environment effect) and the feeding system (H. Larroque, INRA UMR 1388 GenPhySE, Toulouse, France, personal communication). Within breed and experimental year, nulliparous cows with EBV for milk yield higher than average and EBV for fat and protein contents lower than average constituted a “Milk-Group”. Nulliparous cows with EBV for milk yield lower than average and EBV for fat and protein contents higher than average constituted a “Content-Group”. The others nulliparous cows (with high EBV for milk yield and high EBV for fat and protein contents or low EBV for milk yield and low EBV for fat and protein contents) did not enter the experiment. EBV were expressed in deviation from a base population, whose average EBV were set to 0.

Average EBV for production traits

Table 1 *Distribution of the customized Estimated Breeding Values for production traits (milk yield, fat content, protein content and milk solids yield) for Holstein and Normande cows, in the Milk- or the Content-Group.*

	Holstein		Normande	
	Milk-Group	Content-Group	Milk-Group	Content-Group
Centred within breed				
EBV(MY)	+308	-303	+290	-264
EBV(FC)	-1.7	+1.9	-1.9	+1.5
EBV(PC)	-0.5	+0.5	-0.9	+0.8
EBV(MS)	+4.4	-4.4	+5.9	-6.8

Regression of production performance on EBV for production traits

In order to further understand the link between genetic merit for production traits and the actual performance, regression of adjusted production performances (predicted outcomes of the model used in the study) on EBV are presented here.

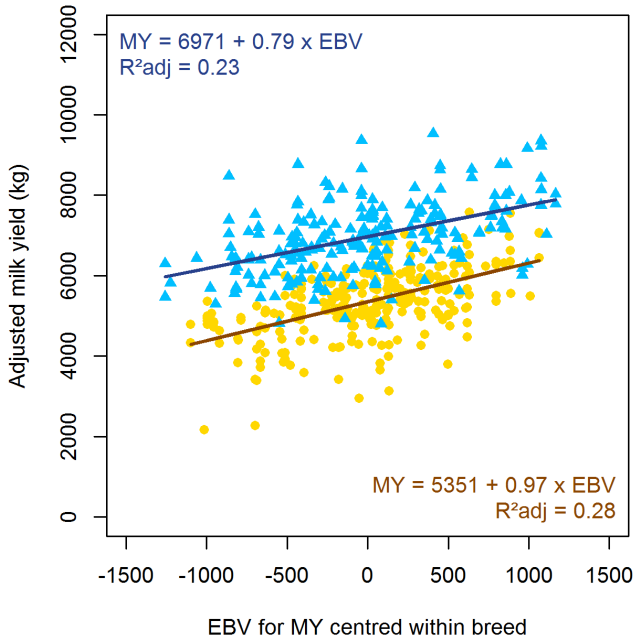

Figure 1 Total milk yield over the 44 weeks of lactation, adjusted from the effect of year, parity, age at first calving, feeding system and lactation length, for Holstein in blue (each filled triangle represent a cow/year) and for Normande in orange (each filled circle represent a cow/year), and regression line of prediction on customized EBV for milk yield for the 217 Holstein and 283 Normande lactations recorded.

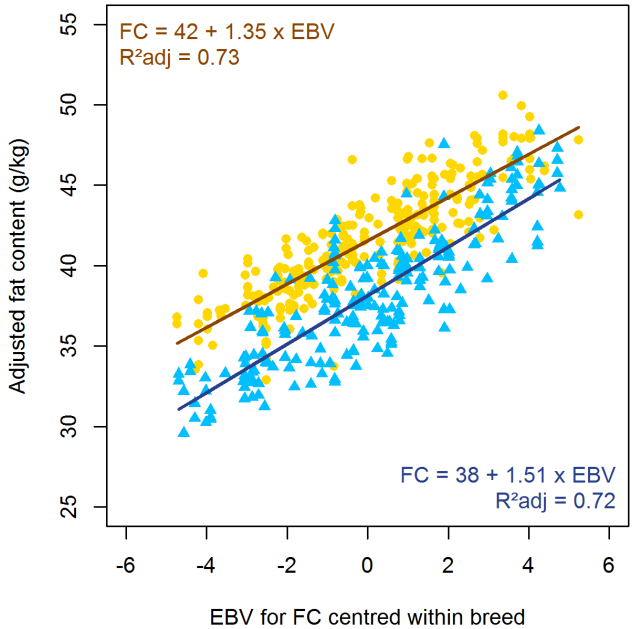

Figure 3 Average milk fat content over the 44 weeks of lactation, adjusted from the effect of year, parity, age at first calving, and feeding system, for Holstein in blue (each filled triangle represent a cow/year) and for Normande in orange (each filled circle represent a cow/year), and regression line of prediction on customized EBV for fat content for the 217 Holstein and 283 Normande lactations recorded.

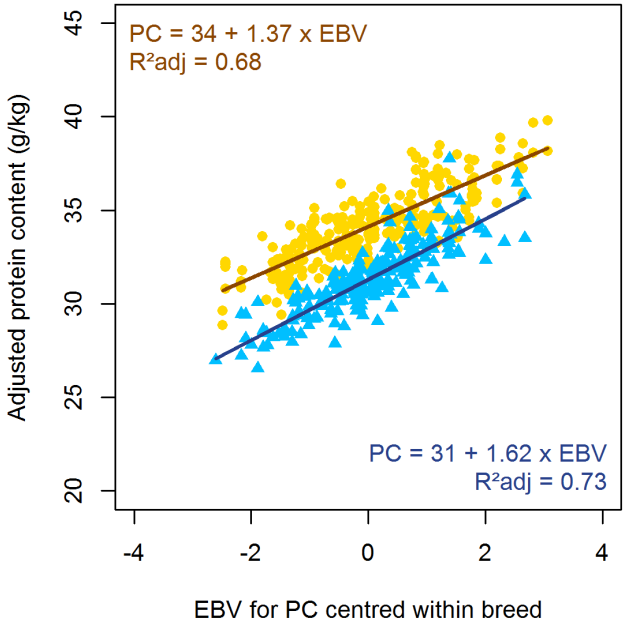

Figure 4 Average milk protein content over the 44 weeks of lactation, adjusted from the effect of year, parity, age at first calving, and feeding system, for Holstein in blue (each filled triangle represent a cow/year) and for Normande in orange (each filled circle represent a cow/year), and regression line of prediction on customized EBV for protein content for the 217 Holstein and 283 Normande lactations recorded.

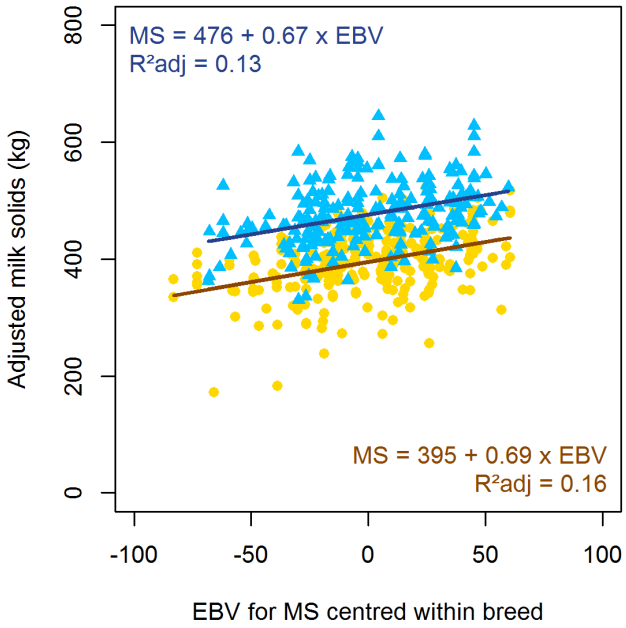

Figure 5 Total milk solids yield over the 44 weeks of lactation, adjusted from the effect of year, parity, age at first calving, feeding system, and lactation length, for Holstein in blue (each filled triangle represent a cow/year) and for Normande in orange (each filled circle represent a cow/year), and regression line of prediction on customized EBV for milk solids for the 217 Holstein and 283 Normande lactations recorded.

Supplementary Table S1

Composition of the diets and amount of feed in the High and Low feeding systems fed to the dairy cows during indoor, pasture and dry period.

	High	Low
Indoor diets		
Maize Silage (% DM)	55	· · ·
Dehydrated Alfalfa pellets (% DM)	15	· · ·
Grass Silage (% DM)	· · ·	50
Haylage (% DM)	· · ·	48
Concentrates + Minerals and Vitamins (% DM)	30	· · ·
Minerals and Vitamins (% DM)	· · ·	2
Pasture feeding		
Rotational Grazing (ha/cow)	0.35[1]	0.55[2]
Concentrates (kg)	4.0	· · ·
Minerals and Vitamins (kg)	0.25	0.50
Dry period		
Grass Silage (% DM)	100	100

[1] in the case of grass shortage, cows received maize silage. This occurred often around mid-July.

[2] in the case of grass shortage, cows received grass silage. This occurred rarely during summer and late-October.

Supplementary Table S2

Adjusted body weight (BW) and weekly BW change over the first 14 lactation weeks, total BW change over the whole lactation (44 weeks) and plasma concentration of glucose, urea and NEFA for Holstein and Normande cows, in the Milk- or the Content-Group, under either the High or Low feeding system.

	Holstein				Normande				Model		Significance levels[2]			
	Milk-Group		Content-Group		Milk-Group		Content-Group							
	High	Low	High	Low	High	Low	High	Low	σ_a[1]	σ_e[1]	B	B:G	FS	B×FS
number of lactations	41	61	60	55	73	57	67	86						
Over first 14 weeks														
BW at calving (kg)	678^{cd}	632^{ab}	657^{bc}	611^{a}	700^{d}	654^{bc}	703^{d}	656^{bc}	43.5	38.8	***	+	***	0.78
BW change (kg/wk)	-3^{bc}	-6^{a}	-3^{bc}	-6^{a}	-2^{cd}	-5^{ab}	-2^{d}	-4^{b}	1.2	2.7	***	0.45	***	0.33
Over 44 weeks														
Total BW change (kg)	$+12^{b}$	-44^{a}	$+4^{b}$	-51^{a}	$+53^{c}$	-3^{b}	$+55^{c}$	0^{b}	23.2	41.4	***	0.58	***	0.17
at 20 days *post-partum*														
Glucose (mg/dl)	61.6^{c}	57.4^{ab}	62.0^{c}	57.9^{ab}	60.7^{bc}	56.6^{a}	61.9^{c}	57.7^{ab}	3.50	5.72	0.55	0.45	***	0.76
NEFA (mg/dl)	415^{c}	418^{c}	381^{c}	410^{c}	333^{bc}	269^{ab}	237^{a}	282^{ab}	0.07	0.59	***	0.11	0.84	0.59
Urea[3] (mg/dl)	23.1^{abc}	19.9^{a}	25.3^{cd}	19.9^{a}	24.5^{bcd}	21.8^{ab}	27.6^{d}	21.9^{ab}	1.10	6.33	**	+	***	0.92
at 60 days *post-partum*														
Glucose (mg/dl)	64.2^{de}	60.6^{abc}	65.2^{e}	61.6^{bcd}	63.9^{cde}	60.3^{ab}	62.3^{bcd}	58.7^{a}	2.35	6.49	**	0.15	***	0.28
NEFA (mg/dl)	223^{bc}	252^{c}	201^{abc}	227^{bc}	197^{ab}	222^{bc}	175^{a}	197^{ab}	0.11	0.48	**	*	**	0.78
Urea (mg/dl)	26.3^{b}	20.6^{a}	26.3^{b}	20.6^{a}	27.0^{b}	21.3^{a}	27.2^{b}	21.5^{a}	2.58	7.53	0.29	0.98	***	0.12

[1] Standard deviation of the random terms, animal genetic and non-genetic effect (σ_a) and residuals (σ_e)

[2] Effects of Breed (B), Genetic group within Breed (B:G), Feeding System (FS), Breed*Feeding System (B×FS).

Significant levels: *** $P<0.001$; ** $P<0.01$; * $P<0.05$; + $P<0.1$

[3] The estimation of the animal variance component σ_a failed. As an alternative, an empirical Bayesian approach was implemented, assuming that a fixed prior for σ_a. The robustness of the other estimated was tested by checking the impact of σ_a on the other estimates when it was multiplied or divided by 2

[a - e] distinguish adjusted means that are different between breeds, genetic groups, and feeding systems ($P < 0.05$, Tukey's pairwise comparison).

CHAPTER 5: Ability of dairy cows to ensure pregnancy according to genetics and breeding system.

Objectifs

Ce dernier chapitre de résultats a pour objectif d'étudier les effets de la race, du type génétique et du régime alimentaire sur l'aptitude des vaches laitières à assurer la gestation. Les hypothèses testées sont (1) les vaches ayant des caractéristiques génétiques (race, type et index génétique) favorables à la production laitière ont une aptitude à assurer la gestation dégradée ; (2) le régime alimentaire affecte la production laitière et la gestion des réserves corporelles, ce qui est lié à cette aptitude.

L'essentiel

Les vaches Normande ont un meilleur taux de revêlage (70 % *vs* 52 %) que les vaches Holstein. Elles ont tendance à avoir un intervalle mise à la reproduction - IA gestante plus court que les Holstein. Ceci s'explique en partie par un meilleur taux de fertilité à l'IA (68 % *vs* 50 % à la 1ère et 2ème IA combinées) lié à une moindre proportion de non-fécondations / mortalités embryonnaires précoces (24 % *vs* 30 % des IA1&2) et moins de mortalités embryonnaires tardives (6 % *vs* 12 % des IA1&2).

Chez la Normande, aucun effet du type génétique n'a été observé sur l'aptitude à assurer la gestation. Chez la Holstein, les vaches de type Taux ont plus de problèmes de fertilité que celles de type Lait. Ce résultat est inattendu. De plus, la source du problème dépend du système alimentaire : dans le système Bas, les vaches Holstein de type Taux ont eu plus de non-fécondations

/ mortalités embryonnaires précoces (47 % *vs* 22 % des IA1&2) ; et dans le système Haut, les vaches Holstein de type Lait ont eu plus de mortalités embryonnaires tardives que celles du type Taux (19 % *vs* 8 % des IA1&2).

Le système alimentaire n'a pas eu d'effet sur le taux de revêlage. Cependant, des indicateurs de statut énergétique (taux protéique et note d'état corporel) étaient positivement associés à de meilleures chances de fécondation et de gestation.

Cette étude montre qu'il existe un lien défavorable entre les index génétiques de taux butyreux et protéique et la fertilité des races laitières. Ceci montre tout d'abord que les étapes de la reproduction sont partiellement découplées : certains leviers peuvent être bénéfiques à certaines étapes et néfastes à d'autres. De plus l'ensemble de ces 3 études soulèvent de nouvelles questions : Comment les caractéristiques génétiques favorables aux taux butyreux et protéique sont défavorables à la fertilité ? Quelles corrélations génétiques ? Quelles associations génomiques ?

Concernant leurs trajectoires adaptatives : les animaux du type Lait produisent plus de lait et mobilisent autant que celles du groupe Taux. Les effets du type Taux sont controversés. Comment étudier ce nouvel aspect de la compétition lactation reproduction ? Quels sont les liens métaboliques ou physiologiques entre les deux fonctions pouvant expliquer ces échecs de gestation ?

Valorisation

Article dans une revue internationale à comité de relecture :

Bedere, N., C. Disenhaus, V. Ducrocq, S. Leurent-Colette, L. Delaby. Ability of dairy cows to ensure pregnancy according to breed and genetic merit for production traits under contrasting pasture based systems. Manuscript accepted the 12/12/2016 for publication in the Journal of Dairy Science.

Actes de conférences internationales - Affiche

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2016. Selecting on fat and protein content instead of milk yield would not improve dairy cows' fertility, in: 67th Annual Meeting of the European Federation of Animal Science. Belfast, Ireland.

Actes de conférences nationales - Affiche

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2016. L'aptitude des vaches laitières à assurer chaque étape de la reproduction dépend de leurs caractéristiques génétiques, in: Rencontres Recherche Ruminants. Paris, France.

ABILITY OF DAIRY COWS TO ENSURE PREGNANCY

Ability of dairy cows to ensure pregnancy according to breed and genetic merit for production traits under contrasted pasture based systems.

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Interpretive summary
Fertility of dairy cows has been declining while their milk production was improving. The present study aimed to assess and measure the effects of breed, genetic merit for production traits, and feeding systems on their ability to ensure pregnancy (fertilization of the oocyte, embryo survival, calving). Rather unexpectedly, our results suggest that there might be a link between genetic merit for fat and protein content and lower ability to ensure pregnancy.

ABSTRACT

The present study aimed to assess and measure the effects of breed, genetic merit for production traits, and feeding systems (FS) on the ability of dairy cows to ensure pregnancy through its components (fertilization, embryonic losses, re-calving). An experiment was conducted over 9 years on Normande and Holstein cows assigned to contrasted FS. Diets were based on maize silage in winter and grazing plus concentrate in spring in the "High FS"; and on grass silage in winter and grazing with no concentrate during spring in the "Low FS". Within breeds, cows were classified into 2 groups with similar estimated breeding values (EBV) for milk solids: cows with high EBV for milk yield were included in a Milk-Group and those with high EBV for fat and protein contents were included in a Content-Group. Holstein cows produced more milk throughout lactation than Normande cows (the differential was greater in the High FS +2,294 kg compared to +1,280 kg in the Low FS) and lost more body condition to nadir (the differential was greater in the High FS -1.00 point compared to -0.80 point in the Low FS). Within breeds, milk solids production was similar between genetic groups. Cows in the High FS produced more milk (+2,495 kg for Holstein cows and +1,481 kg for Normande cows) and had a higher BCS at nadir (+0.40 point for Holstein cows and +0.60 point for Normande cows) than cows in the Low FS. Holstein cows had a lower re-calving rate than Normande cows (-19 percentage units). There was no effect of genetic group and FS on fertility of Normande cows. However, according to FS, Holstein cows in the Content-Group exhibited different fertility failure patterns. In the Low FS, Holstein cows in the Content-Group had more non-fertilizations/early embryo mortality (NF/EEM; +26 percentage units at first and second services) than Holstein cows in the Milk-Group. In the High FS, Holstein cows in the Content-Group had a higher proportion of late embryo mortality (LEM) than in the Milk-Group (+10 percentage units at first and second services). There was no effect of FS on re-calving rate. However, indicators of energy balance (protein content or BCS) were positively associated with successful conception and pregnancy. This study suggested that there may be a link between genetic merit for fat and protein content and lower ability of dairy cows to ensure pregnancy because of more NF/EEM or LEM.

KEY WORDS: dairy cow; genetic merit; fertilization; pregnancy loss

INTRODUCTION

In the past decades, reproductive performance of dairy cows has been declining and the strong genetic selection that was applied on production traits is considered to be responsible for this. Each step of the reproductive step has been impacted: abnormal ovarian activity is more common in the current population (Gautam et al., 2010), the duration and intensity of estrus has dramatically decreased (Kerbrat and Disenhaus, 2004) and the occurrence of pregnancy losses has increased (Grimard et al., 2006). The consensus in the literature is that fertility is impaired by a lack of energy because dairy cows are investing it in milk production, and that this competition is both genetically and nutritionally driven (Royal et al., 2000; Friggens et al., 2010; Walsh et al., 2011).

In various regions, the valuable milk components are fat and protein matters. There are two possible ways to produce fat and protein matters, either through high milk yield or through high fat and protein contents. In the literature, most studies investigating the effect of genetics on production and reproduction performances compared either high and low genetic merit for milk yield (Kennedy et al., 2003; Horan et al., 2004), or high and low genetic merit for milk solids yield (Fulkerson et al., 2001; Pollott and Coffey, 2008). To our knowledge, a comparison of high genetic merit for milk yield and high genetic merit for fat and protein contents at identical global genetic merit for milk solids has never been investigated before. At identical milk solids yield, dairy cows with high genetic merit for fat and protein contents should have a lower peak milk yield, and lower lactose yield than cows with high genetic merit for milk yield. Therefore, cows with high genetic merit for fat and protein contents are expected to invest less energy in milk than cows with high genetic merit for milk yield. It can be hypothesized that dairy cows with high genetic merit for fat and protein content preserve their reproductive performance while producing the same amount of milk solids than cows with high genetic merit for milk yield. A first study showed that primiparous dairy cows with high genetic merit for fat and protein content had an earlier commencement of luteal activity than those with high genetic merit for milk yield, in 2 contrasted breeds (dairy *vs* dual purpose cows) and 2 contrasted feeding systems (high *vs* low inputs; Bedere et al., 2016). This was a promising result to establish strategies to cope with reproductive decline and maintain productive performances. However, there is a lack of knowledge concerning the ability of this type of cows to ensure pregnancy. The present study aimed to assess and measure the effects of breed, genetic merit for production traits, and feeding systems on the fertility of dairy cows. Our hypotheses were that (i) high genetic merit for milk yield is unfavorably associated with the ability to ensure pregnancy, (ii) according to genetic characteristics, dairy cows under nutrient restriction are either preserving body reserve or milk production.

MATERIALS AND METHODS

Experimental Design

An experiment was conducted from 2006 to 2014 at the INRA dairy research farm of Le Pin-au-Haras (48.724986N, 0.185428E, Normandy, France). Dairy cows were equally distributed over 2 breeds and 2 feeding systems (FS) each experimental year. A total of 296 lactations from Normande cows (dual purpose cows) and 240 lactations from Holstein cows (dairy cows) were recorded

throughout the trial. Within breeds, cows were classified into 2 groups according to their estimated breeding values (EBV) for milk yield, fat and protein contents as 2 groups capable of producing the same milk solids quantity in different manners: cows with high EBV for milk yield were included in a "Milk-Group" and those with high EBV for fat and protein contents were included in a "Content-Group" (more details in Table A1 of the Appendix). There were 116 lactations from Holstein cows and 147 lactations from Normande cows recorded under a "High" FS that enabled high milk yield while limiting body condition loss; and 124 lactations from Holstein cows and 149 lactations from Normande cows recorded under a "Low" FS that limited milk yield while inducing a large body condition loss. Diets are presented in Table 1. Cows remained in their FS until they were culled due to lack of pregnancy, severe health problem or accidental death. Among the 536 lactations recorded in this study, 15 were removed because the cows could not be milked and 21 because of severe health problems. Finally, 500 lactations including 207 first lactation cows were included in the analyses of the present study.

Table 1. Composition of the diets of the High and Low feeding systems during stock, pasture and dry periods.

Feedstuffs	High feeding system	Low feeding system
Indoor diet (early lactation)		
Maize Silage (% DM)	55	...
Dehydrated Alfalfa pellets (% DM)	15	...
Grass Silage (% DM)	...	50
Haylage (% DM)	...	48
Concentrates + Minerals and Vitamins (% DM)	30	...
Minerals and Vitamins (% DM)	...	2
Pasture feeding (mid- and late lactation)		
Rotational Grazing (ha/cow)	0.35[1]	0.55[2]
Concentrates (kg)	4.00	...
Minerals and Vitamins (kg)	0.25	0.50
Dry period diet		
Grass Silage (% DM)	100	100

[1] in the case of grass scarcity, cows received maize silage. This occurred often around mid-July.

[2] in the case of grass scarcity, cows received grass silage. This occurred rarely during summer and late-October.

Reproductive Management

The herd was managed under a 3-month compact calving system (January - March). After calving, uterine involution was checked by rectal palpation 25 to 30 d postpartum. When involution was achieved, artificial inseminations were performed on spontaneous estrus, if expressed at least 40 d postpartum and during the breeding period (April - June). If cows were not expressing new estrus in the 35 d following a service, ultrasonography was conducted to diagnose pregnancy status. If the first one was positive, a second diagnosis was performed 60 d after last service. Insemination outcomes were classified by combining information from P4 profiles and ultrasonography examinations (Humblot, 2001; adapted by Cutullic et al., 2011; Table 2) in non-fertilization/early embryo mortality (NF/EEM), late embryo mortality (LEM), fetal death (FD), abortion and calving.

Health events were recorded throughout the lactation with special care on reproductive problems (caesarian, vagina displacement, infectious vaginitis, severe metritis, and retained placenta). Anestrus was not considered to be a pathology and cows that were not seen in estrus before the end of the breeding season were not treated to avoid introducing a bias in the analyses. During this experiment, 107 cows (21 %) experienced reproductive problems. Due to their potential effect on reproductive performances, the effect of having at least one of the reproductive problems described above was accounted for in the analyses as a dichotomous variable (0 = none, 1 = at least one reproductive problem).

Table 2. Decision rules to classify inseminations outcomes by combining information from P4 levels (luteal phase length) and ultrasonography examinations.

Insemination Outcome[1]	luteal phase length	Ultrasonography[2]	Other
NF/EEM	< 25 d		
LEM	≥ 25 d & < 50 d	E. or P. 35 d	
FD	≥ 50 d	P. 35 d & E. 70 d	
Abortion	≥ 50 d	P. 35 d & 70 d	(aborted fetus)
Calving	≥ 50 d	P. 35 d & 70 d	calf

[1] non-fertilization/early embryo mortality (NF/EEM), late embryo mortality (LEM), fetal death (FD), abortion and calving

[2] outcome of the ultrasonography examination can be empty (E.) or pregnant (P.)

Sampling and Measurements

Cows were milked twice daily at 0630 h and 1600 h. Individual milk yields were recorded by flow meters (Metatron, Westfalia, Germany). Fat and protein contents from a.m. and p.m. individual milk samples were determined 3 times a week by infrared analyser (MilkoScan^{TM}, Foss Electric, Denmark). Morning milk samples were taken from calving to either 2 wk after service inducing pregnancy or to the end of July on Monday, Wednesday and Friday and stored at -20°C. Milk progesterone (P4) concentration was determined using commercial ELISA kits (Milk Progesterone ELISA, Ridgeway Science Ltd., England). The coefficients of variation between assays on 5 ng/ml control samples ranged between 8 % and 14 % among experimental years. Two milk P4 thresholds were defined to distinguish the baseline level of progesterone in milk from the luteal phase level (threshold 1) and a low luteal phase level from a high luteal phase level (threshold 2; Petersson et al., 2006; adapted by Cutullic et al., 2011). In short, threshold 1 was the 95 quantile of P4 values of the ovulation days from all cows (based on observed estruses; Petersson et al., 2006). Threshold 2 corresponded to the first quartile value of the points above threshold 1. Across experimental years, their values ranged from 0.01 to 1.49 ng/ml for threshold 1, and from 2.03 to 6.37 ng/ml for threshold 2. Luteal phase started when at least 2 consecutive milk P4 concentrations were above threshold 1 and at least one above threshold 2, and ended when at least 1 value was lower than threshold 1. Blood samples from the caudal vein were taken at 20 and 60 d postpartum to determine plasma concentrations of glucose, NEFA and urea using enzymatic colorimetry (Kone Instruments Corporation, Espoo, Finland). BCS (0-5 scale with 0.25 increments) was evaluated monthly by the same three trained assessors as described by Bazin et al. (1984). Lactation

persistency was defined as the ratio between average daily milk yield from 100 to 200 d in milk and average daily milk yield over the first 100 d in milk.

Statistical Analyses

In this section, all complete models are described, before only significant effects are retained. Effects were kept in the final models if their P-value was lower than 0.10. In some cases, the estimation of variance components failed, probably because of a too limited number of records. In such cases, an empirical Bayesian approach was implemented. All Bayesian mixed models were performed using the *blmer* (linear mixed models) or *bglmer* (generalized linear mixed models) procedures of the R statistical package (R Core Team, 2016).

Production Performances Production performances were studied using the following initial linear mixed model:

$$y_{ijklmn}=\mu+\beta_1 grazing_{ijklmn}+\beta_2 EBV_{ijklmn}+year_i+parity_j+breed_k+(GG|breed)_{kl}+FS_m$$
$$+parity\times breed_{jk}+parity\times FS_{jm}+breed\times FS_{km}+(GG|breed)\times FS_{klm}+cow_n+e_{ijklmn}$$

where y_{ijklmn} was the production performance (e.g. milk yield over the first 14 wk of lactation), μ was the mean of the variable of interest, $grazing_{ijklmn}$ was the covariate describing the effect of days in milk at turnout, EBV_{ijklmn} was the covariate describing the effect of the associated EBV centered within breeds and genetic groups (when the dependent variables were milk yield, fat and protein contents or yields), $year_i$ was the fixed effect of experimental year (i=2006 to 2014), $parity_j$ was the fixed effect of parity (j=1, 2, or ≥3), $breed_k$ was the fixed effect of breed (k=Holstein or Normande cows), $(GG|breed)_{kl}$ was the fixed effect of genetic group (l=Milk-Group or Content-Group) nested within breed, FS_m was the fixed effect of feeding system (m=High or Low), cow_n was the random (genetic and non-genetic) effect of the cow and e_{ijklmn} the random residual effect. All linear mixed model analyses were performed using the *lmer* procedure of the R statistical package (R Core Team, 2016).

Metabolites Plasma concentrations of glucose, non-esterified fatty acids (NEFA) and urea were studied using the following initial linear mixed model:

$$y_{ijklmno}=\mu+out_o+year_i+parity_j+breed_k+(GG|breed)_{kl}+FS_m+parity\times breed_{jk}+parity\times FS_{jm}$$
$$+breed\times FS_{km}+(GG|breed)\times FS_{klm}+cow_n+e_{ijklmno}$$

where $y_{ijklmno}$ was the plasma concentration of interest (e.g. glucose 20 d postpartum), the independent variables as described earlier with the substitution of the covariate $grazing_{ijklmn}$ (days to turnout) by the fixed effect of the type of diet out_o (o=indoor or pasture diet) at the time of blood sampling.

Inseminations Outcomes Inseminations outcomes were studied using the following initial generalized (logistic) linear mixed models:

$$\log\left[\frac{P\left(y_{ijklmno}=1\right)}{1-P\left(y_{ijklmno}=1\right)}\right]=\mu+\beta_1 grazing_{ijklmno}+\beta_2 dim_{ijklmno}+year_i+parity_j+breed_k+(GG|breed)_{kl}+FS_m$$
$$+cpb_o+parity\times breed_{jk}+parity\times FS_{jm}+breed\times FS_{km}+(GG|breed)\times FS_{klm}+cow_n$$

where $y_{ijklmno}$ was the outcome of inseminations as dichotomous variables (NF/EEM: 0/1, LEM: 0/1, FD/abortion: 0/1, calving: 0/1), the independent variables as described earlier with the addition of the covariate $\beta_2 dim_{ijklmno}$ describing the effect of days in milk, and of cpb_o describing the fixed effect of calving problems (o=0 or 1). All generalized linear mixed model analyses were performed using the *glmer* procedure of the R statistical package (R Core Team, 2016).

Days to Conception and Calving Service The times from start of the breeding season (with additional required days: if a cow calved in March the 15th, considering the voluntary waiting period of 40 d, she was allowed to be inseminated from April the 24th only even though the breeding period started in April the 1st) to conception (DAIF: Days to Artificial Insemination resulting in Fertilization of the oocyte) or to successful service (DAIC: Days to Artificial Insemination resulting in Calving) were studied using survival techniques. DAIF and DAIC may be different because of LEM, FD and abortions. A parametric proportional hazard model was used, assuming a Weibull baseline. The hazard for any animal t-days after possible service was modelled as follows:

$$h(t;\mathbf{x},\mathbf{z})=\lambda\rho\,(\lambda t)^{\rho-1}\quad \exp\{\mathbf{x}'\beta+\mathbf{z}'(t)\phi\}$$

where $\lambda\rho\,(\lambda t)^{\rho-1}$ is a Weibull baseline hazard function using 2 parameters (λ and ρ), **x** is an incidence vector relating the hazard function to a set of time-independent effects β including all variables described in the preceding model (with the addition of days from the start of the breeding season to first service to study DAIF; and the effect of DAIF to study DAIC), and **z** is an incidence vector relating the hazard function to time-dependent effects ϕ including protein content in the case of DAIF and milk yield for DAIC. When no artificial insemination resulting in either fertilization of the oocyte or calving was observed, the observation was censored. Censoring means that the event (occurrence of the artificial insemination resulting in fertilization of the oocyte/calving) has not occurred yet by the end of the breeding season. In such cases, DAIF and DAIC were computed as the the time between the start of the breeding season (adjusted for the voluntary waiting period) and its end. Survival analyses make use of this partial information. The estimation of variance components failed, probably because of a too limited number of records. Animal variance was fixed at 0.49 assuming a loggamma distribution for this effect (a common practice in frailty models) with parameter γ equal to 0.4. The robustness of the models was tested by comparing the results with an animal variance equal to 0.22 (γ =0.2) and 1.64 (γ =0.8), and the other results were similar. All survival analyses were performed using the *Survival Kit* statistical package (Mészáros et al., 2013).

Table 3. Adjusted production performances over the 14 firsts lactation weeks or over the whole lactation (44 wk) for Holstein and Normande cows, in the Milk- or the Content-Group, under either the High or Low feeding system.

	Holstein				Normande				Model		Significance levels[1]				
	Milk-Group		Content-Group		Milk-Group		Content-Group								
	High	Low	High	Low	High	Low	High	Low	σ_a	σ_e	B	B:G	FS	B ×FS	B:G ×FS
number of lactations	41	61	60	55	73	57	67	86							
Over first 14 wk															
Milk yield (kg/d)	37.6^{f}	25.6^{d}	35.4^{f}	23.5^{c}	28.1^{e}	20.6^{b}	26.1^{d}	18.7^{a}	1.62	3.16	***	***	***	***	0.11
Peak milk yield (kg/d)	45.2^{e}	29.8^{b}	41.0^{d}	27.6^{b}	32.7^{c}	23.1^{a}	29.5^{b}	21.8^{a}	1.85	3.49	***	***	***	***	†
Fat content (g/kg)	36.8^{a}	37.3^{a}	38.9^{bc}	39.5^{cd}	37.5^{a}	38.0^{ab}	40.2^{de}	40.7^{e}	1.36	2.03	**	***	*	0.87	0.39
Protein content (g/kg)	29.5^{bc}	27.8^{a}	31.0^{de}	29.0^{b}	31.9^{f}	30.2^{cd}	33.9^{g}	31.2^{ef}	0.60	1.27	***	***	***	0.28	*
Milk Solids (g/d)	2,480^{d}	1,664^{b}	2,436^{d}	1,620^{b}	1,938^{c}	1,392^{a}	1,902^{c}	1,356^{a}	111.4	207.4	***	0.26	***	***	0.35
BCS at calving	3.05^{abc}	2.90^{ab}	2.95^{ab}	2.80^{a}	3.60^{d}	3.20^{bc}	3.70^{d}	3.30^{c}	0.258	0.402	***	0.13	***	**	0.11
BCS at nadir	1.95^{bc}	1.50^{a}	1.90^{b}	1.50^{a}	2.80^{e}	2.20^{c}	3.05^{f}	2.45^{d}	0.298	0.323	***	**	***	†	0.29
BC loss (nadir-calving)	-1.20^{b}	-1.55^{a}	-1.20^{b}	-1.55^{a}	-0.65^{c}	-1.05^{b}	-0.50^{d}	-0.85^{c}	0.195	0.320	***	**	***	0.73	0.78
Over 44 wk															
Total MY (kg)	8,857^{f}	6,362^{d}	8,093^{e}	5,598^{c}	6,506^{d}	5,024^{b}	5,857^{c}	4,376^{a}	356.7	620.2	***	***	***	***	0.13
Lactation persistency (%)	78^{abc}	86de	74^{a}	83^{cd}	80^{bc}	88^{e}	76^{ab}	85de	0.042	0.078	*	***	***	0.60	0.91
Total Milk Solids (kg)	584^{d}	416^{b}	568^{d}	401^{b}	463^{c}	354^{a}	448^{c}	338^{a}	23.1	43.3	***	**	***	***	0.58
BCS end of lactation	2.40^{b}	2.00^{a}	2.35^{b}	1.90^{a}	3.30^{d}	2.60^{b}	3.55^{e}	2.80^{c}	0.293	0.340	***	**	***	**	0.80
BC gain (end-nadir)[2]	+0.50	+0.50	+0.50	+0.45	+0.45	+0.40	+0.45	+0.45	0.080	0.325	0.21	0.78	0.36	0.31	0.15

[1] Effects of Breed (B), Genetic group within Breed (B:G), Feeding System (FS), interaction between Breed and Feeding System (B×FS), interaction between Genetic group within Breed and Feeding system (B:G×FS)

[2] The estimation of variance components failed. As an alternative, an empirical Bayesian approach was implemented

[a - f] distinguish adjusted means that are different between breeds, genetic groups, and feeding systems ($P < 0.05$, Tukey's pairwise comparison).

Significant levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; † $P < 0.1$

RESULTS

Production Performances

Effects of Genetic Merit for Milk Yield on phenotypic Milk Yield and Body Reserve Holstein cows produced more milk (+2,294 kg in the High FS and +1,280 kg in the Low FS, $P < 0.001$; Table 3) and more milk solids (+120 kg in the High FS and +62 kg in the Low FS, $P < 0.001$) throughout lactation than Normande cows. Over the firsts 14 wk of lactation, Holstein cows had lower fat (-1.0 g/kg, $P < 0.01$) and protein content (-2.5 g/kg, $P < 0.001$) than Normande cows. Holstein cows had a higher peak milk yield (+12.0 kg in the High FS and +6.3 kg in the Low FS, $P < 0.001$) and a lower persistency (-2 percentage units, $P < 0.05$) than Normande cows. Holstein cows had a lower BCS at calving (-0.70 points in the High FS and -0.35 points in the Low FS, $P < 0.001$), at nadir (-1.00 points in the High FS and -0.80 points in the Low FS, $P < 0.001$), and at the end of lactation (-1.05 points in the High FS and -0.75 points in the Low FS, $P < 0.001$) than Normande cows. At 20 d postpartum, Holstein cows had a similar glycaemia ($P=0.55$; Table 4), higher plasma NEFA concentration (+128 µmol/l, $P < 0.001$) and a lower uremia (-1.9 mg/dl, $P < 0.01$) than Normande cows. At 60 d postpartum, Holstein cows had a higher glycaemia (+1.6 mg/dl, $P < 0.01$), higher plasma NEFA concentration (+28 µmol/l, $P < 0.01$) and similar uremia ($P=0.29$) than Normande cows.

Milk solids production was similar between genetic groups within breeds (+15 kg for cows in the Milk-Group compared to Content-Group within breeds, $P=0.17$ and 0.12 for Holstein and Normande cows respectively) and there was no interaction with FS ($P=0.58$). In both breeds, cows in the Milk-Group produced more milk throughout lactation than those in the Content-Group (+764 kg for Holstein cows and +649 kg for Normande cows, $P < 0.001$). Over the firsts 14 wk of lactation, cows in the Milk-Group had lower fat (-2.1 g/kg for Holstein cows and -2.7 g/kg for Normande cows, $P < 0.001$) and protein content (-1.5 g/kg for Holstein cows and -1.9 g/kg for Normande cows, in the High FS, $P < 0.001$; -1.2 g/kg for Holstein cows and -1.0 g/kg for Normande cows, in the Low FS, $P < 0.01$) than those in the Content-Group. In the High FS, cows in the Milk-Group had a higher peak milk yield (+4.2 kg for Holstein cows and +3.2 kg for Normande cows, $P < 0.001$) and that was not the case in the Low FS ($P=0.11$ for Holstein cows and $P=0.77$ for Normande cows). In both breeds, cows in the Milk-Group had a higher lactation persistency (+4 percentage units, $P < 0.05$) than cows in the Content-Group. In both breeds, cows had a similar BCS at calving between genetic groups ($P=0.13$). Normande cows in the Milk-Group had a lower BCS at nadir (-0.25, $P < 0.01$) and at the end of lactation (-0.20, $P < 0.01$) than in the Content-Group. For Holstein cows, BCS was not affected by genetic groups ($P=0.99$ at nadir and $P=0.90$ at the end of lactation).

Table 4. Adjusted plasma concentrations of glucose, NEFA and urea at 20, 60 d postpartum for Holstein and Normande cows, in the Milk- or the Content-Group, under either the High or Low feeding system.

	Holstein				Normande				Model		Significance levels[1]				
	Milk-Group		Content-Group		Milk-Group		Content-Group								
	High	Low	High	Low	High	Low	High	Low	σ_a	σ_e	B	B:G	FS	B ×FS	B:G ×FS
number of lactations	41	61	60	55	73	57	67	86							
at 20 d postpartum															
Glucose (mg/dl)	61.6^{c}	57.4^{ab}	62.0^{c}	57.9^{ab}	60.7^{bc}	56.6^{a}	61.9^{c}	57.7^{ab}	3.50	5.72	0.55	0.45	***	0.76	0.43
NEFA (µmol/l)[3]	415^{c}	418^{c}	381^{c}	410^{c}	333^{bc}	269^{ab}	237^{a}	282^{ab}	0.07	0.59	***	0.11	0.84	0.59	*
Urea (mg/dl)[2]	23.1^{abc}	19.9^{a}	25.3^{cd}	19.9^{a}	24.5^{bcd}	21.8^{ab}	27.6^{d}	21.9^{ab}	1.10	6.33	**	†	***	0.92	†
at 60 d postpartum															
Glucose (mg/dl)	64.2^{de}	60.6^{abc}	65.2^{e}	61.6^{bcd}	63.9^{cde}	60.3^{ab}	62.3^{bcd}	58.7^{a}	2.35	6.49	**	0.15	***	0.28	0.62
NEFA (µmol/l)[3]	223^{bc}	252^{c}	201^{abc}	227^{bc}	197^{ab}	222^{bc}	175^{a}	197^{ab}	0.11	0.48	**	*	**	0.78	0.40
Urea (mg/dl)	26.3^{b}	20.6^{a}	26.3^{b}	20.6^{a}	27.0^{b}	21.3^{a}	27.2^{b}	21.5^{a}	2.58	7.53	0.29	0.98	***	0.12	0.51

[1] Effects of Breed (B), Genetic group within Breed (B:G), Feeding System (FS), interaction between Breed and Feeding System (B×FS), interaction between Genetic group within Breed and Feeding system (B:G×FS)

[2] The estimation of variance components failed. As an alternative, an empirical Bayesian approach was implemented

[3] plasma NEFA concentrations were log-transformed to perform the analyses

[a - d] distinguish adjusted means that are different between breeds, genetic groups, and feeding systems ($P < 0.05$, Tukey's pairwise comparison).

Significant levels: *** P <0.001; ** P <0.01; * P <0.05; † P <0.1

Effects of Restricted Nutrition on phenotypic Milk Yield and Body Reserve Cows in the High FS produced more milk (+2,495 kg for Holstein and +1,481 kg for Normande cows, $P < 0.001$) and more milk solids (+168 kg for Holstein and +109 kg for Normande cows, $P < 0.001$) throughout lactation than those in the Low FS. The effect of FS on total milk yield and milk solids yield was significantly more pronounced for Holstein than for Normande cows but similar for both genetic groups within breeds. Over the 14 firsts weeks of lactation, fat content was lower (-0.5 g/kg, $P < 0.05$) and protein content was higher (+2.0 g/kg, $P < 0.001$) for cows in the High FS than for those in the Low FS. Cows in the High FS had a higher peak milk yield (+14.4 kg for Holstein and +8.6 kg for Normande cows, $P < 0.001$) and a lower lactation persistency (-9 percentage units, $P < 0.001$) than cows in the Low FS. BCS at calving was not affected by FS for Holstein cows (P=0.53). Normande cows in the High FS had higher BCS than those in the Low FS (+0.40 points, $P < 0.001$). Cows in the High FS had higher BCS at nadir (+0.40 points for Holstein and +0.60 points for Normande, $P < 0.001$) and higher BCS at the end of lactation (+0.45 points for Holstein and +0.70 points for Normande, $P < 0.001$). Cows in the High FS had a higher glycaemia (+4.1 mg/dl at 20 d postpartum and +3.7 mg/dl at 60 d postpartum, $P < 0.001$) and uremia (+4.3 mg/dl at 20 d postpartum and +5.7 mg/dl at 60 d postpartum, $P < 0.001$) than those in the Low FS. Plasma concentration of NEFA was not affected by FS at 20 d postpartum (P=0.84), at 60 d cows in the High FS had a lower plasma concentration of NEFA than cows in the Low FS (-25 µmol/l, $P < 0.05$).

Lactation Performance during the Breeding Season First service was performed on average around 80 d postpartum and second service around 109 d postpartum. While being inseminated, cows were still producing large quantities of milk and milk solids, and had already mobilized a substantial part of their body reserve (Table 5). Holstein cows produced more milk than Normande cows at first (+9.1 kg/d in the High FS and +5.3 kg/d in the Low FS, $P < 0.001$) and second service (+8.7 kg/d in the High FS and +5.2 kg/d in the Low FS, $P < 0.001$). They also produced more milk solids at first (+445 g/d in the High FS and +277 g/d in the Low FS, $P < 0.001$) and second service (+391 g/d in the High FS and +194 g/d in the Low FS, $P < 0.001$) than Normande cows. Holstein cows had a lower BCS at first (-0.90 points, $P < 0.001$) and second service (-0.95 points, $P < 0.001$) than Normande cows.

In both breeds, cows in the Milk-Group produced more milk than those in the Content-Group at first (+2.1 kg/d for Holstein cows, $P < 0.01$ and +2.9 kg/d for Normande cows, $P < 0.001$) and second service (+3.1 kg/d for Holstein cows and +2.3 kg/d for Normande cows, $P < 0.001$). However, milk solids production at service was not affected by genetic groups within breeds (globally significant at first service, but Holstein cows in the Milk-Group produced +59g/d than those in the Content-Group, P=0.62; Normande cows in the Milk-Group produced +52g/d than those in the Content-Group, P=0.64). For Holstein cows, BCS at first and second service was not affected by genetic groups (P=0.84 and P=0.92 respectively). Normande in the Milk-Group had lower BCS at first (-0.25, $P < 0.05$) and at second service (-0.30, $P < 0.05$) than those in the Content-Group.

Table 5. Adjusted production performances at first and second service for Holstein and Normande cows, in the Milk- or the Content-Group, under either the High or Low feeding system.

	Holstein				Normande				Model		Significance levels[1]				
	Milk-Group		Content-Group		Milk-Group		Content-Group								
	High	Low	High	Low	High	Low	High	Low	σ_a	σ_e	B	B:G	FS	B ×FS	B:G× FS
At first service															
number of inseminations	38	50	58	53	72	53	66	80							
days in milk	88±22	79±24	84±24	80±28	79±23	80±26	81±25	75±25							
Milk yield (kg/d)	37.1^{g}	25.3^{d}	35.0^{f}	23.2^{c}	28.4^{e}	20.3^{b}	25.4^{d}	17.4^{a}	1.55	3.50	***	***	***	***	0.16
Fat content (g/kg)	33.5^{a}	35.7^{b}	37.6^{bc}	38.8^{c}	37.2^{bc}	39.1^{c}	41.2^{d}	41.2^{d}	1.43	2.38	***	***	***	0.23	*
Protein content (g/kg)	29.1^{b}	27.3^{a}	31.0^{c}	29.1^{b}	31.7^{c}	29.9^{b}	33.2^{d}	31.3^{c}	0.68	1.46	***	***	***	0.29	0.25
Milk Solids (g/d)	2,337^{d}	1,614^{b}	2,350^{d}	1,626^{b}	1,947^{c}	1,393^{a}	1,849^{c}	1,294^{a}	109.7	237.6	***	*	***	**	0.50
BCS	2.35^{b}	1.80^{a}	2.25^{b}	1.70^{a}	3.05^{de}	2.50^{bc}	3.30^{e}	2.75^{cd}	0.389	0.390	***	*	***	0.25	0.11
At second service															
number of inseminations	17	21	25	32	26	25	28	33							
days in milk	109±22	105±31	111±26	112±30	100±28	114±31	114±29	108±35							
Milk yield (kg/d)	33.4^{g}	25.8^{e}	30.3^{f}	22.7^{cd}	24.3^{de}	20.2^{b}	22.0^{bc}	17.9^{a}	2.06	2.24	***	***	***	***	0.14
Fat content (g/kg)	33.5^{a}	34.6^{ab}	36.6^{bc}	37.7^{cd}	37.8^{cd}	38.8^{d}	41.5^{e}	42.6^{e}	1.48	2.32	***	***	*	0.84	0.85
Protein content (g/kg)[2]	29.3^{b}	28.4^{a}	30.9^{c}	29.9^{b}	32.3^{d}	31.3^{c}	34.0^{e}	33.1^{d}	0.48	1.30	***	***	***	0.39	0.20
Milk Solids (g/d)	2,095^{d}	1,604^{c}	2,036^{d}	1,545^{bc}	1,701^{c}	1,407^{ab}	1,649^{c}	1,355^{a}	152.1	143.7	***	0.24	***	**	0.29
BCS	2.40^{bc}	1.70^{a}	2.35^{b}	1.65^{a}	3.15^{d}	2.45^{bc}	3.45^{d}	2.75^{c}	0.330	0.402	***	*	***	0.28	0.11

[1] Effects of Breed (B), Genetic group within Breed (B:G), Feeding System (FS), interaction between Breed and Feeding System (B×FS), interaction between Genetic group within Breed and Feeding system (B:G×FS)

[2] The estimation of variance components failed. As an alternative, an empirical Bayesian approach was implemented

[a - g] distinguish adjusted means that are different between breeds, genetic groups, and feeding systems ($P < 0.05$, Tukey's pairwise comparison).

Significant levels: *** P <0.001; ** P <0.01; * P <0.05; † P <0.1

Fertility Performances

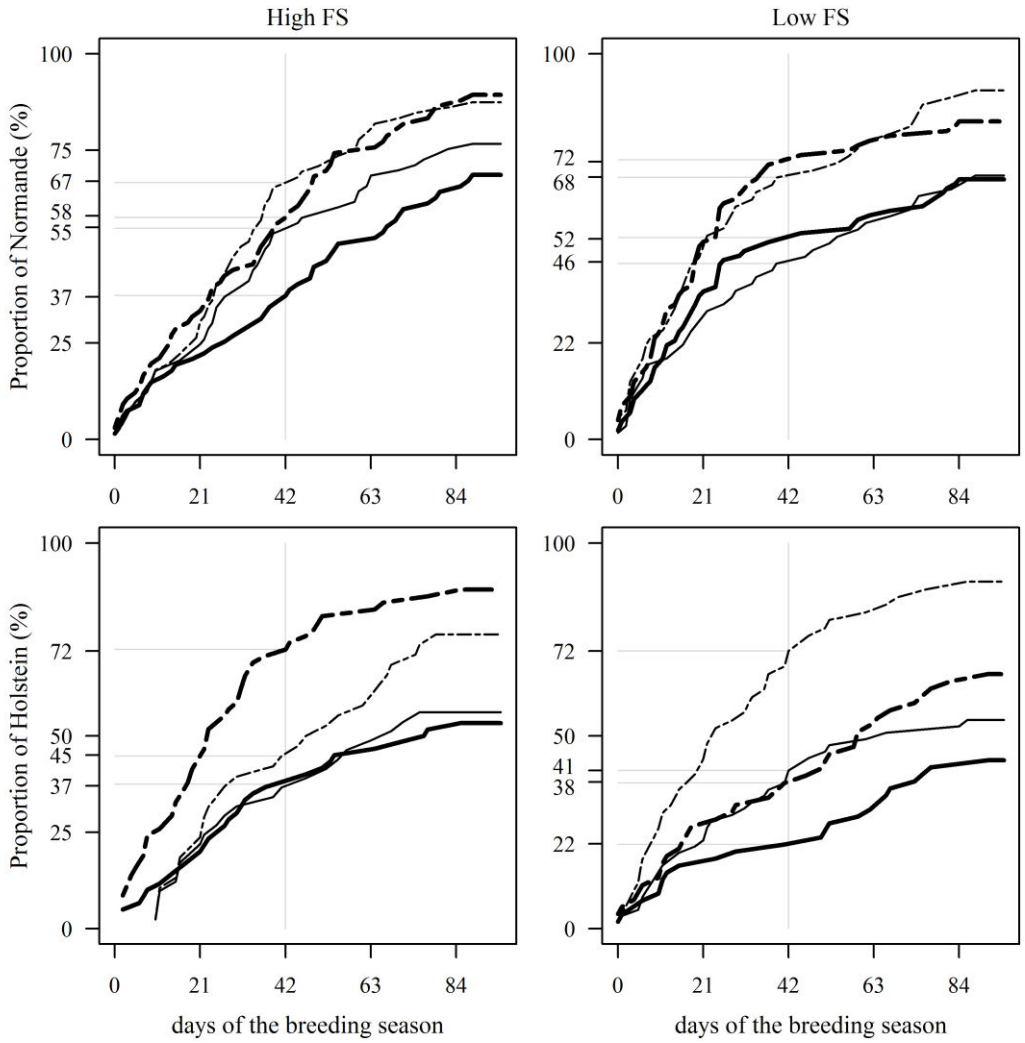

Figure 1: Observed cumulative proportions of Normande (graphs on the top) and Holstein (graphs on the bottom) cows that conceived at service (dashed lines) and that had a service leading to calving (solid lines) from start of the breeding season to the end of the breeding season in the Milk-Group (n=130 Normande and 102 Holstein cows, thin lines) or in the Content-Group (n=153 Normande and 115 Holstein cows, thick lines), under the High FS (n=140 Normande and 101 Holstein cows, graphs on the left) or Low FS (n=143 Normande and 116 Holstein cows, graphs on the right).

Table 6. Time from possible service to conception (DAIF) or to service leading to calving (DAIC) for Holstein and Normande cows, in the Milk- or the Content-Group, under either the High or Low feeding system.

	Holstein				Normande				Model	Significance levels[2]				
	Milk-Group		Content-Group		Milk-Group		Content-Group							
	High	Low	High	Low	High	Low	High	Low	σ_a[1]	B	B:G	FS	B ×FS	B:G ×FS
number of cows inseminated	38	50	58	53	72	53	66	80						
DAIF[3]	47	32	31	49	38	32	38	31	0.70	0.60	0.17	0.48	†	†
$\hat{\beta}$[4]	-0.41	0.36	0.31	-0.71	0.04	0.06	-0.08	0.06						
HR[4]	0.7	1.4	1.4	0.5	1.0	1.1	0.9	1.1						
95% CI[4]	0.25-1.79	1.29-1.60	1.28-1.45	0.43-0.57	0.98-1.09	0.97-1.18	0.86-0.99	1.01-1.12						
number of cows that conceived	29	45	51	35	63	48	59	66						
DAIC[3]	46	43	53	53	39	43	49	35	0.70	†	0.82	0.40	0.76	0.59
$\hat{\beta}$[4]	-0.15	-0.14	-0.68	-0.05	0.17	0.01	0.01	0.36						
HR[4]	0.9	0.9	0.5	0.9	1.2	1.0	1.0	1.4						
95% CI[4]	0.27-2.69	0.86-0.88	0.41-0.62	0.71-1.27	1.13-1.24	0.99-1.03	0.87-1.17	1.25-1.65						

[1] Animal variance was fixed at $\sigma_a^2 = 0.49$, assuming a loggama(0.4) distribution, and the robustness of the model was tested by comparing the results with a variance fixed at 0.22, assuming a loggama(0.2), and 1.64, assuming a loggamma(0.8). The other results were similar.

[2] Effects of Breed (B), Genetic group within Breed (B:G), Feeding System (FS), interaction between Breed and Feeding System (B×FS), interaction between Genetic group within Breed and Feeding system (B:G×FS)

Significant levels: *** p <0.001; ** p <0.01; * p <0.05; † p <0.1

[3] Average DAIF for the 470 cows that were inseminated at least once during their lactation (of which 74 are censored) and average DAIC for the 396 cows that conceived at least once during their lactation (of which 85 are censored)

[4] Estimated effect $\hat{\beta}$, associated Hazard Ratios (HR=exp($\hat{\beta}$)) and 95% Confidence Intervals (CI) constraining the weighted mean of the estimated effects to be 0

Effects of Restricted Feeding on likelihood of Conception of cows Selected for Fat and Protein Contents Over the 500 reproductive sequences (cows/year) involved in the trials, 3 never resumed ovarian activity and 27 were never detected in estrus, which resulted in only 470 inseminated cows. Among those, DAIF ranged from 1 to 92 d, with an average interval to conception of 30 d. Seventy four cows had censored DAIF, with an average censoring time of 74 d. There was a clear genetic x environment interaction for DAIF (Table 6, Figure 1). For Normande cows, genetic group did not affect time to conception (Figure 1). Normande cows in the Low FS had a higher 42-d conception rate than in the High group (+6 percentage units) and overall conception rate was similar in both FS (about 87%). However, Holstein cows in the high FS were more likely to conceive than in the Low FS (+11 percentage units at 42-d, +10 percentage units at the end of the breeding season; Figure 1). In the High FS, Holstein cows in the Milk-Group had the lowest conception rate (45% at 42-d and 76% at the end of the breeding season). The opposite was observed in the Low FS, Holstein in the Content-Group had the lowest conception rate (38% at 42-d, 66% at the end of the breeding season).

Holstein cows had a higher proportion of NF/EEM than Normande cows at first (+7 percentage units, $P < 0.10$; Table 7) and at combined first and second services (+6 percentage units, $P < 0.10$). In the Low FS, Holstein cows in the Content-Group had more NF/EEM (+29 percentage units at first service and +26 percentage units at first and second services) than Holstein cows in the Milk-Group. However, Holstein cows in the Milk-Group under the High FS had an appreciable 53% NF/EEM at first service. EBV for milk yield was not associated with DAIF (P=0.92). Higher protein content was associated with earlier DAIF (estimated effect: $\hat{\beta} = 0.09$, HR = 1.1 for 1g/kg, 95% Confidence Intervals: 95% CI = 1.0-1.2; $P < 0.01$). Each additional week from the start of the breeding season to first service was associated with lower chance of conception or later DAIF ($\hat{\beta} = -0.17$, HR = 0.3 per additional week, 95% CI = 0.2-0.5; $P < 0.001$). Primiparous cows conceived earlier than multiparous cows ($\hat{\beta} = 0.48$, HR = 1.6, 95% CI = 1.2-2.3; $P < 0.001$). Cows without calving problems conceived earlier than cows with calving problems ($\hat{\beta} = 0.40$, HR = 1.5, 95% CI = 1.0-2.3; $P < 0.10$).

Effects of Restricted Feeding on Embryo Mortality of cows Selected for Fat and Protein Contents Over the 470 cows inseminated during this trial, only 396 conceived. Among those, DAIC ranged from 1 to 92 d, with an average interval to the insemination resulting in calving of 34 d. Eighty five cows had censored DAIC, with an average censoring time of 84 d. Holstein cows had a lower re-calving rate than Normande cows (-19 percentage units, P < 0.001; Table 7). Indeed, Normande cows were 1.6 times more likely to have a service leading to calving at a given time than Holstein cows ($P < 0.10$; Table 6, Figure 1). Holstein cows had a lower proportion of service leading to calving (-12 percentage units at first service, $P < 0.001$; -13 percentage units at second service, $P < 0.10$) than Normande cows. Re-calving rate was neither affected by genetic group (P=0.32; Table 7) nor FS (P=0.25). However, within breeds, the higher the EBV for milk yield, the higher the chance to have service leading to calving ($P < 0.10$). At first service, there was a genetics x environment interaction on proportion of calving services ($P < 0.10$; Table 7). The proportion of first service leading to calving was very low for Holstein cows in the Content-Group under the Low FS (24 %) and for Holstein cows in the Milk-Group under the High FS (31 %). Re-calving rate was the lowest for.

Table 7. Proportion of 1st, 2nd or combined 1st and 2nd services leading to non-fertilization or early embryo mortality / late embryo mortatility / foetal death or abortion / calving, and overall re-calving rate for Holstein and Normande cows, in the Milk- or the Content-Group, under either the High or Low feeding system.

	Holstein				Normande				Model	Significance levels[1]				
	Milk-Group		Content-Group		Milk-Group		Content-Group							
	High	Low	High	Low	High	Low	High	Low	σ_a	B	B:G	FS	B ×FS	B:G ×FS
Outcome of 1st service														
number of 1st service	38	50	58	53	72	53	66	80						
Calving (%)²	31	42	36	24	54	42	41	45	0.47	***	0.48	0.62	0.64	†
FD/abortion (%)	3	10	9	6	3	11	9	5	. . .	. . .	. . .	. . .	. . .	. . .
LEM (%)	13	8	22	8	5	9	14	6	. . .	. . .	. . .	. . .	. . .	. . .
NF/EEM (%)	53	40	33	62	38	38	36	44	0.48	†	0.89	0.15	0.81	*
Outcome of 2nd service														
number of 2nd service	17	21	25	32	26	25	28	33						
Calving (%)²	65	38	32	25	46	48	50	55	0.52	†	†	0.46	0.15	0.69
FD/abortion (%)	0	9	8	0	0	8	0	0	. . .	. . .	. . .	. . .	. . .	. . .
LEM (%)	6	10	20	12	12	0	4	15	. . .	. . .	. . .	. . .	. . .	. . .
NF/EEM (%)²	29	43	40	63	42	44	46	30	0.48	0.15	0.18	0.22	0.13	0.45
Combined outcome of 1st&2nd service														
number of 1st service	38	50	58	53	72	53	66	80						
Calving (%)	60	58	50	40	71	64	62	68	0.44	**	†	0.42	0.69	0.57
FD/abortion (%)	3	10	10	4	3	9	5	0	. . .	. . .	. . .	. . .	. . .	. . .
LEM (%)	8	10	19	9	7	2	7	7	. . .	. . .	. . .	. . .	. . .	. . .
NF/EEM (%)	29	22	21	47	19	25	26	25	0.28	†	0.44	0.19	0.64	†
number of lactations	41	61	60	55	73	57	67	86						
Re-calving rate (%)	56	54	53	44	77	68	69	67	0.32	***	0.32	0.25	0.77	0.82

[1] Effects of Breed (B), Genetic group within Breed (B:G), Feeding System (FS), interaction between Breed and Feeding System (B×FS), interaction between Genetic group within Breed and Feeding system (B:G×FS)

Significant levels: *** p <0.001; ** p <0.01; * p <0.05; † p <0.1

Holstein cows in the Content-Group under the Low FS (22% at 42-d and 44% at the end of the breeding season; Figure 1, Table 7). In the High FS, Holstein cows in the Content-Group had a higher proportion of LEM than in the Milk-Group (+10 percentage units at first and second services). Nevertheless, Normande cows in the Content-Group also had more LEM than in the Milk-Group at first service (+9 percentage units). More days from the start of the breeding season to conception (DAIF) was associated with a lower chance of success and later DAIC ($\hat{\beta}$ = -0.25, HR = 0.3 per additional week, 95% CI = 0.1-0.3; $P < 0.001$). Higher BCS at calving was associated with earlier DAIC ($\hat{\beta}$ = 0.38, HR = 1.5 for 1 point, 95% CI = 1.1-1.9; $P < 0.01$).

DISCUSSION

To our knowledge, the association between the balance between milk yield and milk contents and fertility was never studied before. We conjectured that by having a lower peak milk yield and a lower lactose yield cows with positive genetic merit for fat and protein content would have a higher energy status than those with positive genetic merit for milk yield and thus would preserve their reproductive performance while producing the same amount of milk solids.

Genetic Merit for Fat and Protein Content is associated with Reduced Fertility in Holstein cows in Various Environments

Fertility was globally impaired in this study compared to the goals of compact calving systems (e.g. 42-d pregnancy rate of 70 % and final pregnancy rate of 90% in Ireland; Butler, 2014) and was particularly poor in Holstein cows. Holstein cows in the Content-Group had the worst reproductive performance. However, the pattern of failure changed according to FS. Holstein cows in the Content-Group and under the Low FS showed the lowest conception rate (38% at 42-d and 66% by the end of the breeding season). This is not consistent with their submission rate (63% at 21-d; N. Bedere, unpublished data) and the present study showed they had a higher proportion of NF/EEM than other groups (47% at first and second services the range was from 19 to 29% for other groups). They had the lowest BCS at calving, at nadir, at first and second service and at the end of lactation. Low BCS at calving is known to be associated with low conception rate and longer days to conception (López-Gatius et al., 2003; Cardoso et al., 2013). In a previous study, we found that low BCS at nadir was associated with higher risk of NF/EEM (Cutullic et al., 2012). In the present study, higher protein content was associated with higher chance for the cow to conceive. Holstein cows in the Content-Group and under the Low FS had a relatively low protein content (28.9 g/kg over the first 14 wk of lactation) compared to their genetic merit for protein content. Protein content is known to be lower for cows in severe and prolonged negative energy balance (Fulkerson et al., 2001). Negative energy balance is a complex condition and is activating several endocrine signals (fall of IGF-I and leptin blood levels, increased insulin resistance, increased secretion of GH and catecholamine). These signals are impacting nutrient partitioning and are known to promote body reserve mobilization and milk production and to even inhibit reproduction. Mobilization results in high plasma concentrations of NEFA that are used as energy supply but they also damage oocytes and endometrium, causing embryonic death (Santos et al., 2004b; Friggens et al., 2010; Wathes et al., 2013). In our previous study, we showed that primiparous Holstein cows in the Content-Group under the low FS had the lowest body weight (Bedere et al., 2016). According to their BCS, all

Holstein cows suffered of more severe and prolonged negative energy balance than Normande cows. This difference was even larger in the Low FS. When pregnancy was finally established for Holstein cows in the Content-Group and under the Low FS, 9% of first and second services led to LEM which is within the range of most groups (from 2 to 10%). Nevertheless, due to their low proportion of first and second service that induced calving (40%) and longer days to conception, Holstein cows in the Content-Group and under the Low FS had the lowest re-calving rate (22% at 42-d and 44% by the end of the breeding season). This result is below 50% and is underlying the limitations of such genetic characteristics in restrictive FS.

In the High FS, Holstein cows in the Milk-Group had the lowest conception rate (45% at 42-d and 76% by the end of the breeding season). This is partly explained by more NF/EEM (53% of the services) and more LEM (13% at first service). Other results also showed that Holstein cows in the Milk-Group had the lowest submission in both FS (N. Bedere, unpublished data). Their ability to be pregnant at second service balanced this result so that their re-calving rate (56%) is not different from Holstein cows in the Milk-Group under the Low FS (54%) and Holstein cows in the Content-Group under High FS (53%). However, in our study, Holstein cows in the Content-Group under the High FS had a high proportion of LEM (19% at first and second services). LEM are known to be associated with lower lactation persistency (Buckley et al., 2003; Cutullic et al., 2012). Consistently, Holstein cows in the Content-Group under the High FS had the lowest lactation persistency (74%), and this phenomenon was also observed in Normande cows in the Content-Group under the High FS (persistency of 76%). In the present study, persistency was estimated through the ratio between milk yields from 100 to 200 d over 0 to 100 d. A limit of such an indicator is that the effect of persistency may be confounded with the effect of peak milk yield. Other measurements of persistency exist (e.g. parameters of the Wilmink curve, 1987). They could not be estimated in the present study because of the occurrence of atypical lactation profiles (peak milk yield occurring during the last two third of lactation, more than one peak milk yield…). In our experiment, Holstein cows produced daily an extra 10 kg of milk at peak, at first and second services than Normande cows suggesting that low persistency may not impair embryo survival below a certain milk yield threshold. This is consistent with the fact that LEM are more frequent in high producing cows (Grimard et al., 2006). It is also known that cows with high genetic merit for milk production have low genetic merit for BCS (genetic correlation about -0.37, heritability of BCS ranging from 0.20 to 0.50; Bastin and Gengler, 2013). Our result shows that higher BCS at calving was associated with higher chances to have a service resulting in calving. This is also consistent with the fact that BCS is positively associated with probability of pregnancy (Buckley et al., 2003; Santos et al., 2009) and negatively associated with LEM (Silke et al., 2002; Grimard et al., 2006). This association is partly consistent with the positive genetic correlation between BCS and 42-d re-calving rate (0.43; Pryce and Harris, 2006). Morton et al. (2016a, b) showed that higher protein content was associated with higher fertility status. Interestingly, they reported that if protein content is low (i.e. below 30 g/kg) cows with high milk yield are more likely to be inseminated, to conceive and to be pregnant than those with lower milk yield (i.e. 5,000 kg vs 2,000 kg of milk over 120 days in milk). This suggests that the association between protein content and reproduction may also be related to other causes than the extent of negative energy balance. Further studies are required to better understand the

underlying biological mechanisms. Some of our results suggest that there may be a link between genetic characteristics of milk fat and protein contents and fertility. Indeed, we found that cows in the Content-Group perform worst in both FS and breeds (lower conception and pregnancy rates). This is in agreement with another result: EBV for milk yield was associated with a higher chance to have a successful insemination (in this analysis, the effect of genetic group was not significant and therefore not included in the model). Other studies observed results similar to ours: chances of pregnancy can be positively related to high milk yield (Gröhn and Rajala-Schultz, 2000; Buckley et al., 2003). These findings may appear intriguing given the consensus that the higher the milk yield (phenotypically and genetically), the worse the reproductive performance (Royal et al., 2000; Friggens et al., 2010; Walsh et al., 2011). However, they are consistent with the fact that there is a large variability in the direction of relationship between milk yield and fertility at the individual level. Indeed, Nebel and McGilliard (1993) and Bello et al. (2013) found that herds with high genetic merit for milk yield had poor fertility status and that this was not observed between cows within herds. Our results show that in both breeds cows in the Milk-Group have delayed cyclicity compared to the Content-Group (Bedere et al., 2016a). Further studies are needed to confirm whether cows in the Content-Group have impaired fertility because selecting for fat content may have impaired embryo survival or not. This predisposition could explain the high proportion of LEM for Holstein cows in the Content-Group under the High FS. This hypothesis combined with the effects of severe negative energy balance (leading to a poor ability of oocytes to develop themselves) could explain NF/EEM of the Holstein in the Content-Group under the Low FS. Unfortunately, genetic merit for fertility traits of the animals involved in the study was not available which could be another underlying explanation of some of our results. Further investigation on genetic merit for fertility and its association with genetic merit for production traits (yields and contents) is needed.

Ability of Normande to be Pregnant is Preserved by their Adaptive Response to Nutrient Availability

As expected, Normande cows under nutritive restriction produced less milk and limited their body reserve mobilization while Holstein cows limited milk drop and mobilized a larger part of their body reserve (Dillon et al., 2003a; Walsh et al., 2008; Delaby et al., 2009). Consistently with the literature (Dillon et al., 2003b; Michel et al., 2003; Walsh et al., 2008), we found that Normande cows had a higher re-calving rate thanks to more fertilizations and less pregnancy losses than Holstein cows, under both contrasted FS. Results from our previous studies also showed that in both feeding systems, Normande had an earlier resumption of ovarian cyclicity, more normal cyclicity patterns, more ovulations due to shorter cycles, regular ovulation detection rate (about 70%), and higher submission rate (Cutullic et al., 2009, 2011; Bedere et al., 2016). This study also proved that fertility performance of dairy cows exhibits a genetic by environment interaction. There was no significant effect of genetic group and feeding systems on fertility of Normande. But Normande cows in the Low FS conceived earlier than those in the High FS. Our previous studies showed that this difference is notable on submission rate and was related to a higher estrus expression (Cutullic et al., 2011). Normande cows in the Milk-Group under the High FS had the best re-calving rate (77 %) compared to other groups. They calved at 3.60 points of BCS (0 -5 scale), mobilized few body

reserve, had a low peak milk yield (32.7 kg) and a high lactation persistency (80 %). Overall they produced an appreciable 6,498 kg of milk and 460 kg of milk solids. There is a consensus about the fact that these levels of production performance are ideal regarding reproduction success in compact calving systems (López-Gatius et al., 2003; Roche et al., 2009; Friggens et al., 2010). Benefits of breeding such cows should be investigated. Feeding system had no effect on re-calving rate, which is consistent with other studies (Kennedy et al., 2003; Horan et al., 2004; Vance et al., 2013). This suggests that the adaptive strategy of the animals in case of nutrient restriction (support milk yield *vs* preserve body condition) affects more dairy cows' ability to be pregnant than the nutritive uptake. A complementary study was performed on our data from 2006 to 2013 by Cloet et al. (2015), using the approach developed by Ollion et al. (2016). She identified five tradeoffs scenarios based on production, mobilization and reproduction performances of the cows. These scenarios helped us to confirm dairy cows' adaptive strategies to nutrient supply: under high nutrient supply, Holstein cows are investing their energy in milk yield while Normande cows in body reserve and under restricted nutrient supply, Holstein cows are mobilizing their body reserve to support milk production while Normande cows are limiting both mobilization and production. Further investigations on adaptive strategies of dairy cows to nutrient supply and their implication for robustness are needed.

CONCLUSIONS

This study confirmed that Normande cows have a better ability to ensure pregnancy than Holstein cows. There is a genetic x environment interaction on fertility performances. At identical genetic merit for milk solids production, Normande cows with high genetic merit for milk yield and Normande cows with high genetic merit for fat and protein content have a similar fertility. However, Holstein cows with high genetic merit for fat and protein content had lower fertility compared to Holstein cows with high genetic merit for milk yield. Under the restricted feeding system, they had a high NF/EEM rate, whereas under the higher feeding system they had a high proportion of pregnancy losses. We conclude that selecting dairy cows with more emphasis on fat and protein content instead of milk yield may decrease the ability of dairy cows to ensure pregnancy. Further research on genetic merit for fertility and its association with genetic merit for production traits (yields and contents) is needed.

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APPENDIX

The experimental farm is characterized by very heterogeneous and unusual management conditions. Consequently, recorded performances are not routinely included in the national data base and no national EBV are available for these cows. Furthermore, genomic evaluations were not available for the oldest cows of this study because they were not genotyped. Therefore, specific EBV had to be computed. The EBV for each trait was evaluated by combining within herd information analysed with a BLUP animal model with national EBV of the sires and grandsires. The model of analysis of cow performances over three lactations included usual fixed environmental effects (year, lactation number, calving age, calving month, drying off period length, and permanent environment effect) and the feeding system (H. Larroque, INRA UMR 1388 GenPhySE, Toulouse, France, personal communication). Within breed and experimental year, nulliparous cows with EBV for milk yield higher than average and EBV for fat and protein contents lower than average constituted a "Milk-Group". Nulliparous cows with EBV for milk yield lower than average and EBV for fat and protein contents higher than average constituted a "Content-Group". The others nulliparous cows (with high EBV for milk yield and high EBV for fat and protein contents or low EBV for milk yield and low EBV for fat and protein contents) did not enter the experiment. EBV were expressed in deviation from a base population, whose average EBV were set to 0 (Table A1).

Table A1. Customized Estimated Breeding Values for production traits (milk yield, fat content, protein content, fat yield, protein yield, milk solids yield) for Holstein and Normande cows, in the Milk- or the Content-Group

	Holstein		Normande	
	Milk-Group	Content-Group	Milk-Group	Content-Group
EBV for milk yield (kg)	+308	-303	+290	-264
EBV for fat content (g/kg)	-1.7	+1.9	-1.9	+1.5
EBV for protein content (g/kg)	-0.5	+0.5	-0.9	+0.8
EBV for fat yield (kg)	-1.8	+1.1	+1.1	-2.3
EBV for protein yield (kg)	+6.3	-5.5	+4.7	-4.4
EBV for milk solids (kg)	+4.4	-4.4	+5.8	-6.8

CHAPTER 6: Global Discussion

Objectifs

Ce dernier chapitre rappelle les objectifs et hypothèses du projet de recherche. Il permet aussi de réaliser une critique de la démarche scientifique adoptée, une synthèse des résultats obtenus, et leur mise en perspectives.

L'essentiel

Notre travail visait à explorer l'effet sur la reproduction d'un schéma de sélection alternatif (sur les taux) à celui réalisé aujourd'hui sur la production laitière. Nos résultats montrent que sélectionner les vaches laitières sur les taux butyreux et protéique résulterait en une reprise de cyclicité ovarienne *postpartum* plus précoce, n'aurait pas d'effet sur l'intensité des chaleurs, mais dégraderait la fertilité. Nous avons conclu que sélectionner sur les taux butyreux et protéique n'était pas une alternative pertinente afin d'améliorer les performances de reproduction.

Historiquement, la sélection quasiment exclusivement focalisée sur la production laitière a été défavorable à la reproduction. Cet effet est à la fois direct (corrélations génétiques défavorables) et indirect. Elle s'est accompagnée d'une augmentation de la capacité d'ingestion des animaux afin de couvrir leurs besoins de lactation. Or la corrélation génétique entre production laitière et capacité d'ingestion est d'environ 0.5. Un fossé s'est creusé entre la sortie de nutriments via le lait et les entrées via l'alimentation. Les animaux sont donc génétiquement programmés pour maigrir en début de lactation pour combler ce fossé. Or l'amaigrissement est un facteur de risque de dégradation de la reproduction. Comment améliorer les performances de reproduction des animaux sans détériorer leurs performances de production ? Nous proposons des pistes de recherche ou levier d'actions déjà connus pour répondre à ce problème. Par exemple, il serait intéressant de comparer des animaux à haut *vs* bas index génétique d'état corporel à même potentiel de production laitière. Le phénotypage à haut débit permettra de suivre à l'échelle individuelle les animaux sur des critères simples et robustes tel que l'état corporel afin de proposer des actions telle que la monotraite pour préserver leurs performances de reproduction.

1 The effects of milk production and body reserve management differ in importance according to the reproductive step

Our hypotheses were that **the effects of milk yield and body reserve management at each step of the reproductive process (i) differ in importance and that (ii) they can be managed through leverages such as genetics or nutrition.**

Cyclicity is firstly associated with body reserve management. The meta-analysis showed that CLA was associated to body condition score at calving, and that the relationship was curvilinear. Our results suggest that targeting BCS at calving close to 3.00 (ranging from 2.50 to 3.25) would contribute to keep CLA below 25 d postpartum. This optimal BCS at calving for resumption of ovarian activity as well as the quadratic relationship between CLA and BCS in early lactation is consistent with the literature (Roche *et al.*, 2009; Cutullic *et al.*, 2012; Brun-Lafleur *et al.*, 2013). In our experimental approach, CLA was positively associated with body weight at calving and negatively associated with body weight loss in early lactation for primiparous cows. In both the meta-analysis and the experimental approach CLA was not associated with milk yield. Previous results also suggest that slightly over-conditioned cows that experience a larger mobilisation than thinner cows but still have an early resumption of luteal activity are at risk of PLP (Petersson *et al.*, 2006a; Cutullic *et al.*, 2012). Unfortunately, it was not possible to test this hypothesis in the meta-analysis because of the limited data available in the literature. Interestingly, in our experimental approach, Holstein cows in the Milk-Group and under the High feeding system were the cows with both, the highest BCS at calving and the highest occurrence of PLP. This was also true for Normande cows in the Content-Group and under the High feeding system.

Oestrus intensity is firstly associated with milk yield. As expected, we observed in our experiment that the higher the milk yield during the ovulation week, the less intense the oestrus expression (Cutullic *et al.*, 2009; Friggens *et al.*, 2010). The effect of feeding system on oestrus expression was largely explained by differences in milk yield. When milk yield of the ovulation week was included in the statistical model, there was no residual effect of feeding system. Unfortunately, very few studies are reporting data about oestrus expression and duration together with production performance. Thus, the only reproduction trait related to oestrus that was studied in the meta-analysis was the interval from calving to first observed oestrus (COE1). Consistent with the former findings, reducing peak milk yield by 10 kg would result in shortening COE1 by 11 d. No relationship between COE1 and body reserve management was identified.

Overall fertility is firstly associated with body reserve management. The meta-analysis showed that overall pregnancy rate was increased by 21 % for each additional 0.5 BCS unit at calving and by 8 % for each additional 0.5 BCS unit at nadir. Our experimental data confirmed that re-calving rate was positively related to BCS at calving. A previous study suggested that the type of fertility failure was related either to milk yield or to body reserve management (Cutullic *et al.*, 2012). This is consistent with the fact that both body reserve management and milk yield were associated with conception rates in the meta-analysis. Indeed, CRAI1 was increased by 11 % for each additional 0.5

BCS units at nadir and by 17 % for a reduction of 0.5 units of BCS loss to nadir. In our experimental approach, **the occurrence of NF/EEM was more important for cows with the lowest BCS at nadir.** CRAI1 was also increased by 20 % by 10 kg of reduction of milk yield at peak and 22 % by 10 kg of reduction of milk yield at service. High peak milk yield followed by a poor lactation persistency is related to the occurrence of LEM (Buckley *et al.*, 2003; Cutullic *et al.*, 2012). This is consistent with the results from the experiment where **the occurrence of LEM was more frequent in cows with the lowest lactation persistency.** It is also important to keep in mind that with our method it is not possible to separate LEM from PLP following a service. This can potentially bring a bias as PLP and LEM may or may not have the same causes. Each reproductive step is related to the others and the success of the overall reproduction process relies on the success of each individual step. Nonetheless, the fact that the effect of milk yield and body reserve management differs in importance according to the reproductive step suggests that they are also partly disentangled. Thus, improvement of the overall reproduction can not rely on the improvement of a single step. Farming strategies that improve resumption of ovarian activity or oestrus intensity may not be the only solution. Further studies are also required to assess the global benefits and effects of the improvement of each step on the others: the global success may not necessarily result from the optimisation of each single step. Our study is a good example: the genetic experimental factor used improved cyclicity, had no effect on oestrus expression and impaired fertility. Figure 24 provides a proposition of what the lactation curve and body condition curve of a fertile cow may look like according to the results of the meta-analysis. This production profile brings into question the suitability of very high yielding dairy cows.

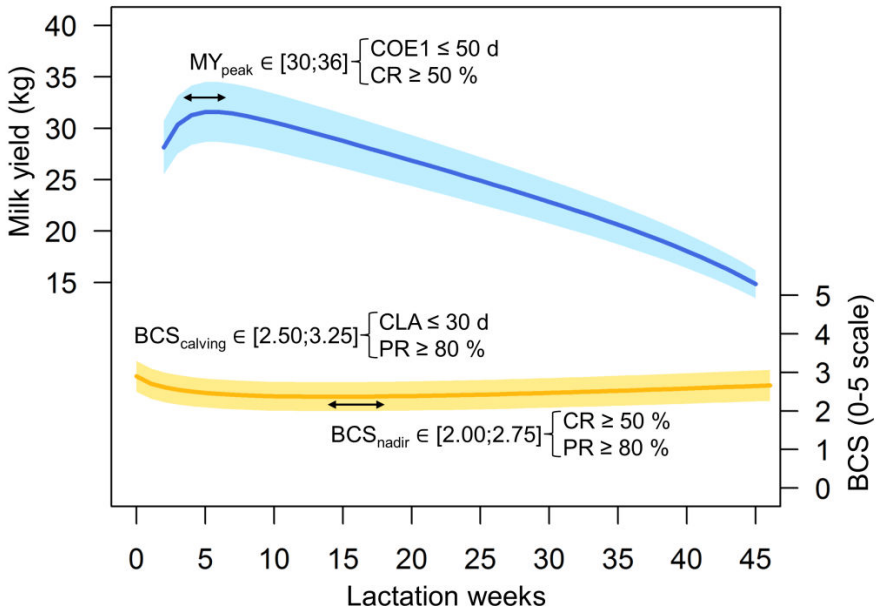

Figure 24: Proposition of ideal lactation and body condition curves for successful reproduction (CLA: commencement of luteal activity, COE1: calving to first observed oestrus interval; CR: conception rate at service; PR: overall pregnancy rate) based on the results of the meta-analysis. The lactation curve was modelled using the equations of prediction of expected milk yield of Faverdin *et al.* (2007) and the BCS curve was obtained using the following model: $BCS_{week} = week + week^2 + log(week)$; assuming that $BCS_{calving} = 2.90$, $BCS_{week17} = BCS_{nadir} = 2.40$; and $BCS_{week44} = 2.75$.

2 Towards selection for fertile and productive cows

2.1 Is selection for high fat and protein content instead of milk yield beneficial to reproduction performance?

The higher the genetic merit for production traits, the more energy that is exported in milk and the less there is for remaining functions (including reproduction). Energy in milk is contained in the fat, protein and lactose. Consequently there are two ways to export the same amount of energy in milk: either through high milk yield or through high fat, protein and lactose contents. Holstein cows are known to have a lower lactose content than other breeds (Dillon *et al.*, 2003a; Walsh *et al.*, 2008). However, variations in lactose content within breeds are less substantial than fat and protein content because it is highly related to milk osmotic pressure. At similar amounts of fat and protein yield, cows producing milk with higher fat and protein contents are supposed to have lower lactose yield than those producing higher milk yield. In such cows, the mammary gland would require less glucose for lactose production and glucose would be more available for other tissues. This glucose can support ovarian activity and thus production of ovarian steroids and development of the oocytes. By having lower milk yield, cows with high fat and protein contents would have a lower liver blood flow and thus a lower sexual hormone catabolism. All these effects could be beneficial to the reproductive process. Thus, **our hypothesis was that, at similar genetic merit for milk solids yield, dairy cows with high genetic merit for fat and protein contents have better reproduction performance than cows with high genetic merit for milk yield.** Many studies investigated the differences between high and low genetic merit for milk yield (Barnes *et al.*, 1990; Snijders *et al.*, 2001; Kennedy *et al.*, 2002, 2003; Windig *et al.*, 2008); or milk solids (Fulkerson *et al.*, 2001, 2008); or the differences between Continental/American and New-Zealand strains of Holstein cows (Horan *et al.*, 2004, 2005a; b; Kolver *et al.*, 2005; Macdonald *et al.*, 2008). **However, to our knowledge, there was no study about the effect on reproduction of genetic merit for high milk yield *vs* high fat and protein content, at identical genetic merit for milk solids.**

In our experiment, in both Holstein and Normande cows, milk solids production was similar between the two genetic groups, but milk yield and milk composition differed. Cows in the Milk-Group produced more milk than cows in the Content-Group. This difference was constant among breeds and feeding systems. Cows in the Content-Group had higher fat and protein contents than cows in the Milk-Group. Interestingly, there was no difference in body weight and body weight change between genetic groups in Normande cows. However, although BCS at calving was similar in both genetic groups, Normande cows in the Milk-Group lost more condition from calving to nadir than the ones in the Content-Group. Holstein cows in the Milk-Group were 20 kg heavier at calving than those in the Content-Group but body weight change was similar between the 2 groups. This difference was already substantial from 6 months of age. Further investigations are required to better understand this phenomenon. It is possible that in the Holstein breed, high genetic merit for milk yield was associated with increased size, globally or for some specific organs like the rumen, the liver or the mammary gland compared to cows with high genetic merit for fat and protein

contents. It does not seem to be due to differences in body fat reserves, since there was no difference in BCS between the 2 genetic groups in Holstein cows.

Table 7: Observed milk lactose content, milk yield and lactose yield over the whole lactation, for Holstein and Normande cows, in the Milk- and Content-Group in the experimental year of 2014 and 2015.

In 2014-2015	Holstein		Normande	
	Milk-Group	Content-Group	Milk-Group	Content-Group
Lactose content (g/kg)	47.1	47.5	47.4	47.4
Total milk yield (kg)	6,909	6,563	5,728	5,178
Lactose yield (kg)	325	312	272	246

Milk lactose content is determined since 2014. The observed data are reported in Table 7. Lactose content was approximately the same among breeds and genetic groups. However, because of the differences in milk production, lactose yields differ. Holstein cows produce an additional 60 kg of lactose compared to Normande cows. Cows in the Milk-Group produced more lactose over the lactation than those in the Content-Group (+13 kg, i.e. 4 % of total lactose yield for Holstein cows and +26 kg, i.e. 10 % of total lactose yield for Normande cows). The difference in milk yield in 2014 and 2015 is different from least-square means reported in the results chapters. Based on the least-square means, the expected difference in lactose yield between genetic groups is about 36 kg in Holstein cows (i.e. 10 % of total lactose yield) and 31 kg in Normande cows (i.e. 11 % of total lactose yield). A difference of 10 % of glucose uptake by the mammary gland is substantial at identical intake (S. Lemosquet and J. Guinard-Flament, personal communication). However, the plasma glucose concentration neither differed at 20 d nor at 60 d postpartum between genetic groups. Plasma glucose concentration is a very well-regulated homeostatic phenomenon. Further investigations on the metabolism (e.g. gluconeogenesis), endocrine status, nutrient partitioning, and fine composition of milk are needed to explore the bioavailability of plasma glucose.

At similar genetic merit for milk solids yield, cyclicity is better in cows with high genetic merit for fat and protein contents than in cows with high genetic merit for milk yield. Indeed, in both breeds, cows in the Content-Group were approximately two times more likely to resume ovarian activity at each additional day postpartum than those in the Milk-Group. There was no difference in occurrence of PLP or in cycle lengths (luteal phase and ovulatory phase) between genetic groups.

Oestrus intensity and ovulation detection rate are similar between cows with high genetic merit for fat and protein contents and cows with high genetic merit for milk yield. Indeed, 70 % of the ovulations were detected in all breeds, genetic groups and feeding systems. However, in both breeds, more ovulations occurred in the Content-Group than in the Milk-Group, thus there was a higher number of ovulations detected resulting in possibly more chance to be inseminated. Surprisingly, the interval between the start of the breeding season and first service was not affected by genetic group, meaning that they had equal chance to have their first service at each additional day of the breeding season. A possible reason is that cows in the Content-Group resumed ovarian activity earlier than the start of the breeding season and that although they cycled and were observed in oestrus they could not be inseminated to maintain compact calving.

Table 8: Genomic estimated breeding values for fertility traits and alleles frequencies for diacylglycerol acyltransferase (DGAT1) for the genotyped cows (51 % of the cows involved in the study) from the Holstein or Normande breed, in the Milk- or Content-Group and under the High or Low feeding system.

	Holstein				Normande			
	Milk-Group		Content-Group		Milk-Group		Content-Group	
	High	Low	High	Low	High	Low	High	Low
Number of cows	24	36	32	36	30	27	35	40
Number of genotyped cows	8	18	16	15	17	15	21	22
GEBV for fertility[1]								
CR Heifer	+15 ±9	+16 ±8	+15 ±8	+12 ±9	+13 ±8	+13 ±7	+12 ±7	+10 ±8
CR Cow	+20 ±11	+14 ±10	+14 ±11	+20 ±11	+18 ±12	+15 ±10	+16 ±10	+16 ±11
CFS	+17 ±10	+13 ±9	+19 ±9	+16 ±11	+10 ±7	+12 ±9	+13 ±9	+14 ±9
Overall reproduction	+16 ±12	+16 ±10	+14 ±11	+16 ±11	+14 ±11	+13 ±10	+13 ±10	+14 ±9
DGAT1 alleles frequencies[2]								
AA/AA	0 (0)	0 (0)	19 (3)	20 (3)	0 (0)	0 (0)	0 (0)	0 (0)
AA/GC	12 (1)	17 (3)	25 (4)	47 (7)	6 (1)	0 (0)	33 (7)	36 (8)
GC/GC	88 (7)	83 (15)	56 (9)	33 (5)	94 (16)	100 (15)	67 (14)	64 (14)

[1]relative GEBV to the reference population, positive GEBV means improvement for conception rate (CR) and overall reproduction [overall reproduction = (0.5 × CR Cow + 0.25 × CR Heifer + 0.25 × CFS) / 0.6965] but deterioration for calving to first service interval (CFS; lengthening the interval)

[2]Percentages with actual numbers in parentheses

Table 9: Some reproduction performance of the 51 % genotyped Holstein and Normande cows according to their genotype for DGAT1

	Holstein				Normande			
	AA/AA	AA/GC	GC/GC	P^1	AA/AA	AA/GC	GC/GC	P^1
Number of lactations	10	27	68		0	38	145	
Normal P_4 profile (% cows)	60 (6)	59 (16)	46 (31)	0.39	···	63 (24)	67 (97)	0.67
PLP (% lactations)	0 (0)	30 (8)	22 (15)	*	···	21 (8)	15 (22)	0.40
Submission rate (% cows)	100 (10)	96 (26)	96 (65)	0.66	···	97 (37)	97 (140)	0.80
Fertility (1st & 2nd AI)				0.95				0.73
NF/EEM (% AI)	30 (3)	27 (7)	26 (17)		···	19 (7)	19 (26)	
LEM (% AI)	10 (1)	12 (3)	5 (3)		···	14 (5)	4 (6)	
Pregnant (% AI)	60 (6)	62 (16)	69 (45)		···	68 (25)	77 (108)	
Re-calving rate (% cows)	70 (7)	44 (12)	65 (44)	0.15	···	79 (30)	76 (110)	0.69

[1] Significant levels: *** P<0.001; ** P<0.01; * P<0.05; + P<0.10

Reproduction performance with generalised model (logistic regression for cyclicity pattern, submission and re-calving rates; and multinomial regression for fertility

At similar genetic merit for milk solids yield, fertility is worse in cows with high genetic merit for fat and protein content than in cows with high genetic merit for milk yield. Indeed, in both breeds re-calving rate was not affected by genetic groups, despite the benefits of the Content-Group on cyclicity and similar oestrus expression and ovulation detection rates. There was no significant effect of genetic group on fertility of Normande cows. **Unexpectedly, Holstein cows in the Content-group had more pregnancy failures than in the Milk-Group (+ 8 % of NF/EEM and + 5 % of LEM).** These results suggest a possible genetic link between milk fat and protein contents and pregnancy losses. By misfortune, was the genetic merit for fertility unbalanced in our data? In France, official EBV for fertility is a combination of genomic EBV (GEBV) for conception rate for each AI (different for cows and heifers) and interval from calving to first AI. Unfortunately, only 51% of our experimental cows have GEBV for fertility trait because of missing information (older cows not genotyped and already culled). The information of those with GEBV for fertility is reported in Table 8. Because of unbalance distribution of the genotyped animal among the experimental factors and very large standard errors, nothing could be concluded. Further knowledge concerning the genetic characteristics of reproduction traits for these animals is required to improve our understanding of the effects described in this study. **Our results suggest that some steps of the reproductive process are genetically uncoupled.** Indeed, cows with high genetic merit for fat and protein contents may have improved cyclicity, identical oestrus expression and degraded fertility. This can also explain why in genome association studies, most of the autosomes are involved in reproduction performance (Khatkar *et al.*, 2014). In recent studies, polymorphism of the major regulator of milk fat content coding for the diacylglycerol acyltransferase (DGAT1) is associated with non-return rates and embryo survival (Demeter *et al.*, 2009; Wathes *et al.*, 2013). The physiological and metabolic causes remain unclear. DGAT1 is highly involved in lipid metabolism, and the K allele (dinucleotide AA) is associated with reduced milk and protein yield but increased fat yield, and higher fat and protein contents (Berry *et al.*, 2010). The K allele is also associated with lower BCS, lower plasma glucose concentrations, and higher NEFA. According to some authors this is the most likely explanation of the association between DGAT1 polymorphism and fertility. However, some authors (e.g. Demeter *et al.*, 2009) found that there were still residual effects of DGAT on fertility when accounting for these side effects. DGAT1 polymorphism or other candidate genes in high linkage disequilibrium may be related to ovary and endometrial cellular function and development (Kaupe *et al.*, 2007, Demeter *et al.*, 2009).

It is quite intriguing to see that the only cows owning the higher K allele frequency and the only K/K genotypes are the Holstein cows from the Content-Group. Table 9 shows preliminary univariate analysis of some reproduction parameters according to genotype for DGAT1 among the genotyped animals of our study. There is no clear-cut effect of DGAT1 genotype in these analyses. There are discrepancies in the literature on this topic: some other studies report unfavourable effects of DGAT1 K allele on 90 d non-return rates (Kaupe *et al.*, 2007) whereas (Oikonomou *et al.*, 2009) found no association with first service conception rate but suggested a possible effect on overall conception rate. Other studies found no link between DGAT1 genotype and fertility traits (Barbosa da Silva *et al.*, 2010; Berry *et al.*, 2010; Wathes *et al.*, 2013; H. Bovenhuis, personal communication). Minozzi *et al.*, (2013) even suggested that DGAT1 may not directly affect fertility but may regulate

other genes associated with production and fertility traits. To sum up, our results suggest a potential genetic relationship between fat and protein content and fertility. A potential explanation is the direct or indirect implication of the polymorphism of DGAT1. In addition, this effect was already suspected and there are discrepancies in the literature. Thus, further studies are required to (i) test the effect of DGAT1 polymorphism *per se* and to (ii) identify other genetic relationship between fat and protein contents and fertility.

2.2 Perspective of genetic selection to safeguard body reserve or for feed efficiency to improve reproduction performance

Despite the low heritability estimates of reproduction traits some countries include fertility traits in their breeding goals (e.g. Sweden, France and Ireland). Two different studies were performed in Ireland and in France that compare the performance of cows with high genetic merit for fertility with cows with low genetic merit for fertility. Their results are intriguing and raise questions about **the genetic relationship between feed efficiency and fertility.**

In France, a program to identify QTLs affecting economic traits was carried out and some were associated with fertility (Boichard *et al.*, 2003). Special care was accorded to a QTL associated with 90 d non-return rate on BTA3. This QTL was mapped and explained about 14 % of the total genetic variance (Guillaume *et al.*, 2007; Ben Jemaa *et al.*, 2008; Druet *et al.*, 2008). Two haplotypes with either a beneficial or deleterious effect on fertility were characterised. The study involved 23 dairy cows homozygous for the favourable haplotype (FR-Ferti +) and 18 cows homozygous for the unfavourable haplotype (FR-Ferti -). FR-Ferti + cows had improved reproduction compared to FR-Ferti - cows: they had an earlier CLA, and improved fertility (Table 10). No data on oestrus expression was reported. Interestingly, FR-Ferti + cows produced more milk, were heavier at first calving and lost less weight in the first 7 weeks of lactation than FR-Ferti - cows. Dry matter intake tended to be higher in FR-Ferti + cows but no difference in energy balance and plasma NEFA concentrations were observed (Coyral-Castel *et al.*, 2013). Unexpectedly, feeding behaviour was affected by genetic merit for fertility: FR-Ferti + cows spent more time at the feeder and tended to have a lower eating rate than FR-Ferti - cows (Coyral-Castel *et al.*, 2013).

In Ireland, the fertility index accounts for 34.8 % (relative emphasis) of the Economic Breeding Index, which is the breeding goal implemented since 2001. The fertility index is made of calving interval (23.2 %) and survival (11.5 %; www.icbf.com). Among them, 26 cows belonged to the top 20 % cows in genetic merit for calving interval (IE-Ferti +) and the other 26 cows belonged to the bottom 5 % of animals in genetic merit for calving interval (IE-Ferti -). IE-Ferti + cows had improved cyclicity (earlier CLA, shorter cycles), a lower occurrence of silent heat and were more fertile than IE-Ferti - cows (Cummins *et al.*, 2012a; b; Moore *et al.*, 2014a). Interestingly, IE-Ferti + cows produced more milk with similar grass dry matter intake and had lower body reserve mobilisation than IE-Ferti - cows (Cummins *et al.*, 2012a). These 2 genetic groups differed in regulation of the somatotropic axis: IE-Ferti + cows produced more IGF-I, its biological availability was higher and stability of circulating levels better than in IE-Ferti - cows (Cummins *et al.*, 2012c).

Table 10: Some production and reproduction performance of cows with high genetic merit for fertility (Ferti +) and others with low genetic merit (Ferti -) from studies in France (FR; Coyral-Castel *et al.*, 2011; Brisard *et al.*, 2012; Coyral-Castel *et al.*, 2012, 2013) and Ireland (IE; .Cummins *et al.*, 2012a; b; c, Moore *et al.*, 2014a; b).

	FR-Ferti +	FR-Ferti -	IE-Ferti +	IE-Ferti -
Number of cows	23	18	26	26
CAI1 (d)	70	71	74	80
CAIF (d)	91	110	86[a]	113.8[b]
Number AI (service/cow)	1.5[(a)]	2.3[(b)]	1.8[a]	2.8[b]
5/6 weeks PR (% of cows)	70[b]	39[a]	72[(b)]	41.2[(a)]
overall PR (% of cows)	65[b]	39[a]	89	72
NF/EEM (% of cows)	17	39		
LEM (% of cows)	16	36	0	11
CI (d)	374	392	392[a]	403[b]
MY (kg/d)	24.0	23.8	19.5[b]	18.7[a]
MY_{peak} (kg)	28.0[b]	26.0[a]	28.8[(b)]	27.3[(a)]
DMI (kg)	16.2	16.4	12.4	12.1
$BCS_{calving}$ (1-5 scale)			3.65	3.55
BCS_{nadir} (1-5 scale)			2.63[(b)]	2.53[(a)]

[a-b] indicates significant difference reported
[(a)-(b)] indicates tendencies of difference reported

Thus, it is possible to genetically improve reproduction without degrading genetic merit for milk production. Genetically fertile cows even had a greater milk production than unfertile ones. These results need to be confirmed on larger numbers of animals and in several reference populations. Still, they suggest a **difference in feed efficiency and nutrient partitioning between genetically fertile and unfertile cows.** These hypotheses needs to be tested to better understand the biological mechanism involved.

GEBV for feed efficiency are available in many countries (e.g. Australia, New-Zealand, United States). They are based either on a conversion ratio trait (milk yield over DMI) or based on the residual feed intake (RFI). The RFI is the difference between the expected DMI and the observed one. The estimation of expected DMI is based on models that may be different among experts. RFI predictors usually include production traits, maintenance (body weight) and the contribution of body reserve management to the available pool of nutrients (BCS change for both mobilisation and accretion of tissues). Depending on the chosen model, RFI has a low to moderate heritability ranging from 0.01 to 0.32 (Pryce *et al.*, 2014b). In Australia, GEBV for feed efficiency have been validated and included in the official breeding goal in 2015 (Pryce *et al.*, 2014a, 2015). Interestingly, **heifers selected for low genetic merit for RFI (efficient animals) had a higher *postpartum* pregnancy rate (about 10 percentage units) than inefficient cows** (Pryce *et al.*, 2014b). Efficient cows would spend more time at the feeder and have lower eating rate (Pryce *et al.*, 2014b). This supports the idea that Ferti + cows may be more efficient than Ferti - ones. Further studies are required on the phenotypic and genetic level to explore the link between feed efficiency and fertility.

Nevertheless, this relationship may be indirect, resulting from differences in body reserve dynamics. Indeed, by definition of the RFI, at equivalent milk yield efficient cows should mobilise less body reserve than inefficient ones. An excellent review from Bastin and Gengler (2013) showed that change in BCS has a low heritability whereas BCS level heritability was high (ranging from 0.20 to 0.50). The highest heritability estimate was obtained in mid-lactation, suggesting high genetic variation in reconstitution of body reserve. The genetic correlation ranged from -0.63 to -0.35 with the interval from calving to first service and from 0.16 to 0.28 with CRAI1. This suggests that **selecting dairy cows for higher BCS in mid-lactation would shorten the interval to first service and increase CRAI1**. However, BCS and production traits are genetically unfavourably correlated (from -0.63 to -0.12 for milk yield). Future studies that compare, at similar genetic merit for milk yield, cows with high and low genetic merit for BCS, can help to identify genetic strategies to cope with reproduction decline. A similar experiment comparing genetically efficient and inefficient cows can also be valuable. Investigations on how to recouple intake capacity and milk yield potential should be considered, in order to limit the genetically programmed body reserve mobilisation.

Given the history of what happened to genetics of fertility, caution should be taken with considering these new traits in selection indices. Studies may investigate the phenotypic difference in reproduction, production, health, behaviour, morphology of cows with high *vs* low genetic merit for feed efficiency or high *vs* low genetic for BCS at identical genetic merit for milk production. Before including them in official breeding goals, many gaps of knowledge or limits of the methods must be further investigated. Indeed, genetic correlations between many traits are still poorly documented (see the section 2.1.1. of the literature review). There are still too many ways and no consensus on how to estimate feed efficiency, what are the variables accounted for in the models for the RFI and about the accuracy of the measure. Similar is also true for BCS that is a subjective measure of subcutaneous body reserve that does not perfectly reflect body reserve and the accuracy of the measure does not allow the detection of small variations.

It is also important to remember that a sire is involved in the reproduction performance of dairy cows and that all the responsibility is not on the dam. In their study, López-Gatius *et al.* (2002) reported when sired by a specific bull, the risk of LEM was 3 times higher than with others bulls. More recently, it was reported that cows sired by bulls with high GEBV for female fertility were less at risk of pregnancy failure than those sired by bulls with low GEBV for female fertility (Ledoux *et al.*, 2015). Still, studies are required on the implication of bulls in the success of reproduction in dairy cows. The service, as the action to inseminate, is a major impact factor of insemination success. This means that the inseminator has to be correctly trained. New technologies such as deep insemination in the uterine horns are becoming available and may improve success rate of the insemination. Another biotechnology available is sexed semen. The benefits of using sexed semen are to increase the number of females, which is good for the herd replacement, and for increasing the selection pressure on the dam of the bulls. This is partly the reason why combining sexed semen with genotyping represents a great opportunity to have phenotype and genotype information. However, sexed semen has a decreased conception rate of 10 to 12 % (Ponsart *et al.*, 2014).

3 Towards farming strategies to cope with reproduction decline

Both the meta-analysis and our experimental approach has shown that farming strategies that limit NEB, body reserve mobilisation and peak milk yield can contribute to improve reproduction. However, only a small number of leverages can successfully manage the trade-off, given the challenge of limiting mobilisation and peak milk yield. The following paragraphs present promising leverages. To conclude a proposition of individualised reproduction management based on our results and these leverages is made.

3.1 What nutritional management can contribute to improving fertility?

***Prepartum* nutrition can contribute to shorten the *postpartum* anovulation period.** Indeed, some studies have investigated the effect of energy density in diets during the drying-off period on production and reproduction performance of dairy cows (McNamara *et al.*, 2003; Pushpakumara *et al.*, 2003; Adrien *et al.*, 2012). In each study, there was no direct benefit of increasing the energy density of drying-off diets on reproduction. However, body reserve at calving, mobilisation, and milk yield were all favourably associated with energy density of the diets. Improved body reserve or limited mobilisation were related to early CLA. This is consistent with the implication of these treatments in the response between BCS at calving and CLA that was established in our meta-analysis. Recent studies have demonstrated that, at identical energy density, glucogenic *prepartum* diets improve EB (van Knegsel *et al.*, 2014) and metabolic status (lower NEFA, higher glucose, higher IGF-I and higher insulin plasma concentrations; Chen *et al.*, 2015a) compared to lipogenic diets. However, these treatments did not affect ovarian activity (Chen *et al.*, 2015b), nor milk yield, nor DMI (van Knegsel *et al.*, 2014).

***Postpartum* nutrition can contribute to improve the overall reproduction performance.** Indeed, different feedstuffs and nutrient lead to different rumen fermentations, coproducts and by-pass elements patterns. As a consequence circulating levels of nutrient (glucose, NEFA, urea…) and hormones may be different. One of the most promising strategies found in the literature is the use of glucogenic/lipogenic sequences (Friggens *et al.*, 2010; Butler, 2014). Rumen fermentation of glucogenic diets is resulting in the production of propionate while that of lipogenic diets results in acetate and butyrate. As a consequence, plasma insulin and glucose concentrations are higher with glucogenic diets than with lipogenic ones. Thus, glucogenic diets result in little body reserve mobilisation (effect of insulin) while lipogenic diets do not limit the genetically programmed mobilisation. However, there may be a paradoxical positive (early CLA) and then deleterious effect (altered oocyte quality) of insulin on reproduction across time. In their study, Garnsworthy *et al.* (2009) switched from a glucogenic to a lipogenic diet at resumption of luteal activity. They showed that such a strategy shortened the anoestrus period without impairing fertility. This promising result needs to be confirmed to implement these kinds of nutritional strategies to cope with reproduction decline.

The unfavourable effect of feeding diets with high crude protein levels on reproduction is known for a long time (Ferguson and Chalupa, 1989). Excessive dietary protein results in high circulating levels of ammonia and its metabolites which are toxic for the oocytes and embryos. This

unfavourable effect is enhanced by the consequent exacerbation of milk production and body reserve mobilisation (Butler, 2000). These effects have been confirmed during the past years even though the toxic effect of circulating levels of urea or the deleterious effects of high dietary crude protein is not consistently observed (Westwood *et al.*, 1998; Butler, 1998; Staples and Thatcher, 2001; Law *et al.*, 2009). Diskin *et al.* (2006) even concluded from their literature review, the most likely plasma level of urea do not have a direct effect on embryo survival. In some studies, no significant effect of dietary level of crude protein or rumen undegradable protein (RUP) on reproduction was observed when the treatment did not induce changes in milk yield (Barton *et al.*, 1996; Bruckental *et al.*, 2000). Limiting the dietary level of RUP has been reported to reduce peak milk yield and body reserve mobilisation (Canfield *et al.*, 1990; Sklan and Tinsky, 1993; Son *et al.*, 1996; Garcia-Bojalil *et al.*, 1998; McCormick *et al.*, 1999; Westwood *et al.*, 2000; Chapa *et al.*, 2001; McCormick *et al.*, 2001). It was also reported that reducing RUP would shorten reproduction intervals (CLA, days to first service, and days open), increase CRAI1 and probably increase pregnancy rate (see the review of Tamminga, 2006). However caution should be taken when reducing nitrogen in the diet as it has also been shown that below 100 g of PDI (protéines digestibles dans l'intestin) per UFL, intake decreases and energy intake is not sufficient thus cows are mobilising body reserve. Above 100 gPDI/UFL, intake does not increase but milk yield does, which may also impact trade-offs (Verite and Delaby, 1998).

To conclude, nutritional strategies that may contribute to improve reproduction in dairy cows would be made of a glucogenic diet during drying-off and the first 5 weeks of lactation followed by a lipogenic source of energy for the rest of the lactation. In addition, the *postpartum* diet should contain moderate levels of RUP. Such a recommendation may not be appropriate to all farming systems. Some systems maximise the efficiency of the use of their resources (e.g. milk production per hectare of grassland) while others the genetic merit for production traits (e.g. milk yield per cow). All intermediate kinds of system also exist. Reducing reproduction intervals together with infertility is a key to the success of some grass-based systems using compact calving whereas it is not adapted to indoor systems with about 15 months calving interval and in which fertility is the only priority (Disenhaus *et al.*, 2005; Friggens *et al.*, 2010).

3.2 Reducing dry period length to shorten the *postpartum* anovulation

The dry period enables the cow to restore sufficient body reserve to face the next lactation and to regenerate the alveolar system of the mammary gland (van Knegsel *et al.*, 2013). It also enables the maximisation of the milk yield of the subsequent lactation and the reduction of subclinical mastitis. The duration of the dry period is about 2 months by custom, which is questionable (Rémond and Bonnefoy, 1997; Rémond *et al.*, 1997). van Knegsel *et al.* (2013) performed a meta-analysis and showed that shortening the standard 2 months dry period to a single month resulted in a reduction in milk production of about 1.4 kg/d (4.5 %) and by omitting the dry period of about 5.9 kg/d (19.1 %) on the overall subsequent lactation. To evaluate the economic impact of shortening dry period, the *prepartum* milk production has to be considered (from 30 to 60 additional days for 30-d and 0-d dry period lengths respectively). In addition, shortening dry period results in increased protein content and has no effects on fat content (van Knegsel *et al.*, 2013). It is also limiting the reduction

in DMI before calving and consequently increasing the resource intake and thus improving energy balance in early lactation (Watters *et al.*, 2008; van Knegsel *et al.*, 2014; Chen *et al.*, 2015a). This is consistent with the fact that shortening dry period results in higher body reserve and limited mobilisation in early lactation (van Knegsel *et al.*, 2013). It was also reported to affect the lactation curves by lowering peak milk yield and improving lactation persistency (Chen *et al.*, 2016a). Consistent with all these elements, shortening the dry period is associated with earlier CLA (Gümen *et al.*, 2005; Rastani *et al.*, 2005; Watters *et al.*, 2009; Chen *et al.*, 2015b). Since no relationship between dry period length and the occurrence of PLP was reported (Gümen *et al.*, 2005), we can conclude that the reported higher proportion of normal cyclicity patterns is linked to the earlier CLA and thus lower proportion of delayed activity (Gümen *et al.*, 2005; Chen *et al.*, 2015b). There is a lack of knowledge concerning the effect of dry period length on oestrus behaviour since, to our knowledge, no data is reported. There are discrepancies on other reproduction traits. No effect on conception rate, number of services per conception, days open and final pregnancy rates could be identified through the meta-analysis of van Knegsel *et al.* (2013). These reproduction steps occur later in lactation and possible effects of shortening or omitting dry period could be hidden behind other major effects (e.g. *postpartum* nutrition). In addition, a large variability is observed in the response of dairy cows to dry period length and diet. Individual characteristics such as parity play an important role. Steeneveld *et al.* (2014) found that multiparous cows had less milk yield loss when omitting dry period than primiparous cows. The interference of parity was also observed in the study of Adrien *et al.* (2012) when comparing energy density of the *prepartum* diet. In addition, for reasons not elucidated yet, some cows are not able to cope with 2 subsequent omissions of dry period (Chen *et al.*, 2016b). In this last study, 48 % of the cows that had no dry period between their first and second lactation naturally dried off after 10 months of lactation (milk yield below 4 kg/d). These cows were almost over-conditioned at calving, experienced the most severe NEB, and had the poorer metabolic status. However, no individual characteristics such as parity are reported. To conclude, in systems where dairy cows are already recovering a sufficient part of their body reserve in late lactation to reach the target of about 3.0 BCS point at calving, reducing dry period to a month can shorten the anovulation period. On the other hand, if cows are still too thin or worse are still mobilising body reserve 3 months before the expected calving date, drying-off should be considered to help the cow recover sufficiently (DairyNZ, 2012).

3.3 Lowering milking frequency to change the trade-offs between lactation and reproduction

Milking cows only once a day instead of twice results in about 30 % of milk yield drop in early lactation and 7 % in late lactation (Rémond and Pomiès, 2005; Pomiès *et al.*, 2008; Stelwagen *et al.*, 2013). Consequently, blood flow drops about 10 to 15 %, the number of mammary epithelial cell

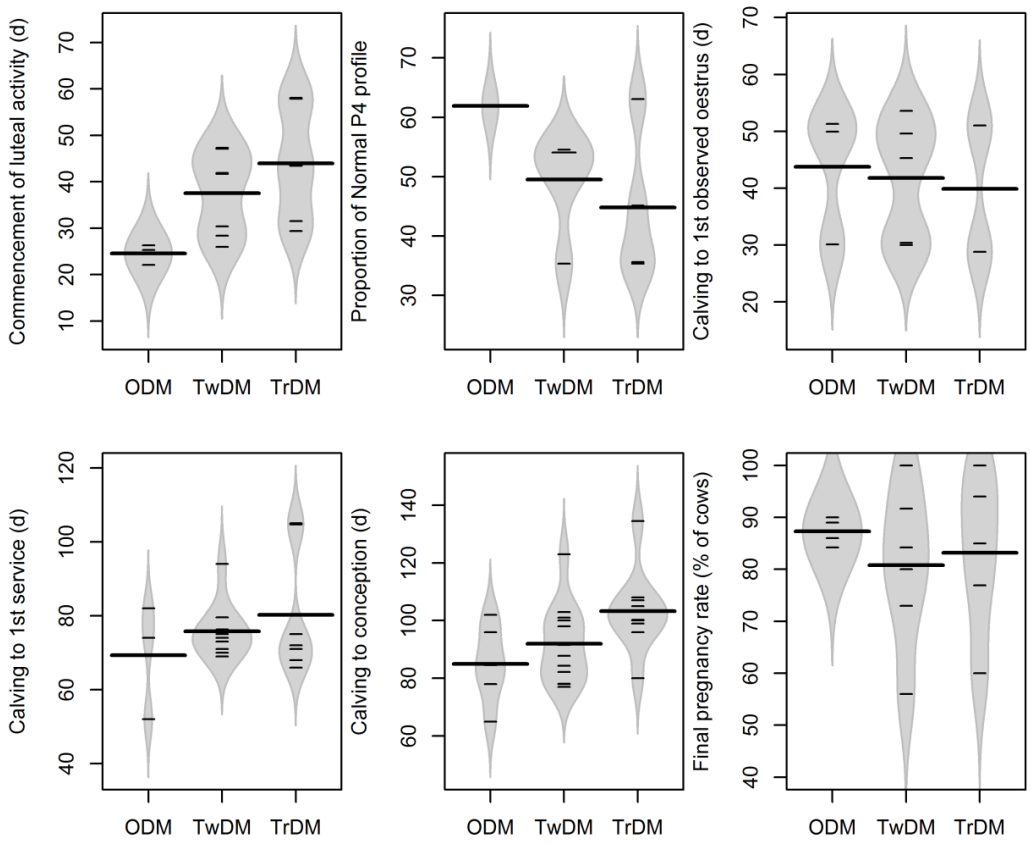

Figure 25: Distribution of some reproductive traits according to daily milking frequencies (ODM: once daily milking, TwDM: twice daily milking, TrDM: trice daily milking). The distribution is shown in grey, small black segments indicates the reported data and the black thick line the estimated mean for the subset (data collected in the studies of Amos *et al.*, 1985; DePeters *et al.*, 1985; Barnes *et al.*, 1990; Disenhaus *et al.*, 2002; Pomiès and Rémond, 2002; Blevins *et al.*, 2006; Clark *et al.*, 2006; Patton *et al.*, 2006, 2007; McNamara *et al.*, 2008; Windig *et al.*, 2008; O'Brien *et al.*, 2009).

decreases and their activity is modified towards fat synthesis (Pomiès *et al.*, 2008). This reduction of milk yield *per se* could be beneficial to reproduction because lower blood flow is associated with lower sexual steroids catabolism in the liver and lower lactose yield to higher glucose availability (Wiltbank *et al.*, 2006). Few data are available on dry matter intake (DMI) and energy balance (EB), mostly because once daily milking (ODM) is a practice used in pasture based systems, where intake is a difficult trait to measure. Still, some studies have been done in controlled conditions, to assess the benefits of ODM with the hypothesis that feed cost would be lower because of a decreased DMI compared to higher milking frequency. All studies failed to show any significant decrease in DMI in cows submitted to ODM (see the review of Stelwagen *et al.*, 2013). Rémond and Pomiès (2005) report a reduction of only 5 % in DMI between twice a day milking and ODM. Consistent with these observations, several studies reported a favourable effect of ODM on body reserve (BW and BCS), decreased plasma NEFA and BHB concentrations (Stelwagen *et al.*, 2013). ODM can be applied to the overall lactation or only during specific periods.

ODM limits milk yield and improves EB, thus benefits on reproduction are expected. However there is no clear-cut scientific support, even though deleterious effects were never reported (Stelwagen *et al.*, 2013). Reproduction data from 11 studies with different milking frequencies are represented

in Figure 25. Very little information on reproduction traits is reported in studies with milking frequency as the experimental factor. The response of these different reproduction traits to milking frequency is highly variable. Treatment groups were made of 7 to 36 cows which is a small number to conclude on reproduction performance. Except in the study of Clark *et al.* (2006) where the number of cows per treatment groups ranged from 120 to 168. Globally, ovarian activity seems to be improved with ODM: CLA is shorter and the proportion of normal P_4 profile higher. The effect on calving to first oestrus and first service is confused. Clark *et al.* (2006) mentioned these results and explained them by fewer opportunities to detect oestrus. Indeed, herd movements to and from the milking parlour are periods during which dairy cows show sexual behaviours (Britt *et al.*, 1986). Further studies are needed concerning the potential effects of applying periods of ODM on each step of the reproductiton process. Hypotheses to be tested in such experiments are that: (i) applying ODM in the 100 first days in milk should shorten CLA, improve oestrus expression, and conception rate, (ii) applying ODM during mid-lactation should lower pregnancy loss (iii) applying ODM in late lactation can improve future reproduction performance through higher BCS at calving.

3.4 Managing rearing and culling to take control over the effects of time

As expected, primiparous cows produced less milk and had later CLA than multiparous ones in our experimental approach (Horan *et al.*, 2004; Meikle *et al.*, 2004; Tanaka *et al.*, 2008; Cutullic *et al.*, 2012). The higher the genetic merit for milk yield and the dietary energy supply, the greater the differences in production between primiparous and multiparous cows. In our study as well as in the literature cited above, primiparous cows had a greater body condition score at nadir and experience larger mobilisation in early lactation. In addition, they are known to have a slower uterine involution and have an additional life function in competition that is growth. In the literature, studies in which primiparous cows had the same body condition at calving than multiparous ones (Friggens *et al.*, 2007) or similar mobilisation (Barton *et al.*, 1996) also had similar CLA. Parity does not seem to influence oestrus intensity (Cutullic *et al.*, 2009). In our study, primiparous cows had an improved re-calving rate than multiparous cows, probably because they are less at risk of NF/EEM and LEM (Cutullic *et al.*, 2012). However, this difference was not significant in other studies (DePeters *et al.*, 1985; Barnes *et al.*, 1990). There may be confounding effects between parity and energy balance, since both the severity and duration of NEB decreases with parity (A. Fischer, personal communication). Because they are still growing, primiparous cows have different metabolic status endocrine responses, and nutrient partitioning (Taylor *et al.*, 2003; Coffey *et al.*, 2006). To disentangle the effect of parity and EB probably due to growth, it could be interesting to study the effect of age at first calving (AFC). Dairy cows usually reach their mature size around 5 years old (Le Cozler *et al.*, 2008). Thus, the growth requirement of cows that are 2 years old at first calving is larger than cows that are 3 years old at first calving. Moreover, their body reserve would be different at calving in quantity and quality (proportion of fat and muscle). Thus further studies should explore the effect of calving closer to the mature size (e.g. difference between 2 years and 3 years AFC), or on the effect of survival and the proportion of cows in third and more lactation in the herd.

3.5 Monitoring body condition for individual reproduction management

Table 11 summarises the expected effects on production and reproduction of the leverages discussed. Our results showed that it is complex to manage dairy cows towards re-calving due to the implication of many interconnected factors and the potential uncoupling of reproductive traits. Before making use of these leverages at the individual scale, it is important to remember that the factors with major impacts are genetic characteristics, the occurrence of health problems, and age. By combining our results and the presented leverages, we built a decision tree for individual reproduction management based on body reserve management of the cow (Figure 26).

Table 11: Expected effects ("+" for favorable, "-" for unfavorable and "?" for potentially but lacking evidences) of the different leverages discussed on production and reproduction of the current lactation in dairy cows.

	Prepartum			*Postpartum*		
	ODM[1]	↘ dry period[2]	↗ UFL diet[3]	Gluco-Lipogenic sequences[4]	↘ PDI diet[5]	ODM[6]
DMI		+ +			-	
$BCS_{calving}$	+ + +	+ +	+ + +			
BCS_{min}	?	+ +	+ +	+ +	+ +	+ + +
MY_{peak}		- -	+		- -	- - -
$MY_{persistency}$		+			+ + +	
Cycliciy	? +	+	? +	+	+	+
Oestrus						?
Fertility					+	? +

[1] as applied to the overall preceding lactation or at the end of the preceding lactation

[2] reduction of the dry period length from 2 months to 1 month

[3] preferably glucogenic diets, the UFL (unite fourragère lait) is the energy contained in 1kg of wheat

[4] turnout from glucogenic to lipogenic diet during the 5th week of lactation

[5] PDI = protéines digestibles dans l'intestin (g) are the combination of RUP and microbial protein

[6] as applied to the overall lactation but can be used on targeted periods

For instance, if the cow has a BCS at calving of 3.0, there is nothing to do yet. However, if her BCS is lower than 2.00 at 17 weeks of lactation, then we recommend the farmer to consider switching to ODM in order to promote BCS gain. If at 30 weeks of lactation, her BCS is higher than 2.50 then she can come back to the initial milking frequency whereas if she did not regain body condition, we advise to switch this cow to ODM and consider early drying-off (3 months before calving) to promote BCS gain. Finally, if this was efficient she can have a regular dry period length of 2 months with a regular *prepartum* diet However, if she still did not recover sufficient body condition, we advise to both (i) shorten her dry period to a month in order to limit the reduction of DMI and to limit future milk production in order to help her to safeguard her body reserve during the subsequent lactation and (ii) to use a *prepartum* diet with high energy content and of glucogenic type. In addition, special care should be given to primiparous cows that are more likely to undergo severe and long periods of NEB.

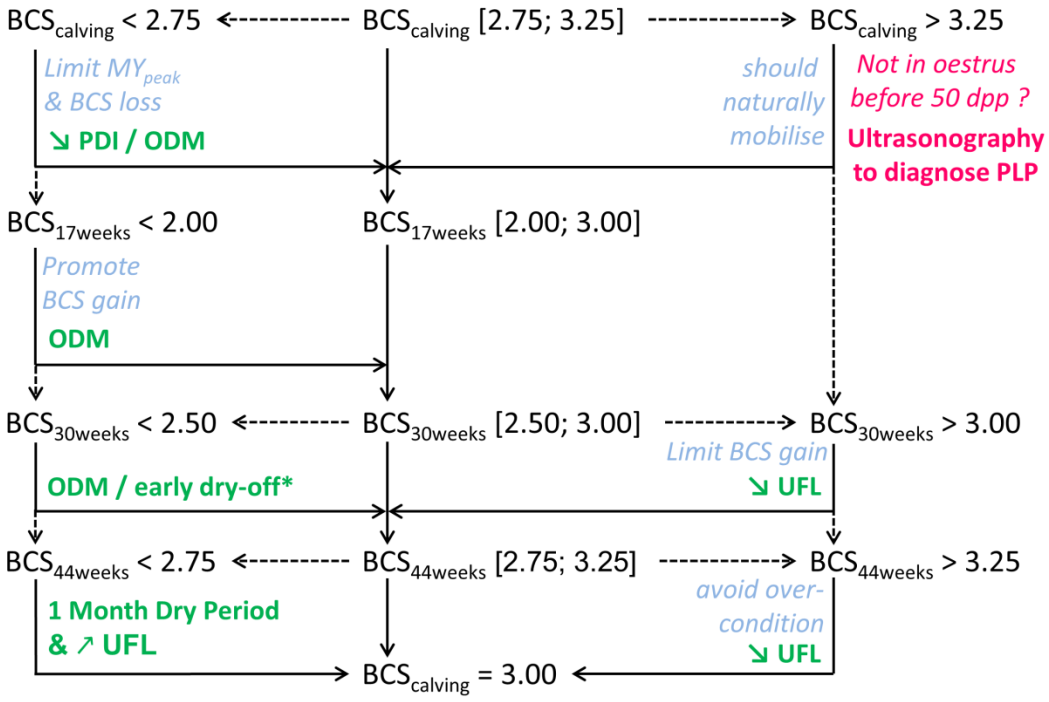

Figure 26: Proposal of a decision tree for individual reproduction management of dairy cows based on the monitoring of their body reserve management. BCS is on a 0-5 scale (Bazin *et al.*, 1984) and based on results in Holstein breed. A solid arrow represent the approach to follow if the cow is in the body condition indicated, dotted arrow the one to follow if she is not. The Ideal profile is the one centred. Objectives are in italic and blue and actions in bold and green. Items in pink are concerning special monitoring of reproduction in fat cows. *If cows are still too thin 3 months before the expected calving date, drying-off should be considered to help the cow recover sufficient.

3.6 The role of precision livestock farming

Precision livestock farming (PLF) tools are starting to be widely used. The existing tools for reproduction mostly focus on heat detection and calving monitoring. Heat detection with PLF is based on physical activity (podometers and accelerometers), mounting detection and in-line progesterone monitoring. They enable the continous recording of the observation and allow detection of oestrus of low intensity, short duration, or occuring during periods when the farmer is away (e.g. by night). However, using PLF does not solve the problem of decreasing oestrus intensity and duration. The time saved not detecting oestrus is not so substantial due to the time required for maintenance of PLF tools. The tools available for detecting the moment of calving are efficient and can help to ensure a correct supervision of this crucial process. However, the farmers have to remember to be the least interventionist possible to reduce risks of health problems (Chanvallon *et al.*, 2016).

As highlighted in the preceding section, individual monitoring of reproduction is possible through a single indicator: BCS. However, BCS is a subjective measurement based on visual and palpation assessment of the animal. By definition, it mainly reflects subcuteneous adipose tissue state and change. There are mainy scales used from 1-5 in Ireland to 1-10 in New Zealand and these scales are not linearly correlated. In France, the 0-5 scale of Bazin *et al.*, (1984) is used. The increments are 0.25 units. Thus, BCS is a subjective measurement of body reserve that does not correctly consider non-subcutaneous body reserve (e.g. intra-muscular fat) and that do not detect

small changes. Moreover, it is only infrequently collected on farm, on a sample of 25 % of the cows of the herd. PLF can help to get an objective measurement of body reserve management through in-line measurement of metabolites (e.g. BHB) or automated estimation of the rear shape in 3D of the cows through in-line image analyses (Faverdin and Fischer, 2016).

4 From G×E interactions to "The cow for the system?"

Adaptation abilities and strategies to cope with environmental constraints rely on genetic characteristics. In the present research project, two distinct genetic characteristics were tested: (i) genetic merit for milk yield (breed, and EBV), and (ii) the genetic merit for the way of producing milk solids. Our hypothesis was that **some genetic characteristics are best suited for a certain kind of system and may not be adapted to others.**

4.1 Adaptation strategies to nutrient scarcity and their consequences on reproduction according to genetics

As expected, in situations of nutrient scarcity, dairy cows produce less milk. Breed by feeding system interactions were observed in our study: the reduction in milk yield and the management of body reserve are different according to breeds. **Adapting to nutrient scarcity by reducing milk production and limiting body reserve mobilisation was associated with better reproduction performance than mobilising body reserve to support milk production.** Indeed, it appeared that Holstein cows were mobilising body reserve to a higher extent than Normande ones in order to limit milk production loss. These strategies are associated with contrasted reproduction performance. Normande cows resume ovarian activity earlier and are more fertile than Holstein cows. However, they express less intense oestrus but this did not impair their ovulation detection rate. Overall, Normande had a better re-calving rate than Holstein cows. These results are consistent with other studies comparing Holstein and Normande cows (Dillon *et al.*, 2003a; b; Michel *et al.*, 2003; Walsh *et al.*, 2008; Cutullic *et al.*, 2009; Delaby *et al.*, 2009; Cutullic *et al.*, 2011). Similar effects were expected between the Milk- and the Content-Group based on the differences in quality and quantity of milk production. However, they were not observed. **There was no difference in body reserve management between the Milk and the Content-Group in a situation of nutrient scarcity.** Still, some "genetic group nested within breed by feeding system" interactions were identified. In situations of nutrient abundance, Holstein cows had a higher peak milk yield than Normande cows and all the more for cows in the Milk-Group than those in the Content-Group. In Holstein cows from the Content-Group, pregnancy failures were different according to the feeding system. In the Low FS, Holstein cows in the Content-Group had more NF/EEM than Holstein cows in the Milk-Group. Conversely, in the High FS, Holstein cows in the Content-Group had a higher proportion of LEM than in the Milk-Group. The occurrence of NF/EEM is known to be associated with low BCS at nadir (Cutullic *et al.*, 2012) and that of LEM with high peak milk yield (Grimard *et al.*, 2006) and low lactation persistency (Buckley *et al.*, 2003; Cutullic *et al.*, 2012). Consistent with this, Holstein cows in the Content-Group under the Low feeding system had lower BCS at nadir, lower peak milk yield and higher persistency than those under the high Feeding system. Normande cows in the Milk-Group under the High feeding system had the best re-calving

rate (77 %) compared to other groups. They calved at 3.60 points of BCS (0 -5 scale) and mobilized few body reserve, they also had a substantial peak milk yield for the breed (32.7 kg) and a moderate lactation persistency (80 %). Overall they produced an appreciable 6,498 kg of milk and 460 kg of milk solids. There is a consensus about the fact that these levels of production performance are ideal regarding reproduction success (López-Gatius *et al.*, 2003; Roche *et al.*, 2009; Friggens *et al.*, 2010). These production performances are also close to our recommendations (Figure 24; section 1). Thus, we can conclude that a production of 6,500 kg of milk over the lactation is a maximum limit to correctly ensure reproduction in compact calving systems. Similar results were observed in Montbéliarde cows, which is a dual purpose breed with a selection history with higher emphasis on milk yield than Normande one. In their study on 273,000 lactations, Roumeas *et al.* (2014) found that at identical total milk yield (6,500 kg), cows with high peak milk yield (32 kg) and a persistency of 76 % had shorter calving to first service interval, fewer services per pregnancy and fewer days open than cows with low peak milk yield (26 kg) and high persistency (94 %). Cows with late and high peak milk yield and low persistency are at risk of LEM, while cows with early peak milk yield and regular persistency are more likely to be pregnant (Buckley *et al.*, 2003). In other words, cows that successfully ensure reproduction and production may be cows that invest in one function at the time: most of their milk in early lactation and then investing in reproduction. This is moderated by the absolute milk yield or breed limitations since in Holstein cows, peak milk yield is associated with degraded fertility (Pryce *et al.*, 2004; Grimard *et al.*, 2006; Friggens *et al.*, 2010; Cutullic *et al.*, 2012). It may also be that cows that have and early peak milk yield and regular persistency are in better EB and health status than those with delayed milk yield and low persistency. Further studies are required to improve our understanding of these phenomena.

4.2 Individual characteristics related to management of trade-offs

We have been wondering if the investment in milk production has been different between genetic groups. This could highlights differences in management of the trade-off between production and reproduction.

As expected, Holstein cows are producing more milk solids using both dietary nutrient and body reserve as resources than Normande cows (Table 12). The relative contribution of the dietary resource to milk solids production (in UFL) was lower for Holstein cows, especially in situation of nutrients scarcity. The difference between the expected and observed performance was more substantial for Holstein than for Normande cows. There was no absolute or relative difference in the source of nutrient, and change of milk solids production (difference between expected and observed milk solids yield) between breeds, genetic groups and feeding systems. Cows under the High feeding system had a similar change than those under the Low feeding system. However, in the High feeding system, the contribution in milk solids production of diet was higher and that of body reserve mobilisation smaller. Once again, a breed by feeding system interaction was observed: Normande cows used preferably the dietary source of resources for milk solids production, even though in situation of nutrient scarcity. These parameters do not allow us to understand the differences between the genetic groups and the partial uncoupling of reproductive

Table 12: Adjusted contributions of diet and body reserve mobilisation in the observed milk solids yield over 44 weeks, and difference between expected and observed milk solids yield for Holstein and Normande cows, in the Milk- or Content-Group, under either the High or Low feeding system

	Holstein				Normande				Model		Significance levels[2]				
	Milk-Group		Content-Group		Milk-Group		Content-Group								
	High	Low	High	Low	High	Low	High	Low	σ_a[1]	σ_e[1]	B	B:G	FS	B×FS	B:G×FS
Expected milk solids (kg)[3]	671	488	661	478	546	420	529	403							
Expected - observed milk solids (kg)[4]	14^{b}	14^{b}	14^{b}	14^{b}	4^{a}	4^{a}	1^{a}	1^{a}	15.4	45.8	*	0.91	0.98	0.78	0.23
	(2 %)	*(2 %)*	*(2 %)*	*(2 %)*	*(1 %)*	*(1 %)*	*(0 %)*	*(0 %)*							
Observed milk solids (kg)	657	474	647	464	542	416	528	402							
Contribution of diet[4]	623^{d}	432^{b}	615^{d}	424^{b}	519^{e}	387^{a}	508^{c}	376^{a}	25.8	46.0	***	0.22	***	***	0.55
	(93 %)	*(89 %)*	*(93 %)*	*(89 %)*	*(95 %)*	*(92 %)*	*(96 %)*	*(93 %)*							
Contribution of body reserve mobilisation[4]	34^{de}	42^{f}	32^{cd}	40^{ef}	23^{ab}	29^{cd}	20^{a}	26^{bc}	2.5	13.0	***	0.12	***	0.47	0.85
	(5 %)	*(9 %)*	*(5 %)*	*(9 %)*	*(4 %)*	*(7 %)*	*(4 %)*	*(7 %)*							

[1]Standard deviation of the random terms: animal genetic and non-genetic effect (σ_a, assuming uncorrelated animal effects) and error (σ_e)

[2]Effect of Breed (B), Genetic group within Breed (B:G), Feeding System (FS), Breed × Feeding System (B×FS) and Genetic group within Breed × Feeding System (B:G×FS). Significant levels: *** P<0.001; ** P<0.01; * P<0.05; + P<0.10

[3]Expected milk yield was estimated by adding the EBV to environmental effects (year, age at calving, month of calving, dry period length, and feeding system) and permanent animal environment.

[4]Calculations are made in a 2-step procedure: milk production is standardised for fat and protein content to 0.44 UFL/kg of milk, 1 point of BCS loss was set to 400kg of standardised milk (P. Faverdin, personal communication). The results were re-transformed to raw milk and contribution of diet was calculated as the observed raw milk minus the estimated contribution of body reserve mobilisation. The difference between expected and observed milk yield was also computed. The percentages are the relative contributions of diet, body reserve mobilisation and the remaining fraction to the expected milk yield.

[a - g] distinguish adjusted means that are different between breeds, genetic groups, and feeding systems (P<0.05, Tukey's pairwise comparison)

Table 13: Adjusted contributions of diet and body reserve mobilisation in the observed milk solids yield over 44 weeks, and difference between expected and observed milk solids yield according to the outcome of some reproduction parameters

	Normal P_4 profile		P^2	Inseminated		P^2	Outcome of 1st&2nd AI			P^2	Re-calving		P^2
	Yes	No		Yes	No		NF/EEM	LEM	Pregnant		Yes	No	
Expected milk solids (kg)[1]	523	524		524	523		523	523	527		525	524	
Expected - observed milk solids (kg)[2]	8	8	0.87	8	8	0.98	15^{b}	28^{b}	2^{a}	**	1^{a}	17^{b}	**
	(1 %)	*(1 %)*		*(1 %)*	*(1 %)*		*(3 %)*	*(5 %)*	*(0 %)*		*(0 %)*	*(3 %)*	
Observed milk solids (kg)	515	516		516	515		508	495	525		524	507	
Contribution of diet[4]	485	485	0.94	485	482	0.77	480^{ab}	464^{a}	494^{b}	**	493^{b}	477^{a}	**
	(93 %)	*(93 %)*		*(93 %)*	*(82 %)*		*(92 %)*	*(89 %)*	*(94 %)*		*(94 %)*	*(91 %)*	
Contribution of body reserve mobilisation[4]	30	31	0.52	31	33	0.28	28	31	31	0.26	31	30	0.46
	(6 %)	*(6 %)*		*(6 %)*	*(7 %)*		*(5 %)*	*(6 %)*	*(6 %)*		*(6 %)*	*(6 %)*	

[1]Expected milk yield was estimated by adding the EBV to environmental effects (year, age at calving, month of calving, dry period length, and feeding system) and permanent animal environment.

[2]Calculations are made in a 2-step procedure: milk production is standardised for fat and protein content to 0.44 UFL/kg of milk, 1 point of BCS loss was set to 400kg of standardised milk (P. Faverdin, personal communication). The results were re-transformed to raw milk and contribution of diet was calculated as the observed raw milk minus the estimated contribution of body reserve mobilisation. The difference between expected and observed milk yield was also computed. The percentages are the relative contributions of diet, body reserve mobilisation and the remaining fraction to the expected milk yield.

[a - g] distinguish adjusted means that are different between breeds, genetic groups, and feeding systems (P<0.05, Tukey's pairwise comparison)

steps observed in our results. However, they bring a new insight on trade-offs management. The relationship between these production parameters and some reproduction ones are presented in Table 13. The contribution of body reserve mobilisation in milk solids production was not related to reproduction success. The proportion of normal P_4 profiles and submission rate was neither related to the magnitude of the change nor to the contribution of diet in milk solids production. However, **high fertility was clearly related to higher contribution of the diet in milk solids production and a lower change**. This may be due to higher feed efficiency and better energy balance, which is consistent with the literature. Future study may estimate the effect of lactation stage on EBV in order to be able to study the change through time of the difference between expected and observed performance. The results of such a study are expected to get close to the concept of priority of Martin and Sauvant (2010a). This effect of time is important to answer the question "the cow for the system?" because the constraints of the systems may change with time and the ability of the cows to quickly adapt is important (e.g. with change of nutritional supply: Delaby *et al.*, 2009; or milking omission: Charton *et al.*, 2016)

Three main profiles based on homeorhesis and homeostasis principles can be defined to characterise adaptation strategies: *"dam now"*, *"future dam"* and *"myself first"*. Indeed, two recent studies using partly the same data and multi-traits statistical approaches have identified these profiles (Cloet, 2015; Ollion *et al.*, 2016). This profiles based on statistical approaches without *a priori* assumption or knowledge on the data structure (e.g. experimental factors) gives similar conclusions on trade-offs management than our results or other studies (e.g. Delaby *et al.*, 2009; Cutullic *et al.*, 2011). The ***"dam now"*** strategy consists in giving the priority to milk production at the expense of safeguarding body reserve and reproducing. The ***"future dam"*** strategy involves reproduction success at the expense of milk production and even sometimes of safeguarding body reserve. These first two strategies are rather based on homeorhetic phenomena. The ***"myself first"*** strategy consists in safeguarding body reserve at the expense of both lactation and reproduction. This last strategy is rather based on homeostatic phenomena. At last, two other profiles were reported in these study. There is a ***"balanced cow"*** profile made of cows with no trade-offs: cows successful manage production, reproduction and correct management of body reserve; and a ***"loser"*** profile with cows failing to ensure all functions. Interestingly, these results show that it is possible to ensure all functions (the *balanced cow*). Cows from the "future dam" profile also produced a substantial quantity of milk, had an earlier peak milk yield, high lactation persistency and a modest body reserve mobilisation (BCS of 3.0 at calving and 2.5 at nadir; Cloet *et al.*, 2015). Further studies are required to confirm these profiles, especially considering feed efficiency in the analyses: the *balanced cow* may be a cow with high feed efficiency or high intake. To do so large datasets are required and future investment on in-line phenotypes should be considered to enable better understanding of trade-offs. Once such profiles are validated, studying their differences of genetic, phenotypic, and individual characteristics could improve current knowledge. It is most likely that fertile cows are cows that experience little trade-offs between life functions and are the most possible ensuring them once at the time.

Table 14: Goals for reproduction and production performances, together with some recommendations for the genetic appropriated to the kind of system as well as systems leverage to manage the trade-offs between lactation and reproduction.

	High inputs indoor systems		High quality forage systems		Grazing-based systems	
	Goals	Comments	Goals	Comments	Goals	Comments
CAI1	100 d	extending lactations	80 d	limit MY_{peak} and mobilisation	variable > 30 dpp	depending on the breeding season > 30 dpp for a correct uterine involution
CR	30-40 %	tolerate a low fertility but work on it	40-50 %	appreciable fertility	50-60 %	90 % of the cows should be pregnant by the end of the breeding season
CAIF	150 d	to limit involuntary culling	100 d	to be monitored	90 d	compact calving system
PR	> 80 %	accept high culling rate remove poorly fertile cows and daughters, genetic progress	> 85 %	avoid involuntary culling	> 90 %	avoid involuntary culling
$BCS_{calving}$	3.0[1]	to limit mobilisation	3.25-3. 50[1]	to promote early CLA and appreciable PR	3.50[1]	for an early CLA and a good PR
BCS_{min}	> 2.0[1]	promote CR	> 2.50[1]	promote CR	> 2.75[1]	promote CR and
MY_{peak}	< 40 kg[2]	promote earlier and smaller	< 35 kg[2]	reduce mobilisation	< 25 kg[2]	milk yield/cow does not matter get used to milk yield/ha
Week of peak	< 14 wk	peaks and higher persistency	< 10 wk	the sooner the better	< 7 wk	the sooner the better, high persistency
Lactation length	15-18 mo	give the cows more time for both functions	< 15 mo	give the cow more time for both functions	< 10 mo	no time, limit the trade-offs by reducing production requirements
Genetics	high yielding dairy cows work on genetic merit for fertility		dairy breeds with high substantial genetic merit for fertility/efficiency		dairy breed with high genetic merit for functional traits, crossbred and dual-purpose	
System	dry period = 1 month use PLF to monitor (return in) oestrus		limit dietary PDI to reduce MY_{peak}		use ODM to reduce MY_{peak} and mobilisation	

[1]BCS targets take into account the type of genetics recommended

[2]these MY_{peak} targets are based on the meta-analysis results. Because of the limited number of study, and the fact that farming systems represented in the response law are mainly grazing-based systems, MY_{peak} targets may be under-estimated for the High inputs indoor and the High quality forage systems.

4.3 What cow and what goals of reproduction performance in each system?

Although very diverse, dairy systems can be categorised in 3 types: intensive, intermediates, and extensive systems (Friggens *et al.*, 2010). Based on existing references (Disenhaus *et al.*, 2005; Friggens *et al.*, 2010; Butler *et al.*, 2012; MacMillan, 2012; Butler, 2014; DairyNZ, 2012) and the results from both the meta-analysis and the experimental approach, we proposed some recommendations for each system to manage dairy cows towards successful reproduction and lactation (Table 14).

High inputs indoor systems are mostly based on high nutritive inputs, with high concentrates levels in the diet. They aim for the maximisation of milk production per animal and use high yielding dairy breeds. In these systems it is important to give the cows more time to reduce trade-offs. Thus, extending lactation should be considered, because cows are failing to reproduce and it may not be worthy to dry cows that are still producing about 20 kg of milk after 10 months of lactation. Therefore, we would recommend to delay the first service in these cows and to start inseminating from a 100 days in milk. During these first 100 d, the farmer should help the cows to limit mobilisation, to have already expressed the peak milk yield and to maintain a good lactation persistency (higher than 75 %). However, giving the cow more time can not solve fertility problems. Special care should be given to genetic merit for fertility of the bulls, and fertility performance should be considered in culling decisions. Reducing the dry period to 1 month in these systems can help to reduce milk yield to biologically acceptable levels and improve *postpartum* EB. Using PLF, such as in-line progesterone measurements or automated heat detection tools (activity meters) can help detect oestrus (low oestrus intensity) and monitor non-return rates.

High quality forage systems aim for low feed costs and maximise the use of forages produced on the farm (maize/pasture). They mostly use high yielding dairy breeds. To reduce trade-offs, it would be more appropriate to aim for a 15 months calving interval in these systems. To help the cow to separate each function through time as much as possible is the key. To do so, we would recommend to stop using high yielding dairy breeds, and use dairy breeds with high genetic merit for functional traits (reproduction, health, survival…). Indeed, in these systems a correct conception rate is required, which is not compatible with high and long peak milk yield. Reducing the PDI of the ration can be the preferred tool to manage peak milk yield and persistency in these systems.

Grazing-based systems aim to maximise milk production and grass utilization. They are based on low inputs and are characterised by the use of seasonal calving and breeding. They mostly use rustic dairy breeds, dairy crossbred and dual-purpose breeds. In these systems, the top priority is to keep seasonal calving to make the nutrient requirements for lactation match the supply of grasslands. First services should occur from the start of the breeding season, and respecting a voluntary waiting period of at least 30-35 d (consistent with the uterine involution, see the Figure 10 of the section 1.4 of the literature review). The use of ODM to manage both milk production and body reserve mobilisation can be considered, even for short periods as a corrective solution. Emphasis should be put on genetics with high merit for early resumption of ovarian activity and high fertility.

Conclusion

Notre travail a permis de préciser l'effet de la sélection sur les taux butyreux et protéique du lait à chaque étape de la fonction de reproduction chez la vache laitière. Aujourd'hui la sélection génétique pour les performances de production est orientée sur la quantité de lait. Sélectionner les vaches laitières sur les taux butyreux et protéique résulterait en une reprise de cyclicité ovarienne *postpartum* plus précoce ; n'aurait pas d'effet sur l'intensité des chaleurs ; mais dégraderait la fertilité. Au final, sélectionner sur les taux butyreux et protéique dans le but d'améliorer les performances de reproduction à même production de matière utile n'apparaît pas comme une alternative pertinente.

Nous pouvons aussi conclure que la cyclicité ovarienne, l'œstrus, et la fertilité sont génétiquement partiellement découplés. Ceci contribue à expliquer pourquoi la seule inclusion d'index génétiques de fertilité dans les schémas de sélection n'avait pas eu l'efficacité escomptée pour freiner voir contrecarrer le déclin global de la reproduction de ces animaux. Aujourd'hui, en France, l'index génétique de reproduction inclut également l'intervalle entre le vêlage et la première insémination. Ce travail n'avait pas pour objectif l'analyse génétique fine de ces caractères, ni l'étude des corrélations génétiques des étapes de la reproduction, entre elles ou avec les caractères de production. Ces éléments doivent être étudiés en priorité afin d'améliorer par la voie génétique les performances de reproduction des vaches laitières sans dégrader d'autres fonctions biologiques. De plus, le découplage génétique partiel suggère également qu'il est possible de créer différents index de reproduction afin de répondre aux exigences de différents systèmes de production. En effet, si l'amélioration de la fertilité est une priorité dans tous les systèmes, raccourcir la durée d'anovulation *postpartum* n'a d'intérêt que dans des systèmes à période d'insémination courte et annuelle.

Nous avons pu conforter et quantifier les relations entre production laitière, gestion des réserves corporelles et reproduction. Notre dispositif expérimental nous a aussi permis d'explorer une large plage de production laitière et d'état corporel. Les vaches laitières ont montré de fortes capacités d'adaptation aux exigences des systèmes, au travers de stratégies diverses. L'essor de l'agriculture de précision va permettre l'émergence de nouveaux phénotypes. La mise au point de la mesure automatisée de l'état corporel et de sa dynamique est prometteuse. Combinée au génotypage, elle permettra une nouvelle alternative de sélection pour améliorer indirectement la reproduction des vaches laitières sans dégrader les performances de production ou de santé des animaux.

L'avenir de l'élevage laitier réside dans la diversité à la fois de ses animaux et de ses systèmes d'élevage. La compréhension des stratégies d'adaptation des animaux aux exigences des systèmes sera une clef pour la sélection d'animaux adaptés à chaque système, et finalement pour le maintien d'une diversité de systèmes de production simples, souples et solides.

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Appendices

International conference proceedings

Oral communications

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2016. The cow for the system: limiting milk yield and body condition loss to ensure reproduction on time, in: 67th Annual Meeting of the European Federation of Animal Science. Belfast, Ireland.

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2015. Towards a better understanding of the effect of genetic merit for milk production on post-partum cyclicity of first lactation dairy cows., in: ADSA-ASAS Joint Annual Meeting. Journal of Animal Science, vol. 93, Suppl.s3 / Journal of Dairy Science, vol. 98, Suppl. 2, Orlando, Florida.

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2015. Resumption of luteal activity in first lactation cows is mainly affected by genetic characteristics, in: 66th Annual Meeting of the European Federation of Animal Science. Warsaw, Poland.

Posters

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2016. Selecting on fat and protein content instead of milk yield would not improve dairy cows' fertility, in: 67th Annual Meeting of the European Federation of Animal Science. Belfast, Ireland.

National conference proceedings

Oral communications

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2015. Les vaches capables de limiter leur production laitière et leur amaigrissement lors d'une restriction alimentaire sont celles qui se reproduisent le mieux, in: Rencontres Recherche Ruminants. Paris, France.

Posters

Bedere, N., Delaby, L., Ducrocq, V., Leurent-Colette, S., Disenhaus, C., 2016. L'aptitude des vaches laitières à assurer chaque étape de la reproduction dépend de leurs caractéristiques génétiques, in: Rencontres Recherche Ruminants. Paris, France.

Popular science

Posters

Bedere, N., Delaby, L., Leurent-Colette, S., Disenhaus, C., 2016. Sélectionner pour la production laitière via les taux, quelles conséquences sur la reproduction des vaches ? Affiche à la Journée Des Doctorants. Saint-Gilles, France.

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The cow for the system: limiting milk yield and body condition loss to ensure reproduction on time

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To study reproductive performances of Holstein and Normande cows managed under a compact calving system (3 months), 2 contrasted grazing-based feeding systems (FS) are used since 2006 in a trial at the INRA farm of Le Pin-au-Haras. The High FS enables cows to produce more milk (MY) while limiting their body condition loss whereas the Low FS limits MY while inducing a large body condition loss throughout lactation (High vs Low: +2,355 kg MY for Holstein, +1,402 kg MY for Normande and +0.40 body condition score in both breeds, P<0.001). It was possible to study the different steps of the reproductive process by combining milk progesterone information (sampled 3 times a week) with intensive oestrous behaviour recording and pregnancy diagnosis (using ultrasonography). Cyclicity of dairy cows was not affected by FS. Normande cows had an earlier resumption of ovarian activity (28.0 vs 31.5 d) and a higher proportion of normal cyclicity patterns than Holstein cows (+22 points, P<0.001). Estruses were more intense in the Low FS than in the High one (+13 points standing to be mounted, P<0.001). For Holstein cows, fertility problems were different in each FS: in the Low FS a higher proportion of inseminations were not-fertilizing or resulted in early embryo mortality (+14 points, P<0.05) whereas in the High FS a higher proportion of inseminations resulted in late embryo mortality (+9 points, P<0.05). Finally, Normande had a higher re-calving rate than Holstein (+19 points, P<0.001), and the High FS tended to be positively associated with re-calving rate (+6 points, P<0.10). The Low FS was very restrictive in nutrients. By limiting their MY to this nutritive constraint Normande do not experience too severe negative energy balance while Holstein tried to maintain the highest MY possible. This resulted in preserved reproduction for Normande cows, and thus animals more robust to compact calving and grazing-based dairy systems.

277 **Towards a better understanding of the effect of genetic merit for milk production on post-partum cyclicity of first lactation dairy cows.** Nicolas Bedere*[1], Luc Delaby[1], Vincent Ducrocq[2], Ségolène Leurent-Colette[3], and Catherine Disenhaus[1], *[1]INRA-Agrocampus-Ouest UMR 1348 PEGASE, Saint-Gilles, France, [2]INRA UMR 1313 GABI, Jouy-en-Josas, France, [3]INRA UE 326 Domaine Expérimental du Pin-au-Haras, Exmes, France.*

Milk genetic merit is known to affect commencement of luteal activity (CLA) in dairy cows. This effect is considered to be due to energy exported in milk production. We hypothesize that cows with genetic predisposition to export milk energy through milk yield would have degraded cyclicity compared with those exporting energy through fat and protein contents. This study aimed to identify and quantify the effect of breed and genetic groups on postpartum cyclicity of primiparous dairy cows. From 2006 to 2013, an experiment was conducted on 194 primiparous dairy (Holstein) and dual purpose (Normande). Within breeds, cows were classified into 2 groups with regards to their estimated breeding value (EBV). Cows with high EBV for milk yield were included in a "Milk" group (MG) and those with high EBV for fat and protein content were included in a "Content" group (CG). Within breeds, exported milk energy and weight loss were similar for cows in both MG and CG groups. Interval from calving to CLA was studied performing survival analyses (Weibull regression). Progesterone profile, milk yield and body condition were analyzed using Chi^2-test and ANCOVA. Holstein cows produced more milk (+1450 kg, $P < 0.001$) and lost more body weight (BW: −1.4kg/wk, $P < 0.01$) than Normande ones. Normande and Holstein cows had different baseline hazard functions for CLA, Normande cows having earlier CLA than Holstein ones. Within breeds, cows in the CG group had earlier CLA (associated HR = 2.0, $P = 0.001$) than cows in the MG group. For Holstein only, BW loss from wk 1 to 14 of lactation tended to be associated with later CLA ($P < 0.1$). Within breeds, no effect of milk yield on CLA was observed. Prolonged luteal phases were frequent (18% of cows) and neither associated with breed nor with differences between MG and CG. Interovulatory intervals were longer for Holstein than for Normande cows (+1.7 d, $P < 0.001$) because of a longer luteal phase and a longer interluteal interval. To conclude, this study showed that cows with genetic predisposition to export milk energy through fat and protein contents had earlier CLA than those exporting milk energy through yield.

Key Words: genetic merit, cyclicity, primiparous cows

Resumption of luteal activity in first lactation cows is mainly affected by genetic characteristics

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Milk genetic merit is known to impact commencement of luteal activity (CLA) in dairy cows. This effect is usually considered to be related to energy exported in milk. The present study aimed to identify and quantify the effects of genetic characteristics (breed and estimated breeding value (EBV) for milk yield and solids content) and feeding system on CLA of primiparous cows. From 2006 to 2013, an experiment was conducted on 194 primiparous dairy (Holstein) and dual purpose (Normande) cows at the INRA farm of Le Pin-au-Haras. Within breeds, 2 groups were created based on EBV: cows with relatively high milk yield EBV (M) and cows with relatively high fat and protein contents EBV (C). Within breeds, exported energy in milk and weight loss were similar for both genetic groups. Two grazing based strategies were used, a High (H) feeding system (maize silage in winter and grazing plus concentrate) and Low (L) feeding system one (grass silage in winter and grazing with no concentrate). CLA was studied performing survival analyses (Weibull regression). Milk yields and body condition were studied using logistic regression, Chi^2-test and ANCOVA. H cows produced more milk (+1,690 kg, $P<0.001$) and lost less body weight (BW) from week 1 to 14 pp (+3.8 kg/wk, $P<0.001$) than the L ones. Holstein cows produced more milk (+1,450 kg, $P<0.001$) and lost more BW (-1.4 kg/wk, $P<0.01$) than Normande ones. As expected, Normande cows had a shorter median CLA than Holstein ones (30 and 33.5 days respectively). However, C cows had shorter CLA (associated RR=2.0, P=0.001) than M ones. No effect of milk yield or feeding system on CLA was found. In conclusion, CLA was only affected by genetic characteristics. Beyond breed, the genetic merit to export energy through milk solids had a beneficial effect on post-partum ovarian recovery.

Selecting for production traits through fat and protein content
What consequences on reproductive performance of Holstein cows?

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Genetic merit for milk yield

Milk production ⚡ Reproduction

Energy exported in milk ?

Is the genetic predisposition to export milk fat and protein through high Milk Yield or high Fat and Protein Contents impacting reproduction ?

Experimental design: The cow for the system ? (INRA Le Pin-au-Haras)

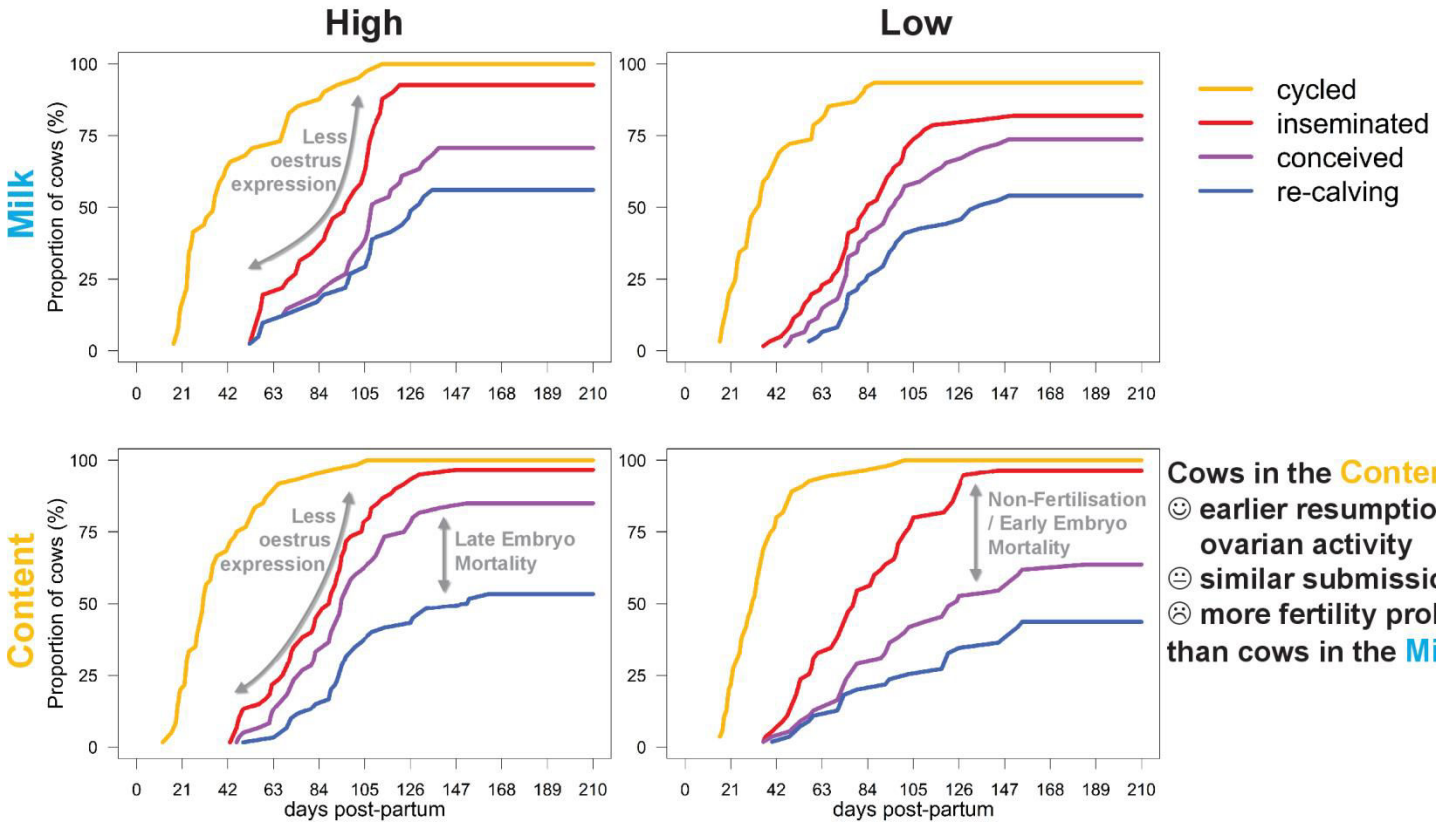
Two Genetic Groups
Similar genetic merit for Fat + Protein Yield

Milk  *VS*  Content

Two Feeding Systems

High  *VS*  Low

217 lactations from 128 Holstein cows between 2006 and 2014



Cows in the Content-Group:
☺ earlier resumption of ovarian activity
😐 similar submission rate
☹ more fertility problems
than cows in the Milk-Group

Selecting Holstein cows for Fat and Protein Content may not be the way leading to more robust cows, capable of yielding the same valuable milk components and coping with reproductive performance decline

The authors are grateful to the technical staff from the dairy section of the INRA farm of Le Pin-au-Haras for managing the herd and to the staff of INRA et AGROCAMPUS OUEST for the laboratory analyses

Selecting for production traits through fat and protein content
What consequences on reproductive performance of Normande cows?

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Genetic merit for milk yield

Milk production — Reproduction

Energy exported in milk ?

Is the genetic predisposition to export milk fat and protein through high Milk Yield or high Fat and Protein Contents impacting reproduction ?

Experimental design: The cow for the system ? (INRA Le Pin-au-Haras)

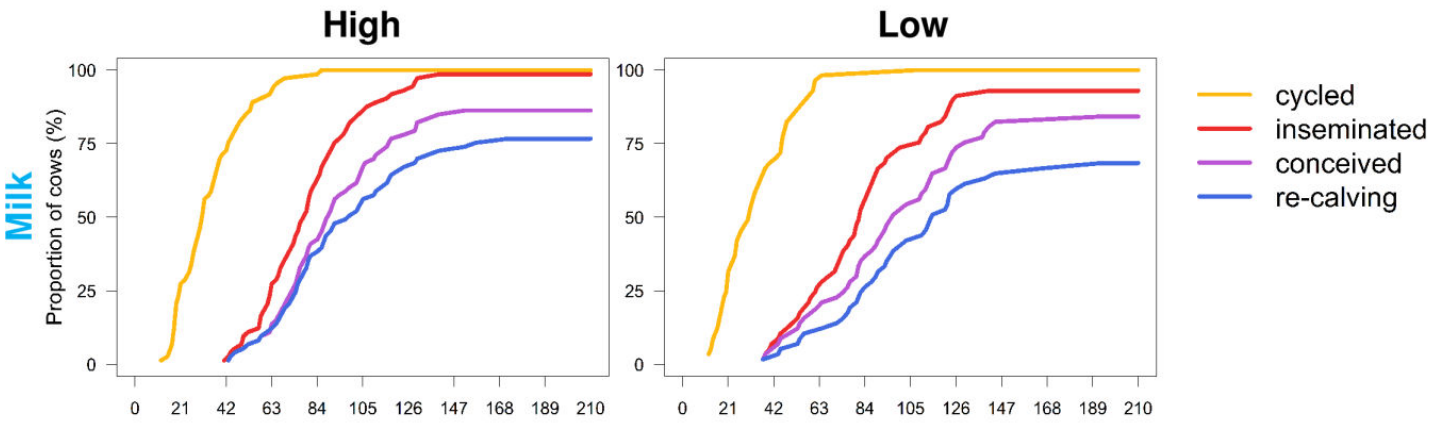
Two Genetic Groups
Similar genetic merit for Fat + Protein Yield

Milk  *VS*  Content

Two Feeding Systems

High  *VS*  Low

283 lactations from 132 Normande cows between 2006 and 2014



Cows in the Content-Group:
- ☺ earlier resumption of ovarian activity
- 😐 similar submission rate
- 😐 similar fertility

than cows in the Milk-Group

Selecting Normande cows for Fat and Protein Content may shorten the anovulation. Given the fertility problems of the Content-Group in Holstein cows, investigations are required before considering such selection strategy to cope with reproduction decline.

The authors are grateful to the technical staff from the dairy section of the INRA farm of Le Pin-au-Haras for managing the herd and to the staff of INRA et AGROCAMPUS OUEST for the laboratory analyses

Les vaches capables de limiter leur production laitière et leur amaigrissement lors d'une restriction alimentaire sont celles qui se reproduisent le mieux

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RESUME

Afin d'étudier les performances de reproduction des vaches laitières Holstein et Normande conduites en vêlages groupés (3 mois), deux systèmes herbagers caractérisés par des apports nutritifs contrastés (Haut et Bas), ont été mis en place depuis 2006 sur le domaine expérimental INRA du Pin-au-Haras. Le système Haut permet aux vaches de produire plus de lait et de limiter leur amaigrissement, tandis que le système Bas bride la production laitière et induit un plus fort amaigrissement au cours de la lactation (Haut *vs* Bas : + 2 131 kg de lait chez les Holstein, + 1 132 kg de lait chez les Normande et + 0,5 point d'état au nadir pour les 2 races). L'étude des différentes étapes de la reproduction est rendue possible grâce à la combinaison du dosage trihebdomadaire de la progestérone du lait, d'un suivi soutenu de l'expression des chaleurs et de diagnostics de gestation par échographie. Le système alimentaire n'a pas eu d'effet sur la cyclicité des vaches laitières. Les vaches Normande sont cyclées plus tôt (- 6 j) et présentent plus de profils de cyclicité normaux que les Holstein. Les chaleurs sont plus intenses dans le système Bas (+ 10 points d'acceptation du chevauchement). Chez les vaches de race Holstein, les problèmes de fertilité diffèrent d'un système à l'autre : les vaches du système Bas subissent plus de non-fécondations / mortalités embryonnaires précoces à l'IA (+ 15 points) alors que celles du système Haut subissent plus de mortalités embryonnaires tardives (+ 7 points). Quel que soit la race et le système d'élevage, les vaches qui revêlent se caractérisent par une durée de la saison de reproduction plus longue (+ 8 j), moins de problèmes au vêlage (- 10 points), et plus de profils de cyclicité normaux (+ 15 points) que les vaches non-gestantes. Le taux de revêlage est plus élevé chez les vaches Normande (+ 16 points), et tend à être plus élevé dans le système Haut (+ 7 points) pour chaque race. Pour conclure, les vaches Normande présentent de meilleures performances de reproduction que les Holstein, quel que soit le système car leur stratégie d'adaptation consiste à limiter leur perte d'état corporel et produire moins de lait en cas d'apports nutritifs insuffisants, ce qui préserve les différentes étapes de la reproduction.

Cows capable of limiting their milk yield and body reserves losses during feed restriction are better ensuring reproduction

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SUMMARY

In order to study reproductive performances of Holstein and Normande cows managed under a compact calving system (3 months), 2 contrasted grazing feeding systems are used since 2006 in a trial at the INRA experimental farm of Le Pin-au-Haras (France). The High system enables cows to produce more milk while limiting their body condition loss whereas the Low system limits milk yield while inducing a great body condition loss throughout lactation (High *vs* Low: + 2 131 kg milk yield for Holstein, + 1 132 kg milk yield for Normande and + 0.5 body condition score in both breeds). It was possible to study the different steps of the reproductive process by combining milk progesterone information (sampled 3 times a week) with intensive estrus behavior recording and pregnancy diagnosis (using ultrasonography). Cyclicity of dairy cows was not affected by feeding system. Normande cows resumed ovarian activity earlier (- 6 d) and show a higher proportion of normal cyclicity patterns than Holstein cows. Estruses were more intense in the Low system than in the High one (+ 10 points standing to be mounted). For Holstein cows, fertility problems were different in each system: in the Low system a greater proportion of inseminations were not-fertilizing or resulted in early embryo mortality (+ 15 points) whereas in the High system a greater proportion of inseminations resulted in late embryo mortality (+ 7 points). Within breed and feeding system, cows that successfully conceived had a longer breeding period (+ 8 d), less problems at calving (- 10 points), and a higher proportion of normal cyclicity patterns (+ 15 points) than non-pregnant cows. Finally, Normande had a higher re-calving rate than Holstein (+ 16 points), and the High feeding system tended to be positively associated with re-calving rate (+ 7 points). To conclude, Normande have better reproductive performances than Holstein because their adaptive strategy is to limit body condition losses and milk yield when nutrient supply is restricted. This is preserving the different steps of the reproductive process.

INTRODUCTION

Dans les régions favorables à la pousse de l'herbe, les systèmes herbagers basés sur des vêlages groupés de fin d'hiver représentent une option intéressante pour l'élevage laitier, dans un contexte de réduction des coûts de production et des impacts environnementaux. Dans ces systèmes, l'obtention de vêlages groupés en hiver est une priorité et de bonnes aptitudes de reproduction des animaux sont nécessaires. Si les systèmes alimentaires à bas intrants ne pénalisent pas inéluctablement les performances de reproduction des vaches laitières, certaines étapes sont sensibles à la stratégie alimentaire alors que d'autres dépendent davantage de la génétique (Delaby et al., 2009, Disenhaus et al. 2009, Cutullic et al., 2011). L'objectif de cet article est d'analyser les performances de reproduction (cyclicité, chaleurs et fertilité) des vaches de 2 races (Holstein et Normande) affectées à 2 systèmes herbagers à forts et

faibles apports nutritifs. Les hypothèses préalables à cette étude sont i) la réussite de certaines étapes de la reproduction dépend de la race ; ii) par leur influence sur la production laitière et les réserves corporelles, les stratégies alimentaires affectent la reproduction des vaches laitières.

1. MATERIEL ET METHODES

1.1. DISPOSITIF EXPERIMENTAL

Depuis 2006, deux troupeaux de 30 à 35 vaches (par an) Holstein et Normande sont affectés à deux systèmes herbagers contrastés (**Figure 1**). Le système alimentaire **Haut** permet un niveau de production laitière élevé et une perte d'état corporel modérée. Le système **Bas**, sans apport de concentré, limite la production laitière et provoque une forte mobilisation des réserves corporelles des animaux. Les vaches restent affectées au système jusqu'à leur réforme (due à un échec de reproduction ou un problème sanitaire). Cette étude repose sur les données issues de 516 lactations, dont 211 premières lactations, durant les 9 années expérimentales réalisées.

1.2. DONNEES COLLECTEES

1.2.1. Production laitière

Les vaches ont été traites 2 fois par jour, leur production laitière a été enregistrée à chaque traite et les taux butyreux et protéique du lait ont été mesurés 3 j par semaine lors des 2 traites. L'état corporel a été noté chaque mois.

1.2.2. Paramètres métaboliques

Des prises de sang à 20 et 60 j de lactation ont été réalisées afin de doser des paramètres métaboliques tels que la glycémie, la concentration plasmatique en Acides Gras Non Estérifiés (**AGNE**) et l'urémie par colorimétrie enzymatique (Kone Instruments Corporation, Espoo, Finlande).

1.2.3. Suivi de la reproduction

Le troupeau est conduit en vêlages groupés sur 3 mois (Janvier-Mars), et donc une saison de reproduction de 13 semaines d'Avril à Juin. A partir du mois de Mars les comportements de chaleurs sont observés 5 fois par jour et de la peinture disposée sur la croupe des animaux a été utilisée comme aide à la détection des chaleurs. Les comportements sont enregistrés tels que définis dans les travaux de Kerbrat et Disenhaus (2004) : les comportements spécifiques (acceptation du chevauchement et chevauchement par l'avant, **AC**), les autres signes sexuels (chevauchement et est chevauchée par l'avant, **Chev.**) ou des signes généraux (pose de menton sur la croupe, reniflement, cajoleries, agitation, glaires filantes à la vulve, meuglement, chute de lait et peinture retirée, **signes Gx.**). A partir de 42 j *post partum* les vaches peuvent être inséminées sur chaleurs spontanées. Des échographies à 35 et 70 j après insémination (**IA**) ont permis un suivi de la gestation.

1.2.4. Paramètres de reproduction déduits des profils de progestérone

Trois fois par semaine du vêlage jusqu'à l'établissement de la gestation ou la fin de la période de reproduction, des échantillons de lait sont prélevés pour déterminer leur concentration en progestérone (**P4**) par kit ELISA (Ridgeway Science Ltd., Lydney, Angleterre). La concentration en P4 du lait reflète l'activité ovarienne : en présence de corps jaune, elle est élevée, sinon elle est faible. Afin d'identifier ces phases sur les profils d'activité ovarienne des seuils sont établis : un premier permet de déterminer le niveau de concentration basal de P4 du lait ; un second seuil permet ensuite de distinguer des concentrations de P4 intermédiaires, induites par la présence d'un corps jaune. Le seuil 1 est calculé de façon à ce que 95 % des concentrations en P4 le jour de l'ovulation se situent en dessous du seuil. Le seuil 2 est calculé de façon à ce que le premier quartile des concentrations en P4 du lait supérieures au premier seuil soit intermédiaire (Cutullic et al., 2011). La durée de l'inactivité ovarienne (anovulation) *post partum* est estimée par l'intervalle entre le vêlage et le premier pic de P4. La cyclicité des vaches est ensuite qualifiée de normale (**N**), retardée (**R** - si la concentration en P4 est inférieure au seuil 1 plus de 60 j chez les primipares et 50 j pour les multipares), présentant au moins une phase lutéale prolongée (**PLP** - si la concentration en P4 est supérieure au seuil 2 plus de 25 j), présentant une interruption de cyclicité (**I** - si la concentration en P4 est inférieure au seuil 1 plus de 10 j entre 2 cycles), et désordonnée (**Z**). Les données de progestérone permettent aussi de valider les chaleurs observées en distinguant les « vraies » chaleurs (en phase ovulatoire) des « fausses » chaleurs (en phase lutéale). Les IA réalisées en phase lutéale ont été exclues des analyses. En combinant les informations des diagnostics de gestation par échographie et les profils de progestérone, les résultats des IA sont classés selon la méthodologie de Humblot (2001) : non-fécondation ou mortalité embryonnaire précoce (**NF/MEP**) ; mortalité embryonnaire tardive (**MET**) ; mortalité fœtale (**MF**) ; **avortement** ou **vêlage** (**Tableau 1**). Le taux de revêlage est calculé comme le nombre de vaches qui revêlent sur le nombre de vaches mises à la reproduction.

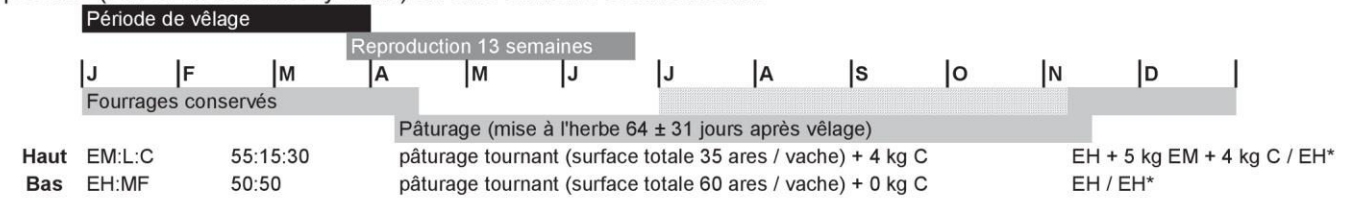
Tableau 1 Règles d'affectation des résultats d'IA en NF/MEP, MET, MF, avortement et vêlage selon le dosage de P4 et les résultats d'échographie (négative : **N.** / positive : **P.**).

Résultat	[P4] élevée	Échographie	Autre
NF/MEP	< 25 j		
MET	≥ 25 j & < 50 j	N. ou P. à 35 j	
MF	≥ 50 j	P. à 35 j & N. à 70 j	
Avortement	≥ 50 j	P. à 35 j & à 70 j	(avorton)
Vêlage	≥ 50 j	P. à 35 j & à 70 j	veau

1.3. ANALYSES STATISTIQUES

Les variables continues (production laitière et taux, état corporel et durée de l'inactivité ovarienne) ont été étudiées par modèles mixtes en incluant la race, le système d'élevage et leur interaction comme facteurs fixes ; l'année, la parité (1, 2, 3 et plus) et la vache en facteurs aléatoires (procédure lmer de R ; R Core Team, 2015). Les effets de ces facteurs sur le type de profil de progestérone ont été analysés par des tests de Chi2. Les variables dichotomiques (détection des chaleurs et résultats des IA) ont été étudiées par modèles linéaires généralisés mixtes en incluant les mêmes facteurs fixes et aléatoires que pour les analyses précédentes.

Figure 1 Calendrier annuel de l'expérimentation comparant deux systèmes à haut ou bas niveau d'apports alimentaires en races Holstein et Normande.,* : ensilage de maïs (EM), bouchons de luzerne déshydratée (L), concentré (C), ensilage d'herbe (EH), ensilage d'herbe mi-fané (MF), ensilage d'herbe (EH) en phase de tarissement. Période hachurée : supplémentation possible (EM ou EH selon le système) si l'offre en herbe est insuffisante



Compte tenu de leur effet, certaines variables supplémentaires ont été ajoutées aux modèles initiaux : l'âge au vêlage, l'index génétique de niveau de production laitière, la présence de problèmes au vêlage (non-délivrance, métrite sévère et vaginite, césarienne, retournement de matrice), la présence d'une congénère en chaleurs et le régime alimentaire à 60 j *post partum* (hivernal/pâturage). Ces modèles initiaux ont fait l'objet d'une sélection pas-à-pas pour déterminer les modèles finaux simplifiés ne contenant que les variables ayant une probabilité d'effet inférieure à 0,10. Afin de caractériser ce qui distingue les vaches gestantes des non-gestantes, l'effet du facteur « gestante » (oui/non) a été ajouté aux modèles finaux. Deux niveaux d'approche sont utilisés dans cette étude : le premier à l'échelle de l'animal, 516 lactations sont analysées ; le second à l'échelle de l'évènement ovulatoire, 1464 ovulations dont 769 suivies d'IA sont analysées.

2. RESULTATS

2.1. ENTRE SYSTEMES, LES VACHES QUI PRODUISENT LE PLUS MAIGRISSENT LE MOINS

Les vaches Holstein ont produit plus de lait, avec des taux plus faibles que les Normande. Elles ont aussi un état corporel inférieur à celui des Normande tout au long de la lactation (**Tableau 1**). Cette relation classiquement observée entre la production laitière et l'état corporel est en partie découplée par les systèmes alimentaires. Les vaches du système Haut ont produit plus de lait que celles du système Bas (+ 2 131 kg pour les Holstein et + 1 132 kg pour les Normande) mais ont perdu moins d'état au cours de la lactation (+ 0,5 point à l'état au nadir pour les 2 races).

Tableau 1 Production laitière (PL - kg/j), taux butyreux et protéique (TB & TP - g/kg) et état corporel (NEC) en fonction de la race (R), du système (S) et de leur interaction (R×S)

	Holstein		Normande		Valeurs P[1]		
	Haut	Bas	Haut	Bas	R	S	R×S
Nb lactations	*113*	*117*	*141*	*146*			
PL 14 sem	35,8^{d}	24,7^{b}	27,0^{c}	19,9^{a}	***	***	***
PL 44 sem	8 277^{d}	5 847^{b}	6 146^{c}	4 715^{a}	***	***	***
TB 14 sem	37,9^{a}	38,3^{ab}	38,9^{bc}	39,3^{c}	*	0,18	0,87
TP 14 sem	30,6^{b}	28,0^{a}	33,0^{c}	30,5^{b}	***	***	0,78
NEC vêlage	3,1^{ab}	3,0^{a}	3,6^{c}	3,2^{b}	***	0,28	**
NEC nadir	2,0^{b}	1,5^{a}	2,9^{d}	2,3^{c}	***	***	0,12
Perte NEC	-1,2^{b}	-1,7^{a}	-0,5^{d}	-1,0^{c}	***	***	0,64
NEC fin	2,3^{b}	1,8^{a}	3,2^{d}	2,5^{c}	***	***	+

[1] *** P < 0,001 / ** P < 0,01 / * P < 0,05 / + P < 0,10
[a – d] distinguent les différences de valeurs 2 à 2 (test de Tukey)

2.2. LA CYCLICITE DES VACHES, UNE AFFAIRE DE GENETIQUE ?

Les vaches Holstein ont une durée d'anovulation *post-partum* de 6 j plus longue que celle des Normande (P < 0,01 ; **Tableau 2**), elles présentent moins de profils de cyclicité normaux, plus de retards (surtout dans le système Haut) et plus de PLP (surtout dans le système Bas). Au sein de chaque race, la durée d'anovulation est similaire pour les 2 systèmes alimentaires (34 et 30 j pour les systèmes Haut et Bas).

2.3. PLUS LA PRODUCTION LAITIERE EST ELEVEE, MOINS LES OVULATIONS SONT DETECTEES

Le taux de détection des ovulations n'est affecté ni par la race, ni par le système alimentaire (**Tableau 2**). Dans les 2 races et systèmes alimentaires, la production laitière le jour de l'ovulation est moins élevée chez les vaches détectées en chaleurs que chez les vaches non détectées (- 1,1 kg, P < 0,001, données non présentés). Les vaches Holstein expriment plus d'acceptations du chevauchement (signe spécifique) que les vaches Normande, qui expriment plus de signes sexuels non spécifiques. Les vaches du système Haut ont exprimé moins d'acceptations du chevauchement que celles du système Bas.

Tableau 2 Durée de l'anovulation *post-partum* (j), proportion de profils de cyclicité normaux et anormaux, taux de revêlage (% des lactations), taux de détection des ovulations et proportions de comportements de chaleurs spécifiques, sexuels ou généraux (% des ovulations) en fonction de la race (R), du système (S) et de leur interaction (R×S)

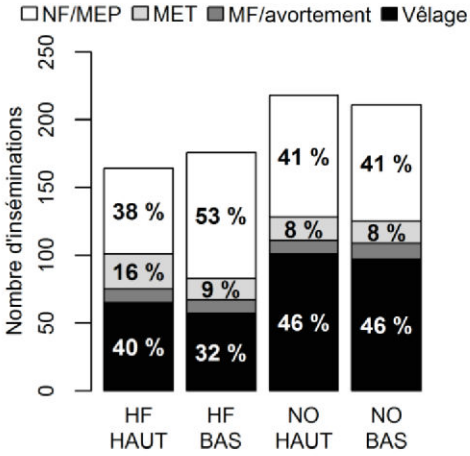
	Holstein		Normande		Valeurs P[1]		
	Haut	Bas	Haut	Bas	R	S	R×S
Nb lactations	*113*	*117*	*141*	*146*			
Anovulation (j)	36,6	33,0	30,7	27,7	**	+	0,52
Cyclicité N	50,4	50,4	66,0	76,6	**	0,40	
Cyclicité R	26,5	17,1	10,6	5,5	***	*	
PLP	18,6	25,6	17,0	12,3	*	0,87	
Tx revêlage	57,5	48,7	72,1	66,9	***	+	0,76
Nb ovulations	*311*	*304*	*422*	*427*			
Tx détection	69,7	73,4	69,0	69,3	0,79	0,28	0,61
AC	37,6	48,7	32,2	40,5	**	***	0,79
Chev.	17,0	13,8	19,7	18,0	*	+	0,51
Signes Gx.	15,1	10,9	17,1	10,8	0,43	***	0,85

[1] *** P < 0,001 / ** P < 0,01 / * P < 0,05 / + P < 0,10

2.4. SELON LEUR ALIMENTATION, LES HOLSTEIN NE FONT PAS FACE AU MEME CHALLENGE DE FERTILITE

Les vaches ont reçu en moyenne 1,5 IA, l'intervalle vêlage - 1ère IA était de 81 ± 25 j et l'intervalle vêlage - IA fécondante de 95 ± 31 j. Le nombre d'IA a été moins important pour les vaches Holstein (**Figure 2**) et leur réussite (proportion d'IA se soldant par un vêlage) moins bonne que celles des vaches Normande (P < 0,01). Le système alimentaire a eu un effet sur les échecs de gestation chez les vaches Holstein : la NF/MEP est plus fréquente dans le système Bas (+ 15 points, P < 0,05) et les MET plus fréquentes (+ 7 points, P < 0,05) dans le système Haut. Il en résulte un meilleur taux de revêlage pour les vaches Normande que pour les vaches Holstein. Ce taux tend à être meilleur dans le système Haut que dans le Bas (**Tableau 2**).

Figure 2 Résultat des IA : taux de Non-Fécondation / Mortalité Embryonnaire Précoce, Mortalité Embryonnaire Tardive, Mortalité Fœtale / Avortement (stable autour de 5 %) et Vêlage par race (HF : Holstein, NO : Normande) et système d'élevage.



2.5. LES VACHES GESTANTES ONT EU MOINS DE PROBLEMES AU VELAGE ET PLUS DE TEMPS POUR SE REPRODUIRE

Les vaches gestantes en fin de campagne ont eu un intervalle entre la date de première IA possible et dernière IA possible plus long (+ 8 j, P < 0,001) car elles vêlent plus tôt en saison et ont moins de problèmes sanitaires au vêlage (-10 points, P < 0,01) que les vaches non-gestantes (**Tableau 3**). Les vaches non-gestantes présentent

Tableau 3 Contexte de la reproduction, performances de cyclicité et paramètres métaboliques en fonction du statut de gestation (G), de la race (R), du système (S) et de leur interaction (R×S)

	Gestantes				Non-Gestantes				valeurs P[1]			
	Holstein		Normande		Holstein		Normande					
	Haut	Bas	Haut	Bas	Haut	Bas	Haut	Bas	G	R	S	R×S
Nb de lactations	*68*	*61*	*103*	*99*	*45*	*56*	*38*	*46*				
DPMR[2] (j)	89	85	88	84	81	77	80	76	***	0,58	*	0,59
Vêlage difficile (%)	30,9	16,4	18,4	8,1	24,4	23,2	26,3	39,1	**	0,57	+	0,44
Cyclicité N (%)	55,9	62,3	63,1	86,9	42,2	37,5	73,7	54,3	*	**	0,40	
Cyclicité R (%)	26,5	13,1	13,6	4,0	26,7	21,4	2,6	8,7	0,49	***	*	
PLP (%)	13,2	18,0	16,5	4,0	26,7	33,9	18,4	30,4	***	*	0,87	
Glucose 60 j (mg/dl)	65,1	61,2	63,1	59,3	65,2	61,3	63,2	59,4	0,87	**	***	0,40
AGNE 60 j (mg/dl)	211,9	242,7	186,5	213,6	204,0	233,6	179,5	205,6	0,44	0,11	**	0,99
Urée 60 j (mg/dl)	25,8	20,3	26,8	21,3	26,9	21,3	27,9	22,3	0,18	0,18	***	0,11

[1] Niveau de significativité : *** P < 0,001 / ** P < 0,01 / * P < 0,05 / + P < 0,10

[2] Durée de la Période de Mise à la Reproduction : intervalle entre la date de première IA possible et dernière IA possible

moins de profils de cyclicité normaux (P < 0,05) et plus de PLP (P < 0,001) que les vaches gestantes. Ni la concentration plasmatique en glucose, ni celle en AGNE, ni celle en urée à 60 j *post partum* ne sont associées au statut de gestation des vaches.

3. DISCUSSION

En accord avec nos précédents travaux, la cyclicité des vaches est dégradée chez les vaches Holstein : elles acquièrent leur cyclicité plus tard et présentent plus de profils de cyclicité anormaux que les vaches Normande (Disenhaus et al., 2009). Dans cette étude, la reprise de cyclicité des vaches tend à être plus courte dans le système Bas, ce qui est cohérent avec une plus forte proportion de profils de cyclicité retardés, mais la proportion de profils normaux et de PLP est identique dans les 2 systèmes alimentaires, d'autres travaux sont en accord avec l'absence d'effet propre de l'alimentation sur la reprise de cyclicité *post-partum* (e.g. Windig et al , 2008). De nombreux travaux ont aussi montré l'importance de l'état corporel au vêlage et de la perte d'état sur la reprise de cyclicité (Friggens et al., 2010).

Le niveau de production laitière a un effet négatif sur le taux de détection des chaleurs ainsi que sur l'intensité des chaleurs exprimées. D'autres études ont montré qu'une forte production laitière diminue la durée et l'intensité des chaleurs (López-Gatius et al., 2005), notamment par une augmentation de la clairance hépatique des hormones sexuelles (Wiltbank et al., 2006) et la diminution de l'activité de synthèse d'œstradiol par les ovaires (Cutullic et al., 2009).

Le taux de revêlage est meilleur chez les vaches Normande et dans le système Haut. Chez les Holstein, les échecs de gestation diffèrent d'un système à l'autre : il y a plus de NF/MEP dans le système Bas et davantage de MET dans le système Haut. Nos précédentes études ont montré que les NF/MEP suite à l'IA seraient pilotées par les réserves corporelles de l'animal, tandis que la MET serait associée à la production laitière exprimée (Cutullic et al., 2012). Les vaches gestantes en fin de campagne ont eu une saison de reproduction plus longue que les non-gestantes, leur offrant plus de chances d'être inséminées. Les vaches non-gestantes ont davantage subi de difficultés au vêlage que les gestantes. Ces difficultés sont connues comme facteurs de risque associés aux anomalies de cyclicité (Opsomer et al., 2000). Dans notre étude, les vaches non-gestantes sont plus sujettes aux PLP que les gestantes, et cette anomalie de cyclicité perturbe la détection des ovulations (Disenhaus et al., 2005).

Contrairement aux idées parfois admises, dans cette étude, l'urémie à 60 j *post partum* n'est pas en cause dans l'infertilité des vaches. En combinant ces résultats avec ceux rapportés par Tamminga (2006) et Diskin et al. (2006), on remarque que le taux de gestation n'est pas systématiquement dégradé pour une urémie au-dessus de 19 mg/dl.

CONCLUSION

Lors de faibles apports nutritifs, les vaches produisent moins de lait. Les vaches Holstein et Normande adoptent néanmoins des stratégies différentes de modulation de la production laitière et de l'état des réserves corporelles. Les vaches Holstein mobilisent de façon importante leurs réserves corporelles de manière à maintenir au mieux leur niveau production laitière. Les vaches Normande profitent de la diminution des besoins en ressources pour la production laitière pour limiter leur perte d'état corporel. Ces stratégies différentes ont des conséquences à chaque étape de la reproduction. Leurs effets se compensent, et les vaches Normande préservent leur aptitude globale à se reproduire alors que les vaches Holstein échouent plus souvent en cas de restriction alimentaire. Les animaux capables de limiter leur production laitière sont donc ceux qui arrivent le mieux à se reproduire en système herbager avec vêlages groupés sur 3 mois.

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L'aptitude des vaches laitières à assurer chaque étape de la reproduction dépend de leurs caractéristiques génétiques.

Ability of dairy cows to ensure each step of the reproductive process according to genetics.

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INTRODUCTION

A même potentiel de synthèse de matières utiles, grasses (MG) et protéiques (MP), les vaches à haut potentiel de production laitière (PL) exportent plus d'énergie dans le lait, ont un pic de lactation plus élevé et une synthèse de lactose plus importante que les vaches à fort potentiel pour les taux butyreux (TB) et protéique (TP). La quantité d'énergie consacrée à la production et le statut énergétique de ces vaches à fort potentiel laitier sont des facteurs connus pour dégrader les performances de reproduction des vaches laitières (Santos *et al.*, 2004 ; Royal *et al.*, 2000 ; Friggens *et al.*, 2010). L'objectif de cette étude est d'identifier et quantifier les effets de la race et du potentiel génétique (favorable à la PL ou aux TB+TP) sur l'aptitude des vaches laitières à assurer ou pas le succès de chaque étape du cycle de reproduction.

1. MATERIEL ET METHODES

Depuis 2006, les performances de reproduction de 60 à 70 vaches/an (500 lactations), conduites sur le domaine INRA du Pin-au-Haras, sont suivies à l'aide de la combinaison du dosage trihebdomadaire de la progestérone du lait, d'un suivi soutenu de l'expression des chaleurs et de diagnostics de gestation par échographie, selon la méthodologie décrite par Bedere et al. (2015). Les effets de la race (Holstein « HF » ou Normande « NO ») et du groupe génétique (à même potentiel de synthèse de matières utiles, les animaux à fort index PL sont assignés au groupe « Lait » et ceux à forts index TB et TP au groupe « Taux ») sur les performances de reproduction sont évalués à l'aide de modèles linéaires/généralisés mixtes. Ils incluaient notamment les effets de l'année, de la parité, de la race, du groupe génétique intra-race, et de la vache (aléatoire).

2. RESULTATS

Les vaches de race HF ont été cyclées plus tard que les vaches NO (+7 jours ; Tableau 1). Dans les 2 races, les vaches du groupe « Taux » ont été cyclées plus tôt que celles du groupe « Lait » (-7 jours en HF, -4 jours en NO). Les HF ont plus d'ovulations détectées sur acceptation du chevauchement (AC) que les NO (+7 points). Les HF ont eu plus de problèmes de fertilité que les NO : plus de non-fécondation/mortalités embryonnaires précoces (NF/MEP ; +6 points) et de mortalités embryonnaires tardives (MET ; +6 points). Les NO « Lait » et « Taux » ont une fertilité à l'IA voisine. Par contre, les HF « Taux » ont plus de NF/MEP (+8 points) et plus de MET (+5 points) que les HF « Lait ». Le taux de re-vêlage des HF est inférieur à celui des NO (-19 points) ; intra race, il n'y a pas de différence entre les groupes « Lait » et « Taux ».

3. DISCUSSION

Les écarts d'aptitude à la reproduction entre les vaches HF et NO sont identiques à ceux décrits précédemment (Delaby *et al.*, 2009). Les vaches « Taux » ont une cyclicité plus précoce que les vaches « Lait » : ceci est expliqué par des différences de niveau de production et d'amaigrissement (Bedere *et al.*, 2016). En effet, la corrélation négative entre la production laitière ou l'amaigrissement et la reproduction est à la fois phénotypique et génétique (Friggens *et al.*, 2010). La dégradation de la fertilité des vaches HF « Taux » par rapport aux « Lait » est par contre assez inattendue. Elle pourrait s'expliquer par le fait que les gènes responsables de la régulation du TB sont négativement associés à la fertilité (Wathes *et al.*, 2013). D'autres études sur les caractéristiques génétiques des capacités de reproduction des animaux de cette étude sont nécessaires pour comprendre les mécanismes physiologiques associés à ce résultat.
Pour conclure, à même potentiel et synthèse de matières utiles, choisir de sélectionner les vaches laitières sur les taux au détriment du lait ne semble pas améliorer leurs performances de reproduction.

Merci à l'équipe du pôle "Lait" INRA – Pin-au-Haras pour le suivi des animaux et au personnel INRA et AGROCAMPUS OUEST pour les dosages. Financements : région Bretagne et projet européen PROLIFIC

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Tableau 1 Délai de reprise de cyclicité, proportion de profil de cyclicité normaux, taux de détection des ovulations, proportion d'ovulation détectées sur acceptation du chevauchement (AC), proportion d'IA1&2 se solvant par une non-fécondation/mortalité embryonnaire précoce (NF/MEP) ou mortalité embryonnaire tardive (MET), et taux de re-vêlage selon la race et le groupe génétique.

Performance	Holstein « Lait »	Holstein« Taux »	Normande« Lait »	Normande« Taux »
Reprise de cyclicité (jours)	39^{b}	32^{ab}	32^{ab}	28^{a}
Profils de cyclicité normaux (% lactations)	40^{a}	53^{a}	66^{b}	71^{b}
Taux de détection des ovulations (% ovulations)	73	69	71	67
AC (% ovulations détectées)	62^{a}	58^{a}	53^{b}	52^{b}
NF/MEP (% IA 1& 2)	25^{a}	33^{b}	22^{a}	25^{a}
MET (% IA1 & 2)	9^{a}	14^{b}	5^{a}	8^{a}
Re-vêlage (% lactations)	55^{a}	49^{a}	73^{b}	68^{b}

$^{a-b}$ distinguent les résultats lorsqu'ils sont significativement différents ($P < 0.05$)

Sélectionner pour la production laitière via les taux, quelles conséquences sur la reproduction des vaches ?

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La sélection génétique favorable à la production laitière s'est accompagnée d'une dégradation des performances de reproduction.
Quelles sont les performances de reproduction de vaches à forts index génétiques Lait ou Taux ?

Dispositif Expérimental : Quelle Vache Laitière pour Quel Système ? (INRA, Le Pin-au-Haras)

2 Races

Holstein (HF)

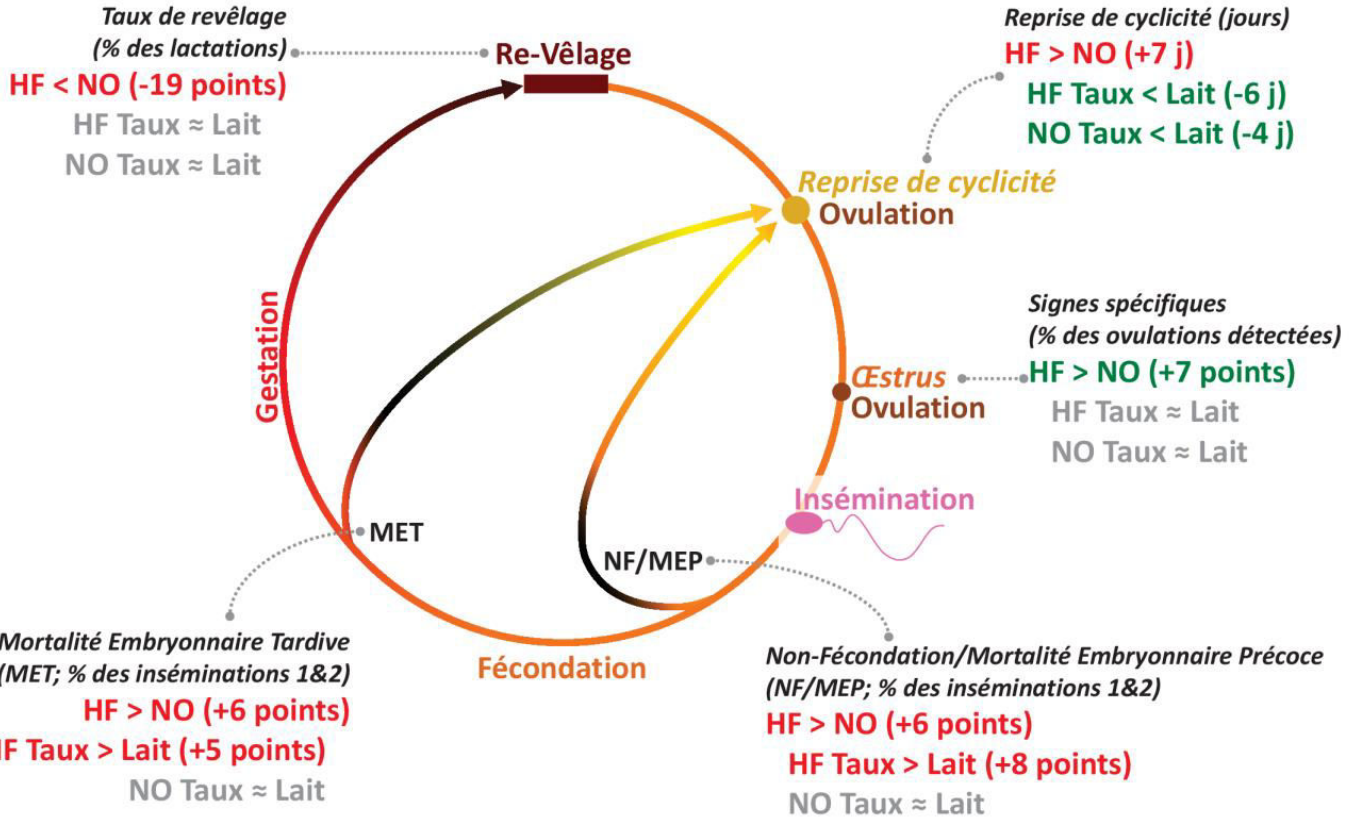
Normande (NO)

2 Groupes Génétiques

À même potentiel de synthèse de Matières Utiles

"Lait" : fort Index Production Laitière

"Taux" : forts Index Taux Butyreux + Protéique (TB et TP)

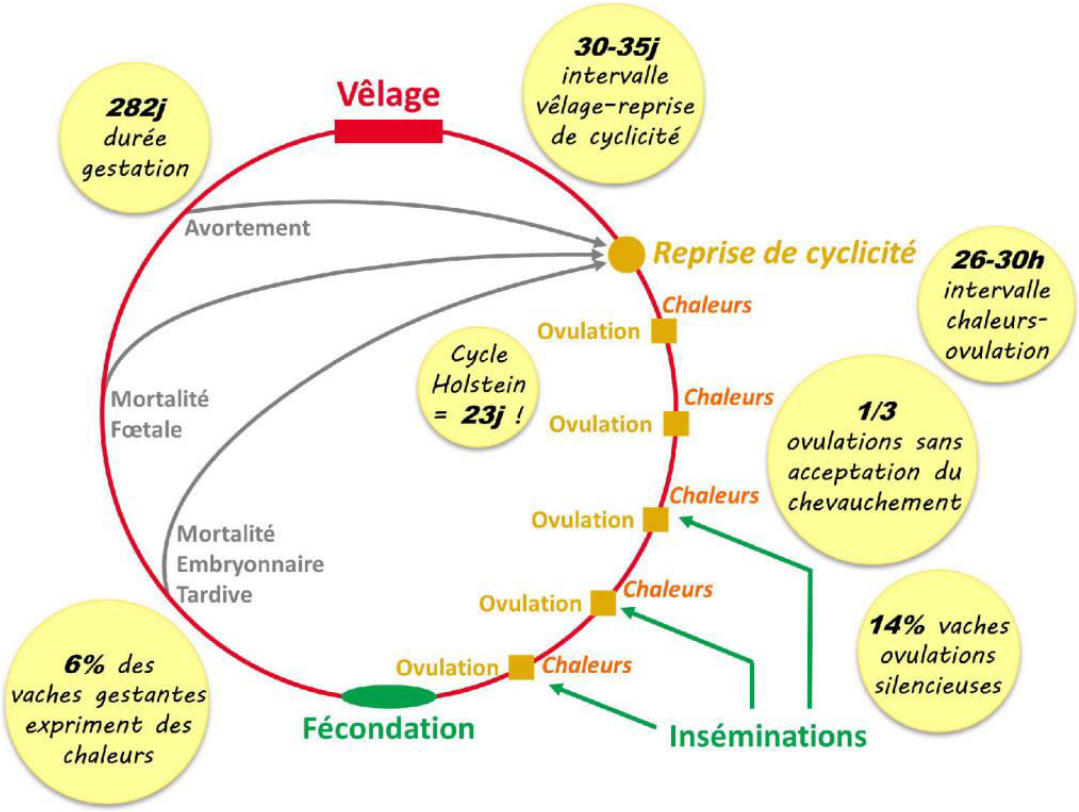


Légende
En vert: amélioration de la reproduction
En rouge : dégradation de la reproduction

Sélectionner les vaches laitières sur les taux (TB et TP) au détriment du lait ne semble pas améliorer les performances de reproduction des vaches laitières.

Merci à l'équipe du pôle "Lait" INRA – Pin-au-Haras pour le suivi des animaux et au personnel INRA et AGROCAMPUS OUEST pour les dosages

Faciliter la reproduction des vaches en temps limité



En amont:

- prévenir évènements **sanitaires** (vêlage difficile, infections utérines, boiteries, mammites...)
- viser **NEC = 3 - 3,5** au vêlage
- limiter **amaigrissement** en début de lactation par des pratiques alimentaires appropriées
- écrêter **pic de lactation** et favoriser la **persistance**

Suite à la mise à la reproduction:

Faciliter la **détection des chaleurs**:

- favoriser le **mouvement** des vaches
- observer aux heures de **traite** et au **crépuscule**
- être attentif aux **signes non spécifiques**
- utiliser des **outils** (peinture, activité-mètre...)

Surveiller les **retours** (calendrier prévisionnel d'évènements, échographies)

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Prairiales Normandie du Pin

Bovins Lait

Se reproduire en temps limité : effets génétiques

Ces dernières années, la **sélection pour la production laitière** s'est accompagnée d'une **dégradation des performances de reproduction**.

Dans l'expérimentation « Quelle vache laitière pour quel système ? » du domaine expérimental du Pin-au-Haras, nous testons, **à énergie exportée dans le lait identique**, les différences de performances de reproduction entre :

Index génétique Lait élevé	Index génétiques TB&TP élevés		Index génétique Lait élevé	Index génétiques TB&TP élevés
34 jours	29 jours	Vêlage → Reprise de cyclicité	45 jours	35 jours
75 %	66 %	Taux de détection des chaleurs	74 %	67 %
65 %	65 %	Taux de réussite à l'IA 1&2	50 %	46 %
12 %	13 %	Taux de mortalité embryonnaire	15 %	28 %
69 %	70 %	Taux de re-vêlage	56 %	48 %

Les animaux à **forts index génétiques TB&TP** ont une **reproduction plus précoce** que ceux dont l'index génétiques Lait est élevé. Cependant semble compensé par une **légère dégradation de la fertilité**.

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RÉSUMÉ *ABSTRACT*

Aptitude des vaches laitières à se reproduire en systèmes pâturants contrastés : quelle vache pour quel système ?

Les performances de reproduction des vaches laitières se sont dégradées parallèlement à l'augmentation de leurs performances de production. L'intense sélection réalisée sur la production laitière est tenue responsable de cela. Chaque étape du processus de reproduction a été affectée : les anomalies de cyclicité ovarienne sont plus fréquentes dans la population actuelle, la durée et l'intensité des chaleurs ont diminué drastiquement, et les échecs de gestation sont plus fréquents. La littérature s'accorde sur le fait que la reproduction est affectée parce que la majeure partie des ressources est investie dans la production laitière. L'objectif de cette thèse de doctorat est d'étudier l'intérêt d'une stratégie de sélection alternative basée sur un fort potentiel génétique de taux butyreux et protéique sur la robustesse des vaches laitières. Les hypothèses testées étaient que par rapport aux vaches à fort potentiel de production laitière, celles à fort potentiel de taux butyreux et protéique (i) exportent moins d'énergie dans le lait et donc préservent leur réserves corporelles, (ii) ont de meilleures aptitudes à se reproduire. Nos résultats montrent que les vaches à fort potentiel de taux butyreux et protéique ont un retour de cyclicité plus précoce, une expression des chaleurs similaire mais plus d'échecs de gestation que celles à fort potentiel de production laitière. Ces résultats intrigants suggèrent que les étapes de la reproduction sont partiellement découplées sur le plan génétique. Sélectionner les vaches laitières sur les taux butyreux et protéique dans le but d'améliorer les performances de reproduction n'apparaît pas comme une alternative pertinente.

Mots-clés : vache laitière, reproduction, production laitière, réserves corporelles, potentiel génétique, cyclicité, œstrus, fertilité

Ability of dairy cows to ensure reproduction in contrasted grazing-based systems: The cow for the system?

The reproductive performance of dairy cows has been declining while their milk production has been improving. The strong genetic selection that was applied on milk production is considered to be responsible for this. Each step of the reproductive process has been impacted: abnormal ovarian activity is more common in the current population, the duration and intensity of oestrus has dramatically decreased and the occurrence of pregnancy failures has increased. The consensus in the literature is that reproduction is impaired because dairy cows are investing most of their resources in milk production. The aim of this Ph.D. thesis is to explore the effect of alternative selection strategies based on milk fat and protein content on robustness of dairy cows. Our hypotheses were that at similar genetic merit for milk solids yield, compared to cows with high genetic merit for milk yield, dairy cows with high genetic merit for fat and protein contents (i) are exporting less energy in milk and are consequently safeguarding their body reserve; (ii) have better reproductive performance (earlier resumption of ovarian activity, more intense oestrus, better ability to ensure pregnancy). Our results suggest that cows selected for production through high fat and protein content instead of high milk yield would resume ovarian activity earlier, have similar oestrus intensity but more pregnancy failures. These intriguing results suggest that the steps of the reproductive process are genetically partly disentangled. Selecting dairy cows for fat and protein content is not a promising way to improve reproduction while maintaining production performance.

Keywords: *dairy cows, reproduction, milk yield, body reserve, genetic merit, cyclicity, oestrus, fertility*

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