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Effects of a difficult calving on the  
subsequent health and welfare of the  
dairy cows and calves

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Thesis submitted for the degree of Doctor of Philosophy

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*To my grandma,*

# Declaration

I declare that I have composed the present thesis. This is my own work and any assistance has been duly acknowledged. The work described has not been submitted for any other degree or professional qualification.

Alice C Barrier

# Acknowledgements

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# Glossary

Commonly used abbreviations are below. Specific abbreviations to this thesis are defined within each chapter.

- ACTH:** Adrenocorticotropin hormone
- AI:** Artificial insemination
- ANOVA:** Analysis of variance
- BCS:** Body condition score
- BMI:** Body mass index
- BVD:** Bovine viral diarrhoea
- CI:** Confidence interval
- COX:** Cyclooxygenase
- d.f.:** Degree of freedom
- DIM:** Days in milk
- DMI:** Dry matter intake
- FPI:** Foeto-pelvic incompatibility
- GLMM:** Generalised linear mixed models
- HDL:** High density lipoprotein
- HPA:** Hypothalamic-pituitary-adrenal
- IQR:** Inter-quartile range
- NSAID:** Non steroidal anti-inflammatory drug
- PI:** Ponderal index
- REML:** Restricted maximum likelihood
- RIA:** Radioimmuno assay
- s.d.:** Standard deviation
- s.e.m.:** Standard error of the mean
- s.e.d.:** Standard error of the differences
- SIA:** Stress induced analgesia
- ZST:** Zinc sulphate turbidimetry

# Abstract

Yearly calvings are essential to the sustainability of modern dairy farming. Currently, calving difficulty (or dystocia) affects one in six calvings among UK dairy herds but vary from 2 to 50% internationally. In dairy cows, despite reports of impaired performance, the extent and threshold of the effect of dystocia on health and performance remains unclear. Over the past years, there has also been increasing concerns about the levels of pain experienced by the dystocial cows. Better understanding of their parturition progress and behaviours is needed so that informed decisions on pain mitigation can be taken. Additionally, the impact of dystocia (besides stillbirth) should also be addressed in dairy calves. The objective of this study was to address the effects of a difficult calving on the health and welfare of both dairy cows and calves.

Retrospective analyses of an experimental farm's detailed records were used to relate calving difficulty with health and performance of the dairy cow. The results showed that after any difficulty at calving, dairy producers incur long-lasting shortfalls in milk sales. Dystocial cows also have impaired fertility, are more likely to leave the herd early and have a higher risk of dystocia at the following calving, thus there is a long-term detrimental impact on dystocial cows.

Video monitoring of calvings allowed detailed investigation of the parturition progress and behaviours of dystocial Holstein cows giving birth to singleton liveborn calves. The study of calving behaviours and parturition progress indicated longer later stages of parturition, increased restlessness and tail raising in the six hours preceding expulsion of the calf, for dystocial cows receiving farm assistance compared with cows calving unaided. This may relate to the expression of higher levels of pain when dystocia occurs. The onset of maternal behaviour was not delayed following calving difficulty, and firm conclusions could not be drawn from investigation of some behavioural indicators of pain in the first three hours postpartum.

Experimental work allowed the monitoring of a cohort of 496 calves born with various degrees of birth difficulty over two years. All but one vet assisted calves were born dead,

and farmer assisted calves were more likely to be stillborn than calves born without assistance. Stillborn dystocial calves displayed larger internal damage, than stillborn eutocial calves, but they did not have a different body shape at birth than dystocial calves that survived. Dystocial dairy calves that survived the birth process had lower vigour at birth, had higher salivary cortisol, acquired lower passive immunity and received more health treatments in the neonatal period. Dystocial heifers also had higher mortality rates by weaning but had similar growth to first service.

Historical records from the farm also showed that dystocial heifer calves were three times more likely to have died by weaning and by first service than calves born without assistance. For those who survived, there was, however, no indication of altered growth to weaning or subsequent impaired fertility. This may be explained by the early mortality of the most badly affected calves or by farm management. However, their high mortality rates still raise welfare concerns. Altogether, results suggest that dairy calves born with any difficulty have poorer welfare in the neonatal period and possibly beyond. The experience of any calving difficulty in dairy cattle therefore not only impairs the welfare of the cow, but also the welfare from their resulting calf. Any strategy implemented to lower the occurrence and mitigate the effects of dystocia will therefore improve the welfare of the cows, their calves and enhance the farm's economic sustainability.

# Publications

## Research articles (peer-reviewed)

**A.C. Barrier**, E. Ruelle, M.J. Haskell, C.M. Dwyer. (2012). Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. *Preventive Veterinary Medicine*, 103 (4): 248-256.

**A.C. Barrier**, M.J. Haskell. (2011). Calving difficulty in dairy cows has a longer effect on saleable milk yield than on estimated milk production. *Journal of Dairy Science*, 94 (4): 1804-1812.

## Conference abstracts (peer-reviewed)

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**A.C. Barrier**, M.J. Haskell. (2011). Calving ease and the subsequent fertility and calving performance of the dairy cow: retrospective analysis from a UK farm. *British Society of Animal Science*, Nottingham, United Kingdom, April 2011. p7.

**A.C. Barrier**, M.J. Haskell. (2011). Calving ease and the subsequent occurrence of mastitis and lameness in dairy cows: retrospective analysis from a UK farm. *British Society of Animal Science*, Nottingham, United Kingdom, April 2011. p208.

**A.C. Barrier**, E Ruelle, M.J. Haskell, C.M. Dwyer. (2010). Calving difficulty in dairy cows results in less vigorous calves but does not affect the onset of maternal behaviour. 44<sup>th</sup> ISAE conference, Uppsala, Sweden, August 2010. p47.

**A.C. Barrier**, E Ruelle, M.J. Haskell, C.M. Dwyer. (2010). Effect of difficulty at calving on the subsequent vigour of the dairy calf, maternal behaviour of the dam and behavioural pain indicators. 14<sup>th</sup> ICPD conference, Ghent, Belgium, June 2010, p52.

**A C Barrier**, M P Coffey, M J Haskell. (2010). Effect of calving difficulty on the saleable milk yield of UK Holstein Friesian dairy cattle at different stages of lactation. *British Society of Animal Science*, Belfast, United Kingdom, April 2010, p17.

# Table of Contents

<b>DECLARATION .....</b>	<b>II</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>III</b>
<b>GLOSSARY .....</b>	<b>VI</b>
<b>PUBLICATIONS.....</b>	<b>IX</b>
<b>TABLE OF CONTENTS.....</b>	<b>X</b>
<b>CHAPTER 1:.....</b>	<b>1</b>
<b>General Introduction .....</b>	<b>1</b>
1.1. Terminology, definition and assessment of calving difficulty .....	2
1.1.1. Terminology .....	2
1.1.2. Assessment of difficulty at parturition and calving difficulty.....	3
1.1.3. Implications for this thesis for defining calving difficulty .....	4
1.2. Prevalence and cost of calving difficulty .....	5
1.2.1. Prevalence and trends of calving difficulty .....	5
1.2.2. Economic cost of calving difficulty .....	5
1.3. Causes and risk factors for dystocia .....	6
1.3.1. Mother-calf physical incompatibility as the major cause of dystocia .....	6
1.3.2. Other factors and the role of calving environment .....	8
1.3.3. Genetic component of dystocia .....	9
1.4. The parturition process and calving difficulty .....	10
1.4.1. The event of parturition .....	10
1.4.2. Parturition when difficulty occurs .....	12
1.4.3. On-farm management of the calving cow and of the dystocial cow. ....	13
1.5. Pain at parturition in cows with dystocia .....	18
1.5.1. Definition of pain .....	18
1.5.2. Concerns for pain experienced by cows with dystocia.....	18
1.6. Effects of calving difficulty on the dairy cow.....	22
1.6.1. Poor survival in the lactation .....	22

1.6.2. Lengthened labour, uterine health and fertility .....	22
1.6.3. Poor milk production .....	23
1.6.4. Feed intake, metabolic dysfunction and behaviour postpartum .....	24
1.7. Effects of calving difficulty on the dairy calf.....	25
1.8. Implications for the welfare of the dairy cow and calf.....	27
1.8.1. What is animal welfare? .....	27
1.8.2. Concerns for the welfare of the dystocial animals.....	28
1.9. Conclusions .....	28
1.10. Thesis objectives and outline.....	29
1.11. Background to the methodology used in this thesis.....	29

**CHAPTER 2:..... 32**

**Milk losses after a difficult calving: should we consider milk produced or saleable milk? ..... 32**

2.1. Interpretive summary .....	33
2.2. Abstract.....	33
2.3. Introduction .....	34
2.4. Materials and methods .....	38
2.4.1. Animals, housing and management .....	38
2.4.2. Datasets description.....	38
2.4.3. Milk production data: saleable milk yields and cow's milk production .....	40
2.4.4. Statistical analyses.....	41
2.5. Results.....	42
2.5.1. Saleable milk yields.....	42
2.5.2. Cow's milk production .....	44
2.6. Discussion .....	46
2.6.1. Detrimental effects of calving difficulty on cow's milk production and saleable milk yields .....	46
2.6.2. Effects of calving difficulty diverged between the herds.....	47
2.6.3. Saleable milk yields best reveal the economic shortfall to the producers and might indicate long-lasting biological stresses in the dystocial cows.....	49

2.7. Conclusion .....	51
2.8. Acknowledgements.....	51
<b>CHAPTER 3:.....</b>	<b>52</b>
<b>Fertility is impaired and dystocia more likely to occur again after a difficult calving in dairy cows.....</b>	<b>52</b>
3.1. Introduction .....	53
3.2. Materials and methods .....	55
3.2.1. Animals, housing and management .....	55
3.2.2. Datasets description.....	56
3.2.3. Statistical analyses.....	59
3.3. Results.....	61
3.3.1. Subsequent calving performance .....	61
3.3.2. Subsequent fertility performance.....	63
3.4. Discussion .....	66
3.4.1. Higher risk of calving difficulty after previous experience of assistance..	66
3.4.2. Impaired fertility at the farm level, following a difficult calving .....	67
3.4.3. Could poor fertility and risks of assistance be worse than the results suggest?.....	68
3.4.4. Implications of long-term impaired performance in dystocial cows .....	69
3.5. Conclusion .....	70
<b>CHAPTER 4:.....</b>	<b>71</b>
<b>Calving difficulty in dairy cows and the subsequent health problems and lactation achieved .....</b>	<b>71</b>
4.1. Introduction .....	72
4.1.1. Mastitis and lameness are major problems in the dairy industry.....	72
4.1.2. Dystocial cows could be at more risk of mastitis.....	73
4.1.3. Dystocial cows might be more predisposed to develop lameness .....	74
4.1.4. Dystocial cows might either be culled early or have stretched lactations	76

4.2. Materials and methods .....	76
4.2.1. Animals, housing and management .....	76
4.2.2. Datasets description.....	77
4.2.3. Statistical analyses methodology .....	83
4.3. Results.....	86
4.3.1. Health records.....	86
4.3.2. Lactation length.....	90
4.4. Discussion .....	96
4.5. Conclusion .....	98

**CHAPTER 5:..... 99**

**Parturition progress and behaviours in Holstein dairy cows with calving difficulty, with an emphasis on expression of behavioural indicators of pain and discomfort ..... 99**

5.1. Introduction.....	100
5.2. Materials and methods .....	103
5.2.1. Animals, housing and calving management .....	103
5.2.2. Video-recording.....	103
5.2.3. Selection of the observed calvings.....	104
5.2.4. Data collection: video scoring.....	106
5.2.5. Datasets, editing and calculation.....	109
5.2.6. Statistical analysis.....	110
5.3. Results.....	112
5.3.1. Calving progress .....	112
5.3.2. Behaviours preceding calving.....	114
5.3.3. Intervention at calving in the dystocial cows .....	118
5.4. Discussion .....	119
5.4.1. Dystocial cows in later stages of labour for longer?.....	119
5.4.2. The birth process with and without intervention.....	120
5.4.3. Behavioural changes prepartum.....	121

5.4.4. Implications of the observed changes in behaviour and progress at parturition: can it reflect more pain and discomfort in the dystocial cows?.....	123
5.4.5. Implications of the observed changes in behaviour and progress at parturition: towards early detection of calving difficulty? .....	125
5.5. Conclusions .....	126

**CHAPTER 6:..... 127**

**Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam ..... 127**

6.1. Abstract.....	128
6.2. Introduction.....	128
6.3. Materials and methods .....	130
6.3.1. Animals, housing and calving management .....	130
6.3.2. Video-recording.....	132
6.3.3. Selection of the calvings and cow-calf pairs .....	132
6.3.4. Data collection: video scoring.....	133
6.3.5. Data manipulation and statistical analysis .....	136
6.4. Results.....	137
6.4.1. Results on the vigour of the calf .....	137
6.4.2. Onset and expression of the maternal behaviour .....	140
6.4.3. Pain-related behaviours in the dams .....	141
6.5. Discussion .....	142
6.5.1. Calves from assisted calvings are less vigorous .....	142
6.5.2. No evidence of an impaired maternal behaviour .....	144
6.5.3. Behavioural indicators of postpartum pain in the dams .....	145
6.6. Conclusion .....	146
6.7. Acknowledgments.....	146
6.8. Conflict of interest statement .....	146

**CHAPTER 7:..... 147**

**Body characteristics and pathology of dairy calves following stillbirth and dystocia ..... 147**

7.1. Introduction .....	148
7.2. Material and methods .....	149
7.2.1. Animals, housing and management .....	149
7.2.2. Study 1: Post-mortem examination of stillborn calves born with/without birth difficulty.....	150
7.2.3. Study 2: Incidence of stillbirth and the body characteristics of calves born dead and following birth difficulty. ....	152
7.3. Results.....	154
7.3.1. Study 1: Post mortem examination of stillborn calves born with/without birth difficulty.....	154
7.3.2. Study 2: Stillbirth and body characteristics of calves born dead and calves born with birth difficulty.....	155
7.4. Discussion .....	157
7.5. Conclusion.....	159

**CHAPTER 8:..... 160**

**Difficulty at birth and the subsequent health, welfare and growth of dairy calves, with a focus in the neonatal period ... 160**

8.1. Introduction .....	161
8.2. Materials and methods .....	164
8.2.1. Farm location and description .....	164
8.2.2. Birth difficulty and rearing of the calves .....	164
8.2.3. Calf recruitment and description of the calves .....	167
8.2.4. Data collection .....	167
8.2.5. Data handling and analysis .....	171
8.3. Results.....	174
8.3.1. Mortality to weaning .....	174

8.3.2. Salivary cortisol .....	174
8.3.3. Milk refusals .....	174
8.3.4. Passive immune transfer .....	176
8.3.5. Non-routine health treatments received.....	177
8.3.6. Rectal temperatures.....	178
8.3.7. Growth of the female calves to first service .....	179
8.4. Discussion .....	180
8.5. Conclusion.....	186

**CHAPTER 9:..... 187**

**Effect of birth difficulty on the subsequent survival, weaning weight, and fertility performance of UK Holstein dairy heifers187**

9.1. Introduction .....	188
9.2. Materials and methods .....	189
9.2.1. Animals, housing and management .....	189
9.2.2. Description of datasets.....	191
9.2.3. Statistical analysis.....	192
9.3. Results.....	195
9.3.1. Stillbirth of dystocial calves .....	195
9.3.2. Survival of the liveborn heifer calves .....	196
9.3.3. Growth of dystocial heifer calves to weaning .....	202
9.3.4. Fertility of dystocial heifer calves.....	203
9.4. Discussion .....	205
9.4.1. Higher stillbirth rates after a difficult birth.....	205
9.4.2. Higher mortality of the heifer calves after a difficult birth beyond the neonatal period .....	206
9.4.3. No evidence of altered growth to weaning or subsequent fertility .....	207
9.4.4. Only the fittest survive? .....	208
9.5. Conclusion .....	209

<b>CHAPTER 10:</b> .....	<b>210</b>
<b>General discussion, recommendations and conclusions</b> .....	<b>210</b>
10.1. The dairy cow after a difficult calving: summary of the results .....	212
10.2. The dairy calf after a difficult birth: summary of the results .....	214
10.3. Are the effects seen true effects of calving difficulty? .....	215
10.4. Any degree of calving difficulty in dairy cows: destined to long-lasting impaired performance? .....	216
10.5. Pain at parturition: higher pain levels for dairy cows with calving difficulty?.....	218
10.5.1. What's the evidence? .....	218
10.5.2. Do the behavioural changes really reflect pain? .....	219
10.5.3. How can we assess pain at parturition in cows?.....	219
10.5.4. Pain at parturition: future work?.....	221
10.5.5. Parturition in cows: should pain relief be given or not? .....	222
10.6. The dairy calf after a difficult birth: condemned to poor welfare? .....	224
10.7. Tackling calving difficulty .....	226
10.7.1. Raising awareness .....	226
10.7.2. Preventing calving difficulty: possible action levers .....	227
10.7.3. Mitigating the effects of calving difficulty when it occurs .....	227
10.8. Recommendations .....	227
10.9. Conclusions .....	228
<b>REFERENCES</b> .....	<b>230</b>
<b>APPENDIX A: BARRIER AND HASKELL (2011)</b> .....	<b>265</b>
<b>APPENDIX B: PRELIMINARY STUDY</b> .....	<b>275</b>
<b>APPENDIX C: BARRIER ET AL. 2012</b> .....	<b>279</b>

## **CHAPTER 1:**

### **General Introduction**

In dairy production, as in most animal production systems, the birth event is essential to the long-term sustainability of the farm. For the dairy cow, the calving marks the start of the lactation. It also provides newborn heifers that will become future lactating animals. Calvings are therefore sources of short and long-term income to producers. However, parturition in most mammal species is a stressful, high risk time for both the mother and newborn. While complex physiological changes are occurring in the mother, the offspring has to make the transition from foetal life to extra-uterine life which can prove to be challenging. Even if this natural process usually goes well in both parties, difficulty in giving birth (also known as “dystocia”) can occur. Dystocia not only increases farm labour when human intervention is necessary, but in other species birth difficulty has adverse effects on the health and performance of both mothers and offspring and this also comes to a price to producers farming cattle (Dematawewa and Berger, 1997; Mee, 2008a), sheep (Cloete et al., 2002; Dwyer, 2003) and pigs (Alonso-Spilsbury et al., 2005; Mainau et al., 2010a). The purpose of this Chapter is to give a general overview of the issues associated with calving difficulty in dairy cattle and introduce the studies presented. Each of the following Chapters contains a review of the relevant literature.

## 1.1. Terminology, definition and assessment of calving difficulty

### 1.1.1. Terminology

Dystocia comes from the greek word “dustokia” composed of dus (difficult) and tokos (birth) and this literary means: “difficulty in giving birth” (Concise Oxford English Dictionary, 2004). There is a wide range of definitions for dystocia as reviewed by Mee (2008a) ranging from need for assistance to considerable force or surgery to extract the newborn. Throughout this thesis, dystocia will be used as a synonym for difficulty at parturition (or specifically, calving difficulty when referring to cattle). Whatever the species considered, difficulty at parturition can be defined as the inability of a dam to give birth by herself without causing overdue distress to either mother or offspring.

### 1.1.2. Assessment of difficulty at parturition and calving difficulty

There are several ways to assess difficulty at parturition (also referred to as calving ease in cattle). Categorical scoring scales that allow for different degrees of difficulty are commonly used across species with ordinal scales with 3 to 5 rating points being popular in cattle (Mee, 2008a). Lower scores are usually given to the easiest births (also called eutocial) and highest scores to the most difficult ones. For example, in the United Kingdom, genetic evaluations in the Holstein Friesian breed are currently performed using the following 4 point scale: “1: easy; 2: assisted; 3: difficult; 4: vet assisted” (Eaglen et al., 2011a).

Assistance and the amount of assistance is commonly used as a proxy measure for the difficulty experienced at parturition, with no or little assistance considered as a “normal” births (or “natural”, “easy”, “eutocial”) and any score above that as being difficult or dystocial (Mee, 2008a). Although assistance is the most common way of grading difficulty (e.g. Dematawewa and Berger, 1997; Lombard et al., 2007; Wathes et al., 2008), other criteria can be used such as the use of a threshold in the duration of labour (Wehrend et al., 2006), a combination of labour duration and assistance, as reported in ewes (Matheson et al., 2011), or even a combination of behaviours, labour length and neonatal outcomes as recently developed in sows (Mainau et al., 2010a).

From a practical point of view, difficult parturitions in farm animals commonly refer to assisted parturitions. The reasons for assistance are subjective and the threshold for provision of assistance may vary between farms and regions (Dargatz et al., 2004), reflecting sociological factors. Assistance itself can take different forms including correction of minor (e.g. folded anterior leg) or major malpresentations (e.g. breech), giving a slight manual pull, the need for an instrumental aid (such as calving/lambing aid) or the need for pharmaceuticals and/or surgery. Current recommendations from the Farm Animal Welfare Council on cattle are that: “*calving aids should not be routinely used to accelerate the delivery of the calf during calving which would otherwise be born naturally*” and only be used when “*delivery per vaginam can be reasonably expected, without causing overdue pain and distress to either mother or offspring*” (FAWC, 1997).

When using assistance as a proxy measurement for difficulty at parturition, a difficult birth would ideally be considered as an assisted birth where:

- from the animal’s point of view there is a need for assistance
- assistance is provided proportionally to requirement
- assistance was always provided when needed.

It is likely that in some cases, assistance may be provided without difficulty occurring at the time of intervention (for convenience for example) although it is impossible to assess whether that same animal would have encountered difficulty if not assisted. Similarly, it is also likely that a proportion of unassisted animals may have benefited from human intervention. However, if good farm practice is observed, the cases where the latest two assumptions are not met should be negligible. Furthermore even when those criteria are not met, it is adequate to use assistance as a proxy measurement for difficulty at parturition. Indeed, it best matches the practical considerations of farmers and the UK industry, because from a practical point of view, level of assistance is the assessment tool which is available.

### 1.1.3. Implications for this thesis for defining calving difficulty

Throughout this thesis, a calving will be considered as difficult (or dystocia), when any kind of assistance is provided at delivery. Within the difficult calvings, different degrees of difficulty will be considered. For purposes of clarity, the term “calving difficulty” will be used when referring to the dams and “birth difficulty” when referring to the newborn but both terms refer to the calving process.

Throughout the studies the scale used to assess calving difficulty is presented in Table 1.1

**Table 1.1** Calving/birth difficulty scores used throughout this thesis

N	Normal calving. No assistance provided	eutocia
FN	Farm assisted - calf showing a normal presentation	difficulty/ dystocia
FM	Farm assisted - calf showing a malpresentation	
VN	Veterinary assistance - calf showing a normal presentation	
VM	Veterinary assistance - calf showing a malpresentation	
VC	Veterinary assistance - caesarean section	

## 1.2. Prevalence and cost of calving difficulty

### 1.2.1. Prevalence and trends of calving difficulty

Because definitions of dystocia vary in the literature, it is not surprising that there is variation in the international prevalence rates of calving difficulty. Internationally, reported prevalence in dairy cattle of severe or considerable difficulty in calving vary from just below 2% to over 22%. However, assistance at calving (including lower degrees of difficulties) is much more prevalent, varying from 10% to over half of the calvings (Mee, 2008a). In the United Kingdom, 16% of calvings are assisted on average (Wall et al., 2010) and 7% of primiparous calvings and 2% of multiparous calvings are thought to result in severe difficulty (Mee, 2008a). In the United States, the average national rate of calving assistance is 20.6% in multiparous animals and 31% in primiparous animals (USDA, 2009) although the rates are also known to reach as much as 29.4% in multiparous and 51.2% in primiparous animals (Lombard et al., 2007). Even though average quotes are of interest, it is worth noting the wide variation in the proportions reported between the farms. Taking the example of Ireland, Mee (2008a) highlights that even though the mean dystocia rate is of 4.2%, the highest quartile of farms were above 12% whereas lowest quartile was below 1%.

Furthermore, the increasing proportion of genes from the Holstein breed in the dairy herds over the years seem to have had negative effects on the ease of calving (Adamec et al., 2006; Heins et al., 2006).

### 1.2.2. Economic cost of calving difficulty

Defining exactly how much dystocia costs UK dairy farmers is complex. Costs suggested range from £110 for the occurrence of a slight difficulty, to £350 to £400 for a very difficult calving (McGuirk et al., 2007). However, these estimates are based on UK dairy herds using beef semen and thus may not be representative of dairy cattle costs. When extrapolating work from Dematawewa and Berger (1997) in the USA to the average UK dairy farm, dystocia would cost around £950 per year to the producer. This estimate is based on the considerations that the average dairy farm counts 112 animals (DEFRA, 2009) of which 24% are heifers (Stott et al., 2005) and that at the herd scale,

dystocia cost \$10 (£6.3) per cow and \$28.53 (£18) per heifer reared on farm. However, this is an underestimate of the real costs as it does not take into account higher culling rates of cows, veterinary costs of treating animals beyond the post-partum period, subsequent additional labour and management costs, and losses of dystocial calves beyond birth.

### 1.3. Causes and risk factors for dystocia

There are various causes and risk factors associated with dystocia in dairy cattle which can result from both maternal and foetal factors. The following section is focussed on cattle, and particularly on dairy cattle.

#### 1.3.1. Mother-calf physical incompatibility as the major cause of dystocia

The most common cause of dystocia results from a physical incompatibility between the pelvic size of the mother and the size of the calf at birth, also called foeto-pelvic incompatibility (or FPI) (Meijering, 1984; Mee, 2008a). This is largely influenced by the weight and morphology of the dam and the calf respectively. The pelvic area available at birth is affected by the size of pelvis but also by fatness of the dam which might partially obstruct the birth canal. The calf's physical factors contributing to a size mismatch between the calf and the dam may include a calf of a big size or malpresentation. These morphological factors are themselves dependant upon various parameters including the age, breed and parity of the dam, twinning, the sex and weight of the calf, the sire and breed of the calf as well as the nutrition of the dam during gestation (Bellows et al., 1971; Meijering, 1984; Hickson et al., 2006; Mee, 2008a).

Calves having high birth weights above 42kg and dams with a small pelvic size are particularly at risk of dystocia (Bellows et al., 1971; Burfening et al., 1978; Meijering, 1984). In a study on Holstein cattle, Johanson and Berger (2003) found that the incidence of dystocia increased by 13% per additional kg of the calf's birth weight and decreased by 11% for an increase of 1 dm<sup>2</sup> of the pelvic size of the dam. Male calves, who are generally heavier at birth, are at higher risk of dystocia. Genetic and

environmental factors affecting calf birth weight are reviewed by Holland and Odde (1992).

Dams with low calving weight as well as abnormally low or high body condition score can result in difficulty at parturition as well (Philipsson, 1976b). Adequate body condition score at calving for both primiparous and multiparous dams (BCS of 2.5 to 3, when assessed on a 5 point scale) ensures optimisation of ease of delivery and subsequent performance.

Primiparous cows are known to have a fourfold greater risk of difficulty than multiparous cows (Nix et al., 1998), partly because of their smaller size and pelvic size. It is recommended that first calving takes place between 22 to 24 months of age to optimise subsequent performance and ease of delivery (Le Cozler et al., 2008; Berry and Cromie, 2009). In order to avoid cases of FPI, it is particularly important for the animal caretaker to mate primiparous animals with bulls that are not expected to sire very large calves. As will be seen in more details (section 1.3.3.), this can be achieved by making an informed choice on their genetic potential for their expected ease of calving.

Pelvimetry can also be used to determine the pelvic area available at calving by measuring set dimensions of the pelvis internally using pelvimeters (conventional methodology) (e.g., Rice pelvimeter) or external proxy measurements (e.g, hip width). This methodology offers the opportunity and ability to detect abnormal pelvic sizes at the time of heifer breeding and proceed to an early cull of animals that may prove problematic and for selecting dams with a larger pelvis. There is however controversy in the accuracy of prediction of dystocia through the use of pelvimetry as explained in Kolkman et al. (2009) and Tsousis et al., (2010). Measurement inaccuracies in field conditions, the lack of full correspondence between the calculated total pelvic area and obstetrically relevant parameter measuring the shape of the pelvis, and the multifactorial etiology of calving difficulty may contribute to lack of sensitivity of the predictions. Pelvimetry has traditionally been implemented in beef breeds and to my knowledge, its use in dairy breeds remains limited. Nonetheless, a recent study in Holstein-Friesian animals Tsousis et al. (2010) correlated the use of external pelvimetry (less invasive and more practical in field conditions) to obstetrically relevant pelvic measurements (pelvic

inlet area and circumferences) which were not accessible by conventional use of internal pelvimetry.

Finally, although they are thought to occur in up to 5-6% of births (Patterson et al., 1987; Mee, 2008a), malpresentations account for 20 to 40% of the dystocial cases (Meijering, 1984). They can occur under various forms such as posterior malpresentations (foetus backwards), leg malpostures (e.g., folded leg or leg back) or cranial malpostures (e.g., head back). The main risk factors for malpresentations are twinning, age of the dam and the choice of the calf's sire (Patterson et al., 1987; Holland et al., 1993).

### **1.3.2. Other factors and the role of calving environment**

Even if FPI is the major reason leading to difficulty at calving, dystocia can result from other causes that interfere with the expulsive forces needed to expel the calf. This includes: lack of uterine contractions (weak labour), incomplete dilation of the cervix and vagina due to stenosis (narrowing and stiffening of the tissue) and uterine torsion. Risk factors for weak labour include hormonal imbalances such as reduction in plasmatic oestradiol concentration (Osinga, 1978), high levels of oestradiol-17 $\beta$  at parturition (Sorge et al., 2008) or high ratios of cortisol to progesterone (O'Brien and Stott, 1977). These imbalances can lower expression of oxytocin receptors in the uterus as well as altering the preparation of the soft tissues, causing weak uterine contractions and weak dilatation of soft tissues (Sorge et al., 2008).

Furthermore, premature assistance (when the cervix and vulva are not fully dilated) can cause stenosis. Therefore, the decision to provide assistance and the timing of assistance should be carefully thought out. It is recommended that assistance should only be provided when foetal feet have been visible for 2 hours at the vulva and no visible progress is made (Mee, 2004).

Finally, environmental disturbance at calving such as overcrowded accommodation can increase the risk for dystocia through occurrence of vulval constriction (Dufty, 1981). Moving of the cow in labour before the foetal feet were seen has also been found to increase risks of stillbirth (Carrier et al., 2006). This suggests that additional stress

introduced to the animal at parturition could favour dystocia and increase risks for stillbirth. Indeed, pain or distress can impair the labour progress through partial blocking of the oxytocin release which is necessary for uterine contractions (Ehrenreich et al., 1985; Taverne, 1992; Lawrence et al., 1997), a phenomenon also called secondary uterine inertia (Mee, 2004). This highlights the importance of an appropriate calving environment and management. By raising awareness of these risk factors, damage caused to the cow and calf could be prevented.

### **1.3.3. Genetic component of dystocia**

Throughout the genetic literature, calving difficulty is usually referred to as calving-ease. Calving difficulty has a genetic underlying component certainly because many factors that are accounting for FPI are also under genetic control. The genetics of calving difficulty are complex because it is a combination of both maternal effects (also called grandsire effects) and effects from the sire of the calf (also called sire effect or direct effect) (Meijering, 1984). Thus, the sire of the calf partly explains the birth weight of the calf and its morphology. Additionally, parameters such as the pelvic size of the dam, the length of gestation and calving weight result partly from the dam's sire. The heritability of calving difficulty (which means the probability of transmission to the following generation) is quite low with estimates of both direct and maternal heritabilities being estimated between 0.03 and 0.20 (Meijering, 1984) but mostly reported below 0.12 in dairy cattle (e.g. Steinbock et al., 2003; Eaglen and Bijma, 2009; Eaglen et al., 2010a) and in beef cattle (Bennett and Gregory, 2001; Eriksson et al., 2004). There are negative correlations between direct and maternal effects (Philipsson, 1976a), which means that genetically, a heifer prone to be born easily will be more likely to have difficulty when she calves herself. It is therefore challenging to find the right balance between the ease with which a cow calves and with which her female offspring will herself calve when she reaches adulthood. This result is also controversial and has not been found consistently across populations (Eaglen and Bijma, 2009). High genetic correlations are also observed for calving difficulty between first and later parities (Thompson et al., 1981; Carnier et al., 2000; Eriksson et al., 2004). This means that similar genes and

mechanisms may be involved in both parity levels and that some cows may be more genetically predisposed to dystocia. However, the heritable variance explains only 10% of the phenotypic variance (Eaglen and Bijma, 2009). As a consequence, although selection against difficult calving is possible and should be encouraged, non-genetic factors (called environmental factors) are also of great importance in the implementation of preventive measures against dystocia. Research into the interactions between genetics and the environment seems to have been overlooked. It is possible that the use of bulls labelled as difficult calvers may lead to different phenotypes depending on the farm management (such as nutrition of the dam for example). Such investigations should be encouraged to facilitate optimised use of genetic potential in the prevention of dystocia.

#### 1.4. The parturition process and calving difficulty

Parturition is a complex process which involves a large number of physiological and behavioural changes in most species. An overview of these changes in cattle is given below.

##### 1.4.1. The event of parturition

###### ***Physiological changes and the three stages of parturition***

Parturition in cows is initiated by the hypothalamus-pituitary-adrenal (HPA) axis of the foetus, which leads to an elevation of foetal cortisol in late gestation. Its action on the placenta consequently increases oestrogen levels and decreases progesterone levels (which are responsible for the maintenance of pregnancy) (Challis and Thornburn, 1975; Lye, 1996; Jenkin and Young, 2004).

The parturition process itself is divided into three stages (Ball and Peters, 2004; Wehrend et al., 2006), during which various physiological mechanisms will ensure the delivery of the calf (Taverne, 1992; Ball and Peters, 2004). As reviewed by Ball and Peters (2004), the first stage of labour begins with dilatation of the cervix and ends with the rupture of the chorioallantois in the vagina. During this stage, which lasts from 6 to 24h, both the dam and the calf prepare for the actual birth process through the dilatation of the cervix and position of the calf with the forelimbs extended. The second stage

starts at that point and continues until the calf is expelled. This stage of labour lasts from 30 min to 4 hours but typically lasts around 40 min (Doornbos et al., 1984; Berglund et al., 1987). It is characterised by regular contractions facilitated by the pressure of the foetus against the cervix and vaginal wall, which releases oxytocin, and in turn, stimulates uterine contractions (Ferguson's reflex). This is also the stage during which the calf is actually born. Finally, the third stage corresponds to the expulsion of the foetal membranes and the placenta at which point the parturition process ends. This usually takes no longer than 6 hours but retention of foetal membranes after 24 hours is considered pathological.

The duration of the calving event varies between individuals as well as between parities, with shorter labour length in multiparae than in heifers (Berglund et al., 1987), the head of the calf taking longer to emerge in heifers (Wehrend et al., 2005). This variation is nicely illustrated in the study of Berglund et al. (1987) in Swedish dairy cows which report on the percentiles for various milestones of parturition. As an example, they observed that 90%, 50% and 10% of the animals delivered their calf within 70, 22 and 5 minutes respectively of the sighting of the calf's feet at the vulva.

Definitions of labour stages can differ between studies, for practical reasons because the start of stage I and II can be difficult to assess. For example, Wehrend et al. (2006) defined stage I as the amniotic sac appearing in the cervix, a criteria that can be assessed by vaginal examination. Berglund et al. (1987) considered the onset of labour to have occurred when "irregular straining was observed and the cow changed her position frequently", which is subjective but less invasive to the cow. The sighting of the part of the calf at the vulva is also sparingly used as a definition of the start of second stage of labour (e.g. Edwards, 1982; Mee, 2004).



Cow in stage II of parturition with the feet of the calf visible at the vulva (left). On the right, cow having just expelled her placenta, which marks the end of stage III of parturition.

### ***Behavioural changes associated with parturition***

Parturition in cows is also associated with behavioural changes. As delivery approaches, cows tend to isolate themselves, choose a delivery site, increase their activity and show more tail raising (Edwards, 1983; Lidfors et al., 1994; Albright and Arave, 1997; Huzzey et al., 2005; von Keyserlingk and Weary, 2007; Miedema et al., 2011b).

Changes in behaviour and physical signs such as relaxation of the pelvic ligaments, udder distension or swelling of the vulva (Berglund et al., 1987) are traditionally used to detect the onset of parturition. With increasing herd sizes and the lower amount of time available for the monitoring of each individual cow, there has been a growing interest in developing automated devices that can accurately detect the onset of parturition in farm animals based on their behaviours (Oliviero et al., 2008; Mainau et al., 2009; Miedema, 2009).

#### **1.4.2. Parturition when difficulty occurs**

The most commonly observed difference in the parturition progress between cows with and without calving difficulty is the longer labour experienced in dystocial cows (Hudson et al., 1976; Civelek et al., 2008; Miedema et al., 2011a). Because of this, labour length is often used to discriminate dystocial from eutocial animals. Indeed, the occurrence of calving difficulty is usually defined as no progress in parturition 2 hours

after the amniotic sac has ruptured (Wehrend et al., 2006) or when no progress has been seen within 2 hours after the feet are visible (Mee, 2004). It is recommended that an internal examination should take place if there has been no progress in labour within the 2 hours following the appearance of the water bag (Leaver, 1999).

Studies relating to behaviours at calving sometimes exclude such cows with difficulty (e.g., Lidfors et al., 1994) and for a majority of them, no mention of calving difficulty is made (Edwards and Broom, 1982; Edwards, 1983; Lidfors and Jensen, 1988; Lidfors et al., 2010). Nonetheless, alongside other elements such as elapsed time since first observed in labour, history and characteristics of the cow (such as parity), behaviours expressed by cows at parturition are essential clues for the farmer to make a judgment on whether or not a calving cow is in difficulty and assistance should be provided. In recent years, there has been a growing interest in documenting the behaviours of dystocial cows at calving, certainly because such changes in behaviours may also be used for early detection of difficulty and may serve as indicators of pain.

However, studies have been inconsistent in the behavioural differences found for dystocial and eutocial cows (e.g. tail raising and self-grooming; Manteca et al., 2010; Mainau et al., 2010b; Miedema et al., 2011a). Therefore, there is a need to further document the behaviours and parturition progress of dystocial cows in relation to cows that calve normally, particularly for behaviours that could relate to the expression of pain.

#### **1.4.3. On-farm management of the calving cow and of the dystocial cow.**

##### ***Housing, supervision and detection of calving***

At the farm level, depending on the farming system and strategy adopted, calvings can occur seasonally or all year round. In the UK, calvings usually take place under housed conditions, with dry cows in their late gestation often housed together in a separate pen to allow for closer supervision until calving. Depending on the building design and farm management, the individual calving animal is sometimes but not necessarily further isolated from its conspecifics at the start, during or shortly after calving. Under The Welfare of Farmed Animals (England) Regulations 2000 (SI 2000/1870, schedule 5),

calving cows should have access to a well-drained and bedded lying area, be separate from livestock other than calving cows and the size of the pen or yard should permit attendance of the cow by a person. This is likely to vary greatly between farms and countries. Approximately a quarter of US dairy farms reported having dedicated calving facilities and 40% of the cows move less than a day prior to calving (USDA, 2010).

Alongside estimation of due dates, there is a variety of indicators which can indicate the imminence of calving and therefore help with the provision of closer supervision. Physical signs include relaxation of the pelvic ligaments, distension of the udder, vaginal secretion, udder oedema, oedema of the vulva, tail relaxation, teat filling and milk losses (Berglund et al., 1987; Mee, 2004; Streyl et al., 2011). Behavioural signs indicating onset of calving are restlessness and separation from the herd (von Keyserlingk and Weary, 2007). There is an increased interest in the use of devices that detect the onset of calving, some of which are available on the market such as Vel'Phone® (Medria, Chateaugiron, France), Agrimonitor® (Databel Trading S.A, Louvain, Belgium) and Alert'Vel (Creavia, Rennes, France). Haematological assessments that assess the drop in the progesterone levels that precede calving have also been developed (described in Streyl et al., 2011). Its high cost and low practicality is a huge limitation to its routine use on farms. This more invasive procedure might nonetheless prove beneficial on certain types of farms, for research purposes or where very high commercial value animals are involved (such as on stud farms, in the equine industry or for cloned animals). Level of supervision during calving might depend on a variety of factors such as the availability of human labour, presence of on-site accommodation, the number of milkings a day and the seasonality of calvings. This is highlighted in the study from Dargatz et al. (2004) on US beef farms, where the frequency of observations of calving animals increased with increasing herd size and for primiparous animals as opposed to multiparous animals. Not surprisingly either, the frequency of observations of calving animals was found to be higher during day time than during night time, with about half of the farms and 18% of the farms allowing less than 3 hours to pass between observations during day time and night time respectively (USDA, 2010). Modern technology that allows remote watching of the calving pens (e.g., cowCam, TM Online

from LUDA Elektronik, Göteborg, Sweden) can also help to ensure the provision of adequate supervision.

As described previously (section 1.3.2.), the quality of the environment at calving can impact on the calving process, which highlights the importance of getting it right. Despite sets of recommendations being in place, there are few objective reports of what actually happens on farms.

### ***Decision for intervention***

Because of the complexity of the calving process and the varied aetiology for calving difficulties, the “one fits all” rule does not necessarily apply when deciding on whether to intervene or not. In his review, Mee (2004) presents management procedures with regards to supervision, examination and provision of assistance during stage I and stage II of labour respectively. As highlighted previously, early intervention will be beneficial during stage I of labour for cases of uterine inertia and during stage II for cases of malpresentations and twinning. In contrast, delaying intervention during stage II is recommended in cases of FPI and cervical or vulval stenosis.

For the most common cases of FPI, Mee (2004) recommends that assistance at calving should be provided when foetal feet have been visible for 2 hours at the vulva and under the condition that no visible progress is made. The importance of observing progress rather than judging solely on the duration since appearance of the feet is also emphasised. In a recent study on a US experimental farm, Schuenemann et al. (2011) recommended that obstetrical assistance should occur 70 minutes after appearance of the amniotic sac or 65 minutes after appearance of calf’s feet. Their reference time is based on the calculation of the mean + 2 standard deviations of unassisted births. However, in this study, assistance was systematically provided after 80 minutes of the amniotic sac appearing at the vulva which, in my opinion, drives the result towards the 70 minute threshold. Getting the timing for intervention right is challenging. While excessive labour lengths are detrimental to calf survival and subsequent fertility (Dornboos et al., 1984), early intervention could also be detrimental. Intervention at less than 1 hour after the onset of stage II has been associated with higher use of a calf puller and incidence of downer cows (Egan et al., 2001).

In practice, there are few studies reporting on farms' practices. In an Irish survey conducted in 1996, 44% and 46 % of the farmers reported intervening at less than an hour and less than two hours respectively, after appearance of the foetal feet (Egan, 2001). Approximately 60% of the US dairies had guidelines on when to intervene at calving (USDA, 2010). Propensity to examine and assist vary between farms (e.g., sizes, location), certainly as a result of underlying socio-economic factors (Dargatz et al., 2004; USDA, 2010). Heifers are usually consistently allowed to labour for shorter periods of time than cows prior to giving assistance. This averaged 2.8h and 3.5h in heifers and cows respectively.

### ***Provision of assistance***

It is not the purpose of this thesis to provide detailed explanations about assistance at calving. As mentioned previously, it is estimated that 1 in 6 cows from the Holstein breed is assisted at calving in the UK but that this figure varies largely nationally and internationally (Mee, 2008). Based on data from the national recording organisations, 25% of the primiparous received assistance in the UK of which 85.2, 12.0 and 2.8 % were classified as moderate, difficult and with veterinary assistance respectively (Eaglen et al., 2011c). As a matter of comparison, in the US, 30% of the primiparous animals received assistance, of which 40, 38 and 22% experienced easy, mild and severe (mechanical and veterinary) difficulty respectively (USDA, 2010).

Assistance at calving can take various forms and is mostly performed by the animal caretakers themselves, with veterinary help sought when this can not be dealt with at the farm level (Egan et al., 2001; USDA, 2010).

Nine out of 10 US dairies reported providing staff training on calving management and this was delivered "on the job" in 90% of the cases (USDA, 2010). During intervention, approximately 60% of the dairies reported isolating the dystocial animal, restraining her and using lubricant (USDA, 2010). About half report washing the perineum area and 3 quarters report disinfecting chains and any equipment inserted in the reproductive tract at calving. When pulling the calf, most (70%) would use tie the calf with chains. The most preferred option is traction from one or two men in more than half of the farms and

the use of a calving jack for 1 out of 5 of the farms. Increasing popularity of the calving jack was confirmed in the Irish survey (Egan, 2001) and its use remains more prevalent in larger operations (USDA, 2010). A calving jack should be used with caution as it can easily exert excessive traction forces of up to 400kg, therefore causing injuries in the dam and the calf (Mee, 2004). As a matter of comparison, during calving, a cow would apply a force of 75kg, and one, two and three men can apply 75, 125 and 175 kg of force respectively (Hindson, 1978). It is recommended that when using a calving jack, pulling is exerted in conjunction with the dam's contractions so that to limit injuries and stenosis. Such practice was reported in 80% of the US farms (USDA, 2010).



Cow giving birth naturally (left) and cow assisted by one person, with the use of a calving jack (right)

Veterinary assistance should be sought when severe abnormality occurs and when farmer assistance is unsuccessful. In the case of a normally presented calf, this should occur after 10 to 15 minutes of assistance without having been able to extract the calf further than the eyes; and for a malpresented calf, if a malpresentation is not corrected after 15 to 30 minutes of manipulation (Mee, 2004). In practice, regardless of the parity of the calving animal, most US farms reported seeking veterinary assistance within 30 to 59 minutes of intervention, with as many as 25% that would wait at least 1 hour.

Guidelines and practical information on how to assist a dystocial cow at calving, techniques for calf extraction and the general management of the dystocial cow and newborn calf will not be developed in this thesis but are described in details in nicely

illustrated books (e.g., Straiton, 2002; Jackson, 2004; Thomas, 2008; Noakes et al., 2009).

## 1.5. Pain at parturition in cows with dystocia

### 1.5.1. Definition of pain

Pain is a complex notion based on both sensory and affective components (see Gregory (2004a) for a review on the physiological mechanisms involved). It is described by the International Association for the Study of Pain (IASP) as “*an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damages*” (Merskey et al., 1979). However, to fit the animal’s need the following has been suggested “*Animal pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues... it changes the animal’s physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery*” (Molony, 1997). Assessing pain in animals can be very challenging. Descriptions of pain and some methods to assess it have been described combining both physiological and behavioural indicators of pain (Molony and Kent, 1997; Rutherford, 2002; Molony et al., 2002). The study of behaviour is a useful tool in the investigation of pain in animals (Bateson, 1991; Rutherford, 2002; Anil et al., 2002; Weary et al., 2006; Vinuela-Fernández et al., 2007). Indicators of pain will differ according to the type of pain in terms of duration, location, origin, species and breed (for a review, see Gregory (2004a)).

### 1.5.2. Concerns for pain experienced by cows with dystocia

#### ***Parturition pain in parturient farm animals: why bother?***

To date, most work on pain in farm animals has generally focussed on mutilations performed for farm management procedures (e.g., tail docking, castration and dehorning) and on a selection of health problems considered as production diseases (e.g., lameness, mastitis). Probably because pain at parturition is natural, pain experienced by parturient farm animals has been given little attention. This is despite births being key drivers to many livestock production systems and the average cow expected to calve and

go through this experience at yearly intervals during her production life. There have been increasing concerns that the experience of dystocia could lead to higher levels of pain, which may be unacceptable. If this is the case, action should be undertaken to relieve pain on ethical and welfare grounds.

Furthermore, negative outcomes are associated with pain states. For example, although it is likely to be innocuous in women with uncomplicated labour, adverse effects of severe labour pain in women include alterations in the maternal respiratory pattern and the catecholamine-mediated stress response, which may trigger metabolic disorders and potentially foetal acidosis (Brownridge, 1995). Also, pain levels achieved after childbirth predict persistent pain and postpartum depression (Eisenach et al., 2008).

### ***Components of pain at parturition***

It is not unreasonable to assume that parturition in cows is a painful experience as it is in women, because of the analogy in the neural structure (Bateson, 1991; Bateson, 2004). Nonetheless, the alleviation of parturition pain in cows has been given little attention (Rushen et al., 2007). In women, labour pain increases with greater dilation of the cervix as well as with more intense, frequent and longer uterine contractions (Corli et al., 1986; Lowe, 2002), which means that pain increases as labour progresses. During childbirth, the first stage of labour triggers abdominal, visceral and lower back pain which is poorly localised and slowly transmitted whereas pain experienced during the second stage of labour, caused by the stretching of the lower birth canal perineum, is sharply intense and localised (Brownridge, 1995; Lowe, 2002).

It is likely that components of parturition pain in cows might be similar to what is encountered by women during childbirth (Gregory, 2004a). However, it needs to be kept in mind that transition from quadrupedalism to bipedalism in human species, was accompanied by narrowing of the pelvis in conjunction with increased foetal-head size, making it riskier for women to give birth without assistance (Trevathan, 1996; Rosenberg and Trevathan, 2002). It is therefore possible that by not being as well adapted to easy birthing, women may experience more intense pain than cattle but also that some components of pain may differ between the two species as a result of anatomical differences in their skeleton.

### ***Primary evidence for pain concerns in dystocial cows***

Across various countries, dystocia is recognised by veterinarians as being a very painful condition, with median pain ratings ranging between 7 and 9 (on a 10 point numerical rating scale) (Huxley and Whay, 2006; Laven et al., 2009; Kielland et al., 2009). These ratings refer to calvings requiring veterinary assistance but ratings for less severe degrees of difficulty do not seem to have been explored. In addition, cows with dystocia experience longer labour and straining compared to cows that are not assisted at calving (Berglund et al., 1987; Gundelach et al., 2009; Miedema et al., 2011a). This means that they may experience more contractions, the occurrence and intensity of which correlates with higher levels of pain rated by women (Corli et al., 1986; Lowe, 2002).



Behaviours performed by calving cows during uterine contractions followed by straining. Note the lateral lying and the tail being raised in both cows. On the left, head and legs are extended. On the right, head is rested, one front leg is bent and, the back is arched.

Higher blood vasopressin concentrations (a hormone secreted in response to stressful/painful stimuli) have been found with higher levels of parturition pain (visually assessed) in dairy goats (Olsson et al., 2004) and in heifers requiring assistance at birth (Hydbring et al., 1999), although it is not clear whether these changes are due to longer labours or to difficulty. The latter study suggested that the hormonal changes observed could relate to pain-stress coping mechanisms. Such mechanisms exist although they do not compensate for the level of pain experienced. Stress-induced analgesia or SIA results in higher pain thresholds around the time of parturition (Jarvis et al., 1997). This is caused by stimulation of the cervix and vagina and leads to an opiate-mediated analgesia

(Gregory, 2004a) which partially inhibits the transmission of pain signals in the spinal cord and brain (Gregory, 2004b).

Finally, intervention itself, although necessary, may lead to additional pain due to further pressure applied to extract the newborn (Scott, 2005) as well as the stretching in the birth canal involved in dystocial deliveries. In support to this, assisted cows have been reported to give a roar when the calf is extracted, coinciding with the passing of the head and forelegs (Gregory, 2004a). When misused, calving aids can result in painful injuries and trauma to the cow and to her reproductive tract such as tears, bruises and fractures (Mee, 2008a; Fishwick, 2011). Furthermore when a caesarean section is necessary, post-surgical pain is expected. Following assistance, whether calf pulling or surgical intervention, cows may suffer from underlying pain resulting from the parturition within the days postpartum (Kolkman et al., 2010a). Therefore an investigation into the level of pain experienced by cows, especially when difficulty in giving birth occurs, is needed.

### ***Alleviation of parturition pain***

Practical considerations and the validity of administering analgesic drugs in dairy cattle to alleviate postpartum pain have been investigated (Richards et al., 2009; Duffield and Newby, 2010; Mainau Brunsó, 2011). In theory, administration of a non-steroidal anti-inflammatory drug (NSAID) postpartum should reduce inflammation and pain associated with parturition. This is because NSAIDs inhibit enzymes (cyclooxygenases COX 1 and COX 2) involved in the synthesis pathways for prostaglandins (deriving from arachidonic acid) which are responsible for pain and inflammation (Richards et al., 2009). However, previous research looking at the use of ketoprofen postpartum (a non steroidal anti-inflammatory drug or NSAID) did not find any advantage of administration after any particular level of dystocia in terms of milk yield and fertility (Richards et al., 2009). Ongoing research is investigating the use of meloxicam postpartum to see whether this would benefit the animals, particularly after dystocia (Duffield and Newby, 2010; Mainau Brunsó, 2011). Meloxicam is a NSAID drug of the oxicam class which specifically targets the inhibition of COX 2 as opposed to COX 1 meaning that some undesirable side-effects on the stomach and kidneys are reduced,

with a longer lasting anti-inflammatory and analgesic effect than the other NSAID drugs (Engelhardt, 1996; EMEA, 2009).

Nonetheless, a better understanding of parturition behaviours prepartum and postpartum in dystocial cows is needed so that behaviours that could serve as behavioural indicators of calving difficulty and of pain can be identified and pain relief administered.

## **1.6. Effects of calving difficulty on the dairy cow**

### **1.6.1. Poor survival in the lactation**

In the most severe cases, dystocia can lead to the death of the cow, usually occurring within 48 hours (Dobson et al., 2008). Even beyond those 48 hours, cows that have experienced dystocia are more likely to die or be culled in early lactation and over the lactating period (Beaudeau et al., 1993; Dematawewa and Berger, 1997; Tenhagen et al., 2007; Lopez de Maturana et al., 2007b; De Vries et al., 2010). This higher culling rate can be explained by various indirect effects of dystocia, as will be investigated later.

Furthermore, the fear that the animal might experience difficulty at her next calving may add weight to the farmer's decision to cull a dystocial cow. As seen previously, some cows may be genetically more predisposed to dystocia. It is however unclear whether at the phenotypic and individual level, a cow with experience of calving difficulty is likely to have difficulty at the next calving. Thompson and Rege (1984) reported small repeatabilities in US dairy cows but this was not large enough to act as good indicator of future calving performance. In a more recent study on Irish pasture-based systems Mee et al. (2011) found that severe difficulty was a risk factor for subsequent assistance but this was not the case for lower degrees of difficulty. Therefore, the phenotypic relation between calving performances from one to the next lactation should be investigated.

### **1.6.2. Lengthened labour, uterine health and fertility**

Cows experiencing difficulty at birth are more likely to suffer from postpartum diseases such as metritis, retained placenta and milk fever (Thompson, 1984; Benzaquen et al., 2007). This could be explained by the possibility of microbial contamination during assistance (Dohmen et al., 2000) combined with a depressed immune status during the

peripartum period. This highlights the importance of good hygiene when intervention at calving is required. Immunodeficiency is probably enhanced in dystocial cows as a consequence of the increased duration of labour and the subsequent higher cortisol levels (Hudson et al., 1976; Civelek et al., 2008). Nakao and Grunert (1990) studied the adrenocortical function of beef cows through adrenocorticotrophic hormone (ACTH) challenge postpartum for different degrees of calving difficulty. They found higher reactivity of the HPA axis for cows having experienced severe dystocia. They suggest that this could increase the susceptibility of the uterus to infection through the anti-inflammatory action of corticosteroids. As well, this might be responsible for causing temporary metabolic disturbances in cows and delaying ovarian recovery and uterine involution. As hormones involved in reproductive function closely interact with HPA axis regulation, this can be related to impaired fertility. In fact, a prolonged second stage of labour resulted in depressed reproductive performance in both beef and dairy cattle (Doornbos et al., 1984; Dobson et al., 2001). An increase in the number of days open, the number of services to conception and a delay to first service has been shown after dystocia (Laster et al., 1973; Thompson et al., 1983; Djemali et al., 1987; Dobson et al., 2001; Lopez de Maturana et al., 2007a). This impaired fertility after dystocia is thought to contribute to 30% of the cow related costs of dystocia (Dematawewa and Berger, 2003).

### **1.6.3. Poor milk production**

It has been shown that calving difficulty reduces milk yield in the cow. It is not clear however, how long the adverse effect on milk production lasts for. In fact, although some authors seem to find a deleterious effect on the overall lactation of cows (Mangurkar et al., 1984; Djemali et al., 1987; Dematawewa and Berger, 1997), some studies have suggested that these effects disappear beyond 14 days in milk (DIM) (Rajala and Gröhn, 1998), 90 DIM (Thompson et al., 1983) or six month postpartum (Tenhagen et al., 2007). Furthermore, the degree of difficulty from which milk losses are reported ranges from slight degrees of difficulty (Djemali et al., 1987; Dematawewa and Berger, 1997) up to only in severe cases when surgery is needed (Tenhagen et al., 2007).

Additionally, the magnitude of losses have been suggested to be greater with increasing degrees of difficulty (Djemali et al., 1987; Dematawewa and Berger, 1997). However, the pattern with which milk losses vary is not always obvious (e.g, Mangurkar et al., 1984) and other factors such as the overall yield or parity of the cow (Rajala and Gröhn, 1998) might influence it.

As well, it is common for studies looking at milk production losses after a difficult calving to restrain their datasets to animals with full lactations or that have survived until a certain lactation stage (e.g., Mangurkar et al., 1984; Djemali et al., 1987). This is nonetheless ignoring a portion of the dystocial population. Approaches for looking at milk losses from the producer's point of view that also fit the animal's perspective should be developed.

#### **1.6.4. Feed intake, metabolic dysfunction and behaviour postpartum**

During the lactating period, dry matter intake was shown to decrease in cows that had experienced dystocia in the months postpartum (Bareille et al., 2003) compared to cows that calved normally, but this was not seen in the first two days postpartum (Proudfoot et al., 2009). This could relate to lower milk production observed in dystocial animals but also to the greater losses in weight and body condition score found in dystocial cows during their subsequent lactation (Berry et al., 2007). According to the authors, this may be related to changes in the metabolic function and lower immunocompetency in these animals. To that extent, the experience of dystocia in Holstein dairy cows is also associated with haematological changes at delivery relating to hepatic function. For example, dystocial Holstein heifers had higher cortisol, cholesterol, glucose, high density lipoprotein (HDL), triglycerides, creatinine and vitamin A levels than eutocial animals, which might reflect higher calving stress in these animals (Civelek et al., 2008). It is possible that such stress but also exhaustion, pain and human intervention during delivery may contribute to reduced or delayed maternal care of the calves in the first hours postpartum, as observed in ewes (Alexander et al., 1988; Dwyer et al., 2001; Fisher and Mellor, 2002). Although altered maternal behaviour in dairy cows may not be relevant for most dairy systems compared to beef rearing systems, this depends on the

farm's policy on the timing of the cow-calf separation. Benefits of appropriate care received by the calf at an early age should be considered and delay in maternal care could support assumptions of underlying pain and exhaustion.

### 1.7. Effects of calving difficulty on the dairy calf

At birth, having to make the transition from foetal life to extra-uterine life is a challenging experience in mammals. Animals born from difficult births are more likely to fail that transition and become stillborn or die within the few days of life (Laster and Gregory, 1973; Nix et al., 1998; Meyer et al., 2001a; Johanson and Berger, 2003; Berglund et al., 2003; Eriksson et al., 2004). Within the group of calves that die perinatally, 90% would be alive at the start of the calving process and three quarters of the deaths occur within an hour of birth (Mee, 2008b; Mee, 2008c), thus emphasising how critical the birth process and early hours of life can be. Dystocial stillbirths usually result from internal and external trauma (Bellows et al., 1987; Agerholm et al., 1993; Berglund et al., 2003; Aksoy et al., 2009) but also from prolonged hypoxia (deprivation of adequate oxygen supply) (Meijering, 1984; Mee, 2008c).

There are also concerns about the beef and dairy calves that survive the birth process. Indeed, survival is not just compromised in the perinatal period but there is also evidence that their survival can be affected in the neonatal period (Wittum et al., 1994a; Johanson and Berger, 2003; Lombard et al., 2007) and for their lifetime for the more severe degrees of difficulty (Henderson et al., 2011). In the neonatal period, severe hypoxia and acidosis (Massip, 1980a; Alonso-Spilsbury et al., 2005; Civelek et al., 2008), impaired breathing (Breazile et al., 1988) and internal injuries (Berglund et al., 2003; Gundelach et al., 2009; Mee, 2010) may contribute to low vigour (Edwards, 1982; Riley et al., 2004) and subsequent poor survival. Additionally, dystocial calves may not thermoregulate properly (Bellows and Lammoglia, 2000) and achieve lower passive immunity (Donovan et al., 1986; Odde, 1988). This may relate to higher morbidity (Wittum et al., 1994a; Steenholdt and Hernandez, 2004; Lombard et al., 2007) and possibly altered growth (Bellows et al., 1988; Goonewardene et al., 2003).

Furthermore, it is likely that the experience of difficulty at birth may have long-term effects on heifer calves. It is well-known that the early life experiences can have long-term implications for the performance, cognition, health and welfare of the individuals among diverse species including ruminants and cattle (Braastad, 1998; Vinuela-Fernandez et al., 2007; Rutherford et al., 2009b). The early experience of pain, stress and hypoxia from the dystocial neonate may have long term effects on the pain sensitivity, neurological and behavioural development of the calves, as shown in rats, piglets and humans (Anand, 2000; Mikati et al., 2005; Bonsignore et al., 2006; Vinuela-Fernandez et al., 2007; Grunau et al., 2009; Rutherford et al., 2009b). However, the effects of dystocia, which can be considered as perinatal stress, have been mostly reported for the neonatal period and there are few studies looking beyond the neonatal period in cattle, especially in dairy breeds. Recently, evidence has emerged from retrospective studies that dystocia could also have potentially long-term effects on dairy heifers. Reduction in survival rates and milk production were seen when they reach an adult age (Eaglen et al., 2010b; Heinrichs and Heinrichs, 2011; Henderson et al., 2011) but further investigation is needed to support those findings. Longitudinal studies linking the early life experience with later performance may also add to this evidence.

Although undesirable effects have been shown on the health and survival of dystocial calves, birth difficulty was not the main focus of most studies, which concentrated either on the first 24 hours of life or on a few physiological indicators only (e.g., Odde, 1988; Vermorel et al., 1989; Civelek et al., 2008; Gasparelli et al., 2009a; Gasparelli et al., 2009b). This means that the overall picture of the outcome of dystocial calves is lacking. Some evidence of adverse effects was also shown for beef breeds (e.g., Bellows et al., 1988; Wittum et al., 1994a; Bellows and Lammoglia, 2000; Goonewardene et al., 2003) but needs further evidence in dairy breeds. Even though dystocia was the focus of a longitudinal study on morbidity and mortality of dairy calves until four months of age (Lombard et al., 2007), there is still a general lack of longitudinal studies looking at the welfare of dystocial dairy calves. Particularly, the neonatal period should be investigated at various levels in terms of behaviour, physiology, health and survival and related with their performance at a later age.

## 1.8. Implications for the welfare of the dairy cow and calf.

### 1.8.1. What is animal welfare?

In the European Union, animals have been officially recognised as “sentient beings” since the ratification of the Treaty of Amsterdam in 1997. The scientific discipline of animal welfare is relatively new and there have been many attempts to define what is meant by animal welfare. Welfare can be seen as “the state of an animal as it attempts to cope with its environment” (Broom, 1988, 1991). Very commonly, welfare has been defined by the 5 Freedoms (FAWC, 1993). Those are: freedom from hunger, thirst and malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom from fear and distress, and freedom from expressing normal behaviour. This definition remains widely used. In the UK, this is currently used as a basis in the ‘Code of recommendations for the welfare of livestock – cattle’ from the Department for Environment, Food and Rural Affairs (DEFRA, 2003a). Fraser (2003) presents animal welfare as deriving from 3 main components: biological functioning, affective states and natural living. This broadly corresponds to being “fit and healthy”, “happy” and to “natural living” (Webster, 2005). Animal welfare can be of varying degrees and this notion is particularly important when referring to quality of life. A good state of welfare can be defined as when the nutritional, environmental, health, behavioural and mental needs of animals are met (Mellor and Stafford, 2001). Emphasis has traditionally focussed more on describing poor or impaired states of welfare which were of primary concerns rather than the achievement of good levels of welfare. More recently, the Farm Animal Welfare Council gave more weight to the positive aspects of animal welfare and adopted the concept of a “life worth living” (FAWC, 2009), the implications of which are extensively discussed by Yeates (2011). Such definition is to a certain extent relatively similar to the definition of health adopted by the World Health Organisation (for humans): “a state of complete physical, mental and social well being, and not merely the absence of disease or infirmity” (WHO, 1946).

In a nutshell, animal welfare refers to the animal’s perspective and to what it feels, that is to say both the physical and mental state of the animal and can not be achieved if one

or the other is impaired. There are various methodologies allowing the assessment of animal welfare and these traditionally focus on physiological, behavioural and production parameters. There is also increasing interest in developing methodologies to assess emotions in farm animals, so that mental states can be further considered (Désiré et al., 2002; Boissy et al., 2007; Mendl et al., 2010).

### **1.8.2. Concerns for the welfare of the dystocial animals.**

As seen in previous sections, there are many potential threats to achieving good standards of welfare for the dam and her calf (or calves) after a difficult calving. These include pain during parturition as a result of calving injuries, painful health conditions, injuries at birth but also distress following poor health, breathlessness, hypoxia and potential hypothermia. These in turn might result in poor production and survival. Possible long-term alteration of the development of the dairy calves following birth difficulty is also likely.

## **1.9. Conclusions**

There appears to be a consensus in the literature that calving difficulty or at least severe difficulty at calving has adverse effects on the health and production of the dairy cow. It is as yet unclear exactly from which degree of difficulty dystocia has adverse effects on the cow, or how long the effects last for and how much it affects the cow. Many studies looking at production parameters have been done using large uncontrolled datasets, with restrictions in place that investigate only a sample of the population. It is felt that although the production approach is necessary, little emphasis was put on the animal's point of view and when investigating welfare aspects of dystocia, such an angle is important. Furthermore, the parturition event in dystocial animals needs to be further investigated in terms of behavioural changes and calving progress so that insights into pain levels can be gained and thereafter early detection and adequate alleviation of pain can be undertaken. Finally, although the incidence of dystocia is likely to have negative outcomes on the dystocial dairy calves, longitudinal studies with a focus on dystocial

calves should be undertaken with regards to the calf welfare, not only in the first few days of life but also beyond.

### 1.10. Thesis objectives and outline

The general objective of the thesis will be to assess the short and long-term effects of a difficult calving both in dairy cows and calves in terms of health and welfare.

The objectives are to:

1. Assess the impact of a difficult calving on the health of the dairy cow and her subsequent performance over the lactating period
2. Improve understanding of the parturition behaviours and progress in dystocia animals, with an emphasis on behaviours that could indicate pain
3. Assess the short and medium-term effects of having had a difficult birth on the subsequent health and welfare of the dairy calf.

Effects of a difficult calving will be assessed in relation to the welfare of the animal. The focus will be on measuring parameters that are direct threats to the maintenance of good or acceptable levels of welfare and which will indicate any impairment in the level of welfare after a difficult calving.

Objective 1 will be addressed by the study of historical data from an experimental farm. Once the impact of calving difficulty has been established, it is expected that this will give more incentive to study in more detail what happens during a difficult calving, with respects to behavioural indicators of pain (Objective 2). Finally, the consequences of a difficult birth will be investigated on the calf's perspective focussing on early life first before moving on to later life (Objective 3).

Chapters 2 to 4, 5 and 6, and 6 to 9 respectively will address objectives 1, 2, and 3. The welfare of the cow will be addressed through Chapters 2 to 6 and calf's welfare through Chapters 6 to 9. Chapter 10 will discuss the outcome of the three objectives altogether.

### 1.11. Background to the methodology used in this thesis

The work will focus on the Holstein breed, which is the major dairy breed represented in the dairy industry in most industrialised countries worldwide. This breed has been very

highly selected over the last decades for its high milk yield potential and is considered by many as being the “formula one” of the existing dairy breeds, with regards to milk production. Apart from their high prevalence, it is felt that cows from this breed are therefore most representative of the modern dairy cow and of her associated welfare challenges. Although, in the following thesis, results will be generalised to dairy cows in general, it needs to be kept in mind that some differences might occur between breeds. Data came from the dairy herd from the Scottish Agricultural College. This experimental herd is commercially run. As will be explained in subsequent chapters, it was previously housed and co-managed with the University of Edinburgh in (Edinburgh, UK) and moved facilities in the 2000s to join the Crichton Royal Farm (Dumfries, UK). As part of an ongoing long-term genetic breeding and feeding trial, this herd contains animals from two genetic groups and split over two diet types (Pryce et al., 1999; Bell and Roberts, 2007). From the start of the trial in the 1990s, thorough daily routine data collection has taken place to gather data on a multitude of production and health parameters of the animals present on the farm (e.g., milk production, fertility, lactation length, any health treatments, diets, calving difficulty scores). The existence of such a database is therefore an invaluable source of high quality data which allows the study of not just the effects of genetics and nutrition but also many relationships between parameters.



Straw-bedded calving sheds from the Crichton Royal Farm. View from the feeder face (left) and from opposite the feeders. Note the separation into two pens for the allocation of the two types of diets. Cows from two genetic groups are housed in each pen.

Work in Chapters 2, 3, 4 and 9 use these high quality historical data. This high quality is in contrast with the huge amount of data generated from national databases, often used in such type of work, and which allow little control and require extensive subjective cleaning procedures. Working with such a database also gives the opportunity to look at what happens at the farm level as opposed to the national level. Although some of the data will refer back to the 1990's, it is felt that it does not invalidate them. This is because half of the animals are from a very highly selected strain and therefore represent the future contemporary animal. The range of animals with various degrees of genetic advancement therefore makes it still relevant to the modern dairy industry.

Work in Chapters 5, 6, 7 and 8 will rely on data collected as part of experimental work on the Crichton Royal Farm. It was not any of the farm's policy to assist animals unless calving difficulty occurred. On both farms, calving difficulty was systematically recorded in a consistent unambiguous manner over the years (Table 1.1.). This limits any drift of scores over the years and ensures the reliability of collection of calving difficulty scores. Calving management, decision and provision of assistance was managed by experienced farm staff. Description of the supervision levels and criteria used for judging calving difficulty on the Crichton Royal Farm are detailed in Chapters 5 and 6.

It is acknowledged that various factors may influence the detection of calving difficulty and the decisions to provide assistance to a calving cow, which may result in variation within scores of difficulty. This is nonetheless reflective of what would routinely happen in practice on any given commercial farm. Therefore, this is expected to be representative of current farm practices.

## **CHAPTER 2:**

### **Milk losses after a difficult calving: should we consider milk produced or saleable milk?**

Adapted from: Barrier, A. C., and M. J. Haskell. 2011. Calving difficulty in dairy cows has a longer effect on saleable milk yield than on estimated milk production. *J. Dairy Sci.* 94(4):1804-1812 (available in Appendix A).

In this Chapter, I was responsible for developing the methodology presented, data extraction, data analysis and writing of manuscript.

## 2.1. Interpretive summary

Calving difficulty raises animal welfare and economic issues. In dairy cows, calving difficulty impaired their milk production as well as the amount of milk saleable by the farmer. However, the degree of calving difficulty from which losses were reported, their magnitude and duration depended on the herd management. The analysis of cumulative saleable milk yields independently of each animal having achieved a full lactation is more representative of the income loss of the dairy producer than the cow's milk production alone. Also, it might be an indicator of the long-term biological stresses the animals experience.

## 2.2. Abstract

A difficult calving has impacts on the welfare of the cow and has economic implications for the farm. The degree of difficulty can vary from no assistance needed through a slight pull required to surgery being needed. With respect to milk production, it is not clear from which degree of difficulty adverse effects occur and how long they last for. Studies usually only consider the milk produced by animals who completed full lactations but the saleable milk production of the whole herd, regardless of each cow having achieved a full lactation, might be a better indicator of the productivity of the cows, the underlying stresses they experience, as well as being more representative of the real losses that producers incur. The objective of this study was to investigate how various degrees of calving difficulty would alter both the cow's milk production and their production of saleable milk over different stages of their subsequent lactation.

The calving difficulty scores and the subsequent milk production were retrieved from an experimental dairy farm (UK) for two herds which contained 2430 and 1413 lactations respectively. To account for milk saleable by the farmer, individual cumulative saleable milk yields, referred as saleable milk yields (SMY) were calculated at 30, 60, 90 and 300 DIM unconditional on the animal having achieved the lactation stage of interest. Lactation SMY were obtained on the basis of the real lactation length achieved by the

animal. Mean daily milk yields were also calculated for the same lactation stages as an estimate of the cow's milk production (CMP).

Calving difficulty impaired milk production of dairy cows in terms of CMP and SMY in both herds, therefore highlighting impaired income for dairy producers as well as detrimental effects to the productivity of the cows and potential impaired health and survival. The management of the herd affected the presence of an effect of each degree of difficulty on SMY and CMP as well as its magnitude and duration. The analysis of SMY, independently of each animal having achieved a full lactation, could be a more sensitive indicator of the subsequent long-lasting biological stresses than the cow's milk production alone.

Key Words: dairy cow, calving difficulty, milk production, dystocia

### 2.3. Introduction

Parturition is intimately interlocked with lactogenesis in all mammals. In dairy cows, it marks the start of the lactation and therefore the beginning of the productive cycle. However, it is also a high risk time for both mother and offspring. Calving difficulty, also known as dystocia, often means that assistance must be provided during delivery. The grading of the amount of assistance required is therefore a widely used measure to identify different degrees of difficulty. In the United Kingdom, severe cases are thought to occur in 7% of the primiparae's births (Mee, 2008a) but assistance rates are much higher reaching about 16% nationally (Wall et al., 2010). Not only does calving difficulty increase farm workload but it has adverse effects on the subsequent survival, health and performance of both mothers and offsprings (Dematawewa and Berger, 1997; Tenhagen et al., 2007; Lombard et al., 2007). For a review on effects and risk factors of dystocia, see Mee (2008a). Thus, calving difficulty raises productivity, economic and animal welfare issues.

It has been shown that calving difficulty reduces milk yield in the cow. It is not clear however, how long the adverse effect on milk production lasts. In fact, although some authors seem to find a deleterious effect on the overall lactation of cows (Mangurkar et al., 1984; Djemali et al., 1987; Dematawewa and Berger, 1997), some studies suggest

shorter-term effects which disappear beyond 14 days in milk (DIM) (Rajala and Gröhn, 1998), 90 DIM (Thompson et al., 1983) or six month postpartum (Tenhagen et al., 2007). Furthermore, the degree of difficulty from which milk losses are reported ranges from slight degrees of difficulty (Djemali et al., 1987; Dematawewa and Berger, 1997) up to only in severe cases when surgery is needed (Tenhagen et al., 2007). Additionally, losses have been suggested to be greater with increasing degrees of difficulty (Djemali et al., 1987; Dematawewa and Berger, 1997). However, the pattern with which milk losses vary is not always obvious (e.g, Mangurkar et al., 1984) and other factors such as the overall yield or parity of the cow (Rajala and Gröhn, 1998) might influence it. The underlying reasons for such variation could be attributed to a range of factors such as different scoring methods, animal genetics, livestock management, calving management or even evaluation methods.

Using milk production to assess performance of dairy cows is straightforward and can be seen as related to the income of dairy producers. This animal orientated approach, combined with other indicators, can also in some extent give an insight into the welfare of the animal since milk yields are known to be lowered in the event of stress (Hasegawa et al., 1997; Bruckmaier and Blum, 1998) or disease because of the redistribution of energy requirements in favour of the immune system (Rajala and Gröhn, 1998; Bareille et al., 2003). However, the use of datasets that contain animals with full lactations only or that have achieved a certain stage of lactation seems to be common practice when calculating effects of calving difficulty on milk production (Mangurkar et al., 1984; e.g, Djemali et al., 1987).

Dystocial animals are more likely to die or be culled in the early stages of their lactation (Tenhagen et al., 2007; Dobson et al., 2008). Therefore, restriction on lactation length could introduce biases in the results. As a consequence, taking into account the whole herd, whatever the lactation stage each animal actually achieves seems preferable to represent the milk losses that producers incur.

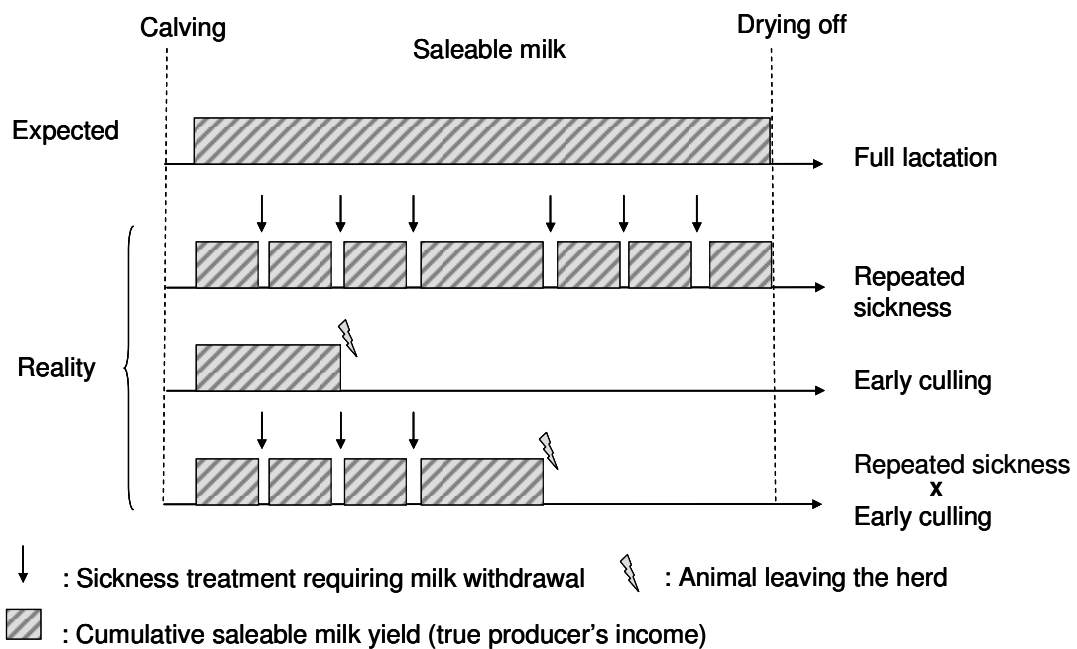
Furthermore, the use of such datasets usually implies extrapolating the milk production from animals to which continuous records are not known (such as test day yields). Because milk production is lowered during a sickness episode, if sampling occurs at or

near that episode, the extrapolation of a cow's milk production can consequently give biased estimations of her total yields. Unfortunately, dystocial cows are known to be more likely to suffer from diseases (Thompson et al., 1983; Benzaquen et al., 2007). Therefore, such cases are more likely to occur and bias to arise in dystocial animals.

Although, the saleable milk yields is related to the animal's milk production, it can account for additional losses due to diseases and their treatment, such as medication requiring a specific milk withdrawal period (e.g, mastitis episode requiring antibiotic treatment when milk produced cannot be sold commercially).

Taking a producer's perspective by assessing cumulative saleable milk yields, regardless of each animal achieving a full lactation, could address both concerns. In fact, such an approach, at the herd level, can take into account the losses due to animals that suffer from diseases or leave the herd early (Figure 2.1). Animals that suffer from diseases may have lowered or null saleable yields for a short period of time whereas cows leaving the herd early don't provide saleable milk any more. Additionally, such a method reflects the dairy producer's true income.

The objective of the study was to investigate how various degrees of calving difficulty would alter the production of saleable milk of UK dairy cattle over different stages of their subsequent lactation as well as their estimated milk production.



**Figure 2.1** Representation of the saleable milk production of an individual dairy cow over her lactation either as expected to be achieved over a full lactation or in the reality. As opposed to expectations, in reality, milk can not always be sold when some treatments for sickness that require a milk withdrawal period occur. As well, when the cow leaves the herd, production of saleable milk will stop. Combination of the two above scenarios can also happen. For each individual cow, at any point in her lactation stage (achieved or not), the cumulative saleable milk produced is more representative of the income she provides to her keeper as opposed to what could have been expected.

## 2.4. Materials and methods

### 2.4.1. Animals, housing and management

Data from lactating animals were obtained from the SAC experimental Holstein Friesian dairy herd (Scotland, United Kingdom). This herd was managed in accordance with the UK regulations on animal care and ethics of experimental animals. Following a long-term genetic breeding and feeding system project, animals were from two genetic groups (S: animals selected toward greater milk solids production; C: animals selected to be the rolling UK average) and split over two diet types (H: high forage diet; L: low forage diet) (Pryce et al., 1999; Bell and Roberts, 2007). Cows from both genetic groups who were not on this long-term trial were fed commercial diets of low forage types. The herd was managed from 1990 to 2001 inclusive at the University of Edinburgh near Edinburgh (Edinburgh herd) where it was shared with their veterinary school, and was then moved to the SAC dairy research centre at the Crichton Royal Farm in Dumfries (Crichton herd) where it was managed from 2002 onwards.

The Edinburgh herd was milked twice a day and the diets were formulated to contain approximately 1500 and 2500kg of concentrate per lactation for the high and low forage diets respectively, representing average practice usage in the UK. Non trial cows were fed a diet close to the low forage diet.

The Crichton herd was managed in two contrasting management systems, with one group kept indoors on a low forage diet and the other group fed with a home-grown high forage diet with summer grazing. Non trial cows were fed a non-trial diet close to the low forage diet. The Crichton herd was milked three times daily from 2003 onwards.

In both herds, calving difficulty was scored as follows: no assistance (N), Farm assistance without / with malpresentation of the calf (FN/FM), Veterinarian assistance without/with malpresentation of the calf (VN/VM) and caesarean section (VC).

### 2.4.2. Datasets description

Data on individual cows lactations were obtained from the farm's database. Data were extracted on the condition that a calving difficulty score was available, the cow had not

aborted and the lactation number was  $\leq 10$ . No restriction was made on a minimum lactation length for the reasons stated above. Prevalence and causes of dystocia may differ among parities, with higher difficulty rates and predominance of cases of fetopelvic incompatibility in primiparae (Mee, 2008). However, the aim of the study was to look at the effects of different degrees of difficulties on milk production at the herd level, no matter how difficulty arises, and therefore, animals from both parities were included.

Lactation yield data of animals having calved from 1990 to 2001 inclusive and from 2003 to 20<sup>th</sup> of August 2009 only were included so that for each herd, all animals would have the same management background, and the transition period between sites was excluded.

The dataset therefore contained data on 3843 lactations: 2430 in the Edinburgh herd completed by 898 animals and 1413 in the CRF herd completed by 555 animals (Table 2.1).

**Table 2.1** Number of lactations per calving difficulty score for both Edinburgh and Crichton herds. For each herd, number of distinct animals and the relative proportion of the scores in % are given in brackets

	N	FN	FM	VN	VM	VC	Total
Edinburgh	1986	265	84	44	33	18	2430
(n=898)	(81.7%)	(10.9%)	(3.5%)	(1.8%)	(1.4%)	(0.7%)	
Crichton	1188	170	36	6	8	5	1413
(n=555)	(84.1%)	(12.0%)	(2.5%)	(0.4%)	(0.6%)	(0.4%)	

For each lactation for each cow, characteristics such as the cow identity, her parity (primiparous vs multiparous), genetic group, diet fed during the lactation, calving season (Summer: April to September; Winter: October to March), calving year, age at calving (in months), sire of the cow and the calving difficulty scores also were extracted from the database. Birth weight of the newborn, sex of the calf, calving weight and calving condition score of the dam at parturition were not considered as candidates to be

included in the statistical model. The reason for this is that the interest was on the effect of calving difficulty in its own right. Those variables are known to be confounded with calving difficulty in the literature (Meijering, 1984; Mee, 2008a) and this was also checked in this study by using data plots and looking at the variation shared if forced into the models.

### **2.4.3. Milk production data: saleable milk yields and cow's milk production**

In both herds, individual daily milk production was recorded automatically at milking conditional on the milk being sent to the tank for sale and, therefore, were considered as being the saleable milk.

Cumulative saleable milk yield (SMY, L) was calculated at the different stages of lactations of interest: 30, 60, 90 and 300 days in milk (DIM). This was calculated regardless of each animal having achieved each of these stages in order to account for the amount of milk the producer was actually able to sell within each period. As shown in Figure 2.1, calculations of SMY were performed by summing the daily amount of milk sent to the tank during those periods, the amount being null when the milk was not saleable because of a medical treatment or from the death/culling of the animal onwards when applicable. Lactation SMY were obtained on the basis of the real lactation length achieved by the animal (rather than truncating at 305 DIM). SMY are being used to reflect a producer's perspective.

To reflect on the cow's prospective, the average daily amount of milk produced by the dam herself, referred to as cow's milk production (CMP, L), was used. For each of the lactation stages of interest, this was calculated as being the ratio between the cumulative saleable milk yield during that period and the number of days when milk was sent to the tank during the same period.

Data from 2239 and 1325 lactations were available for analysis of both the cumulative saleable milk yields and daily mean yields in the Edinburgh and Crichton herd respectively. Because of the data extraction process, some of the cows from the Edinburgh herd appeared in the dataset with artificially truncated lactation implied by the cut-off point of 2002. Therefore, their milk production data was accounted for only

until the lactation stage of interest preceding the truncation. As a consequence, this led to a slight decline in numbers of lactation available towards later lactation stages.

#### **2.4.4. Statistical analyses**

Data from both herds were analysed separately. It was decided to keep the two herds separate for the analysis instead of accounting for a herd effect. In fact, since most of the animals had been transferred from Edinburgh to Crichton, the two herds can not be seen as fully independent and therefore do not comply with the underlying statistical hypothesis of independence of the variables. Furthermore, management practice at calving, especially the threshold for intervention seemed to be different in the two herds, with a higher propensity of the farm staff to provide assistance at calving and to call for a vet in the Edinburgh herd, where the facilities were shared with the University of Edinburgh Veterinary School (Table 2 .1).

Linear mixed models were applied following a Restricted Maximum Likelihood (REML) procedure in Genstat 11<sup>th</sup> Edition (2008, VSN International Ltd) using forward-stepwise techniques (Hosmer and Lemeshow, 2000) as described in previous studies (Haskell et al., 2009; Rutherford et al., 2009a). The random model chosen included the cow identity nested within its sire. All variables were treated as fixed effects (factors) whereas age at calving (age) and calving year were treated as continuous variables. Age at calving did not show a linear relationship with the milk yields and could not be included as such in the model. However, it showed two distinct linear trends for each parity and thus, age at calving was centred on the relevant parity's mean for each lactation (age-C).

An individual model was built for each herd and stage of lactation. Each variable was tested independently as a univariate and became a candidate for the multivariable model if it had a P value less than 0.25. The variables tested as univariates were parity, genetic group, diet, season, year of calving, age at calving and calving difficulty. The candidate variables were then added into the multivariable model using stepwise selection techniques with the most significant variables with the highest Wald statistic being added first. Candidate variables were kept in the model with significance attributed at

$P < 0.05$  (when all other explanatory variables in the models had been fitted) and calving difficulty was always forced in the model and fitted at the end. Interactions were tested once the whole model was set up. This included calving difficulty interacting with respectively genetic group, diet, parity and year, as well as the interaction between year and month, genetic group and diet as well as parity and age. Finally, normality of the residuals was verified. Initially, new models were built for each stage of lactation and outcome considered. However, the contributing factors were very similar for the same outcome measure at each stage of lactation. Therefore, for purposes of clarity, only one model was retained and used to analyse an outcome measure throughout the stages of lactation. These models are presented below:

$$SMY = \mu + parity + genetic * diet + year + season + CD + \varepsilon ; \text{ (Edinburgh herd)}$$

$$CMP = \mu + parity + genetic + diet + year + season + CD + \varepsilon ; \text{ (Edinburgh herd)}$$

$$SMY = \mu + parity * age - C + year + diet + genetic + season + CD + \varepsilon ; \text{ (Crichton herd)}$$

$$CMP = \mu + parity * age - C + diet + genetic + year + season + CD + \varepsilon \text{ (Crichton herd)}$$

Where  $\mu$  = the overall population mean,  $CD$  = calving difficulty and  $\varepsilon$  = the random residual effect

Considering the low numbers of lactations available for the higher degrees of difficulty, the analysis was also run grouping the vet assisted scores together in a single category (Edinburgh herd:  $n=83$ ; Crichton herd:  $n=15$ ).

## 2.5. Results

### 2.5.1. Saleable milk yields

#### ***Edinburgh herd***

Parity, genetic group, diet and calving year affected the saleable yields at all stages of the lactation except at 30 DIM for calving season and at both 300 DIM and over the total lactation for genetic group interacting with diet. Cows experiencing FN and VN scores had decreased cumulative saleable milk production throughout their lactation compared to non assisted animals ( $P < 0.05$ ; Table 2.2). Losses occurred as early as 30 DIM (FN: -5.2%; VN: -8.8%) and persisted until the end of the lactation (FN: -8.1%; VN: -12.5%).

No losses were found for FM, VM and VC births. Grouping the vet assisted score together did not alter the significance of the analysis.

**Table 2.2** Estimated means of the cumulative saleable milk yields (L  $\pm$  s.e.m) at 30, 60, 90, 300 DIM and over the completed subsequent lactation of dairy cattle from the Edinburgh herd following different degrees of calving difficulty. N: no assistance, FN/FM: Farm assistance without/with malpresentation, VN/VM: Veterinarian assistance without/with malpresentation, VC: caesarean section. The number of lactations available for analysis for each calving difficulty score is given in brackets

	Calving difficulty						P value
	N (n=1855)	FN (n=227)	FM (n=74)	VN (n=38)	VM (n=30)	VC (n=15)	
30 DIM	646.7 <sup>a</sup> $\pm$ 5.7	612.9 <sup>b</sup> $\pm$ 11.4	635.0 <sup>ab</sup> $\pm$ 19.1	589.7 <sup>b</sup> $\pm$ 26.7	605.7 <sup>ab</sup> $\pm$ 29.6	571.7 <sup>ab</sup> $\pm$ 42.2	**
60 DIM	1502.0 <sup>a</sup> $\pm$ 12.5	1430.0 <sup>b</sup> $\pm$ 25.3	1479.0 <sup>ab</sup> $\pm$ 42.8	1384.0 <sup>ab</sup> $\pm$ 59.7	1425.0 <sup>ab</sup> $\pm$ 68.4	1377.0 <sup>ab</sup> $\pm$ 93.4	*
90 DIM	2320.0 <sup>a</sup> $\pm$ 19.7	2218.0 $\pm$ 40.0 <sup>b</sup>	2304.0 <sup>ab</sup> $\pm$ 67.2	2110.0 <sup>b</sup> $\pm$ 92.8	2222.0 <sup>ab</sup> $\pm$ 106.1	2165.0 <sup>ab</sup> $\pm$ 145.3	*
300 DIM	6375.0 <sup>a</sup> $\pm$ 70.5	5859.0 $\pm$ 137.8 <sup>bc</sup>	6377.0 <sup>ac</sup> $\pm$ 230.7	5520.0 <sup>b</sup> $\pm$ 305.3	6003.0 <sup>ab</sup> $\pm$ 347.9	5722.0 <sup>ab</sup> $\pm$ 492.0	***
lactation	6857.0 <sup>a</sup> $\pm$ 87.9	6305.0 <sup>bc</sup> $\pm$ 168.0	6931.0 <sup>ac</sup> $\pm$ 280.7	5999.0 <sup>b</sup> $\pm$ 369.4	6578.0 <sup>ab</sup> $\pm$ 419.4	6221.0 <sup>ab</sup> $\pm$ 594.3	**

<sup>a</sup> to <sup>c</sup>: Within a row, means with different superscripts differ (P<0.05).

\*: P<0.05; \*\*: P<0.01; \*\*\*: P<0.001

### ***Crichton herd***

Parity interacting with age-C, calving year, diet, genetic group and calving season were significant at all stages of lactation except for calving season over the lactation. Calving difficulty resulted in lowered saleable milk yields (P<0.05; Table 2.3) for the FM and VC births (FM: -12.4%; VC: -42.8%) compared to non assisted animals only during the first 30 DIM. No significant effect was found at other stages of lactations. Grouping the vet assisted scores together did not alter the significance of the analysis.

**Table 2.3** Estimated means of the cumulative saleable milk yields (L  $\pm$  s.e.m) at 30, 60, 90, 300 DIM and over the completed subsequent lactation of dairy cattle from the Crichton herd following different degrees of calving difficulty. N: no assistance, FN/FM: Farm assistance without/with malpresentation, VN/VM: Veterinarian assistance without/with malpresentation, VC: caesarean section. The number of lactations available for analysis for each calving difficulty score is given in brackets

	Calving difficulty						P value
	N (n=1111)	FN (n=163)	FM (n=36)	VN (n=3)	VM (n=8)	VC (n=4)	
30 DIM	658.9 <sup>a</sup> $\pm$ 10.5	642.0 <sup>a</sup> $\pm$ 18.6	577.3 <sup>bc</sup> $\pm$ 124.3	489.6 <sup>abc</sup> $\pm$ 124.3	607.5 <sup>abc</sup> $\pm$ 76.0	376.6 <sup>c</sup> $\pm$ 107.9	*
60 DIM	1510.0 $\pm$ 25.8	1506.0 $\pm$ 44.0	1406.0 $\pm$ 85.4	1,384.0 $\pm$ 59.7	1067.0 $\pm$ 289.0	1197.0 $\pm$ 251.3	n.s
90 DIM	2304.0 $\pm$ 42.2	2326.0 $\pm$ 71.7	2242.0 $\pm$ 138.7	1619.0 $\pm$ 469.0	2478.0 $\pm$ 286.4	2028.0 $\pm$ 408.8	n.s
300 DIM	6462.0 $\pm$ 147.8	6759.0 $\pm$ 243.3	6658.0 $\pm$ 464.1	4302.0 $\pm$ 1,563.5	5978.0 $\pm$ 955.0	6571.0 $\pm$ 1,361.2	n.s
lactation	7293.0 $\pm$ 189.2	7700.0 $\pm$ 302.8	7466.0 $\pm$ 569.6	4267.0 $\pm$ 1911.5	6443.0 $\pm$ 1165.2	6841.0 $\pm$ 1672.4	n.s

<sup>a to c</sup>: Within a row, means with different superscripts differ (P<0.05).

\*: P<0.05; n.s: non significant

## 2.5.2. Cow's milk production

### *Edinburgh herd*

Cow's milk production was affected by parity, diet, genetic group, calving year and calving season throughout all stages of lactation tested except that the effect of calving season disappeared from 300 DIM onwards. There was no significant effect of calving difficulty on the CMP (P>0.05; Table 2.4) whatever the stage of lactation was. There was a reduction in production of 1.7L/d at 30 DIM (P=0.025) and of 1.6L/d at 60 DIM (P=0.049) for the vet assisted scores grouped together (n=83) compared to the non-assisted animals.

**Table 2.4** Estimated means of the cow's milk production (L/d  $\pm$  s.e.m) at 30, 60, 90, 300 DIM and over the completed subsequent lactation of dairy cattle from the Edinburgh herd following different degrees of calving difficulty. N: no assistance, FN/FM: Farm assistance without/with malpresentation, VN/VM: Veterinarian assistance without/with malpresentation, VC: caesarean section. The number of lactations available for analysis for each calving difficulty score is given in brackets

		Calving difficulty						
		N	FN	FM	VN	VM	VC	P
		(n=1855)	(n=227)	(n=74)	(n=38)	(n=30)	(n=15)	value
30	DIM	26.81	26.52	26.70	24.51	25.79	25.51	†
		$\pm 0.16$	$\pm 0.34$	$\pm 0.57$	$\pm 0.79$	$\pm 0.88$	$\pm 1.26$	
60	DIM	28.50	28.32	28.28	26.07	27.70	27.31	†
		$\pm 0.18$	$\pm 0.36$	$\pm 0.60$	$\pm 0.84$	$\pm 0.96$	$\pm 1.31$	
90	DIM	28.59	28.44	28.37	26.26	28.02	27.53	n.s
		$\pm 0.19$	$\pm 0.36$	$\pm 0.60$	$\pm 0.83$	$\pm 0.95$	$\pm 1.30$	
300	DIM	24.14	24.17	24.11	23.36	23.68	23.49	n.s
		$\pm 0.17$	$\pm 0.30$	$\pm 0.49$	$\pm 0.65$	$\pm 0.73$	$\pm 1.04$	
	lactation	23.42	23.56	23.19	23.04	23.17	22.71	n.s
		$\pm 0.16$	$\pm 0.29$	$\pm 0.48$	$\pm 0.63$	$\pm 0.72$	$\pm 1.02$	

<sup>a to c</sup>: Within a row, means with different superscripts differ ( $P < 0.05$ ).

†:  $P < 0.10$ ; n.s: non significant

### ***Crichton herd***

Cow's milk production was affected by parity interacting with age-C, diet, genetic group, calving year and calving season throughout the stages of lactation tested except that the effect of parity interacting with age-C disappeared from 300 DIM onwards. Compared to non assisted animals, calving difficulty was associated with a decrease in the CMP for FM and VC scores of 7.8% and 25.3% respectively (Table 2.5) at 30 DIM. A decrease of 34.4%, 31.1% and 28.5% of the cow's yields for the VN scores was observed at 30 DIM, 60 DIM and 90 DIM respectively. No effect beyond 90 DIM was shown for any other score of calving difficulty. Grouping the vet assisted score together did not alter the significance of the analysis.

**Table 2.5** Estimated means of the cow's milk production (L/d  $\pm$  s.e.m) at 30, 60, 90, 300 DIM and over the completed subsequent lactation of dairy cattle from the Crichton herd following different degrees of calving difficulty. N: no assistance, FN/FM: Farm assistance without/with malpresentation, VN/VM: Veterinarian assistance without/with malpresentation, VC: caesarean section. The number of lactations available for analysis for each calving difficulty score is given in brackets

	Calving difficulty						P value
	N (n=1111)	FN (n=163)	FM (n=36)	VN (n=3)	VM (n=8)	VC (n=4)	
30 DIM	29.46 <sup>a</sup> $\pm$ 0.28	28.85 <sup>a</sup> $\pm$ 0.51	27.15 <sup>b</sup> $\pm$ 0.99	19.32 <sup>b</sup> $\pm$ 3.37	28.70 <sup>a</sup> $\pm$ 2.05	22.02 <sup>b</sup> $\pm$ 2.98	***
60 DIM	31.32 <sup>a</sup> $\pm$ 0.30	31.09 <sup>a</sup> $\pm$ 0.50	29.61 <sup>a</sup> $\pm$ 0.97	21.57 <sup>b</sup> $\pm$ 3.27	30.32 <sup>a</sup> $\pm$ 1.97	26.12 <sup>ab</sup> $\pm$ 2.93	*
90 DIM	31.55 <sup>a</sup> $\pm$ 0.30	31.39 <sup>a</sup> $\pm$ 0.49	30.05 <sup>a</sup> $\pm$ 0.94	22.57 <sup>b</sup> $\pm$ 3.16	30.52 <sup>a</sup> $\pm$ 1.89	27.25 <sup>ab</sup> $\pm$ 2.88	*
300 DIM	28.94 $\pm$ 0.31	29.08 $\pm$ 0.46	27.81 $\pm$ 0.82	24.10 $\pm$ 2.74	26.90 $\pm$ 1.63	25.91 $\pm$ 2.55	n.s
lactation	28.31 $\pm$ 0.31	28.36 $\pm$ 0.47	27.17 $\pm$ 0.85	23.39 $\pm$ 2.82	26.45 $\pm$ 1.69	25.48 $\pm$ 2.58	n.s

<sup>a to c</sup>: Within a row, means with different superscripts differ (P<0.05).

\*\*\*: P<0.001; \*: P<0.05; n.s: non significant

## 2.6. Discussion

### 2.6.1. Detrimental effects of calving difficulty on cow's milk production and saleable milk yields

Calving difficulty resulted in lowered saleable milk production in both herds but a reduction in the cow's milk production was not found in the Edinburgh herd. However, the increasing variability in the CMP over the scores may have masked such an effect. In fact, when variation was reduced by grouping the vet assisted scores altogether, this group of animals had then a lowered production up to 60 DIM compared to the non assisted animals. A reduction in the milk production of cows experiencing difficulty at parturition is in line with previous studies (Mee, 2008a) and it is therefore not surprising

that SMY are also lowered probably as a consequence of decreased CMP as well as higher odds of being culled or suffering from diseases in dystocial animals (Tenhagen et al., 2007; Dobson et al., 2008).

Impairment of performance following a difficult calving is mainly attributed to the experience of an increased length of labour (Doornbos et al., 1984) and its implications on the adrenocortical function (Hudson et al., 1976; Nakao and Grunert, 1990; Civelek et al., 2008). This could lead to reduced yields in the dams (Dobson et al., 2001). Moreover, such hormonal changes depress their immune system, which, along with higher risks of bacterial contamination during assistance (Dohmen et al., 2000), may increase the animal's susceptibility to disease. Additionally, underlying conditions that put dams at risk of dystocia such as inappropriate body condition score (BCS), poor nutrition over the dry period, and hormonal imbalances might be partly responsible for decreased yields by the animals and affect the development of the udder before the start of the lactation (Berry et al., 2007; Banos et al., 2007; Roche et al., 2009).

Decreased milk production from the dams could also result from lower feed intake postcalving. Although a recent study in dairy cattle did not find differences in the dry matter intake (DMI) between eutocial and dystocial during the first two days postcalving (Proudfoot et al., 2009), a study on beef cattle showed that cows having delivered by caesarian section spent less time eating than cows having delivered naturally during the first three days postpartum (Kolkman et al., 2010a). It is very likely that the increased occurrence of sickness during their subsequent lactation may lower their appetite more often. In the same extent, dystocial cows might experience greater postpartum pain (Barrier et al., 2010) as a result of the traction and associated trauma of the tissues and possible inflammations of the reproductive tract, which in turn, may lower their feed intake.

### **2.6.2. Effects of calving difficulty diverged between the herds**

In this study, although impairment in CMP and SMY was found in both herds, the scores and stages of lactation affected were not the same within the two herds. In the present study, the scale used to rate difficulty at calving was similar between both herds and it is

unlikely that the definitions used may have led to inconsistency of scoring within the years. The level of supervision at calving and the propensity to assist can vary between farms, reflecting sociological factors (Dargatz et al., 2004). It is believed that the threshold for intervention and for calling a vet was lower in the Edinburgh herd than in the Crichton herd, certainly because the former was co-managed by the vet school. As such, a VN calving in Edinburgh could have been scored as a FN calving in Crichton. Additionally, other factors such as how assistance is performed, the pre and post calving care, as well as the overall management of the herd in terms of housing and milking routine could be causing variation in the effects of a difficult calving. Divergences within the two herds were also highlighted in terms of what factors affected milk yields when the statistical models were constructed.

Considering the cow's milk production, no reduction was found in the Edinburgh herd whereas the Crichton herd suffered losses for the FM, VN and VC births. Because estimations for the VN and VC scores rely on a low number of lactations, further evidence from other studies would be needed to support this result. Nonetheless, when grouped together the vet assisted scores showed significantly lower CMP up until 90 DIM. FM dams did not carry over the detrimental effect on CMP for as long as the VN dams whereas VM dams did not suffer losses compared to the other vet assisted dams. Although malpresentation of the newborn can be thought of as increasing the difficulty of the delivery, it is possible that this occurrence is spotted earlier because of the calf getting stuck early in the birth canal or the non-appearance of the feet. Early diagnosis and assistance could then shorten the labour length, therefore diminishing the detrimental effects of prolonged labour on milk production.

In regards to the SMY, in the Edinburgh herd, FN and VN cows had lowered SMY throughout the lactation whereas losses occurred in the Crichton herd for the FM and VC cows only in the first 30 DIM. In both herds, lack of effects on SMY for some of the vet assisted scores might relate to the low number of data available for the analysis, while the dramatic loss found for VC scores in Crichton should be treated with caution for similar reasons. Additionally, in the Edinburgh herd, the absence of effect in FM scores on SMY is in line with the absence of reduction in CMP for this score and might

be explained similarly by shortened labour length. In contrast, FM animals in the Crichton herd showed losses in their SMY. This can be attributed to lower CMP on the one hand but on the other hand, additional manipulation of the calf might have resulted in increased odds of contracting infections (Dohmen et al., 2000). This may explain why, relative to non-assisted animals, losses are much steeper in saleable milk yields than in the dam's yields with a 12.4% decrease in the former and 7.4% in the latter.

Furthermore, in contrast to the Edinburgh herd where SMY losses were predominant throughout the lactation, this was not evident after 30 DIM in the Crichton herd. It is possible that the care and subsequent management of the dystocial dams in the Crichton herd was more favourable for the dams to recover from a difficult calving. Yet, this idea would not be supported by the hypothesis drawn earlier on FM dams. Furthermore, the three times a day milking in the Crichton herd compared to twice a day in Edinburgh may have contributed to the dilution of the milk losses over time. In fact, a 1L reduction of milk production corresponds to an inferior proportional loss when yields are higher. A larger dataset may have helped showing significance if an effect after 30 DIM exists.

As discussed in detail above, the present results support the idea that the way the herd is managed influences how difficulty at calving will affect the performance of the cow in terms of both subsequent cow's yields and cumulative saleable yields.

### **2.6.3. Saleable milk yields best reveal the economic shortfall to the producers and might indicate long-lasting biological stresses in the dystocial cows**

Despite the differences within herds, using the cow's milk production or the cumulative amount of milk saleable by the farmer to assess effects of calving difficulty actually resulted in different effects being shown. This shows that these two methods are distinct from each other. In fact, cow's milk production give an insight into the subsequent production by the animal itself whereas saleable yields of the whole herd will additionally account for the wasted milk and the absence of saleable milk for the animals that leave the herd. therefore being more representative of farmers' losses. It is therefore not surprising that cumulative saleable milk yields were able to show more deleterious

effects than the cow's milk production alone. For example, there was no effect of calving difficulty on CMP in the Edinburgh herd (Table 2.4) whereas SMY were decreased for FN and VN dams. As well, SMY losses could be shown at the lactation level when analysis of SY revealed no significant losses even in the early lactation. It is tempting to infer that the deeper effect reported using SMY compared to CMP could be health-related or due to the early culling / death of the dystocial animals. However, although culling rates and morbidity are higher for cows experiencing difficulty at calving (Thompson et al., 1983; Benzaquen et al., 2007; Tenhagen et al., 2007; Dobson et al., 2008), further investigation into the health and survival data of the two herds would help conclude more firmly in this particular study.

As a consequence, using cumulative saleable milk yields on the whole herd rather than cow's milk production could reveal greater shortfall to the producer because it can account for some of the disease and culling costs. It was not the aim of the study to provide an economic analysis of the herd which would need to be multifactorial. However, to illustrate the income loss experienced by the producer, extrapolation of the results obtained on SMY from the Edinburgh herd, would mean a shortfall of around \$3250 for the average UK dairy farm/year (considering an average farm with 112 lactating animals, a milk price of 24p/L, and 1£=1.6\$) in terms of income drawn from the milk sales.

As explained above, a loss of SMY calculated on the whole herd indicates either reduced production by the animals, and/or health issues and/or animals leaving the herd. To that extent, SMY shall also better indicate the biological stresses that the dystocial animal might encounter during their subsequent lactation than the cow's milk production alone. Therefore, considering the economic shortfall to dairy producers and the deleterious effects on the cow's point of view, using saleable milk production of the whole herd seem to be an attractive approach for improvements to be made. On the other hand, some practicalities should not be ignored and it would not only be very challenging but very costly to collect such data on a larger scale. Furthermore, on a statistic point of view, the study of CMP resulted in less intrinsic variation in the data than the SMY and therefore it is easier to pick up existing differences in the former than the latter.

Nonetheless, the analysis of the cumulative saleable yield of the whole herd best reflect producer's losses following a difficult calving and seem to better address the occurrence of long-lasting biological stresses which can be related to animal welfare.

## 2.7. Conclusion

To conclude, calving difficulty was found to impair the milk production of dairy cows and their production of saleable milk, therefore highlighting reduced income for dairy producers as well as detrimental effects to the cows. Looking at cumulative saleable milk yields of the whole herd, independently of each animal having achieved a full lactation, might give a more sensitive picture of what is happening to the cow and as such, might reflect more accurately the underlying biological stresses experienced by the animals. Finally, the herd management clearly influenced the magnitude, duration of the effects and from which degree of difficulty adverse effects were found. Alleviating difficulty at calving through good herd management practice prior, during and after the parturition, as well as genetic improvement of the animals should therefore improve the dairy herds and the producer's welfare.

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## **CHAPTER 3:**

**Fertility is impaired and dystocia more likely to occur again after a difficult calving in dairy cows.**

In this Chapter, I was responsible for data extraction, data analysis and writing of manuscript.

### 3.1. Introduction

On a dairy farm, calving marks the start of a new lactation and the productive period for the cow, while female calves will contribute to the future lactating herd. Parturition can then be seen as a key event in the dairy production system. However, calving is also a high-risk time for both mother and offspring and difficulty at delivery can occur and human intervention is then needed. The amount of assistance provided is a widely used measure of the degree of difficulty of the calving event. Internationally, reported prevalence in dairy cattle of severe or considerable difficulty in calving vary from just below 2% to over 22% but assistance at calving (including lower degrees of difficulties) is much more prevalent varying, from 10% to over half of all calvings (Mee, 2008a). In the United Kingdom, 16% of the calvings are assisted on average (Wall et al., 2010) and 7% of the primiparae's births are thought to result in severe difficulty (Mee, 2008a). In the United States, the average national rate of calving assistance is 20.6% in multiparous animals and 31% in primiparous animals (USDA, 2009) although the rates are also known to reach as much as 29.4% in multiparous and 51.2% in primiparous animals (Lombard et al., 2007).

By its nature, calving difficulty increases farm workload but it also has adverse effects on the subsequent survival, health and performance of both dams and offspring (Dematawewa and Berger, 1997; Tenhagen et al., 2007; Lombard et al., 2007). For a review, see Mee (2008a). Dystocia is also thought to result in severe pain in dams (Huxley and Whay, 2006; Laven et al., 2009; Kielland et al., 2009). Therefore, a difficult calving has both animal welfare and economic consequences.

In particular, there are concerns about the fertility of the cow after the experience of dystocia. For the dam to return to a fertile state after calving, a series of events is required which includes the involution of the uterus, regeneration of the endometrium, cleansing of any bacterial contamination and return to ovarian cyclicity (Noakes, 1997; Sheldon et al., 2008). However, the experience of difficulty at calving is very likely to compromise one or more of those events. Dystocial cows have been found to have delayed uterine involution, take longer to recover ovarian cyclicity and be more prone to having abnormal cycles (Opsomer et al., 2000; Dobson et al., 2001). In fact, the

lengthened labour (Hudson et al., 1976; Civelek et al., 2008), and the stress and pain experienced at calving interact unfavourably with the endocrine system, thus affecting the start of the new fertility period (Dobson and Smith, 2000; Dobson et al., 2001). It has been suggested that the lengthened labour is the key factor that is primarily responsible for the depressed reproductive performance rather than the giving of assistance per se (Doornbos et al., 1984).

Additionally, cows experiencing difficulty at calving will also experience an increased peripartum relaxation of immunity due to the lengthened labour and the anti-inflammatory effect of cortisol (Nakao and Grunert, 1990) but also are at higher risks of bacterial contamination during the process of assistance (Dohmen et al., 2000). This means those cows are at more risk of developing puerperal health disorders such as milk fever, metritis, endometritis and retained placenta (Peeler et al., 1994; Rajala and Gröhn, 1998; Bruun et al., 2002). The occurrence of uterine disorders will reduce the fertility of the cows. For a review of the underlying mechanisms between uterine disease and infertility, see Sheldon et al. (2008). All of this evidence suggests that improving ease of calving would improve the fertility of dairy cows.

With regards to the economic costs of difficulty at calving, it appears that impaired fertility would account for a third of the overall estimated costs of dystocia (Dematawewa and Berger, 1997; McGuirk et al., 2007; Dobson et al., 2008). In fact, difficulty at delivery results in an increase in days open and the number of services needed to conception, delays in the first service (Thompson et al., 1983; Djemali et al., 1987; Lopez de Maturana et al., 2007a) and decreased conception rate at 200 DIM (Tenhagen et al., 2007). The adverse effects on fertility seem to be enhanced in primiparae and when the degree of difficulty is higher (Dematawewa and Berger, 1997). Dematawewa and Berger (1997) reported an increase of up to 32.6 days open and 0.3 more number of services to conception for the most severe cases. Nonetheless, although there is a consensus that calving difficulty usually has a negative impact on the fertility of the cows, the data on the magnitude of the impairment and from which degree of calving difficulty this occurs is sometimes conflicting. Most of the studies also report at the population level through the use of national datasets or clusters of farms, where farm

management, management at calving and calving assistance thresholds are likely to vary between farms. Therefore, it is not clear if impairment in fertility can be detected at the individual farm level, where it would have a direct impact on the individual farmer.

Furthermore, cows having experienced dystocia are more likely to be culled in their subsequent lactation (Beaudeau et al., 1993; Tenhagen et al., 2007; Lopez de Maturana et al., 2007b). This can be the result of health and fertility problems, but also in anticipation of the re-occurrence of such problems. The fear that the animal might experience difficulty at her next calving may add weight to the farmer's decision to cull a dystocial cow. It would also mean that the dystocial cow might experience a knock-on effect on her production potential in the subsequent lactation but also possibly in the lactation after that. However, there is little evidence to indicate whether cows that have experienced difficulty at calving are more likely to experience difficulty again. Although there is a genetic component to calving difficulty, 90% of the variation seen in the expression of the phenotype is due to the environmental component (Eaglen and Bijma, 2009). Repetition of calving difficulty at the phenotypic level should therefore be investigated. This is an important consideration because, from an animal welfare perspective, the phenotype is what the animal experiences.

The objective of the study was to assess the effect of various degrees of difficulty on the subsequent calving performance and fertility of the dairy cow at the dairy farm level.

It was hypothesised that cows experiencing a difficult calving would be more likely to require assistance at the next calving. Also, dystocial cows would be less likely to conceive at first service, need more services to achieve pregnancy and therefore have a greater number of days open and longer calving intervals. This fertility impairment after a difficult calving would be visible at the farm level, as opposed to the cow population level, meaning that significant direct costs would be imputed to individual farms.

## **3.2. Materials and methods**

### **3.2.1. Animals, housing and management**

Data from lactating animals were obtained from the SAC experimental Holstein dairy herd (Scotland, United Kingdom). This herd is managed commercially and in

accordance with the UK regulations on animal care and ethics of experimental animals. Following a long-term genetic breeding and feeding system project, animals were from two genetic groups (S: animals selected toward greater milk solids production; C: animals selected to be the rolling UK average) and split over 2 diet types (H: high forage diet; L: low forage diet) (Pryce et al., 1999; Bell and Roberts, 2007). Cows from both genetic groups who were not on this long-term trial were fed commercial diets of low forage types. The herd was managed from 1990 to 2001 inclusive at the University of Edinburgh near Edinburgh (hereafter referred to as the EDI herd) where it was milked twice a day. The herd was then moved to the SAC dairy research centre at the Crichton Royal Farm in Dumfries (referred to as the CR herd) where it was managed from 2002 onwards and milked three times daily.

In both herds, assistance at calving was provided following the judgment of experienced farm staff and calving difficulty was scored as follows: no assistance (N), Farm assistance without / with malpresentation of the calf (FN/FM), Veterinarian assistance without/with malpresentation of the calf (VN/VM) and caesarean section (VC).

On both farms, the policy was to calve heifers at 24 months of age and then to aim for yearly calving intervals which is the recommended practice in the UK. Cows were inseminated at the second detected oestrus at an average ( $\pm$  s.d) of  $77.1 \pm 19$  days and  $76.2 \pm 29.5$  days after calving in the EDI and CR herds respectively and were allowed up to 7 services before being removed from the herd. Average number of services to conception ( $\pm$  s.d) was  $1.9 \pm 1.2$  (EDI herd) and  $2.5 \pm 1.7$  services (CR herd). Similar sires were used for insemination of heifers and cows. Sires were of each genetic group were randomly allocated at mating, meaning that there was no cases of assortive mating after a dystocia event.

### **3.2.2. Datasets description**

Data on individual cow lactations were obtained from the farm's database at the end of October 2009. Animals having calved from 1990 to 2001 inclusive (for the EDI herd) and from 2003 to 30 September 2009 (for the CR herd) were included so that for each herd, all animals would have the same management background, and the transition

period between sites was excluded. Data were extracted on the condition that a calving difficulty score was available, the lactation had not been triggered following an abortion, the lactation number was  $\leq 10$  and the cow was still in the herd after 21 days in milk.

The dataset contained data on 3745 lactations: 2345 in the EDI herd completed by 843 animals and 1400 in the CR herd completed by 547 animals. The average lactation numbers ( $\pm$  s.d) were  $2.8 \pm 1.7$  and  $2.6 \pm 1.5$  in EDI and CR herd respectively.

For each lactation of the animals, characteristics such as the cow identity, parity (primiparous vs multiparous), genetic group, diet fed during the lactation, calving season (Summer: April to September; Winter: October to March), calving year, calving month, age at calving (in months), sire of the cow and the calving difficulty scores were also extracted from the database.

Any variable known to be confounded with calving difficulty such as birth weight of the newborn, sex of the calf, calving weight and calving condition score of the dam at parturition (Meijering, 1984; Mee, 2008a) were not included in the statistical model. The reason for disregarding these variables was that the subject of this study was the effect of calving difficulty in its own right. Their confounding in this dataset was confirmed by using data plots and by confirming the sharing of variation when included in the model with calving difficulty.

### ***Subsequent calving performance records***

For each lactation, calving performance for the next calving was retrieved for cows who conceived and remained in the herd until the following calving. Subsequent calving performance of cows were classified as either abortion (AB, calving occurring at least two weeks before the estimated calving date) or calving difficulty as grouped into the following categories: Non-assisted (N), Assisted (A, all scores of difficulty, including vet-assisted). The Vet assisted (VA, all vet assisted scores only) were considered as a separated sub-category. 73.9% and 63.6% of the lactations in the Edinburgh herd and Crichton herd respectively had records for calving performance in the next lactation. Lower retrieval rate in the Crichton herd can be attributed to the fact that some animals had not been given an opportunity to calve yet.

The following underlying questions were addressed: Were animals that required any kind of assistance at calving more likely to be assisted or require veterinary assistance at the next calving, or were they more likely to abort spontaneously? Similarly, were the animals requiring veterinary assistance more likely to require any type of assistance at next calving?

For that purpose, data on calving performance were tabulated for the performance at first and second calving, and at second and third calving, for the following calving performance combinations (presented as calving performance, subsequent calving performance): (A,A); (A,VA), (A,AB) and (VA,A). (VA, VA) and (VA, AB) were not investigated because of the low number of data available.

### ***Fertility performance records***

The following performance records were extracted for each lactation: conception at first service (C1S: presence or absence of pregnancy after a unique insemination assessed either through pregnancy diagnosis by ultrasonography and/or by the absence of further services and the occurrence of a subsequent calving date within the expected range of gestation length); number of services to conception (NSERV: number of services needed for the cow to get in-calf); days open (DO: number of days between calving and the next successful service); and calving interval (CI: number of days between calving and the subsequent calving on the condition the subsequent calving was not an abortion).

Fertility performance records could be retrieved for 73.7% and 74.4% of the lactations in the EDI and CR herd respectively. This means that 1728 and 1041 lactation records were available for analysis in the EDI herd and the CR herd respectively for all the variables, except for calving interval where only 1721 and 876 records could be used. Distribution of the record within each category of calving difficulty can be seen in Table 3.1.

**Table 3.1** Number of lactations per calving difficulty score for both Edinburgh and Crichton herds. For each herd, number of distinct animals and the relative proportion of the scores of calving difficulty in % are given into brackets

	N	FN <sup>†</sup>	FM <sup>†</sup>	VN <sup>†,*</sup>	VM <sup>†,*</sup>	VC <sup>†,*</sup>	Total
Edinburgh (n=678)	1447 (83.7)	181 (10.5)	53 (3.1)	24 (1.4)	15 (0.9)	8 (0.4)	1728
Crichton (n=450)	880 (84.5)	126 (12.1)	24 (2.3)	3 (0.3)	5 (0.5)	3 (0.3)	1041

<sup>†</sup>: denotes the assisted animals (A), <sup>\*</sup>: denotes the vet assisted animals (VA)

### 3.2.3. Statistical analyses

Data from both herds were analysed separately instead of accounting for a herd effect. Since most of the animals had been transferred from Edinburgh to Crichton, the two herds can not be seen as fully independent and therefore do not comply with the underlying statistical hypothesis of independence of the variables. Furthermore, management practice at calving seemed to be different in the two herds, with a higher likelihood for the farm staff to provide assistance at calving and to call for a vet in the EDI herd (Table 3.1), probably because this herd was co-managed with the Royal (Dick) School of Veterinary Studies (Edinburgh, UK).

#### ***Subsequent calving performance analyses***

For each combination of calving performance at first and second, and at second and third calving, odds ratios with a 95% confidence interval were calculated. Statistical significance of each odds ratio was assessed using a Chi-square test.

An odds ratio is a relative measure of risk, measuring how much more likely is a cow that experienced A at calving n, to experience A at calving n+1, as compared to a cow who has not experienced A at calving n. It is calculated as the ratio between the odds of subsequent A when A previously occurred, and the odds of subsequent A when A did not occur previously. An odds ratio greater than 1 indicates that subsequent A is more

likely to occur in either cows having experienced A or cows that did not, and vice versa for odds ratios less than 1.

### ***Fertility performance analyses***

Linear mixed models were built using a forward-stepwise technique (Hosmer and Lemeshow, 2000) and analysed with Restricted Maximum Likelihood (REML) techniques in Genstat 11<sup>th</sup> Edition (2008, VSN International Ltd). The random model chosen was the cow identity. All variables were treated as fixed effects (factors) whereas age at calving ('age') was treated as a continuous variable centred on the relevant parity's mean. An individual model was built for each herd and variate. Each variable was tested independently as a univariate and became a candidate for the multivariable model if it had a P value inferior to 0.25. The candidate variables were then added into the multivariate model using stepwise selection techniques with the most significant variables with the highest Wald statistic being added first. Candidate variables were kept in the model with significance attributed at  $P < 0.05$  (when all other explanatory variables in the models had been fitted) and calving difficulty was always fitted at the end. Biologically relevant interactions were tested once the whole model was set up and normality of the residuals was verified.

Days open and calving interval were analysed using REML after having transformed the data with a logarithm function. Conception at first service and number of services to conception required the use of GLMM (Generalised Linear Mixed Models), following the same procedure, using a binomial distribution with a logit transformation in the former and a Poisson distribution with a logarithm transformation in the later. Days in milk at first service was considered as a continuous variable for the analysis of C1S. The following final models were used for each outcome variable and herd.

Edinburgh herd:

$$C1S = \mu + genetic + season + CD + \varepsilon ;$$

$$NSERV = \mu + genetic + season + diet + CD + \varepsilon ;$$

$$\log(DO) = \mu + genetic + parity * diet + season * diet + CD + \varepsilon ;$$

$$\log(CI) = \mu + genetic * parity + CD + \varepsilon$$

Crichton herd:

$$C1S = \mu + year + month + DIM + CD + \varepsilon ;$$

$$NSERV = \mu + year * season + CD + \varepsilon ;$$

$$\log(DO) = \mu + genetic + month + year + CD + \varepsilon ;$$

$$\log(CI) = \mu + year + month + genetic + CD + \varepsilon$$

Where  $\mu$  = the overall population mean,  $CD$  = calving difficulty and  $\varepsilon$  = the random residual effect

Because of the low number of lactations available in the vet assisted categories (VN, VM, VC), models were also run where they were grouped into a unique category (vet assisted (VA)).

### 3.3. Results

#### 3.3.1. Subsequent calving performance

##### ***EDI herd***

There was no evidence that assistance at first calving resulted in a higher incidence of assistance, veterinary assistance or abortion at the second calving. Neither did veterinary assistance at first calving result in higher odds of assistance at second calving. However, cows that were assisted in their second calving were more likely to be assisted at their third calving (Table 3.2; odds ratio=3.4;  $P \leq 0.01$ ) and, particularly more likely to require veterinary assistance at their third calving (Table 3.2; odds ratio=9.58;  $P \leq 0.05$ ). There was a tendency for animals needing veterinary assistance at their second calving to have a higher probability of requiring assistance at third calving (Table 3.2; odds ratio=7.04;  $P \leq 0.10$ ).

**Table 3.2** Odds ratio and 95% confidence interval (95% CI) following combinations of calving performance (A: assistance, VA, vet assistance, AB, abortion), subsequent calving performance in the Edinburgh herd at first and second, and second and third calving: (A,A), (A,VA), (A,AB) and (VA,A).

	First vs. Second calving		Second vs. Third calving	
	Odds ratio [95% CI]	P value	Odds ratio [95% CI]	P value
(A,A)	1.5 [0.9;2.8]	n.s	3.4 [1.5;7.6]	**
(A,VA)	2.7 [0.8;8.8]	n.s	9.6 [3.2;28.9]	***
(A,AB)	4.3 [0.4;48.2]	n.s	12.0 [0.7;196.8]	n.s
(VA,A)	0.6 [0.1; 2.7]	n.s	7.0 [1.0;51.0]	†

\*\* : P<0.01, \*\*\*: P<0.001, †: P<0.10, ns: P>0.05

### **CR herd**

In the CR herd, vet assistance in first calving resulted in higher odds of being assisted at second calving (Table 3.3; odds ratio=5.34; P≤0.05). Assistance at first calving tended to result in increased risk of assistance at second calving (Table 3.3; odds ratio=1.97; P≤0.10). Assistance at first calving did not result in higher abortion rates at second calving and it could not be estimated whether this resulted in higher incidence of veterinary assistance because of the low number of animals available. Assistance and veterinary assistance at second calving did not result in a greater risk of higher assistance rates in third calving and odds could not be estimated for other parameters due to low numbers of births available in each category.

**Table 3.3** Odds ratio and their 95% confidence interval (95% CI) following combinations of calving performance (A: assistance, VA, vet assistance, AB, abortion), subsequent calving performance in the Crichton herd at first and second, and second and third calving: (A,A), (A,VA), (A,AB) and (VA,A).

	First vs. Second calving		Second vs. Third calving	
	Odds ratio [95% CI]	P value	Odds ratio [95% CI]	P value
(A,A)	2.0 [0.9;4.1]	†	1.4 [0.3;6.5]	n.s
(A,VA)	n.e	n.e	n.e	n.e
(A,AB)	0.8 [0.2;4.1]	n.s	n.e	n.e
(VA,A)	5.3 [1.2;23.5]	*	n.e	n.e

\*: P<0.05, †: P<0.10, n.s: P>0.05, n.e: non-estimable

### **3.3.2. Subsequent fertility performance**

#### ***EDI herd***

No effect of calving difficulty was found on subsequent conception at first service in the EDI herd (Table 3.4;  $P>0.05$ ). The number of services to conception was increased for cows experiencing veterinary assistance with a malpresented calf (+ 1.1 service;  $P<0.05$ ) compared to cows not requiring assistance. Number of days open increased by 29 days following VM births (Table 3.4;  $P\leq 0.05$ ). Calving interval was not lengthened when calving difficulty occurred (Table 3.4;  $P>0.05$ ).

Conception at first service was lower in the highly selected animals compared to the control group (S: 33.6%; C: 40.7%;  $P<0.05$ ) and in winter (winter: 34.4%; summer: 39.8%;  $P<0.05$ ) compared to summer.

When grouping all the vet-assisted scores together, vet-assisted dams required 0.6 more services to conceive ( $P<0.05$ ), had 18 more days open ( $P<0.05$ ). There was no effect seen on conception rate at first service and on calving interval ( $P>0.05$ ).

#### ***CR herd***

No effect of calving difficulty was found on conception at first service, number of services needed to conception, number of days open and calving interval (Table 3.5;  $P>0.05$ ).

**Table 3.4** Adjusted means [lower; upper 95 % confidence interval] of the subsequent fertility performance of dairy cattle in the EDI herd following different degrees of calving difficulty. N: normal; FN/FM: Farm assisted without/with malpresented calf; VN/VM: vet assisted without/with malpresented calf; VC: caesarean section. CIS: conception at first service; NSERV: number of service to conception; DO: number of days open; CI: calving interval. Number of lactations available for analysis for each calving difficulty score is given in brackets.

	Calving difficulty								P value
	N (n=1447)	FN (n=181)	FM (n=53)	VN (n=24)	VM (n=15)	VC (n=8)			
CIS (%)	50.8 [47.9;53.7]	49.9 [42.6;57.3]	50.6 [37.3;63.8]	39.5 [22.2;60]	29 [11.4;56.3]	13 [1.8;55]			n.s
NSERV	1.8 <sup>a</sup> [1.8;1.9]	1.9 <sup>a</sup> [1.7;2.1]	2.2 <sup>a,b</sup> [1.8;2.6]	2.2 <sup>a,b</sup> [1.7;2.9]	2.9 <sup>b</sup> [2.2;3.9]	2.4 <sup>a,b</sup> [1.5;3.7]			*
DO (d)	97.2 <sup>a</sup> [94.6;99.9]	99.7 <sup>a</sup> [94.0;105.7]	106.3 <sup>a,b</sup> [95.4;118.5]	109.6 <sup>a,b</sup> [93.2;128.9]	126.7 <sup>b</sup> [103.4;155.1]	111.9 <sup>a,b</sup> [84.6;147.9]			*
CI (d)	383.8 [380.3;387.3]	384.7 [377.8;391.7]	385.6 [373.6; 97.9]	391.8 [372.9;411.7]	406.5 [383.4;431.0]	397.3 [366.3;430.8]			n.s

\*:P<0.05, \*\*: P<0.01, \*\*\*: P<0.001, ns: P>0.05 ; within a row, numbers without a common letter differ (P<0.05)

**Table 3.5** Adjusted means [lower; upper 95 % confidence interval] of the subsequent fertility performance of dairy cattle from the CR herd following different degrees of calving difficulty. N: normal; FN/FM: Farm assisted without/with malpresented calf; VN/VM: vet assisted without/with malpresented calf; VC: caesarean section. CIS: conception at first service; NSERV: number of service to conception; DO: number of days open; CI: calving interval. Number of lactations available for analysis for each calving difficulty score is given in brackets.

	Calving difficulty								P value
	N (n=880)	FN (n=126)	FM (n=24)	VN (n=3)	VM (n=5)	VC (n=3)			
CIS (%)	39.4 [34.8;44.2]	33.6 [25.5;42.8]	55.2 [34.4;74.4]	0.01 [0;100.0]	17.2 [2;67.6]	69.8 [14.6;96.9]		n.s	
NSERV	2.5 [2.3;2.6]	2.6 [2.3;3.0]	1.9 [1.4;2.6]	3.3 [1.7;6.5]	2.4 [1.3;4.2]	1.6 [0.6;4.0]		n.s	
DO (d)	107.6 [103.3;112.0]	122.1 [111.3;133.6]	106.8 [87.6;130.3]	121.2 [69.0;213.0]	129.6 [83.7;200.7]	91.8 [52.0;162]		n.s	
CI (d)	397.3 [391.9;403.7]	408.4 [397.5;419.6]	391.8 [367.9;417.3]	372.5 [304.1;456.3]	407.4 [357.5;464.3]	367.4 [299.9;450]		n.s	

ns: P>0.05

### 3.4. Discussion

The results of this study suggest that some cows requiring assistance have impaired fertility and are at higher risk of subsequent calving difficulty at next calving. Differences between the farms were seen. Dystocial cows are therefore at risk of long term impaired performance, beyond the subsequent lactation.

#### **3.4.1. Higher risk of calving difficulty after previous experience of assistance**

In the CR herd, any form of assistance at first calving tended to increase the risk of subsequent assistance at next calving. If veterinary assistance was required, primiparous cows were much more likely to be assisted at second calving. In the EDI herd, such associations were not found between first and second parities but assistance at 2<sup>nd</sup> calving made cows more likely to require assistance, and more particularly veterinary assistance, at 3<sup>rd</sup> calving. The idea that a cow with previous history of calving difficulty is at more risk of difficulty at next calving is in agreement with a recent study on Irish pasture-based Holstein-Friesians from Mee et al. (2011). This study found that experiencing dystocia (defined as considerable assistance and veterinary assistance) leads to 1.65 and 2.9 times greater odds respectively of experiencing any assistance and dystocia at next calving, which is lower than the odds found in the present study.

The existence of high genetic correlations ( $>0.70$ ) for calving ease observed between first and later parities (Thompson et al., 1981; Carnier et al., 2000; Eriksson et al., 2004), suggests that similar genes and mechanisms may be involved in both parities. Due to environmental influences, phenotypic expression at the farm level is not evident.

However, subsequent assistance might also be the result of preferential treatment as it exists in heifers (Dargatz et al., 2004; USDA, 2009). It can be hypothesised that an animal that has had problems in the past, especially if her calving incurred veterinary costs, might be under closer supervision at her subsequent calving and that the threshold for decision to intervene could be lower. Delivery by caesarean at first calving might also affect the characteristics of second labour because of physiological changes in the soft tissue of the birth canal after vaginal delivery (Paterson and Saunders, 1991). In

women, when the first child is delivered by caesarean section, second labour resembles labour from a primiparae (Paterson and Saunders, 1991). It is also possible that subsequent dystocia might be the result of carry-over effects. For example, incidence of disorders after a difficult calving might lead to predisposing factors of dystocia such as inappropriate BCS at next calving.

From this study, there was no evidence that calving difficulty can be linked to subsequent abortion, which is probably because spontaneous abortion and calving difficulty do not have the same aetiology.

Discrepancies between the two herds suggest that different management at calving might have had different repercussions. In the CR herd, low availability of data made it difficult to determine whether a cow would repeat her calving performance from her 2<sup>nd</sup> calving in her 3<sup>rd</sup> calving.

#### **3.4.2. Impaired fertility at the farm level, following a difficult calving**

The present study found that following a difficult calving, the subsequent fertility of a proportion of the cows was impaired. This was found at the farm level, in contrast to the majority of other studies which showed effects at the population level, and may give individual farmers more incentive to act on improving the ease of calving. Dams experiencing veterinary assistance in the EDI herd had higher number of days open and number of services to reach conception than cows calving naturally. No effect of calving difficulty on the conception rate at first service was found. Despite an increase in days open, the calving interval was not correspondingly longer. The latter is probably due to greater variances in the calving intervals, which may have reduced statistical power.

Absence of significant effects in the CR herd is believed to be due to lack of statistical power due to the smaller size of the dataset, as suggested by the large confidence intervals. However, plausible differences in the management at calving and the heat detection may have led to absence of effects on fertility of the cows. In fact, in the analysis of milk production records from a previous study on the same farm, it was found that the consequences of calving difficulty on the milk production of the dams were different for each herd although detrimental in both (Barrier and Haskell, 2011).

Impaired fertility following a difficult calving has been widely reported throughout the literature and the effect found in this study is in line with previous findings (Laster et al., 1973; Thompson et al., 1983; Djemali et al., 1987; McGuirk et al., 2007; Tenhagen et al., 2007; Lopez de Maturana et al., 2007a). In particular, the results on fertility are in the same range as reported in a similar work performed on the UK national dataset (Eaglen et al., 2010b). The underlying biological mechanisms that explain why fertility can be impaired following a difficult calving have been reviewed previously and involve delayed return to a fertile state as a consequence of longer labour length, trauma of the tissues and higher susceptibility to uterine diseases (Hudson et al., 1976; Dobson et al., 2001; Sheldon et al., 2008).

### **3.4.3. Could poor fertility and risks of assistance be worse than the results suggest?**

By their nature, the fertility performance indicators that are used in this study and others assume that all cows will be inseminated, get in-calf and calve. For example, to collect data on conception at first service, the cow will have to survive from parturition until a diagnosis of pregnancy is made or return to oestrus occurs. The analysis of number of days open and the count of services to conception rely on the cow achieving pregnancy, while analysis of calving interval requires that the cow gets in calf, maintains pregnancy and survives until the following calving. This means that an infertile cow or a cow that is culled before further insemination will be missing for the calculation of most of the widely used fertility indicators and her subsequent calving performance will remain unknown. This is very relevant for dystocial animals because they are more likely to be culled early in lactation as a consequence of puerperal diseases (Beaudeau et al., 1993; Tenhagen et al., 2007). Inevitably, by not taking into account culled and infertile cows, this means that a slight bias is introduced to the data collected. This probably buffers the effects of dystocia on both fertility and risk of subsequent assistance because generally, dystocial cows are more likely to be culled and probably more likely to be infertile (Thompson et al., 1983; Djemali et al., 1987; Lopez de Maturana et al., 2007a). Nonetheless, even dealing with censored data revealed that experiencing difficulty at

calving would have a detrimental effect on subsequent fertility performance and put animals at higher risks of being assisted again. It is then possible that the biological extent of the problem and the economic implications might be worse than pictured from the results of the present and previous studies.

#### **3.4.4. Implications of long-term impaired performance in dystocial cows**

The results suggest that cows with calving difficulty may not only experience impaired fertility performance but that they may also have a higher risk of calving difficulty at next calving. Calving difficulty also leads to a decreased performance at next lactation including lowered milk production, increased incidence of culling and disease in the postpartum period (Mee et al., 2008). Therefore, experiencing calving difficulty can have a long-term impact which can extend beyond the subsequent lactation to the following lactations. This implies that the production of a dystocial cow might not be optimal in the subsequent lactations. This results in economic shortfalls for the farmer and the dairy industry. Any deviation from optimal production has an environmental impact, because production-efficient and healthy cows have lower methane emissions per unit output, and thus, they have a lower environmental footprint (Bell et al., 2010a). As well, there are welfare concerns for the well-being of the dystocial cow because she would be at higher risk of experiencing greater pain levels during her subsequent parturition (Huxley and Whay, 2006; Laven et al., 2009; Kielland et al., 2009) and during episodes of uterine diseases in the peri-partum period (Peeler et al., 1994; Rajala and Gröhn, 1998; Bruun et al., 2002).

### 3.5. Conclusion

Cows assisted at calving can suffer from subsequent impaired fertility and can be at a higher risk of requiring assistance at next calving. This was shown despite naturally occurring favourable censoring of the data, suggesting that the extent of the effect described might be larger. Dystocial calving, therefore, can have a long-term impact, beyond the subsequent lactation, on the performance of the animals and on the economic losses experienced by the producer. Better calving and breeding management should therefore improve farm profitability, the well-being of the cows but could also contribute to the reduction of the environmental impact of dairy farming by reducing avoidable culls and sub-optimal performance.

## **CHAPTER 4:**

### **Calving difficulty in dairy cows and the subsequent health problems and lactation achieved**

In this Chapter, I was responsible for the methodology, data extraction, analysis and writing of the manuscript.

## 4.1. Introduction

In Chapter 2, it was found that there was a longer-lasting reduction in saleable milk yield in dystocial cows than in the actual overall amount of milk produced by these dystocial cows. It was hypothesised that this might be due to either early culling of dystocial cows, or a higher prevalence of disease, which could lead to milk wastage. It was also suggested earlier in Chapter 3 that dealing with censored data for fertility traits may partly explain why this study found no strong effect of a difficult calving on the fertility of the cows and that the extent of the fertility problem could be worse than described.

From work previously carried out on the same experimental database, it was found that cows were at more risk of developing uterine infection after a difficult calving (Bell and Roberts, 2007) and that a dystocial calving was a risk factor for culling (Bell et al., 2010b).

Both findings would support the hypothesis that culling is higher in dystocial cows than in cows calving without assistance. Diseases of the reproductive tract usually occur in the first months of the lactation (Sheldon et al., 2006) but there might also be longer-term effects of a difficult calving on other organs.

The objective of the following study was to investigate:

- the occurrence in the dystocial dairy cow of the two most prevalent health problems:
  - o mastitis, for which treatment with antibiotics would result in milk produced being discarded,
  - o lameness which has deleterious effect on the amount of milk produced.
- the survival of dystocial cows in their subsequent lactation.

### **4.1.1. Mastitis and lameness are major problems in the dairy industry**

Tackling production diseases such as mastitis and lameness in dairy herds has been a challenge for the dairy industry over the last few decades. Worldwide, mastitis and lameness are the two most prevalent and costly production diseases in dairy cattle (Kossaibati and Esslemont, 1997; Seegers et al., 2003; Huijps and Hogeveen, 2010). Globally, as many as 60% of a herd may become lame at least once a year (Vermunt,

2005; Vermunt, 2007) while in the UK up to 50% of the cows could become lame in a one-year period (Whay et al., 2003; Barker et al., 2010) and 26% of the herd could be affected with mastitis (Kossaibati et al., 1998). In 1997, in the UK, the average cost per affected cow was about £220 for mastitis and £323 for lameness (Kossaibati and Esslemont, 1997; Wilshire and Bell, 2009) but today's costs will have increased. On the basis of these figures, mastitis and lameness would cost over £127 million and £128 million respectively to the UK dairy industry each year (Bennett et al., 1999a; Bennett et al., 1999b; Wilshire and Bell, 2009).

Animals affected by mastitis and lameness have reduced feed intake and a long-lasting reduction in their milk production (Green et al., 2002; Bareille et al., 2003; Seegers et al., 2003; Archer et al., 2010). Lame cows and cows with cases of clinical mastitis in their early lactation also suffer from impaired fertility (Bicalho et al., 2007; Machado et al., 2010; Nava-Trujillo et al., 2010) and are at a higher risk of premature culling (Bicalho et al., 2007; Bell et al., 2010b). Up to half of the involuntary cullings are due to health disorders (Beaudeau et al., 1993) and 12 and 3% of the culls are due to udder problems and lameness respectively (Seegers et al., 1998). There is also evidence that episodes of mastitis and lameness are painful to the animals (Huxley and Whay, 2006). Moreover, lameness can lead to hyperalgesia states which means a greater sensitivity to pain that could last up to a month after lesion occurred (Whay et al., 1997; Whay et al., 1998; Whay et al., 2005). Therefore, occurrence of lameness or mastitis not only triggers huge economic losses but also represents a big problem for animal welfare.

#### **4.1.2. Dystocial cows could be at more risk of mastitis**

After calving, the introduction to the milking parlour and the milking routine represent an exposure to the major causative organisms of mastitis. Unfortunately, the peripartum period is also associated with a lower immunity, a phenomenon also called peripartum relaxation of the immunity. This means that freshly calved cows are at greater risk of contracting diseases. In dystocial cows, the risk could be even higher because lengthened labour at calving time (Doornbos et al., 1984), higher subsequent cortisol levels (Hudson et al., 1976; Civelek et al., 2008), elevated adrenocortical function

(Nakao and Grunert, 1990), and possibly additional stress and pain (Manteca et al., 2010; Barrier et al., 2010; Kolkman et al., 2010a) may result in a greater depression of immunity. Furthermore, entry to the herd increases exposure to disease-causing organisms and poor hygiene during calving assistance are additional challenges to the immune system. For example, dystocial cows are more likely to develop reproductive diseases in the postpartum period than cows who experience non-assisted deliveries (Thompson et al., 1983; Benzaquen et al., 2007; Bell and Roberts, 2007; Beagley et al., 2010; Bell et al., 2010b). Therefore, it can be hypothesised that cows experiencing a difficult calving can be at higher risk of developing mastitis during their subsequent lactation, especially in the early stages.

#### **4.1.3. Dystocial cows might be more predisposed to develop lameness**

There is a higher incidence of lameness within the first 4 months of lactation (Vermunt, 2005). For example, the greatest prevalence of white line disease are found at 9 weeks, claw horn lesions within the first 10 weeks and sole ulcers between 11 and 14 weeks after calving (Tarlton et al., 2002; Blowey, 2005; Hoedemaker et al., 2009). However, the event of calving and its associated challenges appear to be particularly important in the development of lameness (Vermunt, 2005). Indeed, within the hoof, metabolic events associated with calving and the onset of lactation cause a non-inflammatory change which disrupts the structure and weakens the connective tissue of the corium that supports the third phalanx (also called pedal bone) (Tarlton et al., 2002). This alteration in the structural integrity of the claw therefore predisposes the animal to claw horn disruptions which may initiate pathological sequences (e.g, collapse of the bone, bruising) and the development of claw horn diseases such as sole ulcers. This change associated with parturition is of short duration but impairs the resilience of the feet to external stresses encountered during the early lactation period, such as longer standing times on hard floor which lead to higher hoof wear and physiological imbalance (Webster, 2002; Vermunt, 2007; Knott et al., 2007).

Moreover, after calving, the transition to a lactational state induces changes in the cow's energy balance. The change from a low to a high energy diet in the postpartum period

are risk factors for the occurrence of laminitis, which is a type of lameness (Blowey, 1993; Blowey, 2005). Furthermore, the thickness of the digital cushion decreases in line with the diminution in overall body condition score until month 4 in the lactation. As the digital cushion becomes thinner, it loses some of its capacity to dampen the pressure exerted by the third phalanx on the soft tissue beneath, therefore leading to sole ulcers and white line abscesses (Bicalho et al., 2009). Because of this, cows with low BCS who are already at higher risk of dystocia (Berry et al., 2007; Mee, 2008a; Roche et al., 2009) might also be particularly at risk of lameness (Gearhart et al., 1990; Roche et al., 2009; Hoedemaker et al., 2009).

Additionally as mentioned above, disease is more prevalent in the post-partum period and its occurrence can increase the fragility of the corium, which in turn slows down the horn production (Blowey, 2005). Increased standing times during the lactating period (as a result of milking times, longer feeding times to meet the nutritional demand) increases hoof wear, which almost certainly contributes to the increasing risk of lameness (Blowey, 2005). Furthermore, the behaviour of a cow at the time of calving can also contribute to her subsequent risk of lameness. For example, increased standing times and faster feeding could predict the incidence of claw horn lesions and sole ulcers in the subsequent lactation (Chapinal et al., 2009; Proudfoot et al., 2010). Considering that dystocial cows expressed more of these “at risk” behaviours (higher number of standing bouts and reduced feeding time) during the transition period compared to eutocial cows (Proudfoot et al., 2009), it could be possible that dystocial cows are at even higher risk of developing episodes of lameness.

All of this information suggests that dystocial cows might be more predisposed to develop lameness as the result of the experience of additional biological stresses (disease, pain, inappropriate BCS as examples) and their behaviour around calving.

#### **4.1.4. Dystocial cows might either be culled early or have stretched lactations**

In Chapter 2, it was hypothesised that lower cumulative saleable milk yields in the dystocial cows over the lactating period could partly be due to cows not achieving a full lactation due to disease or culling. This is supported by the evidence from the literature that dystocial cows are at greater risk of culling in the early lactation (Tenhagen et al., 2007). This suggests that their length of lactation could be shorter.

Additionally, as seen in Chapter 3, dystocial cows may also suffer from impaired fertility. Although impaired fertility or infertility can certainly increase risks for culling (Seegers et al., 1998), it also means that cows may take longer to achieve pregnancy and consequently have delayed subsequent calving. Because dry off is usually determined by the expected calving date, this means that dystocial cows, when remaining in the herd may also have stretched lactation lengths.

The first objective of the study was to examine if, during set times of their subsequent lactation, cows experiencing a difficult calving would be more prone to experience episodes of mastitis and lameness occurring from either skin diseases or claw horn disease. The second objective of this study was to determine if, following a difficult calving, dairy cows would survive as well in their subsequent lactations as cows who did not need assistance during parturition and if the lactation lengths achieved by cows who complete a lactation would then differ.

## **4.2. Materials and methods**

### **4.2.1. Animals, housing and management**

Data from lactating animals were obtained from the SAC experimental Holstein Friesian dairy herd (Scotland, United Kingdom). This herd was managed in accordance with the UK regulations on animal care and ethics of experimental animals. Following a long-term genetic breeding and feeding system project, animals were from two genetic groups (S: animals selected toward greater milk solids production; C: animals selected to be the

rolling UK average for milk solids) and split over two diet types (H: high forage diet; L: low forage diet) (Pryce et al., 1999; Bell and Roberts, 2007). Cows from both genetic groups who were not on this long-term trial were fed commercial diets of low forage types. The herd was managed from 1990 to 2001 inclusive at the University of Edinburgh's farm near Edinburgh (Edinburgh herd) and was then moved to the SAC dairy research centre at the Crichton Royal Farm in Dumfries (Crichton herd) where it was managed from 2002 onwards.

The Edinburgh herd was milked twice a day and the diets were formulated to contain approximately 1500 and 2500kg of concentrate per lactation for the high and low forage diets respectively, representing average practice usage in the UK. Non trial cows were fed a diet close to the low forage diet.

The Crichton herd was managed in two contrasting management systems, with one group kept indoors on a low forage diet and the other group fed with a home-grown high forage diet with summer grazing. Non-trial cows were fed a non-trial diet close to the low forage diet. The Crichton herd was milked three times daily from 2003 onwards.

In both herds, calving difficulty was scored as follows: no assistance (N), Farm assistance without / with malpresentation of the calf (FN/FM), Veterinarian assistance without/with malpresentation of the calf (VN/VM) and caesarean section (VC). For the purpose of the analysis, vet assisted scores (VN, VM and VC) were grouped together to form a unique vet assisted category (V).

#### **4.2.2. Datasets description**

Data on individual cow's lactations were obtained from the farm's database. Data were extracted for these lactations on the condition that a calving difficulty score was available, the cow had not aborted and the lactation number was  $\leq 10$ . No restriction was made on a minimum or maximum lactation length.

Data of animals having calved from 1990 to 2001 inclusive and from 2003 to 20<sup>th</sup> of August 2009 only were included for the Edinburgh and Crichton herds respectively, so that for each herd, all animals would have the same management background, and the transition period between sites was excluded.

The dataset therefore contained data on 3843 lactations: 2430 in the Edinburgh herd completed by 898 animals and 1413 in the Crichton herd completed by 555 animals.

For each lactation, the characteristics of the cow, her lactation number (Lact N), her parity (primiparous vs multiparous), genetic group (GG), diet fed during the lactation (diet), calving season (Summer: April to September; Winter: October to March), calving year (year), age at calving (in months) (age), calving month (month), sire of the cow, the achieved lactation length, the cumulative milk production during the lactation achieved (CMP) and the calving difficulty scores (CD) were extracted from the database.

### ***Mastitis and lameness data***

Cows were checked for signs of mastitis at milking by the milker (redness, swelling of the gland, change in the textural aspect of foremilk) and treatment was administered accordingly. Lameness was detected as part of a weekly locomotion scoring routine (1: perfect even tracking, no adduction/abduction; 2: adduction/abduction but even tracking or even non-tracking; 3: uneven/short strides; 4: lame; 5: difficulty turning). All cows with high locomotion scores (scores 4 & 5) were examined and treated fortnightly by a veterinarian, with any other treatment being carried out by the farm staff in between when judged necessary. Preventive foot trimming for the whole herd was performed twice a year by an experienced trimmer. As part of the routine data collection on the experimental farm, any health treatment was recorded in the database and therefore only clinical cases could be considered. This included the identity of the animal being treated, the nature of the treatment and the date the treatment was carried out. Records also included the quarter of the udder affected in the event of mastitis, the nature of lameness treated and the identification of the leg affected by lameness.

For each individual's lactation, health records on mastitis and lameness were extracted. An episode of mastitis or lameness was defined as starting on the day of the first treatment. The number of days into the lactation at which the treatment started was calculated. For mastitis, if a treatment occurred again after eight days (whatever the udder quarter affected was), this was considered as a new episode (IDF, 1997). From the lameness records, treatments were categorised as lameness relating to skin disease (SD lameness) (e.g. "foul of foot", digital or interdigital dermatitis), lameness relating to

claw horn disease (CHD lameness) (e.g, laminitis, sole ulcer or white line disease) and lameness on its own, regardless of its nature. The latter would be considered as one case of lameness even if CHD and SD lameness occurred at the same time. The repetition of a treatment for lameness on the same leg for the same nature of lameness within a month was considered as being a continuing episode.

The occurrence of at least one episode (binary data) and the total number of episodes (count data) of mastitis, lameness, SD lameness and CHD lameness during the whole of the lactation were used as measures of the diseases.

Additionally, mastitis occurring at up to 30 DIM was considered as a separate category as it was felt that because of the relaxation of immunity following calving, cows might be more prone to infectious diseases (such as mastitis) in their early lactation, and therefore any effect would be more easily detected. Similarly, cases of lameness occurring at up to 120 DIM were considered as a separate category because CHD lameness tends to develop later in the lactation (Chapinal et al., 2009; Proudfoot et al., 2010).

***Lactation length achieved***

For each lactation, the lactation length achieved was calculated as being the number of days between calving and either the drying off date or the culling date. For the Edinburgh and Crichton herds, lactation lengths were retrieved for 99.5% and 82.4% respectively of the lactations present in the dataset. The distribution of the data over the scores can be found in Table 4.1.

**Table 4.1** Number of lactations with lactation length records (% of the total in brackets) for each herd and for each calving difficulty score

	N	FN	FM	VN	VM	VC	Total
Edinburgh herd	1979 (81.8)	263 (10.9)	82 (3.4)	44 (1.8)	33 (1.4)	18 (0.7)	2419
Crichton herd	948 (81.4)	163 (14.0)	35 (3.0)	6 (0.5)	8 (0.7)	5 (0.4)	1165

Any lactation length above 500 days was double checked, but no indication of errors could be found for these lactations and therefore, they were all kept in the dataset. The rationale for the occurrence of such high values may be due to infertility, which delays the subsequent calving date and therefore the drying off date. This might be exacerbated in this dataset because cows were allowed up to 7 services as part of the protocol for this experimental farm, which means a potential addition of 252 days in milk.

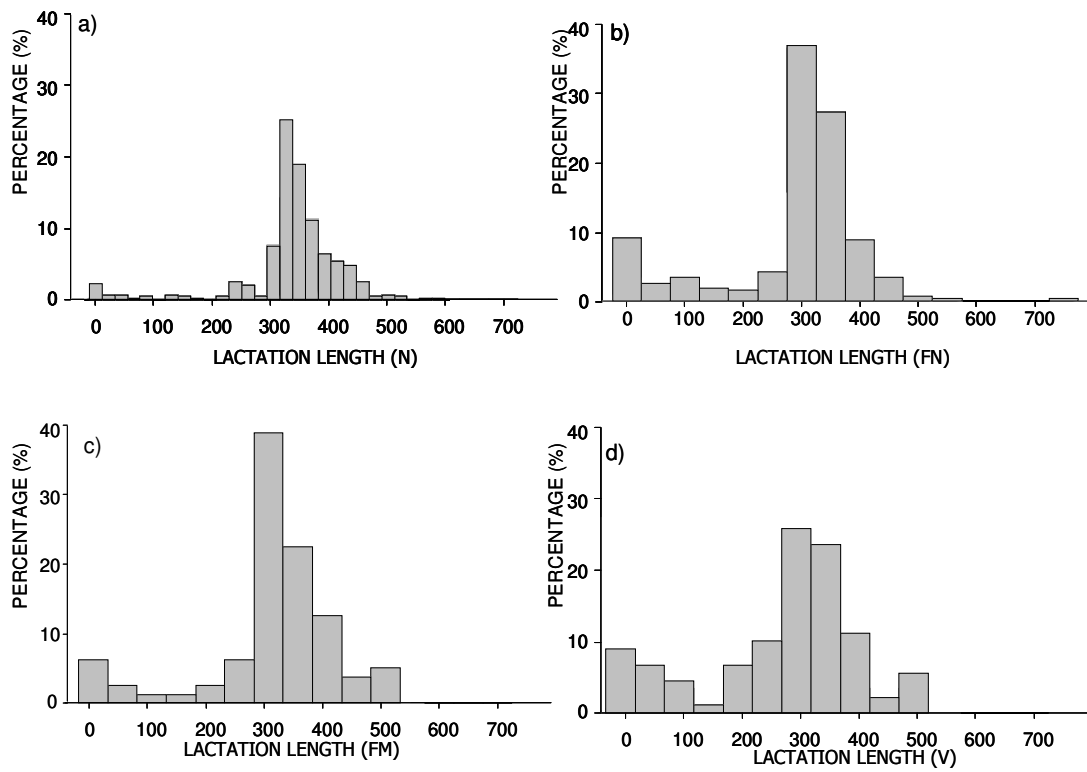
### ***Survival in the lactation until 90 DIM***

From inspection of the frequency distribution of lactation length achieved (Figures 4.1 and 4.2), it seemed that the proportion of animals leaving the herd in the early lactation could be higher following an assisted calving (as indicated by the greater percentage of lactations terminating at less than 100 days for FN, FM and V scores).

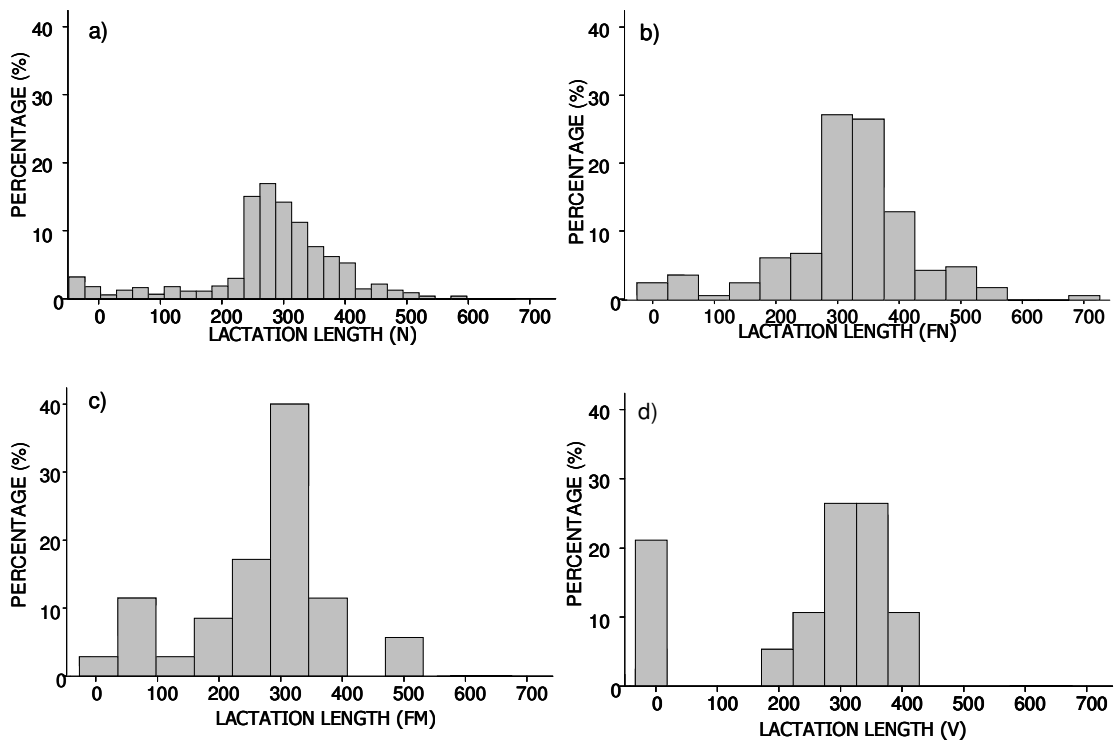
Therefore, it was decided to look at the completion of the first 90 DIM (first trimester of the lactation), regardless of what happens afterwards. For that purpose, three new response variables were created, referred to as survival at 30, 60 and 90 DIM respectively. Those variables took the value 1 when, for a given lactation, the animal was in the herd at a given stage and 0 otherwise (binary data).

### ***Stretched lactations***

To investigate the hypothesis that dystocial cows could also have stretched lactation length, the lactation length achieved for each score of difficulty was investigated for cows with a full lactation (conditional on having achieved at least 305 DIM).



**Figure 4.1** Histograms of the frequency distribution of the achieved lactation length in DIM following a N calving (a), a FN calving (b), a FM calving (c) and a V calving (d) in the Edinburgh herd.



**Figures 4.2** Histograms of the frequency distribution of the achieved lactation length in DIM following a N calving (a), a FN calving (b), a FM calving (c) and a V calving (d) in the Crichton herd.

### **4.2.3. Statistical analyses methodology**

It was decided to keep the two herds separate for the analysis instead of accounting for a herd effect. In fact, since most of the animals had been transferred from Edinburgh to Crichton, the two herds can't be seen as fully independent and therefore do not comply with the underlying statistical hypothesis of independence of the variables. Furthermore, management practice at calving seemed to be different in the two herds, with a higher propensity for the farm staff to provide assistance at calving and to call for a vet in the Edinburgh herd, probably as a result of the co-management of the farm with the Royal (Dick) School of Veterinary Studies (University of Edinburgh, Edinburgh, UK).

Generalised linear mixed models were applied following a Restricted Maximum Likelihood (REML) procedure in Genstat 11<sup>th</sup> Edition (2008, VSN International Ltd) using forward-stepwise logistic regression models (Hosmer and Lemeshow, 2000) as described in previous studies (Haskell et al., 2009; Rutherford et al., 2009a). An individual model was built for the analysis of each of the outcome variables studied. Each explanatory variable was tested independently as a univariate and became a candidate for the multivariable model if it had a P value less than 0.25 according to the methods referred to previously. The candidate variables were then added into the multivariable model using stepwise selection techniques with the most significant variables with the highest Wald statistic being added first. Candidate variables were kept in the model with significance attributed at  $P < 0.05$  (when all other explanatory variables in the models had been fitted) and calving difficulty was always fitted at the end.

#### ***Health records analysis***

The variable 'occurrence of at least one episode of either mastitis or lameness' (incidence) was analysed using generalised linear mixed models using a binomial distribution with a logit link function. Lactation number nested within the cow identity was used as the random model.

For the analysis of the number of episodes, data were restricted to the lactations during which at least one episode of the health issue considered was reported. This was done because of the relatively large number of lactations where no treatments were given for

mastitis or lameness. Number of episodes was analysed using generalised linear mixed models using a Poisson distribution with a logarithmic function as a link function. Lactation number nested within cow identity was used as the random model.

Because of the limited size of the Crichton dataset, only descriptive statistics are presented for this dataset whereas formal statistical tests were used for the Edinburgh herd. Therefore, any data from the Crichton herd presented in the subsequent dataset derive from raw data.

For the Edinburgh dataset, all variables were treated as fixed effects. For that purpose age at calving (AgeQ) and the cumulative milk production (CMP) over the lactation period were divided into quartiles. In order to adjust for the different lengths of the lactations, the adjusted number of episodes for lactation length (by dividing the number of cases by the duration of the lactation) was first considered. However, this led to biased results. This was because animals that were culled very early in their lactations had very high values which led to outliers, pushing the effect of calving difficulty into statistical significance. Therefore, the number of episodes was kept as a variable but lactation length was divided into quartiles (LL) and introduced as a factor when building the models. Variables included in each of the models are presented in the Tables 4.2 and 4.3.

**Table 4.2** Variables included in the final models for the analysis of the incidence of mastitis, and different types of lameness in the Edinburgh herd over the lactation or at specific stages of lactation

Incidence	AgeQ	Year	GG	Diet	LL	CMP	Season	Month	CD
Mastitis	x	x	GG*Diet						x
Mastitis 30 DIM	x	x	x			x	x		x
Lameness	x	x	x		x			x	x
Lameness 120 DIM	x	x							x
SD lameness	x	x	x	x	x				x
SD lameness 120 DIM	x	x		x					x
CHD lameness	x	x			x				x
CHD lameness 120 DIM	x	x		x					x

**Table 4.3** Variables included in the final models for the analysis of the number of episodes of mastitis and different types of lameness in the Edinburgh herd over the lactation or at specific stages of lactation

Episodes	AgeQ	Year	LL	CD
Mastitis				x
Mastitis 30 DIM				x
Lameness	x	x	x	x
Lameness 120 DIM				x
SD lameness				x
SD lameness 120 DIM				x
CHD lameness	x	x	x	x
CHD lameness 120 DIM				x

### ***Analysis of survival in the lactation***

*Lactation length achieved and stretched lactations.* Lactation length achieved and stretched lactations followed a right-skewed distribution. Analysis was attempted using a REML analysis as described previously and by also including age at calving (divided into quartiles for each herd) into the analysis. However residuals did not fulfill the assumption of normality. Therefore, a Kruskal-Wallis one-way analysis of variance was used to analyse those data.

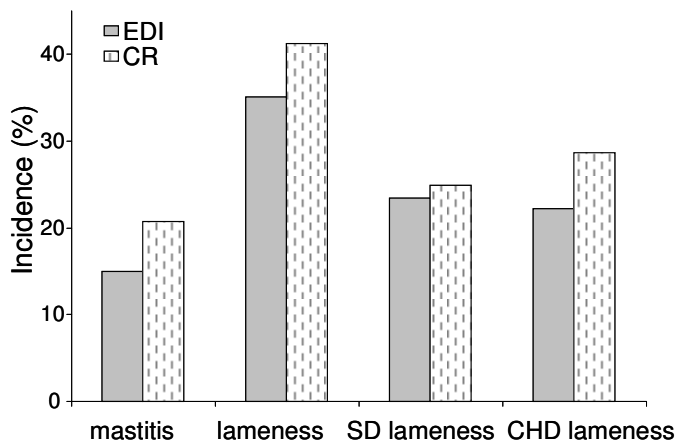
*Survival in the milking herd until 90 DIM.* For each herd, survival in the milking herd (0: absent; 1: present) at 30, 60 and 90 DIM was analysed for both herds using generalised linear mixed models with a binomial distribution and a logit transformation as a link function. Statistical models were built using the same stepwise method as described previously. For the Edinburgh herd, the same model was used across the different time-points. It included diet, parity, calving year, calving month, genetic group and the interaction between parity and calving difficulty. For the Crichton herd, the model included diet, age (in months, divided into quartiles; 1: [20;26]; 2: [27;40]; 3: [41;59]; 4:[60; 115]), calving year, calving month, genetic group and calving difficulty at 60 and 90 DIM. There was no interaction of parity and calving difficulty on the survival of the cows. For the analysis at 30 DIM, a similar model was used but age and calving year were not significant and not included in the final model.

## 4.3. Results

### 4.3.1. Health records

Only the results for calving difficulty are presented unless there was an interesting effect of the other variables that has not been reported previously.

The incidence of mastitis and the different kinds of lameness over the lactation in the two herds is presented in Figure 4.3. It can be seen that the incidence of the diseases follow the same pattern in the two herds.

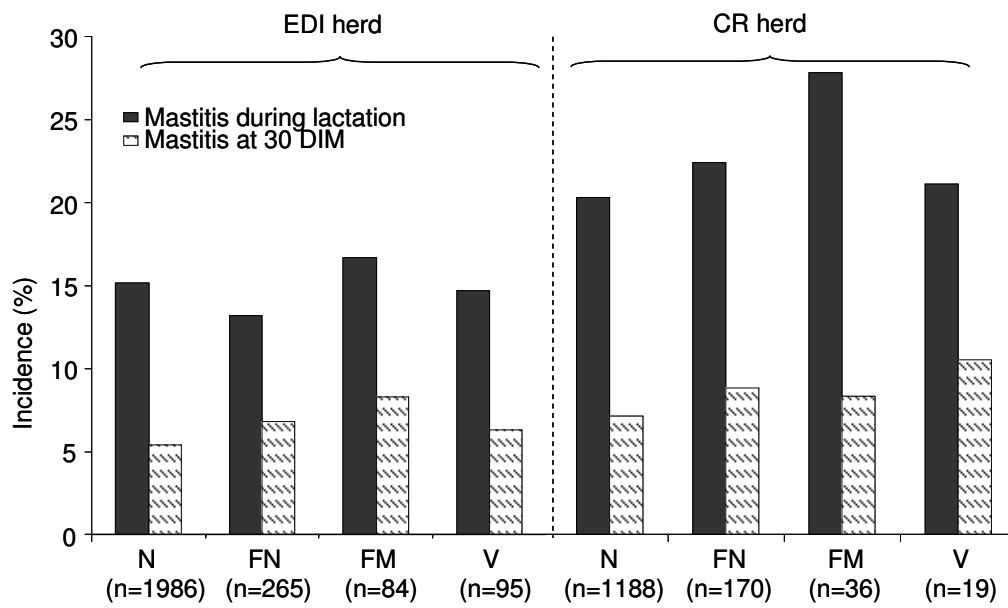


**Figure 4.3** Incidence of mastitis and lameness over the whole lactating period for the Edinburgh herd (EDI, n=2430) and for the Crichton herd (CR, n=1413).

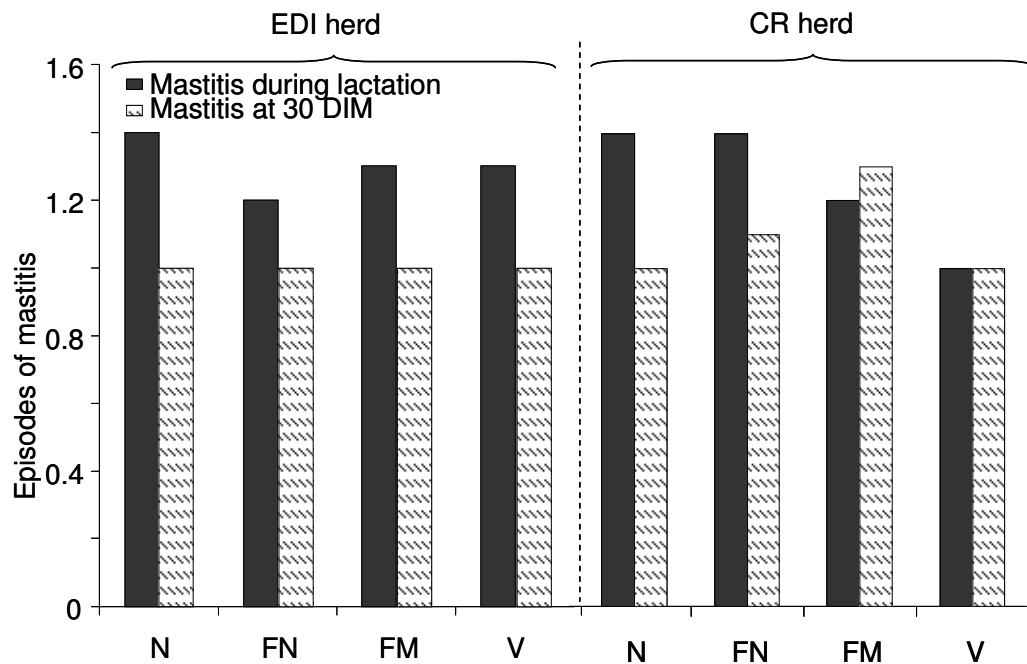
#### ***Mastitis at up to 30 DIM and over the lactation***

***Incidence.*** There was no evidence from the Edinburgh herd that cows having experienced a difficult calving had a higher incidence of mastitis during their first 30 DIM or over their subsequent lactation (Figure 4.4,  $P>0.05$ ). Raw data from the Crichton herd are also presented.

***Number of episodes.*** There was also no evidence that dystocial cows who developed mastitis had more episodes of mastitis at 30 DIM and over their subsequent lactation than cows having calved naturally (Figure 4.5,  $P>0.05$ ).



**Figure 4.4** Incidence (%) of lactations in the Edinburgh (EDI) and Crichton (CR) herd with at least one episode of mastitis at 30 DIM or over their lactating period. Raw data are presented.



**Figure 4.5** Number of episodes of mastitis in the Edinburgh (EDI) and Crichton herd (CR) at 30 DIM and over their subsequent lactation. Raw data are presented.

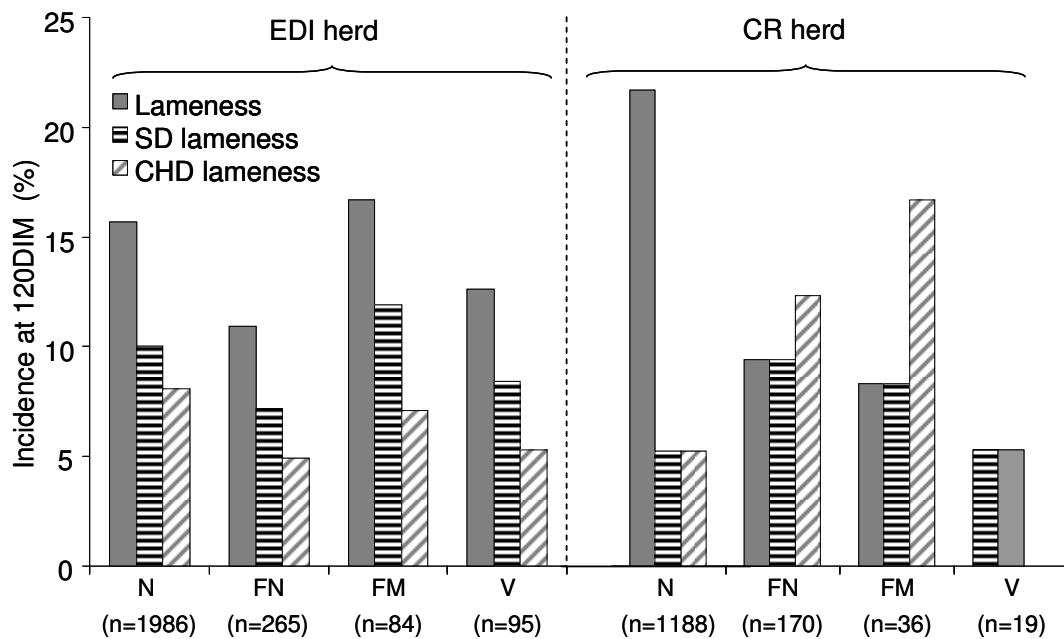
### ***Lameness at 120 DIM and over the lactation***

***Incidence.*** There was no evidence that lameness of any kind was more prevalent among dystocial cows by 120 DIM into their lactations (Figure 4.6,  $P>0.05$ ). There was also no evidence that there was a higher incidence of lameness, SD lameness or CHD lameness in the dystocial cows over the lactating period (Table 4.4,  $P>0.05$ ).

***Number of episodes.*** There was no evidence that dystocial cows who developed lameness, SD lameness or CHD lameness had more episodes of lameness by 120 DIM (Figure 4.7,  $P>0.05$ ) and over their subsequent lactation (Table 4.5,  $P>0.05$ ) than cows having calved naturally.

**Table 4.4** Incidence of lameness, SD lameness and CHD lameness (%) over the lactating period for various degrees of difficulty. Back-transformed estimated means and their 95% confidence intervals are presented for the Edinburgh herd. Calculations from the Crichton herd were done from the raw data. n.s:  $P>0.05$

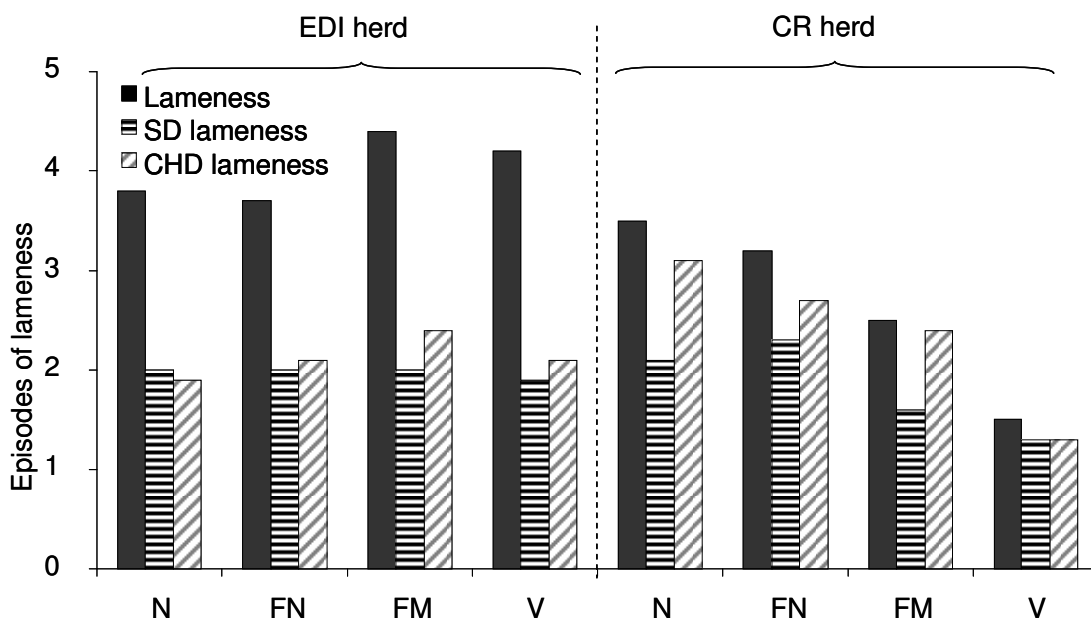
<i>Edinburgh herd</i>	Calving difficulty				P value
	N	FN	FM	V	
Lameness	10.3 [0;99.8]	10.5 [0;99.8]	11.9 [0;99.8]	9.2 [0;99.7]	n.s
SD lameness	14.6 [12.7; 16.6]	13.2 [9.3;18.4]	13.5 [7.9;22.4]	18.7 [11.1;29.7]	n.s
CHD lameness	15.5 [13.5;17.6]	16.3 [11.9;22.1]	12.3 [7.1;20.4]	15.3 [9.0;24.9]	n.s
<i>Crichton herd</i>					
Lameness	41.8	38.2	47.2	31.6	-
SD lameness	25.4	22.9	19.4	21.1	-
CHD lameness	29.0	25.9	36.1	15.8	-



**Figure 4.6** Incidence (%) of lactations in the Edinburgh (EDI) and Crichton herd (CR) with at least one episode of lameness, SD lameness and CHD lameness by 120 DIM in their lactation. Raw data are presented.

**Table 4.5** Number of episodes of lameness, SD lameness and CHD lameness over the lactating period for various degrees of difficulty. Back-transformed estimated means and their 95% confidence intervals are presented for the Edinburgh herd. Calculations from the Crichton herd were done from the raw data.

	Calving difficulty				P value
	N	FN	FM	V	
<i>Edinburgh herd</i>					
Lameness	2.0	2.2	2.1	2.4	n.s
	[1.9;2.1]	[1.9;2.6]	[1.8;2.6]	[1.9;3.0]	
SD lameness	2.0	2.0	2.0	1.9	n.s
	[1.9;2.1]	[1.6;2.4]	[1.5;2.7]	[1.4;2.6]	
CHD lameness	1.5	1.8	1.8	1.7	n.s
	[1.4;1.7]	[1.4;2.1]	[1.4;2.4]	[1.2;2.3]	
<i>Crichton herd</i>					
Lameness	3.5	3.2	2.5	1.5	-
SD lameness	2.1	2.3	1.6	1.3	-
CHD lameness	3.1	2.7	2.4	1.3	-



**Figure 4.7** Number of episodes of all types of lameness, SD lameness and CHD lameness, in the lactations in the Edinburgh (EDI) and Crichton herd (CR) with at least one episode of lameness, over their subsequent lactation. Raw data are presented.

### 4.3.2. Lactation length

#### *Lactation length achieved*

The lactation length achieved did not differ between the categories of difficulty in the Edinburgh herd ( $H=3.064$ ;  $df=3$ ;  $P>0.05$ ) nor in the Crichton herd ( $H=4.594$ ;  $df=3$ ;  $P>0.05$ ). Descriptive statistics of the data by herd can be found in Table 4.6.

**Table 4.6** Descriptive statistics of the lactation length achieved following different degrees of calving difficulty, in both herds.

	Calving difficulty	Count of lactations	Range [min-max]	Median	IQR
<b>Edinburgh herd</b>					
	N	1979	[2;633]	309	288-633
	FN	263	[1;725]	312	282-350
	FM	82	[3;508]	310	290-355
	V	95	[2;513]	304	215-348
	All scores	2419	[1;725]	309	287-344
<b>Crichton herd</b>					
	N	948	[1;824]	315	275-365
	FN	163	[1;703]	326	279-373
	FM	35	[80;506]	336	264-370
	V	19	[2;387]	300	210-329
	All scores	1165	[1;824]	317	275-366

IQR: Inter-quartile range

### ***Survival in the milking herd until 90 DIM***

*Edinburgh herd.* There was an interaction of parity and calving difficulty on the survival of the cows within the first 90 DIM. This means that the presence of the cows in the milking herd until 30, 60 and 90 DIM following a difficult calving was dependent on whether it was a primiparous or multiparous animal (Table 4.7,  $P < 0.05$ ).

Surprisingly in the primiparous animals, vet assisted animals had a higher survival rate at 30 DIM than primiparae calving normally but there was no effect beyond 30 DIM. However in the multiparous cows, FN cows were less likely to be present in the herd by 30 DIM but they had similar survival beyond 30 DIM. Multiparous vet assisted animals had lower survival rates until at least 90 DIM (culls of 8.3%). Graphical representation of the proportion of cows still present in the herd over time for each parity group is available in Figure 4.9.

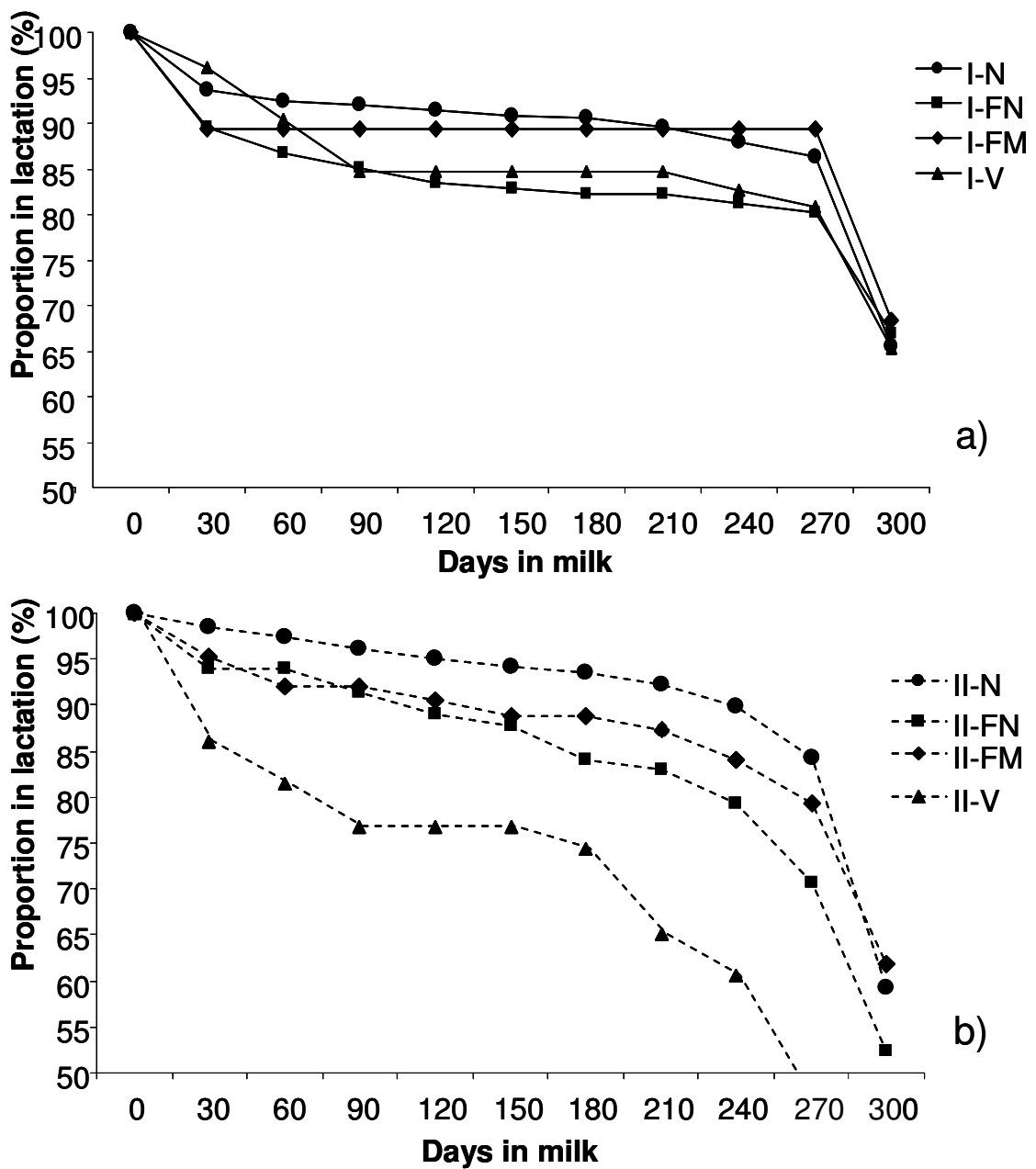
**Table 4.7** Adjusted mean proportion of animals present in the milking herd (%) at 30, 60 and 90 DIM within parity following a normal (N) or a difficult calving (FN/ FM: farm assisted without/with malpresentation; V: vet assistance) in the Edinburgh herd. Primiparous animals (N: n=481; FN: n=181; FM: n=19; V: n=52). Multiparous animals (N: n=1498; FN: n=82; FM: n=63; V: n=43).

		Calving difficulty				P value
		N	FN	FM	V	
30 DIM	Primiparous	99.4 <sup>a,b</sup>	99.1 <sup>a</sup>	94.1 <sup>a</sup>	99.9 <sup>b</sup>	***
	Multiparous	100 <sup>a</sup>	99.8 <sup>b</sup>	99.9 <sup>a,b</sup>	99.4 <sup>b</sup>	
60 DIM	Primiparous	96.9	95.0	92.7	97.9	**
	Multiparous	99.6 <sup>a</sup>	99.0 <sup>a</sup>	98.9 <sup>a,b</sup>	95.5 <sup>b</sup>	
90 DIM	Primiparous	95.7	92.8	91.3	94.6	*
	Multiparous	99.0 <sup>a</sup>	97.6 <sup>a</sup>	98.2 <sup>a</sup>	91.7 <sup>b</sup>	

\*: P<0.05; \*\*: P< 0.01; \*\*\*: P<0.001.

On the same line and within each parity, values without a common letter differ (P<0.05)

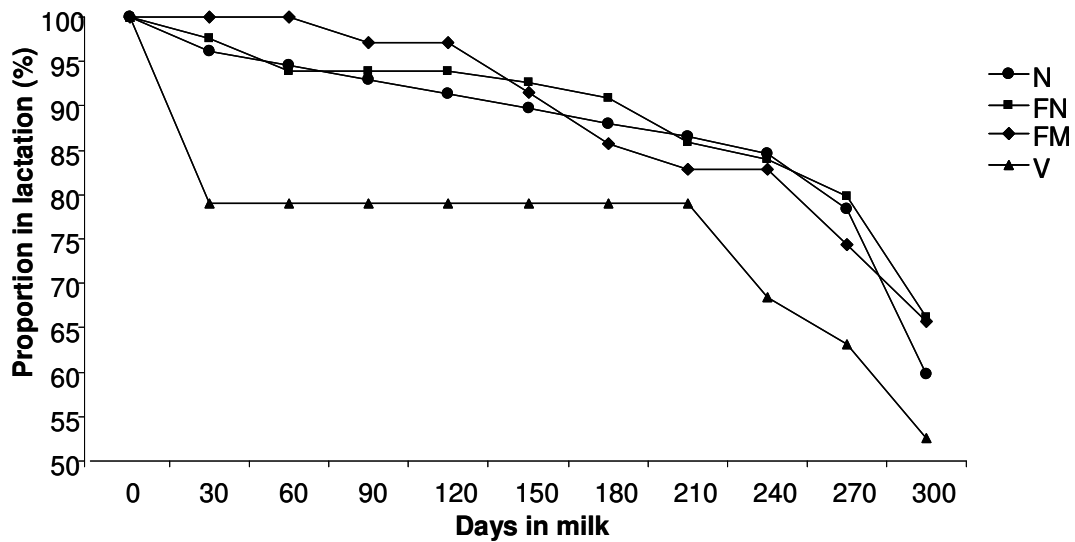
*Crichton herd.* There was no interaction between calving difficulty and parity in the Crichton herd. The vet-assisted animals were more likely to be culled in their first 30 DIM but this was not statistically significant with a 95% confidence interval (Table 4.8, P=0.069). However, this effect disappeared by 60 and 90 DIM. Graphical representation is available in Figure 4.10.



**Figure 4.9** Presence in the milking herd (%) of cows over time (days in milk) in the Edinburgh herd following a normal (N) or a difficult calving (FN/ FM: farm assisted without/with malpresentation; V: vet assistance). Means of the raw data are presented for the primiparous dams (a) and the multiparous dams (b) separately

**Table 4.8** Survival rate (%), calculated from the raw data. at 30, 60 and 90 DIM following a normal (N) or a difficult calving (FN/ FM: farm assisted without/with malpresentation; V: vet assistance) in the Crichton herd. Values without a common letter differed when using a 90% confidence interval (P<0.10)

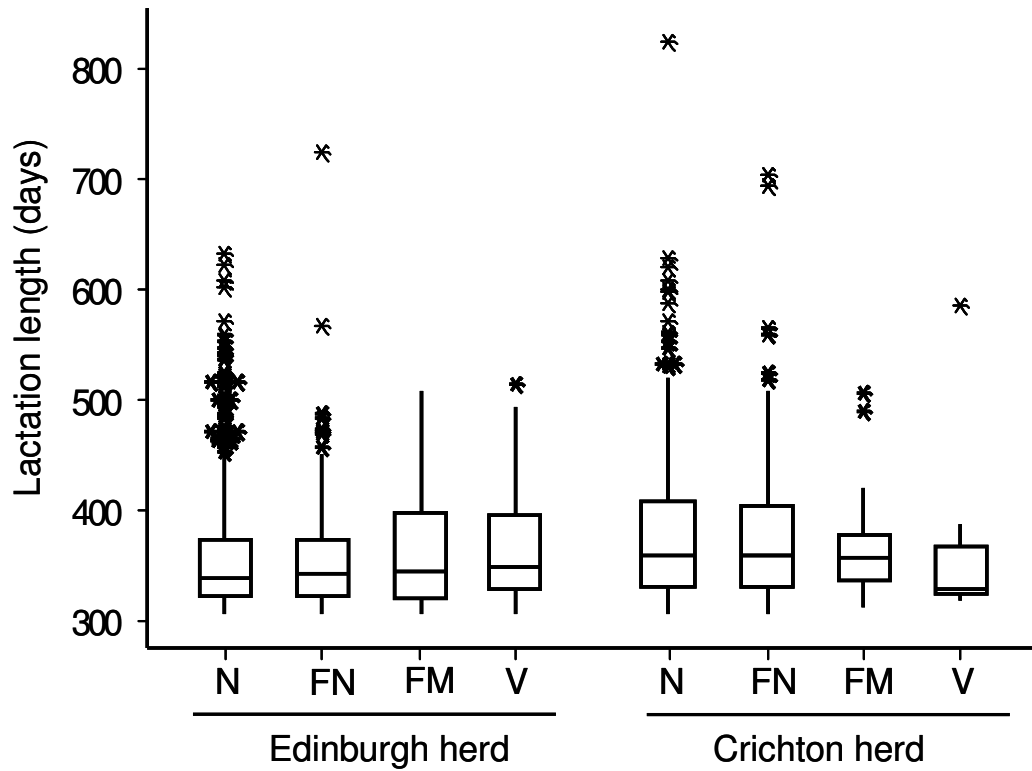
	Calving difficulty				P value
	N (n=948)	FN (n=163)	FM (n=35)	V (n=19)	
30 DIM	96.2 <sup>a</sup>	97.6 <sup>a</sup>	100 <sup>a,b</sup>	79.0 <sup>b</sup>	0.069
60 DIM	94.6	93.9	100	79.0	0.154
90 DIM	92.9	93.9	97.1	79.0	0.164



**Figure 4.10** Presence in the milking herd (%) of cows over time (days in milk) in the Crichton herd following a normal (N) or a difficult calving (FN/ FM: farm assisted without/with malpresentation; V: vet assistance). Means of the raw data are presented.

### Stretched lactations

The lactation length achieved conditional to having reached 305 DIM did not differ between the categories of difficulty in the Edinburgh herd ( $H=1.75$ ;  $df=3$ ;  $P>0.05$ ) nor in the Crichton herd ( $H=4.68$ ;  $df=3$ ;  $P>0.05$ ) (Figure 4.11).



**Figure 4.11** Boxplots of the actual lactation length achieved for the lactating animals reaching at least 305 DIM., following a normal (N) or a difficult calving (FN/ FM: farm assisted without/with malpresentation; V: vet assistance).

#### 4.4. Discussion

In this study, the incidence of mastitis and the number of episodes in any affected lactation was lower than reported for the UK as a whole (Kossaibati et al., 1998; Whitaker et al., 2004; Breen et al., 2009). A quarter of the lactations considered had at least one episode of CHD lameness which is in line with rates reported previously (Machado et al., 2010). The overall incidence of lameness was twice as high as reported in a study on UK farms (Rutherford et al., 2009a) although in their study, incidence of lameness was assessed at a few time-points contrary to the present study where the measure was done much more frequently over a longer period of time. Nonetheless the incidence were in the range reported among other studies in the UK (Barker et al., 2010; Archer et al., 2011).

This study does not support the initial hypothesis that in the lactation following a difficult calving, dairy cows would have a higher incidence of mastitis and lameness. This is in contradiction with the findings of previous studies that dystocial primiparous cows are more at risk of developing at least one case of mastitis (Tenhagen et al., 1999) and that high calf birth weight, which can be associated with occurrence of dystocia (Johanson and Berger, 2003), leads to greater odds of lameness (Linden et al., 2009). It is possible that difficulty at calving has no effect on the occurrence of mastitis and lameness on dairy cows in this herd, or that it is negligible compared to the other stressors that the cows may experience in the peripartum period, which could also affect the subsequent immunity and development of mastitis and lameness. Lameness and mastitis are both multi-etiological conditions, with the former being affected by a wide range of factors including cow cubicle design, nutrition, floor surfaces, foot bathing and animal husbandry in general (Blowey, 2005) and the latter being also affected by the milking parlour routine. However, it is felt that the statistical power for the Edinburgh herd was too low to detect any difference, if they exist, as seen through the large confidence intervals reported. In the study from Tenhagen et al. (1999), an effect was found with a similar herd size but occurrence of dystocia was much higher. Lack of statistical power was problematic when analysing the number of episodes for the

lactations affected, where in most cases, only calving difficulty was forced into the explanatory variables despite no eligibility as a univariate alone. Furthermore, the fact that the disease pressure was not controlled and randomly occurring in the present retrospective study, probably increases the variation in the data. However, although only descriptive statistics were used in the Crichton herd, it is interesting to note that the raw data from that herd suggest higher incidence of diseases in FN and FM cows (Figure 4.4 and 4.6). A study on the same animals as in this study's dataset also showed long-term saleable milk losses in dystocial animals (Chapter 2, Barrier and Haskell, 2011). This was attributed to a decrease in milk production by the cows especially in the early stages of the lactation and it was suggested that longer-term effects on saleable milk yield could be due to higher culling rates and diseases among the dystocial animals (Chapter 2). Moreover, studies on the same experimental farm have shown deleterious effects of being a dystocial animal on fertility (Chapter 3) and on the occurrence of uterine infection (Bell and Roberts, 2007). Dystocial cows are more likely to get culled after a difficult calving (Tenhagen et al., 2007) and this was also shown to be the case on this particular farm (Bell et al., 2010b). It is therefore possible that dystocial cows were culled early (not necessarily for mastitis or lameness) and thus, had not the same opportunity to express such health issues.

The study of lactation lengths in the two herds resulted in slightly different outcomes. However in both herds, the survival of cows following a difficult calving was impaired and the effect was mostly present in the first 30 DIM. This result is in line with previous studies reporting higher culling rates following a difficult calving (Beaudeau et al., 1993; Tenhagen et al., 2007; Bell et al., 2010b). It is interesting to note that in the Edinburgh herd, difficulty at calving for the first parity animals did not lead to impaired survival. Surprisingly, it was found to be improved following a veterinary assistance, when one would expect the exact opposite. It is possible that this result may come from a preferential treatment of the treated animals in their first parities. In fact, it is likely that the intervention threshold might be lower for primiparous animals (Dargatz et al., 2004; Eaglen et al., 2010b), and greater care and additional treatment to aid recovery might be given following vet assistance.

It is not surprising that the early lactation appears as being a critical period for the dystocial cows to remain in the herd. In fact, the post partum period is a high risk period when diseases related to the reproductive tract are more likely to develop such as milk fever, metritis, retained placenta and endometritis. As seen in the preceding Chapters, dystocial cows are at even higher risk of developing such health problems (Joosten et al., 1987; Bruun et al., 2002; Benzaquen et al., 2007), which, in turn, might result in fertility problems and culling (Beaudeau et al., 1993; Seegers et al., 1998; Bell et al., 2010b).

In both herds, there was no difference in the lengths of lactation achieved following a difficult calving or a non-assisted calving. This could be because the effect is diluted when considering the overall lactation. There was also no evidence of stretched lactations among dystocial cows with full lactations despite expectations of infertility in dystocial cows that remain in the herd.

#### 4.5. Conclusion

The hypothesis that cows experiencing calving difficulty would be more prone to mastitis and lameness over their subsequent lactation was not supported by this study. The relationship between calving difficulty and the occurrence of mastitis and lameness merit further exploration due to limitations in the present exploratory study. However, mastitis and lameness and calving difficulty remain a significant economic and welfare problem to the dairy industry. Furthermore, following a difficult calving, cows are at more risk of leaving the herd in the short-term, compared to cows who calved naturally. This may be due to more health and fertility problems in early lactation. However in the longer term, those cows seem to remain in the lactating herd for as much time as cows experiencing normal calvings.

## **CHAPTER 5:**

### **Parturition progress and behaviours in Holstein dairy cows with calving difficulty, with an emphasis on expression of behavioural indicators of pain and discomfort**

In this Chapter, I was responsible for collection of videos, study design, data collection, analysis and writing of the manuscript.

## 5.1. Introduction

On dairy farms, yearly calvings in dairy cows are an essential feature supporting milk production but also for the renewal of the herd. The peripartum period and parturition itself is nonetheless a risky time for the dairy cow and her calf. At parturition, difficulty in giving birth (also called dystocia) often requires human intervention to deliver the calf to avoid unnecessary distress of the dam and reduce mortality risk of one or both parties. In dairy breeds, such interventions occur in nearly 1 in 6 calvings in the United Kingdom (Wall et al., 2010) but there are large variations nationally and internationally (Mee, 2008a) with reports that it can affect up to half of the primiparous cows in the United States (Mee, 2008c). The occurrence of dystocia is associated with decreased performance and health problems in the dairy cows but also higher neonatal mortality and morbidity in their dairy calves (Chapters 2, 3, 4, 8, 9; Lombard et al., 2007; Mee, 2008a). This raises not only economic concerns for the dairy farmers but also animal welfare worries.

It is likely that components of parturition pain in cows might be similar to what is encountered by women at parturition (Gregory, 2004a) although the issue of relieving parturition pain in cows has been given little attention (Rushen et al., 2007). As part of the normal process, labour pain increases with increasing dilation of the cervix as well as with more intense, frequent and longer uterine contractions (Corli et al., 1986; Lowe, 2002), which means that pain increases as labour progresses.

There have been increasing concerns that dystocical calvings could be more painful than normal calvings. Indeed, dystocical cows experience longer labour and straining compared to cows that are not assisted at calving (Berglund et al., 1987; Gundelach et al., 2009; Miedema et al., 2011a). Higher blood vasopressin concentrations (an hormone secreted in response to stressful/ painful stimuli) have been found with higher levels of parturition pain (visually assessed) in dairy goats (Olsson et al., 2004) and in heifers requiring assistance at birth (Hydbring et al., 1999). Furthermore, the intervention itself, although necessary, may lead to additional pain due to further pressure applied to extract the newborn (Scott, 2005) as well as the stretching in the birth canal involved in

dystocial deliveries. In support of this, assisted cows have been reported to give a roar when the calf is extracted, coinciding with the passing of the head and forelegs (Gregory, 2004a). When misused, the use of calving aids can also result in painful injuries and trauma of the cow and her reproductive tract such as tears, bruises and fractures (Mee, 2008a; Fishwick, 2011).

In women, severe pain and distress during childbirth can lead to impaired uterine contractions, metabolic acidemia, tetany and fetal acidosis (Brownridge, 1995). In cows, there is also some evidence that distress and pain can impair the labour process through blocking the oxytocin release that is necessary for uterine contractions (Ehrenreich et al., 1985; Taverne, 1992). Because of this, additional stress and disturbance at calving has been associated with an increased risk of dystocia and stillbirth (Dufty, 1981; Carrier et al., 2006).

Dystocia is recognised by veterinarians as being a very painful condition, with median pain ratings ranging between 7 and 9 (on a 10 point numerical rating scale) across various countries (Huxley and Whay, 2006; Laven et al., 2009; Kielland et al., 2009; Fajt et al., 2011). Yet, Huxley and Whay (2006) report that nearly a quarter of the veterinarians surveyed do not administer pain relief unless a caesarean section is performed, and among those who use analgesics, they are mostly administered in half of the cases encountered or less (Huxley and Whay, 2006). Practical considerations and validation of administering non-steroidal anti-inflammatory drugs (NSAID) such as meloxicam at the time of parturition in dairy cattle to alleviate pain are being investigated (Duffield and Newby, 2010; Mainau Brunsó, 2011).

The study of behaviours is a useful tool in the investigation of pain in animals (Bateson, 1991; Rutherford, 2002; Anil et al., 2002; Weary et al., 2006; Vinuela-Fernández et al., 2007). Behaviours expressed by cows at parturition are essential clues for farmers to make their judgments about the level of distress of the cow and whether to intervene or not, alongside other elements such as elapsed time since first observed in labour, history and characteristics of the cow. Behaviours are also main components in the evaluation of pain when using visual analogue scales or numerical rating scales, which are widely

used in maternity wards and neonatology (Carbajal et al., 1997; Abu-Saad et al., 1998; Currie, 2008; Slater et al., 2008).

Because the behaviour of the cow changes as parturition approaches (Lidfors et al., 1994; Huzzey et al., 2005; Miedema et al., 2011b), behaviours at parturition can also be used for automatic detection of the onset of parturition in farm animals (Mottram, 1997; Oliviero et al., 2008; Mainau et al., 2009). There certainly is advantage in the automated detection of calving to ensure provision of adequate supervision, timely human intervention when difficulty arises and early care to the newborn calf. To that extent, parturition progress and periparturient behaviours have largely been documented in dairy cows that calve normally (for a review, see: von Keyserlingk and Weary (2007)). But there would be increased benefit in being able to automatically detect cows that need assistance, particularly if early diagnosis can be made. There are some documented differences in the prepartum behaviours of dystocial cows compared to cows calving normally (Wehrend et al., 2006; Proudfoot et al., 2009; Miedema, 2009; Mainau Brunsó, 2011), which may be used for early detection of calving difficulty but findings so far, although promising, have been inconsistent.

Therefore, there is a need to document the behaviours and parturition progress of dystocial cows in relation to cows that calve normally, particularly for behaviours that could relate to the expression of pain.

The first objective of the study was to characterise the calving progress of dystocial cows as opposed to cows calving normally. The second objective was to document the intervention at calving. Finally, the third objective was to compare the behaviours of dystocial cows to eutocial cows as labour progresses, with a particular emphasis on behaviours that may indicate pain.

## 5.2. Materials and methods

### 5.2.1. Animals, housing and calving management

The study took place at the SAC dairy Crichton Royal Farm (Dumfries, UK), whose herd is managed in accordance with the UK regulations on animal care and ethics of experimental animals. Preparturient Holstein cows were housed in one of the two contiguous roofed calving sheds (36m x 5.9m; 36m x 5.7m) approximately 3 weeks before they were due to calve. Animals were from two genetic groups (S: animals selected toward greater milk solids production; C: animals selected to be UK average) as part of a long-term genetic breeding and feeding trial (Pryce et al., 1999; Bell and Roberts, 2007). One calving shed was provided with a low forage diet while the other was provided with a high forage diet. Multiparous cows were allocated to a shed dependent upon their diet allocation but heifers were allocated to either shed to balance each feeding group for numbers as they were not allocated to a diet group until they calved. Animals were bedded on straw, provided with ad-libitum access to water and sheds were cleaned regularly. Fresh total mixed ration was delivered at the feeder in the afternoon once every two days.

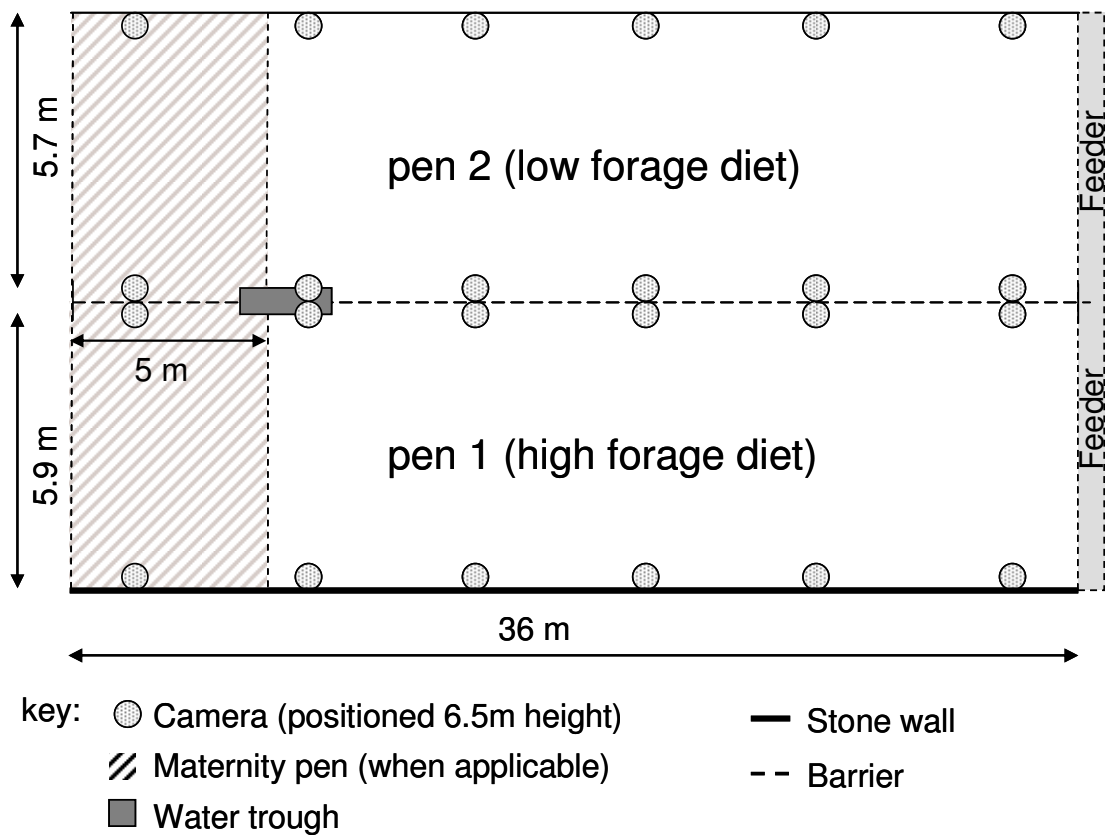
Dependent upon occupancy and space availability, calving animals were isolated from their group-mates by a barrier placed near the entrance of the shed, opposite to the feeders. This created a maternity pen of 5m long within the shed with access to ad libitum water (Figure 5.1).

During the calving itself, the decision to provide assistance and the allocation of the calving difficulty score was taken by experienced farm staff. Scores used were: N (no assistance), FN/FM (Farm assistance without / with malpresentation of the calf), VN/VM (Veterinarian assistance without/with malpresentation of the calf) and VC (caesarean section).

### 5.2.2. Video-recording

The calving sheds were continuously video monitored throughout the trial. For each shed, 12 weather proof infrared CCTV cameras (1/3" Sony Color CCD, EZ420IR-30,

ezCCTV.com Ltd, Herts, UK), were equally distributed around the shed's roof so that all of the pen could be viewed (Figure 5.1.). The twelve cameras were connected to a high memory storage computer using Geovision (version 8, Geovision Inc., Taipei, Taiwan). Animals were labelled with numbers to allow recognition on videos and numbers were remarked as required. To minimise disturbance, the marking procedure was performed before animals entered the pen, or when they were moved out of the pen once a week for husbandry purposes.



**Figure 5.1** Diagram of the calving pens and the set up for video recording.

### 5.2.3. Selection of the observed calvings

Only calvings leading to live singleton purebred Holstein calves were considered and no multiple calvings from the same individuals were included. Retrospectively 38 calvings

(19 N; 12 FN; 7 FM) occurring between November 2008 and February 2010 were selected for the purpose of the study.

Following a paired design, each farm assisted calving was matched in pairs to a non-assisted calving as far as possible for the following criteria by order of importance: parity of the dam (Primiparous vs Multiparous), sex of the calf, calf's birth weight (CBW), genetic group of the dam (GG), calving pen (1: high forage diet; 2: low forage diet) and calving season (Summer: April to September; Winter: October to March). Additional characteristics retrieved for each calving were dam calving body weight (BW; kg), dam calving body condition score on a five point scale (BCS), and calving time (Day: calving time nearest integer from 7h until 19h; Night: otherwise). A summary of the calving characteristics of the observed cows can be found in Table 5.1. For each category of calving difficulty, the mean birth weights  $\pm$  s.d were  $43.1 \pm 5.6$ kg (N),  $42.9 \pm 5.8$ kg (FN) and  $45.3 \pm 4.1$ kg (FM) respectively.

**Table 5.1** Main calving characteristics for the observed animals as for each type of farm assisted calving and their matched normal calvings. For each characteristic, unless otherwise stated, the number of animals is presented.

		FN (n=12)	N (n=12)	FM (n=7)	N (n=7)
Parity	Primiparous	6	6	2	2
	Multiparous	6	6	5	5
Calf sex	Heifer	6	6	2	2
	Bull	6	6	5	5
CBW (kg)	mean $\pm$ s.d	$42.9 \pm 5.8$	$41.8 \pm 6.1$	$45.3 \pm 4.1$	$45.1 \pm 5.4$
GG	Control	6	6	6	6
	Select	6	6	1	1
Pen	1	9	7	5	4
	2	3	5	2	3
Season	Summer	5	3	2	6
	Winter	7	9	5	1
Dam BCS	mean $\pm$ s.d	$2.1 \pm 0.3$	$2.3 \pm 0.3$	$2.4 \pm 0.3$	$2.3 \pm 0.2$
Dam BW (kg)	mean $\pm$ s.d	$538 \pm 69$	$554 \pm 99$	$555 \pm 87$	$599 \pm 74$
Calving time	Day	6	5	3	3
	Night	6	7	4	4

#### 5.2.4. Data collection: video scoring

The recruited calvings were each watched for three distinct continuous observation periods preceding the full expulsion of the calf (A: -6 h to -5h30; B: -4h to -3h; C: -2h to expulsion of the calf) using Observer® XT 9 (Noldus, Wageningen, The Netherlands). The choice for such periods was made following a preliminary study (Appendix B) based on data collected from previous work (Miedema et al., 2011b). After an initial training period, videos were observed by a single observer in a random manner. Intra-observer reliability tests were assessed on nine video files from nine different animals (one per calving difficulty score and per observation period). Cohen's kappa obtained ranged from 0.60-0.87, suggesting a good to a very good agreement (Kaufman and Rosenthal, 2009). The occurrence and duration (when stated) of calving progress and calving behaviours (presented respectively in Table 5.2 and Table 5.3) were recorded.

**Table 5.2** Ethogram used for each of the individual cows and each observation periods. The occurrence and duration (when indicated) of the following behaviours/events were recorded.

<b>Calving progress</b>	
Contraction	Tensing of ventral portion of the abdomen
Calf's feet seen <sup>†</sup>	Calf's feet seen at the vulva
Head out <sup>†</sup>	First appearance of calf's head fully expelled
Shoulder out <sup>†</sup>	Head, forelegs and withers have passed through the vulva
Hips out <sup>†</sup>	Hips have passed through the vulva
Expelled	Calf free from the cow's vulva (Miedema, 2009)
<b>Intervention</b>	
Intervention starts <sup>†</sup>	Time at which at least one human is present in the calving shed to assist the cow without leaving the pen for more than 5 min until the calf is fully expelled or when doing so, having assisted the cow for over 5 min.
Human-assistance <sup>†</sup>	At least one human is in the calving pen. It starts when the human put hands at the back of the cow. Assistance is categorised as: Manual: manual manipulation or pulling of the calf Ropes: Ropes used to pull the calf out. Jack: jack used to pull the calf out.
Number of human	Maximum number of humans involved at any one point during the intervention process

<sup>†</sup> Duration was recorded

**Table 5.3** Ethogram used for each of the individual cows and observation periods.

<b>Cow postures</b>	
Lie lateral, head rested <sup>†</sup>	Cow is lying on the flank, with shoulder touching the ground and at least three legs extended. Head rests on the floor.
Lie lateral, head up <sup>†</sup>	Cow is lying on the flank, with shoulder touching the ground and at least three legs extended. Head not resting on the floor
Lie non-lateral, head rested <sup>†</sup>	Cow is lying but not in a lateral position with her head resting on floor or part of the body
Lie non lateral, head up <sup>†</sup>	Cow is lying but not in a lateral position and her head is not supported.
Standing still <sup>†</sup>	Cow is in a standing position without any forward or backwards movement
Walking <sup>†</sup>	Cow is in a standing position and makes at least two steps forward or backwards.
<b>Head-directed behaviours</b>	
Eating <sup>†</sup>	Cow stands within 1.5 m from the feeder, facing the feeder alternatively taking food in her mouth and chew.
Drinking <sup>†</sup>	Cow moves muzzle within 10 cm of drinking trough.
Self-grooming <sup>†</sup>	Cow licking herself
Turn head	Cow turns her head back facing abdomen
Lick ground	Cow holds muzzle within 10 cm of the ground and licks it.
Sniffing	Cow holds muzzle within 10 cm of the ground
<b>Tail-directed behaviours</b>	
Tail raised <sup>†</sup>	Tail is raised and held away from body (Miedema, 2009) Was scored only when the cow was in a standing position.
Tail switch	Tail swung to the side and then forcefully forward along the flank or directly back down (Miedema, 2009)
<b>Other behaviours</b>	
Stamp	Fore or hind leg raised, foot either reaches forward and down or returned to ground with force (Miedema, 2009)
Rubbing	Cow rubs any part of the body against any solid material
Fidgeting	Change in the weight distribution of the cow's body while being in any of the lying postures but which did not trigger a change in any of the recorded postural categories. For example: cow moving from the left flank to the right flank, cow lifts part of the body, cow changes position of the legs. Any movement occurring within a two second time frame was only counted once.

<sup>†</sup> Duration was recorded



Material used for video recording (left) and for labelling the animals (right)



Cow lying non lateral with the head rested (left) and with the head up (right)



Cow lying lateral with the head rested (left) and with the head up, accompanied by tail switch (right).

## **5.2.5. Datasets, editing and calculation**

### ***General edits and calculations***

Health treatments on the day of or on the following day after parturition were examined. Data from cow 1520 (FM) and cow 1537 (N) were discarded because both cows experienced mastitis and one of them also had a gastrointestinal infection. This may have caused additional pain and may have disrupted their behaviour. Any kind of lying was grouped as a single category (lying). Standing still and walking were also grouped together (standing).

Data were first considered for investigation of calving progress and behaviours preceding calving. Datasets contained data relating to the full duration of each observation period (A: 30min; B: 1h; C: 2h) but in the C period, data was truncated at the start of intervention for the FN and FM cows. This is because some of the behaviours might have been influenced by the presence and handling of the staff, especially when restraint occurred.

Data on intervention at calving for dystocial animals were contained in a separate dataset starting from the start of intervention until the full expulsion of the calf. Across all datasets, durations were converted to percentages of the observation and counts of behaviours to frequencies. This was done to account for the different lengths of observation periods and to allow comparisons over the periods as labour progresses.

For clarity purposes, the data can be divided into three datasets: calving progress, behaviours preceding calving and intervention at calving.

### ***Calving progress***

This dataset contains the information relevant to the progress of parturition as described in the ethogram (Table 5.2). The total number of contractions observed over all three observation periods was also calculated. Labour length was estimated to be the duration from feet being seen until intervention (assisted animals) or the foetus being fully expelled (non-assisted animals). There were no instances when feet were seen between the observation periods and therefore, durations reported are thought to be accurate. Calf's feet were not seen before intervention occurred for cow 1766 (FM), therefore

leading to a null value and could not be accurately scored for cow 1788 (N), therefore leading to a missing value.

Description of the milestones achieved between the feet being seen and the calf being fully expelled was possible in the non-assisted cows only and relative time of feet, head, shoulder, hips seen to birth was calculated. Cow 1778 had missing values except for feet seen to birth as further scoring was unreliable.

### ***Behaviours preceding calving***

For each period, behaviours of interest included: duration of tail raising, lying, lying lateral with head rested (LLHR), self-grooming, drinking and eating; and counts of lick ground, sniffing, rubbing, stamping, turning the head back, tail switching. At each period, the number of transitions from any standing to any lying postures (LS transitions) and a “restlessness” score were also calculated. Restlessness was calculated by adding the counts of postural changes (transitions between the six different cow postures described in the ethogram) and the number of recorded “fidgeting” behaviours.

### ***Intervention at calving***

Intervention at calving concerned dystocial animals only and focussed on the start of intervention until the full expulsion of the calf. The aim was to describe and compare the intervention length, duration of assistance, assistance, calving progress and some of the behaviours expressed by the FN and FM cows during intervention. Percentages of the duration of assistance dedicated to manual assistance, assistance with ropes and assistance with the use of a calving jack were calculated. The total number of contractions visible during intervention and their frequency was also calculated. Occurrence of stamping and turning the head back were also retrieved.

## **5.2.6. Statistical analysis**

### ***Calving progress***

The number of contractions seen for each period was analysed using a REML analysis (Genstat, 11<sup>th</sup> Edition, VSN International Ltd) following a square root transformation. The model included the interaction between calving difficulty and period in the fixed effects and (pair/cow identity)\*period as a random effect. The total number of

contractions was analysed with a REML analysis using calving score as a fixed effect and pair as the random term. Comparison of the labour lengths between the scores was analysed using a non-parametric Kruskal-Wallis test. A Spearman's correlation was used to relate the total number of contractions and labour length. Descriptive statistics were used to describe the calving progress in the non-assisted animals. A Friedman test was used to compare the durations of the different stages of labour. For that purpose, the stage of labour was used as a fixed factor and the cow identity was entered in the random model, and any missing data were excluded from the analysis.

### ***Behaviours preceding calving***

Some behaviours were clustered together. This led to grouping of licking and sniffing (exploratory behaviours), drinking and eating (ingestive behaviours) and rubbing, stamping, tail switching and turning the head back (irritation behaviours) by addition of the appropriate behavioural frequencies and durations.

Duration of tail raising, LLHR, lying, the number of LS transitions, "irritation" behaviours (inverse transformation) and restlessness levels were analysed with a REML analysis. The model included the interaction between calving difficulty and period in the fixed effects and (pair/cow identity)\*period as a random effect. Duration of self-grooming, number of exploratory and ingestive behaviours were analysed for an effect of calving difficulty and period using distinct Kruskal-Wallis tests.

### ***Intervention at calving***

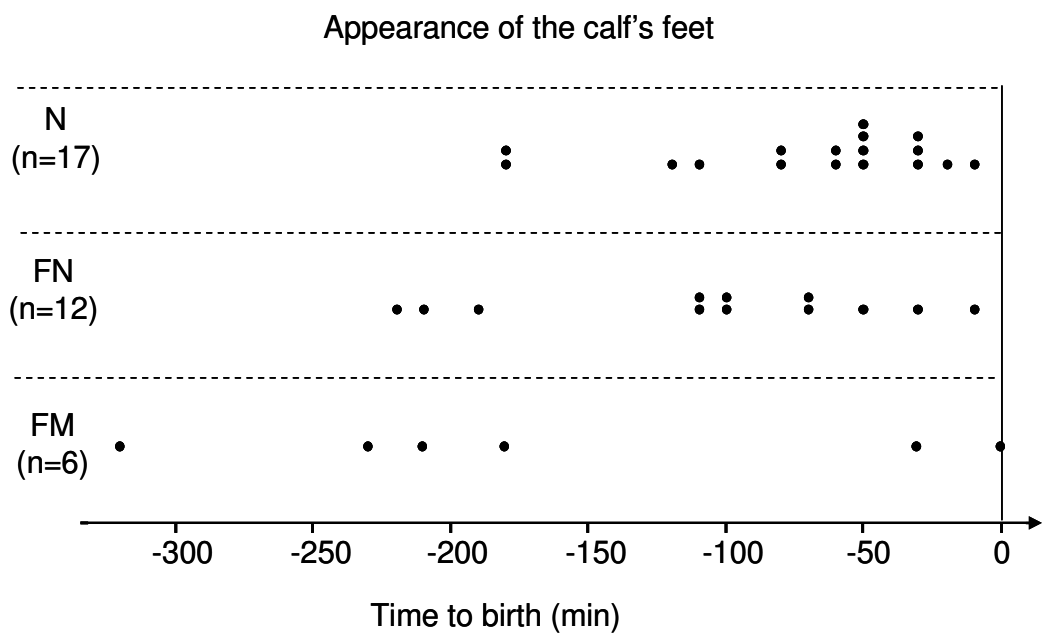
Duration of assistance, number of contractions and their frequency were compared between FM and FN cows using calving difficulty as a fixed effect in a REML analysis. Comparisons for duration of intervention and the percentages of time allocated to each type of assistance were based on Kruskal-Wallis non-parametric tests. Fisher exact tests were used to compare the likelihoods of stamping and turning the head back.

### 5.3. Results

#### 5.3.1. Calving progress

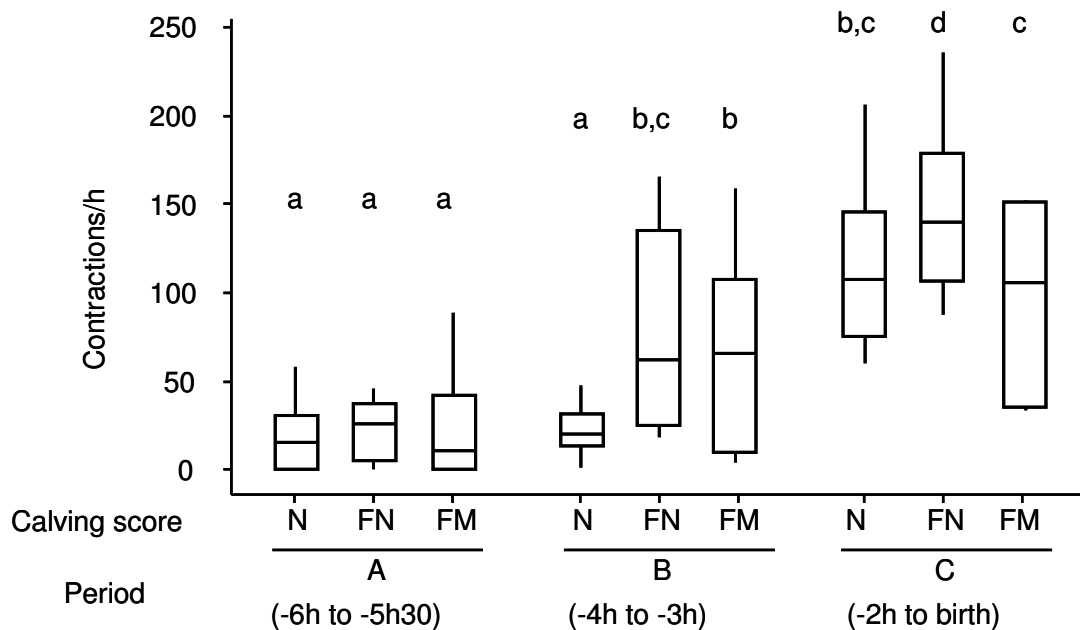
##### *Calving progress in eutocial and dystocial animals*

Labour length (median duration in minutes [inter-quartile range or IQR]) did not differ between the scores of difficulty ( $H_2=3.4$ ,  $P=0.181$ ) (N: 54.7 [27.4; 97.1]; FN: 101.3 [52.0; 167.1]; FM: 194.0 [25; 250.2]) but there were large individual variations within the scores of difficulty (Figure 5.2). The overall number of contractions seen among the periods was positively and significantly correlated with labour length (Spearman's correlation,  $\rho=0.502$ ,  $P=0.002$ ). More contractions were observed in total in the FN cows than in the non-assisted cows, but this was not the case in FM cows (N:  $262.2 \pm 28.8$ ; FN:  $374.0 \pm 35.2$ ; FM:  $254.1 \pm 49.7$ ;  $F_2=3.67$ ;  $P<0.05$ ).



**Figure 5.2** Dotplot showing the first appearance of the calf's feet relative to birth (individual labour lengths) for each cow giving birth without assistance (N), with farm assistance and no malpresentation of the calf (FN) or with the calf being malpresented (FM).

The number of contractions seen depended both on calving difficulty and the observation periods (Figure 5.3.,  $F_4=5.15$ ;  $P<0.01$ ). All cows had similar number of contractions at the early stage of parturition (A period), regardless of calving difficulty. At B period, FN and FM cows had higher number of contractions than N cows, who had no increase in contractions compared to the earlier observation. In the last two hours of parturition (C period), all cows experienced more contractions compared to the B period, regardless of difficulty occurring at calving. FN cows had more contractions than non-assisted animals but this was not the case for FM animals.



**Figure 5.3** Boxplot representing the number of contractions per hour for cows giving birth without assistance (N cows; n=18), with farm assistance without malpresentation of the calf (FN cows; n=12) or with farm assistance and a malpresented calf (FM cows; n=6) across different observation periods as parturition approaches (A: -6h to -5h30; B: -4h to -3h; C: -2h to birth). Boxplots without a common letter differ ( $P<0.05$ ).

### ***Natural calving progress in non-assisted animals***

In the non-assisted cows, the median relative time from birth [IQR], for the feet, head, shoulder and hips to appear were - 56.3min [-113.7;-27.5], -2.9min [-7.4;-2.3], -1.9min [-4.4;-1.0] and -11s [-29;-3] respectively. The stage that took the longest duration to achieve was the appearance of the feet until appearance of the head ( $S_3=37.0$ ,  $P<0.001$ ).

### **5.3.2. Behaviours preceding calving**

#### ***Restlessness***

Level of restlessness depended on the observation period and difficulty at calving (Figure 5.4;  $F_2=3.04$ ;  $P<0.05$ ). Levels of restlessness did not differ between the scores of difficulty in the observation period A. FM cows had similar levels of restlessness throughout the observations while N cows showed an increase in restlessness in the last two hours preceding expulsion of the calf. FN cows showed an increase at each of the observation period and levels of restlessness became higher than those expressed by N and FM cows at periods B and C.

#### ***Tail raising***

There was no interaction between calving difficulty and period of observation on the duration of tail raising in standing animals ( $F_2=1.21$ ;  $P>0.05$ ). Overall, FM cows raised their tail for longer compared to N cows (Table 5.4;  $P<0.05$ ). All cows raised their tail for longer as parturition approached regardless of calving difficulty (Table 5.5;  $P<0.05$ ).

#### ***Lying lateral with the head rested (LLHR)***

FM cows spent longer lying lateral with the head rested (LLHR) during the B observation period and FN cows did so during the C period. However, this was not statistically significant with a 95% confidence interval ( $F_2=2.31$ ;  $P=0.067$ ). Proportion of LLHR was higher in B and C periods compared to A (Table 5.5;  $P<0.05$ ).

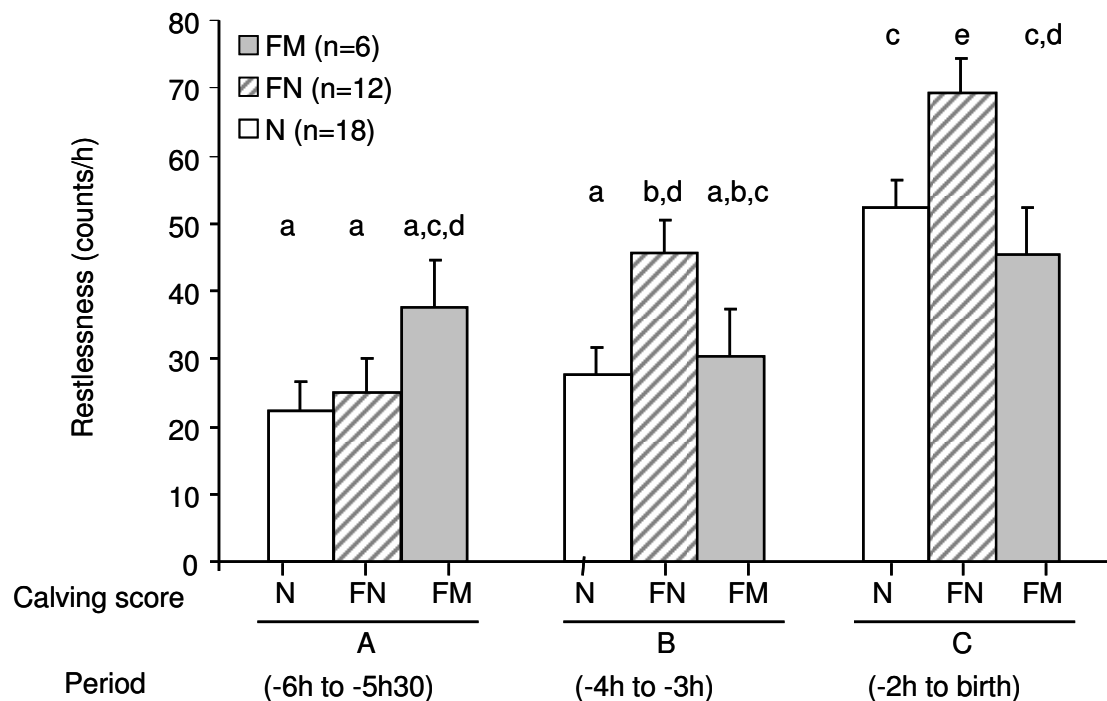
#### ***Transitions from lying to standing***

There was no interaction of observation period and calving difficulty on the number of transitions from lying to standing and on the total duration of lying. Cows performed

more LS transitions in the last two hours preceding calving but there was no difference between cows with different calving difficulty scores (Table 5.4 and 5.5;  $P < 0.05$ ).

### Other behaviours

There was no effect of calving difficulty on the duration of self-grooming, ingestive behaviours, counts of exploratory and of irritation behaviours nor was there an effect of period (Table 5.4 and 5.5;  $P > 0.05$ ) except for ingestive behaviours but almost no cows fed or drank during the last two hours of parturition (Table 5.5;  $P < 0.05$ ).



**Figure 5.4** Predicted means ( $\pm$  se) of the levels of restlessness (nb/h) for cows giving birth without assistance (N;  $n=18$ ), with farm assistance without malpresentation of the calf (FN;  $n=12$ ) or with farm assistance and a malpresented calf (FM;  $n=6$ ) across different observation periods as parturition approaches (A: -6h to -5h30; B: -4h to -3h; C: -2h to birth). Bars without a common letter differ ( $P < 0.05$ ).

**Table 5.4** Effect of calving difficulty (N: no assistance; FN/FM: Farm assistance without/with malpresented calf) on the predicted means  $\pm$  s.e of duration of tail raising (%), lying lateral with the head rested (LLHR), lying, number of transitions from a lying to a standing posture (LS transitions) and on the median [IQR] number of irritation behaviours, self-grooming, exploratory behaviours and duration of ingestive behaviour.

	Calving difficulty			P	
	N (n=18)	FN (n=12)	FM (n=6)		
Tail raising (%)	33.7 $\pm$ 4.2 <sup>a</sup>	42.7 $\pm$ 5.1 <sup>a,b</sup>	54.0 $\pm$ 7.0 <sup>b</sup>	F <sub>2</sub> =3.57	*
LLHR (min/h)	2.4 $\pm$ 0.7	4.3 $\pm$ 0.8	3.2 $\pm$ 1.2	F <sub>2</sub> =2.08	n.s
Lying (min/h)	31.6 $\pm$ 0.3	39.6 $\pm$ 3.1	30.2 $\pm$ 4.4	F <sub>2</sub> =2.39	†
LS transitions (counts/h)	2.9 $\pm$ 0.4	4.0 $\pm$ 0.4	4.3 $\pm$ 0.7	F <sub>2</sub> =2.39	n.s
Irritation <sup>1</sup> (counts/h)	27.6 [13.8;54]	34.8 [24.6;49.2]	30 [17.4;42]	F <sub>2</sub> =0.17	n.s
Self grooming (s/h)	8 [0;28]	2 [0;14]	3 [0;22]	H <sub>2</sub> =1.97	n.s
Exploratory (counts/h)	9 [4.8;16.2]	7.8 [1.8;12.6]	7.8 [7.2;15.6]	H <sub>2</sub> =2.61	n.s
Ingestive (min/h)	0 [0;30.6]	0 [0;1.8]	0 [0;3.4]	H <sub>2</sub> =1.12	n.s

\*\*\*: P<0.001; \*: P<0.05; †: P<0.10; n.s: non significant (P>0.05)

On the same line values without a common letter differ (P<0.05)

<sup>1</sup>: REML analysis performed on transformed data (inverse transformation)

**Table 5.5** Effect of period of observation prior to giving birth (A: -6h to -5h30; B: -4h to -3h; C:-2h to birth) on the predicted means  $\pm$  s.e of duration of tail raising (%), lying lateral with the head rested (LLHR), lying, number of transitions from a lying to a standing posture (LS transitions) and on the median [IQR] number of irritation behaviours, self-grooming, exploratory behaviours and duration of ingestive behaviour.

	Observation period			P	
	A (n=36)	B (n=36)	C (n=36)		
Tail raising (%)	14.1 $\pm$ 4.4 <sup>a</sup>	37.1 $\pm$ 4.4 <sup>b</sup>	79.2 $\pm$ 4.4 <sup>c</sup>	F <sub>2</sub> =96.54	***
LLHR (min/h)	0.4 $\pm$ 0.9 <sup>a</sup>	3.7 $\pm$ 0.9 <sup>b</sup>	5.8 $\pm$ 0.9 <sup>b</sup>	F <sub>2</sub> =12.77	***
Lying (min/h)	29.1 $\pm$ 3.2 <sup>a</sup>	28.2 $\pm$ 3.2 <sup>a</sup>	44.1 $\pm$ 3.2 <sup>b</sup>	F <sub>2</sub> =8.25	***
LS transitions (counts/h)	2.2 $\pm$ 0.7 <sup>a</sup>	3.2 $\pm$ 0.7 <sup>a</sup>	5.8 $\pm$ 0.7 <sup>b</sup>	F <sub>2</sub> =13.97	***
Irritation <sup>1</sup> (counts/h)	32 [10.2;60]	34.8 [23.4;49.8]	37.6 [16.8;41.4]	F <sub>2</sub> =1.10	n.s
Self grooming (s/h)	0 [0;25]	9 [0;22]	4 [0;21]	H <sub>2</sub> =1.10	n.s
Exploratory (counts/h)	7.8 [0;16.2]	10.2 [7.2;13.2]	8.4 [4.8;19.2]	H <sub>2</sub> =1.74	n.s
Ingestive (min/h)	0 [0;4.1]	0 [0;12.5]	0 [0;3]	H <sub>2</sub> =6.20	*

\*\*\*: P<0.001; \*: P<0.05; †: P<0.10; n.s: non significant (P>0.05)

On the same line values without a common letter differ (P<0.05)

<sup>1</sup>: REML analysis performed on transformed data (inverse transformation)

### **5.3.3. Intervention at calving in the dystocial cows**

#### ***Description of the human intervention***

Two cows (FN) were calved without a calving jack, in which case two persons were involved in pulling the calf with the help of calving ropes. For the other 16 jack-assisted cows, 14 cows (10 FN; 4 FM) were assisted by 1 person only and 2 FM cows required the assistance from two persons. Half of the cows (1 FM; 8 FN) were not restrained and were assisted while lying down. The other half of the cows (5 FM; 4 FN) were restrained in a standing position during intervention (6 were forced to stand). This only happened when the calving jack was used.

#### ***Duration of intervention***

Length of intervention ranged from 30s to 35min with a median time of 4.7min. Cows with a malpresented calf needed intervention for longer than when the calves were not malpresented (median in min [IQR]; FN: 2.5[1.3;4.8], FM: 7.3[5.1;19.9],  $H_1=6.88$ ,  $P<0.01$ ). Length of intervention may also be affected by the number of persons involved and the calving type. However, small sample size did not allow such investigation.

#### ***Duration and description of assistance***

There was no difference in the proportion of intervention (in %) dedicated to assistance between FN and FM (FN:  $77.6 \pm 4.2$ ; FM:  $64.4 \pm 6.0$ ;  $F_1=3.27$ ;  $P<0.10$ ). During assistance at calving, there was no difference between the FM and FN animals in the proportion of time (%) allocated to manual assistance (FM: 39.7 [0;75.1]; FN: 0 [0;29.7];  $H_1=2.07$ ;  $P>0.05$ ), assistance with ropes (FM: 31.1 [5.9;46.9]; FN: 2.0 [0;18.2];  $H_1=2.79$ ;  $P>0.05$ ), or assistance with use of a jack (FM: 58.2 [23.0;69.2]; FN: 59.3 [40.3;66.6];  $H_1=0.009$ ;  $P>0.05$ ).

#### ***Labour and behavioural indicators of pain during intervention***

Cows with malpresented calves were not more likely to stamp during intervention, nor to turn their head more often ( $P>0.05$ ). Malpresentation of the calf resulted in less frequent contractions in the dam during intervention than when the calf was presented normally (contractions per min; FN:  $5.5 \pm 0.5$ , FM:  $3.1 \pm 0.8$ ;  $F_1=6.29$ ;  $P<0.05$ ).

## 5.4. Discussion

### 5.4.1. Dystocial cows in later stages of labour for longer?

The observed increase in frequency of contractions as birth approaches is part of the normal physiological process. The frequency of contractions reported in this study was higher than in previous work (Gillette and Holm, 1963; Preliminary study, Appendix B). This certainly is because any tension of the abdomen wall was counted as a contraction in the present study and this also account for successive abdominal presses that follow actual uterine contractions (Gundelach et al., 2009). When comparing with studies reporting on abdominal presses in the expulsive stage of parturition in beef cows (Wehrend et al., 2005), the frequencies reported are in line with their finding.

Assisted cows had more frequent contractions and had contractions for longer than cows calving normally. This means that the assisted cows may have been in later stages of labour for longer. Higher straining may also occur in order to expel the calf, probably as a result of higher discomfort. Another possibility may be a physiological difference in the birth process in dystocial cows, where higher frequency of contractions would be achieved more quickly. However, this is quite unlikely because the main reason for difficulty to occur is foeto-pelvic incompatibility (Mee, 2008a), and this incompatibility would develop at a later stage.

In the last two hours, the FM cows did not have as many contractions as the FN animals. When the calf is malpresented, a different application of pressure from the foetus in the birth canal may lead to a less effective Ferguson's reflex (release of oxytocin following pressure from the foetus on the cervix and vaginal walls, which in turns stimulates contractions). It could also be that additional pain or distress (if exists) in the FM cows impairs the labour process through partial blocking of the oxytocin release which is necessary to uterine contractions (Ehrenreich et al., 1985; Taverne, 1992; Lawrence et al., 1997), a phenomenon also called secondary uterine inertia (Mee, 2004). Alternatively, possible greater exhaustion could result in less straining from the FM cows.

Altogether, the results on contractions show that cows were in the later stages of labour for longer. This is despite no statistical difference seen in terms of length of labour measured in the study, which is also in contradiction with previous research (Hudson et al., 1976; Civelek et al., 2008; Miedema et al., 2011a). The large variability between the individual animals may have led to lower statistical power. Furthermore, the proxy measure used for labour length was the duration between first appearance of the calf's feet and full expulsion of the calf. This is an approximate but not actual measure of the duration of the second stage of labour. In the present study, such proxy measures were used because appearance of the feet at the vulva was the most reliable measurement that could be recorded from the videos, as opposed to the rupture of the chorioallantois and appearance or rupture of the amnion. When malpresentations occur, the hooves or other parts of the calf may not necessarily be visible at the vulva until intervention occurs. Moreover, actual labour length would also include stage 1 of parturition but this was not assessable from video records. Nonetheless, the duration of stage 2 was longer in dystocial animals but not stage 1 (Dwyer et al., 1996).

#### **5.4.2. The birth process with and without intervention**

Not surprisingly, in normal calvings, the full expulsion of the calf followed the appearance of the calf's head in a matter of a few minutes, with time taken for the head to emerge being the longest part of the expulsion phase, which is in accordance with previous studies (Owens et al., 1985; Wehrend et al., 2005). This is because shoulder and hip widths of the calf (both of similar size) are the body parts of the calf that are usually the limiting factors to a natural birth (Kolkman et al., 2010b; Becker et al., 2011).

The length of intervention at calving varied considerably between individuals ranging from 30s to 35min but with a median time of 4.7min. The calving for which intervention occurred for 35min was a FM calving where malpresentation was first corrected, assistance suspended for 20 minutes before full extraction of the calf was resumed. The intervention durations reported are within the recommended times after which a veterinarian should be called (Mee, 2004). Intervention on cows with malpresented

calves took longer, probably as a result of the additional time needed to correct the malpresentation of the calf besides time needed for its extraction. This was also reported previously in sheep (Dwyer and Lawrence, 2005b).

There were no differences in the amount of stamping and turning their head back during intervention between FN and FM cows, which could have suggested higher pain, irritation or discomfort during intervention. The exploration of such features is limited, partly because of animal's restraint during intervention. It is also very likely that all the efforts and attention of the cow are focussed on the expulsion of the calf, and in that extent, half of the observed animals stayed recumbent during the whole intervention and did not need to be restrained.

Only two unassisted animals (one first and one later parity) had a stage 2 of labour of over two hours but delivered their calves without exceeding three hours of labour. This falls under recommended durations where no assistance is required (Mee, 2004). Throughout the study, the unassisted calvings therefore reflect an actual absence of difficulty at calving. This means they are good controls to the study of calving difficulty. There was a large variation in the labour lengths experienced by the animals that received assistance, particularly for the FM calvings. Three quarters of the FN cows were assisted within two hours of appearance of the calf's feet at the vulva. It is likely that in those cases, abnormalities in the progress of labour, foetal oversize or the behaviour of the cow indicated difficulty in delivering a healthy calf unaided, although it can not be fully excluded that in some cases early interventions may have happened, and that the cow may have calved unaided if given more time.

#### **5.4.3. Behavioural changes prepartum**

In the present study, over the last 6 hours of parturition, restlessness was higher in the last two hours of parturition for the eutocial cows whereas in the FN cows, the increase in restlessness was seen earlier. Earlier and increased restlessness in dystocial cows is in agreement with previous studies (Metz and Metz, 1987; Miedema et al., 2011a).

Despite that, the number of transitions from lying to standing and vice-versa, and lying times were similar between assisted and unassisted cows. This contradicts previous

studies which found shorter bouts of lying and standing as well as increased posture transitions in dystocial cows (Misch et al., 2006; Proudfoot et al., 2009) but these studies looked at the 24h preceding birth and not closer to parturition. It can be hypothesised that transitions from lying to standing are less sensitive for detecting the level of restlessness in the calving animals in their final stages of parturition. When straining, the dam may be lying down and re-adjust her posture frequently (probably as a result of increased discomfort) without necessarily standing up.

Across the observations, FM cows spent longer lying lateral with the head rested than the FN and N cows. Time spent in that posture increased as parturition approached, accounting for nearly 80% of the observation duration in the last two hours of delivery. That particular posture is associated with the cow contracting and straining to deliver the calf in the stage 2 of labour (Owens et al., 1985). In the present study the increase in the time spent in that posture follows the increase in the frequency of contractions. To a certain extent, it also follows the level of restlessness of the cows reported earlier on. This is probably because during stage 2 of labour, after a bout of straining while laying recumbent with the head rested, it is not unusual from the cows to change posture or re-adjust their posture until the next contraction and straining bout begins (Owens et al., 1985; personal observations).

Cows raised their tail for longer as parturition approached as reported previously (Owens et al., 1985; Lidfors et al., 1994; Wehrend et al., 2006; Miedema et al., 2011b). FM cows had longer durations of tail raising throughout all the observation periods compared to the FN and N dams but an earlier increase in tail raising was not found in assisted dams as opposed to Miedema et al. (2011a).

Ingestive durations did not differ between cows with or without difficulty which is in line with similar eating duration found in the two hours preceding calf's birth (Miedema et al., 2011a) although feed and drinking intake were reduced 48 and 24h before the calf's birth (Proudfoot et al., 2009). There was also no difference on the duration of self-grooming between dystocial cows and cows calving normally contrary to differences previously seen pre-partum with small degree of difficulty (Manteca et al., 2010; Mainau et al., 2010b) and postpartum (Chapter 6; Barrier et al., 2010; Newby, personal

communication 2011). There was also no evidence of more frequent head turning and kicking among the assisted cows (Manteca et al., 2010; Mainau et al., 2010b), herein contained in the “irritation” behaviours.

The increase in restlessness, tail raising, lying, postural changes from lying to standing as parturition approaches is consistent with previous findings (Huzzey et al., 2005; Wehrend et al., 2006; Miedema et al., 2011a; Miedema et al., 2011b). In the present study, there was no difference in the frequency of exploratory behaviours. This is despite exploratory behaviours being part of the behaviours displayed by cows at calving (Lidfors et al., 1994; Wehrend et al., 2006; von Keyserlingk and Weary, 2007) and that cows performed increased ground licking in the last 6 hours preceding parturition (Miedema, 2009). It could be that exploratory behaviours are associated with stage 1 of parturition and that few changes happen within the last 6 hours.

#### **5.4.4. Implications of the observed changes in behaviour and progress at parturition: can it reflect more pain and discomfort in the dystocial cows?**

The progress of calving observed in the cows with calving difficulty suggest that these cows may be in later stages of labour for longer than the cows that calve naturally. In humans, labour pain increases as labour progresses, with greater dilatation of the cervix, more intense, frequent and longer uterine contractions occur (Corli et al., 1986; Lowe, 2002). Based on the assumptions that this also applies to cows, then it is very likely that the acute pain of calving is experienced for longer in the dystocial cows. In the present study, dystocial cows were also found to be more restless, to lie down more, have their tail raised for longer and to lay laterally with the head rested for longer (posture associated with straining). Those changes in behaviours are consistent with the experience of more discomfort, and possibly higher levels of pain. Behaviours such as tail raising, switching or wagging are commonly used to indicate pain in animals (Gregory, 2004a). During parturition, tail raising is particularly observed during stage 2 of labour, that is to say when the calf enters the birth canal. It is possible that the acute pain experienced in the perineal region might trigger the tail raising behaviour in ruminants. As an example, tail wagging is commonly used as a specific behavioural

indicator of pain following castration in lambs, piglets and calves (Molony et al., 1995; Molony et al., 2002; Hay et al., 2003). In dairy cows, vaginal irritation induced by application of disinfectant is also accompanied by tail raising (Grussel and Busch, 1998). During parturition, injuries such as tears and lacerations of the birth canal can occur, particularly if mechanical aids are used during assistance (Fishwick, 2011). High number of posture changes as well as increased restlessness, such as reported here, are also well demonstrated behavioural indicators of pain at least when tissue damage occurs as in tail docking or castration (Molony and Kent, 1997; Rutherford, 2002; Molony et al., 2002; Gregory, 2004a). In the present study, the behaviour of lying lateral with the head rested was performed during straining. This could be because of the pain associated with straining, discomfort of the calf being in the birth canal or because this posture allows better progress for the expulsion of the calf. Nonetheless, it is interesting to note that in rats, the stretching behaviours (defined as inward turning of the hindpaw, straining and squashing of the lower abdomen on the floor) observed during uterine contractions have been shown to be good behavioural indicators of parturition pain. Indeed, those behaviours have been described frequently in experimental models of visceral pain and were suppressed at parturition when morphine was administered via the epidural route (Catheline et al., 2006).

The behavioural changes observed and the experience of longer later stages of labour suggest higher levels of pain in the dystocial animals than in cows calving naturally. Further studies would be needed to validate those behaviours as behavioural indicators of pain, particularly if they can be suppressed or reduced with the use of analgesics. Physiological indicators of pain could also be investigated although the parturition process itself is an inflammatory response and that ceiling effects of hormonal release may be reached. Nonetheless, higher levels of vasopressin (hormone secreted in response to stressful/ painful stimuli) were found for heifers requiring assistance at birth and related to the amount of pain assessed on a visual analogue scale in dairy goats (Hydbring et al., 1999; Olsson et al., 2004). Higher levels of pain experienced at parturition for dystocial cows would also be consistent with evaluations from veterinarians (Huxley and Whay, 2006; Laven et al., 2009; Kielland et al., 2009).

Traditionally the focus on alleviating pain in farm animals has mostly been drawn to the study of acute pain resulting from farm mutilation (eg, castration or tail docking). Even when pain is recognised in diseased and injured animals such as in mastitis and lameness in dairy cows, this does not necessarily lead to administration of pain relief. This is despite the known benefits of alleviating pain (Barrett, 2004; Weary et al., 2006). Generally in ruminants, there are few studies associated with visceral and abdominal pain. Administration of a non-steroidal inflammatory drug to dairy calves suffering from diarrhoea, which may also trigger gastrointestinal pain, had benefits for the calves including faster recovery and reduction of sickness behaviour (Todd et al., 2010). Despite being of great focus in human obstetrics, there is also very sparse work done to study the pain experienced at parturition in farm animals. Yet, parturition is a key driver to the sustainability of farm systems. Assessing pain in animals is challenging in itself, particularly in prey animals to which cattle belong. The study of pain at parturition is made even more challenging that it involves different types of pain (visceral and somatic; diffused and localised; not necessary involving the same neural pathways) (Brownridge, 1995), that the parturition pain is mostly physiological as opposed to pathological (when it results from injuries or surgery) and that the process of parturition itself is an inflammatory process. Attempts to relieve pain during parturition in dairy cattle by administering NSAID drugs have been compromised by the antagonistic effect they have on the birth process, resulting in longer labour, higher rates of stillbirth and retained membranes (Newby, personal communication). Some research is currently focussing on relieving pain from existing trauma following difficulty at calving (Duffield and Newby, 2010; Mainau Brunsó, 2011), but not before expulsion of the calf; and this should be addressed.

#### **5.4.5. Implications of the observed changes in behaviour and progress at parturition: towards early detection of calving difficulty?**

Similarly to previous work (Wehrend et al., 2006; Proudfoot et al., 2009; Manteca et al., 2010; Miedema et al., 2011a), in the present study, earlier behavioural changes were found in cows experiencing calving difficulty as opposed to cows calving naturally.

Particularly, changes in tail raising and in the activity of the cow seem to be consistent indicators of calving difficulty. However, if using behaviours to automatically detect calving difficulty, emphasis needs to be made on changes over time and at the individual level. Changes in restlessness appeared earlier for dystocial cows but similar levels of restlessness can be reached between dystocial and eutocial animals. Moreover, although this was not the purpose of the study to investigate changes related to parity of the animals, previous studies have emphasised behavioural changes associated with parity of the cow that could potentially be confounded with difficulty at calving and this would also need to be taken into account (Mainau Brunsó, 2011; Miedema et al., 2011a).

## 5.5. Conclusions

Compared to cows that calved by themselves, cows with calving difficulty were in later stages of labour for longer, were more restless for longer and had their tail raised for longer over the course of parturition. This could relate to the expression of higher of pain when dystocia occurs and could also be used to help towards early detection of calving difficulty.

## **CHAPTER 6:**

### **Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam**

Adapted from: Barrier, A.C., E. Ruelle, M.J. Haskell, and C.M. Dwyer. 2012. Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. *Preventive Veterinary Medicine*, 103 (4): 248-256 (available in Appendix C).

In this Chapter, Elodie Ruelle collected the behavioural data from the videos under my supervision. I was responsible for the collection of videos, the design of the study, the statistical analysis, discussion and the writing of the manuscript.

## 6.1. Abstract

The neonate's development and survival is dependent upon being vigorous at birth and receiving appropriate maternal care. However, difficulty at delivery can result in less vigorous offspring and maternal care can be altered, probably as a consequence of exhaustion, pain and human intervention. The first three hours after expulsion of the calf were observed continuously from videos following twelve natural calvings and sixteen calvings assisted by farm staff (including four malpresentations) from Holstein cows. Calvings were balanced within groups for parity of the dam, genetic group, sex and birth weight of the calf, calving pen and calving season. Assisted calves were less vigorous with higher latencies to attempt to stand, achieve standing, walk and reach the udder than unassisted calves ( $P < 0.05$ ). Furthermore, assisted calves also tended to be less likely to stand and walk within the first 3 hours after birth ( $P < 0.1$ ), spent more time lying on their flank ( $P = 0.019$ ) and had more frequent bouts of this behaviour ( $P = 0.033$ ). Assisted dams did not take longer to lick the calf and performed as much licking as unassisted dams ( $P > 0.05$ ), indicating no delayed onset or impaired expression of maternal behaviour in dams given assistance at delivery. Study of potential pain-related behaviours revealed that assisted dams spent less time self grooming ( $P = 0.033$ ) than dams delivering naturally, which could suggest greater pain. However, there were no significant differences in any of the other pain-related behaviours. The results suggest that, although maternal behaviour was unaffected by a difficult delivery, dairy calves born following difficult calvings have lower vigour in the first three hours after birth than unassisted calves. This might have longer-term effects on the health and survival of the calves.

Key words: dystocia, dairy calves, calving ease, vigour, pain, maternal behaviour

## 6.2. Introduction

Parturition is a challenging process and a high-risk time for both the mother and her offspring. Difficulty at birth, also known as dystocia, results in lower vigour in offspring as shown in lambs (Dwyer, 2003; Dwyer and Lawrence, 2005a) and in beef calves

(Riley et al., 2004; Poppe et al., 2006; Hickson et al., 2008). It can be assessed by the speed with which an offspring displays a succession of neonatal behaviours shortly after birth that lead to standing (Dwyer, 2003; Nowak and Poindron, 2006; Baxter et al., 2008), and culminate in a successful bout of sucking.

High vigour at birth is crucial to the neonate's survival and development (Riley et al., 2004) and can result in a better welfare of the newborn (Mellor and Stafford, 2004; Dwyer, 2008b). Early ingestion of colostrum ensure the adequate acquisition of passive transfer of immunity in the immunologically naïve newborn calves, which is important for their survival (Bush and Staley, 1980; Beam et al., 2009; Waldner and Rosengren, 2009). Failure or inadequate passive transfer of immunity has been identified as the major contributing factor to the high morbidity and mortality of dairy heifers (Trotz-Williams et al., 2008; Beam et al., 2009; Vasseur et al., 2010). Dystocial calves are at even higher odds of stillbirth and perinatal mortality and morbidity (Chassagne et al., 1999; Johanson and Berger, 2003; Lombard et al., 2007), which may be related to poor vigour, inadequate colostrums intake and hence inadequate passive transfer of immunity. The rapidity of the onset and the quality of maternal care received contributes to the motivation and success of the calf in performing neonatal behaviours that will lead to ingestion of colostrum (Alexander and Williams, 1964; Dwyer, 2008a). For example, maternal grooming dries, cleans and stimulates the calf to seek the udder (Edwards, 1983; Nowak, 1998; Nowak and Poindron, 2006; von Keyserlingk and Weary, 2007) as well as stimulating the establishment of a durable bond between the two by ingestion and inhalation of amniotic fluid (Poindron, 2005; Poindron et al., 2007; Dwyer, 2008a). Receiving appropriate maternal care as quickly as possible is therefore crucial to the survival of the calf. However, difficulty at delivery can alter maternal care, probably as a consequence of exhaustion and pain and human intervention (Alexander et al., 1988; Dwyer et al., 2001; Fisher and Mellor, 2002).

Little attention has been given to the pain experienced by cows at parturition (Rushen et al., 2007), although components of parturition pain for cows may be similar to what is encountered in women (Gregory, 2004a) and dystocia has been rated as one of the most painful conditions in surveys of veterinarians (Huxley and Whay, 2006; Laven et al.,

2009; Kielland et al., 2009). Dystocial cows remain in labour for longer periods of time compared to a natural calving (Berglund et al., 1987). They may undergo more uterine contractions and straining, the occurrence of which is painful in women (Corli et al., 1986; Lowe, 2002). In dystocial deliveries, additional pain may also be experienced from pressure applied to extract the newborn (Scott, 2005) and stretching in the birth canal. In support of this, assisted cows have been reported to give a roar when the calf is extracted coinciding with the passing of the head and forelegs (Gregory, 2004a). Higher blood vasopressin (hormone secreted in response to stressful/ painful stimuli) concentration have been found with higher levels of parturition pain (visually assessed) in dairy goats (Olsson et al., 2004) and in heifers requiring assistance at birth (Hydbring et al., 1999). Beyond the birth of the calf, the cow still undergoes contractions and might suffer from underlying pain and injuries resulting from the parturition, in the subsequent days postpartum.

High vigour at birth and reception of appropriate care is crucial to the neonate's survival. However, a difficult delivery may lower calf's vigour in conjunction with altering maternal behaviour of the dams and the dam's postpartum pain should be addressed. The objective of the study was to examine the effect of calving difficulty on the subsequent vigour of the dairy calf, the onset and quality of the maternal behaviour and on some behavioural indicators of pain in the dams.

### 6.3. Materials and methods

#### 6.3.1. Animals, housing and calving management

The study took place at the SAC dairy Crichton Royal Farm (Dumfries, UK) in accordance with the UK regulations on animal care and ethics of experimental animals. Animals were from two genetic groups (S: animals selected toward greater milk solids production (fat and protein); C: animals selected to be the rolling UK average) as part of a long-term genetic breeding and feeding trial (Pryce et al., 1999; Bell and Roberts, 2007) and calvings took place all year round.

Preparturient Holstein heifers and cows were housed in one of the two contiguous roofed calving sheds (36m x 5.9m; 36m x 5.7m) approximately 3 weeks before they were due

to calve. There were on average 8 animals housed per shed at any one time and sheds were illuminated round the clock. As part of the long-term on-farm trial, one calving shed was provided with a low forage diet while the other was provided with a high forage diet. Multiparous cows from both genetic lines were allocated to a shed dependent upon their diet allocation but heifers were allocated to either shed to balance each feeding group for numbers as they were not allocated to a diet group until they calved. Animals were bedded on straw, provided with *ad libitum* access to water and sheds were cleaned regularly. Fresh total mixed ration was delivered at the feeder in the afternoon once every two days.

Dependent upon occupancy, when spotted in labour (e.g., cows straining, tail raised, restlessness) calving animals were isolated from their group-mates by a barrier placed within each shed, near the entrance and opposite to the feeders. Within each shed, the barrier set-up allowed contact with group-mates and created a maternity pen of 5m long (respective width of 5.9m and 5.7m) with access to *ad libitum* water.

Supervision of calvings, the decision to provide assistance, assistance and the allocation of a calving difficulty score was made by up to four experienced farm staff. Supervision was routinely ensured between 3h45 am and 11 pm with the possibility of an additional shift if judged necessary. It is farm practice to assist cows only when farm staff judges that a cow is in difficulty. Such judgement is based on criteria such as absence of labour progress (an investigation would be carried out after an hour without visible progress once waterbags have appeared), distress of the cow (e.g., excessive restlessness, vocalisation, cow lying flat, straining without progress) and distress of the calf (e.g., tongue protrusion, swelling of the calf). Assistance was used as a proxy measure for calving difficulty and scores of calving difficulty were: N (no assistance), FN/FM (Farmer assistance without / with malpresentation of the calf), VN/VM (Veterinarian assistance without/with malpresentation of the calf) and VC (caesarean section). Over the last two years, 18.7% of the calvings on this farm were assisted (33% of heifers and 11.9% of cows), which reflects average practice from UK Holstein dairy farms despite slightly higher assistance rates in heifers but lower in cows.

Once born, calves were separated from their mother within 24 hours but rarely spent less than 4-5 hours together. Birth weights were taken at the time of separation. The calf was ear-tagged, its navel disinfected with an iodine solution within the first 8 hours, and fed additional colostrum on judgement of the farm staff by stomach tubing.

### **6.3.2. Video-recording**

The calving sheds were continuously video monitored throughout the trial. For each shed, 12 weather proof infrared CCTV cameras (1/3" Sony Color CCD, EZ420IR-30, ezCCTV.com Ltd, Herts, UK), were equally distributed around the shed's roof so that all of the pen could be viewed at a height of 6.5m. The twelve cameras were connected to a high memory storage computer using Geovision (version 8, Geovision Inc., Taipei, Taiwan).

Animals were labelled with numbers to allow recognition on videos and numbers were remarked as required. To minimise disturbance, the marking procedure was performed before animals entered the pen, or when they were moved out of the pen once of week for farm's husbandry purposes (weight and body condition score).

### **6.3.3. Selection of the calvings and cow-calf pairs**

Only calvings leading to live singleton purebred Holstein calves were considered. Retrospectively, between November 2008 and 2009, 193 N, 26 FN and 4 FM calvings fitted those criteria. Out of the 26 FN calvings, 17 FN could be considered (six had no video footage, two in which the calf could not be observed and one where the cow had a prolapsus) and 12 calvings were chosen at random among them. As only 4 FM calvings were available at the time of the study, they were grouped with the FN category and called subsequently assisted animals (A) as opposed to non assisted animals (N). 12 N calvings were then recruited so that the normal calving group and the assisted calving group were balanced as far as possible for the following criteria by order of importance: parity of the mother (primiparous vs multiparous), sex of the calf, calf's birth weight, genetic group of the mother/ sire of the calf, calving shed (and hence diet) and calving season (Summer: April to September; Winter: October to March). The mean birth weight  $\pm$  s.d was  $42.8 \pm 5.0$ kg (A) and  $42.3 \pm 6.2$ kg (N) respectively.

For each of the recruited calvings, information on the body condition score (assessed on a five point scale by trained farm technicians) of the dam at calving and at conception were also retrieved. A summary of the characteristics of the calvings for both groups can be found in Table 6.1.

**Table 6.1** Summary of the characteristics of the calvings used within the study. Number of animals available (n) within categories are displayed as well as the mean birth weights of the singleton calves and its standard deviation (s.d)

		Assisted	Non-assisted
Parity of the dam	Primiparous	n=8	n=6
	Mutiparous	n=8	n=6
Calf's sex	Bull calf	n=8	n=6
	Heifer calf	n=8	n=6
Birth weight (kg)	Mean $\pm$ s.d	42.8 $\pm$ 5.0	42.3 $\pm$ 6.2
Genetic group	Control line	n=6	n=6
	Select line	n=10	n=6
Calving shed	Shed 1 (high forage)	n=11	n=5
	Shed 2 (low forage)	n=5	n=7
Calving season	Winter	n=10	n=6
	Summer	n=6	n=6
Calving BCS	Mean $\pm$ s.d	2.2 $\pm$ 0.3	2.4 $\pm$ 0.3
Conception BCS	Mean $\pm$ s.d	2.3 $\pm$ 0.3	2.2 $\pm$ 0.3

#### 6.3.4. Data collection: video scoring

The recruited calvings were watched for three hours continuously after the expulsion of the calf using Observer® XT 9 (Noldus, Wageningen, The Netherlands). After an initial training period, videos were observed by a single observer. Three one-hour video-clips, each from a different calving, were watched a second time to assess the intra-observer reliability of video scoring. Cohen's Kappa coefficients were obtained from the software and ranged from 0.61-0.72, suggesting a good agreement within the observer (Kaufman and Rosenthal, 2009).

The latencies of the calves to perform the first bout of the following neonatal behaviours once born were recorded: attempt standing, being supported on two feet, achieve standing, walk, reach the udder, and suck. Additionally, the time to achieve sternal recumbency (Schuijt and Taverne, 1994) as well as the total duration spent lying by the calf was assessed. The number of times and total duration of the calf lying on the flank was also recorded (for definitions, see Table 6.2).

**Table 6.2** Definitions of the calf behaviours scored

Lie flank	Calf is lying on the flank, with shoulder touching the ground and at least 3 legs extended. Head rests on the floor.
Sternal recumbency	The calf lies on his sternum with each front leg positioned on each side of his body
Attempt to stand	The calf has its four legs placed under its body, with the ventral part not touching the ground. Legs don't have to be fully extended
On two feet	The calf is supported by two legs that are extended
Achieve standing	The calf is supported by its four legs, all extended for at least 3 seconds
Walks	The calf is in a standing position and does more than two steps
Reaches the udder	The calf's muzzle is near the udder. Neck and head are curved up as in a sucking position.
Sucks	The calf is under the udder, teat in mouth, in a sucking position for more than five seconds



Neonatal behaviours: sternal recumbency (left), standing (middle) and attempt to suck (right)

To assess the maternal behaviour of the dam, the latency to lick the calf after having given birth as well as the amount of time spent on that activity was recorded. Furthermore the following behaviours that could be pain-related were recorded in the dams: durations of lying, self-grooming, walking and having the tail raised, frequency of posture transition, lying on the flank and tail switching as well as the latencies to drink and to lie down (for definitions, see Table 6.3)

**Table 6.3** Definitions of the pain-related behaviours scored for dams

Lie flank	Cow is lying on the flank, with shoulder touching the ground and at least 3 legs extended. Head rests on the floor.
Lie head rested	Cow is lying but not on the flank with her head resting on floor or part of the body
Posture transition	Cow switching from a lying (body in contact with ground) to standing position (cow on her four legs)
Walking	Cow is in a standing position and makes at least two steps forward or backwards.
Self-grooming	Cow licking herself
Tail switch	Tail swung to the side and then forcefully forward along the flank or directly back down (Miedema, personal communication)
Tail raised	Tail is raised and held away from the body (Miedema et al., 2011b) Was scored only when the cow was standing.
Latency to drink	For the first time after calf is expelled, the cow is standing at water trough, muzzle touching it
Latency to lie down	If the cow calved while standing, latency to lying was used. However, if the cow was already lying when she gave birth, the latency of the cow to lie after having stood for the first time was used.



Cow sniffing her newborn calf (left). Cow about to expel the placenta (right).

### **6.3.5. Data manipulation and statistical analysis**

Two observations (1 N and 1 FM) were shorter than three hours (2h50 and 2h24 respectively) and therefore, percentage of time was used for the statistical analysis rather than duration. Because “tail raised” was scored only when the cow was standing, the duration of that event was adjusted by dividing by the amount of time the cow spent standing before proceeding to analysis. Observations on the tail for dam 1830 were discarded because of the exceptionally high number of tail switches performed probably as a result of a high number of flies present.

Out of the 28 calves observed in the study, three assisted calves had been moved to the front of the cow at birth by the farm staff and ten calves were fed colostrum through stomach tubing within the 3 hour observation period (7 A and 3 N).

Statistical analysis were performed using Genstat 11<sup>th</sup> Edition (2008, VSN International Ltd). Data were first checked for normality using histograms and transformed when not (using logarithm, square-root and inverse function). However, most of the transformed data still did not fulfil this assumption of normality and were therefore analysed using non-parametric statistics.

Duration of licking performed by the dam and the amount of time spent lying on the flank by the calf (transformed with a cube root transformation) were analysed using Mixed Models. Characteristics of the calvings (genetic group, calving condition score, difference in condition score between conception and calving, birth weight of the newborn, sex of the calf, calving season, parity of the dam, calving shed, whether the calf was moved in front of the cow in the case of a difficult calving and whether a human had given additional colostrum to the calf within the observation period) were considered for inclusion in the models as well as biologically relevant interactions and only significant terms and calving difficulty were kept. Duration of licking was analysed using parity of the dam, genetic group of the dam and calving difficulty as fixed effects. Calving season and calving difficulty were used as fixed effect for the analysis of the time spent lying flank.

A Kaplan-Meier analysis using a Wilcoxon Peto-prentice test was performed to analyse the latencies of behaviours when censoring occurred (due to at least one animal not

performing a specific behaviour in the three hour window). One assisted calf was excluded from the analysis on the latency to reach sternal recumbency as it had been put in that position by the farmer at birth.

Fisher's exact tests were applied to determine whether animals from one group rather than the other group were more likely to perform a specific behaviour. Otherwise, Kruskal-Wallis non-parametric one-way-ANOVA tests were used to compare the assisted and non assisted groups, in which case the influence of other calving characteristics was also examined using the same test.

## 6.4. Results

### 6.4.1. Results on the vigour of the calf

Fisher exact tests showed that there was a tendency for calves from the assisted group to be less likely to stand ( $P=0.053$ ) and walk ( $P=0.088$ ) within the first three hours of birth, compared to calves delivered naturally. They were not less likely to suck ( $P>0.05$ ) but only a third of the assisted animals actually achieved successful sucking within that period of time.

Calves assisted at birth took longer to first attempt to stand, achieve standing, walk and reach the udder (Table 6.4). However, there was no difference in the time taken to be in a sternal recumbency position, to be on two feet and to achieve a successful suck ( $P\geq 0.05$ ; Table 6.4).

Furthermore, calves born from an assisted calving lay down on the flank for longer than calves born without assistance and so did calves born in summer compared to winter-born calves ( $P<0.05$ ; Figure 6.1). Assisted calves also performed more bouts of lying flank (median [lower and upper quartile]) (Assisted: 2 [1;4]; Non-assisted: 1 [1;2];  $P<0.05$ ) and tended to spent more time lying overall during the observation period (median [lower and upper quartile]) (Assisted: 163.3min [138.6; >180]; Non-assisted: 146.2 [121.5;156.8];  $P=0.051$ ).

**Table 6.4** Effect of calving difficulty on the median latencies (min), lower and higher 95% confidence intervals (CI) of calves to perform neonatal behaviours after having been expelled, as given following a Kaplan-Meier analysis using a Wilcoxon Peto-Prentice test. Count and percentage of the animals expressing the behaviours within three hours of life is presented for each treatment group.

	Assisted (n=16)		Non assisted (n=12)		P value
	Median [95% CI]	Count (%)	Median [95% CI]	Count (%)	
Sternal recumbency <sup>1</sup>	9.0 [5.7-25.1]	15* (100)	9.0 [5.5-11.1]	12 (100)	0.435
Attempts to stand	36.7 [14.8-49.7]	16 (100)	12.1 [10.5-17.6]	12 (100)	0.009
On two feet	43.3 [31.7-86.1]	15 (93.8)	35.2 [24.3-37.0]	12 (100)	0.106
Stands	99.4 [78.4- >180 <sup>2</sup> ]	11 (68.8)	70.1 [62.6-84.2]	12 (100)	0.025
Walks	166.4 [89.0- >180 <sup>2</sup> ]	9 (56.3)	86.4 [73.5-93.9]	11 (91.6)	0.034
Reaches udder <sup>3</sup>	>180 [128.8->180]	5 (31.3)	102.1 [86.4-112.6]	11 (91.6)	0.016
Sucks <sup>3</sup>	>180 [163.1->180]	4 (25)	170.8 [104.3->180]	6 (50)	0.314

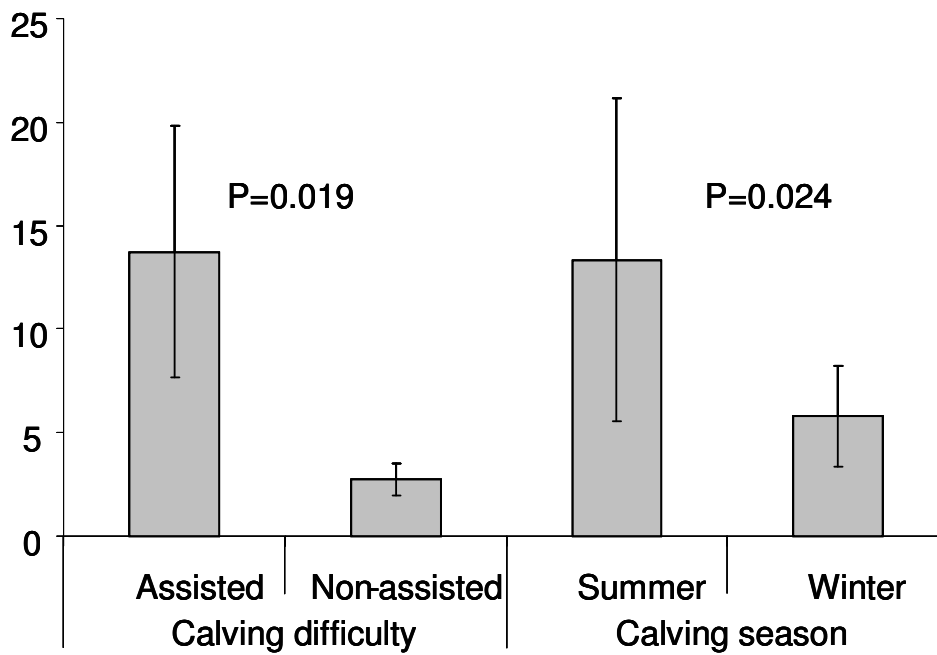
<sup>1</sup> Data presented for sternal recumbency result from a Kruskal-Wallis analysis and the median, lower and upper quartiles are presented.

\* Excludes one calf placed in this position at birth by the farmer.

<sup>2</sup> Values could not be estimated with the Kaplan-Meier analysis and therefore, the upper quartile of the raw data is presented instead.

<sup>3</sup> Values could not be estimated with the Kaplan-Meier analysis and therefore, the median, lower and upper quartiles of the raw data are presented instead.

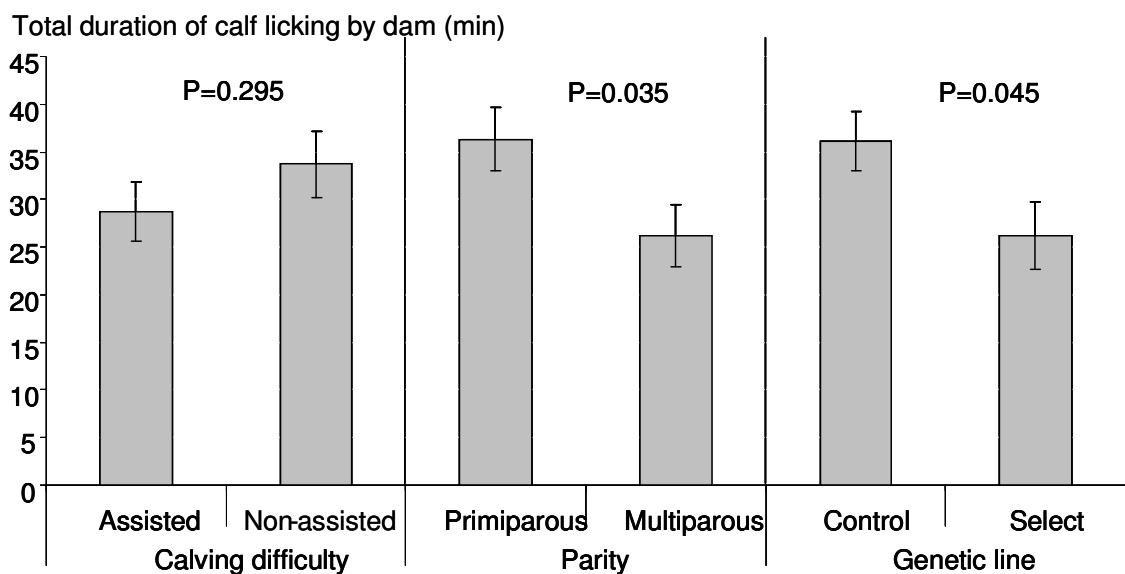
Total duration calf spent lying flank (min)



**Figure 6.1** Effect of calving difficulty (assisted (n=16); non assisted (n=12)) and calving season (summer (n=16); winter (n=12)) on the total time spent lying on the flank by calves. Means and standard error of the means of the raw data are presented. Analysis was performed on transformed percentages of duration of the observation.

#### 6.4.2. Onset and expression of the maternal behaviour

All dams but one assisted dam expressed maternal behaviours. This dam was kept in the analysis and given a censored value of three hours for the latency to lick the calf and a null value for the duration of calf licking. No difference between assisted and non-assisted cows was found on the median latency to lick a calf (in minutes [95% confidence interval]) (Assisted: 7.2 [0.9 -19.6]; Non-Assisted: 2.1 [1.0 - 8.0];  $P>0.05$ ) although cows calving in summer took longer to lick their calf (in minutes [95% confidence interval]) (Summer: 14.8 [2.9 -19.6]; Winter: 1.9 [0.6 – 5.9]; ( $P<0.05$ ). As well, assistance at birth did not affect the amount of time (in % of observation time) spent licking a calf (Figure 6.2;  $P>0.05$ ). However, surprisingly, multiparous cows were found to spend less time licking their calf compared to primiparous cows and as did dams from the genetic group S compared to dams from the C group ( $P<0.05$ ; Figure 6.2)



**Figure 6.2** Effect of calving difficulty (assisted (n=16); non assisted (n=12)), parity (primiparous (n=14); multiparous (n=14)) and genetic line (control (n=16); select (n=12)), on the duration spent by the dam licking her calf. Back-transformed predicted means and their standard errors are presented. Data were analysed using the percentage of duration of the observation.

### 6.4.3. Pain-related behaviours in the dams

Fisher exact tests revealed that there was no effect of calving difficulty on how likely cows were to lie on the flank, lie on the flank more than once and lie with the head rested ( $P \geq 0.05$ ) but cows having had an assisted delivery tended to be less likely to have performed an episode of self-grooming ( $P = 0.088$ ). No effect was found on any other putative behavioural indicators of pain ( $P \geq 0.05$ ; Table 6.5) except that cows having had an assisted delivery performed self-grooming for a shorter period of time than cows who were not provided with assistance ( $P \leq 0.05$ ; Table 6.5).

**Table 6.5** Effect of calving difficulty (assisted (n=16); non assisted (n=12)) on the behavioural indicators of pain postpartum in cows. Medians [lower and upper quartile] of the raw data are reported following Kruskal-Wallis test. Medians [lower and higher 95% confidence intervals] are reported following a Kaplan-Meier analysis using a Wilcoxon Peto-Prentice test

	Assisted (n=16)		Non assisted (n=12)		P value
Self-grooms <sup>1</sup> (s)	3.8	[0-16.7]	25.4	[0-138.8]	0.033
Lying duration <sup>1</sup> (min)	60.4	[20.0-105.5]	53.3	[8.7-72.9]	0.367
Walking <sup>1</sup> (min)	1.8	[1.2-3.5]	3.1	[1.9-5.7]	0.104
Tail raise <sup>1</sup> (% of standing)	75.7	[63.1-94.9]	76.4	[39.6-81.8]	0.367
Tail switches <sup>1</sup> (count)	24.8	[13.5-123.3]	98.2	[26.1-178.2]	0.294
Posture transitions <sup>1</sup> (count)	4	[2.5-6]	4.5	[3-9.5]	0.607
Latency to drink <sup>2</sup> (min)	145.2	[112.2->180 <sup>3</sup> ]	109.8	[97.6-161.9]	0.258
Latency to lie down <sup>2</sup> (min)	97.9	[61.3-117.3]	103.1	[63.7-142.8]	0.926

<sup>1</sup> Kruskal-Wallis analysis.

<sup>2</sup> Kaplan-Meier analysis

<sup>3</sup> Value could not be estimated and therefore the upper quartile of the raw data is presented instead.

## 6.5. Discussion

### 6.5.1. Calves from assisted calvings are less vigorous

Calves from assisted births were slower to express most of the neonatal behaviours leading to reach the udder compared to calves delivered naturally. Some of them did not even stand up during the observation period. They also spent more time lying on the flank and had more bouts of this behaviour, which is a position that does not require a lot of effort to be maintained. Overall, the present study shows that calves from an assisted calving have lower vigour than calves delivered naturally.

This finding is in line with previous reports of difficult delivery on vigour in dystocial dairy calves (Edwards, 1982; Diesch et al., 2004b), beef calves (Adams et al., 1995; Riley et al., 2004; Hickson et al., 2008) and lambs (Dwyer, 2003; Dwyer and Lawrence, 2005a). However, the present study disagrees with a recent study (Vasseur et al., 2009) but this may be due to their higher frequency of assistance. Inclusion of malpresented calves in the present study could perhaps have driven the effect but investigation of the effect of malpresentation from four calves was not possible and calf malpresentation was reported as not having a significant effect on latency to stand and suck (Edwards, 1982). Lower vigour at birth of dystocial calves can be explained by the presence of traumatic lesions (Berglund et al., 2003; Aksoy et al., 2009; Mee, 2010), longer durations of labour (Berglund et al., 1987), (prolonged) obstetrical intervention, and metabolic acidosis as a result of hypoxia or asphyxia (Tyler and Ramsey, 1991b; Mellor and Stafford, 2004; Alonso-Spilsbury et al., 2005). The latter impacts on the calf's adrenal function and metabolism and reduces the muscular tonicity and the ability of the calf to thermoregulate (Vermorel et al., 1989; Adams et al., 1995; Bellows and Lammoglia, 2000).

Despite lower vigour highlighted in assisted calves in our study, latency to the first sternal recumbency did not differ between groups, perhaps because this position does not require as much coordination or muscular effort to achieve in comparison to other behaviours. Only 10 out of the 28 experimental calves sucked within their first three hours of life. Median time to standing in the study (70min in non-assisted animals) was

slightly quicker than reported previously in dairy calves (Edwards and Broom, 1982) (median time of 105min and 130min for first and later parities respectively), but was much longer than the hour within which beef calves were reported to stand (Hyslop et al., 2008; Hickson et al., 2008). Contrary to those studies, we did not find an effect of parity of the dam or sex of the calf on calf's vigour. In the present study, calves spent more time on the flank in summer rather than in winter. It is possible that in cold environments, for thermoregulatory purposes, the calf would be more motivated to lie on the sternum because of the lower energy losses compared to lying on the flank. In other studies, Brahman calves, which may not thermoregulate well in cold environments (Godfrey et al., 1991) are reported to be less vigorous at low temperatures (Riley et al., 2004).

Lower vigour at birth raises longer-term consequences for the calves. Absorption capacity of immunoglobulins decreases rapidly after birth. Prompt sucking after birth maximises adequate acquisition of passive immunity (Beam et al., 2009). Current recommendations suggest that first meal of colostrum should occur within the first three to four hours of life (Morrison et al., 2010b). In the present study, only a third of the assisted calves had reached the udder by three hours. Assisted calves took at least 80 minutes longer to reach the udder than calves born naturally and this may have consequences on their immunity. Although the farmer may feed colostrum quickly after birth, this is largely dependent on calving time, human supervision and labour availability. The ability of the calf to suck quickly is all the more important in dairy systems that allow natural sucking in the first days or even weeks of life (Krohn, 2001). Moreover, when fed colostrum artificially, calves with lower vigour are willing to ingest lower amounts of colostrum (Vasseur et al., 2009). As a consequence, this can result in an inadequate transfer of the immunoglobulins as found in dystocial calves (Vermorel et al., 1989; Waldner and Rosengren, 2009; Gasparelli et al., 2009b), contributing to their poor survivability and higher morbidity compared to calves from a natural birth (Chassagne et al., 1999; Johanson and Berger, 2003; Lombard et al., 2007). Furthermore lower growth at weaning has been reported in dystocial beef calves (Bellows et al.,

1988, Goonewardene et al., 2003) as well as in lambs with low vigour (Matheson et al., 2010).

### **6.5.2. No evidence of an impaired maternal behaviour**

The onset of maternal behaviour, as assessed by the latency of the dam to lick the calf as well as the quantity of maternal behaviour measured through the amount of time the dam spent grooming her calf, was not affected by assistance being given at delivery in this study. This disagrees with the initial hypothesis and the observations made on other species (Alexander et al., 1988; Dwyer et al., 2001; Fisher and Mellor, 2002).

Nonetheless, in the present study, the low number of animals and high individual variation may have masked any underlying effect. Large individual variation could result from various levels of difficulty in the group of assisted animals as well as the potential presence of dams in the non-assisted group that may have benefited from assistance. Maternal behaviour is not a trait used for genetic evaluation and selection in dairy cattle, which might result in larger variation in its expression. Additionally, in three of the assisted births observed, the calf was moved in front of the cow by the farm staff, potentially adding an additional stimulus to the dam and introduced a slight bias.

The presence of the calf was likely to be an important motivator for the cows despite potential exhaustion and pain. A longer time window when time allocation to the care of the calf diminishes may have helped finding differences in the maintenance of maternal behaviour after a difficult calving. However, in sheep, the motivation of the ewe to lick her lamb at birth, reflects her motivation to do so throughout her lactation (Pickup and Dwyer, 2011).

In the present study, cows from the selected line licked their calves less than cows that were selected towards the UK average. This indicates that selection of cows towards milk production might have been unfavourable to the expression of this behaviour. Contrary to the literature (Edwards and Broom, 1982; Dwyer and Lawrence, 2000; von Keyserlingk and Weary, 2007), we found that the primiparae licked their calves more than the multiparae. This might be an artefact from the study.

### **6.5.3. Behavioural indicators of postpartum pain in the dams**

Cows receiving assistance at delivery performed less self-grooming than cows who delivered naturally. Grooming is an important behavioural trait in ruminants. A change in that behaviour is often interpreted as an indicator of pain or discomfort and therefore commonly used as a behavioural measure of animal pain (Molony and Kent, 1997; Rutherford, 2002; Weary et al., 2006). Self-grooming has previously been used as an indicator of postpartum pain in cows (Kolkman et al., 2010a) and was found to decrease in female rats following gynaecologic pain (Tong et al., 2006). Thus, in the present study, a decrease in self-grooming in the dystocial dams could indicate more pain or discomfort than in cows delivering naturally. However, contrary to what was expected, there were no significant differences in any of the other indicators which could have supported this finding. It is therefore believed that this study doesn't give enough evidence to support higher pain levels in dystocial dams in the 3 hours postpartum.

The three hour observation period might have been a limitation in finding any differences between the groups and a longer observation period investigating the recovery of the dams might have been more suitable. However, in a study comparing behavioural indicators of pain in beef cows undergoing delivery by caesarean or per vaginam up to 14 days postpartum, most of the differences were found in the 24 hours following delivery (Kolkman et al., 2010a). This suggests that studies focusing within a day after delivery may be more appropriate. A day long observation would have been compromised by dams entering the milking herd within 24h of the birth. Additionally, we explored only some of the behavioural indicators of pain and other indicators could have shown differences. It is highly likely that the worst of the pain was prior to the calf being expelled and therefore levels of pain afterwards may not differ between assisted and non-assisted dams. For example, behavioural differences have been found during stage 1 of parturition in dystocial dams and were suggested to be related to early expression of pain (Wehrend et al., 2006).

Finally, as for maternal behaviour, there was a large individual variation in the expression of the behavioural indicators of pain observed. This might reflect divergences in the sensitivity to, and expression of, pain between the dams as well as the potential

variability of difficulty that was experienced over the previous stages of parturition. A larger sample size would probably help in detecting any differences.

## 6.6. Conclusion

Calving difficulty, as assessed through assistance provided at the time of delivery, resulted in lower vigour in dairy calves. Low vigour is known to have a longer term impact on the health and survival of the neonates. Therefore, this raises concerns for the welfare of calves from a difficult delivery. Contrary to the expectations, maternal behaviour was not altered and behavioural indicators of pain did not indicate higher level of pains. However, there was a high individual variation in the dams that might have masked differences.

## 6.7. Acknowledgments

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## 6.8. Conflict of interest statement

The authors have no conflict of interest to declare.

## **CHAPTER 7:**

### **Body characteristics and pathology of dairy calves following stillbirth and dystocia**

In this Chapter, I was responsible for the experimental design and implementation of study 1. Postmortem examinations were performed by the SAC veterinary laboratory. I supervised and followed up the data collection of study 2, which were collected by Stephanie Birch, Ainsley Bagnall, David Bell and John Dickinson. I was responsible for the data analysis and writing of the manuscript.

## 7.1. Introduction

Worldwide, perinatal mortality of dairy calves varies from 2 to 10% (Mee et al., 2008). In the UK, as many as 8% of the dairy calves die in the perinatal period (first 48 hours) (Esslemont and Kossaibati, 1996; Wathes et al., 2008; Brickell et al., 2009b), with estimates of mortality rates of 11.6% and 4.3% in Holstein dairy calves, from first and later parities dams respectively (Eaglen et al., 2010b). Such high stillbirth rates raise animal welfare and economic concerns. The cost of stillbirth to the dairy industry is large with estimates of as much as \$125 million a year in the United States (Meyer et al., 2001a), while in the UK, calf mortality was estimated to cost the dairy industry £60 million yearly (DEFRA, 2003b). Ninety percent of calves that die perinatally are alive at the start of the calving process (Mee, 2008b; Mee, 2008c), thus emphasising how critical the birth process can be.

Difficulty at birth, or dystocia, as assessed by the amount of assistance provided at birth, is a major factor contributing to perinatal mortality in cattle with estimates of as many as 50% of the stillborn calves linked to a difficult birth (Meyer et al., 2001a; Berglund et al., 2003; Eriksson et al., 2004). Internationally, assistance at the time of delivery varies from 10% to over half of the calvings (Mee, 2008a), with a 16% rate in the Holstein-Friesian breed in the United Kingdom (Wall et al., 2010). Both birth difficulty and perinatal mortality are heritable although their heritabilities are low (Meyer et al., 2001b; Brickell et al., 2010; Eaglen et al., 2011b).

There are multiple risk factors and underlying reasons for stillbirth in dairy cattle (Nix et al., 1998; Chassagne et al., 1999; Mee et al., 2011) (for reviews, see Mee (2008c) and Meijering (1984)). These include infectious diseases such as BVD (Bovine Viral Diarrhea) or leptospirosis, and non-infectious diseases such as micronutrient deficiencies including iodine (leading to goitre), selenium and vitamin E deficiencies. They can also follow from anoxia, prematurity, growth retardation, placental dysfunctions and premature placental separation. With regards to dystocial stillbirths, they mainly result from trauma and anoxia, although death in-utero and premature placental expulsion can also occur during dystocia (Mee, 2008c).

Traumas following assisted deliveries may result in haemorrhages, injuries of the central nervous system and fractures in farm animals (Wilsmore, 1986; Aksoy et al., 2009) as well as in human babies (Benedetto et al., 2007; Brimacombe et al., 2008; Doumouchtsis and Arulkumaran, 2008). Because postmortems are usually performed for diagnostic purposes, published studies involving necropsies of stillborn calves, are usually aimed at determining the cause of death of those calves (e.g., Berglund et al., 2003; Mee, 2010; Waldner et al., 2010). To that extent, objective reports on the occurrence of specific injuries in relation to the dystocial or eutocial status of the stillborn calves should be investigated.

Because foeto-pelvic incompatibility is the main cause for dystocia (Mee, 2008a), it is not surprising that the size and conformation of calves born with and without birth difficulty can differ (Johanson and Berger, 2003; Kolkman et al., 2010b; Becker et al., 2011). Although an important proportion of dystocial dairy calves are stillborn, a proportion of them still survive the birth process. It is possible that the conformation of the calf may also determine whether a dystocial calf will be dead at birth or alive.

The objective of the following research was to investigate if dystocial stillborn calves present specific pathology compared to eutocial stillborn calves, and whether the dystocial calves that are stillborn differ in their shape from the dystocial calves that survive. The first study aimed at gathering descriptive data on the specific pathologies occurring in calves born dead following birth difficulty. The second study investigated the body weight and conformation of calves born with various degrees of difficulty, born alive or dead.

## 7.2. Material and methods

### 7.2.1. Animals, housing and management

The study took place on the Crichton Royal Farm (Dumfries, UK). This experimental dairy herd is commercially managed and consists of approximately 220 Holstein lactating cows from two different genetic groups (S: animals selected toward greater milk solids and C: animals selected towards the rolling UK average), each kept under two different feeding levels (low forage vs high forage diet) as part of a long-standing

trial (Pryce et al., 1999; Bell and Roberts, 2007). This study was carried out in accordance with the Home Office regulations on the use of experimental animals and also reviewed and approved from the SAC internal Animal Ethics Committee.

Experienced farm staff intervened at calving upon their judgment and each calf was assigned to a birth difficulty score (N: no assistance or normal birth; FN/FM: Calving assisted by farm staff with calf showing a normal / abnormal presentation; VN/VM: Calving assisted by a veterinarian with calf showing a normal / abnormal presentation; VC: Caesarian performed by a veterinarian to deliver the calf). Assistance at birth was used as a proxy measure for birth difficulty. Any calf receiving any kind of assistance (score other than N) was considered as having experienced a difficult birth (or being a dystocial calf).

### **7.2.2. Study 1: Post-mortem examination of stillborn calves born with/without birth difficulty**

#### ***Post-mortem examinations***

Twenty-two full term purebred Holstein calves from various degrees of birth difficulty (N: unassisted, n=10; FN: farmer assistance/no malpresentation of the calf, n=10; V: veterinary assistance, n=2) that were born dead on the SAC experimental farm (Dumfries, UK) between August 2009 and September 2010 were considered for post-mortem examination.

Gross post-mortem examinations were carried out by veterinary surgeons from the SAC Veterinary Laboratory (Dumfries, UK) which is UKAS accredited (Feltham, UK) as soon as possible after death of the calf. During the examination, calves were first weighed and their crown-rump length was measured. A gross post-mortem examination was then performed. Organs were checked for signs of inflation of the lungs (tissue sample floats in water or not), presence, extent and location of meconium stains, petechiae (small blood spots under the skin due to rupture of capillary vessels), bruising (slight or large), haemorrhages (slight: size of a fingerprint; large: at least the size of the palm of a hand), fractures, congestion of the brain and any other obvious abnormality. Presence and location of lesions (any petechiae, bruising or haemorrhage) was reported.

The weight of the thyroid was also noted and the weight ratio between thyroid and body weight was calculated as this can indicate iodine deficiency.



Gross post-mortem examination of a stillborn calf (all). The thoracic wall was inspected for presence of petechiae and bruises (right).

Out of the two vet assisted calves, 1 calf was born by caesarean section and suffered from schistosomus reflexus, a fatal congenital disorder (spine is curved upwards and abdominal viscera are exposed). The sole remaining calf was then excluded from the trial and a postmortem was not carried out. Data will therefore only be reported for the remaining 10 unassisted calves (N) and the 10 farmer assisted calves (no malpresentation) (FN).

### ***Dataset description***

There were two twin calves, a bull and a heifer born from two distinct pairs. In each birth difficulty score (N, FN), there were 7 bull and 3 heifer calves. Approximately half of the stillborn calves were from primiparous dams (11 out of 20) but stillborn calves from primiparae were more prevalent in the FN calves (8 out of 10) than in the N calves (3 out of 10). Two and 4 calves were from the select line (S) in the N and FN calves respectively. There were four missing records for crown-rump lengths of the calves (3 FN; 1 N) and for thyroid weights (2 FN; 2 N) which were considered as missing values.

### ***Data analysis***

T-tests were used to compare the calves' birth weight, length and thyroid/body weight ratios between the two groups of calves. Descriptive statistics are reported on the postmortem examinations.

### **7.2.3. Study 2: Incidence of stillbirth and the body characteristics of calves born dead and following birth difficulty.**

#### ***Birth records of the calves***

Full term purebred, non-deformed calves born between Sept 2008 and July 2010 were enrolled in the study (n=490), of which 23.5% of the calves had experienced birth difficulty. For each calf, the following characteristics were noted: birth weight of the calf, life status at birth (born alive, born dead), birth litter size (single or twin), sex of the calf, parity of the dam (I: primiparous; II: multiparous), genetic group of the calf (S: animals selected toward greater milk solids vs C: animals selected towards the rolling UK average), diet of the dam during her pregnancy (NT: average diet; XE: low forage diet; XM: high forage diet), sire and dam of the calf, the season born (S: April to September; W: October to March), year of birth and month of birth.

#### ***Body characteristics of the calves at birth***

All calves (born alive or dead) were weighed at birth (BW; kg) using a calibrated mechanical scale, measured for crown-rump length (CRL; cm) and heart girth (girth; cm) using a tape measure, and for their height at withers (height; cm) using a height stick (Swali et al., 2008; Brickell et al., 2009a). Up to four recorders took the measurements and were trained to achieve consistency in measurements. For each calf, the ponderal index (PI) and body mass index (BMI) was calculated as being  $\text{weight/length}^3$  ( $\text{kg/m}^3$ ) and  $\text{weight/length}^2$  ( $\text{kg/m}^2$ ) respectively (Baxter et al., 2008).



Stillborn calf (left) and newborn calf being weighed in a calibrated scale (right).

### ***Statistical analysis***

*Life status at birth.* The effect of birth difficulty on the life status of the calf at birth was analysed using a Fisher exact test (grouping all scores of difficulty together).

*Body characteristics of the calves.* BW, CRL, girth, height, BMI and PI were analysed with REML (REstricted Maximum Likelihood) in Genstat 11th Edition (2008, VSN International Ltd, Hemel Hempstead, UK), using a forward-stepwise technique.

Explanatory variables such as calf characteristics and the identity of the recorder were tested independently as univariates and became potential candidates for the multivariate model if they had P values less than 0.25. The candidate variables were then added into the multivariable model using stepwise selection techniques with the most significant variables with the highest Wald statistic being added first. Candidate variables were kept in the model with significance attributed at  $P < 0.05$  (when all other explanatory variables in the models had been fitted) and the interaction between life status at birth and birth difficulty were always forced in the model and fitted at the end. Biologically relevant interactions were tested once the whole model was set up. Calf identity nested within sire of the calf was used as a random model throughout. Unless otherwise stated hereafter, all the models included recorder, birth litter size, parity born, sex of the calf, the interaction between year and season, birth difficulty and stillbirth. Recorder was not included in the final analysis of BW. The interaction between year and season was not included for the analysis of CRL and height. In the latter, only year of birth was included. Birth litter size was not included for the analysis of PI. An interaction between birth litter size and genetic group was fitted for analysis of BW and girth. An interaction between parity born and sex of the calf was fitted the analysis of CRL, PI and BMI. Finally, an interaction was fitted between birth difficulty and genetic group for CRL and girth.

### 7.3. Results

#### 7.3.1. Study 1: Post mortem examination of stillborn calves born with/without birth difficulty

There was no difference in the birth weights, crown-rump length and thyroid/body weight ratios between stillborn calves born unassisted and with farm assistance ( $P>0.05$ ; Table 7.1). A summary of the number of stillborn calves displaying each of the injuries assessed for each group can be found in Table 7.2. Only one calf (1 N) had traces of meconium visible at the macroscopic level and none of the calves examined had obvious fractures. Regardless of level of assistance, half of the calves (6 N, 4 FN) examined had breathed as evidenced by inflated lungs. In terms of displaying lesions (12 calves out of 20), only calves born unassisted had lesions on the legs whereas both assisted and unassisted calves had lesions in the thoracic region and around the neck. Bruising was only reported in the assisted calves (4 out of 10) including two with significant bruising. When petechiae was detected (4 N, 3 FN), this was found mostly on the parietal pleura (mucosa enveloping the lungs) regardless of birth difficulty, with the exception of one FN calf that also had petechiae on the adjacent organs (thymus and heart).

**Table 7.1** Birth weight, length and thyroid/bodyweight ratio of purebred Holstein calves born dead without birth assistance (N; n=10) and born dead with birth difficulty (farmer assistance at birth and calves non malpresented; FN: n=10).

	N (n=10)	FN (n=10)	P value
Birth weight (kg)	43.3 ± 1.8	41.5 ± 1.4	n.s
Length (cm) <sup>1</sup>	96.8 ± 2.8	94.4 ± 2.2	n.s
Thyroid/body weight (g/kg) <sup>2</sup>	0.38 ± 0.02	0.41 ± 0.03	n.s

n.s:  $P>0.05$ ; <sup>1</sup>: N: n=9; FN: n=7; <sup>2</sup>: N: n=8; FN: n=8

**Table 7.2** Number of purebred Holstein calves born dead without birth assistance (N; n=10) and born dead with birth difficulty (farmer assistance at birth and calves non malpresented; FN: n=10) that showed the described conditions upon gross post-mortem examination.

		N (n=10)	FN (n=10)
Inflated lungs		6	4
Meconium staining		1	.
Petechiae	Parietal pleura	4	2
	Left axilla	.	1
	Other <sup>1</sup>	.	1
Bruising	Slight	.	2
	Large	.	2
Haemorrhages	Slight	3	3
	Large	.	2
Lesions	Neck	2	1
	Thorax	4	3
	Leg	2	.
Congested brain		2	1
Fractures		.	.

<sup>1</sup>: located on thymus and heart

### 7.3.2. Study 2: Stillbirth and body characteristics of calves born dead and calves born with birth difficulty.

#### ***Stillbirth following birth difficulty***

In the present study, 7% of the calves were born dead, of which 57% had experienced difficulty at birth. Being born dead was more likely for calves born with birth difficulty (Fisher exact test;  $P < 0.001$ ). Mortality at birth among scores of birth difficulty was as follows: 4% of the N calves, 10.8% of FN calves, 27.8% of the FM calves, and 100% for vet assisted calves.

#### ***Body characteristics of calves***

There was no significant interaction between birth difficulty and life status at birth on any of the body characteristics variables considered ( $P > 0.05$ ). This means that contrary to what was hypothesised, life status at birth did not depend on body characteristics of

dystocial calves. The effect of birth difficulty and life status at birth on the body characteristics of calves are presented in separate categories below.

**Birth difficulty.** FN calves weighed more at birth than calves born without assistance (+ 1.7kg; P<0.01; Table 7.3) but this was not the case for FM and V calves. There was no difference in height, girth and BMI between calves born with or without difficulty. However, FN calves from the Control genetic group were longer than calves born without assistance (+ 3.9cm; P<0.05; Table 7.3) but this was not the case for other scores of birth difficulty and for the Select calves. PI was also lower in FN and FM calves than in calves born naturally (- 5.2kg.m<sup>-3</sup> and - 7.5kg.m<sup>-3</sup> respectively; P<0.05; Table 7.3).

**Life status at birth.** Calves born dead were longer and had lower BMI and PI than calves born alive (Table 7.4; P<0.001). They were taller and larger when considering a 90% confidence interval (Table 7.4; P<0.10) but birth weights were not significantly different (Table 7.4; P>0.05).

**Table 7.3** Predicted means ± standard errors of the birth weight (BW) , height to withers (height), crown-rump length (CRL), body mass index (BMI) and ponderal index (PI) of calves born from various degrees of difficulty (N: no assistance; FN: farmer assistance without malpresentation of the calves; FM: farmer assistance with malpresentation of the calf; V: veterinary assistance). Within a row, means without a common superscript differ (P<0.05).

	Birth difficulty				P value
	N (n=375)	FN (n=92)	FM (n=18)	V (n=5)	
BW (kg)	38.6 ± 0.8 <sup>a</sup>	40.3 ± 0.8 <sup>b</sup>	37.0 ± 1.4 <sup>a</sup>	39.8 ± 2.3 <sup>a,b</sup>	**
Height (cm)	76.0 ± 0.6	76.5 ± 0.7	76.7 ± 1.1	74.4 ± 1.9	n.s
Girth (cm)	75.4 ± 0.7	76.2 ± 0.7	74.3 ± 1.2	73.1 ± 2.0	n.s
CRL (cm)	C 87.1 ± 0.9 <sup>a</sup>	91.0 ± 1.0 <sup>b</sup>	88.8 ± 1.6 <sup>a,b</sup>	87.1 ± 3.9 <sup>a,b</sup>	*
	S 88.8 ± 1.0	88.9 ± 1.2	87.0 ± 3.2	89.1 ± 3.2	
BMI (kg/m <sup>2</sup> )	52.7 ± 1.1	51.9 ± 1.2	49.4 ± 1.9	51.7 ± 3.1	n.s
PI (kg/m <sup>3</sup> )	C 63.5 ± 1.7 <sup>a</sup>	58.3 ± 2.0 <sup>b</sup>	56.0 ± 3.1 <sup>b</sup>	62.3 ± 7.3 <sup>a,b</sup>	*
	S 58.4 ± 1.8	60.0 ± 2.3	62.3 ± 6.0	56.3 ± 6.2	

\*: P<0.05; \*\*: P<0.01; n.s: P>0.05

**Table 7.4** Predicted means  $\pm$  standard errors of the birth weight (BW), height to withers (height), crown-rump length (CRL), body mass index (BMI) and ponderal index (PI) of calves alive or dead. Within a row, means without a common superscript differ ( $P < 0.05$ ).

	Stillbirth		P value
	Born alive (n=455)	Born dead (n=35)	
BW (kg)	39.3 $\pm$ 0.9	38.5 $\pm$ 1.0	n.s
Height (cm)	75.1 $\pm$ 0.7	76.7 $\pm$ 0.8	†
Girth (cm)	75.6 $\pm$ 0.8	74.0 $\pm$ 0.9	†
CRL (cm)	86.4 $\pm$ 1.1 <sup>a</sup>	90.6 $\pm$ 1.2 <sup>b</sup>	***
BMI (kg/m <sup>2</sup> )	54.3 $\pm$ 1.3 <sup>a</sup>	48.6 $\pm$ 1.4 <sup>b</sup>	***
PI (kg/m <sup>3</sup> )	64.2 $\pm$ 2.0 <sup>a</sup>	55.1 $\pm$ 2.3 <sup>b</sup>	***

\*\*\*:  $P < 0.001$ ; †:  $P < 0.01$  n.s:  $P > 0.10$

#### 7.4. Discussion

Stillborn calves born from assisted and unassisted deliveries had similar weight and measurements. There was no enlargement of thyroid, suggesting that there was no iodine deficiency. Half of the calves had inflated lungs and therefore had breathed. This means that half of the calves were not truly born dead but may have lived for at least a few seconds. It is surprising that only one calf had traces of meconium which is indicative of intra-uterine stress but it is possible that in some cases it had washed away following licking from the dam. There was no report of fractures in the assisted calves contrary to previous reports (Aksoy et al., 2009), probably because excessive force was not applied during extraction of the calves. Petechiae, bruising, hemorrhages and brain congestion were present in assisted as well as in unassisted births, but only assisted calves showed large presence of bruising and hemorrhages. This suggests that the extent of foetal stress and trauma may have been more important in cases of dystocia.

There was a higher proportion of stillborn calves following birth difficulty which is in accordance with previous studies (Chassagne et al., 1999; Johanson and Berger, 2003; Lombard et al., 2007). The body measurements reported in the study are in the same range as previously reported in UK dairy calves (Swali and Wathes, 2007). There was

no significant interaction of birth difficulty and life status at birth on any of the body characteristics of the calves. This means that dystocial calves that were stillborn had similar body characteristics to dystocial calves that survived. Nonetheless, calves born dead were longer and had lower BMI and PI than calves born alive and farmer assisted calves were generally heavier, and longer with lower PI than calves born unaided.

Stillborn calves did not differ in weight from calves born alive but they were approximately 4cm longer. This was independent of being born with or without assistance. As a knock-on effect, stillborn calves also had lower Ponderal and Body Mass Indexes than calves born alive. This means that calves born dead were longer and thinner compared to calves born alive. This result is in accordance with previous finding in piglets, where longer length, lower PI and BMI were the best predictors of stillbirth (Baxter et al., 2008).

FN calves were heavier at birth than unassisted calves which is in accordance with increasing birth weight associated with higher risk of dystocia (Johanson and Berger, 2003). This was not the case for higher degrees of difficulty. It is very likely that the cases of malpresentations and veterinary assistance had a different aetiology for calving difficulty compared to FN calves. FN calves were also 4cm longer than N calves but only for calves from the control genetic group. This represents an increase in length of only 4.5%, which might not be biologically relevant in terms of the increased difficulty of the dam to expel the calf during the birth process. Furthermore shoulder and thorax widths rather than length are critical factors for foeto-pelvic incompatibility (Becker et al., 2011), and heart girth did not differ in the present study between dystocial and eutocial calves.

Ponderal and Body Mass Index are measures of both weight and length simultaneously, and therefore relate to the body conformation of the animal. They indicate how weight changes relatively to length during the gestation period (Gluckman and Hanson, 2005). In human neonatal research, such weight:length ratios are used to determine the occurrence of intra-uterine growth retardation. This is a phenomena where intra-uterine growth is not achieved to its potential, and growth asymmetry as opposed to allometry is observed. This occurs when the foetal environment is not optimal, with the main cause

being a compromised nutrient transfer between the foetus and its mother, also known as placental insufficiency. This has implications on immediate survival at birth but also on subsequent growth and development, morbidity and mortality (Bertram and Hanson, 2001).

When extrapolating to calves in the present study, it is possible that underlying placental insufficiency may have played a role to explain why stillborn calves were found to have a slight different shape, although this would not be the sole reason for stillbirth to occur. It can be questioned whether a difference of 4cm is biologically relevant in calves to reflect any foetal restriction. In a study using heifer calves born from primiparous dairy cows as a model for foetal nutritional restriction, those calves were 5cm shorter at birth than calves born from multiparous cows (Swali and Wathes, 2007). However, they were also lighter and there was no difference in their Ponderal Indices at birth.

Another explanation may be that in calves that are longer in shape, the umbilical cord may rupture earlier or be clamped for longer during the birth process, ultimately leading to higher risk of stillbirth.

FN calves from the control genetic group had lower Ponderal Indices at birth regardless of them surviving the birth process, but without difference in Body Mass Index. It is not clear why a lower PI was seen in those calves. Their slight change of shape might have consequences on their future ability to develop, but this would need to be investigated.

## 7.5. Conclusion

Stillborn calves born from difficult calvings did not show evidence of unique types of trauma (or specific trauma), but displayed larger lesions than stillborn calves born naturally, suggesting that dystocial calves may have experienced greater trauma. Relying solely on the presence of haemorrhages, bruising, petechiae and brain congestion when conducting gross post-mortem examinations may not be sufficient to characterise dystocial calves. Body characteristics were related to stillbirth and to dystocia: calves born dead were longer and thinner than calves born alive, and farmer assisted calves were heavier at birth. However, the shape of dystocial calves did not differ between calves that survived the birth process and calves that did not.

## **CHAPTER 8:**

### **Difficulty at birth and the subsequent health, welfare and growth of dairy calves, with a focus in the neonatal period**

In this Chapter, I was responsible for following up and supervising the data collection. Data was collected by Stephanie Birch, Ainsley Bagnall, David Bell and John Dickinson (research technicians), farm staff and myself. I was responsible for the data treatment, data analysis and the writing of the manuscript.

## 8.1. Introduction

Making the transition from foetal life to extra-uterine life at birth is a challenging experience for mammals. During foetal life, the calf's dam provides a stable environment, free of pathogens, in which the calf is provided with oxygen and nutrients, and does not need to maintain its body temperature (for reviews, see Mellor (1988) and Breazile et al. (1988)). After birth, the survival of the calf will depend on its ability to maintain these functions by itself through adequate breathing, sucking, and maintenance of homeothermy. The late gestational period prepares the calf for this transition. As an example, elevated cortisol levels in the foetus near delivery ensure the maturation and development of the organs (Hubbert, 1974), as well as signal the onset of parturition to the dam (Liggins et al., 1979; Taverne et al., 1988; Silver, 1992). However, this transition does not necessarily go smoothly and the birth process, followed by the first hours of life, is a particularly critical time for the calves in terms of their subsequent health and survival. It is estimated that 11.6% and 4.3% of the Holstein dairy calves, from first and later parities dams respectively, die within 48h of birth in the United Kingdom (Eaglen et al., 2011b). Within the group of calves that die perinatally, 90% are alive at the start of the calving process and three quarters of the deaths occur within an hour of birth (Mee, 2008b; Mee, 2008c), thus emphasising how critical the birth process and early hours of life can be.

Difficulty at birth, or dystocia (as assessed by the amount of assistance provided at birth), is a major factor contributing to perinatal mortality in cattle with estimates of as many as 50% of stillbirths linked to a difficult birth (Meyer et al., 2001a; Berglund et al., 2003; Eriksson et al., 2004). Internationally, reported prevalence in dairy cattle of severe or considerable difficulty in calving varies from just below 2% to over 22% but assistance at calving (including lower degrees of difficulties) is much more prevalent, ranging from 10% to over half of the calvings (Mee, 2008a). As examples, in the United Kingdom, 16% of calvings are assisted on average (Wall et al., 2010) while in the United States, the average national rate of calving assistance is 20.6% in multiparous animals and 31% in primiparous animals (USDA, 2009). At the farm level, calving

management when difficulty does occur can impact on the survival of the calves. For instance, reluctance to call a vet when malpresentation occurs results in poorer calf immune function (Beam et al., 2009) but there may be other effects.

The occurrence of difficulty at birth negatively impacts on the production and welfare of the dairy cow as reviewed by Mee (2008a), and also seen previously in Chapters 2 to 5. For calves that survive the birth process despite dystocia, survival is not only compromised in the perinatal period but there is evidence that their health and survival can be affected in the neonatal period (Wittum et al., 1994a; Johanson and Berger, 2003; Lombard et al., 2007). Recently, evidence has emerged that dystocia could also have potentially long-term effects, with reduction in survival rates and milk production seen when they reach an adult age (Eaglen et al., 2010b; Heinrichs and Heinrichs, 2011; Henderson et al., 2011).

There are multiple reasons why dairy calves born from difficult births are at a higher risk of dying in the immediate perinatal period. These include severe hypoxia and acidosis (Massip, 1980a; Civelek et al., 2008), impaired breathing (Breazile et al., 1988) and internal injuries (Berglund et al., 2003; Gundelach et al., 2009; Mee, 2010).

In the neonatal period, it is possible that the experience of dystocia may also affect the ability of the calf to thermoregulate. Previous studies showing impaired thermoregulation have involved beef calves exposed to a cold challenge (Bellows and Lammoglia, 2000) or instrumented dairy calves maintained at a constant temperature in metabolic chambers (Vermorel et al., 1989). But this does not necessarily reflect what happens on dairy farms and other studies performed in ambient environments found no or biologically insignificant effects (Diesch et al., 2004a; Gasparelli et al., 2009a).

Low vigour in dystocial dairy calves (Edwards, 1982; Chapter 6) may be unfavourable to the acquisition of passive immunity through maternal transfer in colostrum. This is absolutely essential in immunologically naïve newborn calves as this is their sole resource to fight off diseases at an early age. Even with human intervention through bottle feeding of colostrum, dystocial calves were found to have lower serum immunoglobulins (Donovan et al., 1986; Odde, 1988); but this decreased transfer of passive immunity has not consistently been found (Stott and Reinhard, 1978). It is

therefore not always clear how dystocia affects the immunity of dairy calves in a farm context where calves are usually reared artificially and colostrum feeding is ensured by the farmer as opposed to the dam.

At a later age, reduced growth to weaning has been found in beef calves assisted at delivery (Bellows et al., 1988; Goonewardene et al., 2003). Nonetheless, in dairy heifer calves, no effects have previously been reported at 3 months of age (Lundborg et al., 2003) or to calving (Heinrichs et al., 2005). In those two studies however, growth was assessed through body measurements of the heifer calves at a unique time point. Similar body weights at calving alone may be due to farm management, so that targeted weights at calving are reached, rather than the demonstration of a similar ability to grow between dystocial and eutocial calves. Additionally, although previous research has reported undesirable effects of a difficult birth on the health and survival of the dystocial dairy calves, many of the effects that were reported were not the primary focus of the research studies (Donovan et al., 1986; Diesch et al., 2004b). Furthermore, many of the studies have focussed on the first 24 hours of life of the calves or on a few physiological indicators only (Odde, 1988; Vermorel et al., 1989; Civelek et al., 2008; Gasparelli et al., 2009a; Gasparelli et al., 2009b), was focussed on beef calves (Bellows et al., 1988; Wittum et al., 1994a; Bellows and Lammoglia, 2000; Goonewardene et al., 2003) or on later life from retrospective analysis of large datasets (Heinrichs and Heinrichs, 2011; Henderson et al., 2011; Eaglen et al., 2011b). Although dystocia was the focus of a longitudinal study on morbidity and mortality of dairy calves until four months of age (Lombard et al., 2007), there is still a lack of longitudinal studies looking at the consequences of a dystocial birth on the welfare of dairy calves.

The objective of the following study was to investigate the physiology, immune competency and survival of liveborn dairy calves following a difficult birth in the neonatal period and their growth in later life, with the use of a longitudinal study.

## 8.2. Materials and methods

### 8.2.1. Farm location and description

The study took place on the Crichton Royal Farm (Dumfries, UK). It was carried out in accordance with the Home Office regulations on the use of experimental animals and also reviewed and approved by the institution's internal Animal Ethics Committee. The experimental dairy farm is commercially managed and the herd consists of approximately 220 Holstein lactating cows. Cows are from two different genetic groups (S: animals selected towards greater milk solids vs C: animals selected towards the rolling UK average), each kept under two different feeding levels (low forage vs high forage diet) as part of a long-standing trial (Pryce et al., 1999) on-going on the farm at the time.

### 8.2.2. Birth difficulty and rearing of the calves

Preparturient Holstein heifers and cows were housed in one of two contiguous roofed calving sheds (36m x 5.9m; 36m x 5.7m) from approximately 3 weeks before they were due to calve. On average there were 8 animals housed per shed at any one time and the sheds were illuminated 24 hours a day. As part of a long-term on-farm trial one calving shed was provided with a low forage diet while the other was provided with a high forage diet. Cows from both genetic lines were allocated to one of the two sheds dependent upon their diet allocation. Heifers were not allocated to a diet group until they calved, and were allocated to either diet group (and appropriate shed) to balance the numbers of animals in each feeding group. Animals were bedded on straw, provided with *ad libitum* access to water and the sheds were cleaned regularly. Fresh total mixed ration was delivered at the feed bunk in the afternoon once every two days.

Dependent upon occupancy, when cows were identified as being in labour (e.g., cows straining, tail raised, restlessness), they were isolated from their group-mates in a maternity pen of 5m long (respective width of 5.9m and 5.7m) created by a barrier placed across one end of the shed, near the entrance and opposite to the feed bunks. The barrier set-up allowed contact with group-mates and access to *ad libitum* water.

Regarding supervision of calvings, the decision to provide assistance, the type of assistance and the allocation of a calving difficulty score was made by four experienced farm staff. Supervision was routinely provided between 3h45 am and 11pm with the possibility of an additional night shift if judged necessary. It was farm practice to assist cows only when a cow was judged to be in difficulty. Such judgment was based on criteria such as absence of labour progress (an investigation would be carried out after an hour without visible progress once the first waterbag has appeared), distress of the cow (e.g., excessive restlessness, vocalisation, cow lying flat, straining without progress) and distress of the calf (e.g., tongue protrusion, swelling of the calf). Assistance was used as a proxy measure for birth difficulty and scores of birth difficulty were: N (no assistance), FN (Farmer assistance with normal presentation of the calf) FM (Farmer assistance with malpresentation of the calf), VN (Veterinary assistance with normal presentation of the calf), VM (Veterinary assistance with malpresentation of the calf) and VC (caesarean section). Over the last two years, 18.7% of the calvings on this farm were assisted (33% of heifers and 11.9% of cows), which reflects average levels from the UK Holstein dairy farms.

Once born, calves were separated from their dam within 24 hours but rarely spent less than 5 hours together. The calf was ear-tagged, its navel disinfected with an iodine solution. Unless calf's sucking had been judged satisfactory by farm staff, calves were fed 2L of colostrum, milked from their dam, through stomach tubing. Calves were then individually housed in straw-bedded pens (1.8 x 1.2m) and fed 2L of milk from newly calved cows twice a day, from a bucket with an artificial teat. They also had *ad libitum* access to water and concentrate (Earlycare Q R D, BOCMS Pauls Ltd, Ipswich, UK) (3.5% oil, 17% protein, 13.5% fibre, 10% ash, 13.8% moisture) and received preventive treatment against diarrhoea caused by cryptosporidium (Halocur, Intervet/Schering-Plough Animal Health, Milton Keynes, UK) during the first six days of life. By approximately day 11 of life, heifer calves were moved to straw bedded group pens (14.3 x 4.3m), which were topped up with straw and cleaned regularly. They were treated at entry against common bovine respiratory diseases (Draxxin®, Pfizer Animal Health, Walton Oaks, UK) and vaccinated against *Trichophyton verrucosum* (Bovilis

Ringvac, Intervet/Schering-Plough Animal Health, Milton Keynes, UK), a skin fungus. In group pens, calves were fed through automatic milk feeders (HL 100, Holm & Laue, Westerrönnfeld, Germany) dispensing milk replacer at 40°C (Gold top, BOCMS Pauls Ltd, Ipswich, UK:18% oil, 23% protein, 0.1% fibre, 8% ash) diluted at 150g/L with an allowance of up to 6L per day. Weaning occurred at 50 days of age with milk allowance restricted by 0.6L/ day from 40 days of age. Calves also had access to *ad libitum* water, straw and the mix described above. Once weaned, heifers were kept and managed in groups of similar age. They were inseminated when their weight was greater than 330kg which was at around 15 months of age.



Newly born calf still with her dam (left) and being fed additional colostrum (right).



Pre-weaning, single housing of the calves in which they are bottle-fed milk (left) before being moved to a group pen (right) with automatic feeders (right).

### 8.2.3. Calf recruitment and description of the calves

All purebred Holstein calves born on the farm between 08<sup>th</sup> Sept 2008 and 13<sup>th</sup> August 2010 were eligible for enrolment in the trial. For each calf, the following characteristics were noted: birth weight of the calf, life status at birth (born alive, born dead), birth litter size (single or twin), sex of the calf, parity of the dam (I: primiparous; II: multiparous), genetic group of the calf (S: animals selected towards greater milk solids vs C: animals selected towards the rolling UK average), diet of the dam during her pregnancy (NT: average diet; XE: low forage diet; XM: high forage diet), sire and dam of the calf, the season born (S: April to September; W: October to March), year of birth and month of birth.

Bull calves took part in the experiment from birth until they were sold or culled. In total, 496 calves (240 heifers and 256 bulls) were monitored throughout the trial, of which 23.8% experienced some difficulty at birth (Table 8.1.).

**Table 8.1** Description of the number of calves monitored throughout the trial in each degree of birth difficulty.

		Birth difficulty					Total (n=496)	
		N (n=378)	FN (n=93)	FM (n=18)	VN (n=2)	VM (n=3)		VC (n=2)
<u>Born dead</u>	Total	15	10	5	1	3	2	36
	Bulls	8	6	3	1	1	1	20
	Heifers	7	4	2	0	2	1	16
<u>Born alive</u>	Total	363	83	13	1	0	0	460
	Bulls	178	50	8	0	0	0	236
	Heifers	185	33	5	1	0	0	224

### 8.2.4. Data collection

#### ***Calf survival to weaning***

Events of sale, death and euthanasia were recorded throughout the trial and survival at weaning determined (alive or dead).

### ***Salivary cortisol***

**Saliva sampling.** To allow assessment of salivary cortisol, saliva samples were obtained for all the calves enrolled on the trial until 1<sup>st</sup> December 2009. Thereafter and until April 2010, only calves born with difficulty (any calf born with assistance) and the next two unassisted calves (N calves) were sampled.

Samples were taken in the morning, between 8h and 11h of the day of birth or the following day. The calf was approached calmly and cotton buds (MP Cotton buds large, Millpledge Veterinary, Clarborough, United Kingdom) were gently rubbed inside the calves' mouths. The cotton buds were then spun in salivettes (Salivette®, Sartsted, Nümbrecht, Germany) and saliva was stored at -20C before further analysis was performed.

**Saliva sample recruiting.** Retrospectively, existing samples from calves with birth difficulty were paired with samples from calves born without assistance using the following criteria: the time from birth to sampling (within 12h if sample taken on the day of birth or between 12 and 24h when taken on the following day), birth litter size, parity of the dam, sex of the calf, birth weight of the calf ( $\pm$  2kg), genetic group of the calf, diet of the dam during her pregnancy and when possible the sire of the calf and the season born. Eighty-four of the samples collected (including controls) could be analysed. All available FM samples and their controls (N sample) followed by singleton FN calves that best matched the allocated control sample were included. This process led to the recruiting of 42 N, 34 FN and 8 FM samples (42 paired samples).

**Salivary cortisol assay.** Samples were assayed, in duplicates using a Radioimmuno assay (RIA) with a Coat-a-Count® kit (RIA Siemens Healthcare Diagnostics, UK) and the guidelines provided. Results were obtained using the software Assayzap (Biosoft, Cambridge, UK). A first assay was unsuccessful due to a technical problem and the assay was therefore run a second time. One N sample could not be rerun and was therefore considered as a missing value. The inter-assay coefficients of variation for low (3.07ng/ml), medium (9.99ng/ml) and high controls (36.6ng/ml) were 4.1%, 6.7% and 6.3% respectively, and the corresponding intra-assay coefficients of variation were 5.7%, 7.8% and 6.3%.

### ***Rectal temperatures***

Rectal temperatures were obtained from all the calves enrolled on the trial until 1<sup>st</sup> December 2009, and thereafter only for calves born with difficulty (any calf born with assistance) and the following two N calves until 1<sup>st</sup> July 2010.

Rectal temperatures of the calves (N: n=294; FN: n=75; FM: n=13) were taken on the day of birth and for the following three days at approximately the same time (between 8 and 10 am) using a digital rectal thermometer. An additional measure was taken for the female calves when they were moved to group pens (median age=11 days).

Calves that received a non-routine health treatment during any of the temperature records were considered as being sick.

### ***Passive immune transfer***

Achievement of passive immune transfer was assessed for calves enrolled on the trial between 17<sup>th</sup> September 2008 and 1<sup>st</sup> July 2010. Enrolment was on the basis of any calf born with difficulty followed by the next available calf born without assistance and alive at time of sampling (N: n=82; FN: n=65; FM: n=11). A blood sample was taken from the jugular vein between 3 and 7 days of age using a vacutainer (BD vacutainer®) and a needle (PrecisionGlide TM, 0.8x 25mm, BD vacutainer®). Samples were chilled at 4°C, spun for 4 minutes at 4000g and plasma isolated. Fresh plasma was analysed for estimates of immunoglobulin levels through the use of ZST tests (Zinc Sulphate Turbidimetry), which precipitates globulins with a zinc sulphate solution (Donovan et al., 1986; Weaver et al., 2000; Morrison et al., 2010a). Analysis was performed by the SAC Veterinary Laboratory (Dumfries, UK) which is UKAS accredited (Feltham, UK).

### ***Milk refusals***

Any occurrence and amount of milk refusals (whole,  $\frac{3}{4}$ ,  $\frac{1}{2}$ , or  $\frac{1}{4}$  of bucket) was noted for the liveborn heifers that spent at least a day in a crate (N: n=181; FN: n=31; FM: n=5).

### ***Health treatments of the calves***

For calves (bulls and heifers) that stayed in the herd for over 24 hours (N: n=357 ; FN: n=80; FM: n=13 ), the number of non routine health treatments received in the first 60

days of age and the number of days with at least one health treatment (days treated) was retrieved. The category of each treatment given was classified as: antibiotic, anti-inflammatory, anticoccidial, supplementation and others.



Sick calf (left) in contrast with an apparent healthy calf (right).

### ***Growth of the female calves to first service***

For female calves enrolled on the trial, additional to birth weights, body weights (BW) were taken at entrance in the group pen, at weaning and monthly thereafter until first service, with the last measurement taken in January 2011. Post-weaning weighing occurred in batches at monthly intervals for all weaned heifers over a maximum two day period.



Weaned heifer calves in their home pen (left) and being weighed on a monthly basis post-weaning (right).

### **8.2.5. Data handling and analysis**

Statistical analyses were performed using Genstat 11<sup>th</sup> Edition (2008, VSN International Ltd, Hemel Hempstead, UK) and Minitab 15 (Minitab Ltd, Coventry, UK). Three N, one FN and one VN calves were premature (gestation length <265d) and one VC calf was deformed at birth (*schistosoma reflexus*). They were therefore excluded from any analysis. Due to availability of the data, the effect of birth difficulty could only be investigated for FN and FM calves and not on vet assisted calves.

#### ***Stillbirth and mortality to weaning***

The effect of birth difficulty on the life status of the calf at birth and at weaning for the heifer calves was analysed using a Fisher exact test (grouping FN and FM scores together). As three heifers (2 N and 1 FN) were sold before weaning, they were excluded from the calculation of mortality rates to weaning.

#### ***Salivary cortisol***

The mean value of the replicates was used for each calf. Log transformed data were analysed using a REML (REstricted Maximum Likelihood) analysis. The model included birth difficulty as a fixed effect and pair number as a random effect.

#### ***Passive Immune transfer***

Data (N: n=82; FN: n=65; FM: n=11) were log transformed and analysed with REML using a forward-stepwise technique. Calf characteristics, age at sampling, additional colostrum received and observation of suckling were tested as univariates and became potential candidates for the multivariate model if it had a P value less than 0.25. The candidate variables were then added into the multivariable model using stepwise selection techniques with the most significant variables with the highest Wald statistic being added first. Candidate variables were kept in the model with significance attributed at P <0.05 (when all other explanatory variables in the models had been fitted) and birth difficulty was always forced in the model and fitted at the end. Interactions were tested once the whole model was set up. The final model included birth difficulty and age at sampling as a fixed effect and birth weight as a covariate.

### ***Rectal temperatures***

Temperature records were analysed using REML with repeated measurements using a power model for correlations within subjects over time. Calves with missing values due to death/culling within that period were included. A similar procedure as described above was followed. Calf characteristics and whether a non-routine health treatment was administered prior to the recording day (yes/no) were considered as potential candidates. Although birth litter size was a significant effect, it was not included in the model. The rationale for that decision was that all liveborn twin calves (n=28) were born from multiparous dams (I: n=118; II: n=239) and it was felt that parity would then better correct for both effects.

Temperatures of all calves (bulls and heifers) during the first four days of life (N: n=294; FN: n=75; FM: n=13) were first analysed. The final model contained the interactions of birth weight and time, parity born and time, sire of the calf, sex of the calf and birth difficulty as the explanatory variables.

Temperatures of heifer calves alone were also analysed in a separate analysis (N: n=149; FN: n=30; FM: n=5), which also included the temperature record taken at entry to group pens (median age =11d), considered as a fixed age of 11 days. 14 calves (12 N, 2 FN) were excluded from the dataset because they had been treated for sickness and this affected the data significantly. For this analysis, the model used the interactions of birth weight and time, and birth difficulty and time as explanatory variables.

### ***Milk refusals***

For this analysis all heifer calves that were assisted (FN and FM) were grouped in a single category. The occurrence of milk refusals was investigated for liveborn heifers that spent at least a day in a pen using a Fisher exact test. Restricting the analysis to heifers with refusals only, the individual total number and total volume of refusals were divided by the number of days spent in the pen to adjust for individual variation in the length of time spent in the pens. Differences between unassisted and assisted animals were analysed using non-parametric Kruskal-Wallis tests.

### ***Health treatments***

As all the considered calves did not stay in the herd for the 60 day period (eg, the sale and culling of bull calves, death of heifers), each individual calf was not exposed to the same probability of receiving a health treatment. Therefore, the number of days that each individual calf stayed in herd was calculated (days exposed). The individual frequencies of treatments received (expressed for 100 days exposed) were calculated by dividing the total number of treatments received by the number of days exposed. They were analysed using a non parametric Kruskal-Wallis test. For each score of difficulty, the sum of days treated and untreated was calculated and differences in the proportions of days with treatment assessed using a chi-square analysis. Each analysis was also performed separately on heifer calves only (N: n=184; FN: n=28; FM: n=5).

### ***Growth of the female calves to first service***

Random coefficient regression models were used to analyse the growth of the heifer calves over time. This type of analysis was chosen because it allows analysis of repeated individual body weight measures despite calves having various ages at recordings post-weaning. It does so by fitting growth curves at individual levels, which means estimations can be obtained if there are unequal numbers of recordings per calf, as it is the case here. Considering the low number of FM female calves still alive at weaning (n=3) that would then have over 3 weight recordings, FN and FM female calves were grouped in a single category (A: birth assisted calves). Female calves with fewer than 3 records were nonetheless kept in the dataset (N: n=15; FN: n=5; FM: n=2). The overall trend of the growth curves was modelled using a quadratic regression of body weight on age of the calf. For the purpose of the analysis, age was rescaled to centred age (ageC) (by dividing by the standard deviation of age) so that convergence could be obtained.

Calf characteristics were considered as potential candidates for inclusion in the model and following the procedure described previously only significant terms were retained. As previously, birth litter size was excluded because of its confounding with parity.

The final model retained centred age squared, parity born and the interaction between centred age and genetic group, and the interaction between centred age and birth difficulty. The random model contained the quadratic regression of body weight on age of the calf.

## 8.3. Results

### 8.3.1. Mortality to weaning

Mortality to weaning in the assisted heifer calves (all scores together) was 2.8 times higher than in non-assisted scores (Fisher exact test;  $P < 0.01$ ). Mortality in the N calves was 4.9%, 9.4% in the FN calves and 40% in the FM calves (Table 8.2).

**Table 8.2** Survival of the full-term liveborn heifer calves from birth to weaning.

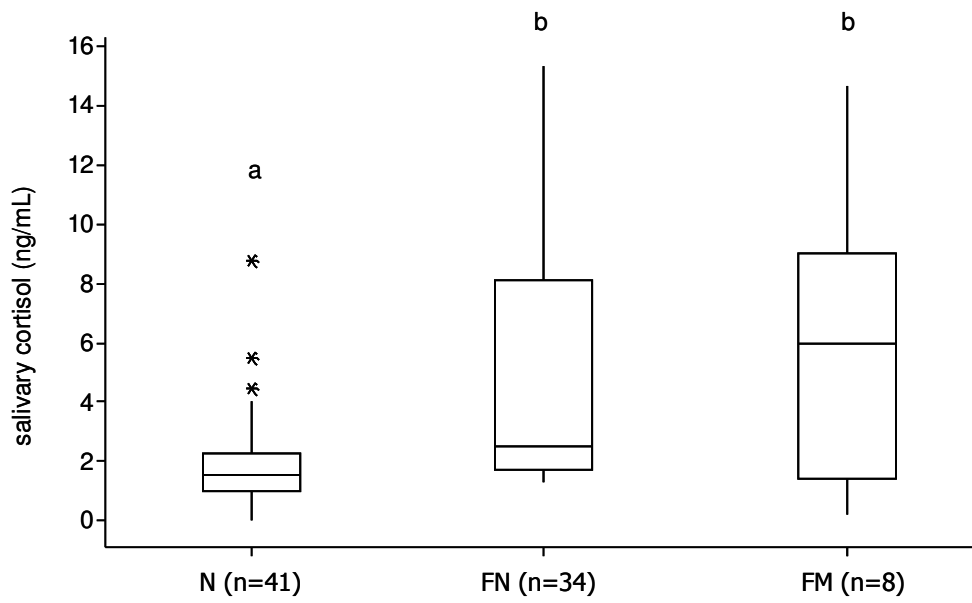
	Birth difficulty				Total
	N	FN	FM	VN	
Liveborn	184	32	5	0	222
Sold	2	1	0	0	3
Died/ put down	9	3	2	0	12
In herd at weaning	173	28	3	1	205
Mortality rate to weaning	4.9%	9.4%	40%	0%	6.7%

### 8.3.2. Salivary cortisol

Calves born with assistance (FN and FM calves) had up to 4 times higher median salivary cortisol levels (ng/mL) in the first 24 hours of life compared to calves born naturally (Figure 8.1;  $P < 0.001$ ).

### 8.3.3. Milk refusals

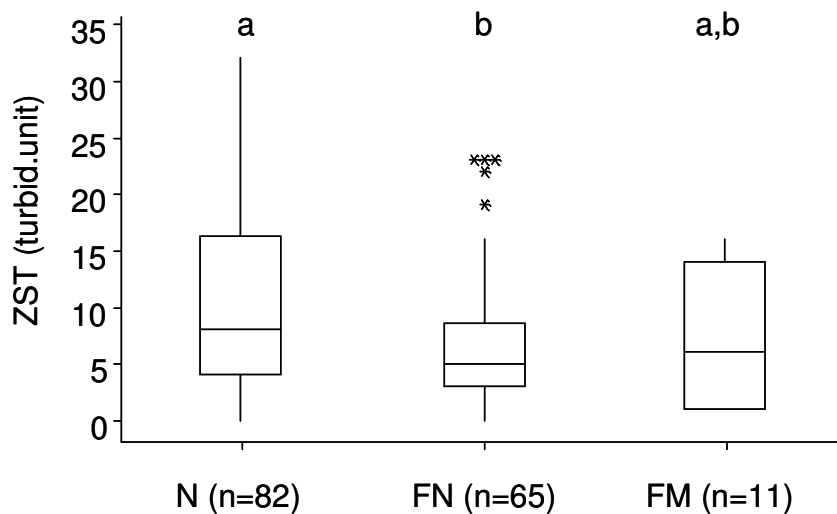
Approximately 20% of the heifer calves had at least one milk refusal when housed in pens (N:  $n=35$ ; FN:  $n=6$ ; FM:  $n=1$ ). Calves born with assistance (FN and FM calves grouped together) were not more likely to refuse milk offered from the buckets ( $P > 0.05$ ) than calves that were not assisted. Among the heifer calves that had refusals, the total number of refusals and the total volume refused (both adjusted for length of time spent in pens) did not differ between the group of calves ( $P > 0.05$ ).



**Figure 8.1** Salivary cortisol (ng/mL) within 24 hours of birth in dairy Holstein calves following different degrees of birth difficulty (N: no assistance; FN: farm assistance without calf malpresentation; FM: farm assistance with calf malpresentation). Median and IQR of the raw data are presented. Medians with without a common letter differ ( $P < 0.05$ ).

### 8.3.4. Passive immune transfer

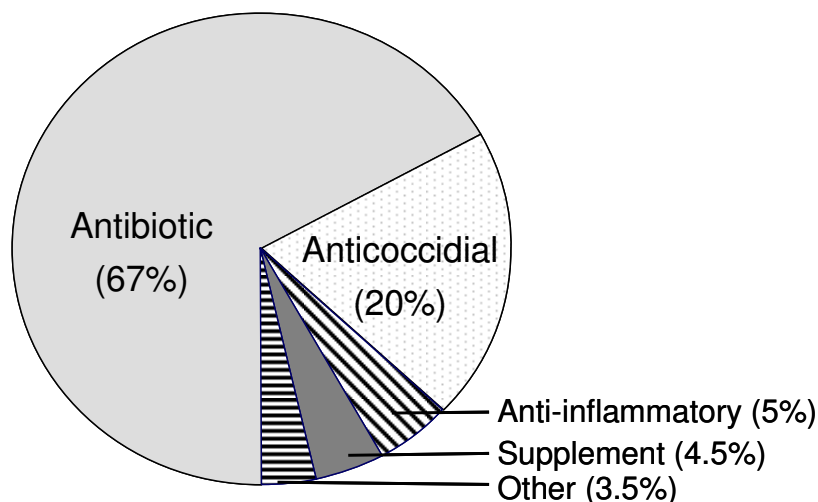
Only 15.9% of the N calves achieved the recommended immunity levels (ZST>19 units) (Knowles et al., 2000) and only 6% of the FN and none of the FM calves achieved recommended levels. Absolute failure of passive transfer (ZST<5 units) was observed in 26.8%, 43.1% and 45.5% of the N, FN and FM calves respectively (34.8% overall). FN calves had lower ZST scores than non assisted calves but this was not seen in the FM calves (Figure 8.2; P=0.03)



**Figure 8.2** Boxplot of the ZST scores (turbidimetry units) within a week of age in dairy Holstein calves following different degrees of birth difficulty (N: no assistance; FN: farm assistance without calf malpresentation; FM: farm assistance with calf malpresentation). Median and interquartile range (IQR) of the raw data are presented. Medians without a common letter differ (P<0.05).

### 8.3.5. Non-routine health treatments received

25.3, 26.8 and 53.8% of the N, FN and FM calves respectively received at least one treatment during the considered 60 day period. When considering heifers only, 80%, 25% and 36.4% of the heifer calves received at least one treatment. The type of non-routine health treatments that were administered is shown in Figure 8.3.



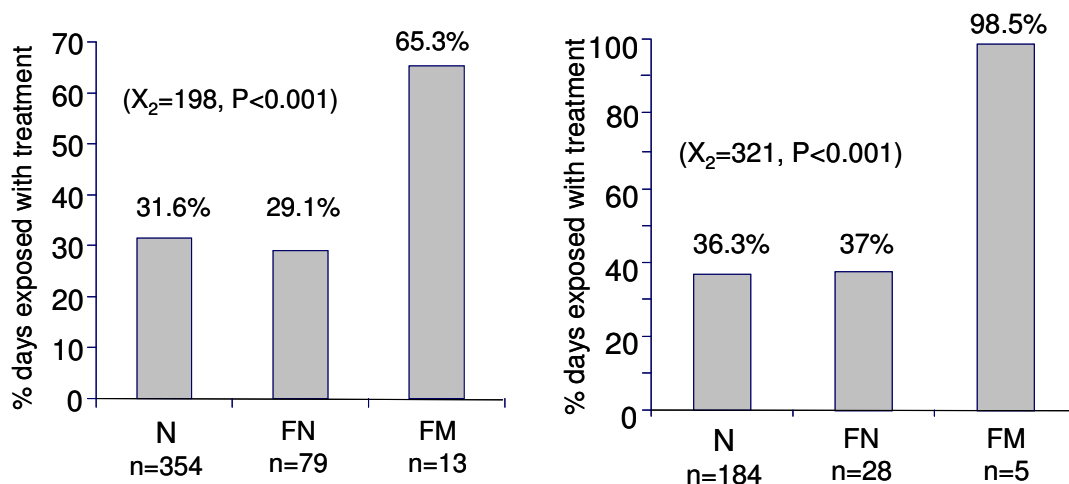
**Figure 8.3** Classification of the treatments received (in %) regardless of the type of calf delivery.

#### ***Frequency of treatments received over days exposed***

The median frequency of non routine health treatments was higher for FM calves than for N or FN calves (N:0; FN:0; FM:1.7; H=6.3; P=0.043). However, when considering heifer calves only, there was no difference in the frequency of treatments administered between calves of different birth difficulty scores (N:0; FN:0; FM:3.39; H=4.4; P=0.112)

#### ***Proportion of days with treatment over days exposed***

FM calves had over twice the proportion of days with at least one treatment compared to N and FN calves during the days exposed to treatment, and this proportion was nearly tripled when considering heifer calves only (P<0.001; Figure 8.4).

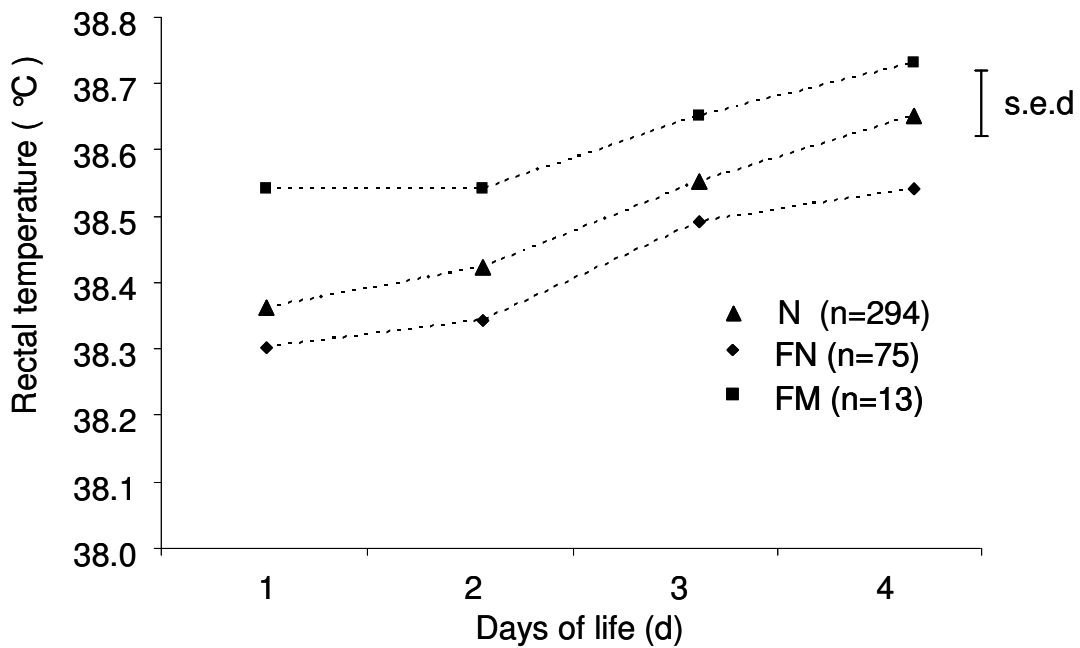


**Figure 8.4** Proportion of days (in %) with at least one non-routine health treatment in the first 60 days of life in bull and heifer dairy calves (left) and heifer calves only (right) following a non-assisted birth (N), farm assistance without malpresentation of the calves (FN) and farm assistance with malpresentation (FM). Number of calves exposed to treatments in each birth difficulty score is presented in brackets.

### 8.3.6. Rectal temperatures

#### ***Bull and heifer calves in the first four days***

The body temperature (°C) of the calves depended on their birth difficulty score (N: 38.5; FN: 38.4; FM: 38.6; s.e.d=0.07; P=0.03) but birth difficulty did not affect the maintenance of temperature over time (Figure 8.5; P>0.05). There was no interaction between birth difficulty and time since birth on the rectal temperatures of the calves (Figure 8.5.; P>0.05) which suggests that, at ambient temperature, dystocial calves did not have a diminished ability to maintain their body temperature. However, FM calves had higher rectal temperatures over the course of the first four days (N: 38.5°C; FN: 38.4°C; FM: 38.6°C; s.e.d=0.07; P=0.03) compared to calves born unassisted.



**Figure 8.5** Rectal temperature (°C) of dairy calves over their first four days of life following a non-assisted birth (N; n=294) or a birth assisted by farm staff without malpresentation of the calf (FN; n=75) or when the calf was malpresented (FM; n=13). Predicted means and the average standard error of the differences (s.e.d) are presented.

#### ***Heifer calves only until entrance in group pens***

Heifer calves born with difficulty had a higher temperature at day 1 than unassisted calves (FN: +0.2°C; FM: +0.5°C) but not in the following days and this was not statistically significant with a 95% confidence interval (P=0.075).

#### **8.3.7. Growth of the female calves to first service**

There was no significant interaction between age (centred data) and birth difficulty on the growth to first service of the female calves (effect presented in kg/centred days of age with N as a reference level; A:  $1.96 \pm 2.0$ ;  $P > 0.05$ ). This means that the growth of female calves born with difficulty was similar to female calves born without assistance.

## 8.4. Discussion

### 8.4.1. Do dystocial calves have more difficulty managing the transition to neonatal life?

#### ***Higher physiological stress response in the dystocial dairy calves?***

When assisted at birth, dairy calves had higher cortisol levels than when delivered naturally. Higher cortisol levels have previously been reported after a difficult delivery in dairy calves (Massip, 1980b; Civelek et al., 2008; Gasparelli et al., 2009a), with levels increasing with the severity of the difficulty (Hoyer et al., 1990). This is also the case in human babies, where assisted deliveries result in higher cortisol levels at birth compared to a vaginal birth or an elective caesarean section (Gitau et al., 2001). However, higher levels have not been consistently found across studies (Stott and Reinhard, 1978; Bellows and Lammoglia, 2000; Gasparelli et al., 2009b).

Higher salivary cortisol levels in the assisted calves reflect likely higher physiological stress in those calves, although an increase in cortisol levels due to other underlying reasons that led to a difficult delivery can not be excluded. Higher cortisol levels may be the result of more stressful deliveries, longer length of labour, the extraction process, or to an adaptive response to the associated hypoxic states and possible pain. Higher cortisol levels following a difficult delivery may also show that the adaptation of the calves to the extra-uterine environment requires a greater adaptive response from the hypothalamic pituitary axis (HPA), towards maintenance of a variety of body functions. Indeed, higher cortisol levels at birth have been associated with low vigour in lambs (Dwyer and Lawrence, 2002). This fits with dystocial calves being less vigorous after birth (Chapter 6) and suggests that those calves may have more difficulty in adapting. Higher cortisol levels in the assisted calves may be seen as a coping mechanism to help in their adaptation by ensuring availability of energy substrate. Indeed, elevated plasmatic glucose levels in calves are associated with high cortisol levels at birth (certainly as a result of its glycogenic effect) (Massip, 1980a; Civelek et al., 2008). The finding of lower cortisol levels after dystocia in some studies may be the result of the

absence of such a response, either because the HPA axis was not activated or malfunctioned, meaning coping mechanisms were not activated.

In the present study, the cortisol levels within 24 hours were lower (median ranging from 2ng/ml for N calves to 6ng/ml in FM calves) than previously reported in dairy calves by Hoyer et al. (1990) (14 to 20ng/ml) and Wooley (2010) (50ng/ml). This is because in the present study, cortisol was measured in saliva and cortisol is present at lower concentrations in saliva than in plasma (Mormède et al., 2007). Further degradation during manipulation may also have occurred when running the assay for a second time. As a matter of comparison with other stressors, in the FM calves, salivary cortisol levels were 9 times higher to levels reached following weaning stress (Loberg et al., 2008) and in a similar range to those recorded in beef calves following castration without anesthesia at 7 months of age (González et al., 2010).

#### ***No evidence of impaired thermoregulation after a difficult birth in unchallenged conditions***

In the present study, FM bull and heifer calves had a higher rectal temperature throughout their first four days of life compared to N and FN calves and so did FM heifers at the first recording but the difference found may not be biologically significant. In studies where calves have been kept at ambient temperature, as it is the case in the present study, no difference was found in the rectal temperature over the first four days of age between dairy calves born assisted or unassisted (Diesch et al., 2004a). Higher rectal temperatures in dystocic Nelore calves (Gasparelli et al., 2009a) at 24h of age was found but the 0.07°C increase reported is unlikely to be biologically significant. Previous studies also found a lowered ability to produce heat as a result of hypoxia in calves (Vermorel et al., 1983; Vermorel et al., 1989), piglets (Herpin et al., 1996; Alonso-Spilsbury et al., 2005) and lambs (Grongnet, 1984; Mellor, 1988). This may explain why previous studies have also found lower rectal temperatures in the first day of life of calves experiencing a difficulty birth compared to calves from an unaided birth; but calves were artificially maintained at a constant temperature of 20°C (Vermorel et al., 1983; Vermorel et al., 1989) or artificially exposed to a cold environment (Bellows and Lammoglia, 2000). It is possible that if challenged with

colder environments, the FM calves may not regulate their body temperature as well as calves born easily. However, this would need to be investigated further.

#### **8.4.2. Poor health and survival after a difficult birth**

##### ***Dystocial calves are immunologically fragile***

In the present study, 34.8% of all calves, dystocial or not, can be classified as having failed to acquire adequate passive immunity. Additional to this concerning rate, FN calves had an even lower acquisition of passive immunity by one week of age, which means their ability to fight off diseases was further impaired. This is in line with studies reporting lower passive immunity transfer in Holstein calves following very difficult and dystocic births (Donovan et al., 1986; Odde, 1988), as well as in beef calves (Vermorel et al., 1989; Waldner and Rosengren, 2009; Gasparelli et al., 2009b). However, this has not been consistently seen in dairy calves and for more moderate degrees of difficulty (Stott and Reinhard, 1978; Burton et al., 1989; Gulliksen et al., 2008).

Such impaired immunity, as shown in FN calves in the present study, can have a long-term impact on the health and survival of the newborn with higher morbidity and mortality in the preweaning period (Wittum et al., 1994a; Chassagne et al., 1999; Lombard et al., 2007) and possible knock-on effects on average daily gain (Weaver et al., 2000). In the present study, low immunity may also explain higher mortality to weaning of the heifer calves and higher morbidity in FM calves, although lower passive transfer in FM calves was not statistically identified and similar weight gains were found.

Lower immunity in the dystocial calves may result from decreased absorption of colostral immunoglobulins (Boyd, 1989; Besser et al., 1990; Tyler and Ramsey, 1991a; Drewry et al., 1999; Jacobsen et al., 2002) as a consequence of acidosis and hypoxic states in dystocial calves (Jacobsen et al., 2000; Taverne, 2008). Also, delay in obtaining colostrum as a result of lower vigour (Chapter 6) as well as possible lower colostrum quality in dystocial dams compared to dams that calve unaided, could also have contributed to lower passive immune transfer. It is not clear how cortisol levels interact with the acquisition of passive immunity. In some instances, elevated cortisol levels

have been found to enhance immunoglobulin absorption in calves (Johnston and Oxender, 1979; Johnston and Stewart, 1986) and in lambs (Hough et al., 1990) although this was found following injection of exogenous cortisol. However, high endogenous serum cortisol levels in eutocial goat kids were also related to higher immunoglobulin acquisition (Chen et al., 1999). On an other hand, no effect of having low or high cortisol at birth was found on the acquisition of immunoglobulins in newborn calves (Jacobsen et al., 2002; Wooley, 2010) and studies have refuted the idea of cortisol being involved in early gut closure (Stott, 1980). There are reports that severe cold stress can also delay or decrease the rate of absorption of immunoglobulins in dairy calves (Olson et al., 1980), suggesting that hypothermic dystocial calves may be at more risk of immunodeficiency. However, in the present study, hypothermia was not a clinical problem.

### ***More health problems following dystocia***

In the present study, approximately 1 in 4 calves received a non-routine treatment during the first 60 days of age. This is in line with reports of morbidity affecting 25% of the heifer calves during their first 8 weeks of age (Wells et al., 1997). A higher proportion of FM calves received at least one non-routine health treatment. FM calves were also administered more treatments and had a higher proportion of treatment days. Altogether, the results highlight that FM calves had more health problems than the N and FN calves. This is in accordance with the higher odds of morbidity, respiratory and digestive diseases reported previously in beef and dairy calves (Wittum et al., 1994a; Lombard et al., 2007).

Occurrence of non-routine treatments as opposed to occurrence of diseases was investigated in this study. Administration of treatment is directly relevant to producers as they account for direct costs. The proportion of days treated also gives an insight into the well-being of the calf under the assumption that a day with at least one treatment may denote a day of “feeling” sick. It is sensible to assume that treatments were administered when the animal was diseased although in this study, cases of prophylactic treatments can not be excluded. Inversely, it is possible that in some cases, a very diseased animal may have been euthanised before treatment occurred (particularly in the case of a bull

calf) or during the treatment period. However, this reflects usual and relevant farm conditions and despite them, a deleterious effect could still be detected on the number of treatments administered to the FM calves.

### ***Higher mortality to weaning in the liveborn animals***

Following on from the previous considerations, there is little surprise that difficulty at birth results in higher mortality. In the present study, mortality rates to weaning were much higher in the female calves that were malpresented (40%) than when there was no malpresentation (12%), which is in line with a previous study (Gundelach et al., 2009). This suggests that from the calf's point of view, being born with malpresentation may result in larger trauma.

Assisted heifer calves that were alive at birth had higher mortality rates to weaning than heifers born without assistance. This is in accordance with the study presented in Chapter 9, where historical data showed that survival of liveborn heifer calves was compromised up to first service after a difficult birth. Higher risk of mortality after a difficult birth has also been found beyond the first days of life in dairy heifer calves (Wells et al., 1996; Lombard et al., 2007; Henderson et al., 2011) and in beef calves from both sexes (Wittum et al., 1994a). It is to be noted that when restricting to liveborn calves only, as done in the present study, this effect disappeared (Wittum et al., 1994a) or subsequently shifted to shorter lifetime period and for more severe degrees of birth difficulty (Lombard et al., 2007). In the present study, 'liveborn' meant 'alive at birth' as opposed to 'alive after 48h', which is commonly used in other studies. This was done because, when taking an animal welfare perspective, it was felt more relevant to consider the outcome of all the calves alive at birth that had therefore reached consciousness levels, rather than ignoring heifer calves that died or were put down within the first 48 hours.

### **8.4.3. An adequate subsequent growth?**

In the present study, there was no evidence of an impaired growth after a difficult delivery in heifer calves until first service. This is in line with the study on historical data reported in Chapter 9 and agrees with previous studies studying growth at 3 months

of age (Lundborg et al., 2003) and growth to calving (Heinrichs et al., 2005). It is somewhat a surprising result because in the present study, dystocial calves also had poorer health, and sickness is associated with decreased growth in calves (Wittum et al., 1994b; Donovan et al., 1998).

In the present study FM calves and FN calves had to be grouped in the same category for the study of growth, although the data collected during the immediate neonatal period suggest that the FM calves may have been more affected than FN calves. It is possible that the dystocial calves that survive weaning may not show impaired performance (Chapter 9); or that the calves that were the most affected by birth difficulty and health problems died early (as suggested by the high mortality rates preweaning), and that the surviving dystocial calves may not have been as affected by a dystocial birth as the ones that died.

#### **8.4.4. Implications for the welfare of the dystocial dairy calves: slowly dying to weaning or survivors?**

Following assistance by farm staff, calves that were alive at birth experienced likely higher physiological stress, poorer immunity, higher morbidity and mortality. This was greater for calves that were malpresented, who received more health treatments and had greater mortality. This was seen despite no effects on their acquisition of passive immunity although this may be due to a larger variability in that smaller group of calves. It is also possible that malpresented calves may have suffered and died from trauma unrelated to poor health. For example, hypoxia levels may have been more intense in those animals (Herpin et al., 1996) and in the case of a breech malpresentation, the umbilical cord, which provides oxygen to the foetus, may rupture while the calf's head has not reached yet reached past the vulva.

The effects reported in the neonatal period on both dystocial bull and heifer calves is of concern for the welfare of those calves. The increase in the number of health treatments and the high calf mortality are imputable costs to farmers. Furthermore, the high morbidity levels in the dystocial heifers may also act as reservoirs for infectious diseases and indirectly impact the health of non-assisted animals.

The present study did not find evidence of impaired growth in the surviving calves. This could be the result of farm management or because the most affected calves may have died in the neonatal and pre-weaning period.

Altogether, this study shows that liveborn dystocial dairy calves have poorer welfare than calves born unassisted, at least in the neonatal period. It is possible that some dystocial calves may have similar welfare levels to the non-assisted calves but this can not be concluded from this study alone. The high mortality and morbidity experienced by the dystocial calves are a direct cost to the calf's welfare and producer.

## 8.5. Conclusion

To conclude, difficulty at birth negatively affects the welfare of the liveborn dairy calves but also incurs costs for producers. Any preventive measure that tackles birth difficulty will therefore benefit the calves' welfare and the economic sustainability of the dairy farms.

## **CHAPTER 9:**

### **Effect of birth difficulty on the subsequent survival, weaning weight, and fertility performance of UK Holstein dairy heifers**

In this Chapter, I was responsible for the data extraction from the database. Ian Nevison (BIOSS, Scotland) gave valuable methodology advice for the use of survival analysis. I was responsible for the data analysis and writing of the manuscript.

## 9.1. Introduction

In dairy systems, the birth of heifer calves represents a long-term investment in the future dairy herd. However, the birth process is known as being a risky time not only for dams but also for the offspring. In UK dairy herds, nearly one in six births are reported as assisted by a farmer or a vet (Wall et al., 2010) while stillbirth occurs in around 8% of the calves (Wathes et al., 2008). The experience of difficulty during the birth process, also called dystocia, increases odds of stillbirth and could explain up to half of the cases of stillbirth (Nix et al., 1998; Chassagne et al., 1999; Meyer et al., 2001a; Eriksson et al., 2004; Lombard et al., 2007). For the liveborn calves that have experienced the trauma of a difficult birth, survival also seems compromised with higher morbidity and mortality being reported for these calves in the neonatal period (Wittum et al., 1994a; Johanson and Berger, 2003; Lombard et al., 2007). More recently, a study found that the survival of Holstein heifers that had experienced at least a hard pull at birth had poorer survival in their lifetime (Henderson et al., 2011), although this was not the case for the lower degrees of difficulty.

It is well-known that early life experiences such as prenatal and perinatal stress can have long-term implications for the performance, cognition, health and welfare of the individuals among diverse species including ruminants and cattle (Braastad, 1998; Vinuela-Fernandez et al., 2007; Rutherford et al., 2009b). This suggests that rearing management in early life can have effects on the subsequent performance of farm animals in terms of growth, fertility and production (Le Dividich and Sève, 2000; Campanile et al., 2001; Le Cozler et al., 2008; Brickell et al., 2009a; Karamitri et al., 2010).

However the effects of dystocia, which can be considered as a perinatal stress, have been mostly reported for the neonatal period and there are few studies looking beyond the neonatal period in cattle, especially in dairy breeds.

There are several clues suggesting that there could be long-term effects of dystocia in dairy calves, beyond higher mortality and morbidity (Wittum et al., 1994a; Lombard et al., 2007). In beef cattle, dystocial calves had a subsequent reduced growth to weaning

(Bellows et al., 1988; Goonewardene et al., 2003). This may have long-term consequences in terms of survival and performance. For instance, in Holstein dairy heifers, growth rate has been found to be a good predictor of survival but also a determinant of subsequent fertility and milk production (Wathes et al., 2008; Brickell and Wathes, 2009; Brickell et al., 2009a; Brickell et al., 2009b), with sub or over-optimal growth being detrimental to the performance of heifers. Furthermore, it is possible that the early experience of hypoxia, as often happens in difficult births, could alter the functioning of the reproductive tract as has been demonstrated in rodents (Ezquer et al., 2008). It has been previously reported that Holstein cows that experienced a difficult delivery themselves as calves had a delayed first calving compared to cows born easily as calves (Heinrichs et al., 2005). It can be hypothesised that animals born with difficulty experienced impaired fertility and/or delayed achievement of the target weights for insemination. This may have resulted in delayed conception, hence delayed first calving. Additionally, the experience of dystocia has been shown to be associated with degraded performance in the longer-term. Recent studies have indeed shown that dystocial Holstein heifer calves have a reduced milk production in their first lactation (Eaglen et al., 2010b; Heinrichs and Heinrichs, 2011).

The objective of this study was to investigate whether the experience of a difficult delivery at birth would alter the survival of dairy heifer calves, the growth of heifer calves to weaning, and alter the fertility of heifers as nulliparous animals.

## 9.2. Materials and methods

### 9.2.1. Animals, housing and management

Data from heifer calves were obtained from the SAC experimental Holstein dairy herd (Scotland, United Kingdom). This herd was managed in accordance with the UK regulations on animal care and ethics of experimental animals.

Calves were reared from 1990 at the University of Edinburgh near Edinburgh (Edinburgh herd) but from 2002 onwards, calves were moved to the SAC dairy research centre at the Crichton Royal Farm in Dumfries (Crichton herd) where they were born and reared.

In the Edinburgh herd, calvings mainly occurred in winter (90% of the records between September and March), whereas calvings took place all year round in the Crichton herd. In both herds, calves were separated from their dams within 24 hours. Thereafter, all calves in the Edinburgh herd were housed in single pens until weaning. They were fed twice a day but once daily from eight days of age. In the Crichton herd, they were single-housed and milk bottle fed twice a day until approximately 6 days of age when they joined group pens with automatic milk feeders dispensing milk replacers. Weaning occurred at around 50 days of age in both herds (s.d=6.5) either abruptly in the Edinburgh herd or with milk allowances decreasing progressively from 46 days of age in the Crichton herd, and weaning weights were taken. Heifers were then reared to become replacement heifers with a target of calving at 24 months of age. In the Edinburgh herd, heifers were served from 13 months of age on condition of weighing at least 350kg, with an allowance of up to two AI services before running with a bull, whereas Crichton heifers were serviced when they had a weight greater than 330kg (at around 15 months of age), with an allowance of up to 7 services before being run with a bull or removed from the herd.

In both herds, birth difficulty was scored as follows: no assistance (N), Farm assistance without / with malpresentation of the calf (FN and FM), Veterinarian assistance without/with malpresentation of the calf (VN and VM) and caesarean section (VC). However, for the purpose of this study and due to the low availability of data, birth difficulty scores were redefined and grouped as follow:

- No assistance (N calves)
- Moderate difficulty: births assisted by farm staff with a normal presentation of the calf (FN calves)
- High difficulty: birth with malpresentation of the calf and/ or veterinary assistance (FM, VN, VM and VC calves).

In this study, FM calves were grouped with the vet assisted calves and not with the FN calves as was previously done in the previous Chapters, where no vet assisted calves were available. This was done because on the calf's point of view, being malpresented

denotes of a more severe degree of birth difficulty. This was supported by the higher mortality and health problems observed in Chapter 8.

### **9.2.2. Description of datasets**

Data from the Edinburgh herd contained calves born between 01.01.1990 and 31.01.2000 and the Crichton herd contained calves born between 01.01.2002 and 26.04.2009. These cut-offs were made to ensure that calves contained in each herd had similar rearing management background.

#### ***Stillbirth***

Data on stillbirths were also extracted for both herds. However, the sex and birth weight of the stillborn calves were not available and data therefore contained calves from both sexes.

#### ***Weaning and fertility performance***

For the liveborn heifer calves (calves alive at 24 hours after birth), the birth characteristics extracted were year of birth, identity of both parents, parity of the dam (primiparous or multiparous), season born (defined as Summer: April to September; Winter: October to March), month of birth, birth weight, genetic group (Select: animals selected toward greater milk solids; Control: animals selected to be the rolling UK average), birth litter size (singleton vs twins) and degree of birth difficulty. Weaning weight and date were retrieved and were used in calculations of age at weaning and growth rate to weaning (g/d). Records of service data allowed calculation of the date and age at first service, the number of services to conception as well as the subsequent date and age at first calving.

#### ***Survival of the liveborn heifers***

The survival of the liveborn heifers was considered up until first calving. Survival age in this period was defined as being either the age of death (true value, uncensored data) or the age of the last record found for this calf either at weaning, first service or first calving (censored value).

The Edinburgh herd contained 1237 records of births, 1151 records of weaning weights, 1011 for service data and 796 for first calving. The Crichton herd contained 721 records of birth weights, 605 records were available for analysis of weaning weights, 375 for service data and 344 for first calving. In the Crichton herd, this drop-out of data over time can be explained not only by disposal of the calves but also by the presence of calves for whom birth records are held but were not old enough to achieve those stages at the time of the data extraction. Discarding records where heifers had not been given the opportunity to achieve first calving yet would have resulted in considering less than half of the dataset. Therefore, no analysis of survival of the liveborn calves in the Crichton herd was performed but counts of heifers present at each stage are presented.

### **9.2.3. Statistical analysis**

As in previous work with the database (see Chapters 2, 3 and 4), it was decided to keep the two herds separate for analysis instead of accounting for a herd effect in the statistical analysis.

#### ***Stillbirth***

Likelihood of being stillborn across the different degrees of birth difficulty was analysed using a Chi-square analysis using Minitab® (15th edition, Minitab Inc, State College, USA).

#### ***Survival analysis***

*Edinburgh herd.* Analysis of the survival of the calves was first considered using a frailty model for survival analysis in R 2.11.1 © (2010, The R foundation for Statistical Computing, Wien, AT). This type of survival analysis was chosen because it can take into account that some survival ages are censored (the exact age of death is not known) and that some heifers are related to each other because of their common parentage (possibility of including a random model). As explained in Friggens and Labouriau (2010), a frailty model is a variant of the Cox proportional model that allows for the presence of a random model.

The event of death is time dependent and can be described by the following hazard function  $\lambda(t)$ :  $\lambda(t) = \lim_{\Delta t \rightarrow 0} \frac{P(t \leq T < t + \Delta t | T \geq t)}{\Delta t}$ , for each  $t > 0$ , where  $T$  is a variable representing the time between birth and death, and  $P(t \leq T < t + \Delta t | T \geq t)$  is the probability of observing death between  $t$  and  $t + \Delta t$  (to the condition that death had not occurred before).

The frailty model is built on the statement that the hazard function can also be written as:  $\lambda(t) = \lambda_0(t) \exp(X\beta + Z\xi)$  where,  $\lambda_0(t)$  is the baseline function,  $X\beta$  are the fixed effects and  $Z\xi$  is the random component. In this particular study, non-assisted animals (N) were set as the reference level. This means the baseline function was defined as:  $\forall t \geq 0$ ,  $\lambda_N(t) = \lambda_0(t)$ .

Global significance of each fixed effect was assessed using a likelihood ratio test, which assumes that twice the difference of the log likelihoods follows a Chi-square distribution (Collett, 2003).

Each fixed effect is expressed as a hazard ratio which is the ratio between the estimated risk of mortality under a particular circumstance and the risk of mortality of the reference level (N animals set as the baseline). Values  $>1$  indicate higher mortality risks (lower survival) associated with that particular factor whereas values  $<1$  indicate a lower risk (higher survival).

Birth litter size, parity, year, season, genetic group, birth difficulty were entered as fixed effects and birth weight (kg) as a covariate (model 1). However, the same model was also run without birth weight (model 2), without birth litter size (model 3) and without parity (model 4). The reason for running four different models was that variation was shared between birth difficulty, birth weight, birth litter size and parity. In support of this, in the present study, 93% of the twins ( $n=86$ ) were from multiparous dams, 25.6% of the twin calves experienced dystocia ( $n=138$ ) By running the models separately and comparing how the results change in relation to each other, this enables a better understanding on their separate effect and the extent of their confounding. It was also decided not to include sire of the calf in the random model because doing so accounted for all the variation shared by birth difficulty and also most of variation shared by birth

weight, birth litter size and parity of the dam. This means the analysis performed is equivalent to using Cox proportional hazard models.

Crichton herd. So that meaningful records could be obtained on survival to calving, records were restricted to calves expected to have reached first calving at the time of data extraction by adding the constraint of the time since birth of the calf being greater than 26 months. This restrained the data to 451 liveborn heifers (no assistance: n=418; moderate assistance: n=27; high assistance: n=6).

### ***Subsequent performance***

Linear mixed models were used to analyse the birth weights, weaning weights, the growth rates to weaning and the age at first calving. A generalised linear mixed model was applied for the analysis of the number of services needed to achieve pregnancy fitting a Poisson distribution and a logarithm link function. In the Crichton herd, sire of the calf interacting with the dam of the calf was chosen as a random model except for the analysis of weaning weights and age at first calving where identity of the sire only could be used. In the Edinburgh herd, sire and dam of the calf were used as the random model.

Models were constructed for each herd and performance indicator using a forward-stepwise technique (Hosmer and Lemeshow, 2000) as described in previous Chapters, and analysed with a Restricted Maximum Likelihood (REML) procedures in Genstat 11<sup>th</sup> Edition (2008, VSN International Ltd). Each variable was tested independently as a univariate and became a candidate for the multivariable model if it had a P value less than 0.25. The candidate variables were then added into the multivariable model using stepwise selection techniques with the most significant variables with the highest Wald statistic being added first. Candidate variables were kept in the model with significance attributed at  $P < 0.05$  (when all other explanatory variables in the models had been fitted) and birth difficulty was always fitted at the end. Biologically relevant interactions were tested once the whole model was set up and normality of the residuals was verified.

In the Edinburgh herd, growth rate to weaning was analysed with birth year interacting with birth season, genetic group, birth litter size and birth difficulty. Weaning weights were analysed with genetic group, year of birth, month of birth, birth litter size and birth

difficulty as well as birth weight and age at weaning as covariates. Birth weight was analysed with parity born, birth litter size, genetic group and birth difficulty. Only birth difficulty was included for number of services to conception. The model for age at first calving included birth season interacting with year of birth and birth difficulty.

In the Crichton herd, growth rate to weaning (g/d) was analysed with year of birth and birth difficulty as fixed effects and birth weight fitted as a covariate. Weaning weights were analysed with year of birth, birth litter size and birth difficulty as fixed effects and birth weight and age at weaning as covariates. Birth weights were analysed for calves with weaning weights only with parity of the dam, birth litter size and birth difficulty as fixed effects. Only genetic group and birth difficulty was included for analysis of number of services to conception. Year of birth interacting with month of birth as well as birth difficulty were taken into account for the analysis of age at first calving.

### 9.3. Results

#### 9.3.1. Stillbirth of dystocial calves

Stillbirth was very significantly influenced by the degree of birth difficulty in the Edinburgh herd ( $\chi=259.8$ ,  $df=2$ ,  $P<0.001$ ) and in the Crichton herd ( $\chi=176.5$ ,  $df=2$ ,  $P<0.001$ ). Stillbirth rates were up to 7 and 8 times higher for calves that experienced high birth difficulty in the Edinburgh herd and in the Crichton herd compared to calves who received no assistance (Table 9.1).

**Table 9.1** Number of heifer and bull calves born live or stillborn among different degrees of birth difficulty in the Edinburgh and Crichton herd. Stillbirth rates are presented for each degree of difficulty.

	No assistance		Moderate difficulty		High difficulty		Total
<i>Edinburgh herd</i>							
Calves born (count; %)	2258	(81.5)	300	(10.8)	214	(7.7)	2772
Stillbirth rate (in %)	3.8		22.7		27.1		7.6
<i>Crichton herd</i>							
Calves born (count; %)	1325	(83.8)	186	(11.8)	71	(4.4)	1582
Stillbirth rate (in %)	6.5		19.9		52.1		10.1

### 9.3.2. Survival of the liveborn heifer calves

#### ***Edinburgh herd***

The survival of the liveborn heifers from birth to weaning is shown in Figure 9.1

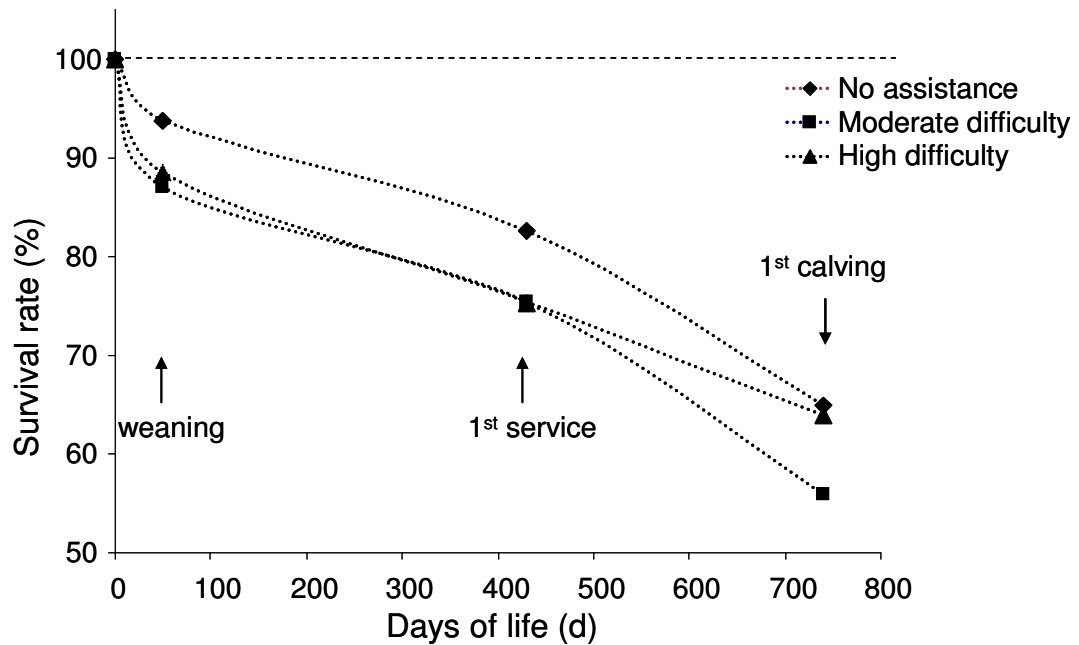
*Survival to weaning.* Dystocial heifers had a threefold greater risk of dying before weaning compared to calves born without assistance when either birth litter size or parity born was excluded from the models (Table 9.2.1;  $P < 0.05$ ) but this effect faded otherwise ( $P > 0.05$ ). This means that the effect seen on birth difficulty also partly accounts for the effect of birth litter size and also for the effect of parity and that taking into account birth weight also partly accounts for birth difficulty.

Calves born from multiparous dams had better survival than those born from primiparous dams except when birth litter size was taken into account (model 3), which resulted in the disappearance of an effect of dam's parity. This is because parity of the dam and birth litter size are highly confounded factors in the present study. Genetic group of the calves and birth season did not affect the survival of the female calves to weaning regardless of the model used. Twin calves had much higher mortality to weaning compared to singleton calves ( $P < 0.001$ ).

*Survival to first service.* Calves born with moderate difficulty had a higher risk of dying before first service, although when birth weight was not taken into account, only a tendency remained (Table 9.2.2). Twin calves had a higher risk of dying before weaning and this risk was not affected by the birth season. There was no effect of the dam's parity ( $P > 0.05$ ) unless birth litter size was excluded (model 3), which resulted in calves from multiparous dams being at higher risk ( $P < 0.05$ ). This is because in the present study, accounting for birth litter size also accounts for effects of dam's parity. The genetic group of the calves also did not affect their survival unless model 1 was used, in which case, risk of dying to first service became slightly higher.

*Survival to first calving.* Survival to first calving was not affected by the difficulty experienced at birth (Table 9.2.3;  $P > 0.05$ ) and twin calves performed worse. However, calves born in winter had a higher risk of dying by first calving and so did calves from

the Select genetic group ( $P < 0.05$ ). Calves from multiparous dams were also at increased risk except when birth weight was excluded ( $P < 0.01$ ).



**Figure 9.1** Survival rate (%) from birth to first calving in heifer calves from the Edinburgh herd born live following different degrees of birth difficulty (no assistance:  $n=1099$ ; moderate difficulty:  $n=77$ ; high difficulty:  $n=61$ ). Raw data presented.

**Table 9.2.1** Hazard ratio of liveborn heifer calves dying from birth to weaning and their 95% confidence interval in the Edinburgh herd, following birth difficulty, parity born, season born, genetic group and birth litter size. Full model (model 1) included birth weight as a covariate. Model 2, 3 and 4 excluded birth weight, birth litter size and parity born respectively.

	Model 1	Model 2	Model 3	Model 4
<i>Birth difficulty:</i>	n.s	n.s	*	*
Normal	Ref.	Ref.	Ref.	Ref.
Moderate	1.7 [0.8;3.5]	1.5 [0.7;3.0]	2.9 [1.4;5.9]	2.8 [1.4;5.5]
High	2.1 [0.5;2.5]	1.0 [0.5;2.3]	1.9 [0.9;4.4]	1.0 [0.5;2.4]
<i>Parity:</i>	***	***	n.s	
Primiparous	Ref.	Ref.	Ref.	n/a
Multiparous	0.3 [0.2;0.5]	0.2 [0.1;0.4]	0.9 [0.5;1.4]	
<i>Birth season:</i>	n.s	n.s	n.s	n.s
Summer	Ref.	Ref.	Ref.	Ref.
Winter	1.2 [0.7;2.1]	1.1 [0.7;1.9]	1.2 [0.7;2.1]	1.2 [0.7;2.1]
<i>Genetic group:</i>	n.s	n.s	n.s	n.s
Control	Ref.	Ref.	Ref.	Ref.
Select	0.8 [0.5;1.3]	0.8 [0.5;1.2]	1.1 [0.7;1.8]	0.9 [0.6;1.4]
<i>Birth litter size:</i>	***	***		***
Singleton	Ref.	Ref.	n/a	Ref.
Twin	14.8 [7.2;30.4]	25.8 [14.7;45.2]		6.9 [3.8;12.7]
<i>Birth weight</i>	**		***	***
	0.9 [0.9;1.0]	n/a	0.8 [0.8;0.9]	0.9 [0.9;1.0]

Ref.: reference level; n/a: not applicable

n.s: P>0.05; †: P<0.10; \*: P<0.05; \*\*: P<0.01; \*\*\*: P<0.001.

**Table 9.2.2** Hazard ratio of liveborn heifer calves dying from birth to first service and their 95% confidence interval in the Edinburgh herd, following birth difficulty, parity born, season born, genetic group and birth litter size. Full model (Model 1) included birth weight as a covariate. Model 2, 3 and 4 excluded birth weight, birth litter size and parity born respectively.

	Model 1	Model 2	Model 3	Model 4
<i>Birth difficulty:</i>	*	†	***	*
Normal	Ref.	Ref.	Ref.	Ref.
Moderate	2.2 [1.3;4.0]	2.0 [1.1;3.5]	3.0 [1.7;5.4]	2.5 [1.4;4.2]
High	0.8 [0.4;1.6]	0.8 [0.4;1.6]	1.4 [0.7;2.7]	0.8 [0.4;1.6]
<i>Parity:</i>	n.s	n.s	*	
Primiparous	Ref.	Ref.	Ref.	n/a
Multiparous	0.8 [0.5;1.2]	0.7 [0.5;1.1]	1.5 [1.0;2.1]	
<i>Birth season:</i>	n.s	n.s	n.s	n.s
Summer	Ref.	Ref.	Ref.	Ref.
Winter	0.9 [0.6;1.3]	0.9 [0.6;1.2]	0.9 [0.7;1.3]	0.9 [0.6;1.3]
<i>Genetic group:</i>	n.s	n.s	n.s	n.s
Control	Ref.	Ref.	Ref.	Ref.
Select	1.1 [0.8;1.6]	1.1 [0.8;1.5]	1.3 [0.9;1.8]	1.1 [0.8;1.6]
<i>Birth litter size:</i>	***	***		***
Singleton	Ref.	Ref.	n/a	Ref.
Twin	7.3 [4.3;12.4]	9.5 [6.4;14.2]		6.6 [4.1;10.5]
<i>Birth weight</i>	n.s		***	*
	1.0 [0.9;1.0]	n/a	0.9 [0.9;0.9]	1.0 [0.9;1.0]

Ref.: reference level; n/a: not applicable

n.s: P>0.05; †: P<0.10; \*: P<0.05; \*\*: P<0.01; \*\*\*: P<0.001.

**Table 9.2.3** Hazard ratio of liveborn heifer calves dying from birth to first calving and their 95% confidence interval in the Edinburgh herd, following birth difficulty, parity born, season born, genetic group and birth litter size. Full model (Model 1) included birth weight as a covariate. Model 2, 3 and 4 excluded birth weight, birth litter size and parity born respectively.

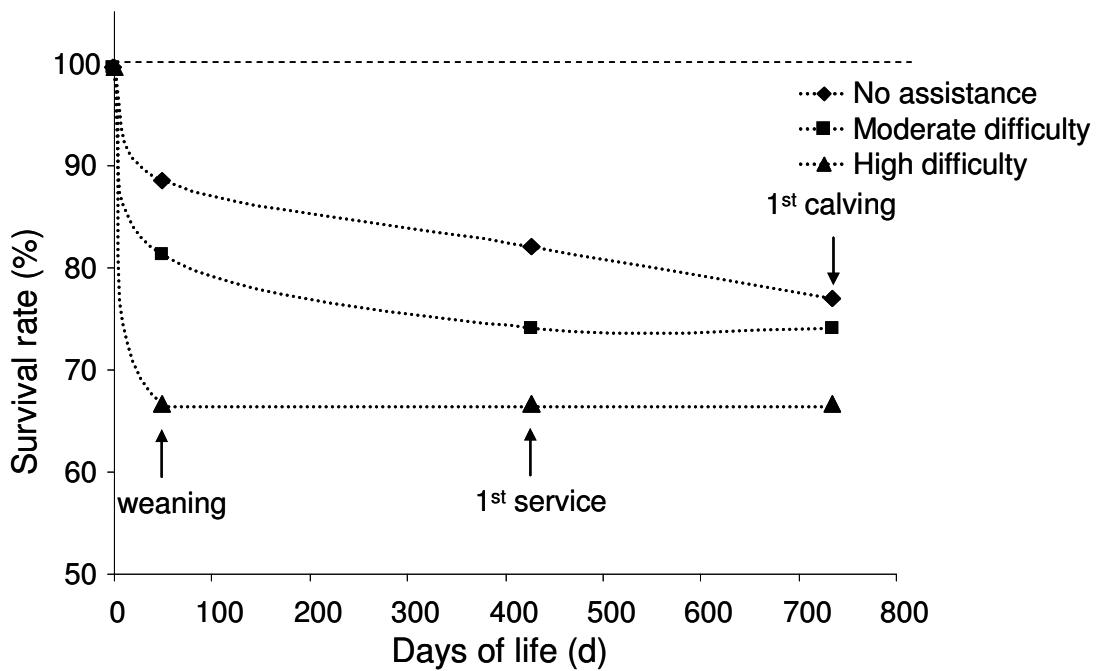
	Model 1	Model 2	Model 3	Model 4
<i>Birth difficulty:</i>	n.s	n.s	n.s	n.s
Normal	Ref.	Ref.	Ref.	Ref.
Moderate	1.4 [0.9;2.2]	1.4 [0.9;2.1]	1.6 [1.1;2.5]	1.4 [0.9;2.1]
High	0.8 [0.5;1.3]	0.8 [0.5;1.4]	1.0 [0.6;1.7]	0.8 [0.5;1.3]
<i>Parity:</i>	***	n.s	**	
Primiparous	Ref.	Ref.	Ref.	n/a
Multiparous	1.1 [0.8;1.4]	1.0 [0.8;1.4]	1.5 [1.1;1.9]	
<i>Birth season:</i>	***	***	***	***
Summer	Ref.	Ref.	Ref.	Ref.
Winter	1.8 [1.4;2.2]	1.7 [1.4;2.2]	1.8 [1.4;2.3]	1.8 [1.4;2.2]
<i>Genetic group:</i>	*	*	***	*
Control	Ref.	Ref.	Ref.	Ref.
Select	1.3 [1.0;1.7]	1.3 [1.0;1.6]	1.4 [1.1;1.8]	1.3 [1.1;1.7]
<i>Birth litter size:</i>	***	***		***
Singleton	Ref.	Ref.	n/a	Ref.
Twin	4.4 [3.0;6.4]	4.7[3.4;6.5]		4.6 [3.2;6.5]
<i>Birth weight</i>	n.s		***	n.s.
	1.0 [1.0;1.0]	n/a	1.0 [0.9;1.0]	1.0 [1.0;1.0]

Ref.: reference level; n/a: not applicable

n.s: P>0.05; †: P<0.10; \*: P<0.05; \*\*: P<0.01; \*\*\*: P<0.001.

### **Crichton herd**

As seen in Figure 9.2, in the Crichton herd, the survival of liveborn calves born without assistance (n=418) were 88.8%, 82.3% and 77% respectively at weaning, first service and first calving. When born with moderate assistance (n=27), their survival rates were 81.5%, 74% and 74%. Calves born with high assistance (n=6) had a steady survival rate of 66.6% from weaning to first calving.



**Figure 9.2** Survival rate (%) from birth to first calving in heifer calves from the Crichton herd born live following different degrees of birth difficulty (no assistance: n=418; moderate difficulty: n=27; high difficulty: n=6). Raw data presented.

### 9.3.3. Growth of dystocial heifer calves to weaning

#### *Edinburgh herd*

Liveborn calves who experienced moderate difficulty at birth were heavier at birth than calves that were not assisted or experienced higher difficulty (Table 9.3,  $P < 0.001$ ). Birth weights (kg) were also lower in calves from primiparous dams ( $n=824$ ) compared to calves from multiparous cows ( $n=312$ ) (primiparous:  $37.4\text{kg} \pm 0.5$ ; multiparous:  $41.6\text{kg} \pm 0.4$ ;  $P < 0.001$ ), lower in twin calves ( $n=47$ ) compared to singleton calves ( $n=824$ ) (twin:  $43.9\text{kg} \pm 0.3$ ; singleton:  $35.1\text{kg} \pm 0.7$ ;  $P < 0.001$ ), and in calves from the Control group ( $n=439$ ) compared to calves from the Select group ( $n=697$ ) (Control:  $39.1\text{kg} \pm 0.51$ ; Select:  $40.0\text{kg} \pm 0.5$ ;  $P < 0.05$ ).

No effect of birth difficulty was found on the subsequent weaning weights and growth rates to weaning (Table 9.3,  $P > 0.10$ ). However, twin calves ( $n=47$ ) had lower weaning weight than singleton calves ( $n=824$ ) (twins:  $69.8\text{kg} \pm 1.0$ ; singleton:  $72.2\text{kg} \pm 0.5$ ;  $P = 0.012$ ) and so did calves from the Control group ( $n=439$ ) compared to calves from the Select group ( $n=697$ ) (Control:  $69.6\text{kg} \pm 0.7$ ; Select:  $72.5\text{kg} \pm 0.6$ ;  $P < 0.001$ ). Twin calves and calves from the Control group also grew slower than singleton calves (twin:  $522.9\text{g/d} \pm 18.2$ ; singleton:  $568.9\text{g/d} \pm 8.6$ ;  $P < 0.01$ ) and than calves from the Select group (Control:  $517.7\text{g/d} \pm 12.8$ ; Select:  $574.0\text{g/d} \pm 11.8$ ;  $P < 0.001$ ).

**Table 9.3** Estimated means  $\pm$  standard errors of birth weights, subsequent weaning weight and growth rate to weaning of heifer Holstein calves of the Edinburgh herd having experienced different degrees of birth difficulty. Number of animals available for analysis is given in brackets.

	No assistance ( $n=1017$ )	Moderate difficulty ( $n=66$ )	High difficulty ( $n=53$ )	P value
Growth rate (g/d)	$542.8 \pm 10.1$	$549.5 \pm 16.9$	$545.4 \pm 17.1$	n.s
Birth weight (kg)	$38.6 \pm 0.4^a$	$41.0 \pm 0.6^b$	$39.2 \pm 0.7^a$	***
Weaning weight (kg)	$71.0 \pm 0.6$	$70.7 \pm 0.9$	$71.3 \pm 0.9$	n.s

n.s: non significant ( $P > 0.05$ ); \*\*\*:  $P < 0.001$

<sup>a-b</sup>: Within a row, means with different superscripts differ ( $P < 0.05$ )

### ***Crichton herd***

Birth weights did not differ within categories of assistance at birth and no effect of the categories of assistance at birth was found on the subsequent weaning weights and growth rates to weaning (Table 9.4,  $P>0.10$ ).

Heifer calves born from primiparous dams ( $n=179$ ) were lighter than calves from multiparous dams ( $n=471$ ) (primiparous:  $40.6\text{kg} \pm 0.7$ ; multiparous:  $43.3\text{kg} \pm 0.6$ ;  $P<0.001$ ). Twin calves ( $n=26$ ) were lighter than singleton calves ( $n=564$ ) at birth (Twin:  $31.7\text{kg} \pm 1.2$ ; Singleton:  $42.1\text{kg} \pm 0.7$ ;  $P<0.001$ ) and at weaning (Twin:  $58.8\text{kg} \pm 1.8$ ; Singleton:  $63.4\text{kg} \pm 1.0$ ;  $P<0.001$ )

**Table 9.4** Estimated means  $\pm$  standard error of the birth weight, subsequent weaning weight and growth rate to weaning of heifer Holstein calves of the Crichton herd having experienced different degrees of birth difficulty. Number of animals available for analysis is given in brackets.

	No assistance ( $n=546$ )	Moderate difficulty ( $n=37$ )	High difficulty ( $n=7$ )	P value
Growth rate (g/d)	$440.3 \pm 8.7$	$444.6 \pm 22.1$	$374.0 \pm 49.9$	n.s
Birth weight (kg)	$36.8 \pm 0.6$	$37.7 \pm 0.9$	$36.1 \pm 1.9$	n.s
Weaning weight (kg)	$62.4 \pm 0.9$	$62.7 \pm 1.4$	$58.3 \pm 2.7$	n.s

n.s: non significant ( $P>0.05$ )

### **9.3.4. Fertility of dystocial heifer calves**

#### ***Edinburgh herd***

No evidence of an effect of birth difficulty was found on the number of services needed to achieve pregnancy and the age at first calving in the Edinburgh heifer calves (Table 9.5;  $P>0.1$ ).

**Table 9.5** Back-transformed means [95% confidence interval] and age at first calving (days) of heifer Holstein calves of the Edinburgh herd having experienced different degrees of assistance at birth. Number of animals available for each analysis is given in brackets.

	No assistance	Moderate difficulty	High difficulty	P value
Number of services to conception	1.5 [1.4 to 1.6] (n=649)	1.7 [1.4 to 2.1] (n=42)	1.5 [1.2 to 2.0] (n=36)	n.s
Age at first calving (d)	753.7 ± 2.9 (n=707)	748.4 ± 8.5 (n=42)	758.8 ± 8.8 (n=39)	n.s

n.s: non significant (P>0.05)

### ***Crichton herd***

No evidence of an effect of birth difficulty was found on the number of services needed to achieve pregnancy and the age at first calving in the Crichton heifer calves (Table 9.6; P>0.10). However, calves from the Select group (n=203) needed 0.3 more services to achieve pregnancy than calves from the Control group (n=172) (P<0.05).

**Table 9.6** Back-transformed means [95% confidence interval] of the number of service to conception (back transformed data) and age at first calving (days) of heifer Holstein calves of the Crichton herd having experienced different degrees of assistance at birth. Number of animals available for each analysis is given in brackets.

	No assistance	Moderate difficulty	High difficulty	P value
Number of services to conception	2.0 [1.8 to 2.1] (n=347)	1.9 [1.4 to 2.6] (n=22)	1.8 [0.9 to 3.3] (n=6)	n.s
Age at first calving (d)	767.3 ± 12.4 (n=319)	774.9 ± 18.2 (n=20)	783.2 ± 28.7 (n=5)	n.s

n.s: non significant (P>0.05)

## 9.4. Discussion

### 9.4.1. Higher stillbirth rates after a difficult birth

In both herds in this study, stillbirth rates were higher for calves born with assistance than for calves born without assistance. This is consistent with the literature and has widely been reported (Philipsson, 1976a; Chassagne et al., 1999; Meyer et al., 2001b; Lombard et al., 2007; Gundelach et al., 2009). Average stillbirth rates are in the range of those reported previously for the UK (Esslemont and Kossaibati, 1996; Wathes et al., 2008; Brickell et al., 2009b) but also worldwide (Philipsson, 1976a; Meyer et al., 2001b; Johanson and Berger, 2003; USDA, 2007). The underlying reason for higher stillbirth rates after a difficult calving can be attributed to traumas in calves experiencing dystocia (Bellows et al., 1987; Agerholm et al., 1993; Berglund et al., 2003), asphyxia or hypoxia or foetal stress (Meijering, 1984; Bleul et al., 2008; Mee, 2008a). As labour length increases, as happens in difficult calvings (Doornbos et al., 1984), foetal stress becomes more likely, which can lead in ingestion of meconium, hypoxia, haemorrhages, or the early clamping or rupture of the umbilical cord (Tyler and Ramsey, 1991b; Jonker et al., 1996; Alonso-Spilsbury et al., 2005; Bleul et al., 2007). Such haemorrhages in stillborn dystocial calves have also been reported in Chapter 7.

The slight increase in the average rate of stillbirth between the Edinburgh herd and the Crichton herd might be partly explained by an increasing trend in stillbirth rates over the years in the Holstein breed (Steinbock et al., 2003; Hansen et al., 2004; Adamec et al., 2006). On the two farms, stillbirth rates were similar for calves that were not assisted and moderately assisted. However, stillbirth rates were much higher in the vet assisted calves in Crichton compared to Edinburgh. In conjunction with the smaller proportion of vet assistance, this supports the hypothesis formulated in Chapter 2 that propensity of to call a vet in Crichton may be lower, which may result in more stillborn calves.

#### **9.4.2. Higher mortality of the heifer calves after a difficult birth beyond the neonatal period**

In the present study mortality rates in the liveborn heifers after a difficult birth could be higher to weaning up to their first service. This poorer survival of dystocial heifers beyond the first two days of life is in line with previous studies on dairy cattle (Wells et al., 1996; Lombard et al., 2007; Henderson et al., 2011) although not always found in beef cattle (Smith et al., 1976). Apart from the study from Henderson et al. (2011) which looked at the lifetime survival, studies mostly focussed on the first months of life. In the present study, increased risk of dying was found following moderate difficulty but not for more severe cases, which is in contradiction with previous work. It is possible that lower statistical power in the highest degrees of birth difficulty may have prevented from finding statistical significance (despite grouping malpresentations and vet assistance together).

Nonetheless in the present study, most of the variation resulting from being born with difficulty was shared with parity of the dam and the calf birth litter size. This means that the effect of birth difficulty in its own right could not be demonstrated and could also partly be attributed to being a twin calf or being born from a primiparous dam. The fact that they share variation can be explained by higher prevalence of difficulty among the primiparous dams and in twin births (Echternkamp and Gregory, 1999; Mee, 2008a). In support of this, in the present study, 93% of the twins (n=86) were from multiparous dams, 25.6% of the twin calves experienced dystocia (n=138) and 48.6% of the dystocial calves were from primiparous dams (n=349). A bigger dataset may have helped to disentangle the effects of birth difficulty, birth litter size and parity of the dam and helped to conclude more firmly.

Poorer survival in twin calves and calves from primiparous dams has previously been reported (Gregory et al., 1996; Lombard et al., 2007; Swali and Wathes, 2007). Calves born in winter had poorer survival to first calving despite birth season not being a significant factor in their survival in early life. This is counter-intuitive because it is expected that calves born in winter are at more risk of dying in the neonatal period (Meyer et al., 2001b; Lombard et al., 2007). But absence of effects of season has also

previously been reported (Brickell et al., 2009b) and could be due to the majority of the Edinburgh calves being winter born. Calves from dams highly selected for milk solids (Select) had poorer survival to first calving than the calves from dams selected for the average genetic merit on milk solids (Control). This did not affect survival to weaning suggesting that this effect of genetic line may express itself at a later age rather than in the neonatal period.

Due to the structure of the dataset, an effect of the calf's sire could not be taken into account in the present study but this should be addressed in studies using larger datasets.

#### **9.4.3. No evidence of altered growth to weaning or subsequent fertility**

Weaning weights and growth rates to weaning did not differ between calves born without help and calves born with difficulty. This does not support the hypothesis formulated initially since, in beef cattle, calves assisted at birth or whose dams experienced long labours had lower growth to weaning (Bellows et al., 1988; Goonewardene et al., 2003). In beef cattle, weaning occurs at a later age and lower weaning weight in the offspring could be attributed to either calf factors (feeding intake and behaviour, metabolism) or to the dam having lowered milk production or reducing maternal care (Dematawewa and Berger, 1997; Mee, 2008a; Barrier and Haskell, 2011). However, absence of effects of birth difficulty has previously been reported on the growth of dairy calves to 3 months of age (Lundborg et al., 2003) and to calving (Heinrichs et al., 2005). This is despite dystocial calves being more likely to suffer from respiratory diseases at least during their 4 first months of life (Wittum et al., 1994a; Lombard et al., 2007) and sickness being associated with decreased growth (Wittum et al., 1994b; Donovan et al., 1998).

Not surprisingly, Select calves grew faster than the Control calves (Coffey et al., 2006; Chapter 8) and similarly, twin calves and calves from primiparous dams achieved lower weaning weights, as previously shown (Swali and Wathes, 2007; Linden et al., 2009).

Predicted birth weights, growth rates to weaning and weaning weights seem to be lower in the Crichton herd compared to the Edinburgh herd while first calving seems to occur at a later age with slightly more artificial inseminations needed. This may be due to

different calf management systems and calving pattern. There was also no evidence that dystocial calves had subsequent impaired fertility in this study, which also contradicts the hypothesis formulated at the start of this study.

#### **9.4.4. Only the fittest survive?**

Similar performance in terms of growth to weaning and subsequent fertility as nulliparous animals could be seen as a relief to farmers as there were no apparent long-term effects of birth difficulty on the performance of their dairy heifers. However, this would be ignoring that they suffer higher health problems beyond weaning (Lombard et al., 2007) and have lower lifetime milk production as adult cows (Heinrichs and Heinrichs, 2011). Most importantly, as shown in this study and reported previously, many calves born with difficulty are either stillborn or have poorer survival, possibly up to their first calving (Henderson et al., 2011), which raises concerns in terms of performance and welfare of the animals but also in terms of economic implications.

It is possible that the most affected dystocial calves die early and that, therefore, only the less affected heifers, on which performance records were collected, remain in the herd. It could also be that good heifer management may have compensated for any harmful effects, if they exist. Effects (if any) may also be negligible at the farm level. But the results from the present study alone do not allow such investigations.

Importantly, many of the dystocial calves do not survive birth and have higher mortality to weaning and to first service. This raises concerns for the welfare of the animals but also is an economic issue for the dairy producers and industry. Heifer mortality, regardless of its causality, is a direct economic cost to the producer but also a major impediment to the long-term economic sustainability of dairy systems. This is because heifers contribute to the renewal of the dairy herd but also deliver to the animals, producers and industry genetic improvement for production and welfare traits. Furthermore, considering the dairy management systems, it is very likely that heifer mortality may be associated with animal suffering, and this is a welfare concern. In that extent, any factor that pushes towards higher heifer mortality, such as birth difficulty, should be tackled.

## 9.5. Conclusion

When difficulty at birth occurs, dairy calves from both sexes were more likely to die perinatally. The dairy heifer calves that survived the birth process had poorer survival in the neonatal period but also beyond, despite their growth to weaning and subsequent fertility not being affected. The results of the present study suggest that the dystocial heifer calves that survive weaning may not be badly affected. However, emphasis should be put on the high number of calves that do not survive, not just in the neonatal period, but also beyond. This raises economic and welfare concerns.

## **CHAPTER 10:**

### **General discussion, recommendations and conclusions**

Regular calving events are essential to the maintenance of modern dairy systems. Parturition is nonetheless a high risk time for both the dam and her calf. Currently 1 in 6 calvings from the Holstein breed are scored as difficult in the United Kingdom (Wall et al., 2010). Dystocia has been associated with lowered performance in dairy cows but with little emphasis on their welfare. It is also unclear exactly from which degree of difficulty dystocia has adverse effects on the dairy cow and the duration of these effects. Little focus has also been given to the parturition event itself in terms of behavioural changes and progress, and whether an understanding of these processes could be used to address concerns of unacceptable levels of pain in the dystocial dairy cow. Although, similarly to other species, negative consequences are expected in dairy calves following difficulty at birth, studies have essentially focussed on the event of stillbirth with little consideration to the surviving calf's welfare. The welfare of the dystocial dairy calf needs to be addressed not only in their first few days of life but also beyond.

The objective of this thesis was to assess the short and long-term effects of a difficult calving both in dairy cows and calves in terms of health and welfare. Particularly, it was aimed to:

1. Assess the impact of a difficult calving on the health of the dairy cow and her subsequent performance over the lactating period
2. Improve understanding of the parturition behaviours and progress in dystocial animals, with an emphasis on behaviours that could indicate pain
3. Assess the short and medium-term effects of having had a difficult birth on the subsequent health and welfare of the dairy calf.

In this thesis, the work was focussed on the Holstein breed. This was done because this breed is the most prevalent and most representative of the modern dairy cow, therefore being a good model study. Thereafter, the results will be discussed with regards to dairy cows in general. However, it must be kept in mind that differences between dairy breeds may occur.

## 10.1. The dairy cow after a difficult calving: summary of the results

The welfare of the dairy cow following a difficult calving was addressed in Chapters 2 to 6. The results obtained from the investigation of the effects of a difficult calving on the health and welfare of the cow are summarised in Table 10.1.

**Table 10.1** Summary of the results obtained on the effects of various degrees of calving difficulty (N: no assistance; FN/FM: Farm assistance without/ with calf malpresentation; VN/VM: veterinary assistance without/ with calf malpresentation; VC: caesarean section) on the subsequent welfare of the cow. Cell shaded in grey: statistical effect found of calving difficulty compared to a non-assisted calving taken as the reference level (Ref.). On the same row, when effect was found for different degrees of difficulty, relative grading of the intensity of the effect (quantitatively) between the scores of difficulty is reported: + (score with lowest effects) to +++ (score with most severe effects)

<i>Effects investigated</i>	<i>Degree of calving difficulty</i>					
	N	FN	FM	VN	VM	VC
Saleable milk losses	Ref.	+	++	++	0	+++
Milk production reduction	Ref.	0	+	++	0	++
Poor fertility	Ref.	0	0	+		
Repetition of calving difficulty	Ref.	+				
Increased lameness	Ref.	0	0	0		
Increased mastitis	Ref.	0	0	0		
Poor survival	Ref.	+	0	++		
Longer later stages of labour	Ref.	+	+	n.i.		
Possible increased pain at calving	Ref.	+	+	n.i.		
Impaired maternal behaviour	Ref.	0			n.i.	
Possible postpartum pain behaviours	Ref.	0			n.i.	

0: no statistical effect found; n.i.: not investigated.

In terms of performance during the subsequent lactation and in comparison with cows that calved unaided, dairy cows that had experienced calving difficulty of nearly any degree had lower milk production in the early lactation, higher losses of saleable milk over the subsequent lactating period and were also at increased risk of repeated difficulty at next calving. Multiparous cows also had poorer survival at 90 DIM except for FM cases. Impaired fertility, as assessed by increased number of days open and number of services to conception, was also a problem for cows that had required veterinary assistance. There was no evidence that the incidence of calving difficulty was related to higher incidence of lameness or mastitis.

Over the course of parturition, despite no differences detected in the time taken from the appearance of the calf's feet at the vulva and birth of the calf between the scores of difficulty, FN and FM cows were in later stages of labour for longer, were more restless for longer and had their tail raised for longer. This could be consistent with higher levels of pain in dystocial animals compared to animals who calved unaided. In the first three hours postpartum, maternal behaviour and behaviours that could be indicative of pain did not differ between the difficulty scores. However, there was a high individual variation between the dams.

Overall, difficulty at calving was associated with poorer performance and survival from the lower degrees of difficulty. These worsened as difficulty increased. Behavioural changes that are consistent with pain were seen in dystocial cows prior to delivery but not postpartum.

## 10.2. The dairy calf after a difficult birth: summary of the results

The welfare of dairy calf following a difficult birth was addressed throughout Chapters 6 to 9. The results obtained on the investigation of the effects of a difficult calving on the health and welfare of the cow are summarised in Table 10.2.

**Table 10.2** Summary of the investigation on the effects of various degrees of birth difficulty (N: no assistance; FN/FM: Farm assistance without/ with calf malpresentation; Vet assistance) on the subsequent welfare of the calf. Cell shaded in grey: statistical effect found of calving difficulty compared to a non-assisted calving taken as the reference level (Ref.). On the same row, when effect was found for different degrees of difficulty, relative grading of the intensity of the effect (quantitatively) between the scores of difficulty is reported: + (score with lowest effects) to +++ (score with most severe effects)

<i>Effects investigated</i>	<i>Degree of calving difficulty</i>			
	N	FN	FM	V
Increased stillbirth	Ref.	+	++	+++
Larger pathology	Ref.	+	n.i.	n.i.
Lower vigour	Ref.	+		n.i.
Likely higher stress	Ref.	+	+	n.i.
Lower passive immunity	Ref.	+	0	n.i.
More health treatments	Ref.	0	+	n.i.
Higher temperature	Ref.	0	+	n.i.
Higher mortality to weaning	Ref.	+	++	n.i.
Higher mortality to first service	Ref.	+	0	
Impaired growth to weaning	Ref.	0	0	
Impaired growth to first service	Ref.	0		n.i.
Impaired subsequent fertility	Ref.	0	0	

0: no statistical effect found; n.i.: not investigated.

From the study experimentally following up calves born from various degrees of birth difficulty, all but one vet assisted calves were born dead, and farmer assisted calves were more likely to be stillborn than calves born without assistance. Stillborn dystocial calves displayed more pathology than stillborn eutocial calves, but they did not have a different body shape at birth than dystocial calves that survived. Dystocial dairy calves that survived the birth process had lower vigour in the first three hours of life, had higher salivary cortisol in their first day of life, achieved lower passive immunity and received more health treatments in the neonatal period. Dystocial heifers also had higher mortality rates by weaning but had similar growth to first service.

Historical records from the farm also showed that dystocial heifer calves were three times more likely to have died by weaning and by first service than calves born without assistance. For those who survived, there was however no indication of altered growth to weaning or subsequent impaired fertility, meaning that performance post weaning was similar to calves born unaided

Altogether, results suggest that dairy calves born with any difficulty have poorer welfare in the neonatal period and possibly beyond. As well, neonatal effects became worse as birth difficulty increased.

### **10.3. Are the effects seen true effects of calving difficulty?**

In the studies presented previously in this thesis, adverse effects were observed in both dairy cows and calves following calving difficulty. The effects observed after a difficult delivery can originate from the underlying causes that led to a difficult calving, during the calving itself or during assistance. In that respect, it should be kept in mind that some of the effects seen may be effects from the difficult delivery itself but that strictly speaking the effects seen are an association rather than a true cause and effect relationship.

As well, there are various factors that are partly or fully confounded with calving difficulty, including the dam's parity, dam's body condition score, twinning, birth weight, sex of the calf and sire of the calf to cite the major ones. In the statistical analyses presented, as much as was possible, the factors that most influenced the trait of

interest were included in the analyses but some others could not be accounted for, particularly when they largely shared variation with calving difficulty. However, in this thesis, the interest was focussed on calving difficulty in its own right and this also includes some of the confounding factors.

Throughout this thesis, assistance at calving was used as a proxy measure for calving difficulty. The validity of assistance as a proxy measure for calving difficulty has previously been discussed in Chapter 1 (section 1.1.2.). This is a widely accepted method to assess difficulty at calving with the majority of scales using no assistance as the “normal” or “easy” calvings and any other degree of difficulty being defined by diverse descriptions of the degrees of assistance (Meijering, 1984; Mee, 2008a). Considering the trait measured is assistance at calving, it could be argued that for accuracy purposes the trait studied should be called calving assistance rather than calving difficulty. Using the terminology “calving assistance”, would be misleading because it would mean that it is the effects of the assistance *per se* which were studied implying the type of assistance or how assistance is performed. In reality, calving difficulty and the underlying reasons that led to assistance being provided, were the focus of the studies rather than the assistance itself.

#### 10.4. Any degree of calving difficulty in dairy cows: destined to long-lasting impaired performance?

In Chapter 2, it was found that cows with calving difficulty had a reduced milk production but also showed greater losses in saleable milk. Investigating cumulative saleable milk independently of each animal having achieved a full lactation enabled longer lasting effects with a lower degree of difficulty threshold to be shown compared to the estimated milk production (milk produced by the cow). It was hypothesised that it might more accurately reflect the underlying biological stresses the dairy cow experiences, by taking into account not only reductions in milk production but also additional losses that can be incurred by health treatments (when milk must be discarded) and early death or culling of the animals. Chapter 3 showed poorer fertility after dystocia but also that, when surviving to the next lactation, dystocial dairy cows

were at more risk of repeating calving difficulty. Chapter 4 did not support the hypothesis that the additional losses of saleable milk seen in dystocial cows throughout their lactation could be due to higher prevalence (and the possible resulting milk wastage), of mastitis or lameness after a difficult calving. Nonetheless, previous work on the same dataset had demonstrated that dystocia was a risk factor for uterine infections (Bell and Roberts, 2007) and this may have contributed to the losses seen. Chapter 4 also supported the statement formulated in Chapter 2 and 3 that dystocial cows were at more risk of leaving the herd in their early lactation. This means that, as highlighted in the study on fertility (Chapter 3), data censoring occurs.

Adverse effects after a difficult calving were seen from the lower degrees of difficulty when farm assistance was given, emphasising that calving difficulty can lead to problems even when there is no need to call for a veterinarian. Also, the extent of the effects seen in the cows worsened with increasing difficulty.

The studies reported in this thesis also support the idea that long-lasting effects are likely in dairy cows after dystocia. Although effects on survival, fertility and the amount of milk produced by the cow were more pronounced in the immediate postpartum period, concerning long-lasting effects were seen on the amount of saleable milk produced by the dystocial cows throughout their lactation. This raises concerns about their health and survival and ultimately about their welfare.

Furthermore, cows with calving difficulty were also more likely to experience calving difficulty at the next lactation. Possible causes are: some cows being more prone to dystocia, lower intervention thresholds at next calving or possible carry-over effects (Chapter 3). Whatever the underlying reasons, this raises concerns for the cows. It primarily means that cow health and performance will also be degraded during the next lactation. Failure to make a full recovery after a difficult calving and carry-over from previous experience of dystocia would be very concerning in terms of welfare of the animal. The experience of dystocia is also likely to be a very painful experience for the dairy cow, as will be discussed further in the following sections. As much as calving difficulty leads to welfare problems, cases where assistance is provided without actual calving difficulty, as might be the case in cows with previous history of dystocia through

overzealous assistance, may also lead to welfare problems. Indeed, such interventions carry avoidable increased risk of uterine infections (which are painful conditions) and possibly unnecessary pain and damage as a result of the intervention process itself.

The reduction of milk production *per se* is more a production problem than a welfare problem and a similar argument applies for reduced fertility. However, the likely underlying causes for these impairments are likely to be related to poor welfare. These include inadequate energy balance, health problems and the possible experience of discomfort, pain and distress. As highlighted in Chapters 5 and 6, dystocial cows may also experience higher levels of pain and discomfort during parturition and the postpartum period. Yet such states may be more prevalent in the subsequent lactation following dystocia. Chapter 4 did not demonstrate higher prevalence of mastitis and lameness, which are two painful conditions (Huxley and Whay, 2006; Hewson et al., 2007; Fajt et al., 2011) in dystocial cows compared to cows calving unaided. Yet, as previously shown in the same herd, dystocial cows are at higher risk of uterine infections (Bell and Roberts, 2007), which are painful and distressing conditions. In dairy cows, failure to stay in the herd is also a proxy measure for poor welfare. Natural death or euthanasia may occur following health problems while the main reasons to cull a dairy cow in early lactation are poor health and fertility (Beaudeau et al., 1993; Seegers et al., 1998).

## 10.5. Pain at parturition: higher pain levels for dairy cows with calving difficulty?

### 10.5.1. What's the evidence?

Chapter 5 and 6 looked at the behaviours of cows with calving difficulty compared to cows that calved naturally. These studies were restricted to calvings that were farm assisted and led to the birth of a singleton liveborn calf or calves and explored behavioural changes from 6 hours prepartum to 3 hours postpartum. Cows with calving difficulty were in the later stages of labour for longer, showed an earlier increase in contraction frequency than cows calving normally but also more frequent contractions.

This result on its own suggests that these dystocial cows experienced the very painful states associated with the later stages of parturition (Corli et al., 1986; Lowe, 2002) for longer. The dystocial cows were also more restless and for longer, than cows that calved naturally, and they also raised their tail for longer. There were, however no behavioural differences seen between dystocial and eutocial cows postpartum but large inter-individual variation was observed (Chapter 6). As discussed in Chapter 5, it is likely that the behavioural changes seen prepartum may relate to higher levels of pain or discomfort until the calf was expelled.

### **10.5.2. Do the behavioural changes really reflect pain?**

Further validation would be needed to accurately demonstrate that the behavioural changes observed prior to birth of the calf are attributable to pain. This could include administration of pain relief to cows with calving difficulty to see if restlessness and tail raising would be similar to or lower than the levels shown by cows without calving difficulty. However, from a practical point of view, it is not possible to predict which cows are experiencing difficulty at calving until it occurs, making it difficult to run such trials. An alternative to that would be to see whether administration of pain relief at the start of parturition would suppress or reduce the expression of such 'pain' behaviours in cows regardless of difficulty occurring. Nonetheless, previous trials using NSAIDs such as meloxicam led to impairment of the labour process and to increased stillbirth rates and retained membranes (Newby, personal communication). This is because this type of drugs inhibits prostaglandins that are responsible for the inflammation and pain response but the same prostaglandins are also involved in the labour process, therefore interfering with the normal progress of birth. Similar problems may also be encountered with opiates as opioids are also components of the process of labour. Other drugs or techniques should therefore be considered.

### **10.5.3. How can we assess pain at parturition in cows?**

Regarding the assessment of pain at parturition, combining behavioural observations with hormonal assays may also have helped to reach a conclusion. Nevertheless, this would have been difficult for several reasons. From a practical point of view, it is not

possible to know whether a cow is having a difficult calving before she actually is in the late stages of calving, making sampling time and experimental decisions challenging. The physiological sampling processes and the disturbance they create could also interfere with the calving progress and behaviours. The parturition event itself is an inflammatory process and the resulting pain is physiological as opposed to pathological. This makes it difficult to dissociate the effects attributable to the experience of pain to those attributable to the parturition process. Some studies have previously reported higher levels of vasopressin in animals with difficulty (Hydbring et al., 1999; Olsson et al., 2004) but it is unclear whether changes seen are attributed to pain or longer labour durations. It is also possible that past a certain threshold, some hormones may reach ceiling effects, independently of the pain experienced. Qualitative behavioural assessments and visual analogue scales could also be used for evaluations of pain but validation would also be needed.

Despite being at the core of the definition of pain, the emotional component of pain is overlooked (Merskey et al., 1979; Molony, 1997). This is rather speculative but all physiological components apart, it is possible that the way the cow perceives her environment may also influence how much pain that same cow feels. In women, there is evidence that poor emotional state increases the rated levels of pain at parturition (Lowe, 2002). In that respect, a primiparous cow may find the calving process more distressing, because of the novelty of the experience, the unfamiliarity with the calving pen, possibly unfamiliar congeners and their lower hierarchical ranking. There are also physiological differences in the parturition progress and behaviours between heifers and cows (Mainau et al., 2010b; Miedema et al., 2011a), some of which may suggest that calving is a more painful experience for heifers than it is in cows. Additionally, the opiate-mediated response that mitigate pain during calving (Gintzler and Komisaruk, 1991; Jarvis et al., 1997) is enhanced in multiparae compared to primiparae. In line with this, in women, pain is ranked higher for the first childbirth than it is for the successive ones (Melzack, 1993).

#### **10.5.4. Pain at parturition: future work?**

The work presented in this thesis relating to pain at parturition comes within the general scope of investigating claims of higher levels of pain in dystocial cows. The overall thematic that this work feeds into, although not addressed in this thesis, is to find whether there are effective ways of relieving pain in dystocial animals, and benefits of doing so. The studies presented in this thesis made a preliminary investigation into the parturition progress and behaviours pre and postpartum for cows with or without calving difficulty and identified some behaviours that could be indicative of higher pain.

Nonetheless, many questions remain unanswered. The present work excluded twin calvings, calvings leading to stillborn calves and did not look at calvings that required veterinary assistance. It is possible that cows bearing twin calves may experience more discomfort than cows carrying a singleton, while the experience of dystocial calvings leading to a stillborn calf and veterinary assistance may constitute a poorer experience in terms of length of labour, exhaustion and pain. This means that extension of the present work is required to fully address the population of dystocial animals.

The intervention itself, although necessary, may lead to additional pain and discomfort (Gregory, 2004a; Scott, 2005), as a result of the pressure and stretching involved during assisted deliveries but also the possibility of lacerations and injuries. There is, to my knowledge, no study (other than anecdotal evidence) looking at pain levels during intervention in farm animals. Preliminary work presented in Chapter 5 did not lead to conclusive results. Such studies face practical challenges as cows are usually restrained during intervention and efforts of the cow may be focussed on the expulsion of the calf. It can also be hypothesised that the experience and attitude of the stockman may also influence the outcome.

Postpartum pain should also be explored further. Work conducted in Chapter 6 on potential indicators of pain did not lead to strong conclusions but large inter-individual variation was found. In theory, administration of a non-steroidal anti-inflammatory drug (NSAID) postpartum should reduce associated inflammation and pain (Richards et al., 2009). The use of ketoprofen did not show however any advantage after dystocia in terms of milk yield and fertility (Richards et al., 2009). Results from a study on eutocial

calvings showed that primiparous cows receiving meloxicam postpartum performed more steps than primiparous cows without the meloxicam treatment but no differences in treatments were seen on other behaviours, health, production, rectal temperature and proteins indicative of inflammation (Mainau Brunsó, 2011).

#### **10.5.5. Parturition in cows: should pain relief be given or not?**

##### ***Practicalities, ethical and welfare challenges***

Disregarding the amount of work needing to be done regarding assessment of pain and pain relief at parturition for cows, the debate on the administration of pain relief to cows at the time of parturition brings with it lots of questions and challenges. Even in women, the supply of pain relief during childbirth is a challenging issue (Melzack, 1984; Brownridge, 1995). The first challenges are the practicalities of administration of pain relief in terms of how, when, to whom and with which drugs, and whether this can be done efficiently. From a practical point of view, both the welfare of the cow and the newborn calf also need to be taken into account for informed decisions to be made.

If cows experience what we think are unacceptable levels of pain, pain relief should be administered on ethical and welfare grounds. Whether unacceptable levels of pain apply to dystocia cows only, all calving cows or even primiparous cows is an open question. To administer pain relief may seem, at least theoretically, an obvious decision in dystocia animals if additional pain is experienced, as is likely to be the case during labour and possibly during assistance and the postpartum period.

When considering pain relief administration to all cows and not just to dystocia animals, some may suggest that this pain is natural and is therefore a normal, acceptable experience that the cow has to go through. Others might think otherwise, arguing that although parturition pain in itself is natural, the repeated experience of calving in modern dairy cows is due to the production system; and in that respect, the “non naturalness” makes calving without pain relief less acceptable. It is however difficult to know what “naturalness” or “natural living” exactly means in the context of modern dairy animals as highlighted by von Keyserlingk et al. (2009). In the wild, many ungulate species would also give birth annually. It is also unclear how domestication of

cattle and the resulting selection have shaped not only the labour process, expression of behaviours and the intensity of pain experienced at parturition.

Adopting an evolutionary perspective, inability to give birth, as happens on farms, would naturally be selected against in the wild. Meanwhile, the adequate balance between a “quick and easy” parturition and adequate maturity of the young at birth would be favourable to the survival of both mother and young and to the fitness of the species. Selection for production traits has resulted in higher incidence of dystocia in production animals, with foeto-pelvic incompatibility being the main reason for dystocia in beef cattle and dairy cattle (Meijering, 1984; Mee, 2008a). Cattle are considered as prey species which means that, for survival purposes, it is in their best interest to show as little behavioural indication as possible of pain or sickness (hence displaying signs of vulnerability to their predators). In terms of predation, parturition in open environments is a particularly vulnerable time because of the presence of physiological liquids and the impaired ability to escape that may attract predators. It is unclear how much of this propensity to minimising expression of pain has remained in modern dairy cattle but also how selection has shaped pain sensitivity and the labour process.

### ***Socio-economic barriers***

Beneficial effects of pain relief are well-known in terms of post-surgical recovery, animal performance, not to mention animal well-being (Mellor et al., 2007; Vinuela-Fernández et al., 2007). But the other challenge faced is: would that be used on farms?

Despite known benefits to the animals of the use of analgesics, there are barriers limiting their use. Disregarding licensing, or drug residues issues and the subsequent milk and meat withdrawal periods, the cost and benefits of giving pain relief should be explored in terms of short and long-term well-being of the animal but also in terms of economic value. At the farm level, the latter is likely to drive whether this will be taken up or not.

As well, the attitude of the stockman and practitioners to animal pain should not be underestimated in a move towards the use of pain relief. Many sociological factors determine the attitude of veterinarians to pain and to their use of analgesics. This includes gender, age, year of graduation, being raised on a farm or even political affiliation (Huxley and Whay, 2006; Laven et al., 2009; Kielland et al., 2009; Fajt et al.,

2011). Despite ranking dystocia and caesarean section as very painful conditions, pain relief is not systematically administered when attended by vets. When attending difficult calvings that did not require surgery, three quarters of the UK vets surveyed declared administering analgesics in some of the cases (mostly NSAID) but the proportion of animals treated is unknown (Huxley and Whay, 2006). Similarly, a third of the Canadian vets surveyed provided some kind of analgesia, with a quarter of the cows receiving some kind of pain relief (ketoprofen and lidocaine mostly) (Hewson et al., 2007). Shockingly, in a survey done in the United States, 3.7 and 6.4% of the vets surveyed reported no use of analgesic drugs when performing a caesarean section on dairy and beef cattle respectively (Fajt et al., 2011). These figures illustrate some of the challenges that may be faced in considerations of giving pain relief to parturient cows. Attitudes of farmers to providing pain relief when difficulty occurs without veterinary assistance should also be investigated.

#### 10.6. The dairy calf after a difficult birth: condemned to poor welfare?

In Chapter 7 and 9, it was emphasised that one major outcome of calving difficulty is the birth of dead calves. Stillbirth rates were high even for the lower degrees of difficulty and increased dramatically when the calf was malpresented or a veterinarian was called out. Calves born dead following an assisted birth showed larger internal damage compared to calves that were stillborn but had an unaided delivery (Chapter 7), which means there was increased trauma in those animals.

In itself, stillbirth may not be a welfare issue from the calf's point of view on the assumption that consciousness has not been achieved and that therefore suffering is not possible (Mellor and Stafford, 2004). Even so, there is debate whether the foetus is capable of some degree of consciousness and of feeling pain (Smith et al., 2000; White and Wolf, 2004; Mellor et al., 2005; Derbyshire, 2006; Weber, 2010). In all cases, stillbirth is a waste from an economic point of view but also in terms of being a barrier to genetic improvement.

For the dystocial calves that survived birth, calves had lower vigour (Chapter 6), higher cortisol levels, lower passive immunity and were administered more health treatments in

the neonatal period (Chapter 8). This means that following a difficult birth, calves may have more difficulty making the transition from foetal to extra-uterine life. They also are less able to mount an immune response to diseases, making them more likely to experience sickness and this was verified by the higher percentage of days when dystocial calves received non routine health treatments compared to calves born unaided (Chapter 8). As well, when investigating survival, heifer calves were more likely to have died by weaning (Chapter 8 and 9) but also, risk of mortality was higher at least until first service (Chapter 9).

Altogether, the results obtained from these studies indicate that dystocial calves have poorer welfare compared to calves that were unaided at birth. This was particularly evident in the neonatal period where results indicated likely higher physiological stress, poor health and survival. In those studies however, the dystocial heifers that survived to weaning performed as well as the eutocial heifers in terms of growth to weaning and first service, and in terms of subsequent fertility as nulliparous animals (Chapters 8 and 9). It is possible that only the fittest dystocial animals survived to weaning, therefore explaining similar performance. Alternatively, similar growth and performance may be explained by good farm management. Nonetheless the dystocial heifers were still more likely to die until first service and this suggests that those animals may have been affected beyond weaning.

Altogether, the results presented in this thesis suggest that dystocial calves have poorer welfare in the neonatal period and possibly beyond. Further investigation of the health of those calves beyond weaning would be needed to conclude more firmly on the welfare outcome of the dystocial heifers. Recent studies on dairy cattle have claimed that being born following a difficult birth could have adverse effects on the survival and production of those animals, when they become adult cows (Eaglen et al., 2010b; Heinrichs and Heinrichs, 2011; Henderson et al., 2011). Yet, there is a lack of longitudinal studies investigating the long-term effects on the welfare of these animals in relation to their experience in early life and whether this is an animal welfare issue.

Although the work presented throughout this thesis supports the hypothesis that dystocial calves have poorer welfare, further investigation would be needed to look at

how the experience of an assisted birth may have affected their mental state, cognitive abilities and stress reactivity but also their subsequent sensitivity to pain. The experience of pain, stress and hypoxia, as likely to be experienced in the dystocial neonate, has been related to long-term effects on the neurological and behavioural development of the neonate as well as the subsequent stress reactivity and pain sensitivity in various species, including cattle (Braastad, 1998; Anand, 2000; Bonsignore et al., 2006; Vinuela-Fernández et al., 2007; Derbyshire, 2008; Rutherford et al., 2009b). For example, human babies born from assisted deliveries showed an higher stress response at 8 weeks of age than babies born naturally or through an elective caesarean section (Taylor et al., 2000) while high cortisol levels at birth are possible contributors of metabolic disorders (Bertram and Hanson, 2002).

Finally, alongside preventive measures to reduce birth difficulties, the question of whether action can be taken following a difficult birth to alleviate the adverse effects on the health and survival of the dystocial calf can be implemented, would be of great benefit to the welfare of the calf but also from an economic perspective.

## 10.7. Tackling calving difficulty

From previous research and the research presented throughout this thesis, it is evident that the incidence of calving difficulty should be tackled in dairy cattle. Any action taken to tackle this issue will help improve welfare of dairy cows and calves but also improve the farm's sustainability.

### 10.7.1. Raising awareness

Most farmers would agree that calving and the early lactation period is a critical period for dairy cows. Despite this, it should not be taken for granted that there is no need to raise awareness about how detrimental difficult calvings can be. The following few points should be emphasised:

- Calving difficulty is a problem to the dairy industry and not just a problem to the beef industry.
- Any necessary assistance duly provided at the time of calving should be regarded as calving difficulty and not just cases where a veterinarian was called out.

- Calving difficulty is not just a problem for the cow but it also is a problem for the calves that will later on constitute the lactating herd.
- It is not just an animal welfare problem but also has adverse economic implications.

### **10.7.2. Preventing calving difficulty: possible action levers**

Prevention of difficult calvings is essential if we are to limit its subsequent adverse effects. The causes, risk factors and current recommendations to avoid dystocia have been highlighted in section 1.3.

Although heritability of calving difficulty is low, selection against calving difficulty in dairy cattle is possible and offers long-term prospects of improving the current level of calving difficulty. Nonetheless, although this should certainly be taken forward, emphasis should also be placed on the importance of the management at the system level, during pregnancy, in the peripartum period and during calving, which are so-called environmental effects. At the farm level, optimal growth to first calving, age and body condition score at first calving, the use of pelvimetry, the adequate choice of the sire used for insemination taking into account potential for calving difficulty, and calving environment are all important factors to be taken into account in the prevention of calving difficulty.

### **10.7.3. Mitigating the effects of calving difficulty when it occurs**

Adequate timely detection of calving difficulty (through direct supervision and/or the use of automated devices) and skilled hygienic assistance when difficulty occurs could also help in mitigating the adverse effects seen. Administration of pain relief to the cows may also alleviate some of the effects seen, through quicker recovery. Diet supplementation and quick provision of high quality colostrum to dystocial calves may be possible to dampen some of the adverse effects seen, but this would need further investigation.

## **10.8. Recommendations**

Following this research, the following recommendations were made to the stakeholders of the project.

- Focus and awareness should be given to the impact that calving difficulty has on the calves, as well as to the cows.
- Any calving difficulty (any calving requiring any type of assistance) should be tackled in future prevention programs. Under the current scale used for genetic evaluations in the United Kingdom, this means that any calving score > 1 should be penalised.
- It was also recommended that calving difficulty should nonetheless be scored on a multiple scale as opposed to a binary scale, so that weights on severity can be applied. Further recommendations on how this should be done were transmitted.

This thesis also gives incentive for more research to be done. Pain at calving should be further considered in view of the scarcity of information available in cattle but also more generally in periparturient farm animals. Particularly, methodologies to assess pain in the parturient animal should be developed and validated. There is also an incentive to find suitable drugs for alleviation of pain and test their efficacy. In the calves, the underlying mechanisms responsible for the adverse effects seen, their emotional states and cognitive abilities should be investigated. Further information on welfare should be gathered in heifer calves that survived weaning. Finally, the management of the dystocial calf should be considered.

## 10.9. Conclusions

This piece of work provided increased insight into the health and welfare of the dairy cow and the dairy calf after a difficult calving. Adverse effects were seen from the lower degrees of difficulties and were increasingly more severe with higher degrees of difficulty. The severity of the effects observed after a difficult birth were even more pronounced for the dairy calves than for their dams.

The work developed a new approach to look at milk losses in the dairy cow that is thought to better reflect the biological stresses experienced after a difficult calving. An animal orientated approach was also taken to address the performance of the cow after a difficult calving and what it implies in terms of welfare. This thesis also provided a better understanding of the parturition progress and behavioural changes when calving

difficulty occurs. The implications in terms of pain experienced by the cows at parturition were discussed. This thesis offers more comprehensive studies on the welfare outcome of dairy calves that are born following an assisted birth, in the neonatal period, as well as at a later age. It also provides evidence that dystocia is not just a problem for the cows but that dystocia is also an issue for the resulting calves which is not limited only to the issue of stillbirth.

Preventive measures should focus on all degrees of calving difficulty and not just on the cases that involved veterinary assistance. Any strategy designed to reduce the occurrence of dystocia or to mitigate its effects will therefore improve the welfare of the dairy cows, their calves and enhance the farm's economic sustainability.

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## **Appendix A: Barrier and Haskell (2011)**

This Appendix contains a copy of the following research article:

Barrier, A. C., and M. J. Haskell. 2011. Calving difficulty in dairy cows has a longer effect on saleable milk yield than on estimated milk production. *J. Dairy Sci.* 94(4):1804-1812.

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## Calving difficulty in dairy cows has a longer effect on saleable milk yield than on estimated milk production

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### ABSTRACT

A difficult calving affects the welfare of the cow and has economic implications for the farm. The degree of calving difficulty can vary from no assistance needed through a slight pull required to surgery being needed. With respect to milk production, it is not clear at which degree of calving difficulty adverse effects occur or for how long they last. Studies usually only consider the milk produced by animals who completed full lactations but the saleable milk production of the whole herd, regardless of each cow having achieved a full lactation, might be a better indicator of the productivity of the cows and the underlying stresses they experience, as well as being more representative of the real losses that producers incur. The objective of this study was to investigate how various degrees of calving difficulty would alter both the cow's milk production and their production of saleable milk over different stages of their subsequent lactation. The calving difficulty scores and the subsequent milk production were retrieved from an experimental dairy farm (in the United Kingdom) for 2 herds that contained 2,430 and 1,413 lactations. To account for milk saleable by the farmer, individual cumulative saleable milk yields, referred to as saleable milk yields (SMY), were calculated at 30, 60, 90, and 300 d in milk unconditional on the animal having achieved the lactation stage of interest. Lactation SMY were obtained based on the real lactation length achieved by the animal. Mean daily milk yields were also calculated for the same lactation stages as an estimate of the cow's milk production (CMP). Calving difficulty impaired milk production of dairy cows in terms of CMP and SMY in both herds, highlighting impaired income for dairy producers as well as detrimental effects to the productivity of the cows and potentially impaired health and survival. The management of the herd affected the presence of an effect of each degree of difficulty on SMY and CMP as well as its magnitude and duration. The analysis of SMY, independently of

each animal having achieved a full lactation, could be a more sensitive indicator of the subsequent long-lasting biological stresses than CMP alone.

**Key words:** dairy cow, calving difficulty, milk production, dystocia

### INTRODUCTION

Parturition is intimately interlocked with lactogenesis in all mammals. In dairy cows, it marks the start of the lactation and therefore the beginning of the productive cycle. However, it is also a high-risk time for both mother and offspring. Calving difficulty, also known as dystocia, often means that assistance must be provided during delivery. The grading of the amount of assistance required is therefore a widely used measure to identify different degrees of difficulty. In the United Kingdom, severe cases are thought to occur in 7% of primiparous births (Mee, 2008) but rates of assisted calvings are much higher, reaching about 16% nationally (Wall et al., 2010). Not only does calving difficulty increase farm workload, it also has adverse effects on the subsequent survival, health, and performance of mothers and offspring (Dematawewa and Berger, 1997; Lombard et al., 2007; Tenhagen et al., 2007). For a review on effects and risk factors of dystocia, see Mee (2008). Thus, calving difficulty raises productivity, economic, and animal welfare issues.

It has been shown that calving difficulty reduces milk yield in the cow. It is not clear, however, how long the adverse effect on milk production lasts. In fact, although some authors seem to find a deleterious effect on the overall lactation of cows (Mangurkar et al., 1984; Djemali et al., 1987; Dematawewa and Berger, 1997), some studies suggest shorter term effects that disappear beyond 14 DIM (Rajala and Grohn, 1998), 90 DIM (Thompson et al., 1983), or 6 mo postpartum (Tenhagen et al., 2007). Furthermore, the degree of difficulty at which milk losses are reported ranges from slight degrees of difficulty (Djemali et al., 1987; Dematawewa and Berger, 1997) to only in severe cases when surgery is needed (Tenhagen et al., 2007). Additionally, losses are thought to be greater with increasing degrees of difficulty (Djemali et al., 1987; Dematawewa and Berger,

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1997). However, the pattern with which milk losses vary is not always obvious (e.g., Mangurkar et al., 1984) and other factors such as the overall yield or parity of the cow (Rajala and Grohn, 1998) might influence it. The underlying reasons for such variation could be attributed to a range of factors such as different scoring methods, animal genetics, livestock management, calving management, or even evaluation methods.

Using milk production to assess the performance of dairy cows is straightforward and can be seen as being related to the income of dairy producers. This animal-oriented approach, combined with other indicators, can give some insight into the welfare of the animal, because milk yields are known to be lowered in the event of stress (Hasegawa et al., 1997; Bruckmaier and Blum, 1998) or disease because of the redistribution of energy requirements in favor of the immune system (Rajala and Grohn, 1998; Bareille et al., 2003). The use of data sets that contain animals with full lactations only or that have achieved a certain stage of lactation seems to be common practice when calculating effects of calving difficulty on milk production (Mangurkar et al., 1984; Djemali et al., 1987).

However, dystocical animals are more likely to die or be culled in the early stages of their lactation (Tenhagen et al., 2007; Dobson et al., 2008). Therefore, restriction on lactation length could introduce bias in the results. Consequently, taking into account the whole herd, whatever the lactation stage each animal actually achieves, seems preferable to represent the milk losses that producers incur.

Furthermore, the use of such data sets usually implies extrapolating the milk production from animals to which daily continuous records are not known (such as test-day yields). Because milk production is decreased during a sickness episode, if sampling occurs at or near that episode, the extrapolation of a cow's milk production can consequently give a biased estimation of her total yields. Unfortunately, dystocical cows are known to be more likely to suffer from diseases (Thompson et al., 1983; Benzaquen et al., 2007). Therefore, such cases are more likely to occur and bias to arise in dystocical animals.

Although saleable milk yield is related to the animal's milk production, it can account for additional losses due to diseases and their treatment, such as medication requiring a specific milk withdrawal period (e.g., a mastitis episode requiring antibiotic treatment when the milk produced cannot be sold commercially).

Taking the producer's perspective by assessing cumulative saleable milk yields, regardless of each animal achieving a full lactation, could address both concerns. In fact, such an approach at the herd level can take into

account the losses due to animals that suffer from diseases or leave the herd early (Figure 1). Animals that suffer from diseases may have lowered or null saleable yields for a short period, whereas cows leaving the herd early do not provide any more saleable milk. Additionally, such a method reflects the dairy producer's true income.

The objective of the study was to investigate how various degrees of calving difficulty would alter the production of saleable milk of UK dairy cattle over different stages of their subsequent lactation as well as their estimated milk production.

## MATERIALS AND METHODS

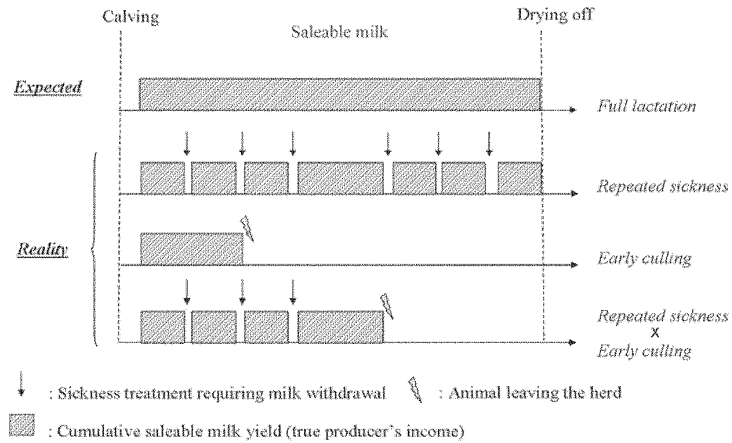
### *Animals, Housing, and Management*

Data from lactating animals were obtained from the Scottish Agricultural College experimental Holstein-Friesian dairy herd (Scotland, United Kingdom). This herd was managed in accordance with the UK regulations on animal care and ethics of experimental animals. Following a long-term genetic breeding and feeding system project, animals were from 2 genetic groups (animals selected toward greater milk solids production and animals selected to be the rolling UK average) and split over 2 diet types (high-forage and low-forage diets; Pryce et al., 1999; Bell and Roberts, 2007). Cows from both genetic groups that were not on this long-term trial were fed commercial diets of low-forage types. The herd was managed from 1990 to 2001 inclusive at the University of Edinburgh near Edinburgh (hereafter, the Edinburgh herd) where it was shared with their veterinary school, and was then moved to the Scottish Agricultural College dairy research center at the Crichton Royal Farm in Dumfries (the Crichton herd), where it was managed from 2002 onward.

The Edinburgh herd was milked twice a day and the diets were formulated to contain approximately 1,500 and 2,500 kg of concentrate per lactation for the high- and low-forage diets, respectively, representing average practice usage in the UK. Nontrial cows were fed a diet similar to the low-forage diet.

The Crichton herd was managed in 2 contrasting management systems, with one group kept indoors on a low-forage diet and the other group fed a home-grown, high-forage diet with summer grazing. Nontrial cows were fed a nontrial diet similar to the low-forage diet. The Crichton herd was milked 3 times daily from 2003 onward.

In both herds, calving difficulty was scored as follows: **N** = no assistance; **FN/FM** = farm assistance without/with malpresentation of the calf; **VN/VM** =



**Figure 1.** Representation of the saleable milk production of an individual dairy cow over her lactation either as expected to be achieved over a full lactation or actually produced (reality). Unlike expectations, in reality, milk cannot always be sold when some treatments for sickness occur that require a milk withdrawal period. Moreover, when the cow leaves the herd, production of saleable milk will stop. A combination of the above scenarios can also happen. For each individual cow, at any point in her lactation stage (achieved or not), the cumulative saleable milk produced is more representative of the income she provides to her keeper rather than what could have been expected.

veterinarian assistance without/with malpresentation of the calf; and VC = caesarean section.

**Data Set Description**

Data on individual cows lactations were obtained from the farm's database. Data were extracted on the condition that a calving difficulty score was available, the cow had not aborted, and the lactation number was <10. No restriction was made on a minimum lactation length for the reasons stated above. Prevalence and causes of dystocia may differ among parities, with higher difficulty rates and predominance of cases of feto-pelvic incompatibility in primiparous animals (Mee, 2008). However, the aim of the study was to look

at the effects of different degrees of difficulty on milk production at the herd level, regardless of how the difficulty arose, and therefore, animals from both parities were included.

Lactation yield data of animals having calved from 1990 to 2001 inclusive and from 2003 to August 20, 2009, only were included so that for each herd, all animals would have the same management background, and the transition period between sites was excluded. The data set therefore contained data on 3,843 lactations: 2,430 in the Edinburgh herd completed by 898 animals and 1,413 in the Crichton herd completed by 555 animals (Table 1).

For each lactation of each cow, characteristics such as cow identity, parity (primiparous vs. multiparous),

**Table 1.** Number of lactations per calving difficulty score (relative proportion of scores, %, in parentheses) for both Edinburgh and Crichton herds

Herd	Calving difficulty <sup>1</sup>						Total
	N	FN	FM	VN	VM	VC	
Edinburgh (n = 898)	1,986 (81.7)	265 (10.9)	84 (3.5)	44 (1.8)	33 (1.4)	18 (0.7)	2,430
Crichton (n = 555)	1,188 (84.1)	170 (12.0)	36 (2.5)	6 (0.4)	8 (0.6)	5 (0.4)	1,413

<sup>1</sup>N = no assistance; FN/FM = farm assistance without/with malpresentation; VN/VM = veterinarian assistance without/with malpresentation; VC = caesarean section.

genetic group, diet fed during the lactation, calving season (summer: April to September; winter: October to March), calving year, age at calving (mo), sire of the cow, and calving difficulty scores were extracted from the database. Birth weight of the newborn, sex of the calf, calving weight, and calving condition score of the dam at parturition were not considered as candidates to be included in the statistical model. The reason for this is that our interest was on the effect of calving difficulty in its own right. Those variables are known to be confounded with calving difficulty in the literature (Meijering, 1984; Mee, 2008) and this was checked in our study by using data plots and looking at the variation shared if forced into the models.

#### **Milk Production Data: Saleable Milk Yields and Cow's Milk Production**

In both herds, individual daily milk production was recorded automatically at milking conditional on the milk being sent to the tank for sale and, therefore, considered as being saleable milk.

Cumulative saleable milk yield (SMY, L) was calculated at different stages of the lactation of interest: 30, 60, 90, and 300 DIM. Saleable milk yield was calculated regardless of each animal having achieved each of these stages to account for the amount of milk the producer was actually able to sell within each period. As shown in Figure 1, calculations of SMY were performed by summing the daily amount of milk sent to the tank during those periods, the amount being null when the milk was not saleable because of a medical treatment or from the death or culling of the animal onward when applicable. Lactation SMY were obtained based on the real lactation length achieved by the animal (rather than truncating at 305 DIM). Saleable milk yields were used to reflect a producer's perspective.

To reflect on the cow's perspective, the average daily amount of milk produced by the dam, referred to as cow's milk production (CMP), was used. For each of the lactation stages of interest, this was calculated as being the ratio between the cumulative SMY during that period and the number of days when milk was sent to the tank during the same period. Data from 2,239 and 1,325 lactations were available for analysis of both the cumulative SMY and daily mean yields in the Edinburgh and Crichton herds, respectively.

Because of the data extraction process, some of the cows from the Edinburgh herd appeared in our data set with artificially truncated lactation implied by the cut-off point of 2002. Therefore, their milk production data were accounted for only until the lactation stage of interest preceding the truncation. Therefore, this

led to a slight decline in numbers of lactation available toward later lactation stages.

#### **Statistical Analyses**

Data from both herds were analyzed separately. It was decided to keep the 2 herds separate for the analysis instead of accounting for a herd effect. In fact, because most of the animals had been transferred from Edinburgh to Crichton, the 2 herds cannot be seen as fully independent and therefore do not comply with the underlying statistical hypothesis of independence of the variables. Furthermore, management practice at calving, especially the threshold for intervention, seemed to be different in the 2 herds, with a higher propensity of the farm staff to provide assistance at calving and to call for a veterinarian in the Edinburgh herd, where the facilities were shared with the University of Edinburgh Veterinary School (Table 1).

Linear mixed models were applied following a REML procedure in Genstat (11th ed., 2008, VSN International Ltd., Hemel Hempstead, UK) using forward-stepwise logistic regression models (Hosmer and Lemeshow, 2000) as described in previous studies (Haskell et al., 2009; Rutherford et al., 2009). The random model chosen included the cow identity nested within its sire. All variables were treated as fixed effects (factors), whereas age at calving (age) and calving year were treated as continuous variables. Age at calving did not show a linear relationship with milk yields and could not be included as such in the model. However, it showed 2 distinct linear trends for each parity and thus, age at calving was centered on the relevant parity's mean for each lactation (age-C).

An individual model was built for each herd and stage of lactation. Each variable was tested independently as a univariate and became a candidate for the multivariable model if it had a  $P$ -value  $< 0.25$ . The variables tested as univariates were parity, genetic group, diet, season, year of calving, age at calving, and calving difficulty. The candidate variables were then added into the multivariable model using stepwise selection techniques with the most significant variables with the highest Wald statistic being added first. Candidate variables were kept in the model with significance attributed at  $P < 0.05$  (when all other explanatory variables in the models had been fitted), and calving difficulty was always forced in the model and fitted at the end. Interactions were tested once the whole model was set up. This included calving difficulty interacting with, respectively, genetic group, diet, parity, and year, as well as the interaction between year and month, genetic group, and diet as well as parity and age. Finally, normality of the residuals

**Table 2.** Estimated means of the cumulative saleable milk yields (L  $\pm$  SEM) at 30, 60, 90, and 300 DIM and over the completed subsequent lactation of dairy cattle from the Edinburgh herd following different degrees of calving difficulty

Item	Calving difficulty <sup>1</sup>						P-value
	N (n = 1,855)	FN (n = 227)	FM (n = 74)	VN (n = 38)	VM (n = 30)	VC (n = 15)	
30 DIM	646.7 $\pm$ 5.7 <sup>a</sup>	612.9 $\pm$ 11.4 <sup>b</sup>	635.0 $\pm$ 19.1 <sup>ab</sup>	589.7 $\pm$ 26.7 <sup>b</sup>	605.7 $\pm$ 29.6 <sup>ab</sup>	571.7 $\pm$ 42.2 <sup>ab</sup>	**
60 DIM	1,502.0 $\pm$ 12.5 <sup>a</sup>	1,430.0 $\pm$ 25.3 <sup>b</sup>	1,479.0 $\pm$ 42.8 <sup>ab</sup>	1,384.0 $\pm$ 59.7 <sup>ab</sup>	1,425.0 $\pm$ 68.4 <sup>ab</sup>	1,377.0 $\pm$ 93.4 <sup>ab</sup>	*
90 DIM	2,320.0 $\pm$ 19.7 <sup>a</sup>	2,218.0 $\pm$ 40.0 <sup>b</sup>	2,304.0 $\pm$ 67.2 <sup>ab</sup>	2,110.0 $\pm$ 92.8 <sup>b</sup>	2,222.0 $\pm$ 106.1 <sup>ab</sup>	2,165.0 $\pm$ 145.3 <sup>ab</sup>	*
300 DIM	6,375.0 $\pm$ 70.5 <sup>a</sup>	5,859.0 $\pm$ 137.8 <sup>b,c</sup>	6,377.0 $\pm$ 230.7 <sup>bc</sup>	5,520.0 $\pm$ 305.3 <sup>b</sup>	6,003.0 $\pm$ 347.9 <sup>ab</sup>	5,722.0 $\pm$ 492.0 <sup>ab</sup>	***
Completed lactation	6,857.0 $\pm$ 87.9 <sup>a</sup>	6,305.0 $\pm$ 168.0 <sup>b,c</sup>	6,931.0 $\pm$ 280.7 <sup>bc</sup>	5,999.0 $\pm$ 369.4 <sup>b</sup>	6,578.0 $\pm$ 419.4 <sup>ab</sup>	6,221.0 $\pm$ 594.3 <sup>ab</sup>	**

<sup>a-c</sup>Within a row, means with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>N = no assistance; FN/FM = farm assistance without/with malpresentation; VN/VM = veterinarian assistance without/with malpresentation; VC = caesarean section. The number of lactations available for analysis for each calving difficulty score is given in parentheses.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

was verified. Initially, new models were built for each stage of lactation and outcome considered. However, the contributing factors were very similar for the same outcome measure at each stage of lactation. Therefore, for purposes of clarity, only one model was retained and used to analyze an outcome measure throughout the stages of lactation. These models are presented below:

$$SMY = \mu + \text{parity} + \text{genetic} \times \text{diet} + \text{year} \\ + \text{season} + CD + \varepsilon \text{ (Edinburgh herd),}$$

$$CMP = \mu + \text{parity} + \text{genetic} + \text{diet} + \text{year} \\ + \text{season} + CD + \varepsilon \text{ (Edinburgh herd),}$$

$$SMY = \mu + \text{parity} \times \text{age-C} + \text{year} + \text{diet} + \text{genetic} \\ + \text{season} + CD + \varepsilon \text{ (Crichton herd),}$$

$$CMP = \mu + \text{parity} \times \text{age-C} + \text{diet} + \text{genetic} \\ + \text{year} + \text{season} + CD + \varepsilon \text{ (Crichton herd),}$$

where  $\mu$  = the overall population mean,  $CD$  = calving difficulty, and  $\varepsilon$  = the random residual effect.

Considering the few lactations available for the higher degrees of difficulty, the analysis was also run grouping the veterinarian-assisted scores together in a single category (Edinburgh herd:  $n = 83$ ; Crichton herd:  $n = 15$ ).

## RESULTS

### SMY

**Edinburgh Herd.** Parity, genetic group, diet, and calving year affected saleable yields at all stages of the lactation except at 30 DIM for calving season and at both 300 DIM and over the total lactation for genetic

group interacting with diet. Cows experiencing FN and VN scores had decreased cumulative SMY throughout lactation compared with unassisted animals ( $P < 0.05$ ; Table 2). Losses occurred as early as 30 DIM (FN:  $-5.2\%$ ; VN:  $-8.8\%$ ) and persisted until the end of the lactation (FN:  $-8.1\%$ ; VN:  $-12.5\%$ ). No losses were found for FM, VM, and VC births. Grouping the veterinarian-assisted scores together did not alter the significance of the analysis.

**Crichton Herd.** Parity interacting with age-C, calving year, diet, genetic group, and calving season were significant at all stages of lactation except for calving season over the lactation. Calving difficulty resulted in decreased SMY ( $P < 0.05$ ; Table 3) for the FM and VC births (FM:  $-12.4\%$ ; VC:  $-42.8\%$ ) compared with unassisted animals only during the first 30 DIM. No significant effect was found at other stages of lactations. Grouping the veterinarian-assisted scores together did not alter the significance of the analysis.

### CMP

**Edinburgh Herd.** Cow's milk production was affected by parity, diet, genetic group, calving year, and calving season throughout all stages of lactation tested except that the effect of calving season disappeared from 300 DIM onward. Calving difficulty did not affect CMP ( $P > 0.05$ ; Table 4) at any stage of lactation. A reduction in production of 1.7 L/d was observed at 30 DIM ( $P = 0.025$ ) and of 1.6 L/d at 60 DIM ( $P = 0.049$ ) for the veterinarian-assisted scores grouped together ( $n = 83$ ) compared with the unassisted animals.

**Crichton Herd.** Cow's milk production was affected by parity interacting with age-C, diet, genetic group, calving year, and calving season throughout the stages of lactation tested, except that the effect of parity interacting with age-C disappeared from 300 DIM onward. Compared with unassisted animals, calving difficulty was associated with a decrease in the CMP for FM

**Table 3.** Estimated means of the cumulative saleable milk yields ( $L \pm SEM$ ) at 30, 60, 90, and 300 DIM and over the completed subsequent lactation of dairy cattle from the Crichton herd following different degrees of calving difficulty

Item	Calving difficulty <sup>1</sup>						P-value
	N (n = 1,111)	FN (n = 163)	FM (n = 36)	VN (n = 3)	VM (n = 8)	VC (n = 4)	
30 DIM	658.9 ± 10.5 <sup>a</sup>	642.0 ± 18.6 <sup>a</sup>	577.3 ± 124.3 <sup>bc</sup>	489.6 ± 124.3 <sup>abc</sup>	607.5 ± 76.0 <sup>abc</sup>	376.6 ± 107.9 <sup>a</sup>	*
60 DIM	1,510.0 ± 25.8	1,506.0 ± 44.0	1,406.0 ± 85.4	1,384.0 ± 59.7	1,067.0 ± 289.0	1,197.0 ± 251.3	NS
90 DIM	2,304.0 ± 42.2	2,326.0 ± 71.7	2,242.0 ± 138.7	1,619.0 ± 469.0	2,478.0 ± 286.4	2,028.0 ± 408.8	NS
300 DIM	6,462.0 ± 147.8	6,759.0 ± 243.3	6,658.0 ± 464.1	4,302.0 ± 1,563.5	5,978.0 ± 955.0	6,571.0 ± 1,361.2	NS
Completed lactation	7,293.0 ± 189.2	7,700.0 ± 302.8	7,466.0 ± 569.6	4,267.0 ± 1,911.5	6,443.0 ± 1,165.2	6,841.0 ± 1,672.4	NS

<sup>a</sup> Within a row, means with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup> N = no assistance; FN/FM = farm assistance without/with malpresentation; VN/VM = veterinarian assistance without/with malpresentation; VC = caesarean section. The number of lactations available for analysis for each calving difficulty score is given in parentheses.

\*  $P < 0.05$ .

and VC scores of 7.8 and 25.3%, respectively (Table 5), at 30 DIM. A decrease of 34.4, 31.1, and 28.5% of the cow's yields for the VN scores was observed at 30, 60, and 90 DIM, respectively. No effect beyond 90 DIM was shown for any other score of calving difficulty. Grouping the veterinarian-assisted scores together did not alter the significance of the analysis.

## DISCUSSION

### Detrimental Effects of Calving Difficulty on CMP and SMY

Calving difficulty resulted in decreased saleable milk production in both herds but a decrease in CMP was not observed in the Edinburgh herd. However, the increasing variability in CMP over the scores may have masked such an effect. In fact, when variation was reduced by grouping the veterinarian-assisted scores together, this group of animals then had decreased production up to 60 DIM compared with the unassisted animals. A reduction in the milk production of cows experiencing difficulty at parturition is in line with previous studies (Mee, 2008). It is therefore not surprising that SMY were also lowered, probably because of decreased CMP as well as higher odds of being culled or suffering from

diseases in dystocia animals (Tenhagen et al., 2007; Dobson et al., 2008).

Impairment of performance following a difficult calving is mainly attributed to the experience of an increased length of labor (Doombos et al., 1984) and its implications on adrenocortical function (Hudson et al., 1976; Nakao and Grunert, 1990; Civelek et al., 2008). This could lead to decreased yields in the dams (Dobson et al., 2001). Moreover, such hormonal changes depress their immune system, which, along with higher risks of bacterial contamination during assistance (Dohmen et al., 2000), may increase the animal's susceptibility to disease. Additionally, underlying conditions that put dams at risk of dystocia such as inappropriate BCS, poor nutrition over the dry period, and hormonal imbalances might be partly responsible for decreased yields by the animals and affect the development of the udder before the start of the lactation (Banos et al., 2007; Berry et al., 2007; Roche et al., 2009).

Decreased milk production from the dams could also result from lower feed intake postcalving. Although a recent study in dairy cattle did not find differences in DMI between eutocia and dystocia during the first 2 d postcalving (Proudfoot et al., 2009), a study on beef cattle showed that cows having delivered by caesarian section spent less time eating than cows having delivered

**Table 4.** Estimated means of the cow's milk production ( $L/d \pm SEM$ ) at 30, 60, 90, and 300 DIM and over the completed subsequent lactation of dairy cattle from the Edinburgh herd following different degrees of calving difficulty

Item	Calving difficulty <sup>1</sup>						P-value
	N (n = 1,855)	FN (n = 227)	FM (n = 74)	VN (n = 38)	VM (n = 30)	VC (n = 15)	
30 DIM	26.81 ± 0.16	26.52 ± 0.34	26.70 ± 0.57	24.51 ± 0.79	25.79 ± 0.88	25.51 ± 1.26	‡
60 DIM	28.50 ± 0.18	28.32 ± 0.36	28.28 ± 0.60	26.07 ± 0.84	27.70 ± 0.96	27.31 ± 1.31	‡
90 DIM	28.59 ± 0.19	28.44 ± 0.36	28.37 ± 0.60	26.26 ± 0.83	28.02 ± 0.95	27.53 ± 1.30	NS
300 DIM	24.14 ± 0.17	24.17 ± 0.30	24.11 ± 0.49	23.36 ± 0.65	23.68 ± 0.73	23.49 ± 1.04	NS
Completed lactation	23.42 ± 0.16	23.56 ± 0.29	23.19 ± 0.48	23.04 ± 0.63	23.17 ± 0.72	22.71 ± 1.02	NS

<sup>1</sup> N = no assistance; FN/FM = farm assistance without/with malpresentation; VN/VM = veterinarian assistance without/with malpresentation; VC = caesarean section. The number of lactations available for analysis for each calving difficulty score is given in parentheses.

‡  $P < 0.10$ .

**Table 5.** Estimated means of the cow's milk production (L/d  $\pm$  SEM) at 30, 60, 90, and 300 DIM and over the completed subsequent lactation of dairy cattle from the Crichton herd following different degrees of calving difficulty

Item	Calving difficulty <sup>1</sup>						P-value
	N (n = 1,111)	FN (n = 163)	FM (n = 36)	VN (n = 3)	VM (n = 8)	VC (n = 4)	
30 DIM	29.46 $\pm$ 0.28 <sup>a</sup>	28.85 $\pm$ 0.51 <sup>a</sup>	27.15 $\pm$ 0.99 <sup>b</sup>	19.32 $\pm$ 3.37 <sup>b</sup>	28.70 $\pm$ 2.05 <sup>a</sup>	22.02 $\pm$ 2.98 <sup>b</sup>	***
60 DIM	31.32 $\pm$ 0.30 <sup>a</sup>	31.09 $\pm$ 0.50 <sup>a</sup>	29.61 $\pm$ 0.97 <sup>a</sup>	21.57 $\pm$ 3.27 <sup>b</sup>	30.32 $\pm$ 1.97 <sup>a</sup>	26.12 $\pm$ 2.93 <sup>ab</sup>	*
90 DIM	31.55 $\pm$ 0.30 <sup>a</sup>	31.39 $\pm$ 0.49 <sup>a</sup>	30.05 $\pm$ 0.94 <sup>a</sup>	22.57 $\pm$ 3.16 <sup>b</sup>	30.52 $\pm$ 1.89 <sup>a</sup>	27.25 $\pm$ 2.88 <sup>ab</sup>	*
300 DIM	28.94 $\pm$ 0.31	29.08 $\pm$ 0.46	27.81 $\pm$ 0.82	24.10 $\pm$ 2.74	26.90 $\pm$ 1.63	25.91 $\pm$ 2.55	NS
Completed lactation	28.31 $\pm$ 0.31	28.36 $\pm$ 0.47	27.17 $\pm$ 0.85	23.39 $\pm$ 2.82	26.45 $\pm$ 1.69	25.48 $\pm$ 2.58	NS

<sup>ab</sup>Within a row, means with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>N = no assistance; FN/FM = farm assistance without/with malpresentation; VN/VM = veterinarian assistance without/with malpresentation; VC = caesarean section. The number of lactations available for analysis for each calving difficulty score is given in parentheses.

\*\*\* $P < 0.001$ ; \* $P < 0.05$ .

naturally during the first 3 d postpartum (Kolkman et al., 2010). It is very likely that the increased occurrence of sickness during the subsequent lactation may lower their appetite more often. Dystocia cows might experience greater postpartum pain (Barrier et al., 2010) as a result of the traction and associated trauma of the tissues and possible inflammations of the reproductive tract, which in turn, may lower their feed intake.

#### Effects of Calving Difficulty Diverged Between the Herds

In our study, although impairment in CMP and SMY was found in both herds, the scores and stages of lactation affected were not the same within the 2 herds. In our study, the scale used to rate difficulty at calving was similar between both herds and it is unlikely that our definitions led to inconsistency of scoring within the years. The level of supervision at calving and the propensity to assist can vary between farms, reflecting sociological factors (Dargatz et al., 2004). It is believed that the threshold for intervention and for calling a veterinarian was lower in the Edinburgh herd than in the Crichton herd, certainly because the former was co-managed by the veterinary school. As such, a VN calving in Edinburgh could have been scored as an FN calving in Crichton. Other factors such as how assistance was performed, the pre- and postcalving care, as well as the overall management of the herd in terms of housing and milking routine could have caused variation in the effects of a difficult calving. Divergences within the 2 herds were also highlighted in terms of what factors affected milk yields when the statistical models were constructed.

Considering CMP, no decrease was found in the Edinburgh herd, whereas the Crichton herd suffered losses for FM, VN, and VC births. Because estimations for the VN and VC scores relied on only a few lactations, evidence from other studies would be needed to support

this result. Nonetheless, when grouped together, the animals with veterinarian-assisted scores showed significantly lower CMP until 90 DIM. The FM dams did not carry over the detrimental effect on CMP for as long as the VN dams, whereas the VM dams did not suffer losses compared with the other veterinarian-assisted dams. Although malpresentation of the newborn can be thought of as increasing the difficulty of the delivery, it is possible that this occurrence is spotted earlier because of the calf being stuck early in the birth canal or by the nonappearance of the feet. Early diagnosis and assistance could then shorten the labor length, thus diminishing the detrimental effects of prolonged labor on milk production.

In regards to SMY, in the Edinburgh herd, FN and VN cows had decreased SMY throughout the lactation, whereas losses occurred in the Crichton herd for the FM and VC cows only in the first 30 DIM. In both herds, lack of effects on SMY for some of the veterinarian-assisted scores might relate to the few data points available for the analysis, and the dramatic loss found for VC scores in the Crichton herd should be treated with caution for similar reasons. In the Edinburgh herd, the absence of an effect in FM scores on SMY is in line with the absence of a reduction in CMP for this score and might be explained similarly by the shortened labor length. In contrast, FM animals in the Crichton herd showed losses in SMY. This finding could be attributed to lower CMP or the additional manipulation of the calf might have resulted in increased odds of contracting an infection (Dohmen et al., 2000). This may explain why, relative to unassisted animals, losses were much steeper in SMY than in CMP, with a 12.4% decrease in the former and 7.4% in the latter.

Furthermore, in contrast to the Edinburgh herd, where SMY losses were predominant throughout the lactation, SMY losses were not evident in the Crichton herd after 30 DIM. It is possible that the care and subsequent management of the dystocia dams in the

Crichton herd was more favorable for the dams to recover from a difficult calving. However, this idea would not be supported by the hypothesis drawn earlier on FM dams. Furthermore, the 3-times-a-day milking in the Crichton herd compared with twice-a-day milking in Edinburgh may have contributed to the dilution of milk losses over time. In fact, a 1-L reduction in milk production corresponds to an inferior proportional loss when yields are higher. A larger data set may have helped to show significance if an effect after 30 DIM exists.

As discussed in detail above, our results support the idea that the way the herd is managed influences how difficulty at calving will affect the performance of the cow in terms of both subsequent cow's yields and cumulative saleable yields.

***SMY Best Reveal the Economic Shortfall to the Producers and Might Indicate Long-Lasting Biological Stresses in Dystocial Cows***

Despite the differences within herds, using CMP or SMY to assess effects of calving difficulty actually resulted in different effects being shown. This shows that these 2 methods are distinct from each other. In fact, CMP gives insight into the subsequent production by the animal itself, whereas SMY of the whole herd will additionally account for the wasted milk and the absence of saleable milk for the animals that leave the herd and therefore be more representative of farmers' losses. It is therefore not surprising that cumulative SMY were able to show more deleterious effects than CMP alone. For example, no effect of calving difficulty on CMP was observed in the Edinburgh herd (Table 4), whereas SMY were decreased for FN and VN dams. Moreover, SMY losses could be shown at the lactation level when analysis of SMY revealed no significant losses even in early lactation. It is tempting to infer that the deeper effect reported using SMY compared with CMP could be health-related or due to the early culling or death of the dystocial animals. However, although culling rates and morbidity are higher for cows experiencing difficulty at calving (Thompson et al., 1983; Benzaquen et al., 2007; Tenhagen et al., 2007; Dobson et al., 2008), further investigation into the health and survival data of the 2 herds would help reach a firmer conclusion in this particular study.

Therefore, using cumulative SMY on the whole herd rather than CMP could reveal greater shortfall to the producer because it can account for some of the disease and culling costs. It was not the aim of the study to provide an economic analysis of the herd, which would need to be multifactorial. However, to illustrate the income loss experienced by the producer, extrapolation

of the results obtained on SMY from the Edinburgh herd would mean a shortfall of around \$3,250/yr for the average UK dairy farm (considering an average farm with 112 lactating animals, a milk price of 24 pence/L, and £1 = \$1.6) in terms of income drawn from milk sales.

As explained above, a loss of SMY calculated on the whole herd indicates decreased production by the animals, health issues, animals leaving the herd, or a combination of these. To that extent, SMY also indicate better the biological stresses that the dystocial animal might encounter during the subsequent lactation than CMP alone.

Therefore, considering the economic shortfall to dairy producers and the deleterious effects on the cow, using saleable milk production of the whole herd seems to be an attractive approach for improvements to be made. On the other hand, some practicalities should not be ignored and it would be not only very challenging but also very costly to collect such data on a larger scale. Furthermore, from a statistical point of view, the study of CMP resulted in less intrinsic variation in the data than SMY and therefore it is easier to pick up existing differences in the former. Nonetheless, the analysis of the cumulative saleable yield of the whole herd best reflected the producer's losses following a difficult calving and seemed to better address the occurrence of long-lasting biological stresses that can be related to animal welfare.

## CONCLUSIONS

Calving difficulty was found to impair the milk production of dairy cows and production of saleable milk, resulting in reduced income for dairy producers as well as detrimental effects to the cows. Looking at cumulative saleable milk yields of the whole herd, independently of each animal having achieved a full lactation, might give a more sensitive picture of what is happening to the cow and as such, might reflect more accurately the underlying biological stresses experienced by the animals. Finally, herd management clearly influenced the magnitude and duration of the effects and at which degree of difficulty adverse effects were found. Alleviating difficulty at calving through good herd management practice before, during, and after parturition, as well as genetic improvement of the animals, should therefore benefit both dairy herds and producers.

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## **Appendix B: Preliminary study**

It has previously been shown that most of the behavioural changes observed at parturition occur in the 6 hours preceding the expulsion of the calf (Miedema et al., 2011b). Furthermore, despite individual variability, most of the animals start labour within the 6 hours preceding the birth of the calf (Berglund et al., 1987; Lidfors et al., 1994; von Keyserlingk and Weary, 2007; Hyslop et al., 2008). Therefore, when investigating the calving progress and the behaviours of assisted and non-assisted dairy cows, it seems a wise option to focus observations on the 6 hours preceding birth of the calf.

### **B.1. Objective of the preliminary study**

The purpose of the preliminary study was to explore the possibility of sampling behaviours at parturition rather than scoring behaviours continuously for the 6 hours preceding expulsion of the calf. The implication of this refinement would be to reduce labour associated with video-watching and therefore, to offer the possibility of increasing the sample size.

### **B.2. Material and methods**

Continuous focal observation of the 6 hours preceding parturition of 44 dairy cows (22 first parities, 22 multiparous, half of each either giving birth naturally or being jack assisted) were gratefully obtained from a previous research project (Miedema et al., 2011b) and used for the purpose of this study.

For the main behaviours of interest (Table B.1), continuous data were extracted for the six hours prior to calving, as well as continuously for the last two hours and in 30 minute segments for the first four hours. Continuous data were also extracted in 30 minute segments for the 6 hours prior to calving and graphed to facilitate decisions on the sampling scenarios (Figure B.1).

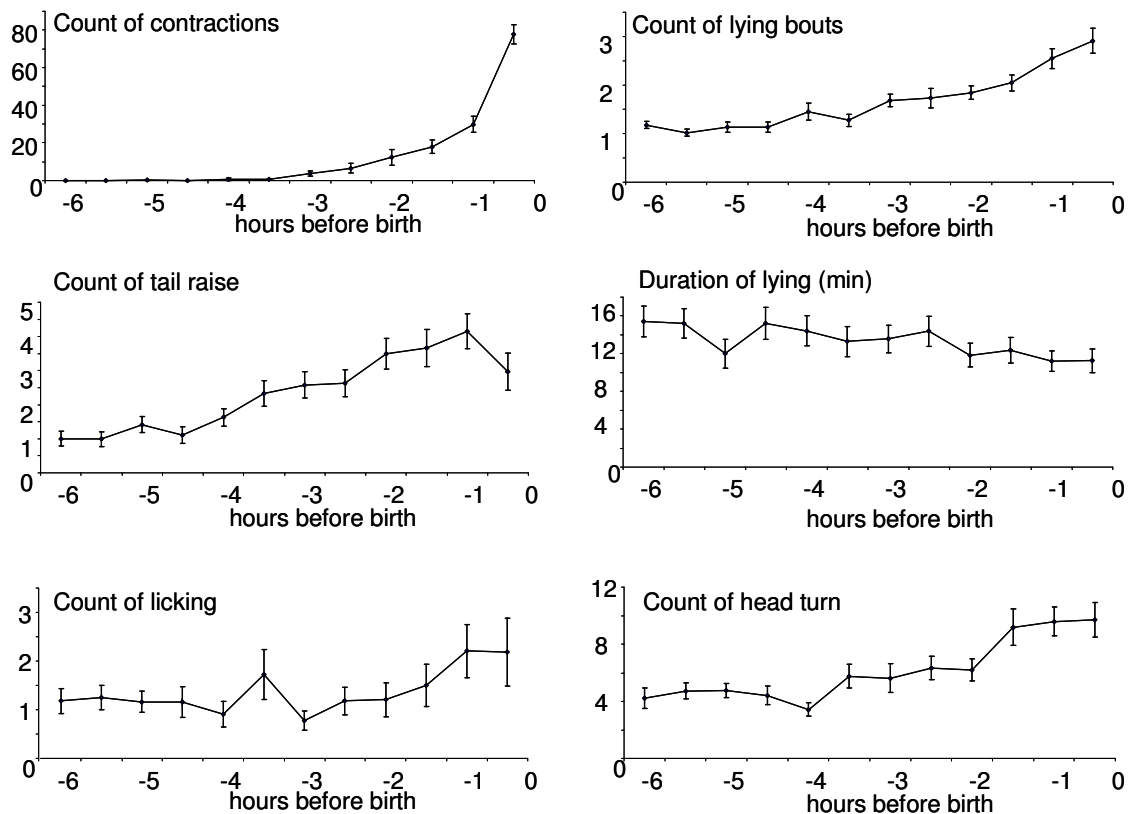
Two sampling scenarios were considered:

- Scenario 1: the first 30 minutes of each hour for the first four hours and the last two hours continuously
- Scenario 2: the first 30 minutes of the observation, a continuous hour from 4 to 3 hours before birth and the last two hours.

Data obtained from each scenario were then extrapolated to predict the six hours of continuous behavioural observations through the appropriate calculations. For each variable, predicted values from each scenario were compared with the observed values using a Mann-Whitney U test (Genstat, 11<sup>th</sup> Ed.). The null hypothesis tested was that the predicted values would not differ from the observed values.

**Table B.1** For each scenario, and behaviour, P values obtained for each Mann-Whitney U test comparing the predicted and the obtained values. P<0.05: predicted values differ from the obtained values (in bold).

Bouts of behaviours	Scenario 1			Scenario 2		
	Total number	Total duration	Mean duration	Total number	Total duration	Mean duration
Walking	0.789	0.899	0.564	0.870	0.800	0.581
Standing	0.751	0.687	0.787	0.794	1.000	0.509
<b>Lying</b>	<b>&lt;0.001</b>	0.663	<b>&lt;0.001</b>	<b>0.006</b>	0.917	<b>0.027</b>
Head up	0.719	0.931	0.718	0.929	0.878	0.826
Head down	0.995	0.997	0.997	0.899	0.681	0.858
Head turn	0.513	-	-	0.811	-	-
Eating	0.693	-	-	0.560	-	-
Drinking	0.214	-	-	0.110	-	-
Lick ground	0.684	-	-	0.935	-	-
Lick self/other	0.658	-	-	0.824	-	-
Tail raise	0.896	-	-	0.903	-	-
Contraction	0.955	-	-	0.213	-	-



**Figure B.1** Mean ( $\pm$  s.d) count of contractions, lying bouts, tail raising, licking, head turn and duration of lying for each 30 min slot from 6 hours preceding birth of the calf until birth of the calf.

### B.3. Results and discussion

Both scenarios were good at predicting the continuous observed data, with only number and average duration of lying bouts being badly predicted ( $P < 0.05$ ). However, the number of lying bouts was deemed an important behavioural indicator that needs to be estimated in a reliable manner. Therefore, none of the two scenarios could be used to predict the continuous six hours of observations.

### B.4. Implications and decisions made from this preliminary study

None of the scenarios investigated can be used to predict continuous data. However, it is still possible to refine the observation periods to key periods when most changes are

expected to happen without attempting to extrapolate the data to continuous observations.

According to the graphs and the literature, the last two hours contain most of the start of labour (Berglund et al., 1987), time spent standing (von Keyserlingk and Weary, 2007) and appearance of the waterbag (Hyslop et al., 2008). Furthermore the median time for restlessness has been reported to be around 3 hours preceding birth of the calves (Berglund et al., 1987) while waterbags could appear as early as 4 hours before (Hyslop et al., 2008). From the graphs, it looks like the period of 4 hours to 3 hours preceding birth would be a good target to discriminate changes between assisted and non-assisted cows. In fact, it is expected that assisted animals might show changes earlier than non-assisted animals and therefore, while some changes may have occurred in the assisted animals at that time, they may not have occurred yet in the eutocial animals. In conclusion, for the rest of the study, no prediction of continuous data on the last 6 hours prior to birth will be made. Instead, behaviours will be compared between the scores of calving difficulty over the observation periods described in scenario 2.

## **Appendix C: Barrier et al. 2012**

This Appendix contains a copy of the following research article:

Adapted from: Barrier, A.C., E. Ruelle, M.J. Haskell, and C.M. Dwyer. 2012. Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. *Preventive Veterinary Medicine*. *Preventive Veterinary Medicine*, 103 (4): 248-256.

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## Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam

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### ARTICLE INFO

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### ABSTRACT

The neonate's development and survival is dependent upon being vigorous at birth and receiving appropriate maternal care. However, difficulty at delivery can result in less vigorous offspring and maternal care can be altered, probably as a consequence of exhaustion, pain and human intervention. The first 3 h after expulsion of the calf were observed continuously from videos following twelve natural calvings and sixteen calvings assisted by farm staff (including four malpresentations) from Holstein cows. Calvings were balanced within groups for parity of the dam, genetic group, sex and birth weight of the calf, calving pen and calving season. Assisted calves were less vigorous with higher latencies to attempt to stand, achieve standing, walk and reach the udder than unassisted calves ( $P < 0.05$ ). Furthermore, assisted calves also tended to be less likely to stand and walk within the first 3 h after birth ( $P < 0.1$ ), spent more time lying on their flank ( $P = 0.019$ ) and had more frequent bouts of this behaviour ( $P = 0.033$ ). Assisted dams did not take longer to lick the calf and performed as much licking as unassisted dams ( $P > 0.05$ ), indicating no delayed onset or impaired expression of maternal behaviour in dams given assistance at delivery. Study of potential pain-related behaviours revealed that assisted dams spent less time self-grooming ( $P = 0.033$ ) than dams delivering naturally, which could suggest greater pain. However, there were no significant differences in any of the other pain-related behaviours. Our results suggest that, although maternal behaviour was unaffected by a difficult delivery, dairy calves born following difficult calvings have lower vigour in the first 3 h after birth than unassisted calves. This might have longer-term effects on the health and survival of the calves.

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### 1. Introduction

Parturition is a challenging process and a high-risk time for both the mother and her offspring. Difficulty at birth, also known as dystocia, results in lower vigour in offspring as shown in lambs (Dwyer, 2003; Dwyer and Lawrence, 2005) and in beef calves (Riley et al., 2004; Poppe et al., 2006; Hickson et al., 2008). It can be assessed by the speed with which an offspring displays a succession

of neonatal behaviours shortly after birth that lead to standing (Dwyer, 2003; Nowak and Poindron, 2006; Baxter et al., 2008), and culminate in a successful bout of sucking.

High vigour at birth is crucial to the neonate's survival and development (Riley et al., 2004) and can result in a better welfare of the newborn (Mellor and Stafford, 2004; Dwyer, 2008b). Early ingestion of colostrum ensures the adequate acquisition of passive transfer of immunity in the immunologically naïve newborn calves, which is important for their survival (Bush and Staley, 1980; Beam et al., 2009; Waldner and Rosengren, 2009). Failure or inadequate passive transfer of immunity has been

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identified as the major contributing factor to the high morbidity and mortality of dairy heifers (Trotz-Williams et al., 2008; Beam et al., 2009; Vasseur et al., 2010). Dystocial calves are at even higher odds of stillbirth and perinatal mortality and morbidity (Chassagne et al., 1999; Johanson and Berger, 2003; Lombard et al., 2007), which may be related to poor vigour, inadequate colostrums intake and hence inadequate passive transfer of immunity.

The rapidity of the onset and the quality of maternal care received contributes to the motivation and success of the calf in performing neonatal behaviours that will lead to ingestion of colostrum (Alexander and Williams, 1964; Dwyer, 2008a). For example, maternal grooming dries, cleans and stimulates the calf to seek the udder (Edwards, 1983; Nowak, 1998; Nowak and Poindron, 2006; von Keyserlingk and Weary, 2007) as well as stimulating the establishment of a durable bond between the two by ingestion and inhalation of amniotic fluid (Poindron, 2005; Poindron et al., 2007; Dwyer, 2008a). Receiving appropriate maternal care as quickly as possible is therefore crucial to the survival of the calf. However, difficulty at delivery can alter maternal care, probably as a consequence of exhaustion, pain and human intervention (Alexander et al., 1988; Dwyer et al., 2001; Fisher and Mellor, 2002).

Little attention has been given to the pain experienced by cows at parturition (Rushen et al., 2007), although components of parturition pain for cows may be similar to what is encountered in women (Gregory, 2004) and dystocia has been rated as one of the most painful conditions in surveys of veterinarians (Huxley and Whay, 2006; Laven et al., 2009; Kielland et al., 2009). Dystocial cows remain in labour for longer periods of time compared to a natural calving (Berglund et al., 1987). They may undergo more uterine contractions and straining, the occurrence of which is painful in women (Corli et al., 1986; Lowe, 2002). In dystocial deliveries, additional pain may also be experienced from pressure applied to extract the newborn (Scott, 2005) and stretching in the birth canal. In support of this, assisted cows have been reported to give a roar when the calf is extracted coinciding with the passing of the head and forelegs (Gregory, 2004). Higher blood vasopressin (hormone secreted in response to stressful/painful stimuli) concentrations have been found with higher levels of parturition pain (visually assessed) in dairy goats (Olsson et al., 2004) and in heifers requiring assistance at birth (Hydbring et al., 1999). Beyond the birth of the calf, the cow still undergoes contractions and might suffer from underlying pain and injuries resulting from the parturition, in the subsequent days postpartum.

High vigour at birth and reception of appropriate care is crucial to the neonate's survival. However, a difficult delivery may lower calf's vigour in conjunction with altering maternal behaviour of the dams and the dam's postpartum pain should be addressed. The objective of the study was to examine the effect of calving difficulty on the subsequent vigour of the dairy calf, the onset and quality of the maternal behaviour and on some putative behavioural indicators of pain in the dams.

## 2. Materials and methods

### 2.1. Animals, housing and calving management

The study took place at the SAC dairy Crichton Royal Farm (Dumfries, UK) in accordance with the UK regulations on animal care and ethics of experimental animals. Animals were from two genetic groups (S: animals selected towards greater milk solids production (fat and protein); C: animals selected to be the rolling UK average) as part of a long-term genetic breeding and feeding trial (Pryce et al., 1999; Bell and Roberts, 2007) and calvings took place all year round.

Preparturient Holstein heifers and cows were housed in one of the two contiguous roofed calving sheds (36 m × 5.9 m; 36 m × 5.7 m) approximately 3 weeks before they were due to calve. There were on average 8 animals housed per shed at any one time and sheds were illuminated round the clock. As part of the long-term on-farm trial, one calving shed was provided with a low forage diet while the other was provided with a high forage diet. Multiparous cows from both genetic lines were allocated to a shed dependent upon their diet allocation but heifers were allocated to either shed to balance each feeding group for numbers as they were not allocated to a diet group until they calved. Animals were bedded on straw, provided with *ad libitum* access to water and sheds were cleaned regularly. Fresh total mixed ration was delivered at the feeder in the afternoon once every two days.

Dependent upon occupancy, when spotted in labour (e.g., cows straining, tail raised, restlessness) calving animals were isolated from their group-mates by a barrier placed within each shed, near the entrance and opposite to the feeders. Within each shed, the barrier set-up allowed contact with group-mates and created a maternity pen of 5 m long (respective width of 5.9 m and 5.7 m) with access to *ad libitum* water.

Supervision of calvings, the decision to provide assistance, assistance and the allocation of a calving difficulty score was made by up to four experienced farm staff. Supervision was routinely ensured between 3:45 am and 11 pm with the possibility of an additional shift if judged necessary. It is farm practice to assist cows only when farm staff judges that a cow is in difficulty. Such judgement is based on criteria such as absence of labour progress (an investigation would be carried out after an hour without visible progress once waterbags have appeared), distress of the cow (e.g., excessive restlessness, vocalisation, cow lying flat, straining without progress) and distress of the calf (e.g., tongue protrusion, swelling of the calf). Assistance was used as a proxy measure for calving difficulty and scores of calving difficulty were: N (no assistance), FN/FM (Farmer assistance without/with malpresentation of the calf), VN/VM (Veterinarian assistance without/with malpresentation of the calf) and VC (caesarean section). Over the last two years, 18.7% of the calvings on this farm were assisted (33% of heifers and 11.9% of cows), which reflects average practice from UK Holstein dairy farms despite slightly higher assistance rates in heifers but lower in cows.

Once born, calves were separated from their mother within 24 h but rarely spent less than 4–5 h together. Birth

weights were taken at the time of separation. The calf was eartagged, its navel disinfected with an iodine solution within the first 8 h, and fed additional colostrum on judgement of the farm staff by stomach tubing.

## 2.2. Video-recording

The calving sheds were continuously video monitored throughout the trial. For each shed, 12 weather proof infrared CCTV cameras (1/3" Sony Color CCD, EZ420IR-30, ezCCTV.com Ltd, Herts, UK), were equally distributed around the shed's roof so that all of the pen could be viewed at a height of 6.5 m. The twelve cameras were connected to a high memory storage computer using Geovision (version 8, Geovision Inc., Taipei, Taiwan).

Animals were labelled with numbers to allow recognition on videos and numbers were remarked as required. To minimise disturbance, the marking procedure was performed before animals entered the pen, or when they were moved out of the pen once a week for farm's husbandry purposes (weight and body condition score).

## 2.3. Selection of the calvings and cow–calf pairs

Only calvings leading to live singleton purebred Holstein calves were considered. Retrospectively, between November 2008 and 2009, 193 N, 26 FN and 4 FM calvings fitted those criteria. Out of the 26 FN calvings, 17 FN could be considered (six had no video footage, two in which the calf could not be observed and one where the cow had a prolapsus) and 12 calvings were chosen at random among them. As only 4 FM calvings were available at the time of the study, they were grouped with the FN category and called subsequently assisted animals (A) as opposed to non-assisted animals (N). 12 N calvings were then recruited so that the non-assisted calving group and the assisted calving group were balanced as far as possible for the following criteria by order of importance: parity of the mother (primiparous vs multiparous), sex of the calf, calf's birth weight, genetic group of the mother/sire of

the calf, calving shed (and hence diet) and calving season (Summer: April to September; Winter: October to March). The mean birth weight  $\pm$  s.d. was  $42.8 \pm 5.0$  kg (A) and  $42.3 \pm 6.2$  kg (N), respectively.

For each of the recruited calvings, information on the body condition score (assessed on a five point scale by trained farm technicians) of the dam at calving and at conception were also retrieved. A summary of the characteristics of the calvings for both groups can be found in Table 1.

## 2.4. Data collection: video scoring

The recruited calvings were watched for 3 h continuously after the expulsion of the calf using Observer<sup>®</sup> XT 9 (Noldus, Wageningen, The Netherlands). After an initial training period, videos were observed by a single observer. Three 1-h video-clips, each from a different calving, were watched a second time to assess the intra-observer reliability of video scoring. Cohen's Kappa coefficients were obtained from the software and ranged from 0.61 to 0.72, suggesting a good agreement within the observer (Kaufman and Rosenthal, 2009).

The latencies of the calves to perform the first bout of the following neonatal behaviours once born were recorded: attempt standing, being supported on two feet, achieve standing, walk, reach the udder, and suck. Additionally, the time to achieve sternal recumbency (Schuijt and Taverne, 1994) as well as the total duration spent lying by the calf was assessed. The number of times and total duration of the calf lying on the flank was also recorded (for definitions, see Table 2).

To assess the maternal behaviour of the dam, the latency to lick the calf after having given birth as well as the amount of time spent on that activity was recorded. Furthermore the following behaviours that could be pain-related were recorded in the dams: duration of lying, self-grooming, walking and having the tail raised, frequency of posture transition, lying on the flank and tail switching as well as

**Table 1**  
Summary of the characteristics of the calvings used within the study. Number of animals available (n) within categories are displayed as well as the means of the birth weights of the calves, calving BCS, conception BCS and their standard deviation (s.d.).

		Assisted	Non-assisted
Parity of the dam	Primiparous	n = 8	n = 6
	Multiparous	n = 8	n = 6
Calf's sex	Bull calf	n = 8	n = 6
	Heifer calf	n = 8	n = 6
Birth weight (kg)	Mean $\pm$ s.d.	42.8 $\pm$ 5.0	42.3 $\pm$ 6.2
Genetic group	Control line	n = 6	n = 6
	Select line	n = 10	n = 6
Calving shed	Shed 1 (high forage)	n = 11	n = 5
	Shed 2 (low forage)	n = 5	n = 7
Calving season	Winter	n = 10	n = 6
	Summer	n = 6	n = 6
Calving BCS	Mean $\pm$ s.d.	2.2 $\pm$ 0.3	2.4 $\pm$ 0.3
Conception BCS	Mean $\pm$ s.d.	2.3 $\pm$ 0.3	2.2 $\pm$ 0.3

**Table 2**  
Definitions of the calf behaviours scored.

Lie flank	Calf is lying on the flank, with shoulder touching the ground and at least 3 legs extended. Head rests on the floor.
Sternal recumbency	The calf lies on his sternum with each front leg positioned on each side of his body.
Attempt standing	The calf has its four legs placed under its body, with the ventral part not touching the ground. Legs do not have to be fully extended.
On two feet	The calf is supported by two legs that are extended.
Achieve standing	The calf is supported by its four legs, all extended for at least 3 s.
Walk	The calf is in a standing position and does more than two steps.
Reach the udder	The calf's muzzle is near the udder. Neck and head are curved up as in a sucking position.
Suck	The calf is under the udder, teat in mouth, in a sucking position for more than 5 s.

the latencies to drink and to lie down (for definitions, see Table 3).

### 2.5. Data handling and statistical analysis

Two observations (1 N and 1 FM) were shorter than 3 h (2:50 h and 2:24 h, respectively) and therefore, percentage of time was used for the statistical analysis rather than duration. Because "tail raised" was scored only when the cow was standing, the duration of that event was adjusted by dividing by the amount of time the cow spent standing before proceeding to analysis. Observations on the tail for dam 1830 were discarded because of the exceptionally high number of tail switches performed probably as a result of a high number of flies present.

**Table 3**  
Definitions of the pain-related behaviours scored for dams.

Lie flank	Cow is lying on the flank, with shoulder touching the ground and at least 3 legs extended. Head rests on the floor.
Lie head rested	Cow is lying but not on the flank with her head resting on floor or part of the body.
Posture transition	Cow switching from a lying (body in contact with ground) to standing position (cow on her four legs).
Walking	Cow is in a standing position and makes at least two steps forward or backwards.
Self-grooming	Cow licking herself.
Tail switch	Tail swung to the side and then forcefully forward along the flank or directly back down (Miedema, personal communication).
Tail raised	Tail is raised and held away from the body (Miedema et al., 2011). Was scored only when the cow was standing.
Latency to drink	For the first time after calf is expelled, the cow is standing at water trough, muzzle touching it.
Latency to lie down	If the cow calved while standing, latency to lying was used. However, if the cow was already lying when she gave birth, the latency of the cow to lie after having stood for the first time was used.

Out of the 28 calves observed in the study, three assisted calves had been moved to the front of the cow at birth by the farm staff and ten calves were fed colostrum through stomach tubing within the 3 h observation period (7 A and 3 N).

Statistical analyses were performed using Genstat 11th Edition (2008, VSN International Ltd.). Data were first checked for normality using histograms and transformed when not (using logarithm, square-root and inverse function). However, most of the transformed data still did not fulfil the assumption of normality and were therefore analysed using non-parametric statistics.

Duration of licking performed by the dam and the amount of time spent lying on the flank by the calf (transformed with a cube root transformation) were analysed using Mixed Models. Characteristics of the calvings (genetic group, calving condition score, difference in body condition score between conception and calving, birth weight of the newborn, sex of the calf, calving season, parity of the dam, calving shed, whether the calf was moved in front of the cow in the case of a difficult calving and whether a human had given additional colostrum to the calf within the observation period) were considered for inclusion in the models as well as biologically relevant interactions and only significant terms and calving difficulty were kept. Duration of licking was analysed using parity of the dam, genetic group of the dam and calving difficulty as fixed effects. Calving season and calving difficulty were used as fixed effect for the analysis of the time spent lying flank.

A Kaplan–Meier analysis using a Wilcoxon Peto–prentice test was performed to analyse the latencies of behaviours when censoring occurred (due to at least one animal not performing a specific behaviour in the 3 h window). One assisted calf was excluded from the analysis on the latency to reach sternal recumbency as it had been put in that position by the farmer at birth.

Fisher's exact tests were applied to determine whether animals from one group rather than the other group were more likely to perform a specific behaviour. Otherwise, Kruskal–Wallis non-parametric one-way-ANOVA tests were used to compare the assisted and non-assisted groups, in which case the influence of other calving characteristics was also examined using the same test.

## 3. Results

### 3.1. Results on the vigour of the calf

Fisher exact tests showed that there was a tendency for calves from the assisted group to be less likely to stand ( $P=0.053$ ) and walk ( $P=0.088$ ) within the first 3 h of birth, compared to calves delivered naturally. They were not less likely to suck ( $P=0.243$ ) but only a third of the assisted animals actually achieved successful sucking within that period of time.

Calves assisted at birth took longer to first attempt to stand, achieve standing, walk and reach the udder (Table 4). However, there was no difference in the time taken to be in a sternal recumbency position, to be on two feet and to achieve a successful suck ( $P \geq 0.05$ ; Table 4).

**Table 4**

Effect of calving difficulty on the median latencies (min), lower and higher 95% confidence intervals (CI) of calves to perform neonatal behaviours after having been expelled, as given following a Kaplan–Meier analysis using a Wilcoxon Peto–Prentice test. Count and percentage of the animals expressing the behaviours within 3 h of life is presented for each treatment group.

	Assisted (n = 16)		Non-assisted (n = 12)		P-value
	Median [95% CI]	Count and (%)	Median [95% CI]	Count and (%)	
Sternal recumbency <sup>a</sup>	9.0 [5.7–25.1]	15 <sup>b</sup> (100)	9.0 [5.5–11.1]	12 (100)	0.435
Attempt standing	36.7 [14.8–49.7]	16 (100)	12.1 [10.5–17.6]	12 (100)	0.009
On two feet	43.3 [31.7–86.1]	15 (93.8)	35.2 [24.3–37.0]	12 (100)	0.106
Achieve standing	99.4 [78.4–>180 <sup>c</sup> ]	11 (68.8)	70.1 [62.6–84.2]	12 (100)	0.025
Walk	166.4 [89.0–>180 <sup>c</sup> ]	9 (56.3)	86.4 [73.5–93.9]	11 (91.6)	0.034
Reach the udder <sup>c</sup>	>180 [128.8–>180]	5 (31.3)	102.1 [86.4–112.6]	11 (91.6)	0.016
Stack <sup>c</sup>	>180 [163.1–>180]	4 (25)	170.8 [104.3–>180]	6 (50)	0.314

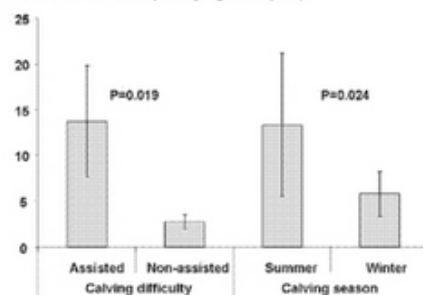
<sup>a</sup> Data presented for sternal recumbency result from a Kruskal–Wallis analysis and the median, lower and upper quartiles are presented.

<sup>b</sup> Values could not be estimated with the Kaplan–Meier analysis and therefore, the upper quartile of the raw data is presented instead.

<sup>c</sup> Values could not be estimated with the Kaplan–Meier analysis and therefore, the median, lower and upper quartiles of the raw data are presented instead.

<sup>d</sup> Excludes one calf placed in this position at birth by the farmer.

#### Total duration calf spent lying flank (min)



**Fig. 1.** Effect of calving difficulty (assisted (n = 16); non-assisted (n = 12)) and calving season (summer (n = 16); winter (n = 12)) on the total time spent lying on the flank by calves. Means and standard error of the means of the raw data are presented. Analysis was performed on transformed percentages of duration of the observation.

Furthermore, calves born from an assisted calving lay down on the flank for longer than calves born without assistance and so did calves born in summer compared to winter-born calves ( $P < 0.05$ ; Fig. 1). Assisted calves also performed more bouts of lying flank (median [lower and upper quartile]) (Assisted: 2 [1;4]; Non-assisted: 1 [1;2];  $P = 0.033$ ) and tended to spend more time lying overall during the observation period (median [lower and upper quartile]) (Assisted: 163.3 min [138.6; >180]; Non-assisted: 146.2 [121.5; 156.8];  $P = 0.051$ ).

#### 3.2. Onset and expression of the maternal behaviour

All dams but one assisted dam expressed maternal behaviours. This dam was kept in the analysis and given a censored value of 3 h for the latency to lick the calf and a null value for the duration of calf licking. No difference between assisted and non-assisted cows was found on the median latency to lick a calf (in min [95% confidence interval]) (Assisted: 7.2 [0.9–19.6]; Non-assisted: 2.1 [1.0–8.0];  $P = 0.259$ ) although cows calving in summer took longer to

lick their calf (in min [95% confidence interval]) (Summer: 14.8 [2.9–19.6]; Winter: 1.9 [0.6–5.9]; ( $P = 0.046$ )). As well, assistance at birth did not affect the amount of time (in % of observation time) spent licking a calf (Fig. 2;  $P = 0.295$ ). However, surprisingly, multiparous cows were found to spend less time licking their calf compared to primiparous cows and as did dams from the genetic group S compared to dams from the C group ( $P < 0.05$ ; Fig. 2)

#### 3.3. Pain-related behaviours in the dams

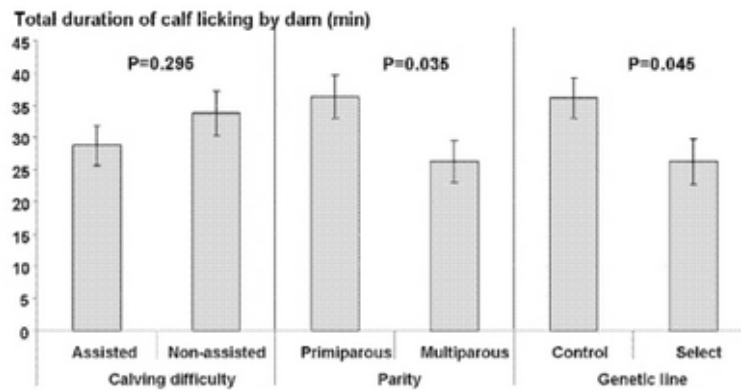
Fisher exact tests revealed that there was no effect of calving difficulty on how likely cows were to lie on the flank, lie on the flank more than once and lie with the head rested ( $P \geq 0.05$ ) but cows having had an assisted delivery tended to be less likely to have performed an episode of self-grooming ( $P = 0.088$ ). No effect was found on any other putative behavioural indicators of pain ( $P \geq 0.05$ ; Table 5) except that cows having had an assisted delivery performed self-grooming for a shorter period of time than cows who were not provided with assistance ( $P \leq 0.05$ ; Table 5).

## 4. Discussion

#### 4.1. Calves from assisted calvings are less vigorous

Calves from assisted births were slower to express most of the neonatal behaviours leading to reach the udder compared to calves delivered naturally. Some of them did not even stand up during the observation period. They also spent more time lying on the flank and had more bouts of this behaviour, which is a position that does not require a lot of effort to be maintained. Overall, our study shows that calves from an assisted calving have lower vigour than calves delivered naturally.

This finding is in line with previous reports of difficult delivery on vigour in dystocia dairy calves (Edwards, 1982; Diesch et al., 2004), beef calves (Adams et al., 1995; Riley et al., 2004; Hickson et al., 2008) and lambs (Dwyer, 2003; Dwyer and Lawrence, 2005). However, our study disagrees with a recent study (Vasseur et al., 2009) but this may be due to their higher frequency of assistance. Inclusion of malpresented calves in the present study could perhaps



**Fig. 2.** Effect of calving difficulty (assisted ( $n=16$ ); non-assisted ( $n=12$ )), parity (primiparous ( $n=14$ ); multiparous ( $n=14$ )) and genetic line (control ( $n=16$ ); select ( $n=12$ )), on the duration spent by the dam licking her calf. Back-transformed predicted means and their standard errors are presented. Data were analysed using the percentage of duration of the observation.

have driven the effect but investigation of the effect of malpresentation from four calves was not possible and calf malpresentation was reported as not having a significant effect on latency to stand and suck (Edwards, 1982).

Lower vigour at birth of dystocical calves can be explained by the presence of traumatic lesions (Berglund et al., 2003; Aksoy et al., 2009; Mee, 2010), longer durations of labour (Berglund et al., 1987), (prolonged) obstetrical intervention, and metabolic acidosis as a result of hypoxia or asphyxia (Tyler and Ramsey, 1991; Mellor and Stafford, 2004; Alonso-Spilsbury et al., 2005). The latter impacts on the calf's adrenal function and metabolism and reduces the muscular tonicity and the ability of the calf to thermoregulate (Vermorel et al., 1989; Adams et al., 1995; Bellows and Lammoglia, 2000).

Despite lower vigour highlighted in assisted calves in our study, latency to the first sternal recumbency did not differ between groups, perhaps because this position does not require as much coordination or muscular effort to achieve in comparison to other behaviours. Only 10 out of our 28 experimental calves sucked within their first 3 h of life. Median time to standing in our study (70 min in non-assisted animals) was slightly quicker than reported

previously in dairy calves (Edwards and Broom, 1982) (median time of 105 min and 130 for first and later parities respectively), but was much longer than the hour within which beef calves were reported to stand (Hyslop et al., 2008; Hickson et al., 2008). Contrary to those studies, we did not find an effect of parity of the dam or sex of the calf on calf's vigour. In our study, calves spent more time on the flank in summer rather than in winter. It is possible that in cold environments, for thermoregulatory purposes, the calf would be more motivated to lie on the sternum because of the lower energy losses compared to lying on the flank. In other studies, Brahman calves, which may not thermoregulate well in cold environments (Godfrey et al., 1991) are reported to be less vigorous at low temperatures (Riley et al., 2004).

Lower vigour at birth raises longer-term consequences for the calves. Absorption capacity of immunoglobulins decreases rapidly after birth. Prompt sucking after birth maximises adequate acquisition of passive immunity (Beam et al., 2009). Current recommendations suggest that first meal of colostrum should occur within the first 3–4 h of life (Morrison et al., 2010). In the present study, only a third of the assisted calves had reached the udder by 3 h.

**Table 5**

Effect of calving difficulty (assisted ( $n=16$ ); non-assisted ( $n=12$ )) on the putative behavioural indicators of pain postpartum in cows. Medians [lower and upper quartile] of the raw data are reported following Kruskal–Wallis test. Medians [lower and higher 95% confidence intervals] are reported following a Kaplan–Meier analysis using a Wilcoxon Peto–Prentice test.

	Assisted ( $n=16$ )		Non-assisted ( $n=12$ )		P-value
Self-grooming <sup>a</sup> (s)	3.8	[0–16.7]	25.4	[0–138.8]	0.033
Lying duration <sup>a</sup> (min)	60.4	[20.0–105.5]	53.3	[8.7–72.9]	0.367
Walking <sup>a</sup> (min)	1.8	[1.2–3.5]	3.1	[1.9–5.7]	0.104
Tail raise <sup>a</sup> (% of standing)	75.7	[63.1–94.9]	76.4	[39.6–81.8]	0.367
Tail switches <sup>a</sup> (count)	24.8	[13.5–123.3]	98.2	[26.1–178.2]	0.294
Posture transitions <sup>a</sup> (count)	4	[2.5–6]	4.5	[3–9.5]	0.607
Latency to drink <sup>b</sup> (min)	145.2	[112.2–180 <sup>c</sup> ]	109.8	[97.6–161.9]	0.258
Latency to lie down <sup>b</sup> (min)	97.9	[61.3–117.3]	103.1	[63.7–142.8]	0.926

<sup>a</sup> Kruskal–Wallis analysis.

<sup>b</sup> Kaplan–Meier analysis.

<sup>c</sup> Value could not be estimated and therefore the upper quartile of the raw data is presented instead.

Assisted calves took at least 80 min longer to reach the udder than calves born naturally and this may have consequences on their immunity. Although the farmer may feed colostrum quickly after birth, this is largely dependent on calving time, human supervision and labour availability. The ability of the calf to suck quickly is all the more important in dairy systems that allow natural suckling in the first days or even weeks of life (Krohn, 2001). Moreover, when fed colostrum artificially, calves with lower vigour are willing to ingest lower amounts of colostrum (Vasseur et al., 2009). As a consequence, this can result in an inadequate transfer of the immunoglobulins as found in dystocial calves (Vermorel et al., 1989; Waldner and Rosengren, 2009; Gasparelli et al., 2009), contributing to their poor survivability and higher morbidity compared to calves from a natural birth (Chassagne et al., 1999; Johanson and Berger, 2003; Lombard et al., 2007). Furthermore lower growth at weaning has been reported in dystocial beef calves (Bellows et al., 1988; Goonewardene et al., 2003) as well as in lambs with low vigour (Matheson et al., 2010).

#### 4.2. No evidence of an impaired maternal behaviour

The onset of maternal behaviour, as assessed by the latency of the dam to lick the calf, as well as the quantity of maternal behaviour measured through the amount of time the dam spent grooming her calf, was not affected by assistance being given at delivery in our study. This disagrees with our initial hypothesis and the observations made on other species (Alexander et al., 1988; Dwyer et al., 2001; Fisher and Mellor, 2002).

Nonetheless, in our study, the low number of animals and high individual variation may have masked any underlying effect. Large individual variation could result from various levels of difficulty in the group of assisted animals as well as the potential presence of dams in the non-assisted group that may have benefited from assistance. Maternal behaviour is not a trait used for genetic evaluation and selection in dairy cattle, which might result in larger variation in its expression. Additionally, in three of the assisted births observed, the calf was moved in front of the cow by the farm staff, potentially adding an additional stimulus to the dam and introducing a slight bias.

The presence of the calf was likely to be an important motivator for the cows despite potential exhaustion and pain. A longer time window when time allocation to the care of the calf diminishes may have helped finding differences in the maintenance of maternal behaviour after a difficult calving. However, in sheep, the motivation of the ewe to lick her lamb at birth, reflects her motivation to do so throughout her lactation (Pickup and Dwyer, 2011).

In our study, cows from the selected line licked their calves less than cows that were selected towards the UK average. This indicates that selection of cows towards milk production might have been unfavourable to the expression of this behaviour. Contrary to the literature (Edwards and Broom, 1982; Dwyer and Lawrence, 2000; von Keyserlingk and Weary, 2007), we found that the

primiparae licked their calves more than the multiparae. This might be an artefact from our study.

#### 4.3. Behavioural indicators of postpartum pain in the dams

Cows receiving assistance at delivery performed less self-grooming than cows who delivered naturally. Grooming is an important behavioural trait in ruminants. A change in that behaviour is often interpreted as an indicator of pain or discomfort and therefore commonly used as a behavioural measure of animal pain (Molony and Kent, 1997; Rutherford, 2002; Weary et al., 2006). Self-grooming has previously been used as an indicator of postpartum pain in cows (Kolkman et al., 2010) and was found to decrease in female rats following gynaecologic pain (Tong et al., 2006). Thus, in our study, a decrease in self-grooming in the dystocial dams could indicate more pain or discomfort than in cows delivering naturally. However, contrary to our expectations, there were no significant differences in any of the other indicators which could have supported this finding. We suggest that our study does not give enough evidence to support higher pain levels in dystocial dams in the 3 h postpartum.

The 3 h observation period might have been a limitation in finding any differences between the groups and a longer observation period investigating the recovery of the dams might have been more suitable. However, in a study comparing behavioural indicators of pain in beef cows undergoing delivery by caesarean or per vaginam up to 14 days postpartum, most of the differences were found in the 24 h following delivery (Kolkman et al., 2010). This suggests that studies focusing within a day after delivery may be more appropriate. A day long observation would have been compromised by dams entering the milking herd within 24 h of the birth. Additionally, we explored only some of the behavioural indicators of pain and other indicators could have shown differences. It is highly likely that the worst of the pain was prior to the calf being expelled and therefore levels of pain afterwards may not differ between assisted and non-assisted dams. For example, behavioural differences have been found during stage 1 of parturition in dystocial dams and were suggested to be related to early expression of pain (Wehrend et al., 2006).

Finally, as for maternal behaviour, there was a large individual variation in the expression of the behavioural indicators of pain observed. This might reflect divergences in the sensitivity to, and expression of, pain between the dams as well as the potential variability of difficulty that was experienced over the previous stages of parturition. A larger sample size would probably help in detecting any differences.

#### 5. Conclusion

Calving difficulty, as assessed through assistance provided at the time of delivery, resulted in lower vigour in dairy calves. Low vigour is known to have a longer term impact on the health and survival of the neonates. Therefore, this raises concerns for the welfare of calves from a difficult delivery. Contrary to our expectations, maternal

behaviour was not altered and behavioural indicators of pain did not indicate higher level of pains. However, there was a high individual variation in the dams that might have masked differences.

#### Conflict of interest statement

The authors have no conflict of interest to declare.

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